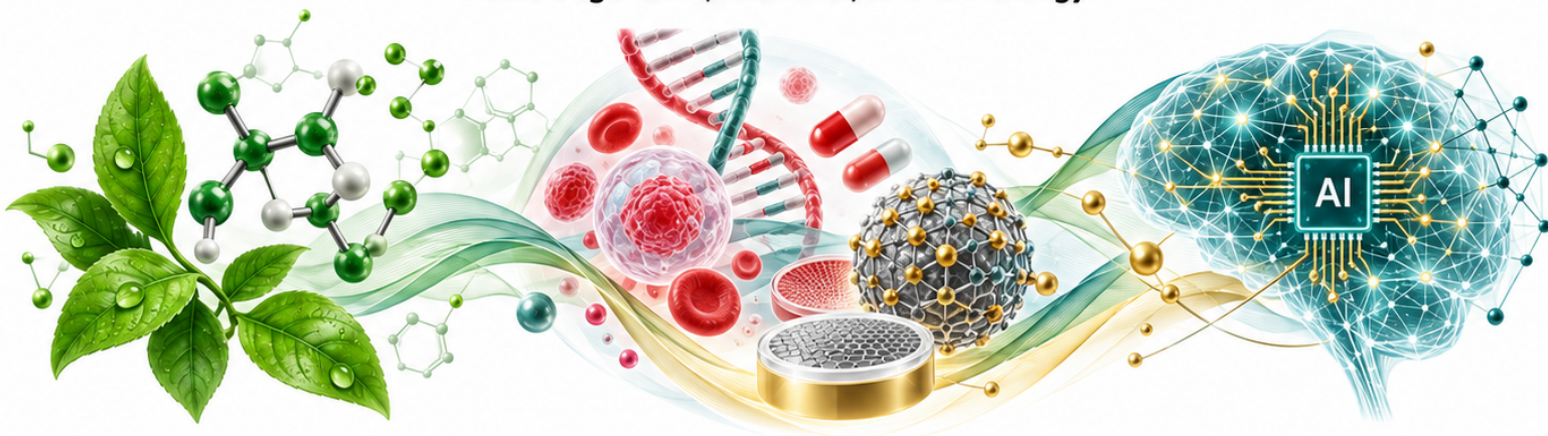




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Copper Oxide–Based MOS Capacitors for High-Frequency Applications

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Abstract

Cu₂O is an important material for optoelectronics and communication technologies because of its suitable bandgap, strong light absorption and potential for low-cost device fabrication. Improving its crystallinity is essential to reduce defects, enhance charge transport and achieve better optical and electronic performance, leading to more efficient and reliable devices. Herein, copper oxide thin films deposited onto glass and n-Si silicon substrates and partially recoated with molybdenum nanosheets were employed as high frequency metal-oxide-semiconductor (MOS) capacitors. As a result, Mo-coating improved the crystallinity and lowered the defect density in CuO. Optically, Mo nanosheets widened the energy band gap without significant variation in absorption spectra of the glass/CuO films. The MOS capacitors also displayed accumulation-depletion-inversion switching characteristics in the frequency domain of 1.0-100 MHz allowing for ultrafast charging/discharging cycle within 10 ns. In addition, Mo-coating lowered the built in potential and tuned the accumulation-inversion switching mode. Moreover, treating the MOS devices as high frequency bands filters in the range of 1.0 M- 6.0 GHz showed the ability of the devices to transmit/reject signals in three high frequency bands centered at 1.02 GHz, 1.47 GHz and 3.30 GHz. The ideal reflection coefficient values approaching zero, return loss characteristics exceeding -43 dB and voltage standing wave ratios (VSWR) equal 1.0 were obtained at notch frequency of 3.30 GHz; the bandwidth at this frequency reaches 2.0 GHz. The features of the CuO-based MOS capacitors presented by ultrafast mode switching, high frequency filtering, wideband-frequency control, large values of return loss and ideality of VSWR nominated them for 6G technology applications.

Keywords: n-Si/p-CuO, Mo nanosheets, MOS, Band filter, 6G, High frequency.

1. Introduction

Copper oxide thin films attracted the interest of researchers and industry sector due to their smart applications and CeO₂-doped CuO performed as supercapacitors exhibiting areal capacitance of 0.14 F/cm² [1]. These capacitors displayed superior electrochemical stability showing retention of 100% and displayed Coulombic efficiency of 99.4% [1]. More specifically, CuO based solar cells showed open circuit voltage of 0.59 V, short circuit current density of 16 mA/cm² and filling factor of 60% indicating a promising characteristic for future solar cell applications [2]. In addition, hydrothermally grown copper oxide thin films illustrated memristive phenomenon presented by pinched hysteresis loop applicable as resistive random access memories [3]. Moreover, copper oxide thin films deposited onto Si substrates by chemical spray pyrolysis technique exhibited photodetectors characteristics showing current responsivity of 2.85 A/W, external efficiency of 279 % and specific detectivity of 2.7×10^{12} Jones when exposed to visible light illumination [4]. As an important feature, CuO was employed to enhance the reception of terrestrial broadcasting and integrated serviced digital broadcasting in the 470-770 MHz frequency band [5]. CuO modified antennas showed average gain of -1 dBi at notch frequency of 600 MHz [5]. The bandwidth of this device reached 300 MHz.

In spite of its important technological applications, copper oxide demonstrated short diffusion lengths between 100 nm and 200 nm [6]. Such a property prohibits charge separation process leading to photodegradation effects [6]. Copper oxide suffers from rapid capacity fading at high levels of charging/discharging rates negatively affecting the charge storability [7]. Different physical and chemical methods were employed to enhance the electrical conductivity and chemical stability of CuO thin films [7]. One of these methods employed the molybdenum as a doping agent to enhance the electrical properties of CuO [8]. Mo doping enhanced the electrical conductivity and specific capacitance as well. Mo-doped CuO nanosheets exhibited specific capacitance of 1392 F/G at 2A/g, revealed energy density of 36 Wh/kg at 810 W/kg and displayed cycle life of 81% after retention of 5000 cycles.

The important applications of CuO in wide range of technology and the enhancements achieved via Mo-corporation into the structure of CuO represented a motivation for the production of a multichannel device that can perform as Metal-Oxide-Semiconductor (MOS) capacitors and as high frequency band filters. Particularly, the work here was concerned with the production of multichannel device made of CuO films deposited onto n-Si substrates. One of the channels was coated with Mo nanosheets of thickness of 100 nm to enhance the electrical performance as suggested in literature [8]. The produced devices were studied by means of X-ray diffraction, impedance spectroscopy and network analysis spectroscopy. The effect of Mo nanosheets on the accumulation, depletion and inversion properties of the capacitance -voltage characteristics in the frequency domain of 1.0-100 MHz was explored to determine the shortest possible charging/ discharging time scale so that the constructed MOS device could be utilized in high frequency applications. In addition, the band filtering characteristics of the device channels were tested in the frequency domain of 1.0 M-6.0 GHz to determine the notch frequency. The pass/reject bands and their return loss value and voltage standing wave ratios were investigated so that their suitability for 6G applications could be determined. As an important information needed to proof the role of the energy band gap widening on the MOS device performance, the optical transmission, reflection and absorption of the CuO and CuO/Mo structures were studied and discussed.

2. Experimental details

Copper oxide thin films ($1.0\ \mu\text{m}$) were deposited onto ultrasonically and chemically cleaned glass and n-Si substrates ($300\ \mu\text{m}$ thick wafers) using NORM-600 VCM thermal evaporator under a vacuum pressure of 10-5 mbar. The thickness of the CuO films was in situ monitored using Inficon-STM-2 thickness monitor installed in the thermal evaporator. The substrate temperature was 300 K and no annealing process were carried out. The source material was CuO powders of 99.99% purity ordered from Alfa Aesar firm. Some of the produced glass/CuO and n-Si/CuO films were masked (square masks) and installed in an ion coater to deposit a molybdenum layer of thickness of 100 nm. The Mo target was of very high purity (99.99%, Alfa Aesar). The thickness of the nanosheets was determined by a SOLID INC. II profilometer. Some of the n-Si/CuO and n-Si/CuO/Mo films were point contacted with Ag (99.999%) point contacts to fabricate capacitors of contact area of $0.0314\ \text{cm}^2$. The rear side (n-Si) of the device arrays was coated with Ag. The schematic of the device was shown in inset-1 of Fig. 1. The structural properties of the produced films were explored using Miniflex 600 XRD unit, and the capacitance-voltage characteristics were recorded in the frequency range of 1.0-100 MHz using an Agilent 4291B-1.0M-1.80 GHz impedance analyzer. The magnitude of the reflection coefficient spectra in the frequency domain of 1.0M-6.0 GHz was measured using Rohde & Schwarz network analyzer 9 kHz-6 GHz. The optical spectra were measured using Thermo-Scientific Evolution 350 spectrophotometer equipped with VEE-MAX-II PIKE variable angle reflectometer. The p-conductivity type of CuO was measured by the hot probe technique.

3. Results and Discussion

The current study targeted the design and fabrication of high frequency ($\sim 100\ \text{MHz}$) depletable metal-oxide-semiconductor (MOS) capacitors made of copper oxide thin films deposited onto n-Si wafers. The design considered the presence of two channels. One was as it was grown composed of n-Si/Cu₂O (abbreviated as SCM-00) and the other was n-Si/CuO top coated with 100 nm thick Mo nanosheets (SCM-100). The schematic for the two channels is shown as inset-1 in Fig. 1. Fig. 1 also shows the X-ray diffraction patterns for the n-Si substrates, n-Si coated with CuO and n-Si/CuO coated with Mo nanosheets. Better details about the observed patterns are presented as inset-2 in Fig. 1. While SCM-00 films displayed very broadened peak centered at $2\theta = 31.78^\circ$ and assignable to the monoclinic phase of CuO ($a = 4.83\ \text{\AA}$, $b = 3.76\ \text{\AA}$, $c = 5.74\ \text{\AA}$, $\beta = 99.18^\circ$ and space group of C_2 (group no: 5)), films coated with 100 nm thick Mo nanosheets displayed much sharper and narrower peak centered at $2\theta = 32.85^\circ$. Both of the peaks were assigned to the same crystal structure along the (111) direction for the peak of SCM-00 and along (110) for SCM-100. The lattice parameters used to explore the observed XRD patterns were partially related to the international center for diffraction data PDF card no: 05-0661). They were also consistent with literature data [9].

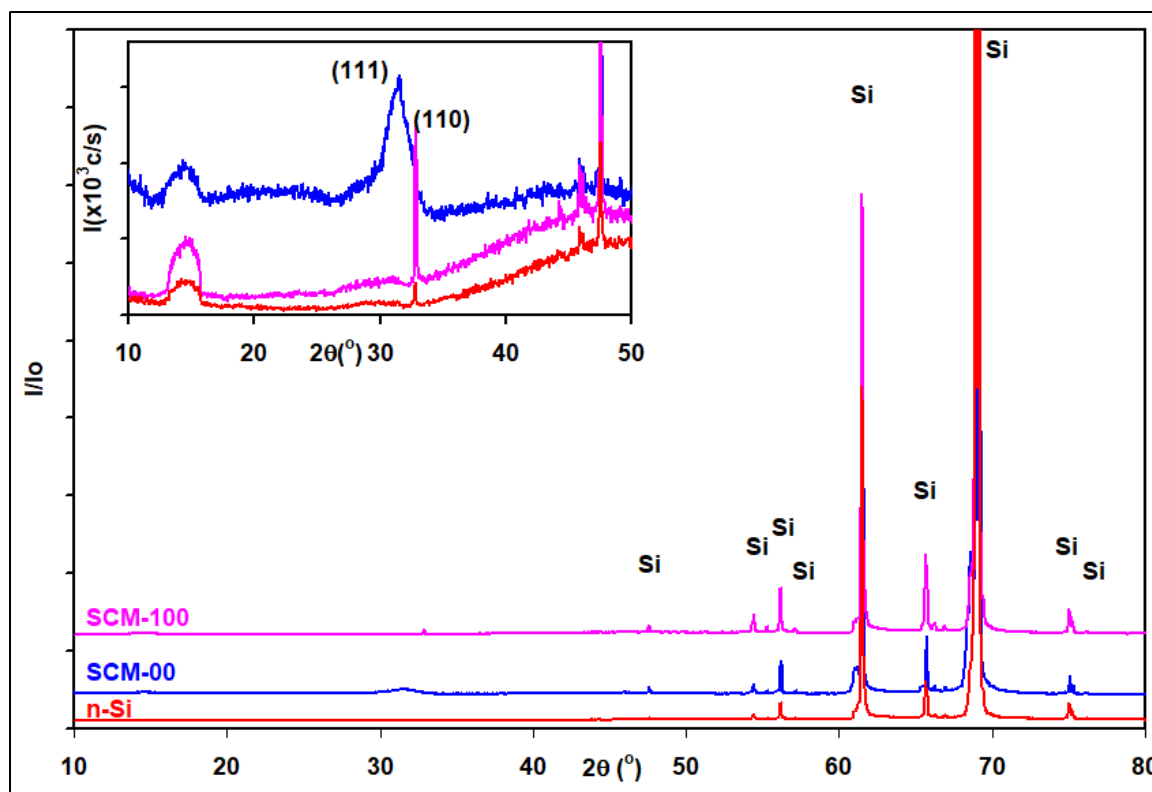


Figure 1. The X-ray diffraction patterns for Si/CuO and Si/CuO/Mo films. Inset-1 showing the schematic for the device and inset-2 showing an enlargement of the XRD patterns in the low diffraction angles ranges (25-40 o).

Most of the peaks relating to the monoclinic structure were absent from the XRD profiles due to the high degree of disorder in the CuO layer. Attempts to explore the role of Mo nanosheets on the structural properties of CuO included calculation of the crystallite size ($D = 0.94\lambda/(\beta \cos(\theta))$), the microstrain ($\epsilon = \beta/(4 \tan(\theta))$) and the dislocation density ($\delta_c = 15\epsilon/(aD)$) alongside one of the crystal axis [10]. Mo nanosheets increased D and decreased ϵ and δ_c values of CuO from 5.0 nm, 2.85×10^{-2} and 1.59×10^{13} lines/cm² to 43 nm, 2.96×10^{-3} and 1.78×10^{11} lines/cm², respectively. The remarkable enhancement in the crystallite size, decreased in microstrain and in defect density by two orders of magnitude, indicated that Mo nanosheets played a vital role in the structural properties of CuO films. The most probable reason for these enhancements was the ionic substitution of Mo+6 (ionic radius is 62 pm [11]) or Mo+4 (68 pm [11]) in sites of Cu+2 (73 pm [12]). Diffusion of Mo ions in sites of CuO, whose bond length was 192.8 pm [13], established bonding between Mo and O forming shorter bonds of Mo-O (bond length is 182.5 pm [14]). Increased number of bonds decreased the defect density because the defects mostly originate from Cu cation interstitials or oxygen atoms occupying four coordinated sites in CuO [15]. The crystallinity enhancements, achieved via Mo nanosheets, resulted in improved carrier confinement within the active region of electronic device [16]. The confinement enhanced the efficiency of charge carrier transport leading to higher carrier mobility [16].

Fig. 2 (a), (b) and (c) show the capacitance (C)- voltage (V) characteristic curves for the device under study (DUT) being recorded at various frequencies in the range of 1.0-100 MHz. It is evident from the figures that the DUT can be depleted showing a frequency and voltage dependent depletion regions marked by pinky shadows in Fig. 2(a)-(c). The slope of the $C - V$ curve in that region was sharper for SCM-100 channels than that of SCM-00. Notably, the higher the imposed ac signals frequency are the less the difference between the

accumulation and inversion regions and the narrower the depletion range are. For a standard MOS device, the surface potential $\phi_s \approx 0.0$ eV in the accumulation region, where the applied voltage, was less than the flat band voltage (V_{fb}) [17]. As the applied voltage exceeded V_{fb} (shown in Fig. 2 (a)), the device was in the depletion region and the surface potential increased with increasing applied voltage reaching a value of $\phi_s = 2\phi_b$ at the inversion region ($V = V_t$ in Fig. 2 (a)) [17]. With increasing ac signal frequency, the accumulation and inversion regions widened, whilst the depletion region narrowed. Numerically, for SCM-100 channel, where the $C - V$ variation, was more pronounced, V_{fb} exhibited values of -0.72 V, -0.45 V and -0.38 V whilst V_t exhibited values of $+0.49$ V, $+0.28$ V and $+0.13$ V, at signal frequency values of 1.0 MHz, 10 MHz and 100 MHz, respectively. Physically, negative applied voltage leads to flow of more holes that accumulate on the surface of the channel till the applied voltage become slightly positive repelling holes hence forming a depletion region with few free charge carriers. More positive voltage makes the flow of the minority carriers (electrons) more dominant at the surface leading to the inversion mode. Remarkably, this behavior is important for the onset of the digital MOS field effect transistors (MOSFET). MOSFET is “ON” and current flows between drain and source when the device reaches the inversion mode. The high frequency operation mode reaching 100 MHz indicates high switching speed. Practically, this means the MOS device at 100 MHz which corresponds to 10 ns period of time must charge/discharge every 5 ns per half cycle. Such property makes the device suitable as $5G/6G$ radio frequency amplifiers and in digital circuits the support high speed digital logic “CPU” unit. SCM-100 channels with these features can also be nominated for fast voltage-controlled tuning in antennas and band filters [18].

In order to understand the physics laying beyond the performance of the device, an abrupt depletion approximation analysis was held assuming the validity of the equation [17],

$$C^{-2} = \frac{2(V - V_{bi} - \frac{kT}{q})}{qA\epsilon_r N} \quad (1)$$

Here $A = 0.0314$ cm² is the contact area, V_{bi} is the flat band potential and ϵ_r is the dielectric constant. $\epsilon_r = 29.5$ and 17.1 for SCM-00 and SCM-100, respectively, was measured by an impedance analyzer at 1.0 GHz (high frequency limit). N is the reduced charge carrier concentration ($N = \frac{N_A N_d}{N_A + N_d}$; N_A , N_d are the acceptor and donor concentrations, respectively). Representative linear fitting of the $C^{-2} - V$ variation at 1.0 MHz is illustrated in Fig. 2 (d). The linear plots allowed calculation of the built in potential and the reduced charge carrier density as function of signal frequency. The frequency dependent V_{bi} and N is illustrated in Fig. 2 (e) and (f), respectively. For SCM-00 channel, the figures indicated a slow decrease in the built in potential with increasing signal frequency in the range of 1.0 - 50.0 MHz. For the same channel, similar behavior of $N - f$ variation was also observed in the frequency range of 2.0 - 100 MHz. The built in potential increased from 1.06 eV to 1.36 eV as the frequency increased from 50 MHz to 100 MHz. For this channel, the inability of the free charge carriers to follow the ac signals lay beyond the decrease in N and increase in V_{bi} at high frequencies (50 - 100 MHz). On the other hand, SCM-100 channel (Fig. 2 (e) and (f)) show an increasing V_{bi} and N with increasing signal frequency. Further, the value of the built in potential in SCM-100 channel was much less than at the SCM-00 channel. It was also noted that the value of N decreased upon coating the surface of CuO with Mo nanosheets.

The effect of Mo nanosheets as a doping agent presented by diffusion of Mo ions into sites of Cu+2 led to the decrease in the built in potential at a particular frequency. The relation of the work function of CuO (ϕ_{CuO}) to the reduced carrier concentration N could be read from the equation [17],

$$\phi_{CuO} = \chi_{CuO} + \frac{E_g}{2} + \frac{kT}{q} \ln\left(\frac{N}{n_i}\right) \approx 5.40 \text{ eV} \quad [19] \quad (2)$$

$$V_{bi} = |\phi_{Mo}(4.6 \text{ eV}) - \phi_{CuO}(5.40 \text{ eV})| = 0.80 \text{ eV} \quad [20] \quad (3)$$

Here, $\chi_{CuO} = 4.07 \text{ eV}$ [19], $E_g = 2.05 \text{ eV}$ (measured in the laboratory) and n_i is the intrinsic carrier concentration in CuO. Eqn. (2) and Eqn. (3) indicated that $V_{bi} \propto -\ln(N)$ which means the doping should increase N . However, this is not the case observed in Fig.2 (f). Even after Mo coating, N of Si/CuO is still larger than Si/CuO/Mo structure. Considering Mo+6 ions replacing Cu+2 in p-type CuO, then excess four electrons are donated into CuO [21]. As the defect density decreased (XRD analyses) owing to the filling of copper vacancies and completed bonding of Mo with oxygen interstitials, adding electrons to the p-type CuO compensates holes, converts the material to n-type and introduce localized states that in turn narrows impurity bands [22]. Narrowing of the impurity bands results in an increase in the energy band gap width [22].

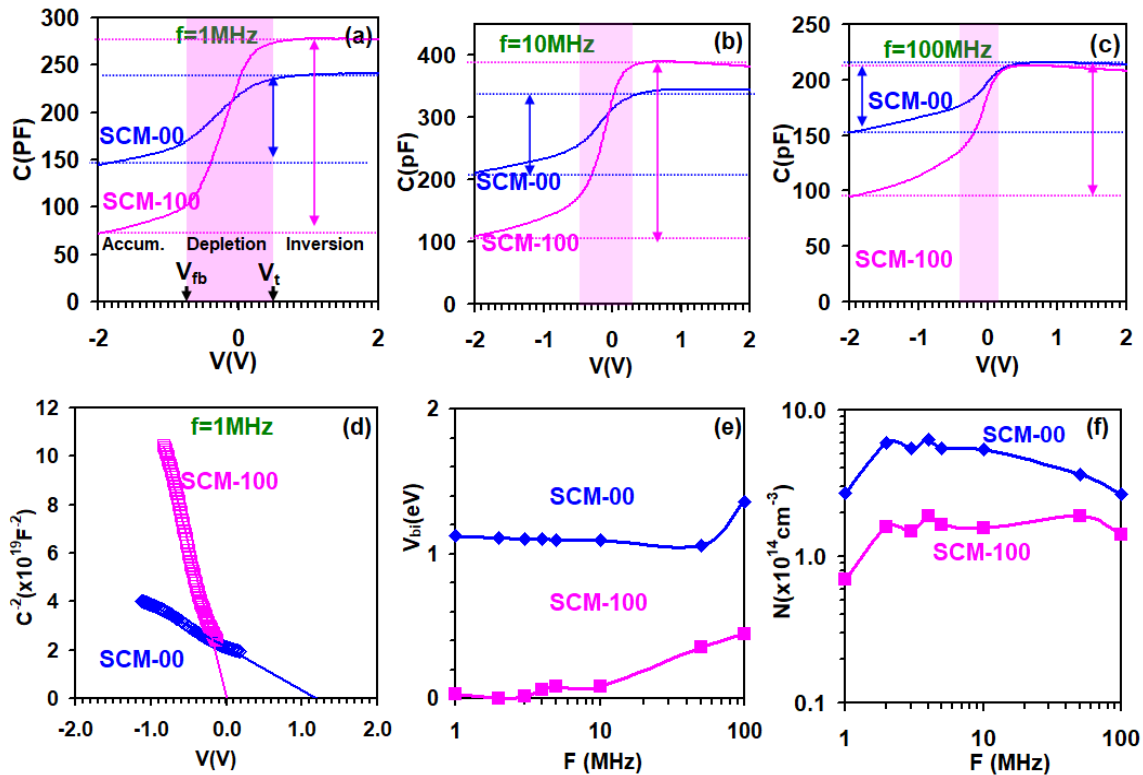


Figure 2. the capacitance-voltage characteristics for CuO-based devices measured at (a) 1.0 MHz, (b) 10.0 MHz and (c) 100 MHz. (d) are the abrupt depletion analyses presented by $C^{-2} - V$ variation for one of the $C - V$ curves and the frequency dependence of (e) the built in and (f) the reduced carrier concentration of CuO based MOS devices.

The band gap widening was confirmed from the absorption coefficient spectra and the related analyses, which are presented in Fig. 3 (a) and (b). The absorption coefficient (α) spectra, which were determined from the measured transmittance (T) and reflectance (R) spectra for films coated onto glass substrates (abbreviated as GCM-00, GCM-100; G is glass) to reserve light transmission were calculated from the relation [10],

$$T = (1 - R_{glass})(1 - R_{CuO})(1 - R_{CuO/Mo}) \exp(-\alpha d) \quad (4)$$

Here d is the total film thickness. T and R spectra displayed in Fig. 3 (a) indicated slight effect of Mo nanosheets on the transmittance and reflectance spectra of CuO. T spectra were slightly redshifted, whilst reflectance spectra were blue-shifted showing larger R values for GCM-100 films. The absorption coefficient spectra, which are illustrated in Fig. 3 (b), showed large decay of α values with decreasing incident photon energy exhibiting a local absorption peak centered at photon energy of 1.79 eV and 1.71 eV for GCM-00 and GCM-100, channels, respectively. The local peak and the blue shift in α spectra were better visible from the inset of Fig. 3 (b). This peak was different from the peaks appearing in T spectra at 2.18 eV and in R spectra at 1.61 eV and 1.68 eV for GCM-00 and GCM-100, respectively. The maximum transmittance value occurring at $E = 2.18$ eV corresponds to the limit of “yellow” excitonic series in Cu₂O revealing optical transitions originating from $\Gamma_{7v}^+ - \Gamma_{6c}^+$ of the first Brillouin zone of Cu₂O [23]. The energy values near 1.60 eV were attributed to the oxygen vacancies situated at the surface of the films [24]. The energy values revealing local absorption maxima at 1.79 eV correspond to transitions in CuO rather than Cu₂O [25]. On the other hand, the Tauc's equation $((\alpha E)^2 \propto (E - E_g))$ [10]) fittings illustrated in Fig. 3 (c) indicated that the E –axes crossings revealed an energy band gap of value of 2.05 eV that blue-shifts to 2.20 eV upon coating of CuO with 100 nm thick Mo nanosheets. Hence, by experiment, the energy band gap widened because of Mo diffusion resulting in an increase in the concentration of electrons and forcing compensation of holes.

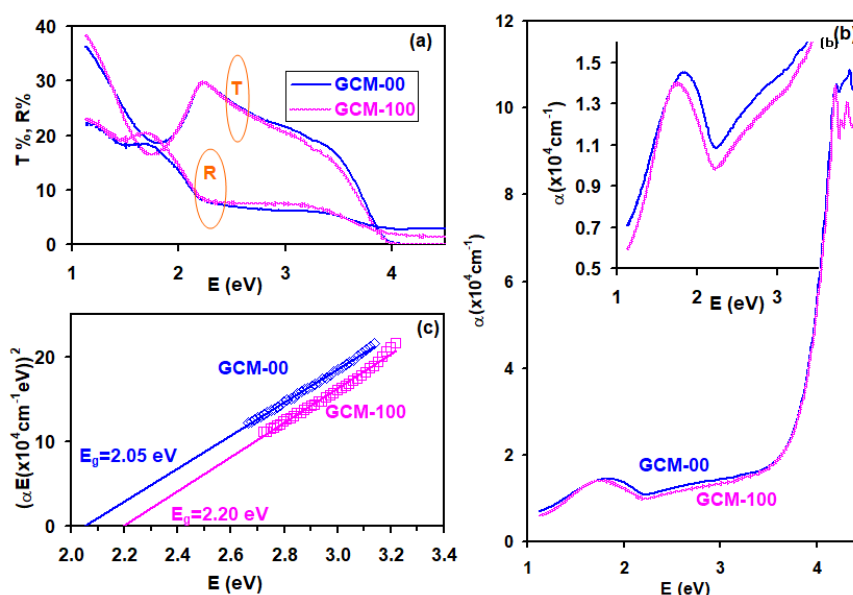


Figure 3. (a) the transmittance and reflectance spectra, (b) the absorption coefficient spectra and (d) the Tauc's equation fittings for glass/CuO films before and after coating with Mo nanosheets. Inset of (b) showing the low absorption range.

In addition to the ultrafast charging/discharging facility of the SCM-xx devices suiting 6G technology applications, the electrodes of the device channel were imposed between the terminals of a Rohde & Schwarz R&S ZVL network analyzer workable in the range of 9 kHz to 6 GHz. The measured absolute value of microwave reflection coefficient “ S_{11} ” spectra are illustrated in Fig. 4 (a). It is evident from the figure that a good correlation between the S_{11} parameters of both channels existed. Three main minimal peaks were detected in the spectra of SCM-00 channels, namely S_{11} of SCM-00 exhibited values of 0.146, 0.17 and 0.02 at 1.02 GHz, 1.47 GHz and 3.27 GHz, respectively. Coating the channel with 100 nm Mo nanosheets increased the respected values to 0.121, 0.189 and 0.0074. The absolute minima detected at 2.27 GHz in SCM-00 were

observed at 3.30 GHz in the S_{11} spectra of SCM-100 channels. The return loss ($L_r = 20 \log(S_{11})$ [26]) spectra for the DUT are displayed in Fig. 4 (b). The first local minima observed at 1.02 GHz and 1.47 GHz exhibited L_r values of 16.6 dB and -15.6 dB, respectively, whilst the absolute minima centered at 3.27 eV exhibited L_r value of -32.4 dB. Coating the CuO layer with Mo nanosheets increased the return loss values at 1.02 GHz and at 3.30 GHz to -18.3 dB and -42.59 dB, respectively. In addition, the voltage standing wave ratios ($VSWR = \frac{1+S_{11}}{1-S_{11}}$) spectra presented in Fig. 4 (c) revealed values of 1.28, 1.46 and 1.01 at 1.20 GHz, 1.47 GHz and 3.30 GHz, respectively.

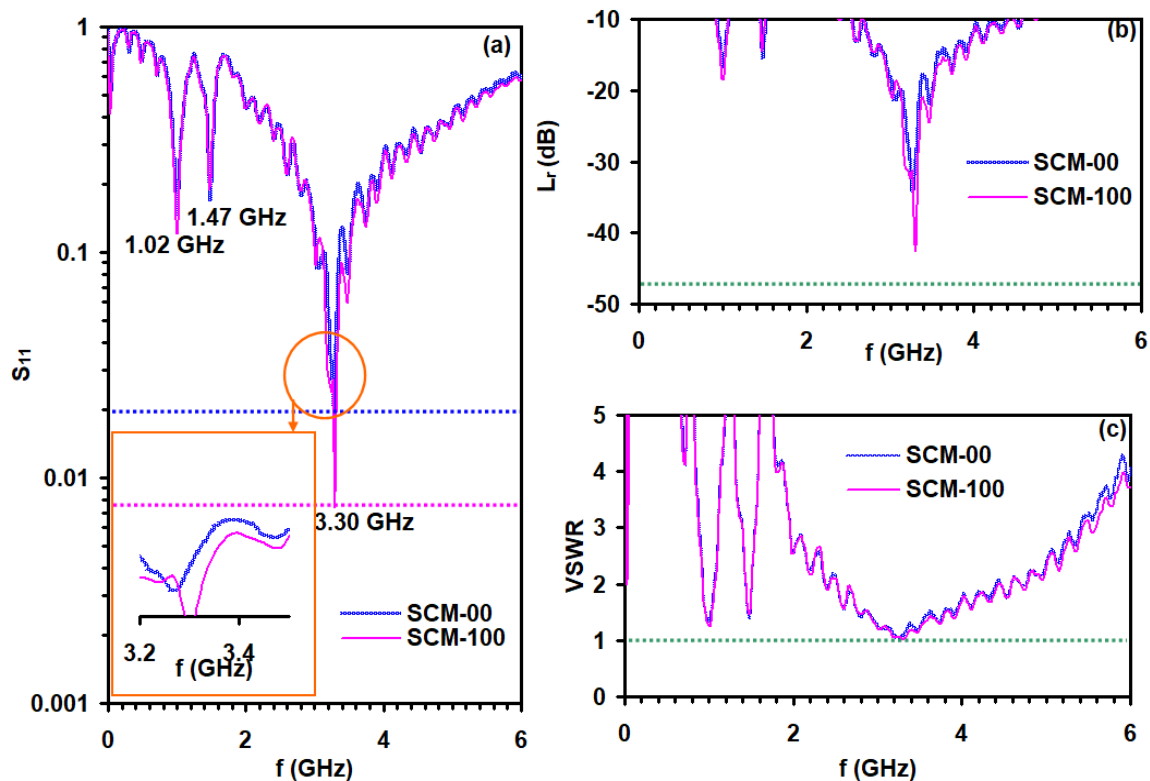


Figure 4. (a) the magnitude of the reflection coefficient spectra, (b) the return loss spectra and (c) the voltage standing wave ratios for SCM-xx devices being studied in the microwave frequency range of 1.0 M-6.0 GHz. Inset of (a) showing the center frequency shift of the absolute minima.

S_{11} parameter represents the value of the power reflected back at the notch frequency (stop band frequency). The return loss parameter measures the power that is not absorbed by the SCM-xx device and therefore is returned to the network analyzer. Acceptable values of L_r exceed -10 dB whilst excellent values are assumed to be less than -20 dB [26], [27]. $VSWR$ values represent the peak voltage that can be found on a line under non-ideal match conditions. It measures the “goodness” of the match [27]. Excellent impedance match and full signal transition occur at S_{11} , L_r and $VSWR$ values of 0.0, -20 dB and 1.0, respectively. The values were recorded at the notch frequencies 3.27 GHz and 3.30 GHz, for SCM-00 and SCM-100, respectively, which are also shown in Table-I, indicated that the proposed SCM-xx channels are ideal for 5G/6G communication technologies. Based on -10 dB thresholds, the bandwidths (BW) of the peak detected at 3.30 GHz was 2.0 GHz. It indicated that the DUT covers a large frequency span. This is a property of wideband notch filter employable in multiband 5G/6G radios, Radar and jamming resistance [28] and EMI (electromagnetic interference)/RFI (radio frequency interference) suppression [29].

It is evident from table-I that SCM-00 and SCM-100 device channels are one of the most impressive channels employable in 5G/6G technology applications. The filter model named RF-Lambda RNFL3351K and the filter model NEDITEK- NDMF02001 is commercial filters sold in market and designed as cavities for 5G and near 6GHz filtering and for 5G interference suppression of C band, respectively, exhibited filter parameters highly combatable with the currently reported device channels indicating the novel properties of the currently proposed devices. The currently proposed devices show the widest bandwidth ever seen in this technology sector.

Table 1. show some of the band filters employed in high frequency technology.

Filter Model	Center Freq (GHz)	S_{11}	Return Loss [dB]	VSWR	Bandwidth (GHz)	Notes	Ref .
RF-Lambda RNFL3351 K	3.35	0.0024	54.2	1.007	0.234	Cavity notch for 5G NR sub-6 GHz filtering	[30]
NEDITEK NDMF02001	4.6	0.006	45.0	1.017	0.100	Band-stop for 5G interference suppression (C-band)	[31]
Square open loop resonator	2.4	0.316	10.0	1.09	0.56	WLAN/Bluetooth/Zigbee/RFID/ISM applications	[32]
MIMO array antenna	3.56	0.33	9.54	2.0	0.68	5G smartphones and IoT	[33]
SCM-00	3.27	0.02	34.21	1.06	2.0	6G technology	*
SCM-100	3.30	0.007	42.59	1.02	2.0	6G technology	*

*Current work

4. Conclusions

In the current work, the researchers showed the possible fabrication of novel metal-oxide –semiconductor devices employable as ultrafast rechargeable capacitors and as band stop filters. The physical design of the devices included coating of copper oxide layer with molybdenum nanosheets to enhance their conductivity. The structural investigations showed the induced crystallinity of copper oxide via Mo nanosheets coating resulting in reduced defect density by two orders of magnitude. The optical analysis showed that Mo coating widens the energy band gap without affecting the absorption spectra of the oxide films. It was observed that the device under study could be depleted at high frequency reaching 100 MHz. In addition, as bandstop filters the uncoated and Mo coated channels exhibited smart bandstop characteristics presented by ideal reflection coefficient, ideal return loss parameter and ideal voltage standing wave ratios. The respective values reaching 0.007, 42.59 dB and 1.02 at a center frequency of 3.30 GHz with ultra-wide bandwidth made the Si/CuO/Mo devices compatible with those released in 2025 for 5G/6G applications. The currently reported device exhibited much wider bandwidth than those used in electronic markets.

5. Acknowledgments

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6. Declarations

Funding

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Conflict of interest/Competing interests

The authors declare that they have no conflict of interest

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author(s) Contribution

Prof. A.F. Qasrawi, prepared literature review, guided the work, edited the article explored all parts of qualitative research. He also analyzed the optics and the XRD parts. Prof H. Khanfar designed a MATLAB based software packages to evaluate the transport parameters and to register the impedance spectra from the analyzer memory.

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Sensitive and Eco-Friendly Determination of Parabens in Toothpaste Using Ionic Liquid-Based DLLME Coupled with HPLC-PDA

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Abstract

An environmentally friendly ionic liquid-based dispersive liquid-liquid microextraction (IL-DLLME) method was developed and validated for the simultaneous determination of four parabens: methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) in commercial toothpastes using high-performance liquid chromatography with photodiode array detector (HPLC-PDA). Hydrophobic ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate [C₄mim][PF₆] and acetonitrile were chosen as extractant and dispersion solvent, respectively. The parameters affecting the extraction efficiency such as pH of the sample solution, dispersion time, volume of the ionic liquid, volume of the dispersion solvent, ionic strength and centrifugation time were optimized by the univariate method. Calibration curves were linear in the range of 100–1000 µg L⁻¹ under optimum conditions with correlation coefficients (R²) of 0.9949–0.9988. The Limits of detection and quantification ranged from 0.69 to 1.01 µg L⁻¹ and 1.23 to 1.70 µg L⁻¹, respectively. Relative standard deviations for intra- and inter-day analyses (n = 5) were less than 8.4%. The method provided enrichment factors of 120–135 and recovery values ranging from 90.4% to 101.5%.

Keywords: Toothpaste, ionic liquid dispersive liquid–liquid microextraction, parabens; HPLC-PDA, preservatives.

1. Introduction

Dispersive liquid-liquid microextraction (DLLME), introduced by Rezaee et al. [1], is one of the most popular miniaturized sample preparation techniques in modern analytical chemistry. The method involves the fast injection of a small volume of water-immiscible extraction solvent, diluted in a miscible disperser solvent, into the aqueous sample to form a fine emulsion of the two phases, which provides an increased interfacial surface area and thus a faster analyte

transfer. Centrifugation is then used to sediment the enriched extraction phase. DLLME provides very short extraction times, high enrichment factors, simple operation, and significantly less organic solvent consumption in comparison with conventional liquid-liquid extraction [1,2].

However, the traditional version of DLLME uses extractants that are dangerous to the human health and environment, such as chlorinated extractants. Therefore, an important step toward more environmentally friendly analytical techniques is the substitution of ionic liquids (ILs) for such solvents. The ionic liquids are molten salts at room temperature that have very low vapor pressure, good thermal stability, and physicochemical characteristics that may be adjusted by combining the right cation and anion [3, 4]. Non-polar ILs with the $[\text{PF}_6]^-$ anion sediment easily in aqueous conditions after centrifugation, which makes them very useful for DLLME. Because of its high density, π - π interactions with aromatic analytes, and moderate hydrophobicity, 1-butyl-3-methylimidazolium hexafluorophosphate $[\text{C}_4\text{mim}][\text{PF}_6]$ has been used extensively as an IL-DLLME extractant [3,5].

Alkyl esters of para-hydroxybenzoic acid, or parabens, are commonly employed as antimicrobial preservatives in food, medicine, cosmetics, and personal hygiene items like toothpaste [6, 7]. The four most often reported parabens are: MP ($\log P = 1.46$), EP ($\log P = 1.80$), PP ($\log P = 2.29$), and BP ($\log P = 2.71$); their variances in alkyl-chain length result in variations in lipophilicity and antibacterial activity [8]. Table 1 summarizes physicochemical characteristics that are important for extraction behavior.

Concerns over long-term paraben exposure have been raised by an increasing amount of toxicological data. Numerous investigations have shown estrogenic activity and possible endocrine-disrupting consequences, including support for hormone-sensitive cancers, including breast cancer [10], and links with reproductive damage [9]. Residual parabens may be absorbed systemically through buccal or gastrointestinal channels after toothpaste comes into direct contact with the mouth mucosa [11]. Sensitive analytical monitoring is crucial because European Cosmetics Regulation No. 1223/2009 does not set any necessary concentration limits for parabens in toothpaste despite the known health risks [12].

Solid-phase extraction (SPE) [13] and cloud-point extraction [14] are two previously documented paraben detection techniques. Toothpaste matrices comprising pulverized silica and surfactants provide special analytical difficulties, as shown by Zotou et al. [11]. When this work was started, no publicly accessible study had ever used IL-DLLME to determine the amount of parabens in toothpaste. Therefore, the goal of the current work is to create, refine, and validate an IL-DLLME-HPLC-PDA method for the simultaneous measurement of MP, EP, PP, and BP in commercial toothpaste products.

2. Related Work

Numerous extraction and detection platforms are used in analytical techniques for paraben measurement. Limits of detection (LOD) of 100–300 $\mu\text{g L}^{-1}$ were attained by SPE-LC-UV methods applied to saliva and toothpaste [11], which are sufficient for routine screening but insufficient for tracking trace-level contamination. Sensitivity was increased to the 10–200 $\mu\text{g L}^{-1}$ range by cloud-point extraction in conjunction with HPLC-MS/MS [14] and DLLME in conjunction with capillary electrophoresis [15], however these methods usually ask for large sample quantities or time-consuming tuning. Although it requires nanomaterial manufacturing, activation procedures, and magnetic separation equipment, magnetic solid-phase extraction employing magnetic cellulose nanoparticles (MSPE-MCNPs) [16,8] achieved LODs similar to the current work (0.69–1.01 $\mu\text{g L}^{-1}$).

Although IL-DLLME has been used on medicinal chemicals, insecticides, and polycyclic aromatic hydrocarbons [3], its use on parabens in toothpaste has not been reported before our work yet. By utilizing the high density and selective solvation characteristics of imidazolium IL, without the need for chlorinated solvents, the combination of $[\text{C}_4\text{mim}][\text{PF}_6]$

as extractant and MeCN as disperser positions, IL-DLLME, as an environmentally friendly substitute, simultaneously satisfies sensitivity requirements and minimizes laboratory chemical waste [17].

3. Methodology

3.1. Standards, Solvents and Reagents

All reagents were of analytical grade. MP ($\geq 99\%$), EP ($\geq 99\%$), PP ($\geq 98\%$), and BP ($\geq 99\%$) reference standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). The ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate $[\text{C}_4\text{mim}][\text{PF}_6]$, ($\geq 97\%$) was purchased from Sigma-Aldrich. LC-MS grade acetonitrile (MeCN) and methanol (MeOH) were obtained from Scharlab (Barcelona, Spain). Sodium chloride (NaCl , $\geq 99.5\%$), hydrochloric acid (HCl , 37 % w/w), sodium hydroxide (NaOH , $\geq 98\%$), and phosphoric acid (H_3PO_4 , 85 %) were from Sigma-Aldrich. Ultrapure water ($\geq 18.2 \text{ M}\Omega \cdot \text{cm}$) was produced by a Milli-Q system (Millipore, Bedford, MA, USA).

3.2. Instrumentation

A Shimadzu Prominence LC-2030C 3D HPLC system (Shimadzu Corporation, Kyoto, Japan) with a photodiode array detector (PDA) was used for chromatographic analysis. At 30°C , separation was accomplished on a Hypersil ODS C18 column ($250 \text{ mm} \times 4.6 \text{ mm i.d.}$, $5 \mu\text{m}$; Thermo Scientific, USA). MeOH:water (60:40, v/v) was used as the mobile phase, and it was supplied isocratically at 1.0 mL min^{-1} . The injection volume was $10 \mu\text{L}$, and the detection wavelength was 254 nm. Within ten minutes, all four parabens were resolved. Phase separation was carried out using a Hettich Universal 320 centrifuge (Germany). For emulsification, an IKA® MS 3 vortex mixer was utilized. An Orion Star A211 pH meter (Thermo Scientific, USA) was used to measure the sample's pH.

3.3. Preparation of Standard Solutions and Toothpaste Samples

All analyte stock standard solutions were made in MeOH at a concentration of 1 mg L^{-1} and kept in the dark at 4°C . Every day, mixed working standards were made by serially diluting ultrapure water. Six commercial toothpaste products: standard fluoride toothpaste (FT), whitening toothpaste (WT), charcoal-based toothpaste (CT), children's toothpaste (ChT), herbal toothpaste (HT), and sensitive toothpaste (ST) were bought from pharmacies in Ramallah, Palestine. A mass of 5 mg of each toothpaste was dissolved in a mixture of 3.0 mL MeOH and 7.0 mL water, sonicated for 10 min after addition of 1.0 mL concentrated HCl, then diluted to 150 mL with ultrapure water. The pH was adjusted to the optimum value and the solution was filtered through a $0.45 \mu\text{m}$ PVDF membrane prior to IL-DLLME.

3.4. IL-DLLME Procedure

A 15 mL glass centrifuge tube with a conical bottom was filled with 10 mL of the aqueous sample solution at the ideal pH and ionic strength. A 1 mL glass syringe was used to quickly inject a mixture of $500 \mu\text{L}$ MeCN (disperser solvent) and $80 \mu\text{L}$ $[\text{C}_4\text{mim}][\text{PF}_6]$ (extraction solvent) into the sample. A fine, stable, hazy emulsion was created by the quick injection. To encourage equilibrium, the tube was vortexed at 2000 rpm for the ideal dispersion period. After that, the emulsion was centrifuged for five minutes at 5000 rpm. Using a $100 \mu\text{L}$ gas-tight microsyringe, the sedimented IL-rich phase (about 75–80 μL) was collected, diluted with $20 \mu\text{L}$ MeCN to minimize viscosity, and then $10 \mu\text{L}$ was injected into the HPLC-PDA system for analysis.

4. Experiments and Results

4.1. Physicochemical Properties of Target Parabens

All four analytes had pK_a values of around 9.0, meaning that they existed mostly in a neutral, undissociated form at pH values much below the pK_a, which greatly favors partition into the hydrophobic IL phase. Due to their shared hydroxyphenyl ester pharmacophore, all four molecules had the same topological polar surface area (tPSA) of 46.53 Å². The steadily rising log P values (1.46, 1.80, 2.29, and 2.71 for MP, EP, PP, and BP respectively) were consistent with the enrichment factors found in the experiments and anticipated growing affinity for [C₄mim][PF₆]. Table 1 compiles pertinent physicochemical characteristics.

Table 1. Physicochemical properties of the four target parabens (data from EPI Suite™/Molinspiration).

Property	MP	EP	PP	BP
Molecular formula	C ₈ H ₈ O ₃	C ₉ H ₁₀ O ₃	C ₁₀ H ₁₂ O ₃	C ₁₁ H ₁₄ O ₃
Mol. weight (g mol ⁻¹)	152.15	166.17	180.20	194.23
Boiling point (K)	548.16	571.04	593.92	616.80
Melting point (K)	359.89	371.16	382.43	393.70
Log P	1.46	1.80	2.29	2.71
pK _a	9.007	8.981	8.970	8.965
tPSA (Å ²)	46.53	46.53	46.53	46.53
CLogP	1.9846	2.5136	3.0426	3.5716
LogS	-1.597	-1.944	-2.218	-2.642
Heat of formation (kJ mol ⁻¹)	-466.51	-487.15	-507.79	-528.43

MP: methylparaben; EP: ethylparaben; PP: propylparaben; BP: butylparaben.

4.2. Optimization of IL-DLLME Conditions

Using a univariate approach, all extraction parameters (pH 7, 80 µL IL, 500 µL MeCN, 10 s dispersion, 0% NaCl, 5000 rpm/5 min centrifugation) were adjusted by changing one factor at a time while keeping the others at their starting values. The optimization response was extraction recovery (ER):

$$ER (\%) = (C_n \times V_n / C_i \times V_i) \times 100 \quad (1)$$

where C_n and C_i are the concentrations in the extract and original sample, respectively, and V_n and V_i are the corresponding volumes. All experiments were carried out in triplicate and mean data were reported.

4.2.1. Effect of Sample pH

The pH range of 2–10 was examined in relation to ER (Figure 1a). The parabens resided mostly as neutral molecules at pH levels substantially below the pK_a values (around 8.97–9.00 for all four analytes; Table 1), which significantly favors their partition into the hydrophobic [C₄mim][PF₆] phase through hydrophobic and π–π interactions. ER values were moderate at pH 2–6 (37–68%) and significantly rose at pH 8, when all analytes showed maximum (MP: 80%; EP: 84 %; PP: 84%; BP: 85%). As the analytes started to ionize toward their phenolate forms above pH 8, ER gradually decreased, decreasing attraction for the IL phase. As a result, pH 8 was determined to be the ideal sample pH.

4.2.2. Effect of Dispersion Time

Vortex periods ranging from 5 to 40 seconds were studied at 2000 rpm (Figure 1b). ER increased from 60–62 % at 5 s to a maximum of 85–89 % at 10 s, with no further substantial improvement noted. The rapid achievement of equilibrium confirmed the excellent mass transfer aided by the fine IL emulsion formed during strong agitation. A dispersion time of 10s was chosen.

4.2.3. Effect of Ionic Liquid Volume

The volume of [C₄mim][PF₆] varied from 5 to 50 µL (Figure 1c). ER reached a plateau at 10 µL for all analytes (MP: 86.2.2 %; EP: 88.4.5 %; PP: 87.2.6 %; BP: 89.1 %). Larger IL volumes did not improve ER but reduced the enrichment factor (EF) by diluting extracted analytes into a larger phase volume. Therefore, 80 µL was selected as the optimal volume to balance recovery and EF. The theoretical EF (10 mL / 0.075 mL $\approx$ 133) was consistent with the experimentally observed range of 120–135.

4.2.4. Effect of Disperser Solvent Type and Volume

MeOH, MeCN, and MeCN:MeOH (1:1, v/v) were the three potential disperser solvents that were assessed (Figure 2a). Due to its better elution strength toward aromatic esters and its capacity to distribute [C₄mim][PF₆] into a homogenous fine emulsion, MeCN provided the highest ER for all analytes (MP: 85%; EP: 88%; PP: 88%; BP: 88%). The ideal disperser solvent volume was determined to be 500 µL of MeCN.

4.2.5. Effect of Ionic Strength and Desorption Time

From 1 to 30 minutes, the impact of desorption time under vortex agitation was assessed (Figure 2b). 10 minutes were chosen since ER reached its peak at the time and then stabilized. Up to 1% NaCl, the addition of NaCl at 0–5.0% (w/v) indicated a modest improvement in salting-out. However, at higher concentrations, there was a slight decline attributed to the increased aqueous viscosity. The ideal ionic strength condition was achieved by adding 1.0% (w/v) NaCl.

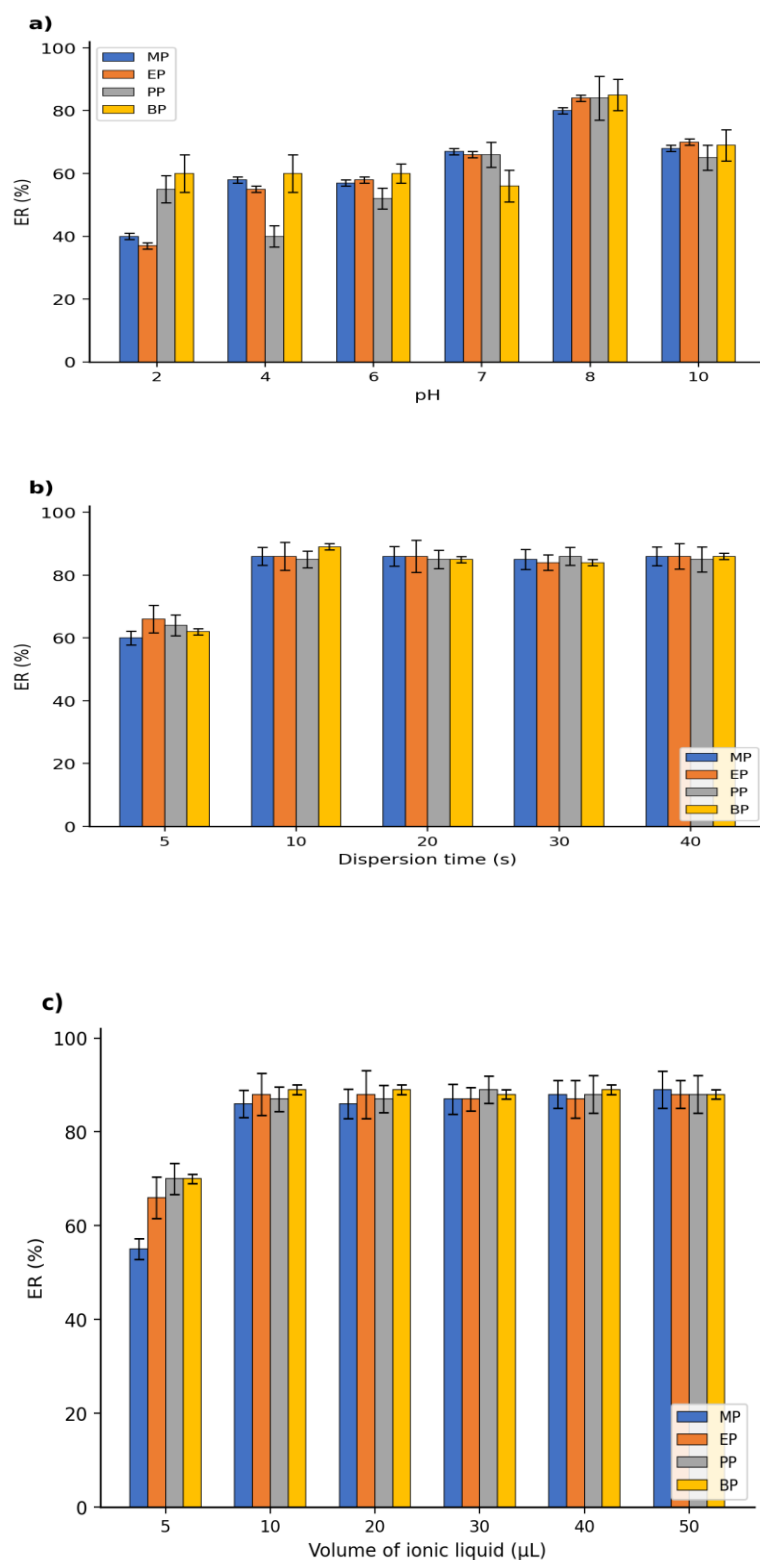


Figure 1. Effect of IL-DLLME extraction parameters on extraction recovery (ER, %) for MP, EP, PP and BP. (a) Effect of sample pH (2–10); (b) effect of dispersion time (5–40 s); (c) effect of [C₄mim][PF₆] volume (5–50 µL). Error bars represent standard deviations (n = 3).

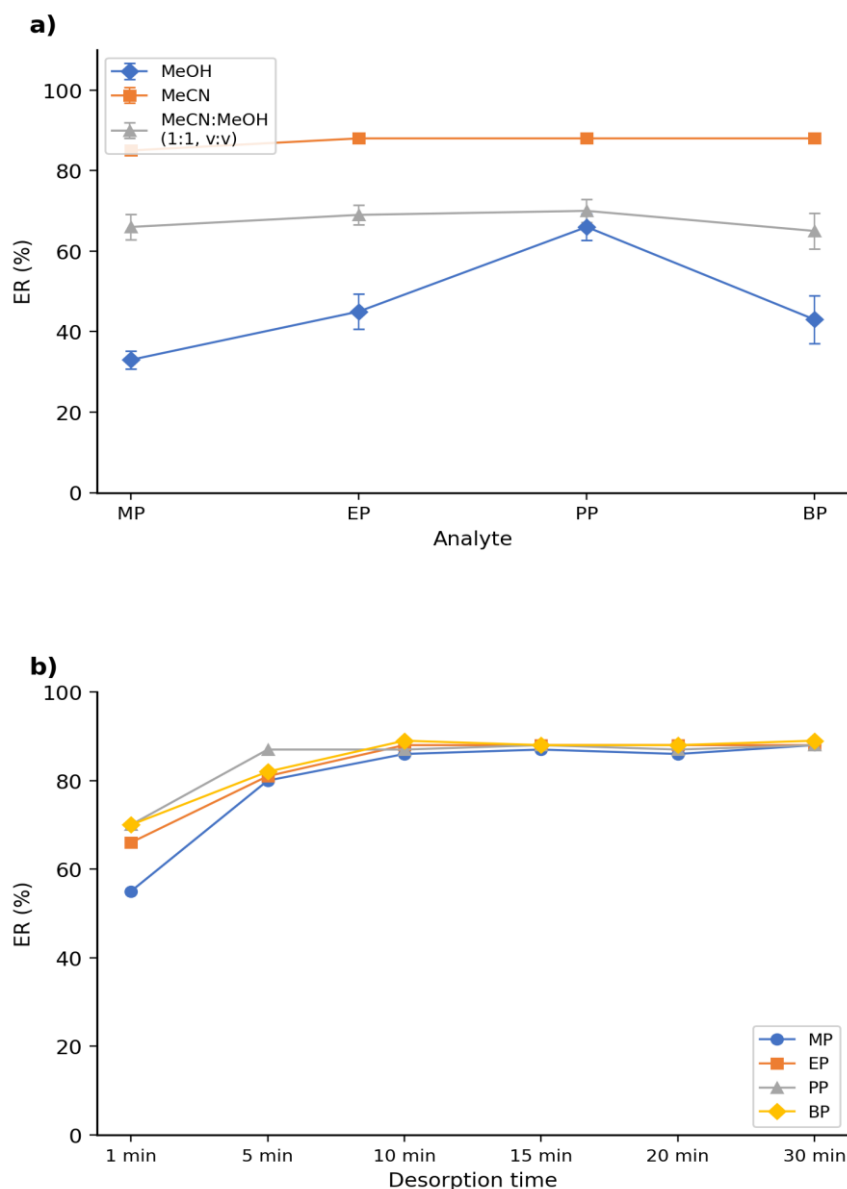


Figure 2. Effect of desorption parameters on ER (%) for MP, EP, PP, and BP. (a) Effect of desorption solvent type (MeOH, MeCN, MeCN:MeOH 1:1 v/v); (b) effect of desorption time (1–30 min). Error bars represent standard deviations (n = 3).

5. Discussion

5.1. Analytical Figures of Merit

Table 2 provides an overview of the optimized IL-DLLME-HPLC-PDA method's analytical performance. The calibration curves had R^2 values of 0.9949–0.9988 and were linear over 100–1000 $\mu\text{g L}^{-1}$. LOD values varied from 0.69 $\mu\text{g L}^{-1}$ (MP) to 1.01 $\mu\text{g L}^{-1}$ (BP), and LOQ values from 1.23 to 1.70 $\mu\text{g L}^{-1}$, derived from S/N ratios of 3 and 10, respectively, on a 50 $\mu\text{g L}^{-1}$ standard exposed to the entire extraction procedure. The repeatability and reproducibility of the intra-day RSD values (2.9–4.8%) and inter-day RSD values (7.6–8.4%; n = 5 over four consecutive days) were satisfactory. Near-quantitative extraction into the IL phase was confirmed by enrichment factors of 120–135.

Table 2. Analytical figures of merit for the IL-DLLME-HPLC-PDA method.

Analyte	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Linear range ($\mu\text{g L}^{-1}$)	R ²	Intra-day RSD % ^a	Inter-day RSD % ^a
MP	0.69	1.23	100–1000	0.9949	3.1	7.6
EP	0.85	1.45	100–1000	0.9988	2.9	8.4
PP	0.78	1.35	100–1000	0.9985	4.8	7.6
BP	1.01	1.70	100–1000	0.9955	4.7	7.8

^a Precision at 100 and 500 $\mu\text{g L}^{-1}$ ($n = 5$); values shown are the higher RSD across both concentration levels.

5.2. Reusability Assessment

Over the course of several extraction cycles, the IL extraction phase's reusability was assessed. Before reuse, the collected IL was cleaned twice with 1 mL of pH 7 ultrapure water and dried for 30 minutes at 50°C. The same IL aliquot was used for seven consecutive cycles of analysis of samples spiked at 50 and 100 $\mu\text{g L}^{-1}$. Reliable reuse was confirmed with ER values of 86% through the fifth cycle. Due to the gradual build-up of matrix co-extractants and slight IL breakdown into the aqueous phase, a discernible decrease was seen in the sixth cycle (~75%) and persisted in the seventh. As a result, the IL phase could be consistently reused for up to five cycles without experiencing appreciable performance degradation, significantly lowering solvent usage in comparison to single-use workflows.

5.3. Analysis of Real Toothpaste Samples

Six commercial toothpaste products made in accordance with Section 3.3 were subjected to the optimized procedure. Since every analyte in every unspiked product was below the limit of detection, aliquots spiked at 100 and 500 $\mu\text{g L}^{-1}$ ($n = 3$ each) and put through the entire IL-DLLME process. Table 3 displays the results. Excellent accuracy and consistent precision in the complex toothpaste matrix are demonstrated by ER values of 90.4–101.5% with RSD < 9.7% for all analytes and matrices. Four well-defined paraben peaks at retention periods of 3.62 minutes (MP), 5.16 minutes (EP), 6.54 minutes (PP), and 7.28 minutes (BP) with no conflicting matrix signals can be seen in representative HPLC-PDA chromatograms of an unspiked and a spiked extract (Figure 3).

Table 3. Extraction recovery (ER $\pm$ SD, %; $n = 3$) and enrichment factor (EF) for parabens in six toothpaste products spiked at two concentration levels.

Sample	Analyte	ER (%) at 100 $\mu\text{g L}^{-1}$	RSD (%) at 100 $\mu\text{g L}^{-1}$	ER (%) at 500 $\mu\text{g L}^{-1}$	RSD (%) at 500 $\mu\text{g L}^{-1}$	EF
FT	MP	93.5 $\pm$ 5.8	6.3	97.4 $\pm$ 7.9	8.2	133
	EP	92.1 $\pm$ 5.5	6.0	94.6 $\pm$ 6.8	7.3	130
	PP	99.8 $\pm$ 3.0	3.0	101.5 $\pm$ 4.6	4.6	135
	BP	91.2 $\pm$ 8.6	9.5	96.0 $\pm$ 6.3	6.6	128
WT	MP	90.4 $\pm$ 5.3	6.0	101.5 $\pm$ 8.8	8.7	133
	EP	90.2 $\pm$ 6.5	7.4	96.3 $\pm$ 5.6	5.9	128
	PP	91.0 $\pm$ 5.9	6.5	100.5 $\pm$ 3.5	3.5	133
	BP	90.4 $\pm$ 4.2	4.7	90.8 $\pm$ 8.5	9.6	120
CT	All	90.1–100.2	3.4–9.1	92.0–101.3	3.7–9.5	120–134
ChT	All	90.6–100.5	3.0–8.7	93.0–101.5	3.9–8.9	121–135

HT	All	90.0–99.2	3.7–9.3	91.4–101.0	4.1–9.4	120–133
ST	All	90.3–99.5	3.1–8.8	92.1–101.5	3.6–9.2	122–135

FT: fluoride toothpaste; WT: whitening toothpaste; CT: charcoal toothpaste; ChT: children's toothpaste; HT: herbal toothpaste; ST: sensitive toothpaste. EF: enrichment factor.

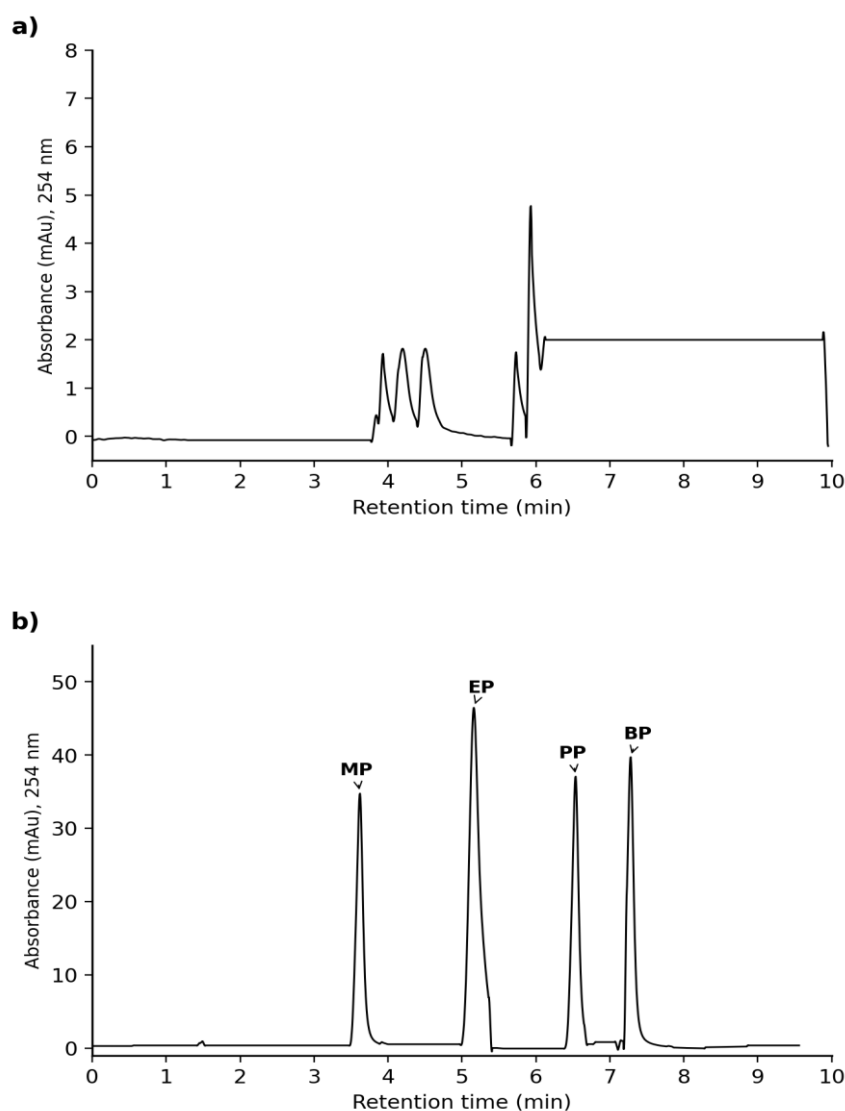


Figure 3. Representative HPLC-PDA chromatograms (254 nm) of toothpaste extracts after IL-DLLME under optimized conditions. (a) Unspiked toothpaste sample; (b) toothpaste sample spiked at $500 \mu\text{g L}^{-1}$ of each paraben. Peak identification: MP = methylparaben ($R_t = 3.62$ min); EP = ethylparaben ($R_t = 5.16$ min); PP = propylparaben ($R_t = 6.54$ min); BP = butylparaben ($R_t = 7.28$ min).

5.4. Comparison with Literature

The suggested approach and previously published methods for determining parabens in toothpaste and similar matrices were contrastive in Table 4. That is, the technique yields LODs of $0.69\text{--}1.01 \mu\text{g L}^{-1}$, which were competitive with CPE-HPLC-MS/MS [14] and significantly lower than SPE-LC-UV applied to toothpaste [11]. IL-DLLME offered comparable sensitivity with much simpler preparation—no material synthesis, activation, or magnetic separation—and completed the extraction in around 20 minutes when compared to MSPE processes that required nanomaterial production [16,8]. Minimal reagent usage and less environmental effect were ensured by using only $80 \mu\text{L}$ of ionic liquid and $500 \mu\text{L}$ of MeCN, together with the IL phase's proven reusability.

Table 4. Comparison of the proposed IL-DLLME-HPLC-PDA method with previously reported procedures for paraben determination in toothpaste and related matrices.

Extraction technique	Detection	Matrix	LOD ($\mu\text{g L}^{-1}$)	Recovery (%)	Linear range ($\mu\text{g L}^{-1}$)	RSD (%)	Ref.
SPE	HPLC-MS/MS	Water	12–24	70–115	80–5000	<20	[9]
SPE	LC-UV	Saliva & toothpaste	100–300	86–113	300–5000	<4	[11]
CPE	HPLC-MS/MS	Water	13–38	71–98	9–730	<0.86	[14]
DLLME-CE	CE-UV	Food & milk	100–200	87–103	700–6000	<3.5	[15]
MSPE-MCNPs	HPLC-PDA	Cream products	0.19–0.31	87.9–101.8	20–1000	<8.7	[16]
IL-DLLME	HPLC-PDA	Toothpaste	0.69–1.01	90.4–101.5	100–1000	<8.4	This work

CPE: cloud-point extraction; DLLME-CE: dispersive liquid–liquid microextraction with capillary electrophoresis; MSPE-MCNPs: magnetic solid-phase extraction with magnetic cellulose nanoparticles.

6. Conclusions and Future Work

An IL-DLLME-HPLC-PDA method was developed and validated for the first time for the simultaneous determination of MP, EP, PP, and BP in commercial toothpaste. Systematic optimization of key extraction parameters—sample pH, dispersion time, IL volume, disperser solvent type, ionic strength, and centrifugation conditions—yielded a robust analytical procedure. The method exhibited excellent linearity ($R^2 = 0.9949\text{--}0.9988$), low detection limits ($0.69\text{--}1.01 \mu\text{g L}^{-1}$), satisfactory precision ($\text{RSD} < 8.4\%$), and high enrichment factors (120–135) even in the complex toothpaste matrix. Replacement of halogenated solvents with an ionic liquid extractant, combined with the demonstrated potential for up to five reuse cycles, substantially reduced reagent consumption and environmental impact, making the procedure both analytically powerful and aligned with the principles of green analytical chemistry.

The validated method was successfully applied to six commercial toothpaste products without paraben detection in any unspiked sample, which indicated compliance with current regulatory limits. Future work should explore extending the analyte scope to include additional preservatives (e.g., phenoxyethanol, benzyl alcohol) and investigating alternative bio-based ionic liquids that offer further improvements in environmental profile. Scaling up to a high throughput format and coupling with UHPLC to improve analysis speed are also promising directions.

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8. Declarations

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Conflict of Interest / Competing Interests

The authors declare no conflict of interest.

Ethics Approval and Consent to Participate

Not applicable. This study involved only commercial products and no human participants.

Consent for Publication

All authors have read and approved the final manuscript and consent to its publication.

Data Availability

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Materials Availability

All chemicals and reagents are commercially available as described in Section 3.1.

Code Availability

Not applicable.

Authors' Contributions

F. Siouri: Conceptualization, Methodology, Formal Analysis, Writing–Original Draft. F. Abujaber: Conceptualization, Supervision, Writing–Review and Editing, Funding Acquisition. A. Mubarak: Methodology, Resources, Writing–Review and Editing. All authors have read and agreed to the published version of the manuscript.

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Designing Around Aldehyde Oxidase: Structural Modifications in Brain-Penetrant Heterocycles: Structure–Activity Relationship, Strategies and Mechanistic Insights

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Abstract

The review critically examined the metabolic liability of aldehyde oxidase (AO), an important consideration in drug discovery, particularly for CNS drug candidates that must cross the blood-brain barrier (BBB) while remaining metabolically stable. The review compared the structural modification strategies adopted to overcome AO-mediated metabolism in brain-penetrant heterocyclic compounds, with emphasis on the mechanistic rationale and level of evidence, and the translational scope of each approach. Structure–activity relationship (SAR) strategies examined included strategic fluorination, ortho-substitution, and scaffold-hopping methods, notably switching a pyridine ring to a pyrimidine. The analysis of the experimental, computational, and case-study literature indicated that site-specific substitutions (especially at the C2 position of azaheterocycles) consistently reduce AO-mediated oxidation, and that strategic fluorination modulates physicochemical properties relevant to BBB penetration by altering pKa, lipophilicity, and interactions with efflux transporters. An alternative kinetic reduction of metabolic turnover is the incorporation of deuterium at oxidized sites. Crystallography, molecular docking, and quantum chemical modelling have been used to study the mechanistic aspects of the AO reaction and its dynamics of binding to the active site, which led to the elucidation of AO active site architecture and rational drug design. Integrated SAR strategies are demonstrated in case studies with β -secretase (BACE1) inhibitors, GluN2B selective negative allosteric modulators, and c-Met kinase inhibitors, which led to the discovery of potent, brain-penetrant molecules with better PK properties. The problems that still lie ahead are interspecies variability in AO activity, underprediction of AO clearance in vitro, and lack of clinical validation data. Increased attention to multiparametric

optimization processes involving both AO metabolic liability and CNS penetration, using enhanced computational models and standardized in vitro phenotyping tools, would be warranted for future research.

Keywords: aldehyde oxidase, brain-penetrant heterocycles, structure-activity relationship, pyridine to pyrimidine, drug metabolism fluorination.

1. Introduction

1.1 The role of Aldehyde Oxidase in Drug Metabolism

Aldehyde oxidase (AO; EC 1.2.3.1) is a hydroxylase enzyme that contains molybdenum and is expressed primarily in the liver and in the intestine, lungs, and brain. Over the past two decades, AO has been recognized as an important enzyme involved in the metabolism of a large and structurally diverse set of azaheterocycles and aldehydes [1,2]. In contrast to the cytochrome P450 (CYP) enzymes, AO does not require molecular oxygen as a primary electron donor in the classical sense, but it uses water as the source of oxygen for hydroxylation, which produces hydrogen peroxide as a byproduct. With its exceptional substrate promiscuity, this difference in mechanism makes it a very difficult enzyme to account for during drug development [3].

The clinical relevance of AO was highlighted by several major drug failures, such as BIBX1382, an epidermal growth factor receptor inhibitor, and CP-724,714, a HER-2 kinase inhibitor, that both had very high levels of AO-mediated oxidation in human liver compared to preclinical rodent hepatic preparations [4–6]. What these failures revealed, and what remains insufficiently emphasized in subsequent literature, is not merely that AO is a metabolically active enzyme, but that the entire framework of preclinical pharmacokinetic validation is structurally biased against detecting AO liability: rats have lower and differently distributed AO activity than humans, in vitro cytosol preparations suffer from enzyme instability under standard incubation conditions, and no validated mechanistic in vitro-in vivo (IVIVE) model existed at the time of these failures or exists today in a form adequate for prospective clinical prediction [7,8]. This review treated that gap not as a peripheral limitation but as a central organizing problem for the field.

1.2 Particular Challenges in CNS Drug Discovery

The problem of AO liability is especially critical in drug discovery for CNS drugs. Brain penetration is a key requirement for any drug to be metabolically stable while meeting certain physicochemical requirements, including low molecular weight, logP, polar surface area, and resistance to efflux pumps like P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) [9]. Nitrogen-containing aromatic heterocycles (including pyridines, pyrimidines, imidazoles, or quinolines) are widely used in drug candidates for CNS drug design, and it is these functional groups that are the targets of preferential AO-catalyzed oxidation [2]. Thus, medicinal chemists developing brain-permeable therapeutics are left with the paradox of achieving inhibition of AO-mediated metabolism, while at the same time maintaining or optimizing BBB penetration, requiring sophisticated and counterintuitive SAR strategies [2,9].

This challenge is compounded by significant interspecies variation in the expression and activity of AO. In rats, which are the most commonly used preclinical species for pharmacokinetic studies, the hepatic AO activity is significantly lower than in humans, and such pharmacokinetic data therefore systematically underpredict AO-mediated clearance when used in human clinical predictions [7,8]. Cynomolgus monkeys have also been

identified as a more suitable surrogate species for human AO metabolism, but due to the cost and ethical considerations, they are not routinely used in early drug discovery [10]. The consequence is that during typical preclinical development, AO clearance is understated and only comes to surface during human pharmacokinetic studies.

A critical gap in the existing literature is that although numerous individual studies address the reduction of AO liability, there is no published, prospective, head-to-head comparison of the strategies (fluorination, C-2 blocking, scaffold hopping, deuterium substitution) applied to the same series of compounds under the same assay conditions. The field consequently rests on a catalogue of precedents, rather than a ranked evidence base from which to inform decisions on strategy selection; this review aims to make that limitation explicit and to partially correct it through critical, comparative analysis.

1.3 Scope and Objectives of the Review

This review contributes to the literature on AO metabolism mitigation in CNS drug discovery in three ways. First, it gives a critical assessment of the evidence that supports each major structural modification strategy, distinguishing strategies supported by primarily empirical precedent from those validated by mechanistic data. Second, it characterizes the translation gap between in vitro AO clearance measurement and in vivo human pharmacokinetics by providing sufficient published IVIVE data to show the size and systematic direction of the IVIVE translation error. Third, it pinpoints three unresolved evidence gaps, which are the absence of comparative head-to-head SAR data, the limitations of existing IVIVE frameworks, and the scarcity of integrated multiparametric datasets, as the key objectives for future methodological investments.

To aid the selection of strategies for a specific scaffold and CNS program, a decision-tree framework was proposed here as a synthesis of the reviewed literature. This framework was provided as an organizational tool and not as a definitive rule to be followed. Each strategy was scaffold-dependent, required certain physicochemical conditions for use, and a recommended sequence of experiments to validate — aspects that have been highlighted separately in previous reviews but have not previously been consolidated in a single practical tool.

1.4 Literature Search Methodology

This is a narrative review in which the literature was searched in PubMed, Web of Science and Google Scholar using combinations of the following terms: "aldehyde oxidase", "AO metabolism", "AOX1", "azaheterocycle oxidation", "molybdenum hydroxylase", "brain-penetrant heterocycles", "blood-brain barrier permeability", "CNS drug design", "structure-activity relationships AO", "fluorination metabolic stability", "pyridine pyrimidine bioisostere", "deuterium kinetic isotope effect drug metabolism", "scaffold hopping AO", "BACE1 inhibitor metabolism", "in vitro in vivo extrapolation aldehyde oxidase", "AO clearance prediction", "cynomolgus monkey AO surrogate", and "efflux transporter pKa CNS". These terms were used in a variety of combinations and with Boolean operators (AND, OR) as per the requirement of specificity for each topic area. There was no date restriction. Older work from the early 2010s remained relevant and was cited together with more recent literature.

The papers were chosen based on their relevance to the key topics: AO mechanism and substrate scope, strategies to minimize metabolism by AO, optimization of BBB permeability, computational prediction of AO sites, and case studies of drug candidates in which the AO liability was deliberately minimized. Review articles,

primary research papers, and computation-based studies were all included when they contributed substantially to topics not otherwise covered in primary literature. Experimental papers (in vitro metabolite profiling, in vivo pharmacokinetics, structural biology or quantitative SAR) were preferred over speculative or opinion-based commentaries.

2. Aldehyde Oxidase: Biology, Substrate Scope, and Mechanism

2.1 Enzyme Structure and Catalytic Mechanism

Human AO (hAO1, encoded by AOX1) is a homodimeric enzyme with each monomer (~150 kDa) comprising three functional domains: an N-terminal domain harboring two [2Fe-2S] iron-sulfur clusters, a central flavin adenine dinucleotide (FAD)-binding domain, and a C-terminal domain that accommodates the molybdenum cofactor (Moco) at the active site [11]. The molybdenum center, in its reduced Mo(IV) state, accepts electrons from the substrate and transfers them sequentially through the iron-sulfur clusters to FAD, where molecular oxygen is ultimately reduced to hydrogen peroxide. Structurally, the AO active site is characterized by a large, predominantly hydrophobic substrate-binding pocket adjacent to the Mo–OH catalytic center, with key residues Phe919, Val811, and Leu799 defining the substrate-binding geometry in the human enzyme [11,12].

Computational and crystallographic investigations have revealed that AO preferentially oxidizes electron-deficient carbon atoms situated adjacent to ring nitrogen atoms in aza-heterocyclic substrates, particularly those at positions that allow optimal coordination with the molybdenum center. [12,13]. The identification of these oxidation-prone positions has been central to guiding rational structural modification strategies.

Recent structural biology studies have provided insights into the mechanisms governing AO dimerization and the functional consequences of AOX1 genetic polymorphisms, including the T755I (rs35217482) variant, which reduces the rate of AO dimerization and the rates of substrate metabolism, revealing the potential for interindividual variability in AO activity and pharmacokinetic variability in clinical populations [14]. This polymorphism has not yet been included in PBPK models of AO substrates, which is a methodological gap that may account for some of the unexplained variability in human pharmacokinetic predictions for this enzyme class. Figure 1 illustrates the three-domain architecture of the human AO1 homodimer and the sequential electron transfer pathway from the molybdenum cofactor through the iron-sulfur clusters to FAD, providing the structural context for understanding how substrate binding at the Mo center initiates the catalytic cycle discussed above.

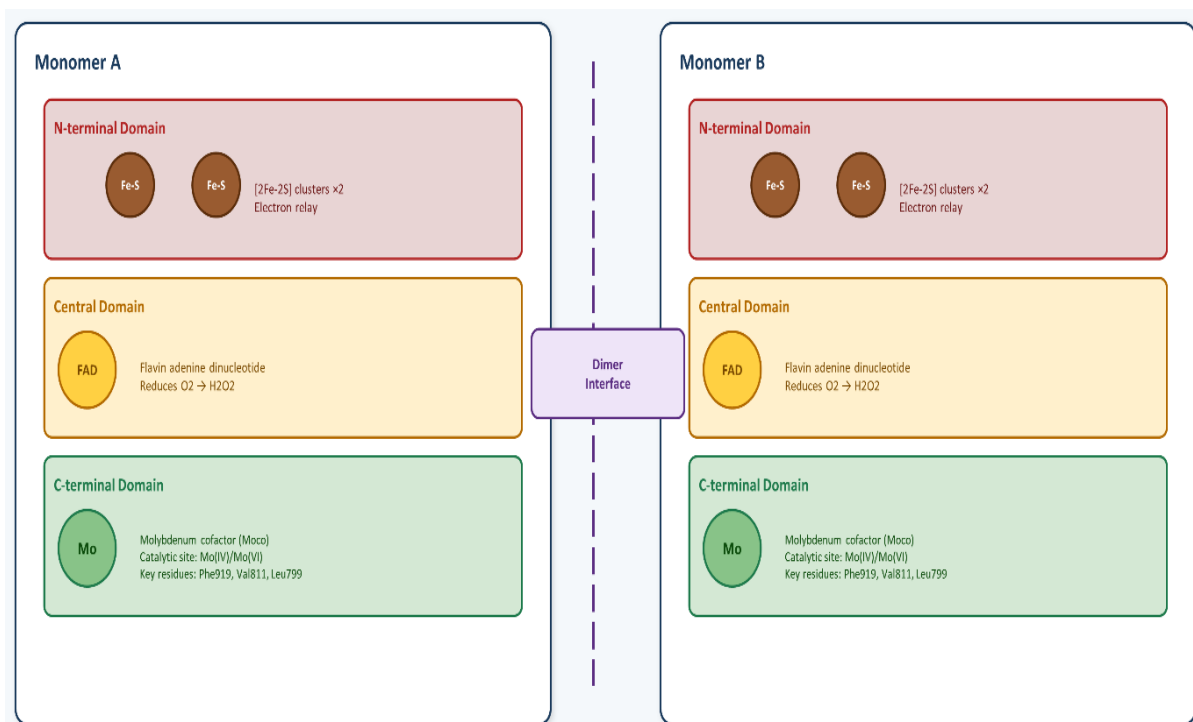


Figure 1. Schematic of the human AO1 homodimer architecture, showing the three functional domains per monomer: [2Fe-2S] iron-sulfur cluster domain (N-terminal), FAD-binding domain (central), and molybdenum cofactor (Moco) domain (C-terminal). The electron transfer pathway from substrate oxidation at the Mo center through FE-S clusters to FAD is indicated by arrows. Structural data adapted from Ferreira et al. [11]. The author prepared this figure using Microsoft PowerPoint (Microsoft Corporation, Redmond, WA, USA) as an original schematic for this review.

2.2 Substrate Scope and Species Differences

AO accepts a very broad range of structurally diverse drug candidates as substrates, including quinolines, phthalazines, purines, pyridines, pyrimidines, imidazopyrimidines, and others [2,3]. This promiscuity arises from the large and flexible active site of the enzyme, which can hold structurally different substrates. However, kinetic parameters vary significantly based on the electronic and steric characteristics of the substrate, as seen in systematic studies of zoniporide analogues, where structural changes yielded significant differences in AO oxidation rate [15]. The common scaffolds are summarized in Table 1 below.

The interspecies difference in AO expression and activity constitutes perhaps the most consequential unresolved challenge in the field. Humans have only a single AO isoform (AOX1) expressed at high levels in the liver, whereas rodents have several AO isoforms with different substrate specificities and activities [16]. Standard rodent pharmacokinetic models often underestimate human AO clearance, a discrepancy that has contributed to drug development failures at late stages of development. Empirical analyses have further demonstrated that current in vitro systems—including liver cytosol preparations and recombinant enzyme assays—systematically underpredict AO-mediated clearance in humans, due in part to enzyme instability during cytosol preparation and incubation and variable protein content across preparations [7,17,18]. Critically, none of these sources of error is resolved by the empirical scaling factor approaches currently in use, which improve prediction accuracy for well-characterized substrates but cannot correct for fundamental mechanistic gaps in the extrapolation model [8,17]. Table 1 summarizes different heterocyclic scaffold classes commonly encountered in CNS drug

candidates, showing the primary AO oxidation site, a relative susceptibility rating derived from structure-metabolism relationship studies, the key blocking strategy with established precedents, the anticipated effect of that strategy on BBB penetration, and illustrative literature references.

Table 1. Common heterocyclic scaffolds in CNS drug discovery, their primary AO oxidation sites, relative susceptibility, established blocking strategies, and illustrative references.

Heterocyclic Scaffold	Primary AO Site	AO Susceptibility*	Key Blocking Strategy	Effect on BBB	Illustrative Reference
Imidazo[1,2-a]pyrimidine	C-2/C-8	High	Systematic site blocking, trifluoromethylation	Neutral if small group	[13,19]
Pyrido[3,4-d]pyrimidinone	C-2	High	C2-substitution (steric + electronic)	Neutral	[20]
Pyridine (monoaza)	C-4/C-2	Moderate-High	Pyrimidine ring switch. Fluorination	Often positive	[2,21]
Quinoline	C-2	Moderate	Steric/electronic blocking, deuterium at C-2	Context-dependent	[22,23]
Pyrimidine (diaz)	C-2 (context-dependent)	Low-Moderate	Fluorination/ ortho-substitution	Largely neutral or positive	[18,24]
Phthalazine	C-1	High	Scaffold hopping dimer interface modulation	Requires re-evaluation	[14,25]
Benzimidazole	C-2	Moderate	C-2 substitution; fluorination	Often positive if logP controlled	[2,26]

*AO susceptibility rating reflects general trends from structure-metabolism relationship studies; individual compounds within each scaffold class may deviate depending on substitution pattern.

3. Structure- Activity Relationship Strategies to Mitigate AO Metabolism

The following critical qualifications apply to all SAR strategies discussed in this section. No prospective, side-by-side comparisons of competing strategies applied to the same scaffold series under the same assay conditions have been published, except for a few case studies. The efficacy data for each approach thus come from different series of compounds and different assay platforms (human liver cytosol vs. recombinant enzyme vs. in vivo rodent clearance), and different measures of the BBB impact. Without a matched control series, causal

attribution of pharmacokinetic improvement to any specific structural modification should be made cautiously. Table 2 presented in section 5.1, provides a comparative summary of the principal AO-blocking strategies alongside the two ortho-substitution variants, rating each on AO reduction magnitude, BBB impact, pKa and efflux effect, evidentiary quality, and main caveat drawn from the reviewed literature.

3.1 Blocking Reactive Sites: C2-Substitution and Ortho-Effects

The most direct approach to reducing AO-mediated metabolism is the introduction of steric or electronic blocking groups at the site(s) of preferred oxidation. Systematic studies on imidazo[1,2-a]pyrimidine scaffolds demonstrated that targeted structural modifications at positions susceptible to AO-catalyzed hydroxylation—identified through metabolite profiling, LC- MS/MS analysis, and molecular modelling—effectively reduced AO metabolism while maintaining the pharmacological activity of the parent scaffold [13]. Similarly, investigation of pyrido[3,4-d]pyrimidin-4(3H)-one derivatives established that C2-substitution is particularly effective at blocking AO-mediated oxidation at this preferred site, with both experimental and molecular docking data corroborating the steric blockade mechanism [20,27].

The role of ortho-substituents in modulating AO metabolism has been explored across several compound series. Steric hindrance introduced by substituents at positions flanking the oxidation-prone carbon disrupts substrate positioning within the AO active site, reducing catalytic efficiency. Docking studies of zoniporide analogues provided mechanistic rationalization, correlating the electronic character of substituents with AO active site binding energetics and demonstrating that electron-donating groups at certain positions markedly affect oxidation rates. [15]. In the quinoline-containing c-Met kinase inhibitor series, electron-donating and steric effects at positions adjacent to the AO oxidation site were similarly shown to modulate metabolic half-life in a predictable fashion [22]. Importantly, the effectiveness of site-blocking approaches is scaffold- dependent; modifications that effectively block AO metabolism in one heterocyclic framework may prove ineffective or reduce target potency in another, necessitating empirical evaluation for each new chemotype [2,28].

A limitation that isn't consistently acknowledged in the literature is the scaffold-dependence of blocking effectiveness. A C-2 methyl group provides effective steric protection in one heterocyclic system, while being geometrically mispositioned in another because of differences in ring nitrogen geometry, bond angle, and active site docking geometry. No published study has systematically explored the geometrically constrained factors that contribute to blocking efficacy across more than one scaffold class at a time, forcing medicinal chemists to conduct their own empirical testing for each new scaffold series. This is a huge inefficiency in current practice, which one could potentially overcome by the use of computational structure-based design, making use of the high-resolution hAO1 co-crystal structures [11,12].

3.2 Strategic Fluorination

Fluorination has emerged as one of the most versatile and widely employed tactics in medicinal chemistry for modulating both metabolic stability and physicochemical properties [19,28]. With respect to AO metabolism specifically, the introduction of fluorine atoms at or near AO oxidation sites reduces electron density at the susceptible carbon, decreasing substrate reactivity towards the electrophilic AO molybdenum center.

In an effort to attenuate AO-mediated hydroxylation, strategic fluorination has been reported to increase the metabolic stability of several heterocyclic scaffolds, including imidazo[1,2-a]pyrimidines, in which

trifluoromethylation proved to be very effective in enhancing metabolic stability but not compromising biological activity [19].

In addition to its effect on AO-mediated metabolism, fluorination has important secondary effects on properties of particular relevance for BBB penetration. Substitution of fluorine can decrease the basicity (pKa) of adjacent nitrogen containing functional groups, which decreases ionic interactions with efflux transporters and reduces P-gp-mediated efflux, thereby improving CNS exposure [29]. A systematic study of phosphodiesterase 9 (PDE9) inhibitors revealed that strategic fluorination predictably modulated pKa, and further systematic changes in pKa resulted in a predictable change in their bidirectional Caco-2 permeability assays, the efflux ratio [29]. The program described by Hilpert et al. is a good example of the use of the BACE1 inhibitors, which they elegantly showed could be substituted with fluorine to diminish AO liability, decrease efflux ratios, and boost brain exposure in rodent PK studies, and thus make the compounds suitable for further development for clinical trials in Alzheimer's disease [30]. However, the effects of fluorination are not always positive, though. Often, over- or mis-fluorination of a target molecule leads to a rise in its polarity, thereby increasing its susceptibility to efflux or decreasing its potency, which is thus considered as a 'Goldilocks Effect', wherein an optimal balance between steric and electronic perturbation needs to be achieved [31]. This principle was elegantly illustrated in the optimization of phenylethanolamine N-methyltransferase (PNMT) inhibitors, where β -fluorination of a tetrahydroisoquinoline scaffold was found to only result in the desired reduction of AO metabolism, potency, and receptor selectivity when the fluorine was at the exact optimum site [31]. All these observations together highlight the need for fluorination to be used in a mechanism-aware and SAR-driven manner, and not as a one-size-fits-all approach.

This positional criticality is the most important and least appreciated aspect of fluorination strategy. The literature is replete with examples of beneficial fluorination, but the cases where fluorination worsened the pharmacokinetic profile, through polarity increase, P-gp substrate recognition, or potency loss, are underreported due to publication bias. Medicinal chemistry programs that screen multiple fluorine positions and report only the successful variants create an impression of fluorination as a reliably beneficial strategy, when the underlying data frequently show that the majority of fluorine positions tested produced inferior compounds. A systematic, unbiased analysis of fluorine position– outcome relationships across a broad set of CNS scaffolds would be a valuable contribution to the field and does not currently exist. Summary of the effects of fluorination is presented in Figure 2 below.

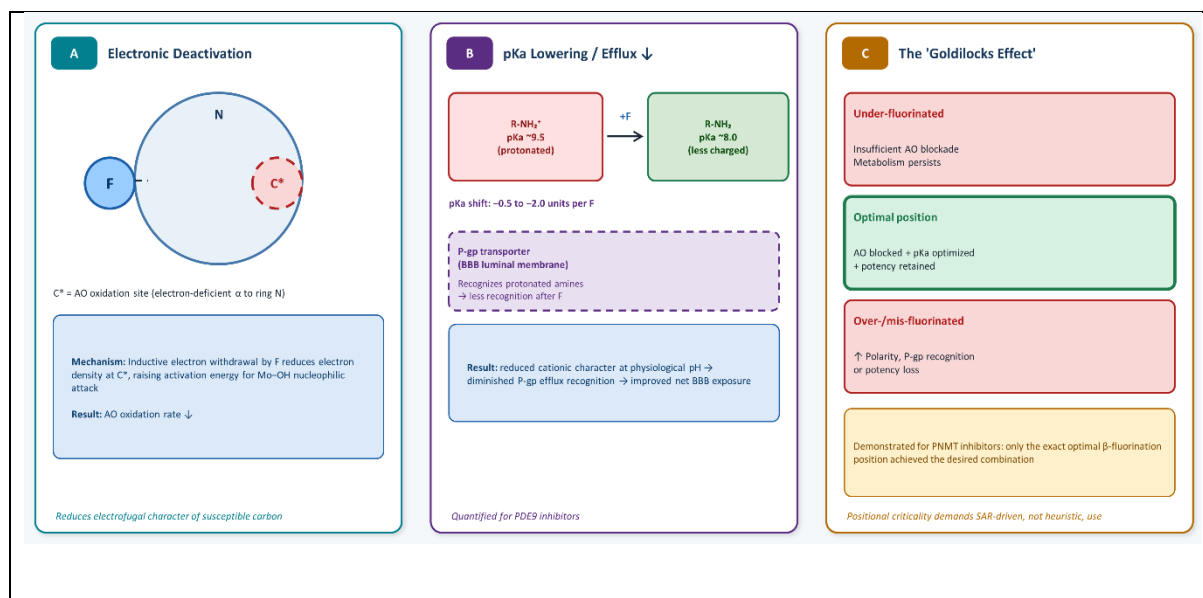


Figure 2. The dual role of strategic fluorination in CNS-targeted AO substrates. (A) Electronic deactivation of the AO oxidation site by fluorine-induced reduction of electron density at the susceptible aromatic carbon. (B) Concurrent lowering of amine pKa by 0.5-2.0 units following fluorine substitution adjacent to the basic nitrogen, reducing cationic charge at physiological pH and diminishing P-gp efflux recognition. (C) The "Goldilocks Effect": Optimal positioning of the fluorine atom for both AO blockade and pKa reduction and target potency — positioning of the fluorine atom is critical, and only the position that achieves the optimal combination of all these three parameters is acceptable; errors in placement may benefit some parameter while harming others. Dotted arrows indicate both changes occurring within the same structural modification event, as documented for BACE1 and PDE9 inhibitor series [30,31]. The author prepared this figure using Microsoft PowerPoint (Microsoft Corporation, Redmond, WA, USA) as an original schematic for this review

3.3 Heterocycle Switching: Pyridine to Pyrimidine Scaffold Hopping

Replacement of metabolically labile pyridine rings with pyrimidine or other electron- deficient heterocycles represents a particularly powerful SAR strategy for mitigating AO liability, because it simultaneously alters the electronic environment of the ring and, in many cases, removes or repositions the oxidation-prone carbon relative to the ring nitrogen [2,28].

Pyrimidines are generally less susceptible to AO-mediated oxidation than pyridines, owing to the presence of two ring nitrogen atoms that inductively reduce electron density at susceptible positions. Scaffold hopping from pyridine to pyrimidine has been successfully employed in multiple CNS drug programs, including the development of S3-truncated aminothiazine BACE1 inhibitors by Wu et al., where pyrimidine ring introduction both addressed AO soft spots identified by metabolite profiling and improved oral bioavailability, ultimately enabling demonstration of brain amyloid- β reduction in rodent models [21].

Similarly, in the discovery of GluN2B-selective negative allosteric modulators for CNS indications, iterative substitution of a pyrimidine core, guided by metabolite identification and AO liability assessment, yielded a series of potent, brain-penetrant compounds with improved metabolic stability [24]. Metabolic improvement via scaffold switching was directly validated through metabolism studies in the respective species, which showed that AO is the main enzyme oxidizing pyrimidine at the soft spot position, and through in vivo pharmacokinetic studies showing the increased CNS exposure in rodents after scaffold modification [24,32]. Other structures,

such as other aromatic rings replaced by non-AO-substrate bioisosteres, have been investigated as a way to remove AO liability completely, not just encourage its reduction [25]. Lazzara and Moore showed that scaffold hopping away from the electron-rich aromatic systems to more electron-deficient alternatives can be done to achieve both AO and CYP metabolic liability, going beyond the scope of specific pyridine-to-pyrimidine substitution [25]. However, such scaffold modifications should be carefully assessed to make sure the pharmacophoric groups necessary for the interaction towards the target are retained.

A limitation that is not consistently acknowledged in the scaffold-hopping literature is that pyrimidine rings are not universally AO-resistant. Crouch et al. [18] demonstrated that the mGlu5 negative allosteric modulator VU0424238 (Auglurant), which contains a pyrimidine ring, was nonetheless metabolized by AO in a species-specific manner — with the pyrimidine itself serving as the AO substrate [18]. This finding demonstrates that the assumption of pyrimidine AO resistance is a statistical generalization, not a mechanistic guarantee, and that pyrimidine-containing compounds require explicit AO metabolite profiling before AO resistance can be claimed. The failure to make this distinction in the SAR literature perturbs a misleading heuristic that pyrimidine switching equals AO resistance.

3.4 Deuterium Substitution as a Kinetic Isotope Approach

The kinetic isotope effect (KIE) of deuterium substitution, exploiting the higher bond dissociation energy of C–D relative to C–H (by approximately 1.2 kcal/mol) to reduce the rate of AO-catalyzed bond cleavage, represents the most mechanistically targeted approach to AO metabolism mitigation [23,33]. The primary KIE for C–H bond cleavage by AO has been measured at 2–7-fold across a range of substrates, depending on the extent to which that bond cleavage contributes to the overall rate-determining step. The c-Met inhibitor MET401 case study showed that deuterium at the quinoline AO oxidation site produced significant improvements in rodent pharmacokinetic parameters, with reduced clearance and prolonged half-life, demonstrating a direct pharmacokinetic consequence of the isotope effect [23].

Two constraints limit the general applicability of this approach that are insufficiently emphasized in the positive case literature. First, the KIE is maximal only when C–H bond cleavage is strictly rate-limiting in the AO catalytic cycle. When other steps — substrate binding, product release, or electron transfer — are partially rate-limiting, the observed KIE will be attenuated (a phenomenon termed the 'commitment to catalysis'), potentially reducing the metabolic improvement below the level required for pharmacokinetic rescue. The magnitude of the commitment to catalysis for AO is substrate-specific and has not been systematically characterized across the scaffold types commonly encountered in CNS discovery. Second, deuterium substitution only affects the metabolic rate at the labeled carbon and does not change any of the underlying physicochemical liabilities that may individually limit BBB penetration, such as P-gp efflux, polar surface area, passive permeability, and thus cannot be used alone as a CNS optimization strategy. In the specific scenario that AO-mediated clearance is the main pharmacokinetic hurdle to CNS penetration with all other parameters for CNS penetration optimized, then its utility is limited.

4. Mechanistic Insights into AO Substrate Recognition and Metabolism

4.1 Computational Prediction of AO Oxidase Sites

The rational implementation of SAR strategies to block AO metabolism requires accurate prediction of which molecular positions are susceptible to AO-catalyzed oxidation. Computational approaches to this challenge have advanced considerably over the past decade [12,34]. Early structure-based models utilized the available AO crystal structures to identify the geometric and electronic requirements for productive substrate binding within the molybdenum-containing active site [13]. More recent computational workflows have employed quantum chemical transition state analyses to predict the intrinsic reactivity of aromatic carbon atoms towards the AO molybdenum center, providing atom-level predictions of oxidation site regioselectivity for a broad range of azaheterocyclic substrates [12]. Levine et al. conducted a large-scale computational survey of AO substrate reactivity, demonstrating that quantum mechanically derived activation energies for model AO oxidation transitions correlate well with experimentally observed metabolite formation rates [12]. Complementing this atom-level approach, Zhao et al. developed an integrated computational model combining atom-level and molecule-level features—including molecular descriptors encoding electronic properties, steric accessibility, and structural context—to predict both AO substrate classification and site-of-metabolism, validating the model against an extensive curated dataset of in vitro AO metabolism experiments [34]. These predictive tools enable prospective identification of AO liabilities at the hit or lead identification stage, supporting early decision-making regarding structural modification needs.

Lepri et al. [26] assembled a comprehensive SAR dataset by performing structure-metabolism relationship studies on a large structurally diverse set of aza- aromatic and amide compounds, using density functional theory (DFT) calculations together with experimental metabolite identification [26]. The dataset showed that systematic electronic and steric effects govern AO oxidation susceptibility across chemical space, which may be useful for predicting oxidation susceptibility in novel scaffolds found in drug discovery programs [26].

A critical limitation of all current computational AO prediction tools is that they have been validated primarily against in vitro intrinsic clearance data from human liver cytosol or recombinant AO, not against in vivo human clearance data. Given the documented failure of in vitro data to predict in vivo AO clearance accurately (Section 4.3), computational tools trained on and validated against in vitro endpoints inherit the same systematic prediction error. A computational model that accurately predicts in vitro AO clearance may therefore still fail to predict human clinical pharmacokinetics. This limitation is not acknowledged in any of the computational prediction papers reviewed here, and represents a fundamental gap between the performance metrics reported for these tools and the translational objective for which they are intended. Future tool development should prioritize validation against in vivo human pharmacokinetic data where available.

4.2 Enzyme-Substrate Interactions from structural and kinetic studies

In addition to computational methods, information on AO–substrate interactions have been obtained through direct experimental studies, which have been very helpful in providing mechanistic information. Dalvie et al. [15] used a molecular docking protocol with kinetic evaluation of a series of zoniporide analogues to establish correlations between the properties of the substrates and the binding affinity of the substrates to the AO and their catalytic efficiency [15]. Such seemingly conservative changes in structure, such as the introduction of methyl or methoxy substituents, were shown to significantly alter the orientation of a substrate in the active site,

and to accompany changes in regioselectivity and metabolic rates [15]. Ferreira et al. [11] reported insights into the conformational dynamics of the AO monomer and homodimer, and how the structural architecture of the enzyme affects the binding and catalytic activity of the substrate [11]. In particular, the homodimeric interface was found to be a region that may regulate substrate access and productive binding, which may be a physiological control mechanism of AO activity [11]. Characterization of AO inhibition mechanisms has also shed mechanistic insight into drug design: Barnes et al. showed that hydralazine inactivates AO by generation of reactive intermediates, a process that is time-dependent and that glutathione can partially protect against, which suggests the possibility of developing selective inhibitors of AO and also allows for the assessment of drug–drug interaction potential [35].

Despite this body of mechanistic data, a fundamental structural gap persists: no high-resolution co-crystal structure of hAO1 in complex with a CNS-relevant drug candidate has been published. The available structural biology data are largely derived from bovine or murine AO homologues or from computational models of the human enzyme, both of which differ in active site geometry from hAO1 in ways that may be pharmacologically significant. Structure-guided design of AO-resistant CNS scaffolds — analogous to the cryo-EM-guided design now possible for amyloid targets — is therefore not yet feasible in a rigorous sense. This represents the most consequential unresolved methodological need in the field.

4.3 Persistent Challenges of In-Vitro and In-Vivo Extrapolation: A Quantitative Assessment

Although great progress has been made towards computational prediction and mechanistic understanding of AO metabolism, relevant in vivo clearance data in humans remains one of the most formidable challenges in the field [7,8,17]. This challenge is due to several factors. First, the activity of AO enzymes in cytosol preparations from liver is unstable, and significant losses of activity occur during preparation and storage of such samples, which adds to interspecies and inter-laboratory variations [7]. Second, the protein content of AO in human liver cytosol is very donor-dependent, and thus the forward scaling of in vitro intrinsic clearance to in vivo hepatic clearance is inherently uncertain [17]. Third, AO metabolism in extrahepatic tissues, such as the intestine, may account for in vivo clearance in a manner not accounted for by the typical hepatic cytosol assay [8].

Some empirical scaling factor methods have been suggested to resolve these shortcomings to some extent. Izat et al. [8] determined system-specific scaling factors for predicting the clearance of an AO, and showed that the use of calibrated empirical correction factors improves the accuracy of in vitro–in vivo extrapolation (IVIVE) of the clearance of AO substrates, which was underestimated with the standard scaling factors [8]. Subash et al. [17] also examined the role of activity loss and protein content variability in the underprediction of AO clearance, suggesting a protein-normalized activity factor approach that combines proteomics data with physiologically based pharmacokinetic (PBPK) modelling for more accurate human clearance predictions [17]. These methodological advances are useful, but it should be recognized that empirical scaling factors will be dependent on restricted datasets and may not be generally applicable to the entire chemical space of AO substrates that typically occur in drug discovery [8,17].

The field is therefore in a situation where no validated prospective IVIVE method for AO clearance exists. The magnitude of prediction error is large enough to cause program-level decisions based on incorrect clearance

estimates, and the methodological investment required to close this gap, such as standardized enzyme-stable cytosol preparations, proteomics-normalized scaling, and extrahepatic contribution characterization, has not been made in a systematic, cross-organizational fashion. This is the single highest-priority methodological gap in the field.

5. Integrating AO Metabolism Mitigation with BBB Optimization

5.1 Physicochemical Determinants of BBB Penetration

In CNS drug design, both BBB penetration and metabolic stability must be simultaneously optimized, a target that is often in conflict and requires a multiparameter optimization [36,37]. Passive BBB permeability depends on the following properties: molecular weight (<450 Da), logP 1–3, polar surface area (<90 Å²), and hydrogen bond donor count (<3), which are collectively represented by CNS-specific analogues of the Lipinski rules. In addition to passive permeability, efflux by P-gp and BCRP at the BBB is also a significant obstacle to CNS penetration, and compounds with good passive permeability can have very low brain penetration due to active efflux [38,39].

In practical terms, AO-blocking modifications can be classified by their expected impact on CNS MPO score. Fluorination, C-2 methylation, and pyrimidine switching are 'CNS- neutral or positive' modifications; they typically reduce AO liability without substantially worsening CNS MPO parameters and may improve them through pKa effects (fluorination) or reduced MW (pyrimidine replacing substituted pyridine) [29,30]. By contrast, the introduction of the polar blocking groups (hydroxymethyl, amino, or carboxamide) at oxidation sites improves AO stability but increases topological surface area (TPSA) and HBD count, typically reducing CNS MPO score and increasing P-gp efflux recognition [2,28]. Deuterium substitution has no impact on CNS MPO score by construction, since it does not alter any physicochemical parameter.

Table 2. Comparative effectiveness of AO metabolism mitigation strategies and their effect on CNS drug-like properties. The assessments reflect the authors' synthesis of published data; no head-to-head comparison under identical conditions exists.

SAR Strategy	AO Reduction	BBB Impact	pKa/Efflux Effect	Evidentiary Quality	Main Caveat
C-2/ ortho-substitution	Strong; site-specific block	Neutral (if small group)	No direct pKa effect, efflux impact depends on group polarity	Moderate-consistent in vitro precedent across imidazo[1,2-a] pyrimidine and pyrido[3,4-d] pyrimidinone scaffolds; mechanistic validation by docking; limited in vivo data	Scaffold-dependent; may reduce the potency

Strategic Fluorination	Moderate-Strong	Often improved (lower pKa, less efflux)	Lowers adjacent amine pKa by 0.5-2 units, reduces P-gp efflux ration in a position-dependent manner	High mechanistic rationale established; quantitative pKa efflux correlation documented; in vivo brain exposure improvement confirmed in BACE1 series	Position-critical; risk of over-fluorination
Pyridine to Pyrimidine Switch	Strong; electronic deactivation	Largely neutral or positive	Minimal direct pKa effect unless adjacent to basic amine; can reduce MW, positively affecting CNS MPO	Moderate — multiple CNS programme precedents; mechanistic basis established; pyrimidine AO resistance is not universal	Pharmacophore may shift, pyrimidine itself may be AO substrate
Deuterium Substitution	Moderate (2-7×KIE)	Neutral	No pKa efflux effect	Low-Moderate—single well-characterized case study (MET401); KIE magnitude substrate-specific; commitment to catalysis not systematically characterized	Applies only when C-H cleavage is rate-limiting, metabolic shifting risk
Scaffold hopping (broad)	Variable; can eliminate liability	Depends on replacement ring	Variable; depends on replacement scaffold physiochemistry	Low-Moderate — case-by-case precedent; no systematic cross-scaffold comparison	Risk of losing target engagement
Ortho-Substitution	Moderate; steric blockade	Mixed (depends on group polarity)	Polar ortho-groups may increase TPSA and reduce	Low-Moderate — mechanistic rationale from docking studies;	Electronic context matters

			passive permeability; non-polar groups neutral	limited in vivo validation	
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KIE: kinetic isotope effect, TPSA: topological polar surface area, CNS MPO: CNS multiparameter optimization.

5.2 Pyrimidines, Morpholines, and Other CNS-Favorable Scaffolds

Several specific scaffold types have emerged as particularly compatible with the dual requirements of AO metabolism resistance and BBB penetration. Pyrimidine-containing scaffolds offer reduced AO susceptibility relative to pyridines and can be designed with the physicochemical properties required for CNS exposure. In the context of multi-target CNS drug design, pyrimidine and pyrrolidine core combinations have been reported to exhibit BBB penetration validated by in vivo efficacy in neuroinflammation models, alongside balanced inhibition of multiple CNS targets [40].

Morpholine-containing heterocycles represent another compound class of particular relevance to CNS drug discovery. Lenci et al. [41] conducted a comprehensive analysis of morpholine-containing CNS drugs, demonstrating that the morpholine ring contributes to favorable BBB permeability through modulation of lipophilicity, pKa, and hydrogen bond acceptor capacity, while also conferring conformational constraints that can improve target selectivity. The physicochemical properties conferred by morpholine make it a privileged scaffold for CNS applications, and its incorporation has been strategically employed in multiple successful CNS drug programs [9,41]. More broadly, Do et al. [42] demonstrated that systematic optimization of lipophilicity and pKa through structural modification of a 2-aminopyridine scaffold yielded potent neuronal nitric oxide synthase (nNOS) inhibitors with low efflux ratios and high brain exposure in rodent pharmacokinetic studies. The study illustrated that multiparametric optimization—simultaneously tracking potency, AO metabolism, and physicochemical/permeability parameters—is both feasible and necessary for the development of efficacious brain-penetrant CNS drugs [42]. Table 3 translates the CNS MPO framework into a practical reference by listing five key physicochemical parameters alongside their established CNS target ranges, the predicted direction of change imposed by common AO-blocking modifications, and the key design consideration for each parameter.

Table 3. CNS drug-like physicochemical targets and predicted impact of common AO-blocking structural modifications

Parameter	CNS Drug Target Range*	Effect of AO-Blocking Mods	Key Considerations
Molecular weight (Da)	< 450	Minimal change expected	Deuterium and F add negligible MW
LogP	1-3	Fluorination can raise logP slightly	Over-fluorination risks P-gp recognition
Polar Surface Area (Å ²)	< 90	Polar blockers increase PSA unfavorably	Prefer small non-polar blocking groups
pKa (basic amine)	7.5-9 (amine); lower for CNS	Fluorination lowers pKa 0.5-2 units	Lower pKa reduces P-gp efflux

p-gp efflux ratio (Caco-2)	< 2 preferred	Strategic F reduces efflux ratio	Efflux reduction often parallels AO benefit
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*Target ranges reflect CNS MPO (Multiparameter optimization) criteria adapted from [6], pKa ranges reflect practical CNS-penetrant compound experience across multiple therapeutics programs

5.3 Efflux Transporter Considerations and pKa Improvement

The relationship between molecular basicity (pKa) and P-gp efflux susceptibility is a recurring theme in CNS drug design. Basic amines, which are common pharmacophoric elements in CNS drugs, are recognized substrates for P-gp when in their protonated form; reduction of amine basicity through structural modification can therefore reduce P-gp efflux and improve CNS penetration [9,29]. Fluorination—as discussed in the context of AO metabolism—additionally reduces pKa of adjacent basic amines, providing a dual benefit of AO metabolism reduction and efflux minimization [29,30]. These effects have been quantitatively documented in a systematic study by Fjelbye et al. [29], which showed a strong correlation between positioning of the fluorine atoms, the pKa shifts, and the in vitro P-gp efflux ratios of the compounds [29].

In addition to fluorination, a modulating physicochemical properties study of tetrahydropyridine-2-amine BACE1 inhibitors was conducted, where, with systematic modification of electron-withdrawing groups, both pKa and efflux susceptibility were predicted and optimized [41]. Such observations highlight the need for simultaneous optimization of all physicochemical properties (metabolic stability, solubility, permeability, and efflux) in the design of brain-penetrant drugs, rather than sequential optimization of each property. Figure 3 represents this multiparametric optimization challenge as a four-axis space, identifying the central green zone that an ideal candidate should occupy and mapping the three most common failure modes as peripheral positions that specific SAR strategies are known to correct.

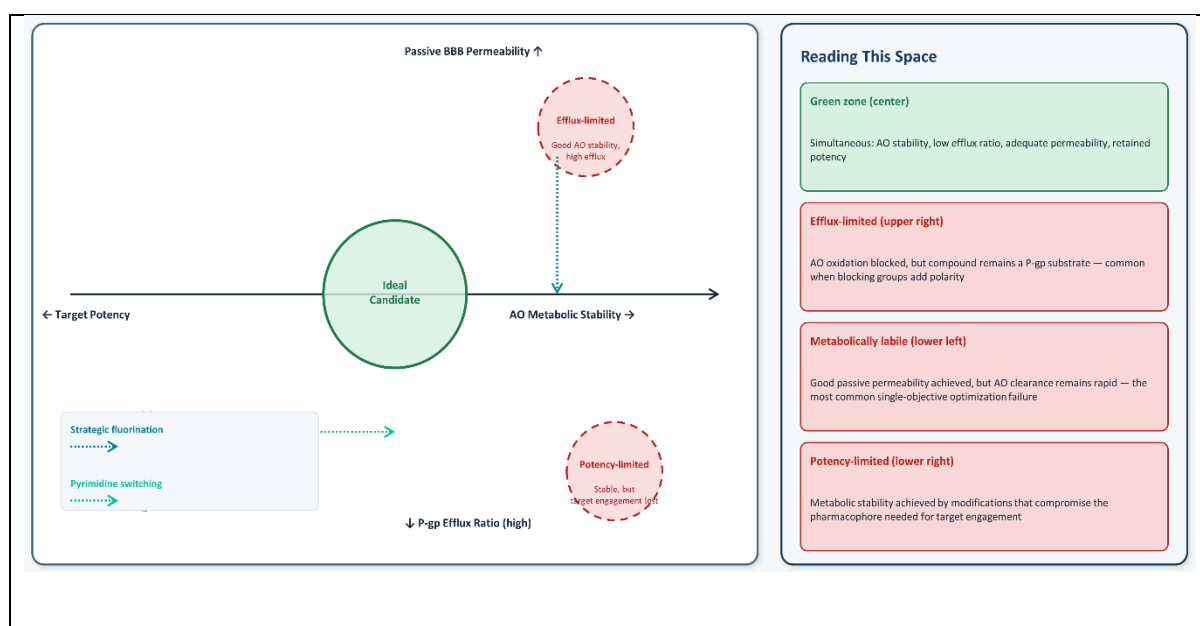


Figure 3. Schematic of the multiparametric optimization space for AO-resistant, brain-penetrant CNS drug candidates. Each axis represents a key parameter (AO metabolic stability, P-gp efflux ratio, passive BBB permeability, target potency). An idealized candidate occupies the center of the optimization window (green zone). Common failure modes are shown: compounds with good AO resistance but high efflux (efflux-limited; upper right), adequate permeability but rapid AO clearance (metabolically labile; lower left), and compounds

that sacrifice potency to achieve metabolic stability (potency-limited; lower right). Strategic fluorination and pyrimidine ring switching tend to move compounds toward the center from multiple directions simultaneously, explaining their prevalence in successful CNS programs. The author prepared this figure using Microsoft PowerPoint (Microsoft Corporation, Redmond, WA, USA) as an original schematic for this review

6. Case Studies of Successful Drug Candidates

The case studies presented below represent the best-available evidence for integrated AO mitigation strategies. They are interpreted critically; where evidence supports causal attribution of pharmacokinetic improvement to a specific structural modification, this is stated; where confounding factors limit causal interpretation, these are made explicit

6.1 BACE1 Inhibitors for Alzheimer's Disease

Perhaps the most well-documented case studies of successful AO metabolism mitigation in the area of CNS drug discovery are those from the β -secretase (BACE1) inhibitor program. The major AO-mediated oxidation point in BACE1 inhibitors is found in their amino-containing heterocyclic scaffolds, which are inherent hot spots for AO oxidation. Hilpert et al. [30] reported the discovery of a series of highly potent BACE1 inhibitors that were developed by using fluorine-scanning of a CNS-targeted lead series, which resulted in a combination of several properties: blocking AO oxidation, decreasing the efflux by P-gp, lowering the pKa to within an optimal CNS range, and increasing in vivo brain exposure in rodent pharmacokinetic studies. The optimized inhibitors showed high in vivo efficacy and were considered to be suitable for clinical development in Alzheimer's disease, being an example of the potential of fluorination to be a multifunctional SAR tool in CNS drug design [30].

The complementary strategy of scaffold hopping has been used in the BACE1 inhibitor space, for example, by replacing a metabolically labile pyridine ring with a pyrimidine at the primary AO site identified by metabolite profiling (which led to an improvement in oral bioavailability), but also resulted in good brain amyloid- β reduction in rodent models. The study is a good example of the ability of pyridine-to-pyrimidine switching, with careful metabolite identification to remove potential AO liability without loss of pharmacological efficacy [21]. The physicochemical aspects of BACE1 inhibitor optimization were further developed in the tetrahydropyridine-2-amine study by systematically varying pKa and efflux with electron-withdrawing substituents, while retaining activity [43].

An important caveat contextualizing both BACE1 case studies is that the BACE1 inhibitors class as a whole failed in Phase III clinical trials for Alzheimer's disease, not only for pharmacokinetic reasons, but also for pharmacodynamic reasons related to on-target toxicity and the inadequacy of amyloid reduction as a therapeutic endpoint [44]. The pharmacokinetic success of these programs is therefore real and instructive, but it did not translate to clinical efficacy, which is a reminder that resolving AO liability is a necessary but not sufficient condition for therapeutic success.

6.2 GluN2B Modulators and Neuropsychiatric Drug Candidates

An interesting discovery program in a CNS context where AO requirements are managed, the modulators of GluN2B are an intriguing therapeutic field because the NMDA receptor subunit composition is involved with depression, neuropathic pain, and other neurological diseases [24]. Gelin et al. [28] reported a triazole-

methoxypyrimidine GluN2B modulator series in which iterative SAR-guided AO metabolic profiling and successive structural changes yielded potent, brain-penetrant compounds with improved metabolic stability and in vivo CNS exposure [28]. For the specific neuroinflammation and cholinergic deficit disease area relevant to Alzheimer's, the pyrimidine and pyrrolidine core study showed multi-target inhibition, good BBB penetration, and effective in vivo activity, while maintaining a balance of the properties of the core that must be stable to AO while also meeting the physicochemical properties required for CNS delivery [40]. Such multi-target directed ligand (MTDL) strategies are becoming even more relevant in the field of neurodegeneration, where polypharmacology could provide therapeutic benefits for therapeutic molecules more selective than MTDLs. Within the series, AO-resistant scaffold replacement was achieved by attaching the pharmacophoric element of GluN2B selectivity to the pyrimidine core, a nice convergence of target and metabolic design considerations that bears out the benefit of taking a close look at the metabolic stability of a scaffold early in a CNS program.

6.3 c-Met Kinase Inhibitors and Deuterium Strategy

The case study for the c-Met kinase inhibitor MET401 is a good example of an approach to AO metabolism mitigation using deuterium substitution. In vitro studies, which have been carried out, determined that AO is the main enzyme involved in oxidative metabolism of MET401 in rat liver cytosol and that the quinoline ring is the major site of AO-mediated hydroxylation. The incorporation of deuterium in the site of AO oxidation resulted in a deuterated analogue, which demonstrated significantly enhanced pharmacokinetic parameters in rats, such as decreased clearance, prolonged half-life and increased systemic exposure, with no obvious effect on target activity or selectivity. This example is important as it shows that the kinetic isotope effect can lead to substantial improvement in the in vivo PK parameters, and not just the rate reduction, as reported by (Robbins et al.,...). The synthetic methodology contributions of Choi et al.,... (deuterium labeling via dimethylformamide dimethyl acetal-d₃) and Wright (deuterated enamines with high isotopic fidelity) are important enabling technologies that make the deuterium strategy practically accessible for a broader range of CNS scaffold types. Without reliable synthetic routes to high isotopic purity, the utility of the strategy is limited by isotopic dilution that reduces the effective KIE in vivo. [23,33,45].

A limitation that the MET401 study does not address is the potential for metabolic shifting, which is where blocking an AO oxidation site by deuterium directs metabolism to an alternative site, potentially generating a metabolite with worse toxicological, pharmacokinetic, or pharmacodynamic properties than the AO-mediated product. Metabolic shifting has been documented for deuterium-substituted drugs targeting CYP enzymes (most notably deutetrabenazine/tetrabenazine) and must be considered a contingent risk for the AO deuterium strategy that requires metabolite profiling of the deuterated analogue before its pharmacokinetic improvement can be considered a net benefit [23,46].

6.4 Species Surrogacy and Translational Validation

One of the key issues that is sometimes overlooked in the process of drug development for an AO is the identification of a suitable preclinical species for AO metabolism. Hutzler et al. [10] have systemically studied the AO metabolism of BIBX1382, an EGFR inhibitor that is extensively metabolized by AO in humans, but not in rats. The study showed that the hepatic cytosol of cynomolgus monkeys can exactly replicate the human AO metabolite profile and AO clearance rate, making the cynomolgus monkey a valid surrogate species for the assessment of human AO metabolism. This finding has practical implications for the drug development pipeline

and shows that the use of cynomolgus monkeys as a metabolic profiling species, for compounds that are AO-active in human cytosol, can significantly enhance the translatability of preclinical pharmacokinetic information [10].

Due to the practical constraints associated with the use of routine cynomolgus monkey for early drug discovery (cost, ethics, throughput), this is usually used for compounds that passed initial rodent screening and further advanced through candidate selection. The availability of surrogate systems to better mimic the predictive value of the cynomolgus monkey assay, with the ability to incorporate recombinant human AO preparations with normalized enzyme content or fresh hepatocyte systems that retain AO activity, with increased throughput and reduced cost, would be a welcome methodological advance. Currently, there are no validated systems.

7. Comparative Analysis of SAR Strategies

7.1 Convergent Findings and Robust Strategies

Across the reviewed literature, several consistent themes arose. There is a wide range of compounds that have been site-specifically blocked, either by addition of C2-substituents at the site of the oxidation or by ortho-substituents on scaffolds that do not contain the AO positions, or by scaffold hopping to less AO-prone heterocycles [13,20,28]. The pervasiveness of fluorination as a modification that can simultaneously tackle the dual issues of AO metabolism and BBB permeability is always highlighted, although its most effective application relies on the strategic consideration of positioning [29–31]. Switching of pyridines to pyrimidines is an especially pure and widely applicable strategy, often affording metabolic stability enhancements with little to no impact on drug activity [21,24,25]. Computational and experimental techniques, including metabolite identification, molecular docking studies, quantum chemical reactivity prediction, and in vitro pharmacokinetic characterization, are all deemed essential for successful AO-driven SAR efforts [12,13,26,34]. The SAR conclusions, generally obtained through studies using more than one methodological approach, and utilizing complementary methodologies, were more predictable and more generalizable than studies using one analytical method [12,20,34].

7.2 Divergent Findings and Context-Dependence

Although there are some similarities, literature showed significant differences. The ability of a specific structural change to be efficient in blocking AO metabolism is clearly scaffold-dependent, and a change that is efficient in one chemical series can be ineffective or even harmful in another, depending on the difference in active site binding geometry or electronic context [2,28,31]. Likewise, the effects of AO-blocking modification on BBB permeability cannot be universally predicted: modifications that increase metabolic stability by increasing polarity can also decrease passive BBB penetration and/or increase efflux, as seen in some studies where extensive fluorination has caused BBB permeability to drop, even when metabolic stability has been enhanced [29]. It can also be seen that there are inconsistencies in the reliability of in vitro–in vivo extrapolation. Empirical scaling factors provide better prediction of the clearance in well-characterized substrates and enzyme preparations; however, they cannot overcome the basic uncertainty arising from enzymes that are not stable in vivo, interspecies differences in enzyme structure and/or the presence of extrahepatic AO metabolism [7,8,17,18]. Even the most stringent in vitro evaluations have been reported to not be predictive of the degree of AO-mediated clearance found in vivo, suggesting the need for better predictive models [8,17].

The most significant divergence found in the literature, however, is the systematic disagreement provided in the parameters measured from one study to another. Some report only in vitro AO clearance, others report only in vivo rodent pharmacokinetics, and few report BBB permeability, efflux ratio, and target potency data at the same time along with metabolic stability. This form of measurement heterogeneity renders the ability to make cross-study conclusions concerning the effectiveness of the strategies methodologically questionable, and is a systematic weakness in the way the field produces an AO SAR.

7.3 Toward Multiparametric Integration

The most important lesson drawn from the comparative analysis of literature from the SAR is that AO metabolism mitigation cannot be effectively addressed without consideration of other pharmacokinetic and pharmacodynamic goals. Optimization of AO metabolic stability while simultaneously neglecting BBB permeability, efflux susceptibility, target potency, or off-target liability risks shifting the bottleneck from one parameter to another without improving overall compound quality [2,9,28,42]. The most successful drug candidates described in the reviewed literature are those for which AO-blocking strategies were implemented within a multiparametric optimization framework, with simultaneous tracking of metabolic stability, physicochemical properties, efflux, and in vivo pharmacokinetics [21,24,30].

Computational tools—including PBPK models that integrate AO clearance predictions with BBB permeability estimates, efflux parameters, and target exposure models—are increasingly enabling prospective multiparametric optimization, though their reliability depends critically on the quality of the underlying experimental data and the accuracy of the component prediction models [12,17,34].

Figure 4 presents a decision-tree framework proposed here as the first synthesis-level tool for strategy selection in AO-CNS drug design, integrating the mechanistic and SAR evidence reviewed in this paper. The framework encodes four key inputs — scaffold class, primary oxidation site, current CNS MPO score, and available assay infrastructure — and maps these to recommended strategy prioritization with explicit rationale and validation requirements.

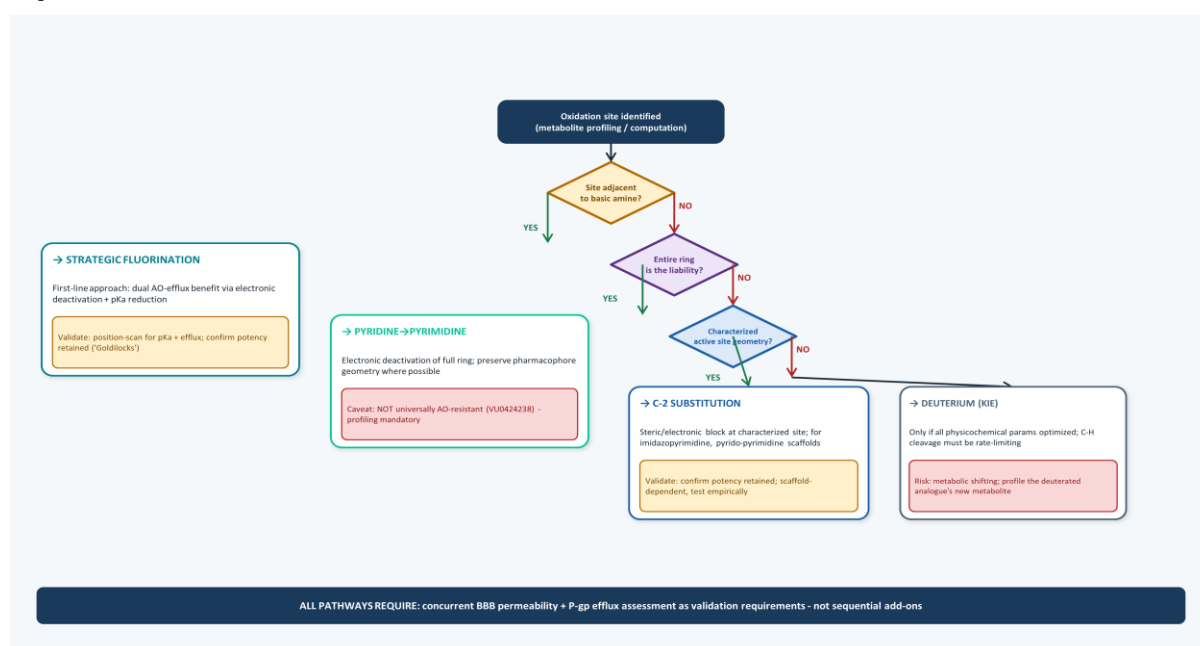


Figure 4. Proposed decision-tree framework for AO mitigation strategy selection in CNS drug discovery programs. Starting from scaffold class and oxidation site identification (from metabolite profiling and/or

computational prediction), the framework guides strategy selection based on: (i) current CNS drug-likeness status (MPO score and key parameters); (ii) nature of the oxidation-prone position (electronic vs. steric blockade feasibility); and (iii) available assay infrastructure. Strategic fluorination is recommended as the first-line approach when the oxidation site is adjacent to a basic amine, due to its dual AO–efflux benefit. Pyrimidine switching is recommended when the entire ring system is the metabolic liability and pharmacophore preservation allows. C-2 substitution is recommended for imidazopyrimidine and pyrido- pyrimidine scaffolds with characterized active site geometry. Deuterium is reserved for scaffolds where all physicochemical parameters are optimized, and only the AO rate at a single C–H position requires reduction. All pathways require concurrent BBB permeability and efflux assessment as validation requirements, not sequential add-ons.

This framework is proposed as a synthesis-level organizational tool by the authors based on the reviewed literature; it does not represent validated prospective guidance and should be tested against novel scaffold series. The author prepared this figure using Microsoft PowerPoint (Microsoft Corporation, Redmond, WA, USA) as an original schematic for this review.

8. Limitations of the Current Literature and Future Direction

8.1 Limitations

The literature on AO metabolism mitigation in brain-penetrant heterocycles is characterized by five systematic limitations that collectively constrain the development of an evidence-based, generalizable framework for CNS program design.

First and most fundamentally, no prospective head-to-head comparison of the four principal SAR strategies (fluorination, C-2 blocking, scaffold hopping, deuterium substitution) was applied to the same scaffold series under standardized, concurrent assay conditions has been published. This absence means that strategy selection remains precedent-based rather than evidence-based, and that the superiority claims made for individual approaches in case studies cannot be extrapolated to general applicability. Second, the IVIVE problem for AO clearance remained unresolved. Despite methodological advances in empirical scaling and proteomics-normalized approaches, prediction errors of 3–10- fold persisted for structurally diverse AO substrates — errors large enough to cause incorrect go/no- go decisions in drug development programs.

Third, high-resolution co-crystal structures of hAO1 with CNS-relevant drug candidates did not exist in the public literature, limiting the rigor of structure-based drug design for AO-resistant scaffolds.

Fourth, the majority of AO SAR studies conducted on a restricted set of pyrido- pyrimidine and imidazopyrimidine scaffolds, and the SAR conclusions could not be generalized to the broader chemical space of CNS drug candidates.

Fifth, the impact of AO-blocking modifications on BBB permeability is inconsistently assessed alongside metabolic stability, creating an incomplete evidence base for multiparametric optimization decisions.

8.2 Future Research Directions

Based on the analysis of the current literature, the papers and experiments identified several priority areas for future research. High priority should be placed on the development and validation of improved in vitro AO assay systems with improved enzyme stability and standardized protein content normalization to reduce the uncertainty in IVIVE and enable more reliable prediction of human AO clearance [8,17,18]. In parallel,

systematic comparative studies of AO metabolism across species using standardized substrates could be conducted to better characterize species differences and validate the cynomolgus monkey surrogate model more broadly [10,32]. High-resolution structural biology studies of human AO1 in complex with diverse substrates and inhibitors would also provide the mechanistic framework needed for structure-based drug design of AO-resistant scaffolds [11,12]. Additionally, integrated multiparametric SAR studies that simultaneously characterize AO metabolism, BBB permeability, efflux susceptibility, and target engagement for the same compound series would provide holistic datasets to support multiparametric optimization [9,30,42].

Further research should also focus on the refinement and validation of computational prediction models for AO substrate reactivity and site of metabolism, incorporating machine learning approaches trained on increasingly comprehensive experimental datasets [12,34]. Systematic evaluation of the long-term safety and metabolic fate of fluorinated and deuterated drug candidates in humans should be applied to assess the potential for unforeseen liabilities introduced by these modifications [28,33,45]. Moreover, characterization of the functional impact of AOX1 polymorphisms and isoform variants on substrate metabolism and interindividual pharmacokinetic variability to inform personalized medicine approaches and clinical pharmacokinetic predictions [14]. Standardization of selective AO inhibitors and establishment of validated phenotyping protocols would also have a significant impact on the field, as these would facilitate more robust AO contribution to a drug's metabolism in complex biological matrices.

In the future, organ-on-a-chip and 3D hepatocyte culture with native AO activity could provide a more physiologically relevant in vitro system for assessing AO metabolism than existing cytosol-based systems, which would help to minimize the gap between prediction and experiment. The application of machine learning models trained on large, curated AO metabolism datasets incorporating structural, electronic, and three-dimensional molecular descriptors represents a promising avenue for improving the speed and accuracy of AO liability prediction at the earliest stages of drug discovery, enabling prospective compound design rather than reactive structural modification.

9. Conclusion

Aldehyde oxidase has evolved from a metabolic curiosity to a recognized and consequential enzyme in drug discovery with particular significance for the design of brain- penetrant therapeutics. The body of literature reviewed herein demonstrated that diverse and complementary structural modification strategies—site-specific blocking of AO oxidation through C2-substitution and ortho-substituents, strategic fluorination, pyridine-to-pyrimidine scaffold hopping, and deuterium substitution—can effectively attenuate AO-mediated metabolism in heterocyclic drug candidates. The mechanistic basis for these strategies is increasingly well- understood through the convergence of computational modelling, structural biology, and experimental metabolite profiling, enabling more rational and prospective implementation of AO- resistant designs.

However, the dual requirement for AO metabolic stability and BBB penetration in CNS drug candidates demands an integrated multiparametric approach that transcends the mitigation of any single liability in isolation. None of these liabilities by themselves. Strategic fluorination is a case in point in the context of the foregoing, as it can effectively decrease the metabolism of the AO, affect its pKa to limit efflux, and increase its passive BBB permeability, resulting in higher levels in the CNS. These principles, if applied with rigor, can be demonstrated in case studies of BACE1 inhibitors and GluN2B modulators and in c-Met kinase inhibitors, to

generate brain-penetrant drug candidates with the desired pharmacokinetic properties for CNS drug development. Ongoing difficulties, such as under-prediction of human AO clearance from in vitro studies and the restriction of preclinical rodent pharmacokinetic models, highlight the need for ongoing methodological advances in AO metabolism evaluation. To bridge the gap between in vitro characterization and in vivo pharmaceutical prediction, improved in vitro systems, validated computational prediction tools, and standardized phenotyping protocols will prove key in the future. Finally, a rational design of AO-resistant and brain-penetrant heterocycles will rely on the ongoing development of a multiparametric design optimization, which is driven by mechanistic enzymology and computational chemistry, and will require further systematic development and validation, as detailed in the reviewed literature.

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Explainable Multi-Target Machine Learning Framework for Predicting Thermophysical Properties of Chemical Elements Using Periodic Table Descriptors

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Abstract

The thermophysical properties of the chemical elements underpin materials selection, process design, and fundamental chemistry, yet their experimental determination remains difficult for rare, radioactive, or synthetic species. This study developed an explainable, multi-target machine learning framework that predicted four key properties, namely melting point, boiling point, density, and atomic radius, directly from readily available periodic-table descriptors. Twelve descriptors, including atomic number, atomic mass, period, group, electronegativity, ionization energy, electron affinity, and valence-shell electron count derived from electron configurations, were compiled for the elements. Eight regression algorithms spanning linear, kernel, instance-based, and tree-ensemble families were systematically benchmarked using repeated five-fold cross-validation, with all targets modeled on a natural-logarithmic scale and missing descriptors handled by leakage-free iterative imputation. Tree-based ensembles considerably outperformed the linear models, which did not account for the nonlinear nature of the periodic relationships. The best mean performance was provided by the Extra Trees algorithm, which served as the framework base learner. After optimization, the framework reached coefficients of determination of 0.72, 0.76, 0.88, and 0.74 for the melting point, boiling point, density, and atomic radius, respectively, alongside low values of root-mean-square error. Model behavior was interpreted using Shapley additive explanations and permutation importance. Ionization energy emerged as the dominant descriptor for melting point, boiling point, and density owing to its correlation with bonding strength and electron localization, whereas the period governed atomic radius in line with the shell structure. The largest prediction errors were associated with elements exhibiting anomalous bonding (such as carbon, boron, and phosphorus) and strong relativistic effects (such as gold and mercury).

Keywords: machine learning, explainable artificial intelligence, thermophysical properties, periodic descriptors, ensemble learning, Shapley additive explanations.

1. Introduction

The physical and chemical behavior of every material ultimately traces back to the properties of its constituent elements. Among these, thermophysical properties such as the melting point, boiling point, density, and atomic radius are particularly significant because they govern phase stability, heat-transfer considerations, alloying behavior, separation techniques, and many other engineering choices [1]. Melting and boiling points give the temperatures at which solid–liquid and liquid–gas phase transitions occur and reflect the degree of interatomic bonding; density expresses the mass per unit volume and is critical in structural and nuclear applications; and the atomic radius influences solid-state atomic packing, coordination, and substitution. Knowledge of all these properties is necessary before designing novel materials. Traditionally, elemental properties have been measured directly or estimated from first-principles approaches. Direct measurement is more accurate but challenging, hazardous, or practically infeasible for rare, highly radioactive, or synthetic elements, of which only a few atoms may ever be produced. First-principles methods based on density functional theory [2] can predict several physical properties from the electronic configuration but are computationally expensive and difficult to apply, especially for finite-temperature properties such as the melting point, which is governed by complex collective behavior [9]. This has motivated the development of data-driven alternatives for estimating elemental properties from simple descriptors.

Despite considerable progress, most studies focus on a single property at a time and on complex, composition-based descriptors that require specialized software to compute. There remains a need for a unified, transparent framework that predicts several fundamental elemental properties simultaneously from a small set of intuitive periodic-table descriptors, and that explicitly explains its predictions in chemical terms. Addressing this gap, the present work develops an explainable multi-target machine learning framework for the joint prediction of melting point, boiling point, density, and atomic radius of the chemical elements.

The main contributions of this study are fourfold. First, a compact set of twelve periodic-table descriptors, several of which are engineered from electron configurations, is assembled and shown to be sufficient for accurate property prediction. Second, eight regression algorithms are systematically benchmarked under a rigorous repeated cross-validation protocol, establishing extremely randomized trees as the most effective base learner across all four targets. Third, the optimized framework is shown to achieve high predictive accuracy despite the small size of the periodic table and the presence of missing descriptor values, which are handled by a leakage-free imputation strategy. Fourth, and most importantly, Shapley additive explanations (SHAP) and permutation-importance analyses are used to interpret the framework, revealing chemically meaningful descriptor–property relationships and rationalizing the residual errors in terms of anomalous bonding and relativistic effects.

2. Related Work

Over the past decade, machine learning has emerged as a powerful third paradigm in the chemical and materials sciences, complementing experiment and simulation [3]. Data-driven models learn statistical relationships between the composition or structure of a material and its properties, enabling rapid screening of vast chemical spaces [4]. Comprehensive reviews have documented the rapid growth of materials informatics and its successful application to formation energies, band gaps, mechanical properties, and many other targets [5,6]. Representative studies include the prediction of molecular atomization energies with sub-kilocalorie accuracy [7], the acceleration of property predictions for polymers and inorganic solids [8], the estimation of melting temperatures of single- and binary-component solids from density functional theory data [9], and the prediction of band gaps of inorganic compounds [10]. Collectively, these works

demonstrate that carefully chosen descriptors combined with flexible regression models can reproduce quantum-mechanical and experimental data at a small fraction of the cost.

Ensemble methods based on decision trees are among the most effective and widely used algorithms for tabular property prediction. Random forests [11], gradient boosting machines [12], extreme gradient boosting [13], and extremely randomized trees [14] combine many weak learners to capture nonlinear relationships and feature interactions while remaining robust to noise and requiring relatively little tuning. Their high performance, however, is accompanied by a loss of interpretability, since a large number of trees makes the model behave like a black box, which is problematic in scientific contexts where the interpretability of predictions is important.

The field of explainable artificial intelligence has developed rigorous tools to address this problem. Explanations based on Shapley values (SHAP), which rely on cooperative game theory, provide a locally faithful and consistent attribution of a model output to its input features [15], and an exact and efficient method exists for SHAP computation in tree ensembles [16]. Local interpretable model-agnostic explanations [17] and the broader suite of interpretable machine learning techniques [18] now make it feasible to look inside the black box and relate model predictions to underlying domain knowledge. This offers an opportunity not only to generate accurate predictions but also to validate that the discovered relationships are consistent with established periodic trends. To the best of our knowledge, no prior study has combined a compact periodic-descriptor representation with multi-target ensemble learning and explainability analysis for the joint prediction of these four elemental properties.

3. Methodology

3.1 Dataset

The study is based on a publicly available periodic-table dataset containing the 118 known chemical elements and their tabulated physical and chemical characteristics (PubChem Periodic Table Dataset). For each element the dataset provides the atomic number, symbol, name, atomic mass, ground-state electron configuration, electronegativity (Pauling scale), atomic radius, first ionization energy, electron affinity, common oxidation states, standard state, and the four target thermophysical properties considered here: melting point (K), boiling point (K), density (g cm^{-3}), and atomic radius (pm), together with a qualitative element category (for example alkali metal, transition metal, or noble gas). The melting and boiling points are expressed as absolute temperatures, and densities correspond to standard conditions.

As is characteristic of the periodic table, the data are incomplete for the heaviest elements: the superheavy synthetic elements beyond curium have few or no measured thermophysical properties, and several descriptors such as electron affinity are undefined or unmeasured for a number of elements. To construct a consistent multi-target learning problem, elements possessing measured values for all four target properties were retained, yielding a modeling set of 93 elements spanning atomic numbers 1 to 96. This complete-target requirement was necessary because multi-output evaluation requires every target to be defined for each element.

3.2 Feature Engineering

Twelve periodic-table descriptors were used as model inputs. Eight are tabulated directly: atomic number, atomic mass, electronegativity, first ionization energy, and electron affinity, together with the period and group, which were assigned from the atomic number using the standard structure of the periodic table (lanthanides and actinides were placed in group 3). The remaining four descriptors were engineered from the ground-state electron configuration by parsing the occupancy of the valence subshells, giving the numbers of valence s, p, d, and f electrons; their sum defines the total number of valence electrons. These configuration-derived features compactly encode the position of an element within its block and

its bonding capacity. The complete descriptor set and its definitions are summarized in Table 1. The four target properties were deliberately excluded from the descriptor list, since they were to be predicted by the framework from fundamental atomistic descriptors alone and not from any other thermodynamic property.

Table 1. Periodic-table descriptors used as inputs to the machine learning framework.

Descriptor	Symbol / unit	Description
Atomic number	Z	Number of protons in the nucleus
Atomic mass	u	Relative atomic mass
Period	—	Horizontal row of the periodic table (1–7)
Group	—	Vertical column (1–18); f-block assigned to group 3
Electronegativity	χ	Pauling electronegativity
Ionization energy	eV	First ionization energy
Electron affinity	eV	Electron affinity
Valence electrons	—	Total number of valence-shell electrons
Valence s-electrons	—	Electrons in the valence s subshell
Valence p-electrons	—	Electrons in the valence p subshell
Valence d-electrons	—	Electrons in the valence d subshell
Valence f-electrons	—	Electrons in the valence f subshell

3.3 Data Preprocessing

Two preprocessing steps were essential. First, the target properties span several orders of magnitude; the density, for example, ranges from about $9 \times 10^{-5} \text{ g cm}^{-3}$ for hydrogen to more than 22 g cm^{-3} for osmium, and the melting and boiling points range from a few kelvin for the light noble gases to several thousand kelvin for refractory solids. Modeling such heavy-tailed targets directly biases the models toward the largest values. Each target was therefore transformed to its natural logarithm before training, and predictions were back-transformed to the original scale for evaluation. A preliminary comparison confirmed that the logarithmic transformation improved the cross-validated accuracy of every target relative to modeling on the original scale.

Second, a minority of descriptor values were missing within the modeling set, most notably electron affinity (about 41% missing) and electronegativity (about 9% missing). Rather than discarding these informative descriptors or the affected elements, missing values were reconstructed by multivariate iterative imputation, in which each descriptor with missing entries is modeled in turn as a function of the others [19]. Crucially, the imputer was fitted only on the training partition within each cross-validation fold and then applied to the held-out partition, so that no information from the test elements leaked into training. The target values were never used during imputation. The strong mutual correlations among periodic descriptors make such imputation reliable, as an element's position in the periodic table largely determines its atomic properties.

3.4 Regression Algorithms

Eight regression algorithms representing four distinct methodological families were evaluated. Two linear baselines were included: ordinary multiple linear regression and ridge regression, the latter adding an L2 penalty to stabilize coefficient estimation [20]. An instance-based k-nearest-neighbors regressor [21] and a kernelized support vector regressor with a radial basis function kernel [22] captured local and nonlinear structure, respectively. Four tree-ensemble methods were

then assessed: the random forest [11], which averages many decorrelated bagged trees [23]; the gradient boosting regressor, which fits trees sequentially to the residuals of the current model [12]; extreme gradient boosting, a regularized and highly optimized boosting implementation [13]; and the extremely randomized trees (Extra Trees) ensemble, which introduces additional randomization by selecting split thresholds at random [14]. For the linear, distance-, and kernel-based models, descriptors were standardized to zero mean and unit variance within the pipeline; the tree ensembles are scale-invariant and required no such scaling.

3.5 Model Evaluation

Predictive performance was estimated by repeated five-fold cross-validation with ten repetitions (fifty train–test splits in total), which provides a robust estimate of generalization on a small dataset and quantifies the variability of the estimate. Within every fold the complete pipeline, comprising imputation, optional scaling, target transformation, and model fitting, was refitted on the training elements only. Three complementary metrics were computed on the original (back-transformed) scale: the coefficient of determination (R^2), the root-mean-square error (RMSE), and the mean absolute error (MAE). The R^2 measures the fraction of variance explained, whereas RMSE and MAE express the typical prediction error in the physical units of each property. Cross-validation as a principle of predictive model assessment follows established statistical practice [24].

3.6 Hyperparameter Optimization

The algorithm with the best mean cross-validated performance across the four targets was selected as the framework base learner and subsequently optimized. The most influential Extra Trees hyperparameters were tuned by grid search, considering the number of features evaluated at each split, the minimum number of samples at a leaf node, and the minimum number of samples required to split a node. Grid search was carried out using an inner five-fold cross-validation loop while keeping the number of trees fixed at 500. The best configuration for each target was then re-evaluated using the same cross-validation protocol employed for model comparison.

3.7 Model Interpretation

The optimized framework was interpreted using two complementary techniques. Shapley additive explanations assign to each descriptor, for each element, a contribution to the predicted value such that the contributions are locally accurate and satisfy consistency axioms derived from cooperative game theory [15,25]. For tree ensembles these values can be computed exactly and efficiently using the TreeSHAP algorithm [16]. The mean absolute SHAP value of a descriptor, averaged over all elements, quantifies its global importance, while the distribution of signed SHAP values reveals the direction and nonlinearity of its effect. As an independent cross-check, permutation importance was also computed by measuring the decrease in model performance when each descriptor was randomly shuffled. Because the models operate on log-transformed targets, SHAP contributions are expressed on the logarithmic scale.

3.8 Implementation

All computations were performed in Python. The regression algorithms, cross-validation, iterative imputation, and permutation importance were implemented with the scikit-learn library [26]; extreme gradient boosting used the XGBoost library [13]; and Shapley explanations used the SHAP library [16]. Numerical computation and data handling relied on NumPy [27] and pandas [28], and figures were produced with Matplotlib [29] and seaborn [30]. Fixed random seeds were used throughout to ensure reproducibility. The overall workflow, from data acquisition and feature engineering through model training, evaluation, selection, and interpretation, is summarized in Figure 1.

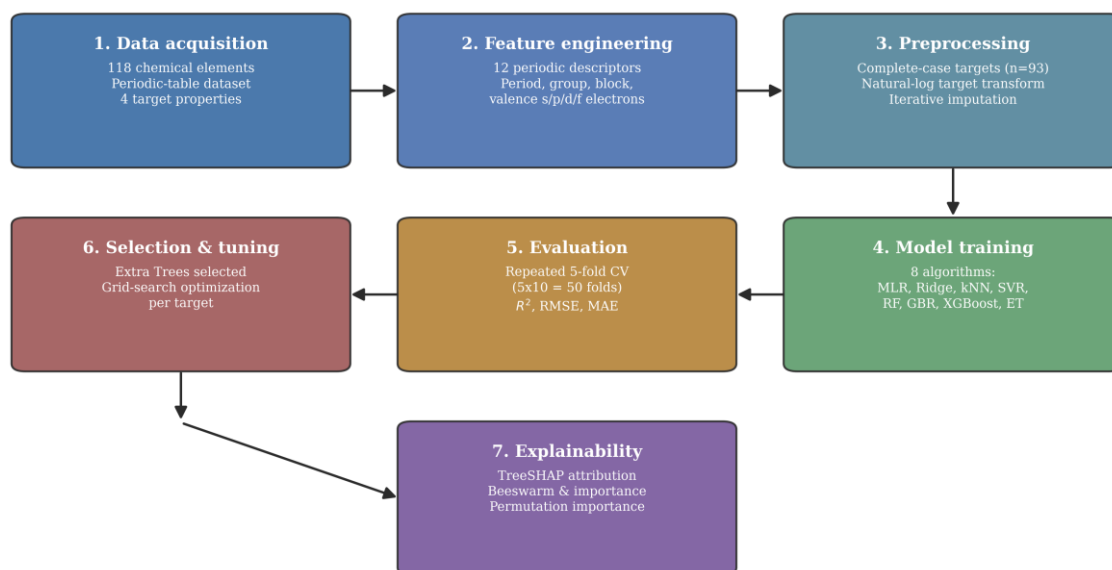


Figure 1. Overview of the explainable multi-target machine learning workflow, from data acquisition and feature engineering through model training, evaluation, selection, and interpretation.

4. Experiments and Results

4.1 Exploratory Analysis of Descriptor–Property Relationships

Table 2 summarizes descriptive statistics for the four target properties across the modeling set of 93 elements. All four target variables display the broad right-skewed distributions expected for the chemical elements (Figure 2, top row). Melting temperatures vary from 0.95 K in helium to 3823 K in carbon; boiling temperatures from 4.22 K to 5869 K; densities vary by more than five orders of magnitude; and atomic radii vary from 120 pm to 343 pm. The logarithmic transform (Figure 2, bottom row) narrows these ranges and produces much more symmetric distributions, which validates its appropriateness for regression analysis.

Table 2. Descriptive statistics of the four target thermophysical properties for the modeling set of 93 elements.

Property	Unit	n	Minimum	Maximum	Mean	SD
Melting point	K	93	0.95	3823	1279.0	921.1
Boiling point	K	93	4.22	5869	2536.2	1588.4
Density	g cm ⁻³	93	9 × 10 ⁻⁵	22.57	7.46	5.88
Atomic radius	pm	93	120	343	206.6	36.3

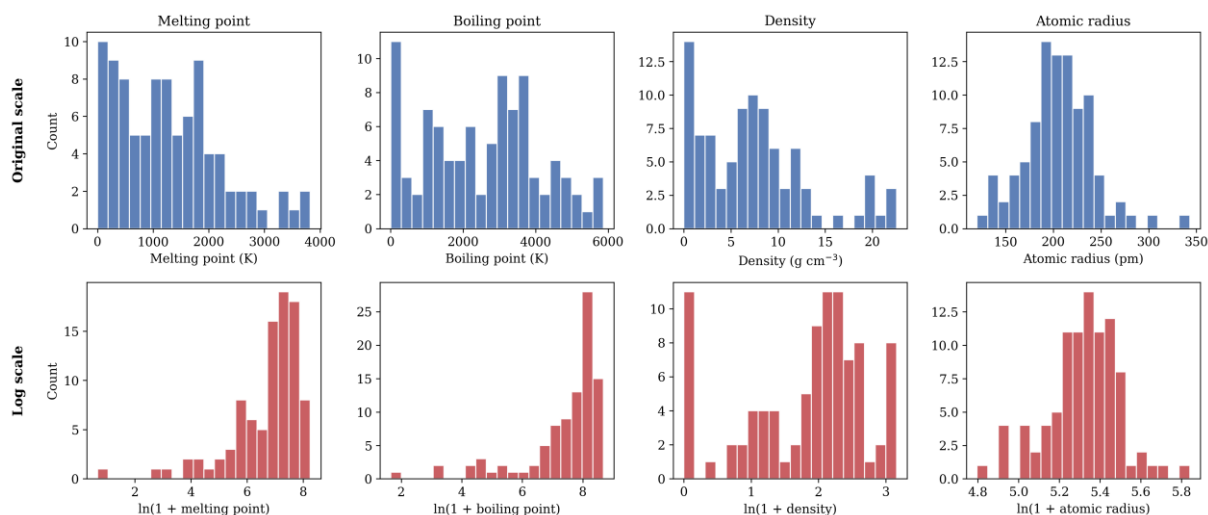


Figure 2. Distributions of the four target properties on their original scale (upper row) and after natural-logarithmic transformation (lower row) for the 93 modeled elements.

The linear correlation structure between descriptors and targets (Figure 3) is informative but limited. Atomic radius correlates most strongly and negatively with ionization energy and electronegativity (Pearson coefficients of about -0.64), consistent with the fact that tightly bound, electronegative atoms are compact. The density of elements is directly related to atomic number, atomic mass, and period, increasing as the mass of the element increases. Melting and boiling points show a very weak linear relationship with any single descriptor; the most significant correlation is observed with the number of p electrons in the valence shell and with ionization energy. The weakness of these linear correlations indicates that the descriptor–property relationships are nonlinear and interactive, and that linear modeling alone is inadequate.

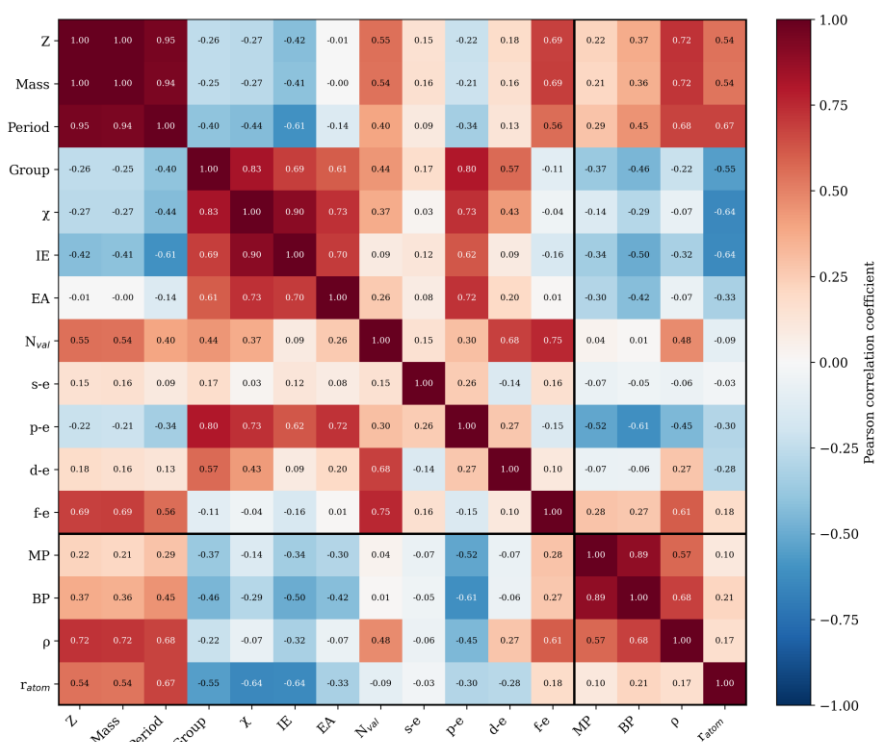


Figure 3. Pearson correlation matrix among the periodic descriptors and the four target properties. The target block is outlined. Symbols: Z, atomic number; χ , electronegativity; IE, ionization energy; EA, electron affinity; MP, melting point; BP, boiling point; ρ , density; r_{atom} , atomic radius.

4.2 Comparison of Regression Algorithms

The eight algorithms were benchmarked against one another using repeated cross-validation under identical conditions (Table 3 and Figure 4). The two linear algorithms performed poorly and even yielded negative coefficients of determination for melting point, boiling point, and density, indicating that they made predictions worse than simply predicting the mean of the data; multiple linear regression on the logarithm of density gave $R^2 = -3.28$. This drastic drop in predictive ability confirms that the relationship between periodic descriptors and thermophysical properties is highly nonlinear and cannot be captured by linear terms. Incorporating nonlinearity through the k-NN and SVR algorithms improved the coefficients of determination to the range 0.42–0.74.

The tree-ensemble models clearly outperformed the other model types for every target. The random forest, gradient boosting, extreme gradient boosting, and Extra Trees models produced coefficients of determination ranging from 0.70 to 0.90. Extreme gradient boosting gave the best performance for density and atomic radius, with R^2 values of 0.90 and 0.75, respectively, while Extra Trees performed best for melting point and boiling point, with R^2 values of 0.72 and 0.76, respectively. Across the four properties, Extra Trees achieved the highest average coefficient of determination (0.78), followed closely by extreme gradient boosting and random forest with averages of 0.77 and 0.75, respectively. The Extra Trees model was therefore chosen as the base learner because of its ability to minimize variance on small datasets.

Table 3. Cross-validated coefficient of determination (R^2) for the eight regression algorithms, reported as mean $\pm$ standard deviation over repeated five-fold cross-validation. The best value for each property is shown in bold.

Algorithm	Melting point	Boiling point	Density	Atomic radius
Multiple linear regression	-0.07 ± 0.54	-0.08 ± 0.72	-3.28 ± 5.30	0.65 ± 0.14
Ridge regression	-0.07 ± 0.48	-0.03 ± 0.63	-2.35 ± 3.71	0.62 ± 0.14
k-nearest neighbors	0.48 ± 0.25	0.42 ± 0.23	0.74 ± 0.15	0.56 ± 0.13
Support vector regression	0.54 ± 0.31	0.55 ± 0.32	0.63 ± 0.33	0.66 ± 0.12
Random forest	0.70 ± 0.27	0.72 ± 0.22	0.87 ± 0.08	0.71 ± 0.11
Gradient boosting	0.68 ± 0.28	0.70 ± 0.21	0.89 ± 0.10	0.72 ± 0.12
Extreme gradient boosting	0.70 ± 0.29	0.71 ± 0.20	0.90 ± 0.07	0.75 ± 0.10
Extra Trees	0.72 ± 0.29	0.76 ± 0.22	0.90 ± 0.09	0.75 ± 0.10

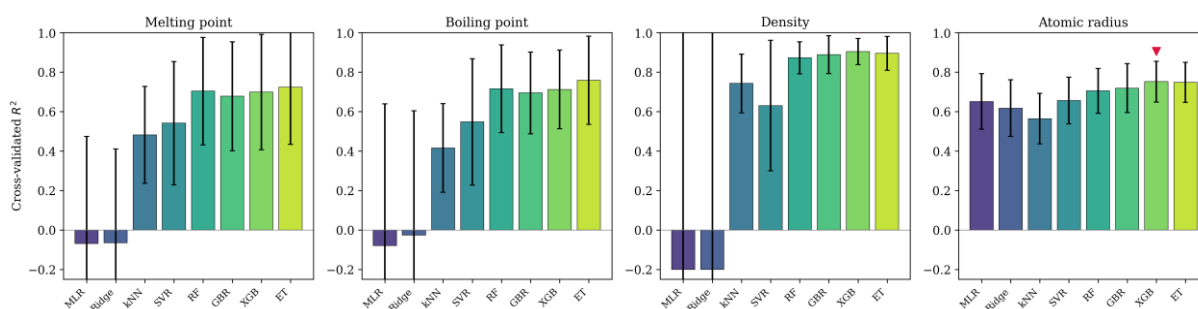


Figure 4. Cross-validated coefficient of determination for the eight regression algorithms across the four properties. Bars show the mean and error bars the standard deviation over repeated five-fold cross-validation; the best model for each property is marked. Abbreviations: MLR, multiple linear regression; kNN, k-nearest neighbors; SVR, support vector regression; RF, random forest; GBR, gradient boosting; XGB, extreme gradient boosting; ET, Extra Trees.

4.3 Performance of the Optimized Framework

Following hyperparameter tuning, the framework was assessed again using repeated cross-validation (Table 4). It accounted for 72% of the variance in melting point ($RMSE \approx 434$ K), 76% of the variance in boiling point ($RMSE \approx 683$ K), 88% of the variance in density ($RMSE \approx 1.84$ g cm $^{-3}$), and 74% of the variance in atomic radius ($RMSE \approx 16.7$ pm). Given the chemical complexity of the periodic table and the relatively small number of training samples, these figures represent a good fit, especially for density, which shows near-quantitative behavior. The associated errors are also small: the mean absolute errors correspond to 12–19% of the mean values of the respective properties. In the observed-versus-

predicted diagrams generated from out-of-fold predictions (Figure 5), all points lie close to and evenly around the ideal line, with no curvature or systematic bias.

Table 4. Performance of the optimized Extra Trees framework, reported as mean $\pm$ standard deviation over repeated five-fold cross-validation (RMSE and MAE in the physical units of each property).

Property	Unit	R ² (mean $\pm$ SD)	RMSE (mean $\pm$ SD)	MAE
Melting point	K	0.72 $\pm$ 0.28	434 $\pm$ 232	271
Boiling point	K	0.76 $\pm$ 0.19	683 $\pm$ 225	474
Density	g cm ⁻³	0.88 $\pm$ 0.08	1.84 $\pm$ 0.73	1.13
Atomic radius	pm	0.74 $\pm$ 0.10	16.7 $\pm$ 3.7	12.7

The optimized model also demonstrated the stability of the base estimator: the best-performing parameter sets for all four targets used all descriptors at each node with minimal leaf regularization, a configuration very close to the default Extra Trees settings. Tuning therefore brought only marginal improvements over the default, indicating that the strong performance arose from the ensemble learning method and the descriptor set rather than from the tuned parameters.

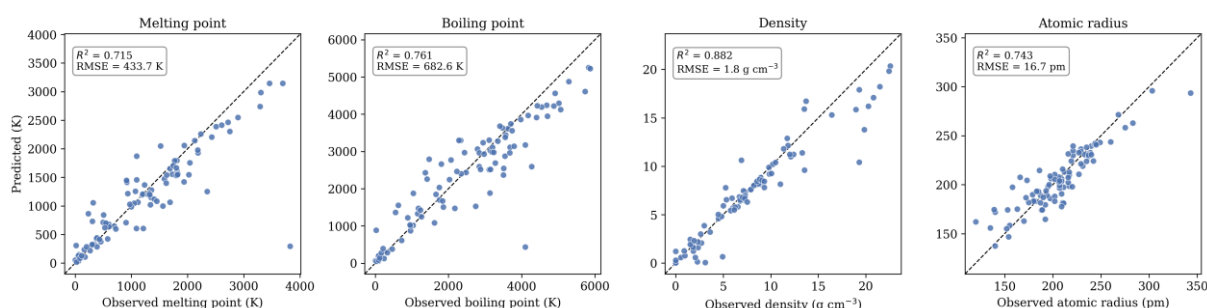


Figure 5. Predicted versus observed values for the optimized framework. Predictions are out-of-fold estimates averaged over ten resampling repeats; the annotated statistics are the repeated five-fold cross-validation averages of Table 4. The dashed line denotes perfect prediction.

5. Discussion

5.1 Model Interpretation and Chemical Insight

The central aim of an explainable framework was to reveal why the model makes its predictions. The global importance of descriptors derived from SHAP are shown in Figure 6, and the leading descriptors for each property are listed in Table 5. A clear and chemically coherent picture emerges. Ionization energy is the single most important descriptor for melting point, boiling point, and density, with the largest mean absolute SHAP value in each case. This is physically reasonable: the first ionization energy reflects how tightly the valence electrons are bound to the nucleus, which correlates with the strength of metallic and covalent bonding, and hence with the cohesive energy that must be overcome to melt or vaporize an element, and with the compactness that governs density. The beeswarm plots confirm the expected directions: high ionization energy pushes the predicted density upward, whereas for atomic radius high ionization energy pushes the prediction downward, since strongly bound electrons yield smaller atoms.

The group number and electronegativity are consistently the next most influential descriptors for the melting and boiling points, together with the number of valence p electrons. This reflects the systematic variation of bonding character across a period, from strongly bonded metals and network solids on the left and center of the table to weakly bonded molecular and monatomic species on the right. For density, the period and electronegativity join ionization energy as the dominant descriptors, encoding the position of an element within the table and thus its mass-to-volume ratio. For atomic radius, the ordering changes: the period becomes the most important descriptor, in direct agreement with the fundamental periodic trend that atomic radius increases with the principal quantum number of the valence shell down a group, followed by ionization energy, group, and electronegativity. The overall SHAP magnitudes for atomic radius are smaller than for the other properties because atomic radius varies over a comparatively narrow range on the logarithmic scale.

It is noteworthy that descriptors expressing similar underlying information, such as atomic number, atomic mass, and period, tend to share importance among themselves, a well-understood consequence of feature correlation in additive attribution. The broad agreement between the SHAP rankings and the independently computed permutation importances, both identifying ionization energy, group, electronegativity, and period as the key descriptors, increases confidence that the framework has learned genuine periodic relationships rather than spurious correlations. In this sense the explainability analysis serves as a scientific validation of the model.

Table 5. The five most important descriptors for each property, ranked by mean absolute SHAP value (given in parentheses, on the natural-logarithmic scale).

Rank	Melting point	Boiling point	Density	Atomic radius
1	Ionization energy (0.34)	Ionization energy (0.42)	Ionization energy (0.69)	Period (0.043)
2	Group (0.23)	Group (0.17)	Period (0.27)	Ionization energy (0.024)
3	Electronegativity (0.13)	Valence p-electrons (0.13)	Electronegativity (0.26)	Group (0.023)
4	Valence d-electrons (0.11)	Electronegativity (0.13)	Valence p-electrons (0.19)	Electronegativity (0.019)
5	Valence p-electrons (0.10)	Valence d-electrons (0.10)	Group (0.19)	Atomic mass (0.019)

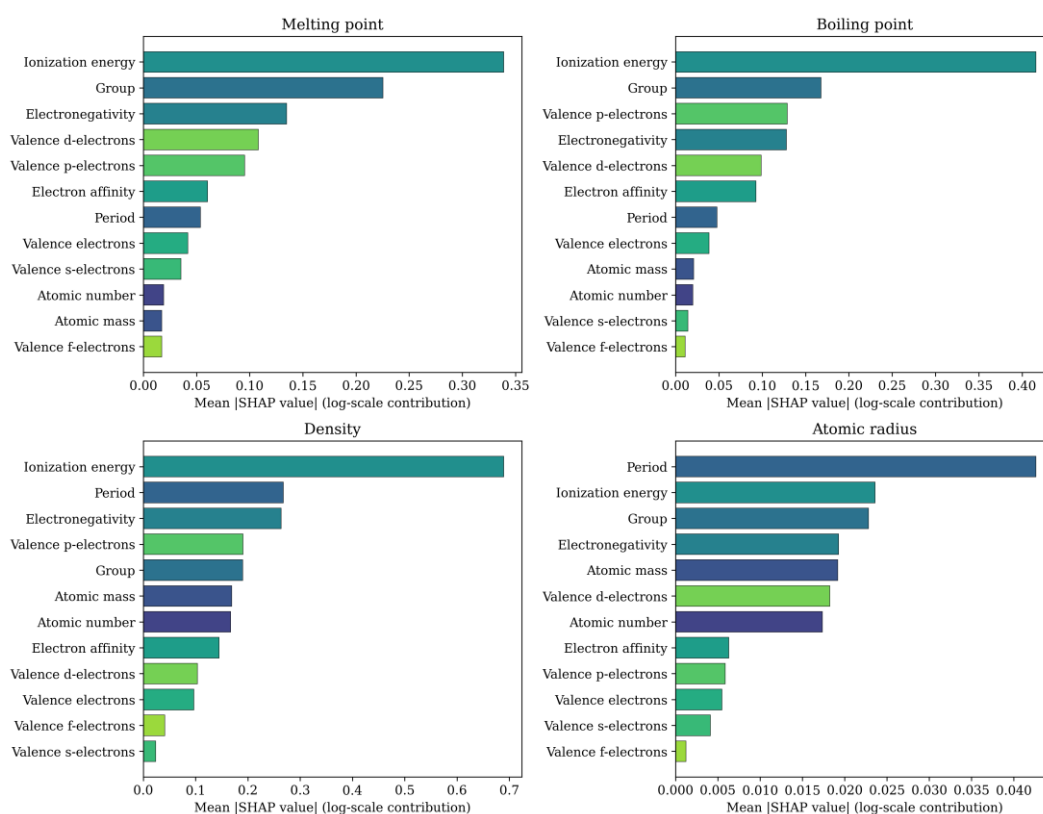


Figure 6. Global descriptor importance for each property, quantified by the mean absolute SHAP value (contribution on the natural-logarithmic scale).

5.2 Error Analysis

Examining the elements with the largest prediction errors provides further insight and, reassuringly, the outliers are precisely those that chemistry would flag as anomalous. For melting point, the most severely underestimated element is carbon, whose measured value of 3823 K vastly exceeds the framework's estimate because carbon, in its diamond and graphite forms, possesses a three-dimensional covalent network with exceptionally strong bonding that is not encoded by single-atom descriptors; boron and silicon, which also form network solids, are underestimated for the same reason.

Conversely, phosphorus is overestimated because its low melting point arises from a discrete molecular structure. For density, gold and mercury are underestimated, reflecting the pronounced relativistic contraction of the 6s orbital in the heaviest elements, an effect absent from the descriptor set; mercury is further exceptional as the only metal that is liquid at room temperature. For atomic radius, the alkali metal cesium, which has the largest radius in the dataset, lies at the extreme of the descriptor space and is underestimated.

These residual errors are not failures of the methodology so much as manifestations of chemistry that transcends the periodic descriptors, namely allotropy, molecular versus network bonding, and relativistic effects. The fact that the interpretable framework isolates exactly these physically meaningful exceptions underscores the value of coupling accurate prediction with transparent explanation, and it indicates where richer, structure-aware descriptors would be required to improve accuracy further.

5.3 Limitations and Outlook

Several limitations should be acknowledged. The periodic table provides only about one hundred fully characterized elements, so the training set is inherently small, which limits the achievable accuracy and widens the uncertainty of the performance estimates, as reflected in the standard deviations in Table 4. The descriptors are single-atom quantities and therefore cannot capture crystal structure, allotropy, or relativistic effects, which set a ceiling on the accuracy attainable for structure-sensitive properties such as melting point. The imputation of the substantial fraction of missing electron-affinity values, although performed without leakage, introduces some additional uncertainty. Finally, the framework is interpolative and should be applied with caution to the superheavy elements that lie outside the range of the training data.

Future work could extend the framework in several directions: incorporating structural or cohesive-energy descriptors to better model network solids; adopting genuinely joint multi-output learning that exploits correlations among the four properties; enlarging the dataset with compounds and alloys; and quantifying predictive uncertainty on a per-element basis. Nevertheless, within its intended scope the framework already provides accurate, transparent, and essentially instantaneous estimates of four fundamental elemental properties, and the explainability analysis confirms that its reasoning aligns with established chemical principles.

6. Conclusions and Future Work

An explainable multi-target machine learning framework was developed to predict the melting point, boiling point, density, and atomic radius of the chemical elements from a compact set of twelve periodic-table descriptors, several of which were engineered from electron configurations. A systematic comparison of eight algorithms showed that tree-based ensembles vastly outperform linear models on this nonlinear problem, and the extremely randomized trees method delivered the best mean performance across the four properties. After optimization and leakage-free handling of missing descriptors, the framework explained between 72% and 88% of the variance in the four properties with low absolute errors, using only tabulated atomic information and negligible computation. Shapley and permutation analyses revealed chemically coherent descriptor–property relationships, identifying ionization energy as the principal driver of melting point, boiling point, and density, and the period as the principal driver of atomic radius, while the largest residual errors were traced to elements with anomalous bonding or relativistic effects.

By uniting predictive accuracy with transparent, chemically meaningful explanations, the proposed framework offers a practical and trustworthy complement to experimental measurement and first-principles calculation for the rapid estimation of fundamental elemental properties. Future efforts should focus on incorporating structure-aware and cohesive-energy descriptors to capture network solids and relativistic effects, adopting fully joint multi-output learning, extending the dataset to compounds and alloys, and providing per-element uncertainty estimates to further broaden the applicability of the framework.

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8. Declarations

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Conflict of Interest / Competing Interests

The authors declare no conflict of interest.

Ethics Approval and Consent to Participate

Not applicable. This study involved only publicly available data and no human participants.

Consent for Publication

All authors have read and approved the final manuscript and consent to its publication.

Data Availability

The dataset analyzed in this study is derived from publicly available periodic-table data (<https://pubchem.ncbi.nlm.nih.gov/rest/pug/periodictable/CSV>). The engineered features and analysis code are available from the corresponding author upon reasonable request.

Materials Availability

Not applicable.

Code Availability

The analysis code is available from the corresponding author upon reasonable request.

Authors' Contributions

F. Abujaber: Conceptualization, Supervision, Methodology, Writing–Review and Editing, Funding Acquisition. F. Siouri: Methodology, Software, Formal Analysis, Visualization, Writing–Original Draft. All authors have read and agreed to the published version of the manuscript.

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In Silico Investigation of Flavonoid Polyphenol binding to AKT1, PRKCA, and PIK3CA Kinases and the P2RY12 GPCR: Potential Implications for Coronary Heart Disease

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Abstract

Coronary heart disease (CHD) is a major cardiovascular condition contributing substantially to global mortality and is closely associated with atherosclerosis and thrombotic platelet activation. This study explored the binding interfaces of selected phytochemicals with key proteins involved in platelet activation. Using molecular docking, six flavonoid polyphenols—morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin—were evaluated for their predicted binding to the kinases AKT1, PRKCA, and PIK3CA and the P2RY12 G-protein-coupled receptor. Binding was assessed using predicted free energies, inhibition constants, and polar and non-polar contacts. The compounds showed variable predicted interactions across the four targets. In silico findings, these identify flavonoid–target interactions that may merit further experimental investigation in the context of platelet aggregation and CHD.

Keywords: flavonoid polyphenols, docking, in silico, platelet aggregation, coronary heart disease

1. Introduction

Cardiovascular diseases are serious conditions affecting a large proportion of the global population [1]. Coronary heart disease (CHD) is regarded among the most common cardiovascular diseases and remains a major cause of mortality worldwide [2]. It is primarily associated with narrowing of the coronary vessels due to atherosclerosis and may be complicated by thrombus formation involving platelet aggregation [3].

1.1. Pathway and protein targets, and the mechanisms of platelet aggregation

Several mechanisms can enhance platelet aggregation in the presence of agonists rather than directly activating platelets. For example, platelet priming can involve phosphatidylinositol 3-kinase (PI3K) signaling pathways, insulin-like growth factor I, and thrombopoietin. Adrenaline can also modulate platelet responsiveness through platelet receptors and cyclic AMP (cAMP)-related signaling [4]. During thrombus formation, fibrin accumulates around platelets and red blood cells and may contribute to vessel obstruction [5].

A balanced hemostatic state is maintained by inhibitory and activating factors in blood [6]. Platelet aggregation is influenced by multiple factors and signaling pathways [7]. Two major classes of platelet activation pathways involve G-protein-coupled receptors (GPCRs) and immunoreceptor tyrosine-based activation motif (ITAM)-linked receptors. GPCRs for ADP, thrombin, and TXA₂ stimulate platelets through signaling that includes phospholipase C β and protein kinase C (PKC). The ADP receptor P2Y₁₂ promotes integrin activation through pathways involving phosphoinositide 3-kinases (PI3K), protein kinase B (AKT), and glycogen synthase kinase (GSK) [8].

Several medicinal plants and the active phytochemicals found in them are currently being thoroughly researched for their potential action against CVDs [9].

1.2. Platelet Activation Affected by Flavonoid Polyphenols

In this study, the researchers tested six compounds (morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin), called flavonoid polyphenols. These compounds form most of phenolic compounds in fruits, vegetables, nuts, drinks, and therapeutic herbs [10]. These water-soluble flavonoids contain two aromatic rings, each containing at least one hydroxyl group, connected by a three-carbon bridge to form a six-membered heterocyclic ring. Flavonoids are further categorized into subclasses based on the combination of the aromatic ring with the heterocyclic ring, the oxidation state of the heterocyclic ring, and their functional groups. Specific compounds within each subclass are differentiated by unique patterns of conjugation and hydroxylation [11].

Anthocyanidins, including malvidin and cyanidin, are the primary pigments that give berries, flowers, and fruits their pink to purple colors. They are found in large quantities in the epidermal cells of these plant parts [12]. Protection against obesity or diabetes as a result of antioxidant, anti-inflammatory, or hypolipidemic properties is one of the partially recognized health claims [13].

Hesperidin and naringenin possess a reactive structure, are susceptible to hydroxylation, glycosylation, and O-methylation, and have a ketone group. Hesperidin and naringenin are found in oranges and in grapefruits, respectively, as glycosides [12]. Several studies have been conducted on the phytochemicals to investigate their

potential antioxidant and lipid-lowering properties [14]. Morin and apigenin, both of which have a phenylchromenone backbone, are glycosides found in spices as well as yellow/orange fruits and vegetables. They were discovered to have antioxidant or anti-inflammatory properties [15].

Anthocyanidins and their metabolites possess anti-thrombotic properties suggesting them as potential promoters of cardiovascular health. Anthocyanidins at 1 μM , colonic metabolites at 10 μM , and a combination of phenolic acids and anthocyanidins (at 1 μM and 0.1 μM , respectively) demonstrated significant platelet-sedating and desensitizing effects. Specifically, P-selectin expression, an indicator of platelet activation, was reduced by 10-40% in resting, TRAP-activated, hydrogen peroxide-stressed, and epinephrine pre-activated platelets compared to controls. The dose-response curve for the weak agonist TRAP showed a 0.8 μM increase in the threshold concentration required for aggregation. Interestingly, the mixture was effective even at ten times lower concentrations than the individual components, indicating a synergistic effect. However, the reactivity of platelet to strong agonists like collagen and ADP remained unaffected [16].

Naringenin, a flavonoid found abundantly in citrus fruits, has protective effects against atherothrombotic diseases by reducing platelet activation, a key factor in athero-thrombogenesis. In vitro, naringenin dose-dependently inhibited platelet aggregation, showing greater efficacy on ADP-induced aggregation. It also suppressed ADP-induced platelet α -granule secretion, fibrinogen binding, intracellular calcium mobilization, and adhesion to collagen. Additionally, naringenin inhibited platelet spreading on fibrinogen and clot retraction, processes mediated by outside-in integrin signaling. Mechanistically, naringenin suppressed PI3K-mediated signaling and phosphodiesterase activity, increased cGMP levels, and induced VASP phosphorylation. These effects were reversed by a PKA inhibitor. In vivo, naringenin reduced thrombus formation in a rat carotid arterial thrombus model without prolonging bleeding time in mice, suggesting its potential as a safe antiplatelet agent targeting PI3K and cyclic nucleotide signaling [17].

Out of all the anthocyanidins tested in whole blood, it was discovered that cyanidin-3-glucoside, at 10⁻⁶ M downregulated ADP-induced integrin activation [18]. Likewise, cyanidin-3-glucoside, at 10⁻⁵ M, antagonized GPVI-induced signaling (Syk, Src). It was additionally found to inhibit collagen-dependent thrombus formation in vivo in mice [19].

Flavonoids might inhibit platelet function by binding to the thromboxane receptor (TP) and subsequently blocking downstream signaling. This binding has been observed in human myometrium and TP-transfected HEK 293T cells, suggesting that the antagonism of TP could mediate the effects of flavonoids across various tissues. Specifically, apigenin, a flavone with yellow color, is suggested to inhibit the activation of platelets through PARs and other agonists by binding to the TXA₂ receptors [20], and/or by interfering with intracellular Ca²⁺ signaling [21]. Morin hydrate can also prevent platelet-mediated thrombosis by decreasing TXA₂ production and integrin $\alpha\text{IIb}\beta_3$ activation while increasing cAMP generation, which inhibits clot retraction. This suggests that morin hydrate may have therapeutic potentials for treating diseases related to platelet activation [22].

Several controlled intervention studies with phytochemical-enriched food components have been conducted in order to discover antithrombotic effects via platelets [12]. A controlled 8-week study was conducted, in which hypertensive subjects were given flavonoid-containing grape seed extract. No evidence was found in alleviating

hypertension. Antiplatelet aggregation effects were only mildly observed [23]. Regular consumption of Kiwi, which also contains polyphenols, resulted in a lower platelet aggregation caused by collagen/ADP [24].

Anthocyanidins can be found in dark grape juice or as an anthocyanin supplement. Eleven small to medium-sized controlled studies showed that anthocyanin-rich food additives have surprisingly strong antiplatelet effects. For one week, drinking juice of purple grape rather than orange juice decreased collagen-induced platelet aggregation [25]. Anthocyanin capsules taken for four weeks by obese subjects realized a reduction in ADP- and collagen-induced platelet activation parameters [26].

Anthocyanin supplementation reduced plasma levels of platelet secretion products in hypercholesterolemic individuals [27]. Healthy volunteers were given either orange juice or dark (anthocyanin-rich) plum juice for a duration of 1 to 4 weeks. These trials discovered a reduction in mortality after intervention. Platelet responses to GPVI and ADP/P2Y₁₂, but not of PAR-dependent responses.

Previous studies examined the *in vitro* and *in vivo* effects of several phytochemicals on platelet aggregation. However, the potential binding of the selected flavonoids to multiple protein targets within the ADP/P2Y₁₂-related platelet signaling pathway remains incompletely characterized. We therefore investigated their plausible interactions with these proteins *in silico*. This work provides a computational basis for prioritizing flavonoid–target interactions for subsequent experimental investigation in thrombosis-related cardiovascular disease.

2. Results

The researchers followed the docking protocol for apigenin, morin, malvidin, cyanidin, hesperidin, and naringenin to scan the surfaces of AKT1 (PDB ID: 1h10 [28]), P2RY12 (PDB ID: 4ntj [29]), PRKCA (PDB ID: 4ra4 [30]), and PIK3CA (PDB ID: 2enq [31]). Polar and non-polar contacts were examined as components of the predicted binding interfaces. The interacting residues and contact types are shown in Figures 1–4. Tables 1–4 summarize the predicted binding free energies, inhibition constants, RMSD values, numbers of polar contacts, and amino acids involved in polar interactions.

Based on the criteria adopted from a previous docking study, the researchers prioritized poses with predicted binding free energies below -5 kcal/mol and inhibition constants below $100 \mu\text{M}$ [32]. Flavonoids meeting these criteria were considered for further structural interpretation.

2.1. Binding results for the flavonoids to AKT1 protein

According to Table 1, all the investigated phytochemicals met the adopted thresholds of $K_i < 100 \mu\text{M}$ and predicted binding free energy < -5 kcal/mol [32]. Several polar contacts were identified at the predicted binding interfaces, supporting further investigation of these flavonoid–AKT1 interactions.

Table 1. Binding free energies, inhibition constants (Ki), root mean square deviation (RMSD), number of polar bonds, and amino acids involved in the polar interaction for docking phytochemicals to the AKT1 protein.

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, Ki (μM)	RMSD for the ligand from the reference structure ($\text{\AA}$)	Number of Polar contacts	Amino acids involved in the polar interaction
Apigenin	-5.50	93.11	17.697	6	ARG-41, LYS-8, TRP-11, GLU-91
Morin	-6.02	38.89	18.404	7	GLN-47, 3 in GLU-40, 2 in LYS-39, LEU-52
Malvidin	-5.96	43.05	34.960	1	GLN-104
Cyanidin	-6.01	39.03	30.153	3	2 in LEU-62, ASP-108
Hesperidin	-6.74	11.34	31.843	5	SER-56, GLU- 116, ARG-76 2 in ASP-3
Naringenin	-6.70	12.35	31.519	4	VAL-83, ARG- 15, GLU-17

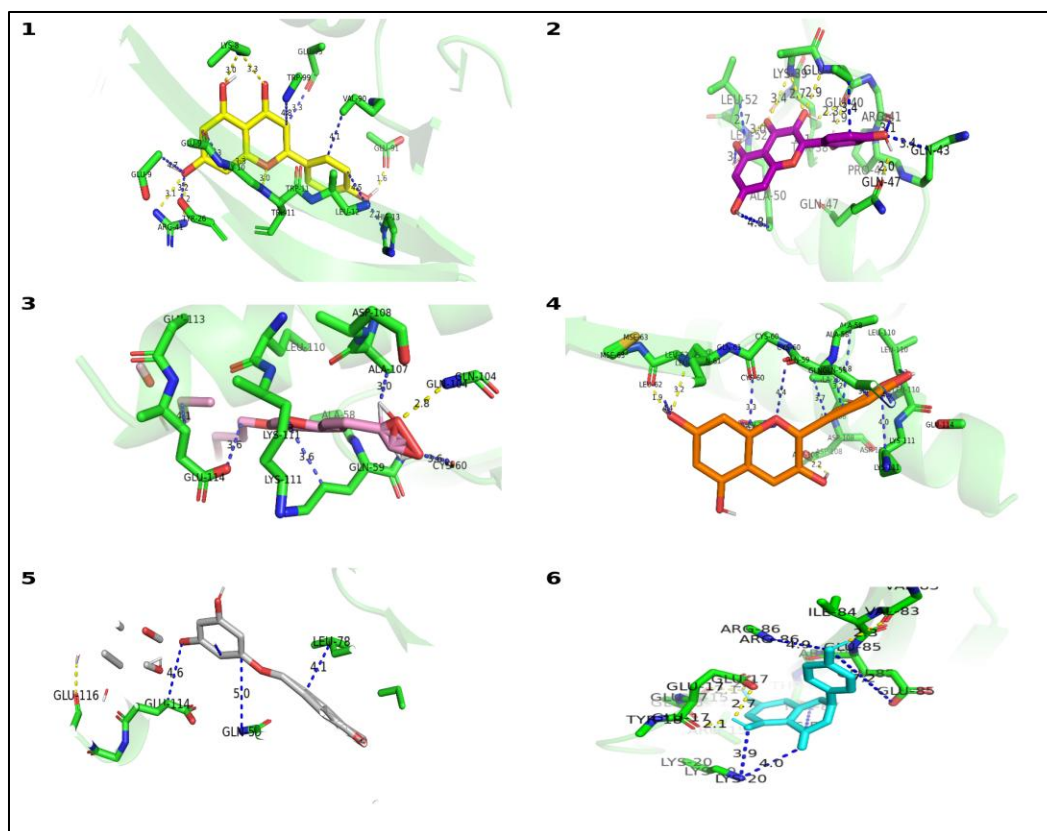


Figure 1. Binding sites for the phytochemicals on the surface of AKT1 (PDB ID: 1h10 [28]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with six polar bonds. 2) Morin with seven polar bonds. 3) Malvidin with one polar bond. 4) Cyanidin with three polar bonds. 5) Hesperidin with five polar bonds. 6) Naringenin with four polar bonds.

Table 2. Binding free energies, inhibition constants (K_i), root mean square deviation (RMSD), number of polar bonds, and amino acids involved in the polar interaction for docking phytochemicals to the P2RY12 protein.

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, K_i (μ M)	RMSD for the ligand from the reference structure ($\text{\AA}$)	Number of Polar contacts	Amino acids involved in the polar interaction
Apigenin	-4.23	793.59	_____	3	GLN-195, ASN-159, GLN-98
Morin	-5.87	49.61	111.418	2	THR-283, TYR-105
Malvidin	-8.80	353.02	123.873	2	HIS-187, ASN-159

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, Ki (μM)	RMSD for the ligand from the reference structure (Å)	Number of Polar contacts	Amino acids involved in the polar interaction
Cyanidin	-6.79	10.48	121.489	5	SER-101, GLN_98, LYS-280, ASN-191, GLN-195
Hesperidin	-4.34	663.00	111.05	3	ASN-201, TYR-192, ARG-256
Naringenin	-6.55	15.83	72.988	2	ARG-128, LYS-131

2.2. Binding results for the flavonoids to P2RY12

According to Table 2, the shaded phytochemicals met the adopted thresholds of $K_i < 100 \mu\text{M}$ and predicted binding free energy $< -5 \text{ kcal/mol}$ [32]. Several polar contacts were identified at the predicted binding interfaces, including contacts involving THR, SER, LYS, GLN, and ARG residues.

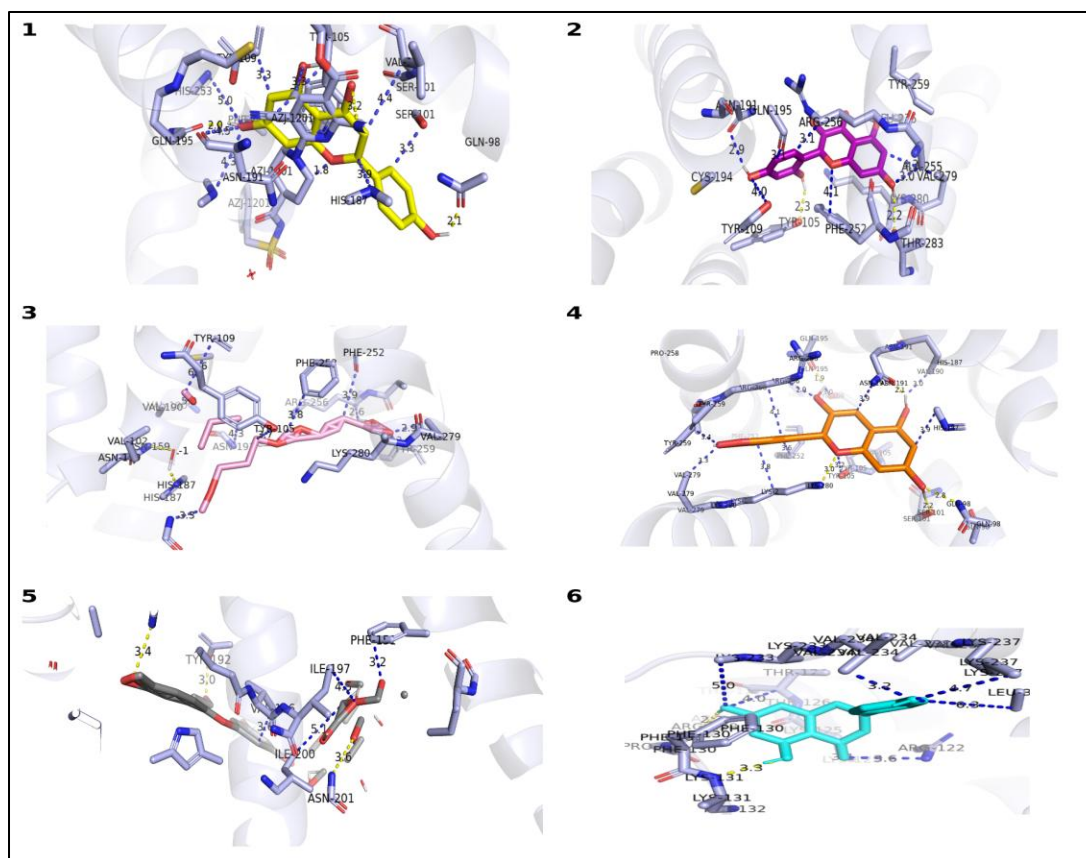


Figure 2. Binding sites for the phytochemicals on the surface of P2RY12 (PDB ID: 4ntj [29]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with three polar bonds. 2) Morin with two polar bonds. 3) Malvidin with two polar bonds. 4) Cyanidin with five polar bonds. 5) Hesperidin with three polar bonds. 6) Naringenin with two polar bonds.

2.3. Binding results for the flavonoids to PRKCA

According to Table 3, the shaded phytochemicals met the adopted thresholds for the inhibition constant and predicted binding free energy [32]. Several polar contacts were identified at the predicted binding interfaces, involving residues including LYS, ASP, LEU, and ALA.

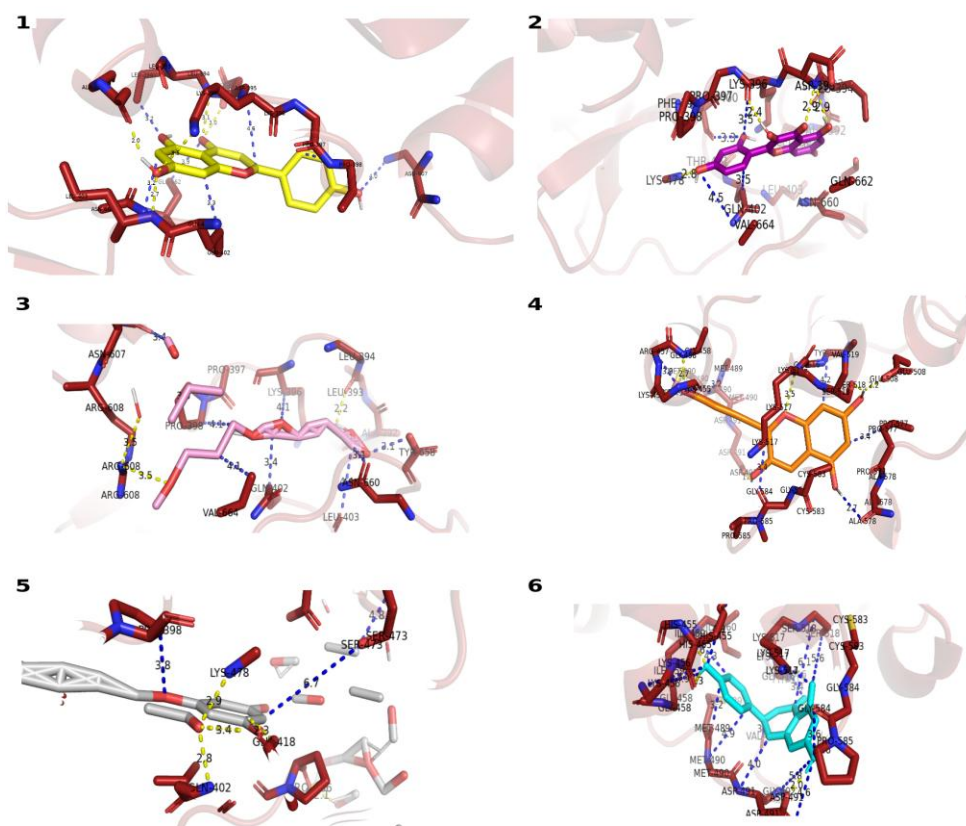


Figure 3. Binding sites for the phytochemicals on the surface of PRKCA (PDB ID: 4ra4 [30]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with five polar bonds. 2) Morin with five polar bonds. 3) Malvidin with three polar bonds. 4) Cyanidin with six polar bonds. 5) Hesperidin with six polar bonds. 6) Naringenin with three polar bonds.

2.4. Binding results for the flavonoids to PIK3C protein

According to Table 4, the shaded phytochemicals met the adopted thresholds for the inhibition constant and predicted binding free energy [32]. Polar contacts involved residues including ASP, SER, ARG, THR, and ASN.

Table 4. Binding free energies, inhibition constants (K_i), root mean square deviation (RMSD), number of polar bonds, and amino acids involved in the polar interaction for docking phytochemicals to the PIK3CA protein.

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, K_i (μ M)	RMSD for the ligand from the reference structure ($\text{\AA}$)	Number of polar contacts	Amino acids involved in the polar interaction
Apigenin	-5.91	46.23	25.505	4	ASP-111, SER-6, ARG-75

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, Ki (μM)	RMSD for the ligand from the reference structure (Å)	Number of polar contacts	Amino acids involved in the polar interaction
Morin	-5.27	136.79	9.472	5	ASN-105, ASN-103, GLY-140, 2 in SER-115
Malvidin	-6.50	17.12	28.577	3	ARG-75, ASP-111, THR-112
Cyanidin	-5.20	154.72	13.890	6	ASP-108, GLY-140, ASN-103, 2 in ASN-105, LYS-117
Hesperidin	-4.92	249.32	11.707	6	2 in ASN-24, VAL-23, VAL-21, GLU-146, ASN-144
Naringenin	-6.60	14.54	16.340	6	ASP-131, LEU-132, ASN-47, GLY-41, GLU-42

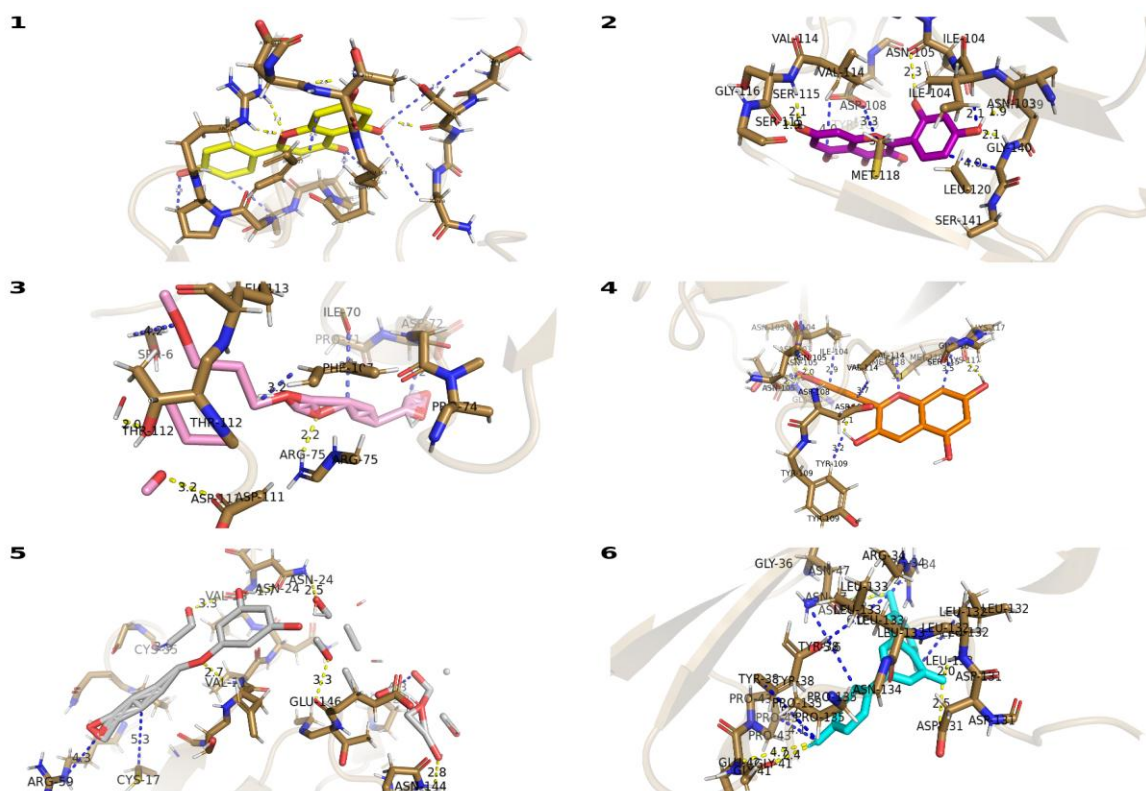


Figure 4. Binding sites for the phytochemicals on the surface of PIK3CA (PDB ID: 2enq [31]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with four polar bonds. 2) Morin with five polar bonds. 3) Malvidin with three polar bonds. 4) Cyanidin with six polar bonds. 5) Hesperidin with six polar bonds. 6) Naringenin with six polar bonds.

2.5. Drug likeness of the investigated flavonoid polyphenols

The researchers also investigated the drug-likeness properties of the selected phytochemicals. Lipinski's rule of five was assessed [33]. All phytochemicals, except hesperidin, complied with the rule without violations, whereas hesperidin showed three violations.

Table 5. Lipinski's Rule of Five Analysis of the Selected Phytochemicals

Phytochemical	Apigenin	Morin	Malvidin	cyanidin	Hesperidin	Naringenin
GI absorption	High	High	High	High	Low	High
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	No; 3 violations: MW>500, N or O>10, NH or OH>5	Yes; 0 violation
Bioavailability	0.55	0.55	0.55	0.55	0.17	0.55

Phytochemical	Apigenin	Morin	Malvidin	cyanidin	Hesperidin	Naringenin
Log P	2.11	1.20	0.92	0.32	-1.06	1.84
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	No	No	No	Yes
BBB permeation	No	No	No	No	No	No

3. Discussion

In addition to the reported dietary effects on glucose metabolism, lipid profiles, hormones, and enzymes [34], flavonoids have been associated with platelet signaling pathways involving AKT, PI3K, P2Y12, and PKC [35]. They may also exert antiplatelet effects through mechanisms involving thromboxane A2 (TxA2) generation and receptor antagonism, with structural features such as the C2–C3 double bond and C4 keto group contributing to activity [36]. The researchers' docking results therefore support further investigation of these phytochemicals as potential modulators of platelet-activation signaling.

The researchers studied the predicted interactions of several flavonoid polyphenols with AKT1, PRKCA, PIK3CA, and P2Y12, proteins associated with ADP/P2Y12-mediated platelet signaling. The phytochemicals included malvidin and cyanidin (anthocyanidins), naringenin and hesperidin (flavanones), and morin and apigenin (flavonoids).

The researchers tested the potential binding of the phytochemicals to the proteins in silico. Ligands were analyzed with respect to their predicted binding interfaces, structural interactions, and docking-derived inhibitory estimates. The findings showed a variation in the predicted binding among the phytochemicals and target proteins. Therefore, experimental validation is required before these interactions can be interpreted as functional antiplatelet effects.

All phytochemicals docked to AKT1 formed the predicted polar contacts. Malvidin showed the fewest polar contacts with one. Based primarily on the predicted ΔG and K_i values, morin and apigenin showed favorable docking estimates for AKT1, although apigenin had a higher predicted K_i .

For P2RY12, the whole surface docking produced high RMSD values for the evaluated ligands. Nevertheless, several ligands showed favorable predicted ΔG values and polar contacts. Cyanidin showed the lowest predicted K_i among the tested phytochemicals. The high RMSD values warrant cautious interpretation of these docking poses.

For PIK3CA, all docked phytochemicals formed the predicted polar contacts. Apigenin, malvidin, and naringenin met the adopted ΔG and K_i thresholds, whereas the other compounds showed higher predicted K_i values and therefore less favorable docking-derived inhibition estimates.

For PRKCA, all the phytochemicals formed predicted polar contacts, and most showed favorable ΔG and K_i estimates according to the adopted criteria. However, the relatively high RMSD values require cautious interpretation and experimental validation before functional inhibition can be inferred.

Morin hydrate could be a promising therapeutic agent for treating diseases associated with platelet activation since it prevents platelet-mediated thrombosis by reducing TXA2 production and integrin $\alpha IIb\beta 3$ activation while increasing cAMP generation, leading to the inhibition of clot retraction [22,37].

Apigenin enhances the antiplatelet effect of aspirin by antagonizing TxA2 receptors, suggesting its potential use in combination therapy for better platelet inhibition [21]. To enhance apigenin's potency, it was hybridized with docosahexaenoic acid (DHA), resulting in the 40-DHA-apigenin hybrid. This new compound showed significantly increased inhibitory activity against platelet aggregation induced by TRAP-6, ADP, and AA, demonstrating almost 2 to 12 times greater efficacy compared to apigenin and DHA alone [38].

In a dose-dependent way, cyanidin-3-glucoside (Cy-3-g) suppresses human platelet activation, aggregation, secretion, and thrombus formation. Additionally, it suppresses the collagen-GPVI signaling pathway [19]. Anthocyanins significantly reduce platelet granule secretion, which is crucial for dampening thrombosis and preventing atherosclerosis. In a clinical trial with hypercholesterolemic individuals, anthocyanin consumption markedly lowered plasma levels of β -thromboglobulin, soluble P-selectin, and RANTES, suggesting their potential as potent cardioprotective agents [27].

Hesperetin, found in grapefruits and oranges, inhibits platelet aggregation induced by collagen and arachidonic acid, suggesting a selective blockade of their signal transduction pathways. Its antiplatelet activity is achieved through the inhibition of PLC- $\gamma 2$ phosphorylation and cyclooxygenase-1 activity, highlighting its potential cardiovascular benefits [39], by reducing PDE activity and PI3K-mediated signaling cascades.

Naringenin inhibits platelet activation. As a result of ADP-induced stimulation, there is a rise in cGMP levels and PKA-mediated phosphorylation of VASP [17].

Regarding the predicted ADME properties and drug-likeness, all the studied phytochemicals complied with Lipinski's rule of five, except hesperidin, which showed three violations. Most compounds were predicted not to inhibit several of the assessed CYP enzymes; however, these computational predictions alone are insufficient to establish a low risk of drug interactions.

4. Materials & Methods

PDB files were prepared for the phytochemicals morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin for use in the docking protocol. Systematic IUPAC names were used to generate SMILES structures with

OPSIN [40], and PDB files were generated using Open Babel [41]. For docking, GPF and DPF files were prepared together with PDBQT files for the proteins and phytochemicals. AutoDock was used to predict ligand orientations and estimate binding free energies using its empirical scoring function and genetic algorithm [42,43]. Polar hydrogen atoms were added and water molecules were removed during structure preparation [44]. The target structures were AKT1 (PDB ID: 1h10 [28]), PIK3CA (PDB ID: 2enq [45]), P2RY12 (PDB ID: 4ntj [29]), and PRKCA (PDB ID: 4ra4 [30]). AutoDockTools was used to prepare the docking files [43,44,46,47]. The phytochemicals were also evaluated using SwissADME for predicted GI absorption, Lipinski's rule of five, bioavailability, LogP, CYP-enzyme inhibition, and blood–brain barrier permeation. Following docking, the best-scoring poses were extracted and analyzed in PyMOL [42,48]. The analysis included polar and non-polar interactions, AutoDock-predicted binding free energy (kcal/mol), inhibition constant (K_i), RMSD of the ligand from the reference structure (Å), number of polar contacts, and amino acids involved in polar interactions [49].

5. Conclusion

In conclusion, this study evaluated the predicted binding of six flavonoid polyphenols—morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin—to AKT1, P2RY12, PRKCA, and PIK3CA, proteins involved in platelet-activation signaling. The compounds showed variable docking profiles based on predicted polar and non-polar contacts, binding free energies, and inhibition constants. These results identified specific flavonoid–protein interactions that may be prioritized for further investigation. Because the present findings are computational, biochemical and cellular validation is required before antiplatelet efficacy or therapeutic relevance to CHD can be established.

6. Declarations

Author Contributions

Conceptualization, S. S.; Data curation, J. Z., H. A.-Z., M.D, M. L., and S. A.-M.; Formal analysis, J. Z., H. A.-Z., M.D, M. L., S. A.-M., and S.S; Funding acquisition, H. Z., and S. S.; Investigation, H. Z., and S. S.; Methodology, J. Z., H. A.-Z., M.D, M. L., S. A.-M. and S. S.; Supervision, H. Z. and S. S.; Validation, S. S.; Writing—original draft, J. Z., H. A.-Z., M.D, M. L., S. A.-M., S.S and H.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available upon request from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

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The Relationship between Nurses' Moral Intelligence and Caring Behaviors among Nurses in Neonatal Intensive Care Units

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Abstract

Background: Neonatal intensive care units (NICUs) represent highly complex health care environments, where nurses face ethical dilemmas, emotional stressors, and difficult clinical decision-making processes. Moral intelligence, understood as the capacity to use ethical principles and values in decision-making and professional behavior, might affect nurses' caring behaviors and patient care. Although many pieces of research have been published internationally, there is a scarcity of research on this relationship among NICU nurses in Palestine. **Objective:** To examine the relationship between moral intelligence and caring behaviors among nurses working in NICUs in Palestinian hospitals. **Methods:** A cross-sectional design was carried out among 171 nurses who work in level II and III NICUs in governmental hospitals in the West Bank. Data were gathered through the Moral Intelligence Questionnaire and the Caring Behaviors Assessment Tool Nursing Version–Short Form (CBAN-SF). The data analysis included descriptive statistics, Pearson correlation, independent-samples t-tests, ANOVA, and multiple linear regression analyses using SPSS version 27. **Results:** The participants had relatively high scores for their moral intelligence (mean = 71.8 ± 5.4) and caring behavior (mean = 99.8 ± 12.4). Moral intelligence correlated positively with caring behavior ($r = 0.314$, $p < 0.001$). Caring behavior correlated positively with the age of the participant ($r = 0.266$, $p < 0.001$) and nursing experience ($r = 0.294$, $p < 0.001$). Nurses of the female gender exhibited significantly higher scores in caring behavior than nurses of the male gender ($p = 0.009$). Multiple regression analysis indicated that moral intelligence ($B = 0.701$, $p < 0.001$) and female gender ($B = 5.147$, $p = 0.005$) were significantly related to caring behavior accounting for 23% of the variance. **Conclusions:** Moral intelligence is positively correlated with caring behaviors in NICU nurses. Enhancing the moral intelligence of nurses can help enhance their caring behaviors.

Keywords: moral intelligence, caring behaviors, neonatal intensive care unit, nurses, Palestine.

1. Introduction

Neonatal intensive care units (NICUs) are among the most complicated environments in the medical field because nurses must give highly specialized care to very ill and prematurely born infants [1]. NICU nurses encounter many ethical challenges, stressors, and difficult decisions when it comes to life-sustaining interventions and the care of dying patients [2]. This demands not only clinical expertise but also sound ethics and morals for the maintenance of a high level of patient-centered care [3,4].

1.1 Background

Ethical dilemmas such as restarting ventilation, discontinuing treatment, parental disagreement, and managing health care resource distribution are commonplace among NICU nurses. During such scenarios, nurses are required to make ethical decisions that require them to be empathic and at the same time adhere to professional guidelines. Therefore, the presence of moral intelligence among NICU nurses will contribute positively to their ability to perform their duties ethically and effectively. Knowing about this connection will aid in improving ethical practice among NICU nurses and consequently enhance the outcomes for the babies [4,5].

Though many studies have been conducted internationally on moral intelligence and caring practices in the nursing profession, there is insufficient amount of such studies in the Palestinian healthcare system especially regarding nurses operating in NICUs. Previous studies were conducted in Western nations and other healthcare settings of the Middle Eastern region. Such healthcare settings have considerable organizational differences with those in Palestine due to cultural backgrounds, moral standards and health care facilities in those areas. Consequently, conclusions made by those studies may not fully represent the hardships encountered by Palestinian NICU nurses. Nurses in Palestine encounter various issues regarding politics, economy, and healthcare, such as the shortage of medical supplies, the shortage of healthcare staff, overload, and uncertainties in the field of healthcare. The listed above factors may affect the making of the right ethical decisions, demonstrate moral awareness, and show compassion by nurses in the workplace. Nevertheless, despite the listed above obstacles, there are no numerous studies devoted to the Palestinian nurses' practices of providing compassionate care amid ethical issues and intense emotional situations in intensive care units. In the Palestinian literature, one may find the information concerning job satisfaction, stress, professional burnout, and the quality of nurses' care. However, the aspect of moral intelligence, which is an essential ethical competence of nurses, has not been studied extensively yet. So far, there are no studies on the Palestinian topics concerning the relation between moral intelligence and compassionate care in neonatal intensive care units. This shortage indicates the need for further studies to be done to understand the role of moral intelligence in enhancing caring behaviors and improving neonatal nursing care in Palestinian hospitals. The investigation of such connection in the Palestinian context may aid nursing educators, health professionals, and policy makers in developing better educational and work environments that enhance nurses' ethical decisions and caring behaviors. Therefore, the purpose of this study was to investigate the correlation between the nurses' moral intelligence and caring behaviors among NICUs nurses.

1.2 Objectives

The purpose of this study was to investigate the correlation between the nurses' moral intelligence and caring behaviors among NICUs nurses.

2. Related Work

The concept of moral intelligence is concerned with the use of ethical values and principles in one's personal goals, behaviors, and interactions with other individuals. It entails such aspects as honesty, responsibility, empathy, and forgiveness, and allows one to make proper decisions and behave in a correct manner in the workplace [6]. In nursing, moral intelligence allows nurses to make decisions in complicated healthcare situations in an ethical manner, adhere to professional standards, and safeguard patients' rights and dignity [7]. Research demonstrates that moral intelligence makes nurses competent in coping with difficult ethical situations and being responsible for their behavior in healthcare organizations [5,8].

Nursing care is considered the most significant and primary component of nursing practice. It reflects the way in which nurses act, think and communicate with each other for the purpose of improving the patients' health, emotional and psychological state as well as general well-being. According to the Theory of Human Caring developed by Watson, caring includes such components as empathy, verbalization, emotional support, respect towards patients and commitment to fulfilling their needs [9]. In neonatal intensive care units, the implementation of caring behaviors plays a particularly crucial role since newborns are extremely vulnerable and incapable of expressing their needs, thus the presence of caring nurses ensures the safety and successful treatment of the babies [10]. Currently, more attention is being paid to exploring the relationship between ethical competence and caring behavior in nursing research. Studies prove that nurses who have a high level of moral values and ethical awareness tend to demonstrate kind behaviors and deliver full care to their patients and families [11,12]. At the same time, encountering challenging moral problems and situations and making ethical decisions can have a negative impact on nurses' emotions and affect their caring behavior in a negative way, especially in intensive care units where nurses face numerous stressful situations and work under great pressure [13].

3. Methodology

Design, Setting, Population, and Sample

A cross-sectional study was conducted on nurses working in NICUs in the West Bank hospitals. The study was carried out between 1 June and 1 July 2026. The study's inclusion criteria were met by nurses working in level II and III NICUs in governmental hospitals throughout the West Bank. These hospitals were chosen because they are prominent providers of newborn care, with well-established NICUs that represent nurses working in larger, publicly supported healthcare facilities. Furthermore, the uniform rules and procedures used by these institutions contribute to a consistent work environment, increasing the generalizability of the study's results. As essential foundations of the healthcare system in Palestine, these organizations have also offered an opportunity for gaining important information from nurses whose practice is characterized by acute risks. Information was collected at 11 NICUs with about 150 incubators each.

A convenience sampling technique was adopted to ensure that there was a representative sample of NICU nurses for the study. Raosoft software was used to calculate the sample size taking into consideration a total number of 300 respondents, a response distribution of 50%, a margin of error of 5% and a confidence interval of 95%. This gave an estimated sample size of 169. To minimize attrition, 200 nurses were recruited for the study; however, 171 participated and answered the questionnaires.

The study's inclusion criteria required participants to be full-time NICU nurses with informed consent and at least six months of unit experience. This criterion made sure that participants had enough real-world experience and familiarity with the duties and difficulties of working in a NICU. Nurses who had administrative positions, worked part-time, or were on leave or absent were not included. Administrators were purposefully left out since their duties are mostly managerial and do not directly involve patient care at the bedside.

Study instruments

The instrument comprised three sections. The first section gathered demographic characteristics such as age, gender, educational level, and nursing work experience.

The second section assessed moral intelligence which was assessed by the 40-item Moral Intelligence Questionnaire by Lennick & Kiel [7]. Responses rated on a Likert scale ranging from strongly disagree to strongly agree] produced a total potential score between 40 and 200. Scores were changed to a 100-point scale. Higher scores indicate higher levels of moral intelligence. Also, the total scale may rate based on 90-100 was exceptional, 80-89 was very good, 70-79 was decent, and below 69 was poor. This tool's dependability (Cronbach's alpha) was stated as 0.897.

The third section was the Caring Behaviors Assessment Tool Nursing Version-Short Form (CBAN-SF) [14]. CBAN-SF enables nurses to evaluate their own care behaviors across seven factors: humanism/faith-hope/sensitivity, helping/trust, and expression of positive/negative feelings, supportive/protective/corrective environment, teaching/learning, human needs assistance, and existential/phenomenological/spiritual forces. In addition, the scale proved to be highly reliable with Cronbach's alpha coefficient of 0.97 for all items combined. High ratings on CBAN-SF imply more satisfaction from nurses' care behavior. CBAN-SF was earlier validated for application to nurses from Palestine to ensure the cultural relevance [11,15].

Validation of the questionnaire was conducted by three experts in the medical and nursing education domain to check the content validity of the instrument. In addition to that, a pilot study on 15 NICU nurses was conducted to evaluate the feasibility and reliability of the test instruments used. The value of the Cronbach's alpha for moral intelligence scale and caring behaviors scale is 0.86 and 0.89 respectively. Additionally, the nurses who participated in the pilot study were not included in the main research to lessen the possibility of bias. It took participants 15 to 20 minutes to complete the questionnaire, which is deemed appropriate. The final study also assessed Cronbach's alpha coefficient for moral intelligence and caring behaviors, with findings of 0.90 and 0.92, respectively.

3.1 Data Collection

After securing approval to conduct the study, the researcher visited hospitals and convened with the head nurses overseeing neonatal intensive care units. The researcher outlined the study's aims and requested the preparation of lists containing the names of nurses working in these units along with their duty schedules for subsequent meetings. Following that, the researcher explained the goals of the research to the nurses. Those nurses who agreed to join the research were given informed consent forms that they signed prior to filling out the questionnaire. Questionnaires were administered personally, and the interview session was face-to-face. Since all the participants could speak English, the questionnaire in paper form was provided in English.

3.2 Analysis Methods

Statistical analysis of data was done using Statistical Package for the Social Sciences (SPSS), version 27. Descriptive statistics involving frequencies, percentages, means, and standard deviations were used to provide an overview of demographics and profession, moral intelligence, and caring behavior scores of the participants. Pearson's correlation coefficient was used to examine the correlation among caring behavior, moral intelligence, age, and nursing experience of the participants. Independent samples t-tests and one-way ANOVA were used to compare differences in caring behavior scores based on gender and education level, respectively. Those variables found to be significantly correlated with caring behavior from the bivariate analysis were then included in the multiple linear regression analysis. Statistical significance was set at $p < 0.05$.

4. Experiments and Results

The mean age of the participants was 31.5 years ± 5.6 . Female participants 116 (67.8%) constituted most of the sample. Regarding educational attainment, most participants 121 (70.8%) held a bachelor's degree. In terms of professional experience, the participants had an average of 6.5 ± 4.5 years of nursing work experience, as seen in Table 1.

Table 1. Demographic and professional characteristics of the participants (N = 171)

Characteristics		n	%	M (SD)
Age				31.5 (5.6)
Gender	Male	55	32.2	
	Female	116	67.8	
Educational Level	Diploma	22	12.9	
	Bachelor	121	70.8	
	Master and above	28	16.4	
Work Experience in Nursing				6.5 (4.5)

The analysis revealed that the mean moral intelligence score was 71.8 ± 5.45 on a scale ranging from 20 to 100, indicating a relatively high level of moral intelligence among the participants. The overall mean score for caring behavior was 99.8 ± 12.4 , suggesting that participants generally demonstrated positive caring behaviors in their nursing practice. Among the dimensions of caring behavior, the highest mean score was observed for Supportive/Protective/Corrective Environment ($M = 22.8 \pm 2.7$) while the lowest mean scores were reported for Existential/Phenomenological/Spiritual Forces ($M = 11.06$, $SD = 1.53$), as seen in Table 2.

Table 2. Distribution of the study variables (N = 171)

Variable	M	SD
Moral intelligence	71.8	5.4
Caring behavior	99.8	12.4
Humanism/ faith-hope/ sensitivity	14.5	2.6
Helping/ trust	14.1	2.8
Expression of positive/ negative feelings	11.3	1.5

Teaching/ learning	14.7	2.2
Supportive/ protective/ corrective environment	22.8	2.7
Humans need assistance	11.2	1.9
Existential/ phenomenological/ spiritual forces	11.1	1.5

The analysis revealed that there was a statistically significant positive correlation between caring behavior and moral intelligence ($r = 0.314$, $p < 0.001$), indicating that nurses with higher levels of moral intelligence tended to demonstrate higher levels of caring behavior. Caring behavior was also positively correlated with age ($r = 0.266$, $p < 0.001$) and years of nursing experience ($r = 0.294$, $p < 0.001$), suggesting that older and more experienced nurses exhibited stronger caring behaviors. Significant differences in caring behavior scores were observed according to gender ($t = 2.691$, $p = 0.009$). Female participants reported higher caring behavior scores ($M = 101.8 \pm 10.3$) compared with male participants ($M = 95.7 \pm 15.2$), as seen in Table 3.

Table 3. Association of caring behavior scores with moral intelligence and demographic/professional characteristics

Variable		n	M	SD	Statistical test	p value
Moral Intelligence					$r = 0.314^{**}$	< 0.001
Age					$r = 0.266^{**}$	< 0.001
Gender	Male	55	95.7	15.2	$t = 2.691$	0.009
	Female	116	101.8	10.3		
Educational Level	Diploma	22	103.5	8.7	$F = 2.158$	0.119
	Bachelor	121	100.0	13.0		
	Master and above	28	96.3	11.5		
Work Experience in Nursing					$r = 0.294^{**}$	< 0.001

The overall regression model was statistically significant ($F=12.406$, $p<0.001$), and it explained 23.0% of the variation in caring behavior ($R^2=0.230$; adjusted $R^2=0.212$). Moral intelligence was found to be a significant positive predictor of caring behavior ($B=0.701$, $p<0.001$). Particularly, one unit increase in moral intelligence will lead to about 0.70 increase in caring behavior scores. Also, gender was found to be a significant predictor such that women nurses had higher caring behavior scores than men ($B=5.147$, $p=0.005$). That is, being a woman will bring about an increase in caring behavior score by about 5.15 units, as depicted in Table 4.

Table 4. Predictors of caring behavior: multiple linear regression

Predictor	Caring Behavior					
	B	Beta	t	p value	95% CI	
					Lower	Upper
Moral Intelligence	.701	.309	4.500	.000	.394	1.009
Age	.035	.016	.094	.925	-.775	.705

Work Experience in Nursing	.862	.314	1.844	.067	-.061	1.785
Gender (Female)	5.147	.195	2.829	.005	1.555	8.738
R ² = 0.230, adjusted R ² =0.212, F= 12.406, p=0.000						

5. Discussion

The results of this study have shown that nurses in NICU of Palestine possessed quite high moral intelligence and caring behaviors, which means that all participants had high ethical values and caring practices. Such results can be explained by the peculiarity of neonatal intensive care when nurses must make ethical decisions and provide compassionate care for critically ill babies and their families. The results obtained from this study are in line with the results of other studies conducted previously, which have shown the interconnection between ethical competence and quality nursing care. Karabey reported that nurses had high levels of moral intelligence and high levels of caring behaviors, with a statistically significant positive relationship between the two constructs [16]. The study concluded that nurses who possess stronger moral values and ethical decision-making abilities are more likely to exhibit caring behaviors in clinical practice, which supports the current findings. Similarly, Afrasiabifar et al. found that nurses demonstrated good caring behaviors and that moral sensitivity was positively associated with caring behavior [17]. The authors argued that ethical awareness enhances nurses' ability to provide compassionate, patient-centered care. These findings support the present study by suggesting that stronger ethical attributes are reflected in higher-quality caring practices. Further support comes from Chen et al., who examined nursing interns and found that moral sensitivity was positively associated with caring behaviors [18]. The study also demonstrated that empathy partially mediated this relationship, indicating that ethical awareness contributes to caring practice through enhanced understanding of patients' needs. This evidence reinforces the notion that ethical and moral intelligence are important determinants of caring behaviors among nurses.

The current study is distinguished from Afrasiabifar et al. study in terms of the fact that the former indicated that nurses' moral sensitivity was not high, but moderate [17]. At the same time, caring behavior was assessed as good. The reasons for such a difference between results may lie in differences in settings of care provision, culture, load, organization, and measurement. Specifically, the authors themselves admit the possibility of the significant impact of social and cultural factors on the ethical perception and behavior of nurses that may lead to variations between different studies and even countries. In addition to it, differences between studies may arise due to methodological reasons. In the commentary on the research in the field of caring behavior of nurses, it was said that self-report measure may tend to overrate the actual caring behavior because of the positive self-evaluation of nurses.

In relation to the caring behavior dimensions, it was found that the highest mean scores were recorded for the dimension of Supportive/Protective/Corrective Environment, then Teaching/Learning and Humanism/Faith-Hope/Sensitivity. It should be noted that the findings coincide with the results of a study carried out among NICU nurses conducted by Ayed et al., where the highest mean scores for caring behaviors were recorded for the dimension related to creation of a supportive, protective and corrective environment, followed by the Teaching/Learning dimension and Humanism behaviors [11]. According to the authors, NICU nurses attach importance to creating such an environment for their neonatal patients because such patients are especially vulnerable and need constant monitoring and protection from possible complications. Moreover, education and support of parents is an integral part of neonatal nursing because families need to be prepared to participate in the infant's care both when they stay in the hospital and afterwards.

The importance of the Teaching/Learning dimension can also be attributed to the growing emphasis placed on family-centered care in the neonatal environment. Parents of hospitalized babies usually feel stressed, anxious, and uncertain, which makes it necessary for the nurses to teach them about their baby's problems and treatment as well as feeding techniques and discharge plans. In turn, teaching becomes an essential part of the everyday nursing practice in NICUs and greatly contributes to the impressions of caring behavior. Also, high marks in the Humanism/Faith-Hope/Sensitivity dimension reveal the efforts of nurses to show respect, empathy, and kindness to the infants and their parents regardless of technology-oriented neonatal care. This result is consistent with the Theory of Human Caring, according to which the key role of nursing practice is played by the creation of a healing environment, hope, sensitivity, and the establishment of meaningful interpersonal relationships [19]. On the other hand, lower results in the dimensions of Existential/Phenomenological/Spiritual Forces and Expression of Positive/Negative Feelings have been observed. This result corresponds to research conducted in critical care departments, since the psychosocial and spiritual aspects of care often get less consideration than the physical and technical aspects. The highly specialized and rapid rhythm of NICUs requires that the nurses pay more attention to immediate care, patient monitoring, and issues concerning patients' safety. Besides, the nurses may lack confidence in their ability to address the spiritual needs of patients and talk to them about their emotions, especially in conditions characterized by a high level of acuity and workload. As a result, the manifestation of caring behaviors involving emotional expression and spirituality becomes less common than behaviors involving safety and protection [20].

An important finding from this study was the presence of a significant positive correlation between moral intelligence and caring. It was found that nurses with high levels of moral intelligence possessed significant amounts of caring behavior, thus indicating that ethical competence and moral reasoning can be considered as essential aspects in the development of nursing practices. The results of this study confirm the theoretical perspectives that emphasize that caring is the aspect of ethical and moral nature and not just a technical one. Nurses with high moral values will show empathy, dignity, respect, accountability, and patient-centeredness. The results of the study are consistent with other studies that showed a positive relationship between moral intelligence and caring behaviors of nurses. For example, according to Karabey and Yıldız [21], nurses with high moral intelligence scores showed better caring behaviors, whereas, in turn, Mollazadeh et al. stated that the enhancement of moral intelligence can contribute to the improvement of the quality of nursing care [22]. The results like the ones obtained in this study were found by Afrasiabifar et al. [17].

Another important relationship found in this study is the significant positive correlation among caring behaviors, age, and years of experience in nursing practice. In this case, older nurses and nurses with many years of work experience were observed to exhibit more caring behaviors than younger nurses with less work experience. This can be attributed to the accumulation of professional skills and expertise as well as the development of effective communication skills in experienced nurses. Experienced nurses usually demonstrate high confidence in dealing with complicated clinical settings and can identify the physical, emotional, and psychosocial needs of patients and families.

Differences in gender were also noted in the current study where female nurses had significantly high scores of caring behaviors compared to their male counterparts. In addition, gender was significantly predictive of caring behavior in the regression model implying that the female nurses had high levels of caring behavior despite the adjustment for moral intelligence, age, and experience of nursing. The results of the study are similar to those of

some previous research which indicated significantly high levels of caring behavior among female nurses, possibly due to social and cultural considerations which make women nurture and express themselves emotionally. Caring is one of the competencies in professional nursing which can be learned by all nurses irrespective of their gender. Moral intelligence emerged as the most significant independent predictor of caring behavior through the multiple linear regression analysis conducted. Despite controlling age, gender, and nursing experience, there was still a significant relationship between moral intelligence and caring behavior. It shows how much importance ethical abilities can play a role in shaping nursing behaviors. Nurses who have high moral intelligence will be more inclined to integrate ethical values in their daily behaviors in the form of compassion, responsibility, honesty, advocacy, and show respect towards patients and their families. The findings are in line with previous literature which supports the role of moral intelligence in determining professional nursing behaviors [21,22].

Overall, the regression analysis showed that 23% of the variation in caring behavior could be attributed to moral intelligence and gender, hence showing that moral intelligence and gender significantly determine the caring behaviors of NICU nurses. Nevertheless, a relatively large amount of variance is unexplained, implying that there are other variables like emotional intelligence, organizational culture, leadership support, job satisfaction, workload, and professional commitment which influence caring behaviors. Further research on these variables is recommended to gain better insight into caring behaviors.

Implications of the Study

The results of this study are crucial for nursing education, clinical practice, and healthcare administration in Palestine. It is necessary for nursing educators to ensure that moral intelligence development, ethical decision making, and reflective practices are included in undergraduate and postgraduate training. Moreover, healthcare facilities must ensure that an environment where nurses are provided opportunities to deliver compassionate patient care prevails. Moral intelligence development can help in achieving superior quality care, patient/family satisfaction, and overall better neonatal nursing care. These results emphasize the need for healthcare administrators to invest in nurses' moral and ethical development.

Strengths and Limitations

There are several strengths in this study, among them being the fact that the topic is an under-researched one in the Palestinian healthcare system and that the sample included nurses from various governmental NICUs in the West Bank. Valid and reliable instruments were used in measuring the variables in this study, and there were high levels of internal consistency for these instruments and multivariate analysis used to establish independent predictors of caring behavior. There are several limitations to be aware of in this study. For instance, the cross-sectional design makes it impossible to establish causality between moral intelligence and caring behaviors. There were some forms of bias present because data was collected using self-reported questionnaires. Only 23% of the variance of caring behavior was explained by the regression model.

6. Conclusions and Future Work

Based on the findings from this study, nurses showed relatively high levels of moral intelligence and caring behavior. That is, it was found that there was a significant positive correlation between moral intelligence and caring behavior, implying that those nurses who have higher moral intelligence will also show relatively high levels of caring behavior in the process of practicing their profession. Caring behavior had a positive association

with age and years of practice of nurses while female nurses were found to show significantly higher levels of caring behavior compared to male nurses. Moral intelligence and being female also served as the significant predictors of the caring behavior. Overall, based on the results of this study, it is important to emphasize the significance of the development of moral intelligence of nurses as one way of promoting their caring behavior. As a result, moral intelligence, ethical decision making, reflection, and value-based education need to be incorporated in nursing education programs through undergraduate and professional nursing education curricula. Nursing leaders need to create an ethical environment that nurtures the moral intelligence and caring of nurses. In future, research designs that are either longitudinal or experimental need to be used to find out any causal relationship and intervention measures for increasing moral intelligence among nurses. Also, more research is needed to find other factors associated with caring behavior including emotional intelligence, organizational culture, leadership support, workload, job satisfaction, and professional commitment.

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8. Declarations

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Conflict of interest/Competing interests

Researcher declares no conflict of interest with any organization regarding the materials discussed in this paper.

Ethics approval and consent to participate

Approval was obtained from the Institutional Review Board (IRB) of Arab American University (R-2026/A/90/N). The nurses were made aware of the objective of the research and that participation was voluntary and that they could withdraw at any time without any repercussions by the researcher. Confidentiality was assured by the researcher since no names or any other identifiable information about the participants was collected or disseminated. Participation in the research was strictly voluntary.

Consent for publication

Not applicable.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Materials availability

Materials used in this study are available from the corresponding author upon reasonable request.

Code availability

No custom code was generated for this study.

Author(s) Contribution

Ahmad Ayed conducted the study and wrote the manuscript. The author read and approved the final manuscript.

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Sick-Day Knowledge, Recalled Education, and Intended Medication Management Among Adults Prescribed Sodium–Glucose Cotransporter 2 Inhibitors in Jenin, Palestine: A Cross-Sectional Study

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Abstract

Sodium–glucose cotransporter 2 (SGLT2) inhibitors can precipitate diabetic ketoacidosis with normal or mildly elevated glucose, particularly during acute illness, fasting, volume depletion, or surgery; safe use therefore depends on patients recognizing when to seek advice and temporarily withhold treatment. This clinic-based cross-sectional survey evaluated sick-day knowledge and intended self-management among 207 adults with type 2 diabetes prescribed an SGLT2 inhibitor in Jenin, Palestine. A structured, interviewer-administered Arabic questionnaire assessed five knowledge domains, attitudes, recalled sick-day education, intended action during acute illness, previous continuation during illness, and information sources. Associations were examined using chi-square testing, logistic regression, and HC3-robust linear regression; a strict knowledge-scoring sensitivity analysis was performed. The mean Knowledge Score was 2.44 out of 5 (SD 1.56). Only 48.8% recalled receiving sick-day education, 77.3% reported an appropriate intended action, and 70.4% reported previously continuing treatment during illness. Recalled education was associated with appropriate intended action both before adjustment (OR 5.71, 95% CI 2.59–12.60) and after adjustment for age, gender, educational attainment, and diabetes duration (adjusted OR 5.39, 95% CI 2.39–12.13). Older age was associated with lower knowledge ($b = -0.301$, $p = 0.003$), whereas the positive association with educational attainment was not retained under strict scoring. Recognition of ketoacidosis without marked hyperglycemia and preoperative withholding was limited. These findings support standardized Arabic sick-day counseling, particularly for older adults, using written instructions, teach-back, and involvement of relatives or caregivers, with consent. Prospective studies should assess observed behavior and clinical outcomes rather than relying solely on recalled education and hypothetical intended actions.

Keywords: Sodium-glucose cotransporter 2 inhibitors, sick-day management, diabetic ketoacidosis, patient knowledge, medication safety, type 2 diabetes mellitus.

1. Introduction

1.1 Background

Sodium-glucose cotransporter 2 inhibitors are established therapies for type 2 diabetes because they improve glycemic control and provide important cardiovascular and kidney benefits in appropriately selected patients. Despite these benefits, they are associated with uncommon but potentially serious diabetic ketoacidosis. Unlike conventional diabetic ketoacidosis, SGLT2 inhibitor-associated ketoacidosis may develop when blood glucose is normal or only moderately elevated, which can delay recognition and treatment [1].

Acute illness, reduced oral intake, vomiting, dehydration, prolonged fasting, surgery, and relative insulin deficiency are recognized precipitating circumstances. Current guidance recommends avoiding or temporarily withholding SGLT2 inhibitors during severe acute illness, prolonged fasting, and other situations associated with ketosis or volume depletion. Before elective surgery, treatment should generally be withheld for three days for most agents and four days for ertugliflozin. Therapy should be restarted only after the precipitating risk has resolved and the patient is clinically stable with adequate oral intake [2,3].

Safe implementation of these precautions depends on patients recognizing relevant risk situations, understanding that normal or mildly elevated glucose does not exclude ketoacidosis, knowing when to seek clinical advice, and receiving clear instructions about withholding and restarting treatment. However, sick-day guidance is not consistently delivered across healthcare systems, and the content, format, and implementation of available resources vary. A systematic scoping review found many sick-day educational resources but limited evidence concerning their implementation and effects on clinical outcomes, while subsequent international consensus work emphasized the need for consistent, understandable, and actionable guidance [4].

Clear communication may be particularly important for older adults, who may benefit from simplified explanations, repetition, large-print instructions, and scenario-based teach-back. With the patient's consent, relatives or caregivers who assist with medication management may also need to receive the instructions so that they can support appropriate action during acute illness. Nevertheless, education should be provided systematically to all patients rather than being restricted according to age or educational attainment.

1.2 Objectives

This study aimed to evaluate SGLT2 inhibitor sick-day knowledge, attitudes, recalled sick-day education, intended self-management during acute illness, previous medication continuation during illness, and primary information sources among adults with type 2 diabetes attending diabetes clinics in Jenin, Palestine. The primary analytic objective was to examine the association between recalled sick-day education and a prespecified appropriate intended action during acute illness. Secondary objectives were to examine the associations of age, gender, educational attainment, and diabetes duration with the five-item Sick-Day Knowledge Recognition Score and to evaluate the robustness of these findings using a stricter scoring rule for multiple-response knowledge items.

2. Related Work

Research on sick-day medication management has highlighted substantial variation in the advice provided to patients. Watson et al. identified numerous guidance documents for people with diabetes, kidney disease, or cardiovascular disease but found limited empirical evaluation of how the guidance was implemented or whether

it improved medication-management behavior and clinical outcomes. A subsequent modified Delphi process developed consensus recommendations intended to make sick-day guidance more consistent and usable in clinical practice[4]. These studies established the need for structured education but also demonstrated that the evidence supporting specific delivery strategies remains limited.

SGLT2 inhibitor-specific studies have similarly identified gaps in patient understanding. In a multicenter quality-improvement initiative, baseline awareness of sick-day rules and SGLT2 inhibitor-related ketoacidosis was low, while targeted written information improved several patient-reported knowledge and confidence measures [5]. Because this was a quality-improvement project rather than a randomized effectiveness trial, the findings support feasibility but provide limited evidence of causal effects. The SAKURA cluster-randomized trial evaluated pharmacist-delivered sick-day materials in community pharmacies and demonstrated improved patient knowledge, although significant improvements in anxiety, intended behavior, and attitudes were not observed [6]. This distinction is important because improved recognition does not necessarily ensure appropriate action during a real illness.

Studies conducted among Palestinian adults with type 2 diabetes have documented limitations in general diabetes knowledge, self-management practices, medication beliefs, and adherence. In the West Bank, greater diabetes knowledge was associated with younger age and higher educational attainment, while important gaps remained between knowledge, attitudes, and reported practices [7]. Other Palestinian studies found that knowledge and beliefs about medicines were related to adherence and identified continuing challenges in long-term medication use [8,9]. However, these studies did not examine SGLT2 inhibitor-specific sick-day safety.

Consequently, evidence remains limited regarding whether Palestinian patients receiving SGLT2 inhibitors recognize high-risk clinical situations, understand the possibility of ketoacidosis without marked hyperglycemia, or know when to seek advice and temporarily withhold treatment. There is also limited evidence concerning the association between recalled sick-day education and intended medication management in this setting. The present study addresses these gaps by examining both item-specific and overall knowledge, intended action, recalled previous behavior, and factors associated with knowledge in adults attending diabetes clinics in Jenin.

3. Methodology

3.1 Data Collection

A clinic-based cross-sectional survey was conducted from March 30 to May 23, 2026, at two endocrinology and diabetes care sites in Jenin, Palestine: Dr. Waddah Abed Private Clinic and Dr. Bayan Abu Al-Rub Clinic/Ibn Sina Specialized Hospital.

Adults aged 18 years or older were eligible if they had type 2 diabetes, were currently prescribed an SGLT2 inhibitor, attended one of the participating sites during the recruitment period, and provided informed consent. Potential participants were approached consecutively during routine clinic visits. A total of 207 questionnaires were completed.

For sample size calculation, the Palestinian Ministry of Health dispensing records for Jenin indicated the use of approximately 1,000 dapagliflozin tablets per month. These tablet-consumption data were used only to assess recruitment feasibility and were not treated as a source-population denominator. Consecutive recruitment continued throughout the predefined study period.

Participation was voluntary and anonymous. Before questionnaire administration, potential participants received an explanation of the study and were asked whether they consented to participate. Consent was documented as an affirmative response to the first questionnaire item before the interview proceeded. No directly identifying personal information was collected, and only records with documented consent were included.

3.2 Experimental Setup

This was an observational survey and did not involve an experimental intervention. This subsection therefore describes development and administration of the survey instrument and the prespecified variable definitions. A structured, interviewer-administered Arabic-language questionnaire was developed by the investigators after reviewing SGLT2 inhibitor prescribing information, published sick-day recommendations, and patient-education literature [2,4,6,10]. The questionnaire was not directly translated or adapted from a single validated instrument.

The questionnaire was pilot-tested with 15 adults who met the study eligibility criteria. The pilot assessed item clarity and comprehensibility, cultural appropriateness, suitability of the response options, approximate administration time, and feasibility of interviewer administration. Only minor wording changes were made, and the pilot participants were not included in the main analytical sample.

The instrument collected age, gender, educational attainment, diabetes duration, and the prescribed SGLT2 inhibitor. Five knowledge items assessed recognition of: (a) temporary withholding during severe illness; (b) clinical situations requiring temporary withholding; (c) SGLT2 inhibitor-associated diabetic ketoacidosis despite normal or moderately elevated blood glucose; (d) warning symptoms requiring medical attention; and (e) preoperative withholding. Three attitude items assessed the perceived importance of knowing when to withhold treatment, perceived ability to manage treatment during illness, and preference for written instructions. Additional items assessed recalled sick-day education, intended action during an acute illness involving vomiting or fever, previous continuation of treatment during illness, primary information source, and desire for further information.

The five knowledge items were scored dichotomously and summed up to produce the Sick-Day Knowledge Recognition Score, ranging from 0 to 5, with higher scores indicating greater recognition. Two items used a select-all-that-apply format. Under the primary scoring rule, an item was scored as recognized when at least one clinically appropriate option was selected; otherwise, it was scored as not recognized. Because this rule neither required selection of every appropriate response nor penalized simultaneous inappropriate selection, it represented partial recognition rather than complete mastery. The strict sensitivity rule required selection of all clinically appropriate responses without selecting 'do not know.' No externally validated cutoff was used to classify knowledge as low, moderate, high, adequate, or inadequate; the score was analyzed continuously, and item-level responses were reported separately.

Temporarily withholding the SGLT2 inhibitor or contacting a physician or pharmacist was classified as an appropriate intended action. Continuing treatment or responding 'do not know' was classified as inappropriate. This outcome represented stated intention rather than observed behavior. Recalled sick-day education was considered present when participants reported previously being told when to withhold their SGLT2 inhibitor during illness. Medication names recorded in Arabic or English, including combination products, were harmonized into active-ingredient categories before analysis.

Internal consistency of the five dichotomously scored knowledge items was assessed using the Kuder-Richardson Formula 20 and was 0.69 in the analytical sample. The three dichotomized attitude items showed low internal consistency ($\alpha = 0.27$) and were therefore reported separately without a composite attitude score. Criterion validity, construct validity, and test-retest reliability were not evaluated. Accordingly, the questionnaire was regarded as an investigator-developed, pilot-tested instrument with preliminary reliability evidence rather than a fully validated measure. The administered Arabic questionnaire and its English translation are provided in Supplementary File 1.

3.3 Analysis Methods

Categorical variables were summarized as frequencies and percentages, with available case denominators reported when responses were missing. The Sick-Day Knowledge Recognition Score was summarized using the mean, standard deviation, median, interquartile range, and observed range. Item-level knowledge responses and the three attitude items were described separately. No missing values were imputed.

For the primary analysis, Pearson's chi-square test assessed the association between recalled sick-day education and appropriate intended action. Univariable binary logistic regression estimated the unadjusted odds ratio and 95% confidence interval. A multivariable logistic regression model then estimated the adjusted odds ratio after controlling for age band, gender, educational attainment, and diabetes-duration category.

For the secondary analysis, multiple linear regression with HC3 heteroscedasticity-robust standard errors modeled the Sick-Day Knowledge Recognition Score as a function of age band, gender, educational attainment, and diabetes duration. Age band, educational attainment, and diabetes duration were assigned increasing ordinal values. Their coefficients therefore represent the estimated average change in Knowledge Score associated with moving one category higher and assume a constant increment across adjacent categories. This parsimonious coding was prespecified for the available sample size. Variance inflation factors were examined to assess multicollinearity. Recalled sick-day education was not included as a predictor in this four-predictor knowledge model.

In the sensitivity analysis, the two select-all-that-apply items were rescored using the strict rule, and the same four-predictor HC3-robust linear regression model was repeated using the strict Knowledge Score. All tests were two-sided, and p-values below 0.05 were considered statistically significant. Interpretation prioritized effect estimates and 95% confidence intervals, particularly for secondary analyses.

Analyses were conducted in Python 3.12 using pandas 2.2.3, NumPy 2.3.5, and SciPy 1.17.0. Maximum-likelihood logistic regression and ordinary least-squares regression with matrix-based HC3 covariance estimation were implemented in the analysis code. The final analysis code and prespecified scoring rules should be retained with the study records to support reproducibility.

4. Experiments and Results

This was an observational cross-sectional survey and did not involve an experimental intervention. Accordingly, this section presents the descriptive findings, prespecified association analyses, sensitivity analysis, and interpretation of the results.

4.1 Results Overview

4.1.1 Participant characteristics

The analytical sample comprised 207 adults prescribed an SGLT2 inhibitor. The largest age groups were 51–60 years (30.4%) and 61–70 years (28.5%). Women accounted for 54.6% of the sample, 45.9% had university-level education, and 49.8% had lived with diabetes for more than five years. Dapagliflozin and empagliflozin monotherapy together accounted for 67.6% of reported SGLT2 inhibitor regimens (Table 1).

Table 1. Sociodemographic and Clinical Characteristics of Participants (N = 207)

Characteristic	n	%
Age group		
20–30 years	8	3.9
31–40 years	17	8.2
41–50 years	43	20.8
51–60 years	63	30.4
61–70 years	59	28.5
>70 years	17	8.2
Gender		
Female	113	54.6
Male	94	45.4
Formal educational level		
Primary	35	16.9
Secondary	77	37.2
University	95	45.9
Diabetes duration		
<1 year	25	12.1
1–5 years	79	38.2
>5 years	103	49.8
SGLT2 inhibitor regimen		
Dapagliflozin	74	35.7
Empagliflozin	66	31.9
Dapagliflozin/metformin	37	17.9
Ertugliflozin	19	9.2
Empagliflozin/metformin	8	3.9
Canagliflozin	3	1.4

Note: Values are n (%). Percentages may not total 100% because of rounding.

4.1.2 Knowledge, attitudes, and behavioral indicators

The mean Sick-Day Knowledge Recognition Score was 2.44 (SD = 1.56), with a median of 3 (interquartile range, 1–4) and a range of 0–5. The Kuder–Richardson Formula 20 coefficient for the five dichotomously scored knowledge items was 0.69. Recognition was lowest for SGLT2 inhibitor-associated diabetic ketoacidosis occurring with normal or modestly elevated blood glucose (23.7%) and for presurgical withholding (37.2%); warning symptoms requiring medical attention were recognized most often (77.8%) (Table 2).

Overall, 101 participants (48.8%) recalled receiving sick-day education, and 160 (77.3%) reported that they would temporarily withhold the SGLT2 inhibitor or contact a physician or pharmacist during an acute illness involving vomiting or fever. Among 206 respondents to the previous-use item, 145 (70.4%) reported having continued the medication during at least one previous illness. Because these questions assessed a hypothetical intention and recalled behavior in potentially different circumstances, the aggregate contrast does not establish an individual-level intention–behavior gap. Of 205 respondents to the information-preference item, 195 (95.1%) wanted additional information.

Table 2. Knowledge, Attitudes, Sick-Day Education, Intended Action, and Information Preferences

Domain	Indicator	n/N	%
Knowledge	Recognized temporary withholding during severe illness	97/207	46.9
	Recognized ≥ 1 situation requiring temporary withholding	122/207	58.9
	Recognized ketoacidosis without marked hyperglycemia	49/207	23.7
	Recognized ≥ 1 warning symptom requiring medical attention	161/207	77.8
	Recognized presurgical withholding	77/207	37.2
Attitude	Agreed that sick-day information is important	158/207	76.3
	Agreed that they knew how to manage treatment during illness	98/207	47.3
	Preferred written instructions	189/207	91.3
Education	Recalled receiving sick-day education	101/207	48.8
Intended action	Reported an appropriate intended action	160/207	77.3
Previous behavior	Previously continued medication during illness	145/206	70.4
Information source	Physician	120/206	58.3
	Pharmacist	48/206	23.3
	Internet	38/206	18.4
Information preference	Wanted additional information	195/205	95.1

Note. Values are n/N (%). An appropriate intended action was defined as temporarily withholding the SGLT2 inhibitor or contacting a physician or pharmacist during acute illness involving vomiting or fever. Denominators differ because of missing responses. The three attitude items are reported separately because they were not treated as a single scale.

4.1.3 Recalled education and intended action

An appropriate intended action was reported by 92 of 101 participants (91.1%) who recalled receiving sick-day education and by 68 of 106 participants (64.2%) who did not. The association was statistically significant, $\chi^2(1) = 21.39$, $p < 0.001$. In univariable logistic regression, recalled education was associated with higher odds of an appropriate intended action (OR = 5.71, 95% CI [2.59, 12.60], $p < 0.001$). After adjustment for age band, gender, formal educational level, and diabetes duration, the association remained statistically significant (adjusted OR = 5.39, 95% CI [2.39, 12.13], $p < 0.001$). None of the other covariates were statistically significant (Table 3).

Table 3. Multivariable Logistic Regression of Factors Associated with Appropriate Intended Action (N = 207)

Predictor	Adjusted OR	95% CI	P
Recalled sick-day education (yes vs. no)	5.39	2.39–12.13	<0.001
Age band (per category increase)	0.78	0.52–1.19	0.256
Gender (female vs. male)	1.36	0.66–2.83	0.406
Formal educational level (per category increase)	0.90	0.52–1.58	0.718
Diabetes duration (per category increase)	0.98	0.52–1.86	0.952

Note. CI = confidence interval; OR = odds ratio. All predictors were entered simultaneously. Age band, formal educational level, and diabetes duration were modeled as ordinal variables.

4.1.4 Factors associated with knowledge

The HC3-robust linear regression model was statistically significant, $F(4, 202) = 8.18$, $p < 0.001$, and explained 13.5% of the variance in Knowledge Score ($R^2 = 0.135$; adjusted $R^2 = 0.118$). After mutual adjustment, each one-category increase in age band was associated with a 0.301-point lower score (95% CI [−0.499, −0.104], $p = 0.003$), whereas each one-category increase in formal educational level was associated with a 0.396-point higher score (95% CI [0.056, 0.735], $p = 0.023$). Gender and diabetes duration were not associated with Knowledge Score. All variance inflation factors were below 2.0 (Table 4).

Table 4 HC3-Robust Multiple Linear Regression Predicting Knowledge Score (N = 207)

Predictor	b	Robust SE	95% CI	P
Age band (ordinal, 1–6)	−0.301	0.101	−0.499 to −0.104	0.003
Gender (female = 1)	0.180	0.215	−0.241 to 0.601	0.403
Formal educational level (ordinal, 1–3)	0.396	0.173	0.056 to 0.735	0.023
Diabetes duration (ordinal, 1–3)	0.031	0.156	−0.275 to 0.338	0.841

Note. CI = confidence interval; SE = standard error. All predictors were entered simultaneously. Coefficients for the ordinal variables represent the estimated mean difference in Knowledge Score associated with moving one category higher. Model $R^2 = 0.135$; adjusted $R^2 = 0.118$; all variance inflation factors < 2.0.

4.1.5 Sensitivity analysis

Applying the strict scoring rule to the two multiple-response knowledge items reduced the mean Knowledge Score to 1.39 (SD = 1.41). In the corresponding four-predictor HC3-robust model, age remained associated with

knowledge ($b = -0.303$ per age-band increase, 95% CI $[-0.504, -0.102]$, $p = 0.003$). The coefficient for formal educational level was attenuated and was no longer statistically significant ($b = 0.238$, 95% CI $[-0.065, 0.542]$, $p = 0.126$); gender and diabetes duration remained unassociated with the strict score. Thus, the inverse association with age was robust to the scoring rule, whereas the educational-level association was sensitive to how partial recognition was scored.

5. Discussion

5.1 Principal Findings

This study was a cross-sectional survey conducted in a clinic setting that aimed to identify important gaps in the sick-day knowledge of adults with type 2 diabetes in Jenin. The mean Sick-Day Knowledge Recognition Score was 2.44 points out of 5 points, and fewer than half of participants recalled receiving explicit sick-day education. Two key safety issues were under-recognized: only 23.7% recognized that SGLT2 inhibitor-associated DKA can occur in the absence of significant hyperglycemia, and only 37.2% recognized the need to withhold treatment before surgery. In contrast, 77.8% identified at least one warning sign that needed medical care. This pattern indicates limited recognition of individual safety messages rather than a full understanding of when and why treatment should be suspended.

Recalled sick-day education was strongly associated with selection of an appropriate intended action during acute illness. Participants who recalled receiving sick-day education had 5.39 times the odds of reporting an appropriate intended action. Although clinically relevant, this association cannot be assumed to be causal as the education and intended action were not measured at a different survey visit, and the exposure was self-reported. Age was an independent factor associated with a lower Knowledge Score. Under strict scoring, the association between higher levels of formal education and the Knowledge Score was reduced. In the sensitivity analysis, the age association did not change considerably, and it therefore remained the strongest demographic finding, whereas the association with educational level was more dependent on how partial recognition was scored.

While 77.3% chose the right answer in a hypothetical illness, 70.4% stated that they had taken the medicine during at least one previous illness. The study could not determine when the prior illness merited withholding, whether education occurred before the episode, or whether the same individuals had a safe intention that they did not follow through on. However, the overall contrast highlights the potential for inconsistent translation of stated intentions to actual medication management in the real world and suggests a need to evaluate observed behavior in subsequent research.

5.2 Comparison with previous evidence

It is noteworthy that ketoacidosis without significant hyperglycemia may not be immediately recognized, which may delay diagnosis and treatment of SGLT2 inhibitor-associated diabetic ketoacidosis. Another important safety measure is perioperative interruption. The general guidance is to withhold SGLT2 inhibitors for three days before elective surgery and four days before elective surgery for ertugliflozin, and then restart them when the precipitating risk has passed and the patient is clinically stable with adequate oral intake [3,10]. It is unsurprising that warning symptoms were identified more often than the underlying risk or the need to interrupt treatment. Counseling should provide a single action plan linking the precipitating situation to recognition of warning symptoms, withholding of the medication, urgent assessment, and safe restarting.

The education gap observed is consistent with recent education-specific research on SGLT2 inhibitors, although direct numerical comparisons are not suitable due to varying populations and instruments used to measure education. A study of targeted written information reported low baseline awareness of SGLT2 inhibitor sick-day rules and enhanced patient-reported knowledge following the intervention [5]. Pharmacist-provided sick-day materials in the SAKURA cluster-randomized trial did not significantly affect anxiety or behavioral intention and had a non-significant effect on attitudes [6]. In addition to the current correlation between recalled education and intended action, these studies suggest that structured education is a potential safety measure and that information alone might not be enough to affect behavioral change.

The age pattern was consistent with previous Palestinian diabetes studies, where younger age was associated with higher general diabetes knowledge, as was the educational pattern [7]. Previous research in Palestine has correlated medication knowledge and beliefs with adherence and indicated continued problems with adherence to long-term medication [8,9]. These studies, however, did not look at the safety of using SGLT2 inhibitors specifically for sick days. However, the present sensitivity analysis requires qualification: there was no association with formal educational level when full and not partial recognition was necessary. Formal education should therefore not be assumed to be a good indicator of the ability to follow the SGLT2 inhibitor sick-day guidelines.

5.3 Implications for Practice

The results are consistent with published guidance and consensus recommendations for a standardized sick-day education pathway that is initiated when a SGLT2 inhibitor is first started and reinforced at follow-up visits, medication refills, acute-illness contacts, and preoperative assessment [4]. Common situations that lead to the need for temporary withholding should be identified; the fact that ketoacidosis may occur in the absence of significant hyperglycemia should be explained, warning signs and when urgent assessment is necessary should be explained, agent-specific instructions for pre-operative precautions should be given, and clear explanations of when and how treatment should be resumed should be provided. A simple verbal explanation plus one-page written action plan, teach-back, and documentation in the clinical record would be a simple minimum standard. A large proportion of participants preferred written instructions (91.3%) and desired more information (95.1%), indicating the general acceptability of this intervention. Physicians were most often reported as the primary source of information, followed by pharmacists. Thus, the level of counseling could be enhanced by having coordinated messages sent from diabetes clinics and community pharmacies. Education should be expanded to include short scenarios and rehearsing behavior and should be used in situations beyond recognition questions, such as when patients explain what they would do if they developed vomiting or fever, were unable to keep fluids down, became dehydrated, or were preparing for surgery.

Attention should be paid to older adults because the inverse association between age band and knowledge persisted under both scoring rules. For older patients, counseling should include simple language, large print, repetition, and a scenario-based teaching-back approach. Relatives or caregivers who help with medication management should be given the same written action plan and be involved in rehearsal of the steps to be taken during acute illnesses or before surgery, with the patient's permission. This reinforcement should be complementary to systematic education, not a replacement.

5.4 Strengths and limitations

This study has several strengths, including targeting an under-investigated medication-safety problem, consecutive enrollment in routine clinical care, item-level reporting that included explicit denominators, adjustment for prespecified demographic and clinical variables, and sensitivity analysis with a stricter scoring rule. Separate reporting of the knowledge items also facilitated the detection of clinically specific deficits that would not have been apparent from the overall score alone.

Several limitations should be taken into account. Firstly, the cross-sectional design does not allow conclusions to be drawn about temporal sequence or causality. Participants who remembered education may have had different levels of health literacy, motivation, healthcare contact, cognition, disease complexity, or other unmeasured characteristics. Second, self-reported measures of education, intended action, and previous behavior are susceptible to recall and social-desirability bias, which is likely to be significant in an interviewer-administered survey. The hypothesized outcome of intended action was not observed because the illness episode had occurred in the past, and the survey did not determine if the episode of illness was preceded by education. No clinical outcomes, including ketoacidosis, dehydration, hospitalization, or perioperative complications, were measured.

Third, the questionnaire developed by the investigator was not fully validated. The knowledge scale had only preliminary internal consistency (Kuder–Richardson coefficient 0.69), and there was no criterion validity, construct validity, or test–retest reliability. The most important criterion for the two multiple-response items, which were awarded partial credit, was that no more than one undesirable response could be selected at the same time. Some of the conclusions are sensitive to this choice, including the lower score under strict scoring, as well as the decline in the educational-attainment coefficient. The three attitude items should also be kept separate due to low internal consistency between items, which did not allow the creation of a single composite construct.

Fourth, the five items were bounded and coarse, and age, education, and diabetes duration were ordinal scores with a fixed relationship between adjacent scores. Standard errors were large; however, other models or category-specific estimates may be informative in larger samples. Lastly, the study has limited generalizability to other Palestinian regions, primary care, and those without easy access to care. Assessment of selection bias is further constrained by the inability to measure the number approached, as well as the nonresponse profile.

In summary, the findings highlight a meaningful and potentially targetable gap in the safety of SGLT2 inhibitors in the context of sick days. They assist with the implementation of a standardized Arabic counseling intervention with written instructions and teach-back. Future studies should use a more fully validated instrument, document the timing and content of education, assess retention, and measure observed medication management and clinical outcomes rather than relying solely on recalled education and stated intentions.

6. Conclusions and Recommendations

Among adults with type 2 diabetes receiving SGLT2 inhibitors in Jenin, sick-day knowledge was incomplete, and fewer than half recalled receiving specific sick-day education. Important gaps concerned recognition of ketoacidosis without marked hyperglycemia and preoperative withholding. Recalled education remained associated with appropriate intended action after adjustment, although causality cannot be established. Older age was consistently associated with lower knowledge, whereas the association with educational attainment was

sensitive to the scoring method. These findings highlight the need for systematic SGLT2 inhibitor safety education, with particular attention to older adults and those involved in supporting their medication use.

A standardized Arabic-language sick-day education protocol should be provided when an SGLT2 inhibitor is initiated and reinforced during follow-up visits, medication refills, acute illness contacts, and preoperative assessment. Older adults should receive additional support using plain language, large-print written instructions, repetition, and scenario-based teach-back. With the patient's consent, relatives or caregivers should be included in counseling so that they can recognize warning symptoms and assist with appropriate medication management during illness.

Counseling should explain when temporary withholding is indicated, the possibility of ketoacidosis without marked hyperglycemia, relevant warning symptoms, when urgent medical advice is required, preoperative withholding, and when treatment may be safely restarted. Physicians and pharmacists should provide consistent messages and document education of both the patient and, when applicable, the caregiver. The effectiveness of this approach should be evaluated prospectively using validated measures and observed medication-management behavior.

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8. Declarations

The authors declare that they have no financial or non-financial competing interests related to this study.

Funding

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Conflict of interest/Competing interests

The authors declare that they have no financial or non-financial interests related to this work.

Ethics approval and consent to participate

Participation was voluntary, and participants received information about the study before providing informed consent. Consent was documented through an affirmative response to the first questionnaire item before the interview proceeded. No directly identifying personal information was collected.

Consent for publication

Not applicable. This manuscript contains no identifiable personal information, images, or individual-level case details.

Data availability

The de-identified data supporting the findings of this study are available from the corresponding author upon reasonable request and subject to applicable ethical and institutional requirements.

Materials availability

The Arabic-language questionnaire administered in the study is provided in Supplementary File 1. Additional study materials are available from the corresponding author upon reasonable request.

Code availability

The Python code used for data management and statistical analysis is available from the corresponding author upon reasonable request.

Authors' Contributions

Ikhlas Jarrar, Huda Masri, and Suhad Al-Ahmad contributed to the study conceptualization and design, questionnaire development, methodology, statistical analysis, project supervision, and preparation, critical revision, and editing of the manuscript. 5th-year students Dina Khalaf and Nagham Zakarneh contributed to participant recruitment and data collection as part of their graduation project. All authors reviewed and approved the final manuscript and agreed to be accountable for the work.

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Biostatistical Models for Predicting Mortality in Intensive Care Units

Running Title: Physician Perspectives on ICU Mortality Prediction Models

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Abstract

Intensive care Units clinicians often rely on risk scores. However, the use of a prediction model depends on how it is built, updated, explained and introduced into clinical work. The researchers surveyed 223 physicians working in intensive care settings in the West Bank and asked about eight features of biostatistical models used for mortality prediction: input variables, model type/methodology, calibration and discrimination, temporal resolution, handling of missing data, interpretability, external validation and clinical integration. Accordingly, the responses were analyzed in SPSS using descriptive statistics, Cronbach's alpha, collinearity diagnostics and multiple linear regression. All the eight dimensions showed positive and statistically significant associations with the physician-reported mortality-prediction outcome. The model type/methodology had the largest standardized coefficient ($\beta = 0.288$; $B = 0.256$; $p < 0.001$) and followed by the temporal resolution ($\beta = 0.229$; $B = 0.202$; $p < 0.001$). The full model yielded $R = 0.905$ and $R^2 = 0.819$. The findings showed how the physicians viewed the usefulness of the model characteristics; they did not represent the validation of an ICU mortality model using patient-level outcomes.

Keywords: intensive care unit, mortality prediction, biostatistical models, machine learning, interpretability, external validation, clinical integration.

1. Introduction

Estimating the risk of death is a routine part of intensive care. APACHE, SAPS and SOFA remain familiar tools because they condense several clinical measurements into a severity score that can be used at the bedside. Their simplicity is useful, but an ICU patient can change quickly, sometimes within hours. This has created interest in statistical and machine-learning methods that can use a broader set of measurements and, when data are available, update risk as the clinical picture changes.

Recent ICU studies have explored several such approaches. Khajehali et al. [1] compared machine-learning methods for mortality prediction and reported their best performance with AdaBoost after preprocessing the data. Using MIMIC-IV records from patients with cardiac arrest, Sun et al. [2] evaluated LASSO, XGBoost, logistic regression and NEWS2; LASSO, XGBoost and logistic regression discriminated outcomes better than NEWS2. Jeon et al. [3] examined patients with severe pneumonia and also found better discrimination with machine-learning models than with SAPS II. Taken together, these reports suggest that the modelling method can matter, although its value still depends on the population, the available data and the way the model is used. For clinical use, the data entering a model are just as important as the algorithm itself. Age, comorbidities, vital signs, laboratory results and severity scores are commonly available in ICU records, but their completeness and timing vary. The choice of method then determines how these inputs are combined. Logistic regression has the advantage of being familiar and relatively easy to explain. More flexible methods, including boosting algorithms and neural networks, can model nonlinear patterns, but added complexity is not automatically an advantage at the bedside.

Model quality cannot also be reduced to a single performance number. Discrimination asks whether patients with different outcomes can be separated, whereas calibration asks whether the estimated risks are close to what is actually observed. A model may perform well in the hospital where it was developed and lose accuracy when moved elsewhere. External validation is therefore important, especially in critical care, where case mix, staffing, treatment pathways and resource availability differ between institutions [2,4,5].

Timing creates another difficulty. That is, ICU measurements are collected repeatedly rather than once, so a risk estimate based only on admission data may miss later deterioration or improvement. Studies using real-time vital signs and multivariate time-series data have shown the value of updating predictions as new information becomes available [6,7]. Missing data are equally common in routine records and need to be handled explicitly. Clinicians also need to understand what is driving a prediction and to see the result in a form that fits the existing workflow rather than in a separate research interface [8,9].

Most work in this field has concentrated on the performance of algorithms in patient datasets. Far less is known about which model features physicians themselves consider convincing or workable, particularly in Palestinian hospitals. The researchers, therefore, examined the views of ICU physicians in the West Bank rather than building a new mortality algorithm. The survey covered eight dimensions—input variables, model type/methodology, calibration and discrimination, temporal resolution, missing-data handling, interpretability, external validation and clinical integration—and assessed how each related to the physicians' overall rating of mortality-prediction usefulness.

The conceptual framework used in the study is summarized in Figure 1.

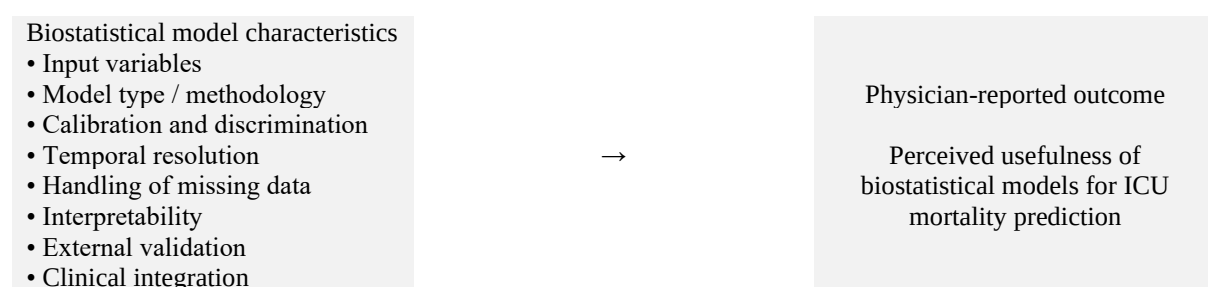


Figure 1. Conceptual framework based on the literature [5,7-12].

Study hypotheses

Main hypothesis: The combined characteristics of biostatistical models are positively associated with the physicians' perceptions of their usefulness for ICU mortality prediction.

H1: Ratings of input variables are positively associated with the physician-reported mortality-prediction outcome.

H2: Ratings of model type/methodology are positively associated with the physician-reported mortality-prediction outcome.

H3: Ratings of calibration and discrimination are positively associated with the physician-reported mortality-prediction outcome.

H4: Ratings of temporal resolution are positively associated with the physician-reported mortality-prediction outcome.

H5: Ratings of missing-data handling are positively associated with the physician-reported mortality-prediction outcome.

H6: Ratings of interpretability are positively associated with the physician-reported mortality-prediction outcome.

H7: Ratings of external validation are positively associated with the physician-reported mortality-prediction outcome.

H8: Ratings of clinical integration are positively associated with the physician-reported mortality-prediction outcome.

2. Materials & Methods

2.1. Study design

The researchers used a cross-sectional survey because the study question was concerned with the physicians' views of mortality-prediction models at one point in time. No patient records were used, and no prediction algorithm was developed or tested in this study.

2.2. Questionnaire development

The questionnaire was prepared after reviewing published work on predictive modelling, interpretability, missing data, temporal modelling, external validation and clinical integration [5,7-12]. It had two sections. The first collected demographic information, including gender, experience and education. The second contained items covering the eight model-related dimensions examined in the study. The physicians recorded their responses on a five-point Likert scale through Google Forms.

2.3. Study setting and participants

The physicians working in intensive care settings in the West Bank, Palestine were population of the study. The researchers invited 290 physicians to participate and retained 223 complete questionnaires after screening, corresponding to a response rate of 76.8%. The sampling frame included government, private, and private or charitable hospitals.

Table 1. Study population source

Source	#
government hospitals	18
private hospitals	18
private or charitable hospitals	22
Total	58

2.4. Statistical analysis

SPSS was used for data screening and statistical analysis. The researchers first described the participants with frequencies and percentages and summarized questionnaire items and dimension scores with means (μ) and standard deviations (σ). Cronbach's alpha was calculated for internal consistency. Tolerance and variance inflation factor (VIF) values were examined before regression analysis. The researchers then fitted a multiple linear regression model with the eight model-related dimensions as predictors of the physician-reported ICU mortality-prediction outcome. A p value below 0.05 was considered statistically significant.

Table 2. Internal consistency of study variables (Cronbach's α)

Variable	α
Input Variables	0.973
Model Type / Methodology	0.938
Calibration and Discrimination	0.948
Temporal Resolution	0.939
Handling of Missing Data	0.928
Interpretability	0.954
External Validation	0.952
Clinical Integration	0.943
Predicting Mortality in (ICU)	0.926

3. Results

3.1. Participant characteristics

Of the 223 respondents, 116 (52.0%) were men and 107 (48.0%) were women. Out of the respondents, 107 physicians (48.0%) were reported to have more than 18 years of professional experience. According to the education categories used in the questionnaire, 151 participants (67.7%) were in the bachelor-level group and 72 (32.3%) were in the high studies category. Table 3 shows the complete distribution.

Table 3. Demographic characteristics of participants

	f	%
Gender		
Male	116	52.0
Female	107	48.0
Experience		
Less than 5 years	33	14.8
6-11	60	26.9
12-17	23	10.3
More than 18 years	107	48.0
Education		
BA	151	67.7
HIGH STUDIES	72	32.3

	f	%
Total	223	100.0

3.2. Questionnaire responses

All of the eight-dimension means were above 3.00, which is the midpoint of the five-point scale. The mean for the overall physician-reported mortality-prediction outcome was 3.840 (SD = 0.897). Input variables received the highest dimension score (mean = 3.819), followed by temporal resolution (3.719), model type/methodology (3.706), external validation (3.611), interpretability (3.527), calibration and discrimination (3.392), clinical integration (3.367), and handling of missing data (3.332). The item-level values are shown in Table 4.

Table 4. Questionnaire item and dimension scores

Statement	μ	σ
The model shows age, sex and comorbidities of patients all the time	3.888	1.018
Vital signs are always flashing including (heart rate, blood pressure, respiratory rate)	3.821	1.080
Indicators of labs values can be seen on screen all the time	3.839	1.044
The Glasgow Coma Scale (GCS) flashes when there are changes	3.848	1.067
Score are always available including APACHE, SAPS, or SOFA scores	3.700	1.228
Input Variables	3.819	1.035
The models have the ability to determine interactions	3.767	1.135
They are able to capture nonlinear relationship	3.381	1.104
Models are all improved in their accuracy	3.803	1.101
They can also improve interpretability of results	3.888	1.163
They are based on logistic regression and XGBoost	3.700	1.129
Model Type / Methodology	3.706	1.009
Models adopted are reliable and able to predict risks	3.350	1.175
They are crucial for clinical utility	3.305	1.222
They depend on calibration plots	3.466	1.273
They can show the Area Under the Receiver Operating Characteristic Curve (AUROC)	3.377	1.224
They are based on Brier Score	3.413	1.193
Calibration and Discrimination	3.392	1.103
Model incorporate time varying data	3.673	1.195
All predictions are updated over time	3.601	1.158
Model are based on real time updating	3.744	1.128
They utilize time-series analysis	3.744	1.108
They operate on recurrent neutral networks (RNNs)	3.834	1.105
Temporal Resolution	3.719	1.021
All models enjoy a good level of robustness and reliability	3.305	1.089
We make sure that models are able to avoid biased mortality prediction	3.256	1.163
Model can ignore and exclude incomplete cases and data	3.121	1.090
Model are always aware of lost information (imputation)	3.345	1.148
ICU environment must be able to handle missing data	3.632	.991
Handling of Missing Data	3.332	.967
There is a continuous risk assessment	3.623	.973
Models aren't based on one-time scoring of data	3.547	1.007
The updating is always in real-time	3.493	.999
This can increase trust and adoption	3.565	1.002
Modifiable factors are always up-to-date	3.390	1.157
Interpretability	3.527	.951
Models can generate data outside dataset	3.668	1.251
Models can prevent overfitting	3.318	1.194
They present a broader clinical application	3.691	1.230
They can generate data from different patients at the same time	3.789	1.289

Statement	μ	σ
Generalizability is possible based on certain conditions that can't be ignored	3.619	1.231
External Validation	3.611	1.131
All model fit with ICU's electronic health record (EHR) systems	3.332	1.158
They provide a real-time decision-making	3.274	1.212
They present highly integrated alert tools	3.408	1.273
They are based on clinicians point of care	3.372	1.193
They make sure that all tools are usable for predictions	3.448	1.176
Clinical Integration	3.367	1.086
ICU environment depends highly on biostatistical models	3.839	1.099
All ICU's tasks are complex and must be maintained in a developed approach	3.816	1.064
Predicting patients' risk of death is highly crucial medical aid	3.946	1.043
Biostatistical models can help ICU make decisions and allocate resources	3.964	.990
Biostatistical characteristics are crucial for effective ICU efforts	4.013	1.002
Predicting mortality rate is important when it comes to clinical integration	3.462	1.093
Predicting Mortality in (ICU)	3.840	.897

3.3. Collinearity assessment

VIF values ranged from 1.519 to 5.142, with corresponding tolerance values of 0.194 to 0.659. None reached the conventional VIF threshold of 10 used in the original analysis. The highest value was observed for model type/methodology (VIF = 5.142), indicating some overlap with the other predictors and suggesting that its individual coefficient should be interpreted with care.

Table 5. Collinearity statistics

	Tolerance	VIF
Input Variables	.535	1.870
Model Type / Methodology	.194	5.142
Calibration and Discrimination	.332	3.008
Temporal Resolution	.659	1.519
Handling of Missing Data	.550	1.818
Interpretability	.406	2.461
External Validation	.294	3.403
Clinical Integration	.288	3.472

3.4. Regression analysis

The eight predictors were strongly associated with the physician-reported mortality-prediction outcome in the multiple regression model ($R = 0.905$; $R^2 = 0.819$). In other words, the model accounted for 81.9% of the variation in the questionnaire outcome. This percentage concerns variation in respondents' ratings; it is not the proportion of real ICU deaths explained or predicted by the model.

Each regression coefficient was positive and significant at $p < 0.05$. Model type/methodology had the largest standardized coefficient ($\beta = 0.288$; $B = 0.256$; $p < 0.001$), and temporal resolution was next ($\beta = 0.229$; $B = 0.202$; $p < 0.001$). The remaining coefficients were external validation ($\beta = 0.136$; $p = 0.012$), calibration and discrimination ($\beta = 0.112$; $p = 0.027$), clinical integration ($\beta = 0.109$; $p = 0.045$), interpretability ($\beta = 0.094$; $p = 0.042$), input variables ($\beta = 0.085$; $p = 0.034$), and handling of missing data ($\beta = 0.083$; $p = 0.036$). The direction of H1-H8 was therefore supported within this questionnaire-based analysis.

Table 6. Multiple regression results for the study hypotheses

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	.292	.126		2.317	.021
Input Variables	.074	.034	.085	2.134	.034
Model Type / Methodology	.256	.059	.288	4.357	.000
Calibration and Discrimination	.091	.041	.112	2.225	.027
1 Temporal Resolution	.202	.032	.229	6.397	.000
Handling of Missing Data	.077	.036	.083	2.112	.036
Interpretability	.088	.043	.094	2.050	.042
External Validation	.108	.043	.136	2.532	.012
Clinical Integration	.090	.045	.109	2.012	.045

4. Discussion

The survey shows that the physicians did not view ICU mortality prediction as a matter of algorithm choice alone. Ratings across all eight dimensions were positively related to the overall questionnaire outcome, with model type/methodology and temporal resolution showing the strongest associations. Because the outcome was based on the physicians' responses, the result is best read as a picture of what clinicians consider useful in a prediction system, not as evidence that a particular model predicts death accurately.

Model type/methodology ranked first in the regression. That is plausible: the modelling approach affects both the patterns a system can capture and how readily a clinician can understand its output. Logistic regression remains attractive when transparency is important, whereas boosting methods and neural networks can represent more complicated relationships. ICU studies have reported good results with AdaBoost, XGBoost, LightGBM and multilayer perceptron models [1-3]. Our survey did not test one method against another; it indicates that physicians pay attention to the method behind the prediction.

Temporal resolution came next. In intensive care, many of the variables that matter—vital signs, laboratory tests, ventilatory support and treatment—change during the admission. A risk score calculated once may therefore become outdated. Work based on real-time vital signs and multivariate time-series modelling supports the value of repeated updating [6,7]. The relatively high score for temporal resolution in this survey suggests that this issue is also recognized by the clinicians working in the study setting.

External validation, calibration and discrimination were also associated with the outcome. A model can separate survivors from non-survivors reasonably well and still give poorly calibrated risk estimates. Likewise, the results obtained in one hospital may not carry over to another. Sun et al. [2] examined both discrimination and calibration, and Hargovan et al. [4] focused specifically on external validation of sepsis mortality models. The responses in our study suggest that physicians see these checks as part of deciding whether a model is credible enough for wider use.

Interpretability and clinical integration were significant as well. A prediction that sits outside the normal clinical workflow, or one that gives a risk number without an understandable basis, is unlikely to be used consistently. EHR integration, clear displays and access to the main contributors to a prediction may help, but they do not

replace validation. Previous work on interpretable machine learning and transparent ICU decision support raises the same concern [5,7,8]. Tools such as SHAP can assist explanation, but the clinical value of an explanation still depends on the quality of the underlying model.

Input variables and missing-data handling produced smaller standardized coefficients, although both remained significant. The researchers do not interpret the smaller coefficients as evidence that these issues are secondary. ICU records often contain irregular or incomplete measurements, and the way missing values are treated can alter model behavior. The same applies to input selection: even a sophisticated method will perform poorly if the variables are unreliable or not clinically relevant. Bias and missingness in ICU electronic records have been discussed repeatedly in the literature [9,11].

The size of R^2 requires particular caution. Predictor and outcome scores came from the same questionnaire, and several items deal with related aspects of the same general idea. Shared wording, overlap between constructs and common-method effects may therefore have increased the observed association. Model type/methodology also had a VIF just above 5. These points do not remove the associations seen in the survey, but they make it unwise to treat the individual coefficients as if they were independent measures of clinical effectiveness.

The local setting is worth noting. Published work on predictive analytics rarely includes ICU physicians from the West Bank. This survey gives a first indication of the features that clinicians in this setting say they would value in a mortality-prediction system. It does not show that any existing model is accurate for Palestinian ICU patients. That would require patient-level data and formal model validation.

4.1. Limitations and future directions

Several limitations follow from the design. The sample was restricted to ICU physicians in the West Bank, and all measures were self-reported at a single time point. Predictors and outcome were answered by the same respondents, while some constructs are closely related, so common-method variance and item overlap may have inflated the regression. No patient-level clinical data were analyzed; consequently, this study cannot provide AUROC, calibration, sensitivity, specificity or evidence of reduced mortality for any algorithm. A next study should test candidate models on local ICU data, compare simpler transparent approaches with more flexible machine-learning methods, and include internal and external validation. If an EHR-based risk tool is later introduced, its effect on decisions, alert burden and possible bias should also be evaluated prospectively.

5. Conclusion

The physicians surveyed in this study associated the usefulness of ICU mortality-prediction models with a combination of technical and practical features. Model type/methodology and temporal resolution had the strongest associations, but external validation, calibration and discrimination, clinical integration, interpretability, input variables and missing-data handling were also significant. For the West Bank, these findings are better used to guide the design and evaluation of future tools than to justify immediate clinical deployment. Candidate models should first be tested on patient-level ICU data and shown to be calibrated, discriminative and reasonably generalizable. Explainable output and EHR integration can then be considered as implementation features rather than substitutes for validation.

6. Declarations

• Clinical trial number

Not applicable

• Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Arab American University, Palestine (Approval No. AAUP-IRB: J-2025/A 95/N). All participants provided written informed consent prior to participation.

• Consent for publication

Written informed consent for publication was obtained from all relevant participants.

• Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

• Competing interests

The authors declare that they have no competing interests.

• Funding

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• Authors' contributions

S.A. conceived and designed the study, collected the data, performed the analysis, and drafted the manuscript. All authors reviewed and approved the final version.

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Mutual synchronization between quantum-dot passively mode-locked laser frequency combs: Theoretical approach

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Abstract

A comprehensive theoretical framework is established for describing repetition-rate synchronization and partial optical coherence in two bidirectionally coupled quantum-dot passively mode-locked lasers (QD-PMLLs). Each laser is modeled as a two-section monolithic device composed of a forward-biased gain section and a reverse-biased saturable absorber. The coupled delay-rate equations incorporate the slowly varying complex optical fields, modal gain dynamics, absorber loss, photon decay, linewidth-enhancement factors, delayed optical coupling, coupling phase, and spontaneous-emission noise. In the weak mutual-injection limit, the full model is systematically reduced to an effective timing-phase description, yielding the locking relation $\Delta\Omega_0 = 2K\cos(\psi - \Omega_s\tau_{\text{ext}})\sin(\delta)$, and hence the locking condition $|\Delta\Omega_0| \leq 2K|\cos(\Omega_s\tau_{\text{ext}} - \psi)|$. Representative simulations establish that two initially detuned 10 GHz pulse trains are pulled into a single shared repetition frequency, with the locked frequency showing pronounced and controllable delay-dependent tunability. The synchronized state exhibits a marked reduction in root-mean-square timing jitter, whereas finite mutual optical coherence appears only when the optical carrier mismatch simultaneously lies within the coherent injection-locking bandwidth. Agreement with recent synchronization experiments on InAs/InGaAs QD-PMLL combs demonstrates that the model not only reproduces the central experimental trends but also clarifies the underlying hierarchy: repetition-rate locking is robust and readily achieved, delay tuning provides an effective control parameter, timing noise is strongly suppressed in the locked regime, and optical phase coherence is an additional conditional constraint rather than an automatic consequence of rate synchronization.

Keywords: quantum-dot laser, passively mode-locked laser, frequency comb, delayed optical coupling, synchronization, timing jitter, mutual coherence.

1. Introduction

Quantum-dot passively mode-locked lasers (QD-PMLLs) represent a highly attractive class of compact semiconductor sources for generating picosecond pulse trains and optical frequency combs. Owing to their low-

dimensional active medium, these devices exhibit discrete carrier states, reduced temperature sensitivity, enhanced differential gain, and rich dynamical behavior that is fundamentally distinct from that of conventional quantum-well lasers. These advantages position QD-PMLLs as promising light sources for integrated photonic interconnects, microwave photonics, coherent optical communication systems, high-speed optical sampling, and chip-scale metrology [1]. Early demonstrations of stable mode locking in two-section QD lasers, together with extensive studies of mode-locked QD and quantum-dash semiconductor lasers, have firmly established low-dimensional gain media as a robust platform for compact comb generation [2–4]. Furthermore, nonlinear dynamical analyses have revealed that carrier reservoirs, carrier capture and escape processes, and the associated time scales critically govern pulse formation, timing stability, and the overall mode-locking dynamics of these devices [5].

Recent experimental demonstrations further support the use of QD active media as practical comb sources for integrated photonics. Two-section InAs QD mode-locked lasers have produced combs with a stable 25.5 GHz spacing over a wide temperature range, while broadband QD devices have been shown to operate in both amplitude-modulated and frequency-modulated comb regimes [6,7]. The direct growth of reflection-insensitive QD lasers on silicon, together with continuing advances in silicon photonics, also strengthens their relevance for scalable photonic integration [8,9]. More recently, high-channel-count passively mode-locked QD lasers monolithically integrated on silicon have enabled multi-terabit transmission, highlighting their potential for integrated communication systems [10]. These advances show that QD comb sources can function not only as short-pulse emitters, but also as stable, broadband, multi-line sources for chip-scale optical platforms.

Synchronization is a critical requirement in systems where multiple optical combs operate together. Optical injection locking offers an all-optical mechanism for aligning the frequency and phase dynamics of semiconductor lasers, avoiding the need for high-power microwave distribution [11]. At the same time, studies of optically injected semiconductor lasers have shown that injection strength, frequency detuning, and relative phase can substantially reshape the nonlinear laser dynamics, including transitions to complex or chaotic behavior [12]. Earlier work on optical-pulse injection locking, together with general frequency-comb injection-locking theory, further indicates that repetition-rate locking and optical carrier-phase compatibility are closely related but not identical synchronization conditions [13–15]. For QD-PMLLs, optical feedback and delayed optical perturbations are particularly important because they can directly influence phase noise, RF linewidth, and pulse-to-pulse timing jitter [16,17]. From a modeling perspective, delayed-field formulations are rooted in the Lang–Kobayashi description of external optical feedback in semiconductor lasers [18], providing the basis for incorporating delayed optical coupling terms into the present rate-equation framework.

In many practical systems, a single optical comb source is not sufficient; multiple combs must operate with matched line spacing, stable relative pulse timing, or controlled mutual coherence. Electrical locking can achieve this synchronization, but it requires microwave driving circuitry and may reduce the scalability and compactness of integrated photonic implementations. Optical synchronization provides a more attractive alternative by allowing the pulse train or comb field emitted by one laser to be injected into another, thereby imposing timing and/or frequency coherence through purely optical coupling. Recent experiments have demonstrated synchronization of QD mode-locked laser combs under unidirectional optical injection [19], followed by bidirectional passive optical coupling between two monolithic InAs/InGaAs QD-PMLLs operating near 10 GHz and 1260 nm [20]. These demonstrations motivate a dynamical modeling framework capable of capturing how

delayed optical coupling, coupling strength, detuning, and mutual phase interactions govern synchronization between QD-PMLLs.

These experimental advances raise a theoretical question of direct relevance to both device design and system integration: what is the minimal physically grounded model that links delayed optical coupling to repetition-rate locking, delay-dependent frequency tuning, timing-jitter suppression, and conditional optical coherence? The approach adopted here is deliberately reduced, yet remains physically traceable. Specifically, this manuscript extends standard delay-rate descriptions of passive mode locking [21,22] by incorporating bidirectional delayed optical injection together with a timing-phase reduction. This formulation is consistent with reduced synchronization frameworks previously used to describe coupled photonic oscillators and micro resonator combs [23].

The objective of this work is to develop a theoretical framework that directly links coupled QD-PMLL rate equations to the synchronization signatures observed experimentally. The model is maintained in symbolic form so that each variable and parameter can be clearly associated with a measurable physical quantity or a design-relevant device parameter. In this form, the framework serves as a compact analytical design tool for estimating synchronization and locking conditions prior to fully calibrated numerical simulations or experimental optimization.

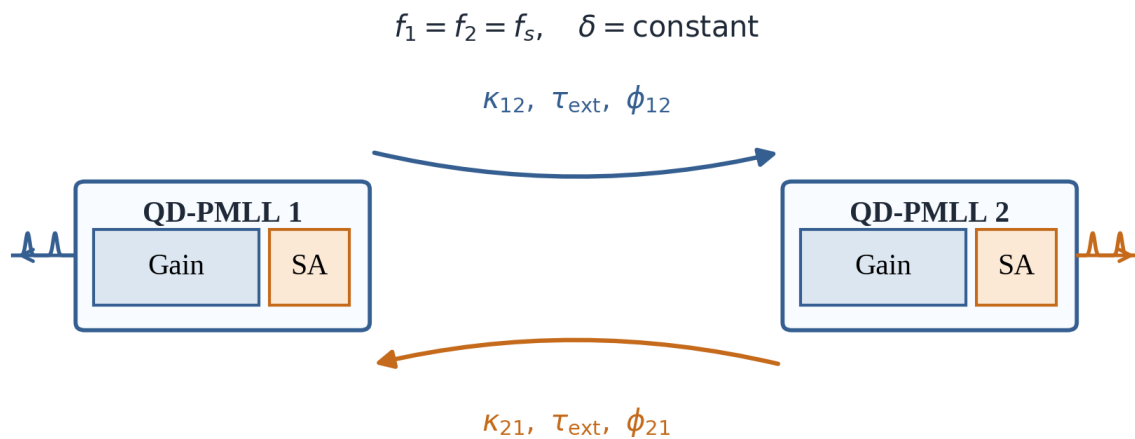


Figure 1. Bidirectionally coupled QD-PMLL frequency-comb system. The two lasers exchange delayed optical fields with coupling coefficients κ_{12} and κ_{21} , external delay τ_{ext} , and phases ϕ_{12} and ϕ_{21} . In the locked state, the repetition frequencies collapse to $f_1 = f_2 = f_s$ with a constant timing offset δ .

Nomenclature

Subscripts i and j stands for the receiving and the injecting lasers, respectively.

Table 1. Nomenclature used in the model.

Symbol	Definition	Unit
i, j	laser indices; j denotes the opposite laser to i	dimensionless
t	time	s
$E_i(t)$	slowly varying complex intracavity field of laser i	normalized field
$P_i(t) = E_i(t) ^2$	intracavity optical intensity or power	normalized power
$g_i(t)$	saturable modal gain	s^{-1} or dimensionless
$q_i(t)$	saturable absorber loss	s^{-1} or dimensionless
g_{0i}, q_{0i}	unsaturated gain and absorber loss	s^{-1} or dimensionless
I_i, V_i	gain-section current and absorber reverse bias	A, V
τ_g, τ_q	gain and absorber recovery times	s
E_g, E_q	gain and absorber saturation energies	J or normalized
γ_p	photon decay rate	s^{-1}
Γ	field-filter or cavity bandwidth parameter	s^{-1}
R_i	linear round-trip transmission in the delay-map form	dimensionless
α_g, α_q	linewidth-enhancement factors of gain and absorber	dimensionless
$T_i, f_{r,i0}$	cavity round-trip time and free-running repetition frequency	s, Hz
$\Omega_{i0} = 2\pi f_{r,i0}$	free-running angular repetition frequency	rad s^{-1}
$\Delta f_0 = f_{r,20} - f_{r,10}$	free-running repetition-rate detuning	Hz
$\Delta\Omega_0 = \Omega_{20} - \Omega_{10}$	free-running angular detuning	rad s^{-1}
κ_{ij}	optical coupling coefficient from laser j into laser i	s^{-1} or normalized
τ_{ij}	external propagation delay from laser j into laser i	s
τ_{ext}	symmetric external propagation delay	s
ϕ_{ij}	optical phase accumulated along the coupling path	rad
$\xi_i(t), D_i$	complex spontaneous-emission noise and strength	model dependent
$\theta_i(t)$	timing phase of the pulse train emitted by laser i	rad
$\eta_i(t)$	timing noise in the reduced phase equation	rad s^{-1}
K_{ij}, ψ_{ij}	effective timing-coupling coefficient and phase shift	rad s^{-1} , rad
K_r	linear timing restoring rate in the locked state	s^{-1}
Ω_s, f_s	synchronized angular and ordinary repetition frequencies	rad s^{-1} , Hz
δ	constant timing-phase offset in the locked state	rad
ϑ_r	small relative timing-phase perturbation about the locked state	rad
$t_{i,n}, T_{i,n}$	arrival time of pulse n and instantaneous period	s
$\sigma_{T,i}$	root-mean-square timing jitter of laser i	s
$\gamma_{12}(\tau_c)$	normalized mutual optical coherence at correlation delay τ_c	dimensionless

Symbol	Definition	Unit
$S_i(\omega)$	optical power spectrum	a.u.

2. Coupled delay-rate model

2.1. Device configuration

The modeled system consists of two edge-emitting QD-PMLLs labelled $i = 1$ and $i = 2$. The optical field inside laser i is denoted by $E_i(t)$, the corresponding intensity is $P_i(t) = |E_i(t)|^2$, the modal gain is $g_i(t)$, and the saturable absorber loss is $q_i(t)$. The gain section is governed by the injection current I_i through the unsaturated gain $g_{0i}(I_i)$, while the absorber section is controlled by the reverse-bias voltage V_i through the unsaturated loss $q_{0i}(V_i)$.

The optical field emitted by laser j is injected into laser i after an external delay τ_{ij} , with optical coupling coefficient κ_{ij} and accumulated phase ϕ_{ij} . Symmetric coupling is recovered under the conditions $\kappa_{12} = \kappa_{21} = \kappa$, $\tau_{12} = \tau_{21} = \tau_{\text{ext}}$, and $\phi_{12} = \phi_{21} = \phi$. The asymmetric coupling configuration is retained in the general formulation to capture the transition from mutual synchronization to unidirectional injection locking.

2.2. Dynamics of Local field, gain, and absorber

For i, j in $\{1, 2\}$ and $j \neq i$, the local coupled rate equation for the complex field is

$$\frac{dE_i(t)}{dt} = \frac{1}{2} \left[(1 + i\alpha_g)g_i(t) - (1 + i\alpha_q)q_i(t) - \gamma_p \right] E_i(t) + \kappa_{ij} \exp(i\phi_{ij}) E_j(t - \tau_{ij}) + \xi_i(t). \quad (1)$$

A minimal gain-absorber representation is

$$\frac{dg_i(t)}{dt} = \frac{g_{0i}(I_i) - g_i(t)}{\tau_g} - \frac{g_i(t)P_i(t)}{E_g}, \quad (2)$$

$$\frac{dq_i(t)}{dt} = \frac{q_{0i}(V_i) - q_i(t)}{\tau_q} - \frac{q_i(t)P_i(t)}{E_q}. \quad (3)$$

Equations (1) – (3) are formulated in a deliberately compact form to emphasize the essential dynamical mechanisms. If required, this reduced description can be extended by replacing the scalar gain and absorber equations with more comprehensive quantum-dot carrier-reservoir rate equations that explicitly account for the wetting layer, excited states, and ground states [24–26]. The delayed coupling contribution is kept at the complex-field level, since repetition-rate locking and optical coherence are intrinsically determined by the simultaneous evolution of the optical amplitude and phase.

2.3. Delay-map interpretation

For strongly pulsed operation, the complex optical field can be formulated in a delay-map representation over a single cavity round trip

$$E_i(t + T_i) = \sqrt{R_i} \exp \left\{ \frac{1}{2} \left[(1 + i\alpha_g)G_i - (1 + i\alpha_q)Q_i \right] \right\} E_i(t) + C_{ij} E_j(t - \tau_{ij}) \exp(i\phi_{ij}). \quad (4)$$

This representation highlights the role of the injected pulse. A delayed pulse emitted by laser j perturbs the pulse-formation window of laser i and consequently shifts the arrival time of the subsequent pulse. Although this timing

shift is small under weak coupling, it accumulates over many round trips and can lead to repetition-rate locking when the coupling-induced correction compensates the free-running detuning.

3. Reduction of timing-phase

Let $\theta_i(t)$ denote or denotes the slowly varying timing phase of the pulse train emitted by laser i . The timing phase advances by 2π each time the laser emits one pulse. Under weak coupling and stable mode locking, projecting the delayed optical perturbation onto the neutral timing mode gives

$$\frac{d\theta_i}{dt} = \Omega_{i0} + K_{ij}\sin[\theta_j(t - \tau_{ij}) - \theta_i(t) + \psi_{ij}] + \eta_i(t), \quad j \neq i. \quad (5)$$

Here $\Omega_{i0} = 2\pi f_{r,i0}$ is the free-running angular repetition frequency, K_{ij} is the effective timing-coupling coefficient, ψ_{ij} is a phase shift determined by the pulse shape and coupling phase, and $\eta_i(t)$ is timing noise. For symmetric coupling,

$$K_{12} = K_{21} = K, \quad \tau_{12} = \tau_{21} = \tau_{\text{ext}}, \quad \psi_{12} = \psi_{21} = \psi. \quad (6)$$

In the locked state both lasers share the synchronized angular repetition frequency Ω_s , and their timing phases are

$$\theta_1(t) = \Omega_s t, \quad \theta_2(t) = \Omega_s t + \delta. \quad (7)$$

Substitution of Eq. (7) into the symmetric timing equations gives the locking relation

$$\Delta\Omega_0 = 2K\cos(\psi - \Omega_s\tau_{\text{ext}})\sin(\delta), \quad (8)$$

and hence the repetition-rate locking condition

$$|\Delta\Omega_0| \leq 2K|\cos(\Omega_s\tau_{\text{ext}} - \psi)|. \quad (9)$$

The synchronized frequency is shifted by the coupling and delay phase. A useful approximate expression is

$$\Omega_s \approx \frac{\Omega_{10} + \Omega_{20}}{2} + K\sin(\psi - \Omega_s\tau_{\text{ext}})\cos(\delta), \quad f_s = \frac{\Omega_s}{2\pi}. \quad (10)$$

Equations (8) – (10) demonstrate that the locking range is not determined solely by the coupling magnitude. Instead, the external delay and optical phase can either enhance the effective timing-restoring strength or weaken it.

4. Numerical implementation

The coupled delay-rate equations were numerically integrated using stored histories of the delayed fields $E_j(t - \tau_{ij})$. The integration time step was selected to resolve the shortest relevant physical time scale, including the absorber recovery time, the internal pulse width, and the delayed feedback contribution. Each simulation was separated into a transient interval and a recording interval. During the recording interval, the complex optical fields, intensities, gain, absorber loss, and pulse-arrival times were recorded.

Representative baseline parameters were selected on the experimental scale of QD-PMLs operating at a repetition frequency of approximately 10 GHz and a center wavelength close to 1260 nm. These parameters are used as illustrative values and are not intended to represent a device-specific parameter extraction.

Table 2. Representative model parameters for the baseline theoretical simulations.

Parameter	Symbol	Representative value
Center wavelength	λ_0	1260 nm
Free-running repetition frequency of laser 1	$f_{r,10}$	9.998 GHz
Free-running repetition frequency of laser 2	$f_{r,20}$	10.012 GHz
Free-running detuning	Δf_0	14 MHz
Gain recovery time	τ_g	800 ps
Absorber recovery time	τ_q	5 ps
Gain linewidth-enhancement factor	α_g	representative
Absorber linewidth-enhancement factor	α_q	representative
External coupling delay	τ_{ext}	12.5 ns
Symmetric optical coupling coefficient	κ	varied
Symmetric timing-coupling coefficient	K	fitted from simulation
Noise strength	D_i	normalized

4.1. Pulse-arrival time and locking

The n -th pulse-arrival time of laser i is denoted by $t_{i,n}$ and is obtained from the local maximum of $P_i(t)$ near that pulse. The instantaneous period is

$$T_{i,n} = t_{i,n+1} - t_{i,n}. \quad (11)$$

The average repetition frequency is

$$f_i = \left\langle \frac{1}{T_{i,n}} \right\rangle_n. \quad (12)$$

The two lasers are considered repetition-rate locked when

$$|f_1 - f_2| < \Delta f_{\text{tol}}, \quad (13)$$

where Δf_{tol} is a selected tolerance. The mutual timing error is obtained by comparing corresponding delayed pulses and removing the nearest integer multiple of the period.

4.2. Timing jitter and mutual coherence

The root-mean-square timing jitter of laser i is calculated from the fluctuations of $T_{i,n}$ around its mean:

$$\sigma_{T,i} = \sqrt{\left\langle \left[T_{i,n} - \langle T_{i,n} \rangle_n \right]^2 \right\rangle_n}. \quad (14)$$

The normalized mutual optical coherence is calculated from the complex fields as

$$\gamma_{12}(\tau_c) = \frac{\langle E_1^*(t) E_2(t + \tau_c) \rangle}{\sqrt{\langle |E_1(t)|^2 \rangle \langle |E_2(t + \tau_c)|^2 \rangle}}. \quad (15)$$

Repetition-rate locking requires bounded pulse-envelope timing. High optical coherence additionally requires carrier-frequency compatibility and a favorable optical coupling phase.

5. Theoretical results

5.1. Unlocked reference case

For the baseline free-running case, the detuning is $\Delta f_0 = 14$ MHz. The relative timing phase $\theta_2 - \theta_1$ therefore drifts approximately linearly, the timing error remains unbounded, and the radio-frequency spectra show two distinct peaks. The optical spectra remain comb-like but exhibit different line spacings.

5.2. Repetition-rate locking map

Increasing the coupling coefficient strengthens the delayed mutual timing correction between the two pulse trains. When K is below the detuning term in Eq. (9), the injected pulse train only perturbs the local pulse-formation dynamics and is insufficient to arrest the relative timing drift. Once K exceeds the effective detuning threshold, the accumulated drift is suppressed and the two pulse trains converge to a common synchronized repetition frequency.

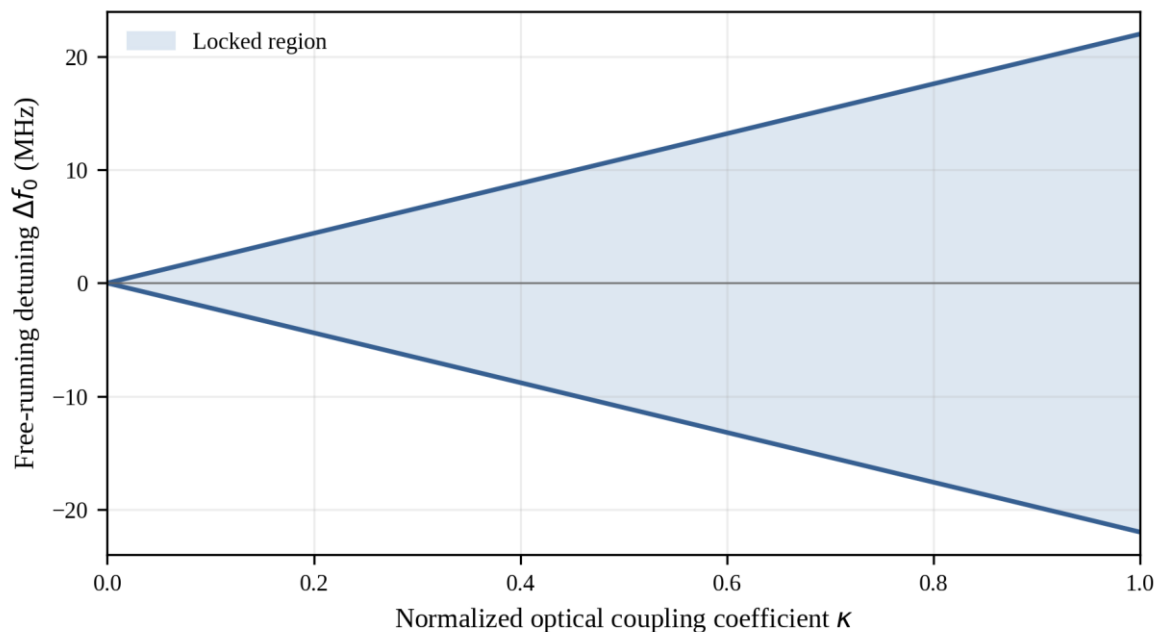


Figure 2. Theoretical synchronization map in the plane of normalized optical coupling coefficient κ and free-running detuning Δf_0 . The wedge-shaped locked region reflects the synchronization boundary predicted by the reduced timing-phase condition in Eq. (9).

The predicted locking region has a wedge-like shape: small κ locks only small $|\Delta f_0|$, while larger κ increases the locking half-width. The shape is modified by the delay phase, so the same coupling magnitude can be effective or ineffective depending on τ_{ext} and ϕ .

5.3. Delay tuning of the common repetition frequency

Eq. (10) predicts that τ_{ext} tunes f_s through the phase $\Omega_s \tau_{\text{ext}} - \psi$. From a physical perspective, a delayed pulse arriving ahead of the local pulse tends to advance its timing, whereas a pulse arriving after the local pulse tends to retard it. This behavior enables an all-optical mechanism for fine-tuning the synchronized microwave repetition frequency.

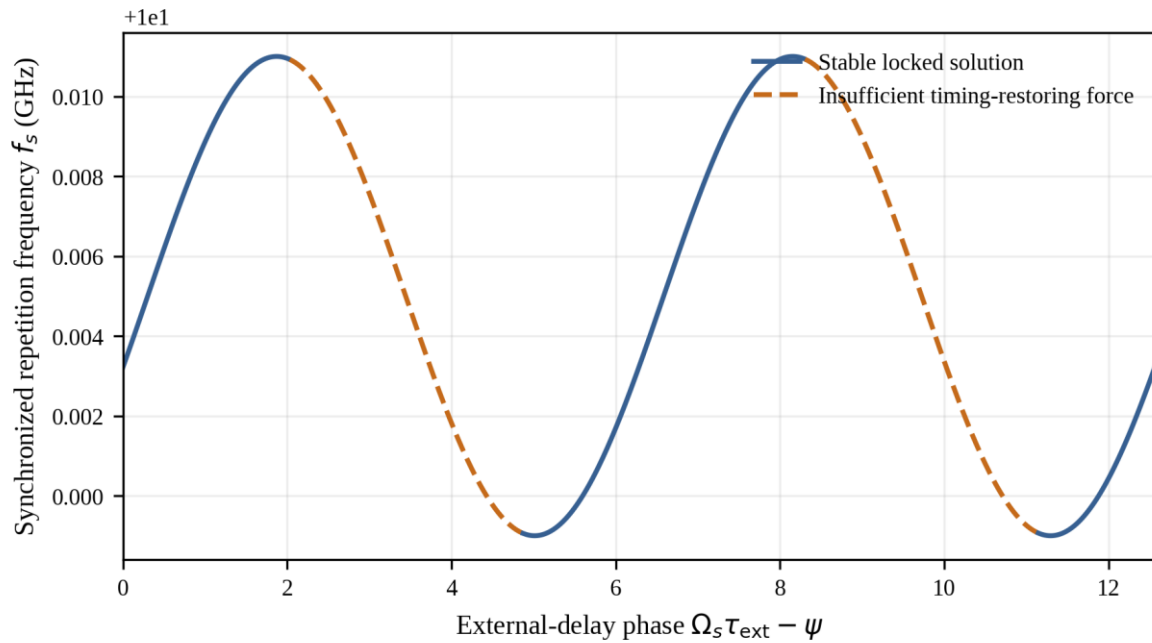


Figure 3. Representative delay-based tuning of the synchronized repetition frequency. The solid branch corresponds to a stable locked solution, while the dashed segments denote delay offsets for which the timing-restoring force is insufficient or has the incorrect sign.

5.4. Timing-jitter reduction

In the unlocked state, spontaneous emission and carrier-induced amplitude-phase coupling produce timing diffusion. In the locked state, the relative timing perturbation ϑ_r obeys the linearized restoring equation

$$\frac{d\vartheta_r}{dt} = -K_r \vartheta_r + \eta_r(t), \quad K_r > 0. \quad (16)$$

The relative timing-phase variance then scales as

$$\text{Var}(\vartheta_r) \approx \frac{D_\vartheta}{K_r}. \quad (17)$$

Thus, stronger positive restoring strength K_r reduces timing fluctuations, while unfavorable delay phases with $K_r \leq 0$ cannot stabilize the locked state.

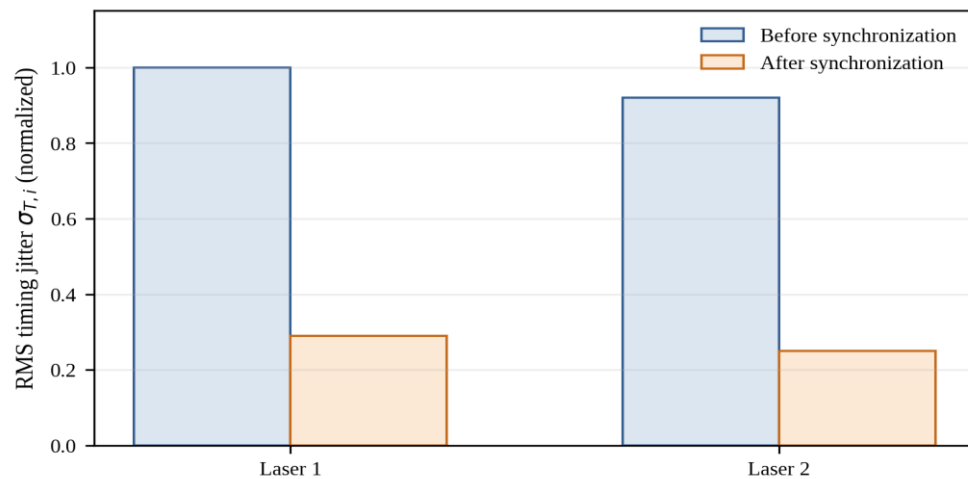


Figure 4. Representative RMS timing jitter $\sigma_{T,i}$ before and after synchronization. These values are intended only as illustrative outputs of the normalized-noise model and must be recalibrated against experimentally measured timing-noise spectra before they can support reliable device-level predictions.

5.5. Mutual optical coherence

Repetition-rate locking constrains the pulse-envelope timing, but it does not by itself enforce stabilization of the optical carrier phase. The condition $|\gamma_{12}|$ approximately equal to 1 requires the optical carrier-frequency mismatch to lie within the coherent injection-locking bandwidth and requires constructive coupling phase. Therefore, the model predicts a hierarchy: repetition-rate locking can occur without strong optical mutual coherence, approximately equal to 1 requires the optical carrier-frequency mismatch to remain within the coherent injection-locking bandwidth and further requires a constructive coupling phase. Accordingly, the model predicts a well-defined hierarchy: repetition-rate locking may be achieved even in the absence of strong optical mutual coherence, whereas high $|\gamma_{12}|$ implies both rate locking and carrier-phase compatibility.

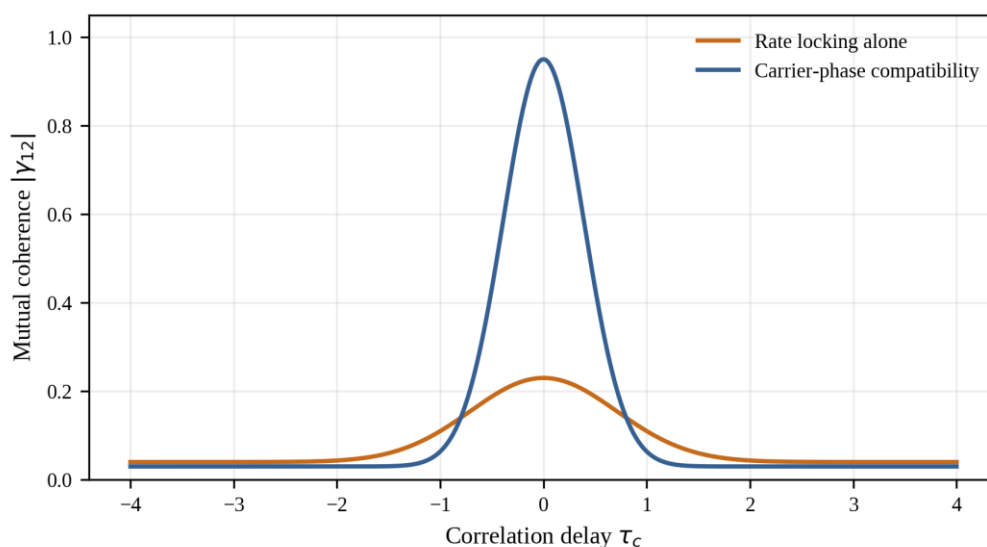


Figure 5. Calculated mutual coherence $|\gamma_{12}|$ for two locked regimes. Rate locking alone gives a small coherence peak, while simultaneous carrier-phase compatibility gives a much stronger peak around the correlation delay τ_c .

6. Comparison with experimental results

The model is evaluated against experimental trends reported for frequency-comb synchronization in QD mode-locked lasers. Both unidirectional synchronization with phase-noise reduction and bidirectional passive synchronization between monolithic InAs/InGaAs QD-PMLLs exhibit the same qualitative hierarchy: repetition-rate locking is robust, delay tuning is experimentally observable, locked states reduce relative timing jitter, and optical phase coherence emerges only when additional carrier-phase conditions are satisfied. The present theory is not intended to serve as a parameter-refitted reproduction of these specific devices; rather, it provides a compact framework that captures the same hierarchy using representative parameters on the relevant experimental scale.

Table 3. Comparison of the theoretical model with QD-PMLL synchronization experiments.

Aspect	Published experiment	Present model	Interpretation
Device scale	Two-section QD-PMLLs; approximately 10 GHz repetition rate; combs near 1260 nm	Baseline uses $f_{r,10} = 9.998$ GHz, $f_{r,20} = 10.012$ GHz, $\lambda_0 = 1260$ nm	Consistent operating scale
Coupling topology	Bidirectional passive coupling through a delayed optical path	κ_{12} , κ_{21} , τ_{12} , τ_{21} , ϕ_{12} , ϕ_{21} included explicitly	Same physical mechanism
Rate locking	Different free-running rates collapse to identical repetition rates	Eq. (9) predicts locking below the delay-weighted coupling bandwidth	Agreement in mechanism
Delay tuning	Common repetition rate can be tuned by external path length	Eq. (10) and Fig. 3 show f_s depends on τ_{ext}	Agreement in trend
Timing jitter	Locked lasers exhibit reduced timing jitter relative to free-running operation	Linearized restoring force gives $\text{Var}(\vartheta_r) \approx D_\vartheta/K_r$	Agreement in sign and mechanism
Mutual coherence	Interferometric fringes appear only under specific conditions	High $ \gamma_{12} $ only with carrier-frequency and phase compatibility	Rate locking does not imply automatic phase locking

6.1. Experimental extensions suggested by the model

First, a systematic scan of the external delay τ_{ext} at fixed optical coupling would allow direct testing of the predicted delay-periodic locking bandwidth and delay-tuned synchronized repetition frequency.

Second, simultaneous measurement of RF linewidth, pulse-arrival-time jitter, and optical mutual coherence would separate envelope synchronization from carrier-phase coherence.

Third, controlled asymmetry between κ_{12} and κ_{21} would test the predicted transition from mutual synchronization to master-slave injection locking.

7. Physical interpretation

Bidirectional optical coupling synchronizes two QD-PMLLs by enforcing mutual pulse-timing correction. A pulse emitted from one laser reaches the other after the external delay, where it perturbs the optical field, gain, and absorber loss within the subsequent pulse-formation window. If these timing corrections are insufficient, the relative timing phase continues to drift because the free-running repetition frequencies remain mismatched. Once the coupling-induced corrections exceed this mismatch, the relative timing phase is driven toward a stable constant offset δ and the two lasers emit pulse trains locked to a common repetition frequency.

The coupling delay serves two distinct functions. First, it sets which earlier pulse from one laser overlaps with the current pulse-formation window of the other laser. Second, it determines the phase factor governing the effective sign and magnitude of the timing correction. Consequently, even small variations in τ_{ext} can shift the synchronization boundary and tune f_s .

Timing-jitter reduction occurs because mutual coupling converts the two lasers from independently diffusing timing oscillators into a jointly stabilized locked system. In the unlocked case, timing fluctuations in each QD-PMLL accumulate independently, leading to relative timing diffusion. In the locked state, however, the delayed optical field provides a restoring action: if a pulse is generated too early relative to the locked timing position, the incoming delayed field from the other laser tends to pull it back; if a pulse is generated too late, the same coupling mechanism tends to advance it. As a result, relative timing fluctuations are suppressed and the root-mean-square timing jitter is reduced. Optical coherence requires an even stricter condition than repetition-rate synchronization, because matching the pulse-envelope timing is not sufficient; the optical carrier phase must also remain stable within the coherent injection-locking bandwidth.

8. Limitations and future work

The reduced model provides a useful framework for interpreting the underlying synchronization mechanisms; however, quantitative prediction for a specific chip requires calibrated gain, absorber, noise, and coupling parameters. Future work should integrate the timing-phase model with measured device transfer functions, optical spectra, RF spectra, and interferometric coherence data. Further refinement may also include separate ground-state and excited-state carrier populations, wetting-layer capture dynamics, polarization effects, thermal drift, and spatial hole burning.

9. Conclusion

A coupled delay-rate and timing-phase theory is developed to describe mutual synchronization between quantum-dot passively mode-locked laser frequency combs. The complex-field formulation captures delayed bidirectional optical coupling between two two-section QD-PMLLs, including the key dynamical contributions of the optical fields, gain recovery, absorber loss, photon decay, coupling phase, and noise. In the weak-coupling regime, the full delay-rate description is reduced to an effective timing-phase model, yielding an explicit repetition-rate locking relation together with a delay-dependent locking condition. The resulting framework predicts the collapse of initially detuned pulse trains into a shared repetition frequency, controlled tuning of the locked frequency through the external delay, suppression of relative timing jitter in the synchronized state, and the emergence of mutual optical coherence only when additional carrier-phase requirements are satisfied. This hierarchy clearly separates repetition-rate synchronization from full optical phase coherence and is consistent with the principal qualitative trends reported in recent QD-PMLL synchronization experiments. The theory therefore provides a compact but physically transparent design framework for analyzing and engineering future chip-scale coupled-comb systems.

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Potential of Propolis for Women's Reproductive Health as The Source of Vitamins and Minerals, Antiemetic, Anti-inflammatory and Analgesic

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Abstract

Women's reproductive health problems, including anemia, emesis gravidarum, and primary dysmenorrhea, are problems that require effective and safe treatment. Conventional therapies often cause side effects, so alternatives based on natural ingredients are needed. Propolis, a natural product from honey bees, contains bioactive compounds with anti-inflammatory, analgesic, antiemetic, vitamins, and minerals that have the potential to be used as complementary therapy. This review aims to evaluate the therapeutic potential of propolis for women's reproductive health problems by synthesizing relevant literature published between 2015 and 2025. The reviewed evidence includes experimental, preclinical, and clinical studies conducted in both human and animal models that explore the effectiveness of propolis in addressing the three conditions. This literature provides the latest review of the potential of propolis as a natural therapy for anemia, emesis gravidarum, and primary dysmenorrhea in women. Existing literature suggests that propolis enhances hemoglobin levels through the content of iron, vitamins, and flavonoids that facilitate the absorption and utilization of iron. Propolis also has an antiemetic effect through compounds that inhibit dopamine and serotonin receptors, and protect the gastric mucosa. This effect is equivalent to antiemetic drugs such as metoclopramide. In addition, propolis shows anti-inflammatory and analgesic effects through inhibition of COX and LOX enzymes and relaxation of uterine smooth muscle, thus effectively reducing menstrual pain. Studies show that propolis contains active compounds such as CAPE, kaempferol, and pinocembrin, which play a role in this mechanism

Keywords: Propolis, Reproductive health, Anemia, Emesis Gravidarum, Primary Dysmenorrhea

1. Introduction

Anaemia, emesis gravidarum, and primary dysmenorrhea represent three prevalent and clinically significant conditions that affect women across different stages of the reproductive life course. These conditions contribute substantially to morbidity, reduced daily productivity, impaired physical performance, and diminished quality of life, and therefore constitute important public health concerns. [1–3]

Based on global data, around 30% of women of childbearing age worldwide suffer from anaemia, with a prevalence ranging from 4.3% to 20% in developed countries and 30% to 48% in developing countries. Children (43%), pregnant women (38%), and non-pregnant women (29%) are among the many vulnerable groups affected by anemia, which is linked to negative consequences such as persistent fatigue, decreased productivity, and an increased risk of pregnancy-related problems.[1,4–6] The main cause of IDA is inadequate iron intake, and the most widely used treatment for this condition is oral iron supplementation.[7] Side effects such as digestive disorders and less than optimal absorption indicate the need to also develop oral iron supplement formulations that are more effective, safe, and easily absorbed by the body.[8]

Emesis gravidarum, or what is often known as nausea and vomiting in pregnancy, is experienced by around 70–80% of pregnant women; 10% continue with symptoms of nausea and vomiting until full term, and even 0.3–2% experience hyperemesis gravidarum, which can cause dehydration and electrolyte disorders and require hospitalization.[9–10] Pregnant women who experience emesis gravidarum will generally receive various forms of therapy from medical personnel, one of which[11] is the administration of vitamin B6 (pyridoxine). Although this therapy is quite effective and safe, recent studies have begun to reveal the potential of complementary therapies based on natural ingredients as a promising alternative[12].

Approximately 50% of adult women and up to 90% of adolescent girls experience dysmenorrhea, and 10–20% of them report that the pain they experience is severe enough to interfere with daily activities.[13–14] Pharmacological management of primary dysmenorrhea is generally with non-hormonal therapies such as acetaminophen, paracetamol, and nonsteroidal anti-inflammatory drugs (NSAIDs)[15] and hormonal therapy. Unfortunately, this therapy often causes side effects, ranging from impaired cognitive function to the risk of drug dependence.[16–17] These limitations highlight the need to explore safer complementary approaches, including therapies derived from natural products with lower adverse-effect profiles.

1.1 Background

In recent decades, natural materials have been proposed for the development of future drugs due to their broad therapeutic effects, rare side effects, and low toxicity.[18–19] Propolis has emerged as a natural product of interest for reproductive health-related conditions.[20]

Propolis is a naturally occurring resinous substance collected by honeybees from plant exudates and is characterized by a complex chemical composition.[20] Traditionally, propolis has been used for health maintenance and the management of various conditions, supported by evidence indicating anti-inflammatory, analgesic, and antiemetic activities. [21–23]

Propolis contains a wide range of bioactive compounds, including polyphenols and flavonoids, as well as essential minerals and vitamins that contribute to its biological properties. It has been reported to provide minerals such as iron, calcium, and magnesium, along with vitamins of the B complex, vitamin C, vitamin E, and provitamin A, which are relevant to physiological and metabolic functions [24–28] Evidence from experimental and preclinical studies indicates that propolis exhibits anti-inflammatory and analgesic activities, primarily demonstrated in in

vivo and laboratory models.[21,29–30] These biological effects have been associated with the modulation of inflammatory responses and pain-related processes, although most supporting evidence remains preclinical in nature.[31] Propolis has also demonstrated antiemetic activity in experimental models, which has been attributed to the presence of multiple bioactive constituents.[22]

1.2 Objectives

Although propolis has been widely used and investigated for various health-related applications, focused reviews examining its relevance to specific women's reproductive health conditions remain limited. Therefore, this review aims to summarize and critically discuss available evidence regarding the potential role of propolis and its chemical constituents in relation to anaemia, emesis gravidarum, and primary dysmenorrhea. Relevant literature published between 2015 and 2025 was identified from multiple scientific sources and synthesized using a narrative review approach. The discussion is organized into thematic sections reflecting the reported effects of propolis on selected reproductive health conditions and includes evidence derived from both human and animal studies. An overview of the included studies and their main findings is presented in Table 1.

2. Related Work

Propolis has been widely researched for its putative therapeutic applications in women's reproductive health. Clinical investigation has demonstrated that providing 500 mg of bee propolis twice daily to women with endometriosis-related infertility resulted in a 60% conception rate compared to 20% in the QuillBot placebo group [32]. Furthermore, propolis possesses antibacterial, anti-inflammatory, anesthetic, and immunostimulatory qualities; additionally, its flavonoid content has anti-allergic, immunomodulatory, anti-cancer, anti-fungal, and anti-hyperlipidemia capabilities [33]. Propolis contains vital vitamins like B1, B2, B6, C, and E, as well as minerals like magnesium, calcium, potassium, sodium, copper, zinc, manganese, and iron. SciELO Brazil [20]. Numerous preclinical investigations have shown ample evidence of propolis's analgesic and anti-inflammatory qualities. Propolis ethanol extract possesses analgesic properties and shows dose-dependent anti-inflammatory effects in models of carrageenan-induced paw edema, Freund's adjuvant-induced arthritis, and foreign body-induced granuloma [34]. A standardized ethanol extract of Bulgarian propolis reduces acetic acid-induced stomach contractions and causes considerable pain relief in rats at relatively modest doses (Springer) [23]. These anti-inflammatory effects are generally related to inhibition of cyclooxygenase and the resultant prostaglandin, as well as the free radical scavenging activity of Hindwi [35]. Although more research is required to determine the safety and effectiveness of propolis specifically as an antiemetic agent during pregnancy, propolis samples from Indonesia have been tested for their high antiemetic activity and potential use in the treatment of nausea and vomiting during pregnancy [22].

3. Methodology

3.1 Study Design

This study was conducted employed a systematic literature review approach following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines to aimed at synthesizing current evidence on the potential role of propolis in women's reproductive health, particularly anemia, emesis gravidarum,

and primary dysmenorrhea. The review focuses on the nutritional properties of propolis, including its vitamin and mineral content, as well as its reported antiemetic, anti-inflammatory, and analgesic effects. This approach was chosen to allow a descriptive and thematic exploration of findings derived from diverse study designs, including experimental, animal, and human studies

3.2 Search Strategy

To identify studies relevant to the narrative synthesis, a literature search was conducted. The databases searched included: PubMed/MEDLINE (via NLM interface), Scopus (via Elsevier), Web of Science (via Clarivate Analytics), ScienceDirect (via Elsevier), Google Scholar

The search strategy was developed using a combination of Medical Subject Headings (MeSH) terms and free-text keywords related to the main concepts of the review. The key search terms included: Concept 1 (Propolis): "propolis" OR "bee propolis" OR "bee glue" OR "propolis extract", Concept 2 (Women's Reproductive Health): "women's health" OR "reproductive health" OR "female reproductive" OR "gynecological" OR "pregnancy" OR "endometriosis" OR "PCOS" OR "polycystic ovary syndrome" OR "infertility" OR "ovarian health" OR "menstrual", Concept 3 (Properties): "vitamins" OR "minerals" OR "nutrients" OR "antiemetic" OR "anti-nausea" OR "anti-inflammatory" OR "analgesic" OR "pain relief" OR "antioxidant",

Boolean operators (AND, OR) were applied as appropriate, and search strings were adapted to the syntax and indexing systems of each database. The search covered articles published between 2015 and 2025.

3.3 Eligibility Criteria

Studies included in this review based on the following criteria. (1) examined propolis or its bioactive compounds; (2) focused on women's reproductive health conditions, specifically anemia, emesis gravidarum, or primary dysmenorrhea; (3) original research articles or relevant review studies; (4) published in the English language; and (5) available in full-text form. Studies that did not align with the research objectives or lacked sufficient methodological or outcome-related information were excluded.

3.4 Study Selection Process

The selection of studies was conducted through a relevance-based screening process appropriate for a narrative review. Retrieved records were screened based on titles and abstracts to identify potentially relevant studies. Full-text articles were then assessed to confirm their relevance to the scope and objectives of the review.

Duplicate records were removed using reference management software. The selection process emphasized conceptual relevance and contribution to thematic synthesis rather than formal risk-of-bias scoring.

3.5 Data Extraction

Relevant information from the included studies was extracted descriptively to support the thematic analysis. The extracted data encompassed authorship, year of publication, and study location, as well as study design and population characteristics, including human, animal, or experimental models. Information regarding the characteristics of propolis, such as its origin, preparation method, and type of extract, was documented when available. Reported outcomes related to nutritional content and antiemetic, anti-inflammatory, and analgesic effects were also collected, along with key findings relevant to women's reproductive health. In addition, data on safety profiles and reported adverse effects were noted where applicable. The data extraction process was intended to facilitate qualitative comparison across studies.

3.6 Data Synthesis

Data synthesis was carried out utilizing a narrative and thematic approach because to the significant heterogeneity between study designs, study populations, interventions, and outcome measures. The synthesis framework was predefined based on the key biological and clinical properties of propolis reported in the literature, including its nutritional composition (vitamins and minerals) and its antiemetic, anti-inflammatory, and analgesic effects. Evidence within each thematic category was synthesized descriptively to allow for structured comparison across studies, with explicit consideration of consistency and divergence in reported outcomes as well as methodological limitations. Findings from animal studies and mechanistic investigations were analyzed separately from those derived from human clinical studies to maintain interpretative clarity.

4. Results

4.1 Chemical Constituents of Propolis

Propolis is a natural resinous product characterized by a highly complex chemical composition. It is mainly composed of resins and vegetable balms (45–55%), along with wax (8–35%), essential oils (5–10%), fatty acids (approximately 5%), pollen (5%), and other minor organic constituents. The color of propolis varies from greenish-yellow to dark brown, a variation influenced by differences in botanical sources and geographic origin, which in turn affect its chemical profile and biological potency [36–38].

The therapeutic properties of propolis are closely linked to its rich content of bioactive compounds, particularly phenolic compounds and flavonoids [39]. Findings show that, out of 16 compounds present at concentrations exceeding 1%, 11 demonstrated antiemetic activity, indicating a broad and cooperative pharmacological mechanism [22]. In addition to its bioactive compounds, propolis contains essential vitamins such as vitamins A, B1, B2, B6, C, and E, which play crucial roles in erythrocyte formation and the maintenance of normal physiological functions. These vitamins are vital for supporting red blood cell production and overall health [40].

Table 1. Summary of potential effect of propolis for women's reproductive health problems

No	Author (Year)	Study Design	Population	Health Outcome	Evidence Type	Key Findings	Notes
1	Rohmatin et al. (2022)	Quasi-experimental study	44 adolescent girls	Anaemia	Human (clinical)	Propolis supplementation significantly increased hemoglobin levels (mean increase: 2.873 g/dL), exceeding the increase observed with conventional iron supplementation.	Effect attributed to minerals and vitamins (Fe, Zn, vitamins B and C) enhancing erythropoiesis and iron absorption.

No	Author (Year)	Study Design	Population	Health Outcome	Evidence Type	Key Findings	Notes
2	Touzani et al. (2022)	Experimental in vivo study	360 streptozotocin-treated fish	Anaemia (functional)	Animal	Propolis, particularly when combined with honey, significantly improved hemoglobin levels by reducing oxidative stress and protecting erythrocyte membranes under hyperglycemic conditions.	Demonstrates antioxidant-mediated hematological protection; indirect relevance to human anaemia.
3	Fikri et al. (2018)	Experimental antiemetic assay	32 young chicks injected with CuSO ₄	Emesis	Animal	Propolis extracts inhibited retching by 44.96–98.51%, showing strong antiemetic activity through modulation of dopamine, 5-HT ₃ , 5-HT ₁ , and NK ₁ receptors.	Widely used preclinical emesis model; mechanistic relevance to nausea in pregnancy.
4	Mahani et al. (2021)	Clinical intervention study	50 tuberculosis patients (12–45 years)	Nausea and vomiting	Human (indirect)	Propolis supplementation significantly reduced nausea prevalence by inhibiting emetic triggers, inducing protective enzymes, and exerting hepatoprotective effects.	Indirect evidence; population not pregnant, but mechanisms relevant to emesis gravidarum.
5	Jenabi et al. (2019)	Randomized controlled trial	86 female students	Primary dysmenorrhea	Human (clinical)	Bee propolis significantly reduced menstrual pain severity compared to	Direct clinical evidence supporting analgesic and anti-

No	Author (Year)	Study Design	Population	Health Outcome	Evidence Type	Key Findings	Notes
						placebo, as measured by visual analog scale scores.	inflammatory effects.
6	Al-Hariri & Abualait (2020)	Experimental in vivo study	18 rats	Pain and inflammation	Animal	Alcoholic extract of Brazilian green propolis significantly reduced nociceptive responses and inflammatory pain induced by formalin and acetic acid.	Supports antinociceptive mechanism relevant to dysmenorrhea.
7	Sokeng et al. (2020b)	Experimental in vivo study	100 Wistar albino rats	Inflammation and pain	Animal	Arachic acid ethyl ester (AAEE) isolated from propolis inhibited acute and chronic inflammation, reduced edema and granuloma formation, and showed dose-dependent analgesic activity.	Mechanistic evidence for anti-inflammatory and analgesic pathways.
8	Sahlan et al. (2019)	Experimental in vivo study with LC-MS/MS analysis	53 male Sprague Dawley rats	Inflammation	Animal	Indonesian Tetragonula propolis contained multiple anti-inflammatory biomarkers and significantly reduced carrageenan-induced paw inflammation via NF- κ B inhibition.	Mechanistic support for inflammation-related menstrual pain modulation.

4.2 Potential of Propolis for Anaemia

Anaemia remains a major public health concern, particularly among women and adolescents, due to its association with impaired cognitive performance, increased maternal and perinatal morbidity, and reduced work productivity. The etiology of anaemia is multifactorial, with iron deficiency related to menstruation, nutritional inadequacy, chronic disease, and impaired iron metabolism being the most common causes.[41] Oral iron supplementation is the primary intervention for iron deficiency anaemia (IDA), [1] by vitamin C which enhances iron absorption.

Evidence from a quasi-experimental human study demonstrated that propolis supplementation significantly increased hemoglobin levels in adolescent girls with anaemia, showing a greater mean increase compared to conventional iron supplementation.[40] This effect is attributed to the rich micronutrient composition of propolis, including iron, zinc, folic acid, and vitamins B and C, which support erythropoiesis and enhance iron absorption. In addition, flavonoids present in propolis may facilitate hemoglobin synthesis through improved iron bioavailability. Propolis also benefits conditions like hyperglycemia, which damages red blood cells and can lead to functional anaemia due to impaired erythrocyte production and iron metabolism.[42–43]

Supporting evidence from animal studies further suggests that propolis may alleviate functional anaemia associated with metabolic disorders. In streptozotocin-induced hyperglycemic models, propolis administration improved hemoglobin levels by reducing oxidative stress and protecting erythrocyte membranes, thereby supporting normal red blood cell function and iron metabolism [44]. These findings indicate that propolis may serve as a complementary intervention for anaemia by enhancing dietary iron utilization, supporting hemoglobin synthesis, and protecting erythrocytes from oxidative damage.

4.3 Emesis Gravidarum

Emesis gravidarum, or nausea and vomiting during pregnancy, is a common issue linked to gastrointestinal, hormonal, or genetic factors and can lead to serious health risks, including depression and preeclampsia [9]. Preclinical and indirect clinical evidence suggests that propolis possesses significant antiemetic activity. Experimental studies using established animal models demonstrated that propolis extracts markedly reduced emetic responses, likely through modulation of neurotransmitter pathways involved in nausea and vomiting, including serotonergic and dopaminergic receptors. In addition, indirect human evidence from clinical populations reported that propolis supplementation reduced the prevalence and severity of nausea, potentially through hepatoprotective effects and inhibition of nausea-inducing stimuli. [22,45] Several studies have reported the antiemetic potential of propolis, which is largely attributed to its bioactive compounds, particularly flavonoids and phenolic substances. These compounds have demonstrated antioxidant, gastroprotective, and hepatoprotective effects in preclinical and experimental models, suggesting a role in reducing gastric irritation and metabolic stress associated with nausea and vomiting.[46–48] Most of the current evidence comes from animal studies or non-pregnant human populations, while direct clinical data in pregnant women remain very limited. As a result, the applicability of these findings to managing emesis gravidarum in pregnancy is still not well established.

4.4 Potential of Propolis for Primary Dysmenorrhea

Propolis has demonstrated significant anti-inflammatory effects that may alleviate menstrual pain associated with primary dysmenorrhea. Clinical evidence from a randomized controlled trial demonstrated that propolis supplementation significantly reduced pain severity in women with primary dysmenorrhea compared to placebo, indicating its potential as an effective natural analgesic.[49–50] These findings are supported by experimental

animal studies showing that propolis exhibits strong anti-inflammatory and antinociceptive effects across various pain models. [51] Studies also have highlighted the analgesic potential of propolis, which is largely linked to its bioactive components, including isoflavonoids and phenolic acids. These compounds have been shown in experimental models to inhibit inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), leading to reduced prostaglandin production. In addition, specific constituents, such as arachidic acid ethyl ester (AAEE), demonstrated both acute and chronic anti-inflammatory effects as well as central analgesic activity in vivo. Propolis also exhibits antispasmodic properties that may facilitate uterine muscle relaxation, further supporting its potential to relieve menstrual pain. [52] Overall, these results confirm the traditional usage of propolis and emphasize its potential as a supplemental treatment approach by suggesting that it may affect inflammatory and pain-related pathways involved in primary dysmenorrhea.

5. Discussion

5.1 Chemical Constituents of Propolis

The components of propolis vary in quantity and include: 45–55% resins and vegetable balms (esters, phenolic acids, and flavonoids); 8–35% wax (mostly beeswax); 5–10% aromatic and essential oils (pinene, viridiflorol, tricosane, and eudesmo); 5% fatty acids (usually from wax, with the remaining amount depending on botanical origins); 5% pollen; and 5% other organic components (sugars, quinones, lactones, ketones, steroids, and microelements (Fe and Zn)).[36–37] Its color ranges from greenish-yellow to brown, reflecting differences in plant sources and maturity.[38]

A wide range of bioactive compounds have been identified in propolis, particularly flavonoids (e.g., quercetin, kaempferol, galangin, pinocembrin), phenolic acids (e.g., caffeic acid, p-coumaric acid, ferulic acid), and esters such as caffeic acid phenethyl ester (CAPE) [39,53]. These constituents are closely associated with the biological activities of propolis, including antioxidant, anti-inflammatory, antiemetic, and hematopoietic effects, which are relevant to women's reproductive health conditions.[39,53]

Propolis also composed of a variety of bioactive compounds, vitamins, and minerals, which contribute to its pharmacological properties. In addition to flavonoids and phenolic acids, propolis contains vitamins A, B1, B2, B6, C, and E, as well as folic acid, which are important for erythrocyte formation and overall hematological health.[40] In propolis, there are a total of 16 constituents with concentrations exceeding 1%, culminating in an aggregate concentration of 84.46%. The role of these constituents has been described, especially their antiemetic properties. Of the 16 constituents with concentrations greater than 1%, 11 constituents showed antiemetic activity, either directly or indirectly; one constituent showed biological activity different from the antiemetic effect; and three constituents had no biological activity whatsoever. Compounds that showed antiemetic effects included (1) Methyl- α -D-glucopyranoside, (2) 5-Azulenemethanol, 1,2,3,3a, 4,5,6,7 octahydro- α , α , 3,8-tetramethyl, (3) 1,2,3-Propanetriol, (4) Hexadecanoic acid, (5) 3-Hydroxy-Tridecanoic acid ethyl ester, (6) 2,6-Dimethoxyphenol, (7) Pentadecanoic acid, 14-methyl-, methyl ester, (8) 1,6-Anhydro-Beta-D-Glucopyranose, (9) (2S, 3R) -3-Allyl-3-methylapfelsaure-4-ethyl ester, (10) 1,4,5-Trimethylnaphthalene, (11) Tetradecanoic acid. Compounds that show biological activity include isosorbide. In contrast, three compounds have not shown any biological activity, namely: (1) 2-Methoxyethyl acetate, (2) 8-Oxabicyclo [5.1.0] octane, and (3) Dimethyl 2-hydroxy-2-methylbutane-1,4-dioate.[48] Propolis exerts its antiemetic effects through various mechanisms.

Hydroxycinnamic acids found in propolis function as δ receptor antagonists (enkephalinergic) and inhibit dopamine receptors. In addition, propolis contains triterpenes, which act as antagonists for 5-HT₃, 5-HT₄, and/or NK1 receptors. These receptors are involved in the stimulation of emesis.[22]

Several flavonoids, cinnamic acid derivatives, phenolic acids, and caffeic acid have been associated with anti-inflammatory and antinociceptive effects in preclinical studies. For instance, alcoholic extracts of propolis have reduced pain responses in experimental models, as indicated by decreased frequency of acetic acid-induced abdominal contractions and hind limb stretching. These effects are likely mediated through modulation of inflammatory mediators and peripheral nociceptive signaling, although detailed mechanisms vary among specific compounds. Most mechanistic evidence regarding these constituents is derived from preclinical and experimental studies, emphasizing the need for further investigation in human clinical settings.[51]

5.2 Propolis for Anemia

Anaemia remains a significant public health concern, particularly among women, due to its adverse impact on cognitive development, maternal and perinatal outcomes, and overall physical productivity.[41] Iron deficiency anaemia (IDA) is the most prevalent form, often resulting from menstrual iron loss, inadequate dietary intake, and deficiencies in vitamin B12 and folate [40,54]. Early identification of iron deficiency and timely intervention are essential to prevent progression to IDA [1].

Oral iron supplementation is the conventional treatment for IDA; however, limitations such as suboptimal absorption and gastrointestinal side effects have motivated the exploration of complementary natural therapies [7–8]. Propolis, a resinous substance produced by bees, has been investigated for its potential to support hemoglobin regeneration through enhanced utilization of dietary iron and overall blood health [42–43]. Limited clinical and experimental studies suggest that propolis supplementation may increase hemoglobin levels more effectively than conventional iron therapy, with mean increases reported at 2.87 g/dL versus 1.39 g/dL in control groups [40].

The hematological benefits of propolis are linked to its rich composition of bioactive compounds, vitamins, and minerals. Propolis contains iron, zinc, and vitamins B1, B6, B12, C, A, and E, which are critical for erythropoiesis and hemoglobin synthesis [40,55]. Analysis of propolis has identified phenolic and flavonoid compounds such as pinobanksin-3-o-acetate, pinocembrin, caffeic acid phenylethyl ester (CAPE), kaempferol, and chrysin, as well as minerals including calcium, magnesium, and sodium, contributing to its overall nutritional value [44]. These constituents support erythrocyte integrity and may improve hemoglobin levels by maintaining red blood cell quality.

Experimental evidence further indicates that propolis may mitigate factors that compromise hemoglobin synthesis, such as oxidative stress and hyperglycemia-induced erythrocyte damage, as observed in streptozotocin-induced rat models [44,56]. Propolis has also been associated with indirect support of erythropoietin (EPO) production, which promotes erythrocyte formation, thereby aiding in hemoglobin regeneration [57].

These findings highlight propolis as a promising complementary agent for improving hemoglobin levels and managing anaemia. However, further rigorously designed human studies with standardized dosing are needed to establish its effectiveness and safety.

5.3 Propolis for Emesis Gravidarum

Emesis gravidarum, often known as nausea and vomiting in pregnancy (NVP), is a common problem in early pregnancy with an unknown origin that may be related to gastrointestinal, hormonal, or genetic factors.[9] Although NVP is often considered a normal pregnancy discomfort, it is associated with a higher risk of major health problems such as depression, preeclampsia, and arterial hypertension.[58–59] Conventional antiemetic drugs are limited in pregnancy due to potential teratogenic effects, prompting interest in natural remedies including propolis.[60–61]

Propolis, a resinous substance collected by bees, contains bioactive compounds such as flavonoids and phenolics with antibacterial, antioxidant, and antiemetic properties.[22,45] Experimental studies have demonstrated significant antiemetic activity. For example, Indonesian propolis reduced nausea in chicks administered CuSO₄, showing effects comparable to the antiemetic drug metoclopramide, with inhibition rates ranging from 44.96% to 98.51%.[22,62]

Propolis exerts its antiemetic effects through several mechanisms, including:

- 1) Direct Inhibition of 5-HT₄ Stimulus

Propolis contains compounds that directly inhibit 5-HT₄ stimulation, which is known to trigger nausea and vomiting. Terpenoids and flavonoids, the main components of propolis, contribute to this direct antiemetic activity. Specifically, 5-Azulenemethanol (a terpenoid) and Methyl- α -D-glucopyranoside (a flavonoid) have been identified to have antiemetic activity through this mechanism.[48]

- 2) Induction of Glycerol Kinase Enzyme

Propolis indirectly reduces nausea by inducing the formation of the enzyme glycerol kinase in the body.[48] Glycerol, which is present in propolis, plays a role in the metabolic pathway of gluconeogenesis in the liver. Glycerol kinase enzyme deficiency can cause nausea and vomiting symptoms, so the ability of propolis to induce this enzyme helps prevent these symptoms.[48,63]

- 3) Hepatoprotective Activity

Propolis acts as a hepatoprotector, protecting the liver from damage caused by toxic compounds, especially those from ATD.(Sulaeman, Fikri, et al., 2018) ATD can cause liver injury, which causes nausea and vomiting due to impaired detoxification.[64] By maintaining liver function, propolis helps minimize vomiting symptoms caused by liver toxicity.[65] Compounds with antioxidant and anti-inflammatory activities, such as 2,6-dimethoxyphenol and various fatty acids, contribute to this hepatoprotective effect.[48,66]

Propolis from *Wallacetrigona incisa* in South Sulawesi, Indonesia, has shown antiemetic activity in clinical contexts, such as reducing nausea in patients receiving antituberculosis drugs (ATD). Phytochemical analysis identified 11 compounds at concentrations above 1% that contribute to antiemetic effects either directly or indirectly, with a total concentration of 75.47% in propolis. [22]

These findings indicate that propolis may serve as a complementary option for the management of nausea and vomiting, acting through mechanisms such as receptor modulation, metabolic support, and organ protection. Thus, direct evidence from studies involving pregnant women with emesis gravidarum remains limited. Further clinical trials are therefore required to determine its safety, effectiveness, and appropriate dosage.

5.4 Propolis for Primary Dysmenorrhea

Propolis is known for its anti-inflammatory, analgesic, and antispasmodic properties, which make it a potential natural agent for managing primary dysmenorrhea. The main cause of menstrual pain is the excessive production of endometrial prostaglandins, particularly $\text{PGF2}\alpha$, during the late luteal phase due to decreased progesterone. These prostaglandins induce uterine smooth muscle contractions, vasoconstriction of spiral arteries, tissue ischemia, and hypoxia, which activate sensory nerves and cause pain. [49–50] Several studies have shown that propolis can modulate this pathway:

1) Anti-inflammatory effects

Flavonoids and phenolic compounds in propolis, such as quercetin, galangin, CAPE, and arachidic acid ethyl ester (AAEE), inhibit key enzymes including cyclooxygenase (COX) and lipoxygenase (LOX), reducing prostaglandin and leukotriene synthesis. Propolis also reduces pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and other mediators involved in nociception (PGE2, nitrite), effectively decreasing local inflammation and pain perception. [52,67–69]

2) Analgesic effects

Propolis demonstrates both central and peripheral analgesic properties. Its anti-nociceptive action has been observed in animal studies, reducing formalin-induced and acetic acid-induced pain, indicating effectiveness in both immediate and prolonged pain phases. Some bioactive compounds in propolis, including AAEE, interfere with inflammatory cascades and modulate neurotransmitters such as serotonin and kinins, contributing to pain relief. [51–52]

3) Antispasmodic activity

Propolis relaxes uterine smooth muscles, reducing cramps associated with dysmenorrhea. Compounds such as p-coumaric acid derivatives regulate calcium channels, decreasing Ca^{2+} influx necessary for muscle contraction. This antispasmodic effect has been supported by studies on smooth muscle tissues in animals. [23,50,69–70]

4) Molecular mechanisms via NF- κ B inhibition

Several compounds identified in propolis (e.g., dehydrogingerdione, alpha-tocopherol succinate, adhyperforin, 6-epiangustifolin, deoxypodophyllotoxin, kurarinone, xanthoxyletin) inhibit NF- κ B, a key transcription factor in inflammatory signaling. By downregulating NF- κ B, propolis reduces the expression of iNOS, COX-2, and TNF- α , further contributing to decreased pain and inflammation. [29]

Clinical studies suggest that propolis reduces menstrual pain scores significantly compared to placebo, supporting its traditional use for dysmenorrhea. [50,69] However, direct comparative studies between propolis and conventional therapies are limited. Further well-controlled studies are required to determine its efficacy relative to standard treatments and to optimize dosing regimens.

6. Conclusions

Propolis's antifungal mechanisms—disruption of cell structure and function, inhibition of biofilm formation and morphogenesis, and robust clinical and in vivo activity—highlight its potential as a versatile therapeutic agent. Its bioactive compounds, including phenolic acids, flavonoids, and benzophenones, target fungal membranes, biofilms, and virulence factors with high specificity and efficacy. The ability to synergize with conventional antifungals and enhance immune responses further amplifies its therapeutic value. From Brazilian red and green

propolis to varieties from Brunei, Bulgaria, and beyond, the global diversity of propolis underscores its adaptability and potency. These findings pave the way for developing novel propolis-based therapies, such as nanoemulsions, hydrogels, and PDT, to address the growing challenge of antifungal resistance and improve clinical outcomes in fungal infections

The current body of research supports propolis as a promising complementary therapeutic agent for women's reproductive health. Its rich content of vitamins, minerals, and bioactive compounds, combined with demonstrated anti-inflammatory and analgesic properties. However, the antiemetic properties specifically require further clinical investigation. Future research should focus on standardizing propolis preparations, determining optimal dosing regimens, conducting large-scale clinical trials, and establishing comprehensive safety profiles for use during pregnancy and reproductive years.

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8. Nomenclature

- AAEE: Arachic Acid Ethyl Ester
- AGEs: Advanced Glycation End-products
- CAPE: Caffeic Acid Phenylethyl Ester
- COX: Cyclooxygenase
- CP/CPPS: Chronic Prostatitis/Chronic Pelvic Pain Syndrome
- EPO: Erythropoietin
- Hb: Hemoglobin
- HbA1c: Glycated Hemoglobin
- ID: Iron Deficiency
- IDA: Iron Deficiency Anemia
- IL: Interleukin
- LOX: Lipoxygenase
- NF-kB: Nuclear Factor kappa B
- NSAIDs: Nonsteroidal Anti-Inflammatory Drugs
- NVP: Nausea and Vomiting in Pregnancy
- OAT/ATD: Antituberculosis Drugs
- RBC: Red Blood Cell
- ROS: Reactive Oxygen Species
- STR: Streptozotocin
- TB: Tuberculosis
- TNF: Tumor Necrosis Factor
- 5-HT: 5-Hydroxytryptamine (Serotonin)

Chemical Compounds:PGF2 α : Prostaglandin F2-alpha

PGE2: Prostaglandin E2

Ca²⁺: Calcium ionsCuSO₄: Copper Sulfate**Units:**

g/dl: grams per deciliter

mg/kg: milligrams per kilogram

mg GAE/100g: milligrams Gallic Acid Equivalent per 100 grams

mg QE/100g: milligrams Quercetin Equivalent per 100 grams

9. Declarations

Conflict of interest/Competing interests

The authors confirm that there are no conflicts of interest associated with this publication.

Author(s) Contribution

ER designed the study, conducted the primary literature search, and drafted the manuscript. MSM contributed to data analysis, visual presentation, and technical editing. MS supervised the interpretation of phytochemical data and provided scientific consultation. HU evaluated clinical relevance and supported methodology development. W revised the discussion section and improved the overall structure of the paper. HH coordinated all project stages, reviewed the final content, and approved the final manuscript. All listed authors have reviewed and approved the publication of this article.

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