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ORIGINAL ARTICLE

Improving camphor solubility and stability through α-cyclodextrin encapsulation: characterization, cytotoxicity investigation, and bull sperm preservation evaluation

Hayet Ahlem Lezrag[1,2,3] · Sofiane Fatmi[1,2,4] · Lamia Taouzinet[1,5] · Zahra Toutou[1,2,5,6] · Katarzyna Pokajewicz[3] · Farid Ait Merzeg[7] · Mohamed Skiba[4] · Piotr P. Wieczorek[3] · Mokrane Iguerouada[1]

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Abstract

Camphor, a crystalline white substance with diverse biological benefits, faces limitations in human health applications due to its volatile and hydrophobic nature, which impedes its solubility and stability. This study addresses these challenges by investigating the encapsulation of camphor within various cyclodextrins (CDs), particularly focusing on α-cyclodextrin (α-CD), to enhance its solubility and protect it from environmental degradation. The formation of camphor-CD inclusion complexes was confirmed through a combination of infrared spectroscopy (FTIR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). Among the CDs tested, α-CD demonstrated the highest efficiency, achieving a solubility coefficient of 23.38 M^{-1} and inclusion efficiencies of 24.37% and 34.63% for 1:1 and 1:2 stoichiometries, respectively. Biological evaluations revealed that the Camphor/α-CD complex exhibited significant preservative effects on sperm motility at low concentrations while demonstrating a toxic effect on red blood cells. Additionally, the complex showed promising radical scavenging activity as assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. These findings suggest that camphor, when complexed with α-CD, holds considerable potential for novel applications in drug delivery and cryopreservation technologies.

Keywords Camphor · Encapsulation · Cyclodextrins · Solubility · Biological properties · Cytotoxicity · Sperm cryopreservation

✉ Sofiane Fatmi
sofiane.fatmi@univ-bejaia.dz

Hayet Ahlem Lezrag
hayetahlem.lezrag@univ-bejaia.dz

Lamia Taouzinet
lamia.taouzinet@crtaa.univ-bejaia.dz

Zahra Toutou
zahra.toutou@univ-bejaia.dz

Katarzyna Pokajewicz
katarzyna.pokajewicz@uni.opole.pl

Farid Ait Merzeg
farid.aitmerzeg@univ-bejaia.dz

Mohamed Skiba
mohamed.skiba@univ-rouen.fr

Piotr P. Wieczorek
Piotr.Wieczorek@uni.opole.pl

[1] Université de Bejaia, Faculty of Nature and Life Sciences, Associated Laboratory in Marine Ecosystems and Aquaculture, 06000 Bejaia, Algeria

[2] Université de Bejaia, Faculty of Technology, Technology Pharmaceutical Laboratory, 06000 Bejaia, Algeria

[3] Institute of Chemistry, University of Opole, Opole 45-052, Poland

[4] Technology Pharmaceutical and Bio Pharmaceutics Laboratory, UFR Medicine and Pharmacy, Rouen University, 22 Blvd. Gambetta, Rouen 76183, France

[5] Centre de Recherche en Technologies Agro-Alimentaires. Route de Targa Ouzemmour, Campus Universitaire, Béjaïa 06000, Algérie

[6] Université de Bejaia, Faculty of Technology, LMPA, 06000 Bejaia, Algeria

[7] Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques (CRAPC), BP 384 Bou- Ismail, Tipaza, RP 42004, Algeria

Introduction

Essential oils (EOs), complex mixtures of volatile compounds derived from plant metabolism, have a long history of medicinal use, particularly in treating respiratory ailments. Their applications have expanded to encompass food preservation, cosmetics, agriculture, and pharmaceuticals. However, their hydrophobic nature and volatility often limit their bioavailability and stability. Camphor, a cyclic terpene with recognized pharmacological properties, exemplifies these challenges. Despite its therapeutic potential, its poor water solubility restricts its development as a drug molecule [1, 2].

Cyclodextrins (CDs), cyclic oligosaccharides, offer a promising strategy to enhance the solubility and stability of hydrophobic compounds. By forming inclusion complexes, CDs can encapsulate guest molecules within their hydrophobic cavity, improving their physicochemical properties [3]. While CDs have been widely explored for drug delivery, their application to camphor remains relatively limited. Previous studies have primarily focused on the characterization of camphor-cyclodextrin complexes or the development of cyclodextrin-based formulations, with limited attention to the biological impact of camphor encapsulation [4–6]. Few studies have investigated the encapsulation of camphor within cyclodextrins, particularly regarding the improvement of its chemical and biological properties. Kokkinou et al. [7], characterized the inclusion complexes of (R)- and (S)-camphor in α-cyclodextrin using X-ray crystallography and molecular dynamics simulations, revealing a specific organization. Celebioglu et al. [8] and Yu et al. [9] studied the electrospinning of nanofibers based on cyclodextrin-camphor inclusion complexes, highlighting improvements in solubility, thermal stability, and controlled release of camphor.

Oxidative stress induced by reactive oxygen species (ROS) is a well-researched factor in sperm dysfunction during cryopreservation. To mitigate these adverse effects, natural cryoprotective agents - in particular essential oils and their derivatives - have emerged as promising therapeutic candidates, and reduces oxidative stress by trapping oxidised lipids (indirect antioxidant effect). In recent years, Benbarkane et al. [10] have notably demonstrated that hydroxypropyl-β-cyclodextrin (HP-βCD) significantly improves sperm motility after thawing by mitigating cryopreservation-induced cell damage. This finding highlights the potential of cyclodextrin-based formulations in protecting spermatozoa from oxidative stress during low-temperature storage [10]. However, to the best of our knowledge, no research has yet explored the effects of camphor encapsulated in α-cyclodextrin on its solubility and toxicity in cellular models such as spermatozoa and red blood cells, particularly regarding its potential use as a cryoprotectant. This study aims to fill this gap by investigating the encapsulation of camphor within α-cyclodextrin and assessing its impact on solubility, stability, cytotoxicity, and effectiveness in sperm cryopreservation. We hypothesize that complexation with α-cyclodextrin will significantly enhance camphor's aqueous solubility, protect it from degradation, and alter its biological activity. By combining detailed physicochemical characterization with comprehensive in vitro biological assays, this research aims to offer a thorough understanding of the camphor-α-cyclodextrin complex and its potential applications in pharmaceutical and cryopreservation technologies.

Materials and methods

Materials and reagents

Camphor (M.W 152.23 g/mol) was provided by Thermo Scientific Chemicals (France), α-CD (972 g/mol), β-CD (1135 g/mol), γ-CD (1297 g/mol), and hydroxypropyl-β-CD (HP-β-CD (KLEPTOSE®), 1488 g/mol) were provided by Roquette Frères (France).

Methanol, ethanol, 2,2-diphényl 1-picrylhydrazyle (DPPH), ascorbic acid and sodium chloride (NaCl) were obtained from Biochem. All chemicals and reagents were of analytical grade and purchased from Sigma-Aldrich and Biochem.

Methods

Phase solubility studies

Phase solubility studies were conducted to investigate the solubility enhancement of camphor in the presence of cyclodextrins. Excess camphor was added to aqueous solutions containing increasing concentrations of α-CD (0-150 mM), β-CD (0–16 mM), γ-CD (0-180 mM) and HP-β-CD (0-467 mM). The mixtures were incubated at ambient temperature for 7 days with constant shaking (200 rpm). Subsequently, the solutions were filtered (0.45 µm), and the camphor concentration was determined spectrophotometrically at 287 nm using a UV-Vis spectrophotometer (Shimadzu) [11]. A standard curve was employed to quantify camphor concentration. The resulting data were plotted as camphor solubility versus cyclodextrin concentration. The stability constant (Ks) of the inclusion complexes was calculated from the slope and intercept of the phase solubility diagram according to the Higuchi and Connors Eq. (1).

$$Ks = \frac{slope}{intercept \times (1 - slope)} \quad (1)$$

Where intercept is the intrinsic solubility of camphor in the absence of CDs.

The preparation of the α-CD/camphor complex

The complex was prepared using the co-evaporation method. Stoichiometric amounts of α-cyclodextrin and camphor (molar ratios 1:1 and 1:2) were dissolved in 40 mL of ethanol. The mixture was stirred for 24 h at room temperature before the solvent was removed under reduced pressure using a rotary evaporator. The resulting white crystalline powder was dried in a desiccator [12]. For comparison, physical mixtures of camphor and α-CD were prepared by homogenization in a mortar and pestle at room temperature. The resulting mixtures were stored in sealed glass containers.

Physicochemical characterizations of the camphor/α-CD inclusion complex

Fourier transform infrared spectroscopy (FTIR) Fourier transform infrared (FTIR) spectroscopy was employed to investigate the interactions between camphor and α-cyclodextrin within the inclusion complex. Approximately 100 mg of KBr and 150 mg of sample (camphor, α-CD, complex, or physical mixture) were ground together and pressed into pellets. FTIR spectra were recorded on a Thermoscientific IR-ATR Nicolet spectrophotometer model iS5 in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}.

Thermal characterization analysis (TGA) Thermogravimetric analysis (TGA) was performed using a PerkinElmer STA 8000 thermogravimetric analyzer to assess the thermal stability and decomposition behavior of the samples. Approximately 10 mg of each sample (α-CD, camphor, complex, and physical mixture) was subjected to a heating rate of 10 °C/min from 25 to 600 °C [13].

X-ray diffraction analysis X-ray diffraction (XRD) patterns were obtained using a Panalytical Empyrean diffractometer equipped with Cu Kα radiation (λ = 1.5418 Å). Samples were scanned from 2° to 70° with a step size of 0.02° and a scan rate of 5°/min. XRD analysis was used to investigate the crystallinity and structural changes upon complex formation.

Antioxidant activity (DPPH assay)

The antioxidant activity of camphor and its inclusion complex was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [14]. Briefly, 2.5 mL of a methanolic solution containing camphor or its complex (at various concentrations) was mixed with 2.5 mL of a 0.3 mM DPPH solution. The mixture was incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu) [14]. Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$Free\ radical\ scavenging\ activity\ (\%) = \frac{(A0 - A1)}{A0} \times 100 \quad (2)$$

Where A0 was the absorbance of the solutions of DPPH without (camphor or camphor/α-CD complex), A1 was the absorbance of the solution containing (camphor or camphor/α-CD).

Cryopreserved bull sperm test

Semen was collected using the retrograde flushing method as described by Benberkane et al. [10]. Briefly, a control sample consisting of 0.9 ml of Tris buffer and 0.1 ml of sperm was prepared, alongside treatment samples where various concentrations of camphor and its inclusion complex (ranging from 0.05 to 0.8 mg/ml) were added to 0.1 ml of sperm. All samples were stored at 4 °C. Sperm motility and quality were assessed over a period ranging from 30 min to 48 h by measuring and plotting parameters such as curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), and average path velocity (VAP, µm/s) [10].

Cytotoxicity effect of camphor and camphor/α-CD IC on bull semen

The semen collection and concentration procedures are described in the previous section (Cryopreserved Bull Sperm Test) [10]. The cytotoxicity of camphor and its inclusion complex on spermatozoa was assessed using the Computer-Assisted Sperm Analysis (CASA) system after 160 min of cold storage at 4 °C. The velocities, including curvilinear velocity (VCL), straight-line velocity (VSL), and average

path velocity (VAP), were then plotted to evaluate the impact of camphor and its complex on sperm motility.

In vitro hemolysis assay

The hemolytic activity of camphor and its inclusion complex was evaluated using a standard hemolysis assay. Human blood was collected and centrifuged at 3500 rpm for 10 min to obtain plasma. A solution containing NaCl and plasma was prepared, and different concentrations of the test samples (camphor, complex) were added. A control group without the test sample was included. The samples were incubated at room temperature for 10 min, followed by centrifugation at 3500 rpm for 10 min. The absorbance of the supernatant was measured at 540 nm using a spectrophotometer to determine hemolysis, as described by Toutou et al. [15]. The percentage hemolysis was calculated using the following equation:

$$Hemolysis\ Assay\ (\%) = \frac{A0 - A1}{A0} \times 100 \quad (3)$$

Where A0 was the absorbance of the control (without camphor or the complex) and A1 was the absorbance in the presence of camphor or the complex.

Results and discussions

Phase solubility diagram

Phase solubility studies were conducted to evaluate the influence of cyclodextrins on camphor solubility. As depicted in Fig. 1, the majority of cyclodextrins exhibited an AL-type phase solubility profile, indicating the formation of 1:1 inclusion complexes, consistent with the Higuchi and Connors classification [16]. The calculated stability constants (Ks) for α-CD, γ-CD, and HP-β-CD were determined to be 23.3, 16.01, and 6.42 M^{-1}, respectively, suggesting a higher affinity of α-CD for camphor compared to the other cyclodextrins. In addition, the camphor aqueous solubility using α-CD was found to be significantly enhanced, yielding a five-fold, this improvement can be attributed to the formation of a stable inclusion complex [8].

β-CD displayed a distinct BS-type profile, indicating complex formation at lower cyclodextrin concentrations

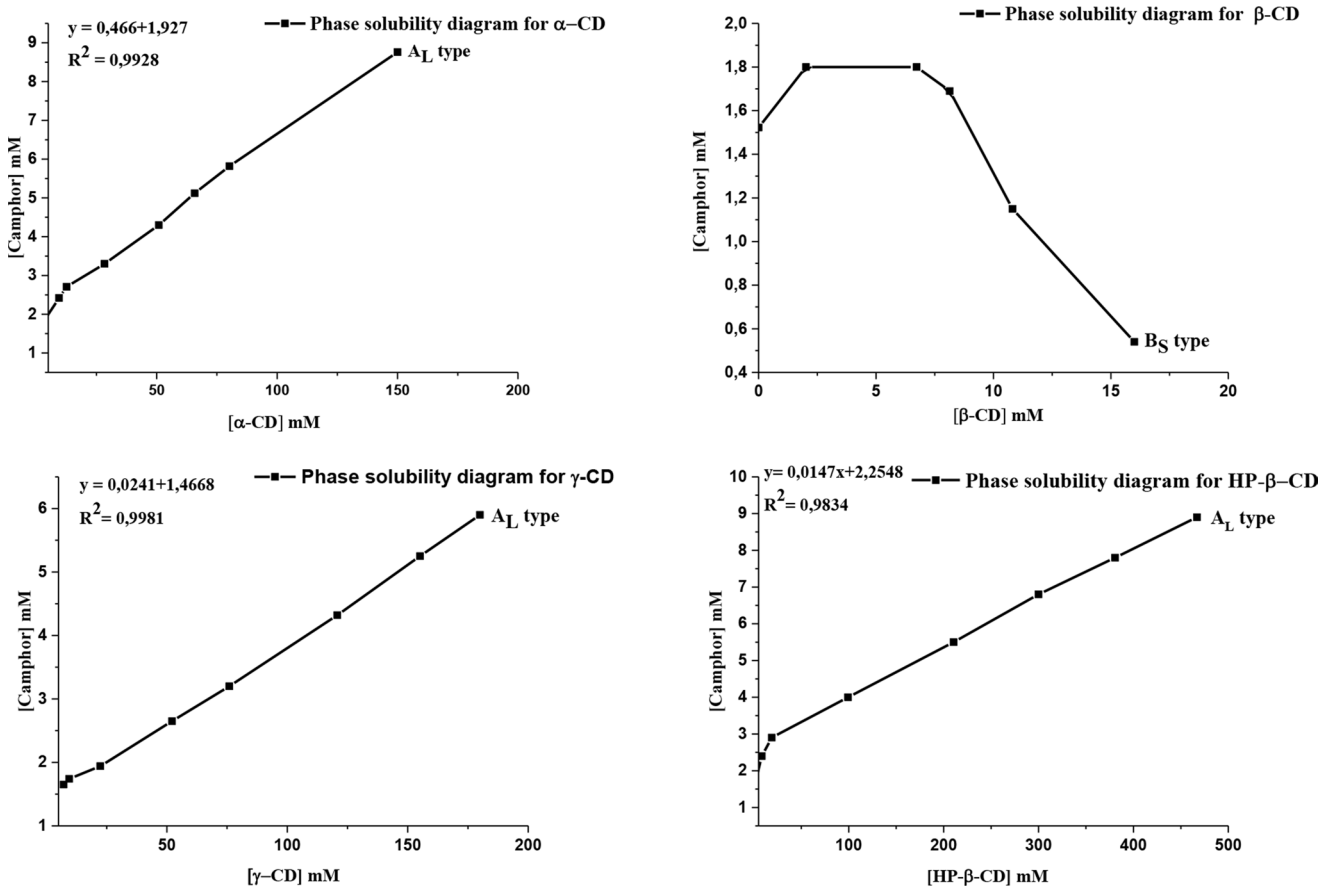

Fig. 1 Phase solubility diagram for camphor using α-CD, β-CD, γ-CD and HP- β-CD

followed by complex saturation, likely due to its limited aqueous solubility [17]. This observation contrasts with the findings of Benchallal et al. [17].

HP-β-CD exhibited an AL-type profile, demonstrating a linear increase in camphor solubility with increasing cyclodextrin concentration, consistent with the formation of 1:1 inclusion complexes [8].

The observed phase solubility profiles provide valuable insights into the complexation behavior of camphor with different cyclodextrins. The formation of 1:1 inclusion complexes with α-CD, γ-CD, and HP-β-CD is evident, with α-CD demonstrating the highest complexation efficiency. The lower complexation efficiency of β-CD can be attributed to its limited aqueous solubility, hindering the formation of stable inclusion complexes. The obtained results highlight the potential of cyclodextrins, particularly α-CD, for enhancing the solubility of camphor. Further investigations into the structural and thermodynamic properties of these inclusion complexes would provide additional insights into the factors influencing complex formation and stability.

Formation of the camphor/α-CD inclusion complex and determination of inclusion efficiency

The inclusion efficiency (IE), representing the percentage of camphor encapsulated within the complex relative to the initial camphor amount, was calculated using Eq. (4).

$$(IE\,\%) = \frac{mass\ of\ the\ encapsulated\ camphor\ (mg)}{initial\ camphor\ mass\ to\ be\ encapsulated\ (mg)} \times 100 \quad (4)$$

The molar ratios 1:1 and 1:2 were investigated to determine the optimal conditions for complex formation. The calculated IE values for the 1:1 and 1:2 ratios were 24.37% and 34.63%, respectively (Table 1). The higher IE value for the 1:2 ratio suggests that a larger proportion of cyclodextrin enhances the encapsulation efficiency, potentially due to a more complete filling of the cyclodextrin cavity and reduced aggregation of camphor molecules. The higher inclusion efficiency observed for the 1:2 molar ratio indicates that an excess of cyclodextrin is beneficial for encapsulating camphor. This finding suggests that a larger proportion of cyclodextrin molecules can effectively accommodate camphor within their cavities, preventing aggregation and improving the overall encapsulation efficiency as indicated by Jansook et al. [18, 19].

Table 1 The inclusion efficiency (IE) of camphor

	Camphor/α-CD (1:1)	Camphor/α-CD (1:2)
Initial camphor mass to be encapsulated (mg)	80	160
Mass of the encapsulated camphor (mg)	19.5	55.4
IE (%)	24.37	34.63

Characterization of the camphor/α-CD inclusion complex

Infrared spectroscopic analysis (FITR)

FTIR spectroscopy was employed to investigate the potential interactions between camphor and α-CD within the inclusion complex. FTIR spectra of pure camphor, α-CD, the physical mixture, and the inclusion complex were recorded in the range of 4000–400 cm^{-1} and are depicted in Fig. 2.

The FTIR spectrum of camphor exhibited characteristic bands at approximately 2900 cm^{-1} (C-H stretching), 1750–1450 cm^{-1} (C=O stretching), 1250 cm^{-1} (C-O stretching), and 900 cm^{-1} (C-O-C stretching). The α-CD spectrum displayed prominent bands attributed to O-H stretching (3500 cm^{-1}), C-H stretching (2900 cm^{-1}), C-O-C stretching (1100–950 cm^{-1}), and C-C stretching (1000–900 cm^{-1}) [11, 15].

The spectrum of the physical mixture exhibited a simple superposition of the individual spectra of camphor and α-CD, indicating the absence of significant interactions between the components. In contrast, the spectrum of the inclusion complex showed significant modifications compared to the individual spectra and the physical mixture. The characteristic bands of camphor, particularly those associated with the carbonyl group, were notably altered or diminished in intensity. These observations suggest the formation of inclusion complexes, where the camphor molecule is encapsulated within the α-CD cavity, leading to changes in the molecular environment and vibrational modes [11, 15].

TGA analysis

Thermogravimetric analysis (TGA) was employed to investigate the thermal behavior of α-CD, camphor, their physical mixture, and the inclusion complex. TGA thermograms are presented in Fig. 3a and b.

α-CD exhibited a minor weight loss of approximately 4,5% between 20 °C and 105 °C, attributed to the removal of adsorbed water. Subsequent thermal decomposition of the α-CD structure occurred at around 73,61%. Pure camphor demonstrated a significant weight loss about 96,85% starting at 40 °C, reaching a maximum rate at 170 °C, consistent with its reported boiling point. This weight loss corresponds to the volatilization of camphor [13, 20].

The inclusion complex displayed a different thermal profile compared to the physical mixture. A slight weight loss observed in the initial stage for the complex suggested the

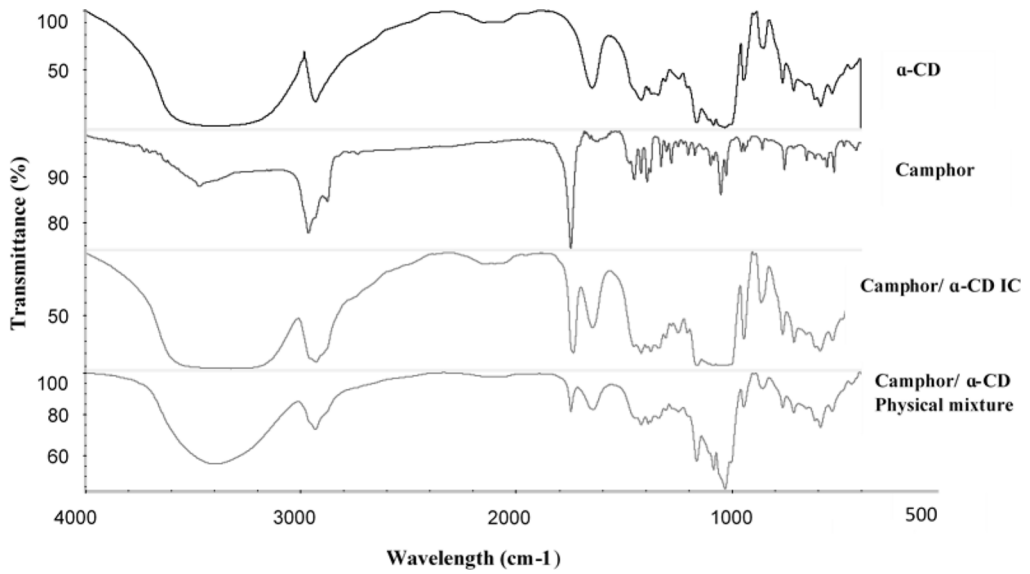

Fig. 2 FTIR analysis spectra of: α-CD, camphor, camphor/ α-CD IC, and physical mixture

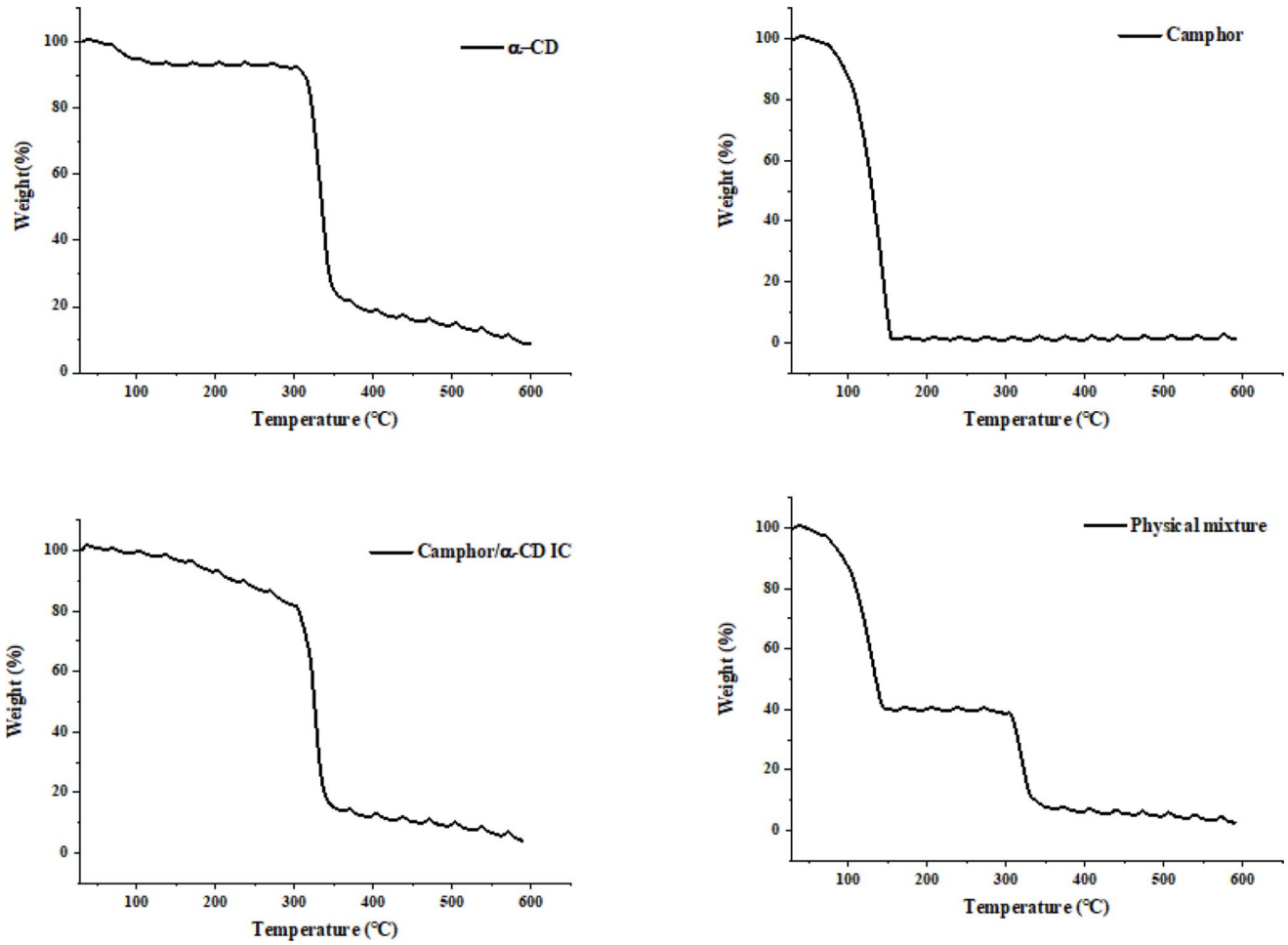

Fig. 3 **a**. Thermograms of α-CD, camphor, camphor/α-CD, and physical mixture. **b**. Combined thermograms of: α-CD, camphor, Camphor/α-CD, and physical mixture

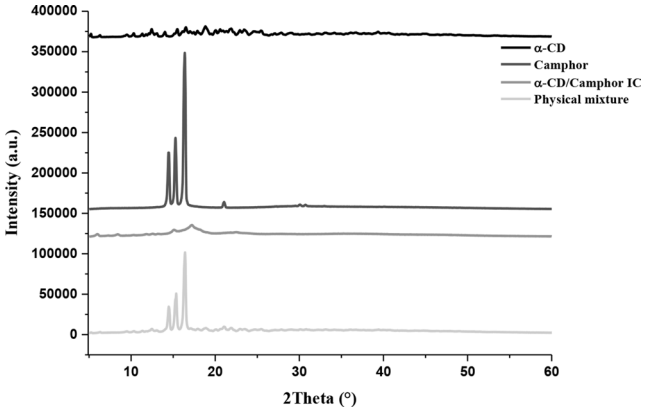

Fig. 4 X-ray patterns of α-CD, camphor, camphor/α-CD, and its physical mixture

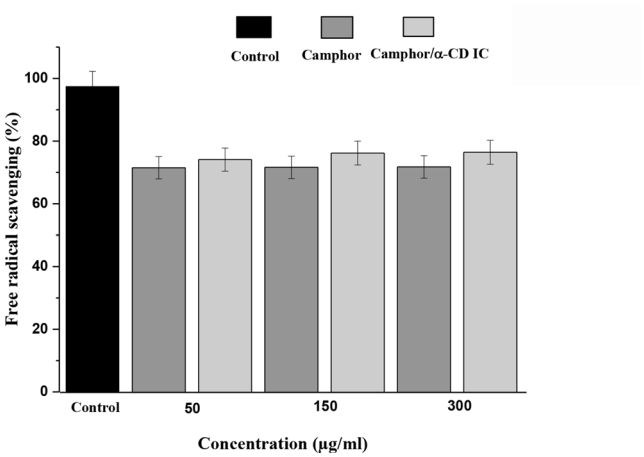

Fig. 5 Antiradical activity of camphor and camphor/α-CD (complex)

removal of adsorbed water or potential interactions between camphor and α-CD. A subsequent weight loss between 250 °C and 380 °C indicated the decomposition of the complex, suggesting a degree of interaction between the components. In contrast, the physical mixture exhibited distinct thermal events corresponding to the individual components, with a minor weight loss attributed to potential weak interactions [21].

X-ray diffraction

The X-ray diffraction (XRD) analysis was performed to investigate the structural changes associated with complex formation. The XRD patterns of pure camphor, α-CD, the physical mixture, and the inclusion complex are presented in Fig. 4.

Camphor exhibited a crystalline structure with distinct diffraction peaks at 14.4°, 15.3°, and 16.7°. The XRD pattern of the inclusion complex showed a significant reduction in the intensity of the characteristic camphor peaks, suggesting a loss of crystallinity upon complexation. These observations strongly support the formation of an amorphous inclusion complex, where camphor molecules are encapsulated within the α-CD cavity, disrupting the crystalline structure of camphor [8]. Indeed, as noted in numerous studies, the diffraction profile of a simple binary blend reflects the additive signals of its individual components, without evidence of host–guest interaction. Specifically, the physical mixture in our work displays an exact superposition of the characteristic peaks of α-cyclodextrin (α-CD) and camphor, confirming the absence of inclusion complex formation in this sample. This behaviour mirrors that reported by Fatmi et al. (2015) [22] in their investigation on camptothecin cyclodextrin encapsulation, where the physical mixture's diffractogram likewise coincided with the sum of the individual CPT and CD patterns, whereas the true inclusion complex exhibited broad amorphous halos and a marked loss of crystallinity. Thus, by contrasting the superimposed diffraction of the physical mixture with the amorphous profile of the inclusion complex, we not only validate the formation of a new amorphous phase but also provide a clear mechanistic rationale for the improved chemical solubility observed in our system.

Antioxidant effect

DPPH radical scavenging method was used to determine the antioxidant activity of camphor and its inclusion complex. Figure 5 summarizes the results obtained by scavenging our free radicals using a methanolic solution of DPPH containing pure camphor and its complex, giving the antioxidant effect. The DPPH radical scavenging capacity of free camphor or encapsulated camphor increased with increasing sample concentrations.

The maximum reducing power was observed in the complex at a concentration of 300 µg/ml achieving 76.43%. This indicates that the camphor solubilized in the cavity of α-CD allowed a higher antioxidant property, reduced volatility and protection against unfavorable interactions with the environment compared to unencapsulated camphor, which had a lower effect at all concentrations (50–300 µg/ml) equal to 72.48%, 71.61% and 71.74%, respectively, these results are probably attributed to the high solubilization of camphor in organic solvents mainly methanol [8], which leads to a reduction of the free radicals. However, the antioxidant effect of the control (ascorbic acid) was found to be 97.41%, affirming its high antioxidant capacity [23].

This leads to the conclusion that the camphor/α-CD complex has greater reducing power than crude camphor and helps fight against oxidative stress.

The antioxidant capacities of camphor, its inclusion complex, and ascorbic acid (positive control) were evaluated using the DPPH radical scavenging assay. The percentage of

DPPH radical scavenging activity was determined spectrophotometrically at 517 nm. As illustrated in Fig. 5, both free camphor and the inclusion complex exhibited dose-dependent antioxidant activity, with the complex demonstrating a higher radical scavenging capacity compared to pure camphor, probably attributed to the high solubilization and low evaporation of camphor in the inclusion complex [8]. The maximum antioxidant activity was observed for the inclusion complex at a concentration of 300 µg/mL, reaching 76.43% inhibition of the DPPH radical. In contrast, the antioxidant activity of camphor ranged from 72.48 to 71.74% at concentrations between 50 and 300 µg/mL. Ascorbic acid exhibited the highest antioxidant activity with a scavenging percentage of 97.41%, which leads to a reduction of free radicals. However, the antioxidant effect of the control (ascorbic acid) was found to be 97.41%, affirming its high antioxidant capacity [23]. Consistent with previous reports on non-phenolic terpenes such as borneol [24], camphor exhibited moderate DPPH activity - weaker than phenolic standards like ascorbic acid but in agreement with literature values found by Lei Chen et al. [24], the antioxidant activity of monoterpenes, strongly depends on the polarity of solvent used [25]. In addition, the study performed by Karolina A. Wojtunik et al. [25] claims that π bonds are responsible for the chain-breaking antioxidant activity of monoterpenes which in in accordance with our study. These findings suggest that the formation of the camphor-α-CD inclusion complex has a positive impact on the antioxidant properties of camphor.

Cellular cytotoxic effect

Cytotoxic effect of camphor and its complex on spermatozoa

Figure 6 illustrates the impact of camphor and its encapsulated form on spermatozoa motility, measured by VCL (curvilinear velocity), VAP (average path velocity), and VSL (straight-line velocity) after 2 h at 4 °C. Encapsulated camphor demonstrated superior motility metrics compared to pure camphor across concentrations of 0.05 to 0.4 mg/mL, indicating reduced cytotoxicity. Specifically, encapsulated camphor at these concentrations preserved sperm motility better, likely due to the controlled release mechanism of the encapsulation, which minimizes direct contact with sperm membranes.

However, at a concentration of 0.8 mg/mL, the encapsulated form exhibited toxic effects, likely due to the high concentration of camphor being released from the inclusion complex, which surpasses the threshold of sperm membrane tolerance. This is consistent with the observations by Njus et al. and Fourmentin et al. [26, 27], which reported that higher concentrations of unencapsulated camphor (≥0.25 mg/mL) lead to sperm toxicity, impairing motility and viability. The negative impact at high concentrations aligns with studies by Troisio et al. and Cavalleri et al. [28, 29], who found that essential oils in excessive amounts are detrimental to sperm cells, likely due to oxidative stress or membrane disruption.

Cytotoxicity assay on human red blood cells (RBCs)

Figure 7 shows the hemolysis data for camphor and its complex (camphor/α-CD) after incubation with RBCs. At

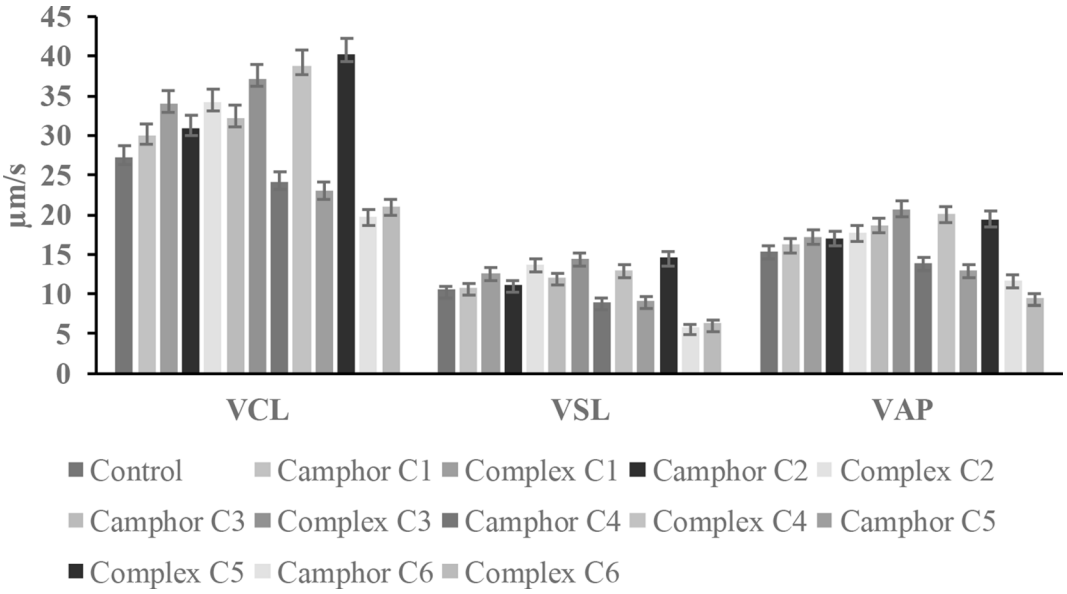

Fig. 6 Cytotoxic effect of camphor and its inclusion complex (0.05–0.8 mg/ml) after 120 min of storage

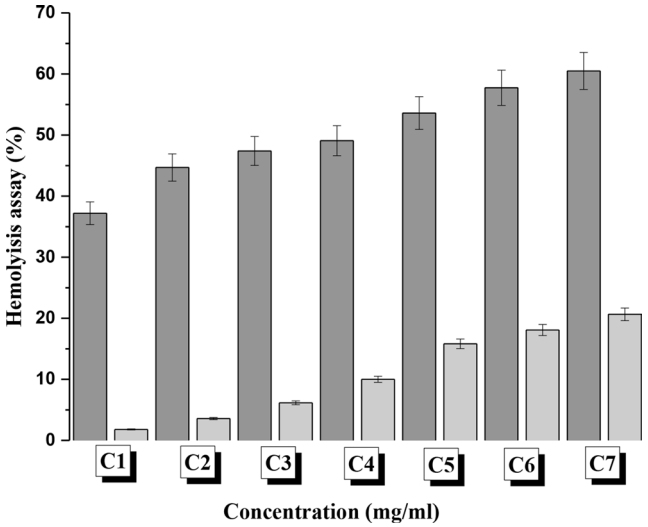

Fig. 7 Hemolysis test investigation on camphor and camphor/α-CD inclusion complex at different concentrations (C1:0,01-C7:0,8 mg/ml)

low concentrations (0.01 and 0.075 mg/mL), both forms of camphor did not induce hemolysis, corroborating literature that considers hemolysis significant only above 10% [30]. However, at higher concentrations (0.4 and 0.8 mg/ml), both camphor and its complex caused considerable hemolysis (> 10%), suggesting that the increased solubilization of camphor in its complex form elevates its effective concentration in solution.

This finding is supported by studies showing that high doses of essential oils can be hemolytic due to membrane disruption or oxidative damage [31–32]. The enhanced solubilization effect observed in this study, resulting from encapsulation, underscores the importance of concentration management to prevent erythrocyte damage. The toxic effect at higher concentrations highlights the need for further research into optimizing encapsulation techniques to balance effective delivery with safety.

Cold storage protection effect on sperm cell motility

Camphor, a monoterpene with notable biological properties, was evaluated for its effect on sperm motility during cold storage at 4 °C. Figure 8 shows that initially (t = 0 min) the control group exhibited superior motility across all kinetic parameters (VCL, VAP, VSL) compared to sperm treated with either pure camphor or its encapsulated form at concentrations ranging from 0.05 to 0.8 mg/ml. This initial observation suggests that, at t = 0, the camphor and its complex had not yet interacted sufficiently with the sperm cells to produce a measurable effect.

As storage time progressed, encapsulated camphor demonstrated enhanced protective effects on sperm motility compared to pure camphor, with optimal results observed at most time points (30, 60, 1440, and 2880 min). However, at higher concentrations (C5: 0.4 mg/ml and C6: 0.8 mg/mL), the control group showed better motility than the encapsulated camphor, likely due to the detrimental impact of excessive camphor solubilization, which may have compromised sperm viability. Encapsulation is known to improve the stability and solubility of volatile compounds, thus shielding them from oxidative stress, thermal shock, and osmotic damage [10, 15, 33–37]. This protective role aligns with findings in the literature, reinforcing the efficacy of encapsulation in maintaining sperm motility under storage conditions.

Conclusion

This study evaluated the solubility of camphor in water using various cyclodextrins, with α-cyclodextrin demonstrating the highest solubility coefficient (Ks). The optimal complexation was achieved at a 1:2 molar ratio, which provided the most efficient inclusion. The formation of the camphor/α-cyclodextrin inclusion complex was confirmed through thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FT-IR), and X-ray diffraction (XRD). The encapsulated camphor exhibited enhanced antioxidant activity, showing a stronger antiradical effect than the free form of camphor. However, at higher concentrations, both camphor and its complex were found to be toxic to spermatozoa and red blood cells (RBCs). Encapsulated camphor demonstrated enhanced protective effects on sperm motility compared to pure camphor, with optimal results observed at most time points (30, 60, 1440, and 2880 min). However, at higher concentrations (C5: 0.4 mg/mL and C6: 0.8 mg/mL), the control group showed better motility than the encapsulated camphor.

Molecular encapsulation of camphor with cyclodextrins presents a promising strategy in the pharmaceutical field. These findings suggest that camphor, when complexed with α-CD, holds considerable potential for novel applications in drug delivery and cryopreservation technologies.

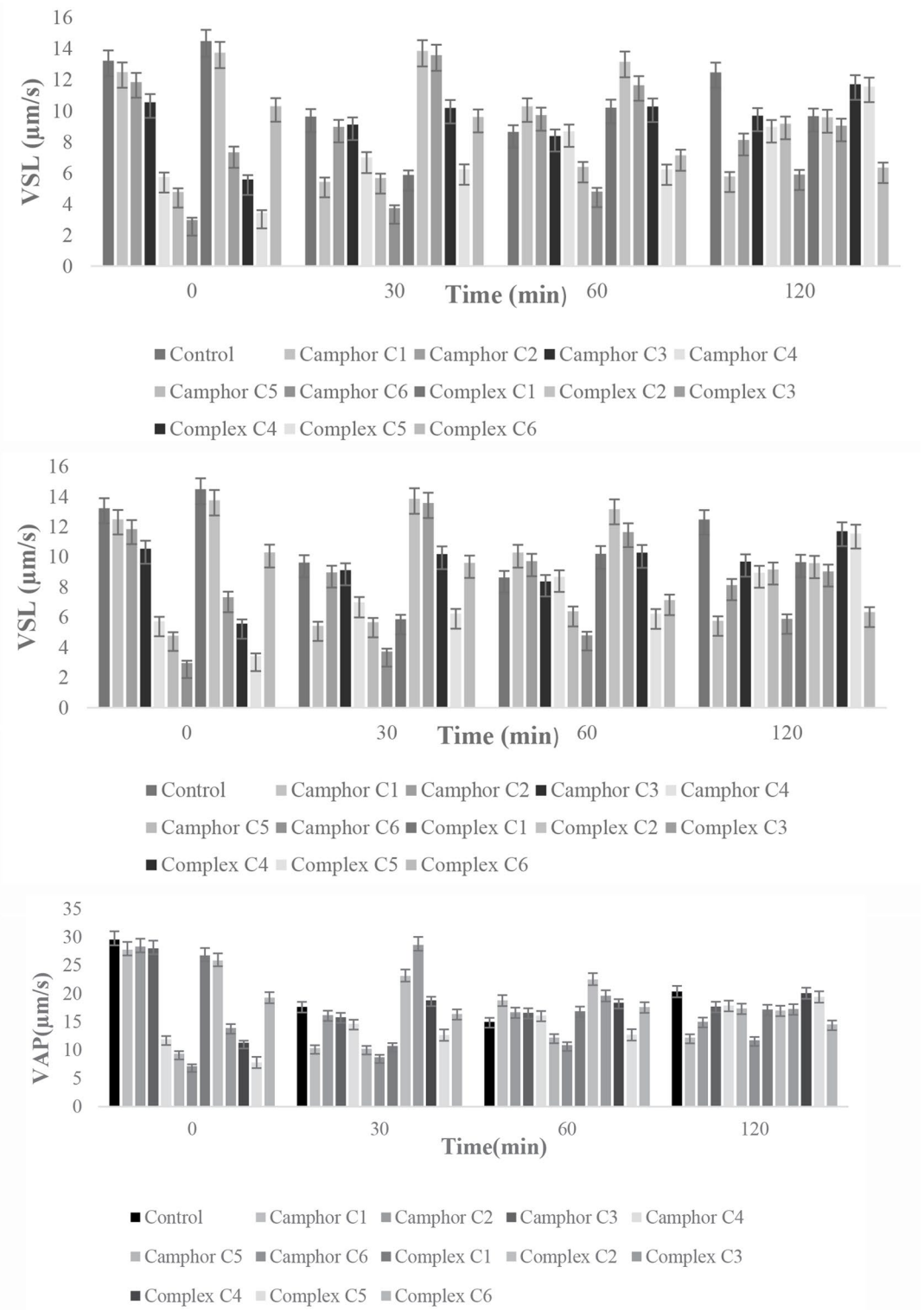

Fig. 8 Preservation effect of camphor and its inclusion complex (0.05–0.8 mg/ml) at time intervals (0, 30, 60, 120,1440 and 2880 min) at 4 °C

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Declarations

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