Journal of Drug Delivery Science and Technology 127 (2027) 108857

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Formulation-dependent physicochemical properties and biological response of DOPC liposomes encapsulating *Opuntia stricta* extracts

Ahlem Ben Saad [a,c], Michela Tozzi [a], Flaminia Cesare Marincola [a], Franca Piras [b], Antonella Rosa [b], Marco Piludu [b], Valeria Sogos [b], Fatma Saad [c], Lotfi Achour [c], Andrea Salis [a,*], Cristina Carucci [a,**]

[a] Department of Chemical and Geological Sciences and CSGI, University of Cagliari, Cittadella Universitaria, S.S. 554 bivio Sestu, Monserrato, CA, 09042, Italy
[b] Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, S.S. 554 bivio Sestu, Monserrato, CA, 09042, Italy
[c] Laboratory of Bioresources: Integrative Biology and Exploiting, Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia

ARTICLE INFO

Keywords:
Opuntia stricta
Betacyanins
Liposomes (DOPC)
Cytocompatibility
HaCaT cells
SH-SY5Y cells

ABSTRACT

The development of liposomes for the delivery of chemically labile natural extracts is often limited by extract instability and poorly controlled biological responses. In this study, zwitterionic 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) liposomes were engineered as platforms for the encapsulation of aqueous extracts from *Opuntia stricta* var. *stricta* (OpS) and var. *dillenii* (OpD), and the role of the formulation medium as a key design parameter was systematically investigated. Liposomes were prepared at pH 5.0 in water, phosphate solution, or acetate buffer and characterised in terms of size, surface charge, morphology, encapsulation efficiency, and loading capacity. While all formulations displayed nanometric dimensions, the ionic composition of the preparation medium markedly influenced liposome organisation and extract retention. Acetate-based formulations showed enhanced encapsulation efficiency (up to 61%) and loading capacity (up to 23%), together with improved size homogeneity compared to phosphate-based systems. Release studies under physiological conditions revealed a sustained and biphasic release profile for both extract varieties. The biological performance of liposomes was evaluated in vitro using human HaCaT keratinocytes and SH-SY5Y neuroblastoma cells. All formulations were cytocompatible towards HaCaT cells, whereas acetate-formulated OpS-Lip and OpD-Lip induced a moderate, formulation-dependent reduction in SH-SY5Y cell viability, highlighting the influence of physicochemical design parameters on cellular response. Overall, these results demonstrate that formulation conditions critically govern the colloidal and biological performance of DOPC-based liposomes, providing design guidelines for the development of lipid carriers for unstable plant-derived bioactives.

1. Introduction

Lipid-based biomaterials are widely investigated as platforms for the delivery of bioactive compounds, owing to their biocompatibility, structural versatility, and ability to modulate cellular interactions [1]. Among these systems, liposomes represent a well-established class of soft biomaterials capable of encapsulating chemically labile payloads, including complex plant-derived extracts [2]. Natural plant-derived extracts have been traditionally employed as colouring agents in food products [3], cosmetics [4], and textile applications [5]. Beyond their traditional use, increasing attention has been devoted to their functional and health-promoting properties [6]. In this context, *Opuntia stricta* (*O. stricta*) is recognised as an important source of water-soluble phytochemicals. Among these components, betalains, which are responsible for the characteristic colouration of *Opuntia* fruits, have been widely investigated for their antioxidant and anti-inflammatory properties [7]. By scavenging free radicals and inhibiting lipid peroxidation, they reduce inflammation and protect against cellular damage induced by reactive oxygen species (ROS) [8]. These antioxidant properties are relevant in the prevention and management of various health conditions, including cardiovascular disease and cancer [9]. A major limitation for the practical exploitation of plant-derived extracts is their chemical instability, which is strongly influenced by environmental factors including pH, temperature, light exposure, oxygen, and solvent

* Corresponding author.
** Corresponding author.
E-mail addresses: andrea.salis@unica.it (A. Salis), cristina.carucci@unica.it (C. Carucci).

https://doi.org/10.1016/j.jddst.2026.108857
Received 20 June 2026; Received in revised form 13 August 2026; Accepted 27 August 2026
Available online 27 August 2026

composition [10–12]. For instance, the stability of betalain chromophores has been shown to depend on the nature of the buffer system, with maximum stability typically observed around pH 5.0 [13]. To overcome these limitations, several strategies have been explored, including complex formation [14], copigmentation [15,16], and encapsulation [17,18], which have been proposed as effective approaches to improve the stability and functional performance of extracts under adverse conditions [19]. These stability limitations motivate the design of carrier systems in which formulation parameters actively contribute to the functional performance of the material.

Liposomes are versatile nanocarriers capable of encapsulating both hydrophilic and hydrophobic bioactive compounds, owing to their amphiphilic structure and the biocompatible, low immunogenic nature of their lipid components, such as phosphatidylcholine and cholesterol [20]. Their ability to protect labile molecules and modulate release profiles makes them particularly attractive for the delivery of plant-derived extracts. Liposome formulation of plant extracts is inherently more challenging than that of single purified compounds because they contain chemically diverse constituents that differ in polarity, ionisation state, molecular size and affinity for the aqueous core and lipid bilayer. As a consequence, formulation must simultaneously ensure efficient encapsulation of a chemically heterogeneous mixture while preserving the stability of labile compounds and maintaining suitable vesicle physicochemical properties. Nevertheless, successful formulation offers important advantages, including protection of chemically unstable constituents, modulation of their release and improved control over their biological availability. For betalain-rich *O. stricta* extracts, the formulation medium therefore plays a critical role in preserving the labile chromophoric fraction while maintaining efficient encapsulation, vesicle stability and controlled release [21]. However, the physicochemical properties and performance of liposomal formulations strongly depend on the preparation environment and on the interactions between the lipid bilayer and the encapsulated active ingredient [22]. In addition to their role as delivery vehicles, liposomes can be regarded as lipid-based biomaterials whose physicochemical organisation and biological behaviour are strongly governed by formulation-dependent parameters. In particular, the ionic composition of the medium plays a central role in modulating bilayer organisation. Buffer composition and electrolyte content modulate lipid headgroup hydration, electrostatic screening, and bilayer packing, resulting in changes in vesicle size, surface charge, and cargo retention [23]. These effects are governed by a complex interplay of osmotic and hydration forces, which influence liposome size and stability even in the absence of a net lipid charge [24,25]. Furthermore, ion-specific interactions can induce structural discontinuities and local rearrangements in phosphatidylcholine bilayers, highlighting the sensitivity of zwitterionic lipid assemblies to the surrounding ionic environment [26]. More broadly, buffer specific effects have been widely documented across soft matter [27], hard matter [28,29], and biomolecular systems [30], where differences in counterion identity modulate interfacial interactions. These observations support the view that the ionic environment acts as an active determinant of phosphatidylcholine bilayer organisation, even in zwitterionic lipid assemblies [27]. However, formulation parameters are often treated as secondary variables rather than as intrinsic design elements governing biological performance. In fact, within lipid-based biomaterials, ion and buffer specific effects represent underexplored but powerful design variables capable of modulating bilayer organisation, cargo retention, and cellular response. Although numerous studies have focused on improving the encapsulation efficiency of plant-derived extracts [17,31], a systematic understanding of how formulation conditions influence the physico-chemical properties of liposomal carriers, extract loading, and the resulting biological response remains limited [32–34]. In particular, the role of electrolyte composition as a formulation variable capable of modulating the performance of extract loaded liposomes has received little attention.

To address this gap, the present work aims to systematically investigate how formulation conditions influence the physicochemical properties and biological behaviour of liposomes composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) loaded with aqueous extracts derived from *O. stricta* fruit juice. DOPC was selected as a zwitterionic phospholipid that forms stable liposomes with fluid bilayers under physiological conditions and is often used as a model single component liposomal system [26,35,36]. Specifically, we examined how the preparation medium, comparing acetate and phosphate based systems, modulates vesicle size, morphology, and colloidal stability at pH 5.0, corresponding to conditions relevant for the stability of betalain extracts [13,19,37]. We further assess the release behaviour at physiological pH (7.4) and evaluate how formulation-dependent modifications translate into cytocompatibility outcomes in human keratinocytes (HaCaT) and neuroblastoma cells (SH-SY5Y). By correlating formulation-dependent physicochemical properties with in vitro cellular response, this study aims to provide design guidelines for DOPC-based liposomes intended for the delivery of chemically labile plant-derived bioactives.

2. Experimental section

2.1. Materials

The plant material used in this study consists of the fruit of two varieties of *O. stricta* (var. *stricta* and var. *dillenii*), harvested in February 2025 in the Falaise region of Monastir, Tunisia. 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC ≥ 99%) was provided by Avanti Polar Lipids (Alabaster, Alabama, USA). Chloroform ($CHCl_3$ > 99%), sodium acetate (99%), acetic acid (99%), Na_2HPO_4 (≥99%), NaH_2PO_4 (≥99%), NaCl (≥99%) were all purchased from Sigma-Aldrich (Italy). Water was purified through a Millipore type 2 purification system. Phosphate buffer saline (PBS) 10 mM NaCl 150 mM at pH 7.5 was used for dialysis. PBS at pH 7.4, employed for release studies, was prepared as follows: NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4 10 mM, KH_2PO_4 1.8 mM. Chemicals for cell culture, including penicillin, streptomycin, fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), and Trypsin 0.25%-EDTA were acquired from EuroClone (Pero, MI, Italy). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Merck Life Science (Milan, Italy).

2.2. Preparation of Opuntia extracts

Extracts were obtained from lyophilized *O. stricta* juice by maceration [38]. Briefly, 1 g of powdered juice was suspended in 40 mL of distilled water at 45 °C for 150 min, using an orbital shaking incubator (150 rpm). Then, the mixture was centrifuged at 10.000 × g for 10 min at 4 °C. The supernatants were collected and freeze-dried. The resulting red-violet dye powder was stored at −20 °C until further analysis.

2.3. UV-Vis analysis

UV–Vis spectra of *O. stricta* var. *stricta* and var. *dillenii* extracts were recorded at different concentrations (0.25, 0.5, 0.75, 1, 1.5, and 2 mg/mL) in three media: water adjusted to pH 5, 50 mM phosphate solution (pH 5.0), and 50 mM acetate buffer (pH 5.0). The spectra showed a characteristic absorption band at λ = 538 nm, associated with betalain chromophoric components responsible for the red–purple colouration of the extracts. The chromophoric species concentration was expressed as betacyanin equivalents (Bc, mg/L) with the following equations [39]

$$[Bc] = \frac{A \times DF \times MM \times 1000}{\varepsilon \times L} \quad (1)$$

where A is the absorbance measured at λ = 538 nm, DF is the dilution factor, MM is the molar mass (550 g mol^{-1}), ε is the molar extinction

coefficient in water ($\varepsilon_{betacyanins} = 60000\ M^{-1}\ cm^{-1}$) [39], and L is the optical path length of the cuvette (1 cm).

2.4. ESI-MS

Positive ESI-MS spectra were recorded with a high-resolution LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The solutions were injected at a flow rate of 5.00 μL/min into the ESI source. Spectra were recorded in the range of *m/z* 300–600 with a resolution of 240,000 (FWHM). The extracts were dissolved in Milli-Q water at 10 mg/mL, kept at 4 °C in dark, and analysed without further purification.

2.5. ^{1}H NMR spectroscopy

^{1}H NMR spectra of aqueous solutions of *Opuntia* extracts (10 mg/mL in 100% D_2O) were recorded on a Varian Unity INOVA 500 spectrometer at 300 K. One-dimensional NOESY presat experiments were performed using an acquisition time of 1.8 s, a relaxation delay of 2 s, and 10000 transients. An external reference was prepared in a capillary tube by adding 5 μL of a 20 mM aqueous solution of 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (100% D_2O). Peak assignment was done by using The Food Database (FOODB, https://foodb.ca).

2.6. DOPC liposome preparation

Liposomes were prepared by the thin film hydration method. This approach was selected because it enables direct hydration of a pre-formed DOPC lipid film with the aqueous OpS and OpD extracts, providing a simple and reproducible preparation procedure for all formulation media [40]. An aliquot of 80 μl of DOPC solution (25 mg/mL in $CHCl_3$) was added into a glass vial. The organic solvent was removed under a gentle N_2 stream to form a thin layer at the vial bottom. The thin lipid film was then rehydrated using 4 mL of the selected formulation medium (water, acetate buffer or phosphate solution) at pH 5.0 either in the absence (Lip) or in the presence of *O. stricta* or *O. dillenii* extracts (25 mg/mL) dispersed in the appropriate medium. The resulting dispersions were homogenised under continuous stirring for 30 min at room temperature. Then, they were left to equilibrate at 4 °C for 1 h before further characterisation. After the equilibration, the liposomal dispersions were sonicated using a Titanium Exponential Microprobe with a 3 mm tip diameter, operating at 23 kHz for 20 min in pulse/pause mode setting (1-s on/1-s off). To avoid sample heating due to the ultrasonic probe, the liposome dispersions were kept in an ice bath throughout the process. Empty DOPC liposomes (Lip) and extract-loaded liposomes (OpS-Lip and OpD-Lip) were dialysed using cellulose membrane (MWCO 14,000 Da) against PBS buffer for 24 h. The suspension inside the membrane was then collected, suspended in the corresponding formulation medium, and characterised by DLS and ELS after dialysis.

2.7. Dynamic light scattering (DLS) and electrophoretic light scattering (ELS)

The hydrodynamic size, polydispersity index (PDI), and zeta potential of the encapsulated extracts were determined by dynamic light scattering (DLS) and electrophoretic light scattering (ELS). Analyses were performed at room temperature using a particle analyser (Malvern Zetasizer Nano ZSP) equipped with a 633 nm laser and a 173° detection angle. Each sample was analysed in triplicate, and the results are expressed as mean ± standard deviation. The hydrodynamic diameter and PDI were used to assess the size distribution and physical stability of the particles, while the zeta potential was used to estimate their electrostatic stability in suspension. These measurements helped confirm the formation and stability of the encapsulation structures obtained. Data are reported as mean of 3 independent samples ± SD.

2.8. Encapsulation efficiency of betanin and loading capacity of liposomes

Encapsulation efficiency (EE) and loading capacity (LC) were determined by separating encapsulated from unencapsulated extracts using static diffusion across a dialysis membrane. Since the aqueous extracts consist of complex mixtures of water-soluble metabolites, encapsulation was quantified through the chromophoric fraction of the extracts, monitored at 538 nm and expressed as betacyanin equivalents. A known volume of the encapsulated sample was placed in a dialysis bag (cellulose membrane, MWCO 14,000 Da) and then immersed in 100 mL of phosphate-buffered saline (PBS, pH 7.5) maintained at room temperature under continuous stirring for 24 h. After dialysis, the absorbances of extract retained inside the membrane (encapsulated fraction) and that diffused into the external medium (non-encapsulated fraction) were measured by spectrophotometry at 538 nm, as described previously. All measurements were performed in triplicate, and results are reported as mean ± standard deviation (SD). Then, the encapsulation efficiency (EE %) and loading capacity (LC %) were determined according to Fadeel et al. [41] using the following equations:

$$EE\% = \frac{[Bc]_f}{[Bc]_i} \times 100 \quad (2)$$

where $[Bc]_i$ and $[Bc]_f$ are the betacyanin concentration (mg/L) before and after encapsulation in liposomes, respectively.

$$LC\% = \frac{m\ Bc}{m\ Lip} \times 100 \quad (3)$$

Where m *Bc* is the betacyanin amount (mg) calculated within the liposome formulation and m Lip is the liposome mass (mg).

2.9. Storage stability assay of OpS-Lip and OpD-Lip

Free and encapsulated extracts were stored at 4 °C (refrigerated conditions) for 21 days. Samples were collected at 1, 7, 14, and 21 days. The physical stability of liposomal formulations was evaluated by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), monitoring changes in hydrodynamic diameter (D_H), polydispersity index (PDI), and zeta potential (ζ) over time. The stability of free and encapsulated extract was assessed by a spectrophotometric assay. At each time point, the absorbance at 538 nm was measured and expressed as betacyanin equivalents (Bc, mg/L) by Eq. (1). Accordingly, the retention (%) was calculated using the formula [38]

$$Retention(\%) = \frac{[Bc]_t}{[Bc]_0} \times 100 \quad (4)$$

where $[Bc]_t$ is the betacyanin content measured after the selected days of storage (mg/L) and $[Bc]_0$ is the betacyanin content (mg/L) measured just after encapsulation. All measurements were performed in triplicate, and results are reported as mean ± standard deviation (SD).

2.10. Release kinetics from OpS-Lip and OpD-Lip

The release kinetics of the encapsulated extract were investigated using a dialysis-based diffusion method (MWCO 14,000 Da). For the OpS and OpD liposomes 4 mL of liposomal formulation was introduced into a semi-permeable membrane and immersed in 50 mL of PBS at pH 7.4. During incubation, a 3.5 mL aliquot of the external solution was taken for analysis at predetermined time intervals and immediately replaced with the same volume of fresh PBS to maintain constant diffusion conditions. The release (diffusion) behaviour of the corresponding free extracts was also evaluated using the dialysis method. All release experiments were performed at 37 °C under gentle stirring (100 rpm) for 26 h. The concentration of the released chromophoric fraction at each time point was determined spectrophotometrically at 538 nm and expressed as betacyanin equivalents. Calibration curves were prepared

independently from serial dilutions of OpS and OpD extracts and used to convert absorbance values into chromophoric concentrations.

The cumulative amount released, corrected for the successive withdrawals, was calculated as:

$$M_t = C_t V_R + V_s \sum_{i=1}^{n-1} C_i \quad (5)$$

where C_t is the concentration measured in the receiver medium at time (t), V_R is the total receiver volume, V_s is the volume withdrawn at each preceding sampling point, and C_i represents the concentrations measured in the previously collected aliquots.

The cumulative release expressed as a percentage of the initially encapsulated amount was fitted using the following equation [42]:

$$\frac{M_t}{M_0}\% = A_{max}\left(1 - e^{-kt}\right) \quad (6)$$

where M_t (mg) is the amount of the released chromophoric fraction at time t, M_0 (mg) is the amount of chromophoric fraction present in the liposomal sample introduced into the dialysis membrane at $t = 0$, A_{max} is the maximum release and k (h^{-1}) is the release rate constant.

2.11. Cell cultures

Human keratinocyte cell line (HaCaT cells) was obtained by CLS-Cell Line Services (Eppelheim, Germany). Human neuroblastoma SH-SY5Y cell line (code# HTL95013) was supplied by the Cell Bank Interlab Cell Line Collection, IRCCS San Martino Policlinico Hospital, Genova, Italy. Cells were grown at 37 °C in DMEM with high glucose, enriched with 2 mM L-glutamine, penicillin (100 units/mL), streptomycin (100 μg/mL), and 10% v/v FBS, in a humidified atmosphere at 5% CO_2. The cells were split once a week before they reached confluency using a Trypsin 0.25%-EDTA solution.

2.12. Cytotoxic activity (MTT assay)

The cytotoxic effects of OpS and OpD extracts and liposomes in HaCaT and SH-SY5Y cells were assessed by the MTT colourimetric assay as previously reported [43,44]. Cells were plated in a 96-well plate at 10^5 cells/mL density in 100 μl of complete culture medium and cultured for 72 h. After medium removal, cells (at 70-80% cell confluence) were treated for 24 h with different concentrations (from 0.05 to 5 mg/mL) of OpS and OpD water extracts and DOPC liposomes (100 μl, corresponding to 50 μg/ml) in fresh medium (treated cells). Control (untreated) cells were incubated for 24 h in fresh medium. After incubation, cells were subjected to the MTT viability test as previously reported, and results were expressed as a percentage of cell viability with respect to control cell viability (100% of viability). Cell morphology after 24 h of incubation with S and D extracts and various types of liposomes was evaluated by microscopic analysis with a ZOE™ Fluorescent Cell Imager (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.13. Statistical analysis

All data are reported as mean ± standard deviation (SD). Statistical analyses of physicochemical data were carried out using SPSS 20 software (Statistical Package for Social Sciences). Differences among groups were evaluated by applying the one-way analysis of variance test (ANOVA) followed by the Duncan's post hoc test. Biological assay data were analysed using GraphPad INSTAT software (GraphPad Software, San Diego, CA). Comparisons between two groups were performed using an unpaired Student's t-test with Welch's correction, while multiple group comparisons were assessed by one–way ANOVA followed by the Bonferroni post hoc test. In all cases, differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Characterisation of Opuntia extracts (UV–Vis, NMR and ESI-MS)

UV-Vis spectroscopy was used to compare the visible absorption characteristics of the *Opuntia* extracts and to monitor the pigment stability during liposome formulation. The spectra of both OpS and OpD exhibited a prominent absorption band at 538 nm, attributed to betacyanins responsible for the characteristic red/purple colouration of Opuntia (Fig. S1) [31]. A significant difference ($p < 0.001$) in chromophoric content between the two varieties was observed with higher yields in var. *dillenii* (1700 ± 12 mg/100 g) compared to var. *stricta* (992 ± 10 mg/100 g) [45]. UV–Vis spectra were recorded before and after ultrasonic treatment to evaluate whether sonication affected the extract chromophoric profile. After 30 min of sonication, a small decrease in absorbance was detected, while peak position and spectral shape remained unchanged, indicating preservation of the chromophoric properties (Fig. S2). These results suggested that sonication does not significantly affect the optical features of the extract, supporting its use for encapsulation into DOPC liposomes. The use of water as extraction medium in the present study was consistent with previous reports indicating enhanced recovery of polar compounds, including betalain components from *Opuntia* fruits [45,46]. This behaviour has been attributed to the ability of water to disrupt hydrogen-bonding interactions between betalains and other components of the plant matrix, facilitating their release. Aqueous extraction represents a simple and effective approach to recover water-soluble phytochemicals from the fruit matrix [37,47]. As confirmed by NMR analysis of OpD and OpS extracts, this extraction strategy yielded complex mixtures of water-soluble compounds. The ^{1}H NMR spectra displayed dominant signals arising from sugars (glucose, fructose, sucrose) together with several low-molecular-weight metabolites, including organic acids and amino acids (Fig. 1). Notable differences were observed in the carbohydrate region, with sucrose signals present in OpS but absent in OpD. Furthermore, in the downfield region, weak resonances were detected at approximately 8.2, 7.0 and 6.2 ppm, compatible with proton resonances reported for betacyanin pigments [48–50]. The same signals appeared less intense in the OpS spectrum than in OpD, suggesting a lower relative contribution of betacyanin-related components in OpS. OpS and OpD extracts were also characterised with ESI -MS. ESI-MS analysis revealed intense ions in the *m/z* 590–600 region, particularly in OpD extracts (Fig. S3). The dominant ion detected at *m/z* 593.16 is consistent with betalain-derived compounds previously reported in cactus fruits, including decarboxylated phyllocactin or isophyllocactin derivatives.

Both extracts show signals in the typical mass range of betacyanin compounds in agreement with previous ESI-MS/MS studies reporting the identification of betacyanin pigments in cactus fruit extracts [51]. In OpD, the main ions are observed at *m/z* 589.23 and 593.16, while OpS is dominated by the ion at *m/z* 593.16, with a minor contribution at *m/z* 589.14 [51]. The higher abundance of different ions in OpD is in agreement with the UV–Vis, NMR and spectrophotometric results, supporting a greater relative abundance of betalain-related compounds in this variety [52].

3.2. Characterisation of empty and extract loaded liposomes

Among the encapsulation strategies explored to improve stability of plant extracts under physiological conditions and to enable controlled release [53–56], nanoscale liposomes (<200 nm) are particularly attractive, as their size favours cellular interaction and uptake [57–62].

To compare the liposomes physicochemical properties under conditions relevant for the encapsulation of the extracts [13], empty DOPC liposomes (Lip) and liposomes loaded with extracts from var. *stricta* (OpS-Lip) and var. *dillenii* (OpD-Lip) were prepared in three different media: Milli-Q water, 50 mM phosphate solution, and 50 mM acetate buffer. The formulation was carried out at pH 5.0, selected as a

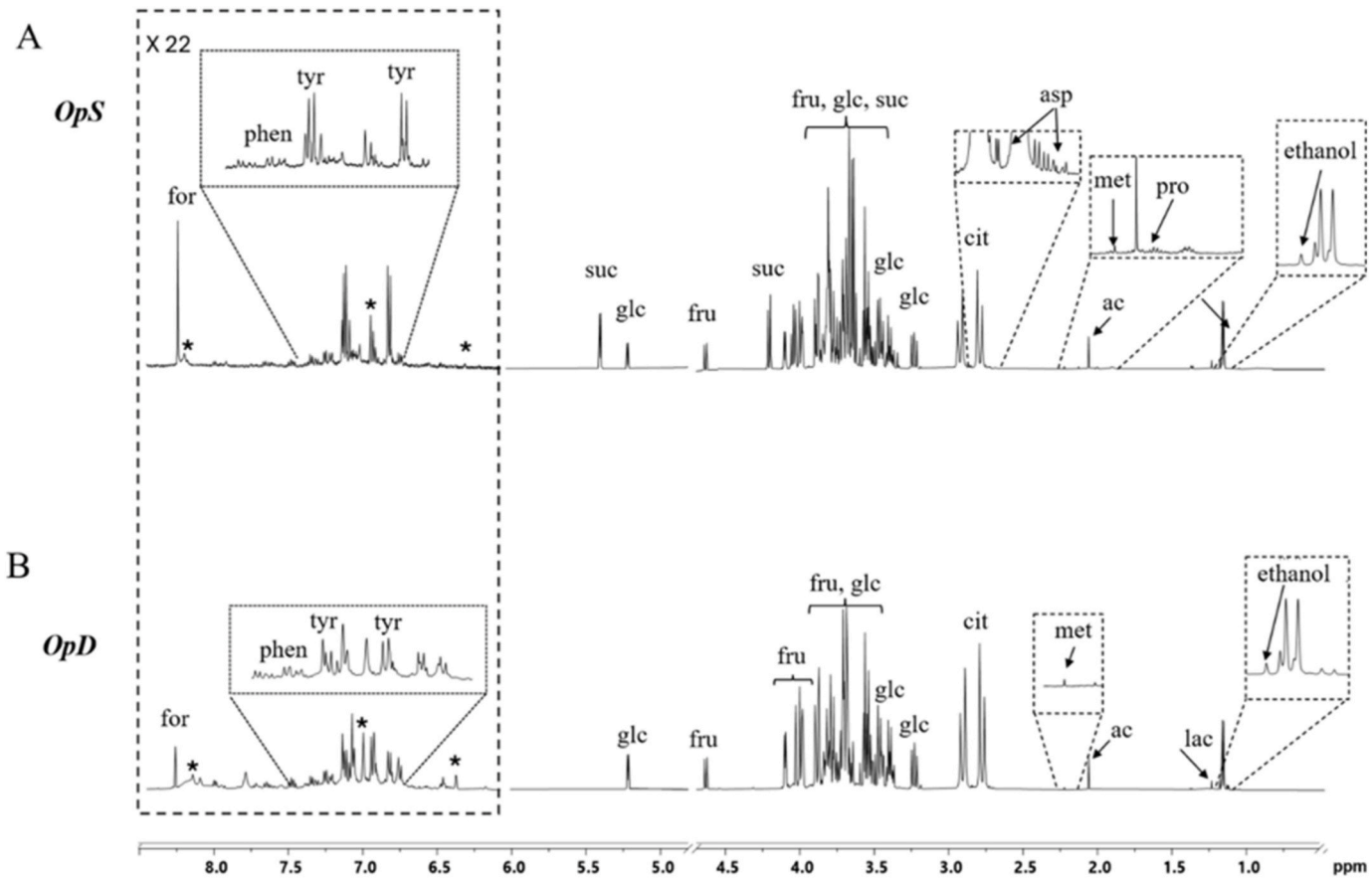

Fig. 1. [1]H NMR spectra of aqueous extract obtained from *O. stricta* fruit juice: var. *stricta* (OpS, A) and var. *dillenii* (OpD, B). Asterisks indicate weak resonances assigned to betacyanin-related structures based on literature data [48]. Abbreviations: ac, acetate; asp, aspartate; cit, citrate; for, formate; fru, fructose; glc, glucose; lac, lactate; met, methionine; phen, phenylalanine; pro, proline; suc, sucrose; tyr, tyrosine.

compromise between maintaining the stability of the extract during preparation and ensuring suitable conditions for liposome formation [19,37]. Mildly acidic conditions are indeed known to favour the preservation of betalains, while also supporting stable bilayer organisation. It is worth noting that phosphate exhibits negligible buffering capacity at pH 5.0, as this value lies outside its effective buffering range ($pK_{a2} \approx 7.2$). For clarity and to maintain consistent terminology across formulations, we refer to this medium as "phosphate solution" throughout the manuscript.

The physicochemical properties of the liposomes were found to depend on both formulation type and electrolyte composition (Table S1 and Fig. 2). Lip showed clear size differences depending on the buffer, indicating that the medium composition affects liposome formation. In particular, formulations prepared in water showed smaller hydrodynamic diameters (31 ± 3 nm) compared to those in phosphate (61 ± 7 nm) and acetate buffer (48 ± 5 nm). These size differences were accompanied by high PDI values (0.58) and nearly neutral zeta potential in phosphate solution ($+0.2 \pm 0.1$ mV). In the case of OpS-Lip, D_H values were relatively consistent across the different media (55–60 nm), with slightly lower PDI values observed in acetate buffer (0.39), indicating

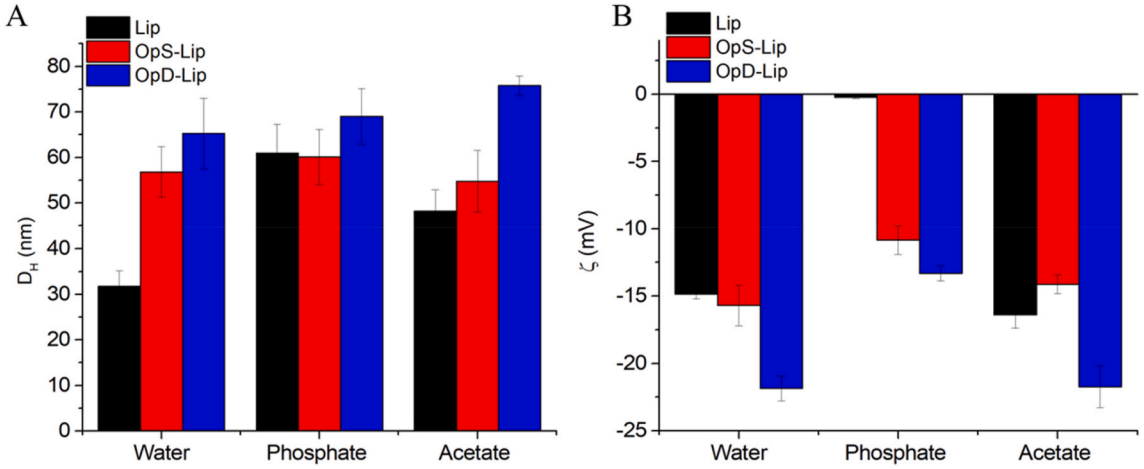

Fig. 2. (A) Hydrodynamic diameter (D_H) and (B) zeta potential (ζ) of empty DOPC liposomes (Lip) and DOPC liposomes loaded with the aqueous extracts of *O. stricta* var. *stricta* (OpS-Lip) and var. *dillenii* (OpD-Lip). Measurements were performed in water, 50 mM phosphate solution (pH 5.0), and 50 mM acetate buffer (pH 5.0).

improved size homogeneity. Zeta potential values remained moderately negative (-14 ± 1 to -16 ± 1 mV), supporting a reasonable degree of colloidal stability. Overall, the presence of the extract reduced the differences in size observed between preparation solution media. Conversely, OpD-Lip exhibited a more pronounced dependence on the formulation medium. While comparable sizes were observed in water and phosphate solution (65 ± 8 nm and 69 ± 6 nm respectively), a marked increase in hydrodynamic diameter was detected in acetate buffer (76 ± 3 nm), as reported in Table S1, accompanied by a slightly more negative ζ potential (-22 ± 2 mV). This behaviour may reflect ion-specific effects at the DOPC/water interface with $H_2PO_4^-$ modulating lipid headgroup hydration and bilayer packing differently from acetate [63].

The D_H data of liposomes prepared in acetate buffer were further supported by TEM analysis performed through negative stain technique (Fig. 3) [64]. TEM images revealed spherical structures for both empty Lip and OpD-Lip and OpS-Lip formulations, with particle sizes ranging from 70 to 120 nm. These dimensions are in good agreement with DLS measurements. No major morphological differences were observed between unloaded and loaded liposomes. Samples prepared in phosphate solution (Fig. S4) showed similar features when compared to those in acetate buffer, except for OpS-Lip, which does not exhibit clearly defined nanostructures (Fig. S4C). The predominantly water soluble extract molecules are expected to reside mainly in the aqueous liposome core without substantially perturbing the DOPC bilayer. Therefore, the moderate loading-dependent changes detected by DLS did not translate into clearly distinguishable alterations of the overall spherical morphology [21,64].

3.3. Encapsulation efficiency and loading capacity of liposomes

Encapsulation efficiency (EE%) and loading capacity (LC%) were evaluated to assess the performance of the liposomal formulations in different media [65,66]. EE% represents the percentage of the

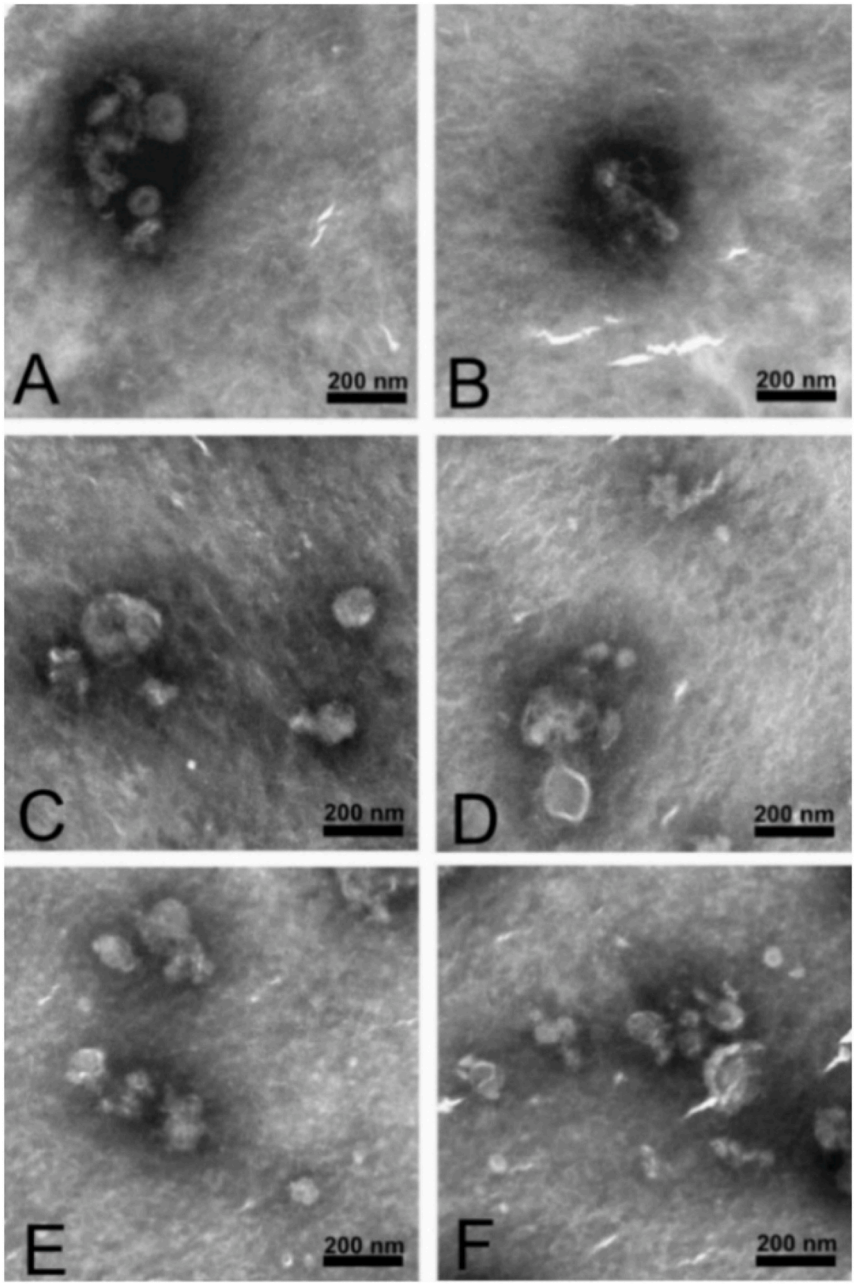

Fig. 3. TEM images of Lip (A-B), OpD-Lip (C-D) and OpS-Lip (E-F) samples stained with phosphotungstic acid (PTA) 2% prepared in acetate buffer 50 mM pH 5.0.

chromophoric fraction retained within the liposomal formulation relative to the initial amount used (Eq. (2)), whereas LC% expresses the amount of encapsulated chromophoric fraction with respect to the total liposome mass (Eq. (3)). Since the aqueous extracts consist of complex mixtures of water-soluble metabolites, encapsulation, stability, and release studies were monitored through the chromophoric fraction of the extracts, quantified spectrophotometrically at 538 nm and expressed as betacyanin equivalents (Fig. S5). Both EE and LC parameters were affected by the formulation medium (Table S2). In particular, liposomes prepared in acetate buffer showed the highest encapsulation efficiencies (up to 61.3%) and markedly higher loading capacities (up to 22.6%) compared to the formulation obtained in phosphate solution. Acetate buffer also favoured the highest loading capacity, confirming its strong effect on encapsulation. The higher EE and LC values observed in acetate buffer may reflect the more homogeneous vesicle organisation shown above, which could promote retention of the extract chromophoric fraction within DOPC liposomes. Conversely, the profile observed in liposomes formulated in phosphate solution may reduce the ability of the formulation to retain the chromophoric fraction. The different encapsulation efficiencies obtained for OpS and OpD indicate that the performance of liposomal formulations is influenced not only by the properties of the lipid carrier, but also by the chemical composition of the encapsulated plant extract [23]. Taken together, these results demonstrate that the physicochemical properties of DOPC liposomes depend on both the formulation medium and extract composition. In the absence of the extracts, phosphate produced larger and more polydisperse empty liposomes than water used as formulation medium, whereas acetate showed an intermediate behaviour. These differences are consistent with specific effects on phosphocholine headgroup and bilayer packing. Extract loading further partially modified these medium-dependent effects: OpS-Lip maintained similar sizes across the investigated media, while OpD-Lip showed larger vesicles and a more negative ζ potential in acetate, suggesting extract interactions at the bilayer interface. Notably, the improved size homogeneity and well-defined morphology of acetate formulations were accompanied by the highest encapsulation efficiency and loading capacity. Therefore, these findings indicate that the formulation medium acts as an active parameter controlling vesicle organization, interfacial properties and cargo encapsulation.

3.4. In-vitro release from OpS-Lip and OpD-Lip

The in-vitro release kinetics of the encapsulated extracts were investigated by dialysis in phosphate-buffered saline (PBS 10 mM at pH 7.4) at 37 °C to mimic physiological conditions. The release was monitored spectrophotometrically at 538 nm and expressed as betacyanin equivalents. As shown in Fig. S6 and summarized in Table 1, both acetate-formulated OpS-Lip and OpD-Lip showed an initial rapid release followed by a slower, sustained phase, typical of encapsulated systems [67–69]. The maximal amount of extract released (A_{max}) was slightly higher for OpS-Lip (28.4 ± 0.5%) compared to OpD-Lip (25.9 ± 0.4%), although the difference is modest. Both formulations showed the same release rate constant (k = 0.26 h^{-1}), indicating similar release kinetics of the two extracts. For acetate-formulated liposomes, encapsulation slowed the diffusion of the extract chromophoric fraction, resulting in a more gradual and sustained release over time.

Compared with acetate-formulated liposomes, free OpD and OpS extracts exhibited higher rate constants (0.50 and 0.35 h^{-1}, respectively) and higher A_{max} values (60.0% and 49.1%). Encapsulation in acetate-formulated DOPC liposomes reduced both parameters, consistent with a more gradual release and greater retention of the chromophoric fraction. In contrast, phosphate-formulated liposomes showed higher rate constants (0.60 h^{-1} for OpS-Lip and 0.79 h^{-1} for OpD-Lip) but lower A_{max} values (15.2% and 24.4%, respectively), indicating rapid diffusion followed by substantial retention. Overall, the formulation medium modulated both the kinetics and extent of extract release from DOPC liposomes.

Table 1
Kinetic parameters obtained by fitting the release profiles of free OpD and OpS extracts and of the corresponding DOPC liposomal formulations prepared in phosphate or acetate media according to Eq. (6).

Samples	Formulation medium	k (h^{-1})	A_{max} (%)	R [2]
Free OpD	-	0.50	60.0	0.97
Free OpS	-	0.35	49.1	0.99
OpS-Lip	Phosphate	0.60	15.2	0.99
	Acetate	0.26	28.4	0.99
OpD-Lip	Phosphate	0.79	24.4	0.99
	Acetate	0.26	25.9	0.99

3.5. Storage stability of free and encapsulated extract

The stability of empty and extract liposomes was evaluated by monitoring changes in D_H, PDI, and ζ potential during storage at 4 °C (Fig. 4).

All formulations maintained sizes below 100 nm during storage, with no signs of aggregation. The stability of both free and encapsulated betalains was further assessed by monitoring dye retention over time using UV-Vis spectroscopy (Table S3). At 4 °C, liposomal encapsulation improved the stability of betalain chromophoric fraction compared to the corresponding free extracts. In particular, OpS-Lip retained 78.0% of the initial betalain concentration after 21 days, whereas the free OpS extract retained 58.8%. Differently, for OpD formulations, comparable retention values were observed for free and encapsulated extracts (68.3% and 70.0%, respectively), indicating a less pronounced protective effect of encapsulation in this system. Stability data confirmed the protective role of liposomal encapsulation, particularly under refrigerated conditions with both free and encapsulated extracts showing higher pigment retention at 4 °C in agreement with previous reports on fruit extracts liposomal systems [38,54]. Within this context, our findings identify the preparation medium at pH 5.0 as a key parameter in the physicochemical design of DOPC liposomes loaded with *Opuntia* extracts. Although no trend in liposome size was observed across all formulations, clear formulation dependent effects were identified. In particular, OpS-Lip prepared in acetate (50 mM, pH 5.0) were associated with smaller sizes and lower PDI values than those prepared in phosphate solution under the same conditions, indicating improved dispersion homogeneity. These differences were accompanied by higher encapsulation efficiency and loading capacity, suggesting a more favourable retention of the chromophoric fraction within acetate-based formulations, a property that is particularly advantageous for the delivery of chemically labile plant derived compounds such as *Opuntia* extracts [20,70].

3.6. Cytotoxicity of loaded liposomes on HaCaT and SH-SY5Y cells

Cultured human HaCaT cells, being physiologically similar to normal human keratinocytes, represent a cell model widely used to assess the biocompatibility (cytotoxicity and proliferative effects) in human epidermal cells and skin pathologies, providing a reliable platform for evaluating candidate topical agents [44,71]. Undifferentiated SH-SY5Y human neuroblastoma cells (neuroblast-like cells), characterised by polygonal cell bodies and short processes, are widely used as an in vitro neuroblastoma-derived cancer cell model and are suitable for evaluating neuroblastoma-targeted therapeutic strategies, including the anticancer activity of natural compounds and nanoformulations [43,72]. The biological effects of free and encapsulated extracts prepared in the two different media were evaluated by MTT assay, measuring cell metabolic activity as an indicator of viability. Fig. 5 shows the viability of HaCaT and SH-SY5Y cells after 24 h exposure to increasing concentrations (0.05-5 mg/mL) of OpS (Fig. 5A) and OpD (Fig. 5B) extracts. Cell viability is expressed as a percentage relative to untreated control cells

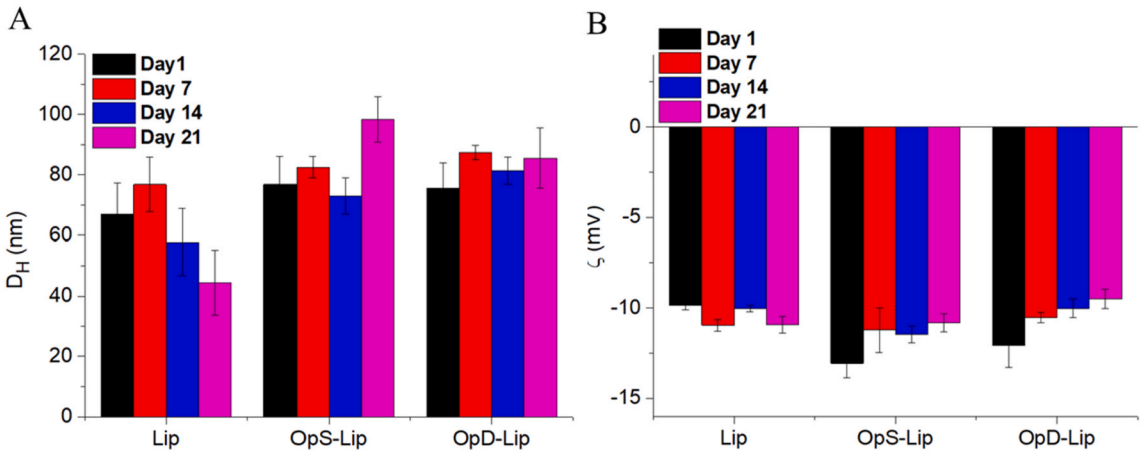

Fig. 4. A) D_H and B) zeta potential of Lip, OpS-Lip and OpD-Lip in acetate buffer (pH 5, 50 mM) stored at 4 °C.

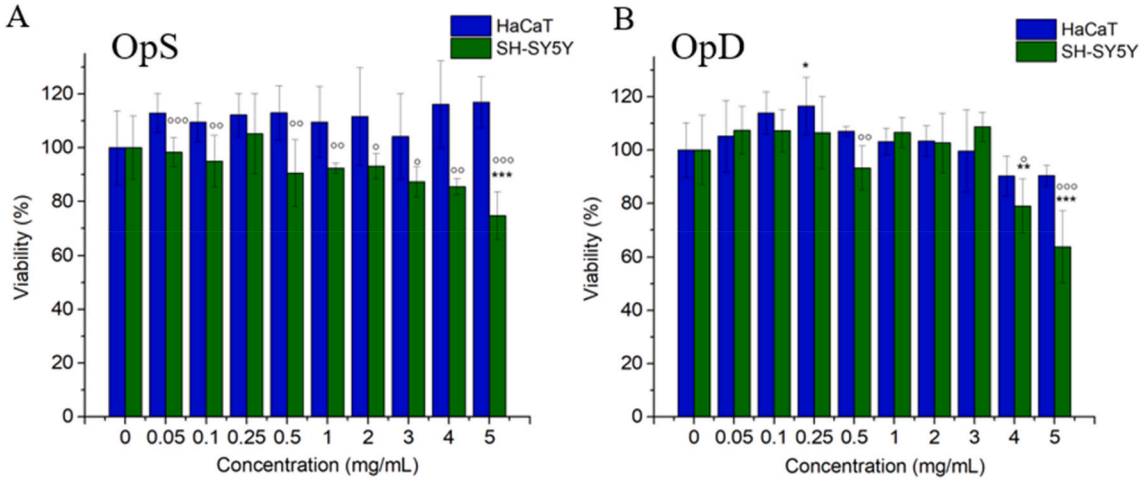

Fig. 5. Viability, expressed as % of the control (untreated cells, 0), induced by incubation for 24 h with different concentrations (from 0.05 to 5 mg/mL) of OpS (A) and OpD (B) extracts in human HaCaT keratinocytes and SH-SY5Y cells (MTT assay). Data are presented as mean and standard deviation (SD) (n = 9). For each series: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ versus control cells (0). For each concentration: °°° = $p < 0.001$, °° = $p < 0.01$, ° = $p < 0.05$ for SH-SY5Y cells versus HaCaT cells.

(set at 100%). As shown, treatment with OpS extract did not induce cytotoxic effects in HaCaT cells over the tested concentration range, with cell viability remaining comparable to, or slightly higher than, control values. In contrast, SH-SY5Y cells exhibited a concentration-dependent reduction in viability, reaching a significant decrease of approximately 25% at 5 mg/mL. Similarly, treatment with OpD extract did not significantly affect HaCaT cell viability at any tested concentration (Fig. 5B).

In SH-SY5Y cells, however, OpD extract induced a significant reduction in viability at the highest concentrations tested (4 and 5 mg/mL), with decreases of approximately 21% and 36%, respectively (Fig. 5B). These results suggest that the biological effects are influenced by differences in extract composition between the two varieties, with OpD showing a stronger effect on SH-SY5Y cell viability than OpS. This behaviour is consistent with compositional differences identified by UV–Vis, ESI-MS and ^{1}H NMR analyses, which indicated a higher relative abundance of chromophoric components in OpD extracts. Phase-contrast microscopy confirmed the absence of morphological alterations in HaCaT cells, whereas reduced cell density and cellular debris were observed in SH-SY5Y cells treated with the highest extract concentrations (Fig. S7). The effects of loaded liposomal formulations were evaluated at a concentration of 50 µg/mL (Fig. 6). No significant reduction in HaCaT cell viability was observed following treatment with Lip, OpS-Lip and OpD-Lip, regardless of preparation medium or encapsulated extract. Consistently, no changes in cell morphology or density were detected by phase-contrast microscopy (Fig. S8). In SH-SY5Y cells, unloaded liposomes and phosphate formulations did not significantly affect cell viability. In contrast, acetate-buffered OpS-Lip and OpD-Lip induced a modest but significant reduction in cell viability (approximately 14%), accompanied by a decrease in cell density (Fig. S8), highlighting a selective and formulation-dependent cytotoxicity effect associated with the buffer used for liposome preparation. Despite their greater cargo retention and slower release compared with the free extracts, acetate-formulated liposomes retained a measurable biological response in SH-SY5Y cells while remaining cytocompatible towards HaCaT cells. Therefore, the added value of encapsulation lies in modulating extract availability without abolishing its biological activity, rather than in simply increasing cytotoxicity. Overall, the results highlight a combined contribution of formulation characteristics and extract composition to the observed cellular responses, underscoring the complexity of plant extract-loaded liposomal systems.

4. Conclusions

In this study, DOPC liposomes were investigated for the delivery of chemically labile plant-derived compounds, with particular emphasis on

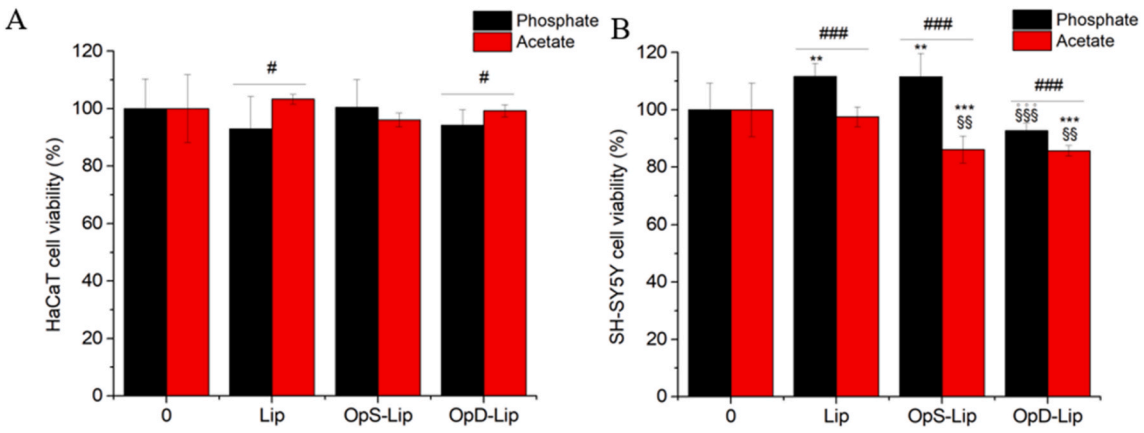

Fig. 6. Viability, expressed as % of the control (untreated cells, 0), induced by incubation for 24 h with different types of unloaded (Lip) and loaded (OpS-Lip and OpD-Lip) liposomes (50 μg/mL) prepared in acetate and phosphate medium, in human HaCaT keratinocytes (A) and SH-SY5Y cells (B) (MTT assay). Data are presented as mean and standard deviation (SD) (n = 9). For each series: *** = $p < 0.001$, ** = $p < 0.01$ versus control cells (0); §§§ = $p < 0.001$, §§ = $p < 0.01$ versus cells treated with Lip; °°° = $p < 0.001$ versus cells treated with OpS-Lip. ### = $p < 0.001$, # = $p < 0.05$ for liposomes prepared in acetate versus those in phosphate.

the role of the formulation electrolyte as an active design parameter. A systematic comparison of water, phosphate, and acetate-based formulations at pH 5.0 demonstrated that the ionic composition of the preparation medium strongly influences the physicochemical properties of lipid-based biomaterials loaded with *O. stricta* extracts. Among the investigated systems, acetate-based formulations exhibited improved structural homogeneity together with enhanced encapsulation efficiency and loading capacity, while maintaining nanometric dimensions and preventing aggregation under the investigated conditions. These formulation dependent behaviours were also reflected in the release performance, with acetate-based formulations showing sustained and biphasic profiles under physiological conditions. Correlations between physicochemical properties and in vitro cellular response revealed that biological performance is directly linked to the organization of the liposomal biomaterial. While all formulations were cytocompatible towards HaCaT keratinocytes, acetate formulated extract loaded liposomes induced a moderate and formulation dependent reduction in SH-SY5Y neuroblastoma cell viability. Although the molecular basis of these effects was not directly investigated, the results highlight the importance of the formulation environment in determining lipid-based biomaterials performance. Overall, these findings demonstrate that electrolyte specific formulation conditions should be regarded as intrinsic design variables rather than passive preparation parameters in lipid-based biomaterials. By establishing clear relationships between formulation environment, structural properties, and cellular response, this work provides practical guidelines for the rational development of DOPC-based carriers for unstable plant derived bioactives.

CRediT authorship contribution statement

Ahlem Ben Saad: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Michela Tozzi:** Methodology, Formal analysis, Investigation, Writing – review & editing, Data curation. **Flaminia Cesare Marincola:** Writing – review & editing, Visualization, Resources, Data curation, Conceptualization. **Franca Piras:** Methodology, Formal analysis, Data curation. **Antonella Rosa:** Writing – original draft, Methodology, Formal analysis, Data curation. **Marco Piludu:** Methodology, Formal analysis. **Valeria Sogos:** Writing – review & editing, Supervision, Resources. **Fatma Saad:** Methodology. **Lotfi Achour:** Supervision. **Andrea Salis:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Cristina Carucci:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

A.B.S., F.S. and L.A. thank the Ministry of Higher Education and Scientific Research and the Doctoral School of Biological Sciences and Biotechnology at the Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia, for their financial support. The authors gratefully acknowledge FIR 2025 for financial support and CESAR (Centro Servizi di Ateneo per la Ricerca), University of Cagliari, Italy, for access to the JEM 1400 Plus TEM microscope and the LTQ Orbitrap Elite mass spectrometer.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2026.108857.

Data availability

Data will be made available on request.

References

[1] S. Mallick, J.S. Choi, Liposomes: versatile and biocompatible nanovesicles for efficient biomolecules delivery, J. Nanosci. Nanotechnol. 14 (1) (2014) 755–765, https://doi.org/10.1166/jnn.2014.9080.
[2] G. Prevete, E. Donati, A.P. Ruggiero, S. Fardellotti, L. Lilla, V. Ramundi, I. Nicoletti, F. Mariani, M. Mazzonna, Encapsulation of Olea Europaea leaf polyphenols in liposomes: a study on their antimicrobial activity to turn a byproduct into a tool to treat bacterial infection, ACS Appl. Mater. Interfaces 16 (50) (2024) 68850–68863, https://doi.org/10.1021/acsami.4c13302.
[3] A. Gürses, M. Açıkyıldız, K. Güneş, E. Şahin, Natural dyes and pigments in food and beverages, in: Renewable Dyes and Pigments, Elsevier, 2024, pp. 49–76, https://doi.org/10.1016/B978-0-443-15213-9.00013-2.
[4] A. Negi, Natural dyes and pigments: sustainable applications and future scope, Sustain. Chem. 6 (3) (2025) 23, https://doi.org/10.3390/suschem6030023.
[5] M.R. Repon, T. Islam, T.K. Paul, S. Jurkonienė, A. Haji, S. Shukhratov, G.F.I. Toki, Natural dyes in textile printing: parameters, methods, and performance, Environ. Sci. Pollut. Res. 31 (35) (2024) 47552–47583, https://doi.org/10.1007/s11356-024-34424-1.
[6] N. Martins, C.L. Roriz, P. Morales, L. Barros, I.C.F.R. Ferreira, Food colorants: challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices, Trends Food Sci. Technol. 52 (2016) 1–15, https://doi.org/10.1016/j.tifs.2016.03.009.

[7] K. Mueangnak, H. Kitwetcharoen, S. Thanonkeo, P. Klanrit, J. Apiraksakorn, P. Klanrit, P. Klanrit, P. Thanonkeo, Enhancing betalains production and antioxidant activity in Celosia argentea cell suspension cultures using biotic and abiotic elicitors, Sci. Rep. 15 (1) (2025) 376, https://doi.org/10.1038/s41598-024-83096-x.
[8] N.P. Nirmal, S. Medhe, M. Dahal, P. Koirala, S. Nirmal, F. Al-Asmari, B. Xu, Betalains protect various body organs through antioxidant and anti-inflammatory pathways, Food Sci. Hum. Wellness 13 (3) (2024) 1109–1117, https://doi.org/10.26599/FSHW.2022.9250093.
[9] R. Abedi-Firoozjah, E. Parandi, M. Heydari, A. Kolahdouz-Nasiri, M. Bahraminejad, R. Mohammadi, M. Rouhi, F. Garavand, Betalains as promising natural colorants in smart/active food packaging, Food Chem. 424 (2023) 136408, https://doi.org/10.1016/j.foodchem.2023.136408.
[10] M.G. Miguel, Betalains in some species of the Amaranthaceae family: a review, Antioxidants 7 (4) (2018) 53, https://doi.org/10.3390/antiox7040053.
[11] A. Kumorkiewicz-Jamro, T. Świergosz, K. Sutor, A. Spórna-Kucab, S. Wybraniec, Multi-colored shades of betalains: recent advances in Betacyanin chemistry, Nat. Prod. Rep. 38 (12) (2021) 2315–2346, https://doi.org/10.1039/D1NP00018G.
[12] R. Castellar, J.M. Obón, M. Alacid, J.A. Fernández-López, Color properties and stability of betacyanins from opuntia fruits, J. Agric. Food Chem. 51 (9) (2003) 2772–2776, https://doi.org/10.1021/jf021045h.
[13] K. Sutor-Świeży, J. Proszek, Ł. Popenda, S. Wybraniec, Influence of citrates and EDTA on oxidation and decarboxylation of betacyanins in red beet (Beta vulgaris L.) betalain-rich extract, Molecules 27 (24) (2022) 9054, https://doi.org/10.3390/molecules27249054.
[14] G. Molina, A. Hernández-Martínez, M. Cortez-Valadez, F. García-Hernández, M. Estevez, Effects of Tetraethyl orthosilicate (TEOS) on the light and temperature stability of a pigment from Beta Vulgaris and its potential food industry applications, Molecules 19 (11) (2014) 17985–18002, https://doi.org/10.3390/molecules191117985.
[15] S. Showkat, A. Rafiq, R. Richa, Q. Sidique, A. Hussain, U.C. Lohani, O. Bhat, S. Kumar, Stability enhancement of betalain pigment extracted from Celosia Cristata L. flower through copigmentation and degradation kinetics during storage, Food Chem. X 26 (2025) 102312, https://doi.org/10.1016/j.fochx.2025.102312.
[16] C. Tan, Y. Dadmohammadi, M.C. Lee, A. Abbaspourrad, Combination of copigmentation and encapsulation strategies for the synergistic stabilization of anthocyanins, Compr. Rev. Food Sci. Food Saf. 20 (4) (2021) 3164–3191, https://doi.org/10.1111/1541-4337.12772.
[17] B. Armendáriz-Barragán, N. Zafar, W. Badri, S.A. Galindo-Rodríguez, D. Kabbaj, H. Fessi, A. Elaissari, Plant extracts: from encapsulation to application, Expert Opin. Drug Deliv. 13 (8) (2016) 1165–1175, https://doi.org/10.1080/17425247.2016.1182487.
[18] B.P. Pratama, B.W. Harsanto, M.A. Razi, B.W.K. Wardhani, A.T. Khalisa, A. R. Khairullah, S. Yuliani, B. Triwiyono, L. Leliana, U.F. Handayani, Y.L. Dewi, R. G. Sativa, N. Harimurti, I. Agustinisari, Encapsulated plant extracts as potential clean-label alternatives to synthetic preservatives in carbohydrate-based foods: mechanisms, efficacy, and industrial prospects, Trends Sci. 23 (1) (2025) 11801, https://doi.org/10.48048/tis.2026.11801.
[19] M.I. Khan, Stabilization of betalains: a review, Food Chem. 197 (2016) 1280–1285, https://doi.org/10.1016/j.foodchem.2015.11.043.
[20] M. Tozzi, F. Piras, A. Rosa, M. Piludu, A. Frongia, V. Sogos, C. Carucci, A. Salis, F. Cesare Marincola, Glycine betaine surfactant-based liposomes: physicochemical characterization and cytocompatibility assessment, Colloids Surfaces A Physicochem. Eng. Asp. 727 (2025) 138318, https://doi.org/10.1016/j.colsurfa.2025.138318.
[21] S. Parralejo-Sanz, Á. Balea, T. Requena, M.P. Cano, Development of liposomal systems for the encapsulation of Opuntia Ficus-Indica Var. Blanca buenavista extracts: characterization and in vitro digestion, Food Bioprocess Technol. 18 (7) (2025) 6634–6649, https://doi.org/10.1007/s11947-025-03846-1.
[22] A.-M. Gaber, M. Tsakiri, H. Katifelis, M. Gazouli, C. Demetzos, Preparation, physicochemical evaluation and in vitro toxicity studies of HSPC and HSPC:DMPC stigmasterol-loaded liposomes, J. Liposome Res. 35 (4) (2025) 345–356, https://doi.org/10.1080/08982104.2025.2502928.
[23] X. Wang, C.J. Swing, T. Feng, S. Xia, J. Yu, X. Zhang, Effects of environmental PH and ionic strength on the physical stability of cinnamaldehyde-loaded liposomes, J. Dispersion Sci. Technol. 41 (10) (2020) 1568–1575, https://doi.org/10.1080/01932691.2019.1627887.
[24] D.F. Parsons, C. Carucci, A. Salis, Buffer-specific effects arise from ionic dispersion forces, Phys. Chem. Chem. Phys. 24 (11) (2022) 6544–6551, https://doi.org/10.1039/d2cp00223j.
[25] J. Sabın, G. Prieto, J.M. Ruso, R. Hidalgo-Álvarez, F. Sarmiento, Size and stability of liposomes: a possible role of hydration and osmotic forces, Eur. Phys. J. E 20 (4) (2006) 401–408, https://doi.org/10.1140/epje/i2006-10029-9.
[26] J.U. De Mel, S. Klisch, S. Gupta, G.J. Schneider, Ion-mediated structural discontinuities in phospholipid vesicles, Langmuir 40 (29) (2024) 14990–15000, https://doi.org/10.1021/acs.langmuir.4c01219.
[27] C. Carucci, J. Philipp, J.A. Müller, A. Sudarsan, E. Kostyurina, C.E. Blanchet, N. Schwierz, D.F. Parsons, A. Salis, J.O. Rädler, Buffer specificity of ionizable lipid nanoparticle transfection efficiency and bulk phase transition, ACS Nano 19 (11) (2025) 10829–10840, https://doi.org/10.1021/acsnano.4c14098.
[28] M. Mura, B. Humphreys, J. Gilbert, A. Salis, T. Nylander, Cation and buffer specific effects on the DNA-Lipid interaction, Colloids Surf. B Biointerfaces 223 (2023) 113187, https://doi.org/10.1016/j.colsurfb.2023.113187.
[29] M. Mura, C. Carucci, E. Caddeo, Š. Sovová, M. Piludu, M. Pekař, B. Jachimska, D. F. Parsons, A. Salis, Specific buffer effects on the formation of BSA protein Corona around amino-functionalized mesoporous Silica nanoparticles, J. Colloid Interface Sci. 677 (2025) 540–547, https://doi.org/10.1016/j.jcis.2024.07.258.
[30] A. Salis, L. Cappai, C. Carucci, D.F. Parsons, M. Monduzzi, Specific buffer effects on the intermolecular interactions among protein molecules at physiological PH, J. Phys. Chem. Lett. 11 (16) (2020) 6805–6811, https://doi.org/10.1021/acs.jpclett.0c01900.
[31] I. Gómez-López, G. Lobo-Rodrigo, M.P. Portillo, M.P. Cano, Characterization, stability, and bioaccessibility of betalain and phenolic compounds from Opuntia Stricta Var. Dillenii fruits and products of their industrialization, Foods 10 (7) (2021) 1593, https://doi.org/10.3390/foods10071593.
[32] R. Pulicharla, C. Marques, R.K. Das, T. Rouissi, S.K. Brar, Encapsulation and release studies of strawberry polyphenols in biodegradable chitosan nanoformulation, Int. J. Biol. Macromol. 88 (2016) 171–178, https://doi.org/10.1016/j.ijbiomac.2016.03.036.
[33] J. Viganó, A.A.D. Meirelles, G. Náthia-Neves, A.M. Baseggio, R.L. Cunha, M. R. Maróstica Junior, M.A.A. Meireles, P. Gurikov, I. Smirnova, J. Martínez, Impregnation of passion fruit bagasse extract in alginate aerogel microparticles, Int. J. Biol. Macromol. 155 (2020) 1060–1068, https://doi.org/10.1016/j.ijbiomac.2019.11.070.
[34] M. Thiruvalluvan, B.P. Kaur, A. Singh, S. Kumari, Enhancement of the bioavailability of phenolic compounds from fruit and vegetable waste by liposomal nanocarriers, Food Sci. Biotechnol. 33 (2) (2024) 307–325, https://doi.org/10.1007/s10068-023-01458-z.
[35] G. Vancuylenberg, A. Sadeghpour, A.I.I. Tyler, M. Rappolt, Planar confined water organisation in lipid bilayer stacks of phosphatidylcholine and phosphatidylethanolamine, Soft Matter 19 (27) (2023) 5179–5192, https://doi.org/10.1039/D3SM00387F.
[36] Y. Gu, B.M. Reinhard, Membrane fluidity properties of lipid-coated polylactic acid nanoparticles, Nanoscale 16 (17) (2024) 8533–8545, https://doi.org/10.1039/D3NR06464F.
[37] P.-J. Tsai, C.-H. Sheu, P.-H. Wu, Y.-F. Sun, Thermal and PH stability of Betacyanin pigment of djulis (Chenopodium Formosanum) in Taiwan and their relation to antioxidant activity, J. Agric. Food Chem. 58 (2) (2010) 1020–1025, https://doi.org/10.1021/jf9032766.
[38] X. Lin, B. Li, J. Wen, J. Wu, D. Tang, Y. Yu, Y. Xu, B. Xu, Storage stability and in vitro bioaccessibility of liposomal betacyanins from red pitaya (Hylocereus Polyrhizus), Molecules 27 (4) (2022) 1193, https://doi.org/10.3390/molecules27041193.
[39] F.C. Stintzing, K.M. Herbach, M.R. Mosshammer, R. Carle, W. Yi, S. Sellappan, C. C. Akoh, R. Bunch, P. Felker, Color, betalain pattern, and antioxidant properties of cactus pear (opuntia Spp.) clones, J. Agric. Food Chem. 53 (2) (2005) 442–451, https://doi.org/10.1021/jf048751y.
[40] M. Aslan, N. Ertaş, M.K. Demir, Storage stability, heat stability, controlled release and antifungal activity of liposomes as alternative fungal preservation agents, Food Biosci. 51 (2023) 102281, https://doi.org/10.1016/j.fbio.2022.102281.
[41] D.A. Fadeel, M. Fadel, A.I. El-Kholy, A.A. El-Rashedy, E. Mohsen, M.I. Ezzat, M. Y. Issa, Nano-Liposomal beetroot phyto-pigment in photodynamic therapy as a prospective green approach for cancer management: in vitro evaluation and molecular dynamic simulation, Pharmaceutics 16 (8) (2024) 1038, https://doi.org/10.3390/pharmaceutics16081038.
[42] C. Carucci, N. Scalas, A. Porcheddu, M. Piludu, M. Monduzzi, A. Salis, Adsorption and release of sulfamethizole from mesoporous Silica nanoparticles functionalised with triethylenetetramine, Int. J. Mol. Sci. 22 (14) (2021) 7665, https://doi.org/10.3390/ijms22147665.
[43] A. Rosa, F. Pollastro, V. Sogos, F. Piras, Comparative evaluation of cytotoxic and apoptotic effects of natural compounds in SH-SY5Y neuroblastoma cells in relation to their physicochemical properties, Molecules 30 (8) (2025) 1742, https://doi.org/10.3390/molecules30081742.
[44] F. Piras, V. Sogos, F. Pollastro, G. Appendino, A. Rosa, Arzanol, a natural phloroglucinol α-pyrone, protects HaCaT keratinocytes against H2O2 -induced oxidative stress, counteracting cytotoxicity, reactive oxygen Species generation, apoptosis, and mitochondrial depolarization, J. Appl. Toxicol. 44 (5) (2024) 720–732, https://doi.org/10.1002/jat.4570.
[45] C. Betancourt, M.J. Cejudo-Bastante, F.J. Heredia, N. Hurtado, Pigment composition and antioxidant capacity of betacyanins and betaxanthins fractions of Opuntia Dillenii (Ker Gawl) haw cactus fruit, Food Res. Int. 101 (2017) 173–179, https://doi.org/10.1016/j.foodres.2017.09.007.
[46] N. Sanchez-Gonzalez, M.R. Jaime-Fonseca, E. San Martin-Martinez, L.G. Zepeda, Extraction, stability, and separation of betalains from Opuntia Joconostle Cv. Using response surface methodology, J. Agric. Food Chem. 61 (49) (2013) 11995–12004, https://doi.org/10.1021/jf401705h.
[47] M.I. Khan, P. Giridhar, Plant betalains: chemistry and biochemistry, Phytochemistry 117 (2015) 267–295, https://doi.org/10.1016/j.phytochem.2015.06.008.
[48] F.C. Stintzing, J. Conrad, I. Klaiber, U. Beifuss, R. Carle, Structural investigations on betacyanin pigments by LC NMR and 2D NMR spectroscopy, Phytochemistry 65 (4) (2004) 415–422, https://doi.org/10.1016/j.phytochem.2003.10.029.
[49] M. Barkociová, J. Tóth, K. Sutor, N. Drobnicka, S. Wybraniec, B. Dudík, A. Bilková, S. Czigle, Betalains in edible fruits of three Cactaceae Taxa—Epiphyllum, hylocereus, and Opuntia—their LC-MS/MS and FTIR identification and biological activities evaluation, Plants 10 (12) (2021) 2669, https://doi.org/10.3390/plants10122669.
[50] M. Chaari, S. Akermi, K. Elhadef, M. Ennouri, L. Jlaiel, M.A. Mosrati, L. Mellouli, W. Elfalleh, T. Varzakas, S. Smaoui, Betalains from Opuntia Stricta peels: UPLC-MS/MS metabolites profiling, computational investigation, and potential

applicability as a raw meat colorant, Heliyon 10 (21) (2024) e39784, https://doi.org/10.1016/j.heliyon.2024.e39784.
[51] J.A. Araujo-León, I. Sánchez-del Pino, L.G. Brito-Argáez, S.R. Peraza-Sánchez, R. Ortiz-Andrade, V. Aguilar-Hernández, In Silico mass spectrometric fragmentation and liquid chromatography with Tandem Mass spectrometry (LC-MS/MS) betalainic fingerprinting: identification of betalains in Red Pitaya, Molecules 29 (22) (2024) 5485, https://doi.org/10.3390/molecules29225485.
[52] R. Bonner, G. Hopfgartner, Annotation of complex mass spectra by multi-layered analysis, Anal. Chim. Acta 1193 (2022) 339317, https://doi.org/10.1016/j.aca.2021.339317.
[53] Q.-H. Tran, H.-K.T. Chu, P.-T. Nguyen, V.-M. Nguyen, Q.-T. Nguyen, C.-D. Tran, T.-D. Nguyen, Double nanoemulsion loading betalains extract of beetroot (beta Vulgaris L.): Ultrasound-assisted synthesis, storage stability, and antioxidant activity, ACS Food Sci. Technol. 3 (12) (2023) 2229–2237, https://doi.org/10.1021/acsfoodscitech.3c00430.
[54] S. Sravan Kumar, A. Singh Chauhan, P. Giridhar, Nanoliposomal encapsulation mediated enhancement of betalain stability: characterisation, storage stability and antioxidant activity of Basella Rubra L. fruits for its applications in vegan gummy candies, Food Chem. 333 (2020) 127442, https://doi.org/10.1016/j.foodchem.2020.127442.
[55] W. Zhang, R. Taheri-Ledari, F. Ganjali, S.S. Mirmohammadi, F.S. Qazi, M. Saeidirad, A. KashtiAray, S. Zarei-Shokat, Y. Tian, A. Maleki, Effects of morphology and size of Nanoscale drug carriers on cellular uptake and internalization process: a review, RSC Adv. 13 (1) (2023) 80–114, https://doi.org/10.1039/D2RA06888E.
[56] C. Bonechi, G. Tamasi, A. Donati, F. Bisozzi, M. Baglioni, M. Andreassi, F. Ietta, G. Leone, A. Magnani, C. Rossi, Cationic liposomes as carriers of natural compounds from plant extract, Biophys. Chem. 318 (2025) 107381, https://doi.org/10.1016/j.bpc.2024.107381.
[57] J. Menge, C. Yang, D.A. Weitz, K. Jahnke, Engineering the biophysical properties of lipid nanostructures for drug delivery, Commun. Mater. 7 (1) (2026) 25, https://doi.org/10.1038/s43246-026-01073-5.
[58] N. Hoshyar, S. Gray, H. Han, G. Bao, The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction, Nanomedicine 11 (6) (2016) 673–692, https://doi.org/10.2217/nnm.16.5.
[59] A.U. Andar, R.R. Hood, W.N. Vreeland, D.L. DeVoe, P.W. Swaan, Microfluidic preparation of liposomes to determine particle size influence on cellular uptake mechanisms, Pharm. Res. 31 (2) (2014) 401–413, https://doi.org/10.1007/s11095-013-1171-8.
[60] S. Dadpour, A. Mehrabian, M. Arabsalmani, E. Mirhadi, A. Askarizadeh, M. Mashreghi, M.R. Jaafari, The role of size in PEGylated liposomal doxorubicin biodistribution and anti-tumour activity, IET Nanobiotechnol. 16 (7–8) (2022) 259–272, https://doi.org/10.1049/nbt2.12094.
[61] C. Bonechi, F.F. Mahdizadeh, L. Talarico, S. Pepi, G. Tamasi, G. Leone, M. Consumi, A. Donati, A. Magnani, Liposomal encapsulation of Citicoline for ocular drug delivery, Int. J. Mol. Sci. 24 (23) (2023) 16864, https://doi.org/10.3390/ijms242316864.
[62] Q. Chen, Y. Huang, C. Wu, X. Pan, C. Yu, J. Wang, W. Wang, Z. Huang, Charge effects: influence of surface charge on protein Corona adsorption behavior on liposomal formulations, Pharmaceutics 18 (1) (2026) 76, https://doi.org/10.3390/pharmaceutics18010076.
[63] X. He, A.G. Ewing, Hofmeister series: from aqueous solution of biomolecules to single cells and nanovesicles, Chembiochem 24 (9) (2023), https://doi.org/10.1002/cbic.202200694.
[64] L.E. Franken, E.J. Boekema, M.C.A. Stuart, Transmission electron microscopy as a tool for the characterization of soft materials: application and interpretation, Adv. Sci. 4 (5) (2017), https://doi.org/10.1002/advs.201600476.
[65] Y. Qin, Medical textile materials with drug-releasing properties, in: Medical Textile Materials, Elsevier, 2016, pp. 175–189, https://doi.org/10.1016/B978-0-08-100618-4.00013-3.
[66] A. Gonzalez Gomez, S. Syed, K. Marshall, Z. Hosseinidoust, Liposomal nanovesicles for efficient encapsulation of staphylococcal antibiotics, ACS Omega 4 (6) (2019) 10866–10876, https://doi.org/10.1021/acsomega.9b00825.
[67] C. Jaudoin, M. Maue Gehrke, I. Grillo, F. Cousin, M. Ouldali, A.-A. Arteni, E. Ferrary, F. Siepmann, J. Siepmann, F. Simelière, A. Bochot, F. Agnely, Release of liposomes from hyaluronic acid-based hybrid systems: effects of liposome surface and size, Int. J. Pharm. 648 (2023) 123560, https://doi.org/10.1016/j.ijpharm.2023.123560.
[68] A. Jain, S.K. Jain, In vitro release kinetics model fitting of liposomes: an insight, Chem. Phys. Lipids 201 (2016) 28–40, https://doi.org/10.1016/j.chemphyslip.2016.10.005.
[69] Y. Wang, S. Tu, A.N. Pinchuk, M.P. Xiong, Active drug encapsulation and release kinetics from hydrogel-in-liposome nanoparticles, J. Colloid Interface Sci. 406 (2013) 247–255, https://doi.org/10.1016/j.jcis.2013.05.081.
[70] A. Luo, Q. Zhao, X. Zhang, J. Chen, Q. Liu, R. Huang, Q. Zheng, S. Zhong, Delivery systems for betalains: from stabilization strategies to precision nutrition applications, Food Res. Int. 226 (2026) 118095, https://doi.org/10.1016/j.foodres.2025.118095.
[71] A. Pessina, A. Raimondi, A. Cerri, M. Piccirillo, M.G. Neri, C. Croera, P. Foti, E. Berti, High sensitivity of human epidermal keratinocytes (HaCaT) to topoisomerase inhibitors, Cell Prolif. 34 (4) (2001) 243–252, https://doi.org/10.1046/j.0960-7722.2001.00214.x.
[72] O. Abd Alkader Alabrahim, A. Maher Abdeldayem, H.M.E.-S. Azzazy, Enhanced cytotoxic activity of Moringa Oleifera -Loaded pharmacosomes against Neuroblastoma, Nanoscale Adv. 8 (6) (2026) 1851–1870, https://doi.org/10.1039/D5NA00473J.