

# Structure-Dependent Incorporation of Terpenes into Amphiphilic Poly(2-oxazoline) Micelles

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## Research Article

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## Structure-Dependent Incorporation of Terpenes into Amphiphilic Poly(2-oxazoline) Micelles

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### Statements and Declarations

**Conflicts:** A.V.K. has issued and pending patents related to this work and is a co-founder, shareholder, and licensor of technology to DeLAQUA Pharmaceuticals, which develops poly(2-oxazoline) micelle formulations.

### Abstract

Poly(2-oxazoline) (POx) amphiphilic block copolymers are highly effective carriers for poorly water-soluble small molecules, yet structural features that promote drug incorporation remain unclear. Motivated by the unusually high loading of diterpene taxanes in POx micelles, we examined whether shared terpene motifs support solubilization with POx. Representative mono-, di-, and triterpenes,  $\alpha$ -pinene, carnosic acid (CA), carnosol (CARN), squalene, lupeol, betulin, and ursolic acid, were tested for micellization with a well-defined POx triblock copolymer. Only the diterpenes formed small, uniform, and stable micelles. CA and CARN showed the best performance: CA produced reproducible 22–29 nm micelles at a 2/10 (w/w) CA/polymer feed, achieving ~17% loading capacity with quantitative efficiency. Higher feed ratios yielded large, polydisperse aggregates by DLS and TEM. Other terpenes displayed minimal or unstable incorporation. CA-loaded micelles remained colloidally stable for 48 h at room temperature, though CA's chemical stability varied by batch. Lyophilization improved CA stability, enabling 67–89% recovery after reconstitution. pH-dependent studies showed increasing particle size with higher pH for CA/POx micelles, consistent with CA ionization and altered hydrogen bonding. Physiological NaCl suppressed this pH sensitivity for CA, indicating ionic-strength-mediated stabilization. Overall, the rigid diterpene scaffold and ortho-hydroxyl groups appear key for POx incorporation, highlighting CA as a promising candidate for further formulation development.

### Keywords

Amphiphilic triblock Poly(2-oxazoline), terpenes, carnosic acid, polymeric micelles.

## Introduction

The development of universal drug delivery systems capable of overcoming physiological barriers and resisting biochemical degradation remains a central challenge in modern pharmaceutics<sup>1-2</sup>. Such systems must accommodate a wide range of therapeutics, from poorly water-soluble small molecules to biologics, including nucleic acids, proteins, peptides, and antibodies. However, most current existing platforms are constrained by limited drug-loading cargo-loading capacity, immunogenicity, off-target distribution, and dependence on cold-chain infrastructure<sup>3</sup>. Our previous work<sup>4</sup> demonstrated that amphiphilic block copolymers from the poly(2-oxazoline) (POx) family can function as highly efficient carriers for diverse small-molecule therapeutics. POx-based micelles loaded with antitumor agents show markedly enhanced tumor accumulation *in vivo* and favorable pharmacokinetic properties<sup>5</sup>. The safety of several POx micellar formulations, including paclitaxel (PTX)-loaded micelles, has been established in both rodent and non-human primate (NHP) models<sup>6</sup>. Notably, unlike polyethylene glycol (PEG), which can elicit pre-existing anti-PEG antibodies in a subset of patients<sup>7</sup>, POx has not been associated with detectable pre-existing antibody to date.

The objective of this study is to assess the feasibility of using POx micelles for terpene delivery. This direction is motivated by the observation that taxanes, which are diterpenes, exhibit exceptionally high incorporation and solubilization within POx micelles<sup>8</sup>, suggesting that other terpenoid structures may similarly benefit from POx-based encapsulation.

Terpenes constitute a broad class of natural products defined by a repeating isoprene (C<sub>5</sub>H<sub>8</sub>) structural motif and encompass numerous bioactive compounds with anti-inflammatory, anticancer, antimicrobial, analgesic, and anxiolytic properties<sup>9</sup>. Many terpenes are also classified as Generally Recognized as Safe (GRAS). In this study, we screened and characterized several mono-, di-, and triterpenes for their ability to load POx micelles.

$\alpha$ -Pinene, a monoterpene, was selected based on its strong safety record and GRAS designation by the U.S. Food and Drug Administration (FDA) for use as a synthetic flavoring substance and adjuvant under the Code of Federal Regulations (CFR) (21 CFR 172.515). Structurally,  $\alpha$ -pinene contains a single carbon–carbon double bond and no additional functional groups, making it highly hydrophobic and practically insoluble in water<sup>10</sup>.

In contrast, pure carnosic acid (CA) is not listed by the FDA as a standalone regulated food additive; however, rosemary extracts standardized to CA content fall under the FDA's food additive and GRAS framework (e.g., 21 CFR 182.20). CA is a phenolic diterpene featuring hydroxyl groups at C-11 and C-12 and a carboxylic acid at C-20. Despite these functionalities, its predominantly hydrophobic backbone renders it sparingly soluble in water and physiological buffers<sup>11</sup>. Carnosol (CARN), another diterpene abundant in rosemary extract, shares CA's core scaffold, including hydroxyl groups at C-11 and C-12, but contains a lactone moiety in the B ring. CARN also forms through oxidative degradation of CA<sup>12</sup>.

The cyclic triterpenes evaluated lupeol, ursolic acid, and botulin, are not currently GRAS or listed in the CFR. Nonetheless, their structural features, including hydroxyl and/or carboxylic acid groups combined with strong hydrophobicity<sup>13</sup>, made them compelling candidates for assessment in POx micelles. Squalene, a linear polyunsaturated triterpene, is regulated as a component of biologic products (e.g., vaccines) rather than as an independent food or cosmetic additive. Its six conjugated double bonds (all-E configuration) and pronounced hydrophobicity<sup>14</sup>, motivated its inclusion in the panel.

Overall, the goal of this work was to identify structural determinants that enable or enhance micellization with POx by systematically evaluating natural organic compounds with established safety profiles.

## Materials

Reagents for micelles formulations and characterization. Amphiphilic triblock copolymers p(MeOx<sub>x</sub>-b-BuOx<sub>y</sub>-b-MeOx<sub>z</sub>) were synthesized as previously described<sup>15</sup> and purchased from deLAQUA (Supplementary **Table S1**). CA was purchased from three different vendors (Supplementary **Table S2**). CARN was purchased from MedChemExpress. Alpha-pinene, Betulin, Lupeol, Ursolic Acid, Squalene were purchased from Sigma Aldrich. HPLC Water and Trifluoroacetic acid (TFA) were purchased from Sigma Aldrich. HPLC Acetonitrile was purchased from Avantor Sciences. Sterile water was purchased from Stericore solutions. Ethanol 200 Proof was purchased from Decon Labs Inc.

Instrumentation. Mettler Toledo XS105 Dual Range Analytical Balance. Malvern OMNISEC GPC/SEC. Bruker AVANCE NEO 400 MHz Spectrometer 1H NMR. Malvern Zetasizer Nano ZS (Malvern Panalytical Ltd., UK). Thermo Scientific Talos F200X scanning/transmission electron microscope (Thermo Fisher Scientific, CZ). Labconco FreeZone® Triad™ Benchtop Freeze Dryer (Labconco, US). Agilent Technologies 1200 High-Pressure Liquid Chromatograph (Agilent, US) equipped with Nucleosil C18 column (4.6 mm × 250 mm, 5 μm) (Macherey-Nagel, Germany). Oakton Ion 700 benchtop pH meter (OAKTON Instruments, US).

## Methods

**Small-scale thin film preparation of polymeric micelles for screening.** For small-scale thin-film preparation, stock ethanol solutions of the tested terpenes were prepared at their maximally ethanol-soluble concentrations, determined by serial dilution and visual confirmation of no precipitation (CA, CARN, squalene, and α-pinene at 10 mg/mL; lupeol, betulin, and ursolic acid at 2.5 mg/mL). The calculated volumes of each terpene stock were combined with 125 μL of polymer stock solution in ethanol (20 mg/mL). The mixtures were transferred into 1.5 mL microtubes at the desired polymer-to-terpene ratios, mixed thoroughly, and placed in a 65 °C heating block. A gentle nitrogen stream was applied to accelerate ethanol evaporation. After complete solvent removal (~15–20 min), the resulting thin films were hydrated with saline and incubated at 65 °C for an additional 10 min to facilitate micelle formation.

**Large-batch thin-film preparation and lyophilization of polymeric micelles.** 200  $\mu$ L of an ethanol solution containing 2 mg of CA (from different vendors) and 500  $\mu$ L of an ethanol solution containing 10 mg of P2 polymer (JR12) were combined in a pre-treated 10 mL round-bottom flask. Prior to use, the flask was heated in an oven at 220 °C and allowed to cool to room temperature for 30 min. Thin films were formed using a rotary evaporator with the water bath set to 65 °C, and ethanol was removed under carefully reduced vacuum (gradually lowered to prevent bumping or “boiling”) until complete solvent evaporation was achieved. The dried thin film was then hydrated with 1 ml saline to form a micellar solution. The flask was returned to the 65 °C water bath and rotated to ensure complete removal of the solvent and homogeneous dispersion. The resulting micellar solution was aliquoted into 15 ml glass vials (250  $\mu$ l per vial), sealed with rubber stoppers, and placed into the freeze-dryer chamber (Labconco FreeZone® Triad™ Benchtop). The lyophilization cycle (Supplementary **Table S3**) was optimized to yield an amorphous solid dispersion (ASD). Upon completion, vials were hermetically sealed and wrapped in aluminum foil to protect CA from light.

**Particle size determination.** The size distribution of POx micelles was measured using a Nano-ZS dynamic light-scattering (DLS) instrument. Prior to analysis, each terpene/POx micelle formulation was diluted 1:6 in sterile 0.9% saline. The hydrodynamic (effective) diameter and polydispersity index (PDI) were obtained using cumulant analysis of the intensity-weighted z-average. Measurements were performed on three independent micelle samples, and results are reported as average values.

**Transmission electron microscopy (TEM).** The morphology of CA/POx micelles was examined using a Thermo Scientific Talos F200X scanning/transmission electron microscope. A drop of each diluted CA/POx micelle solution (500- or 1000-fold dilution in DI water) was deposited onto a copper grid with a carbon support film and allowed to adsorb for 5 min. Excess liquid was gently wicked off using fine filter paper. A drop of 1% (w/v) uranyl acetate staining solution was then applied, allowed to contact the grid for 1 min prior to TEM imaging.

**Drug loading characterization.** Drug concentrations in POx micelles were quantified by reverse-phase HPLC using an Agilent 1200 system equipped with a Nucleosil C18 (5  $\mu$ m, 250 mm $\times$  4.6 mm) column. Samples exhibiting large particle sizes by DLS were first centrifuged for 1 min to remove large aggregates prior to analysis. Each micelle sample was diluted 1:10 in the mobile phase (acetonitrile with 0.01% TFA/water with 0.01% TFA; 60/40, v/v). A 10  $\mu$ L aliquot of the diluted sample was injected. Chromatographic separation was performed at a flow rate of 1.0 mL/min, column temperature of 40°C, and detection wavelength of 240 nm. The analyte was eluted over a 15-min run time. Quantification of CA was performed using a calibration curve (Supplementary **Table S4**).

The drug loading capacity (LC) and loading efficiency (LE) were calculated according to Equations (1) and (2):

$$LC = \frac{[Drug]_{solution}}{[Drug]_{solution} + [Polymer]} * 100\% \quad (1)$$

$$LE = \frac{[Drug]_{solution}}{[Drug]_{feed}} * 100\% \quad (2)$$

where  $[Drug]_{feed}$  and  $[Drug]_{solution}$  are the concentrations of the drug initially added to the mixture and quantified in the final micellar solution, respectively, and  $[Polymer]$  is the concentration of the polymer excipient.

**pH stability test.** A 0.1 M citrate buffer (pH 3) was prepared using citric acid, trisodium citrate dihydrate, and deionized (DI) water. Acetate buffers (0.1 M, pH 4 and pH 5) were prepared from glacial acetic acid, sodium acetate trihydrate, and DI water. Phosphate buffers (0.1 M, pH 6 and pH 7.4) were prepared using sodium phosphate monobasic and dibasic salts in DI water. A 0.1 M borate buffer (pH 9) was prepared using boric acid and sodium borate in DI water. A 0.1 M carbonate buffer (pH10) was prepared using sodium carbonate and sodium bicarbonate in DI water. The pH of each buffer was verified using an Oakton Ion 700 benchtop pH meter. CA/POx and PTX/POx ASDs were prepared using a large-batch thin-film method and a modified large-batch thin-film method, in which the thin films were dispersed in DI water rather than saline, aliquoted, and lyophilized. Aliquots (100  $\mu$ L) of each buffer were then added to the corresponding CA/POx or PTX/POx samples. pH-dependent micelle stability was evaluated at room temperature by monitoring changes in hydrodynamic diameter and PDI using DLS.

## Results

**Diterpenes demonstrated the ability to incorporate into POx micelles.** We evaluated the solubilization of several affordable and readily available pure terpenes in POx micelles. Among the monoterpenes,  $\alpha$ -pinene was tested; among the diterpenes, CA and CARN; and among the triterpenes, linear squalene and the cyclic compounds lupeol, betulin, and ursolic acid (**Figure 1**). Of these terpenes, CA and CARN showed the most favorable incorporation into POx micelles (**Table 1**). Using a POx triblock copolymer, p(MeOx<sub>x</sub>-b-BuOx<sub>y</sub>-b-MeOx<sub>z</sub>) (Supplementary **Table S1**), with ethanol as the solvent for stock solutions and saline for thin-film hydration, we first optimized loading ratios for CA. Our previous work<sup>5-6, 8</sup> showed exceptionally high loading for taxanes - also diterpenes - which informed this strategy. At a CA/polymer feed ratio of 2/10 (w/w), we consistently obtained small, uniform, and reproducible micelles with diameters of 22–29 nm, confirmed across two independent polymer batches and three CA batches from different vendors (**Table 2**). In subsequent experiments unless it is indicated differently the CA V2 and JR12 batch are used. Although CARN also forms uniform micelles with POx, we chose to focus on the CA/POx formulation due to their structural similarity and the potential oxidative conversion of CA to CARN<sup>16</sup>.

An HPLC method<sup>17</sup> was optimized to quantify CA concentration and loading in the micelles. At the 2/10 CA/polymer ratio, the loading capacity (LC) was approximately 17%, and the loading efficiency (LE) remained quantitative (measured LE  $\approx$  106%) (**Table 3**). In contrast, increasing the CA/polymer ratios to 4/10 or 8/10 resulted in a pronounced increase in particle size and polydispersity, accompanied by substantial reductions in LE. Notably, our HPLC procedure included a centrifugation step to remove

large aggregates prior to column injection. We therefore concluded that, at higher feed ratios, CA was largely incorporated into the oversized aggregates detected by DLS; however, these aggregates were removed during centrifugation, and thus the associated CA was separated and not detected in the micellar fraction analyzed by HPLC.

The TEM analysis further confirmed the particle morphology and the presence of aggregates in the studied dispersions (**Figure 2** and **Supplementary Figure S1, S2**). At the 2/10 CA/polymer ratio, the micelles appeared as small, relatively uniform and nearly spherical particles with diameters of approximately 20–30 nm, although occasional aggregates were also observed. These observations are generally consistent with the DLS results (**Table 2**), keeping in mind that TEM of soft polymeric nanoparticles on a solid substrate often yields slightly larger apparent particle sizes than DLS. At higher CA/polymer feed ratios of 4/10 and 8/10, along with individual spherical micelles the micrographs revealed substantially larger, micron-scale aggregates, often exhibiting worm-like structures suggestive of multiple micelles clustered together or fused. These observations were consistent with much larger particle size and polydispersity measured by DLS (**Table 2**). It is noteworthy that TEM imaging was performed ~24 hours after micelle preparation; therefore, storage time may have contributed to the observed morphological changes.

Using the established terpene/polymer loading ratio of 2/10 and the same thin-film formulation approach, we next evaluated representative mono- and triterpenes. However, none of the tested compounds yielded uniform or stable micelle formulations under these conditions (**Table 1**). Among them,  $\alpha$ -pinene showed partial incorporation and may warrant further investigation using alternative loading ratios or formulation parameters, but it is unlikely to be suitable for co-formulation with other drugs in high-loading, stable POx micelles.

**Stability of the CA-containing micelles.** To assess the stability of CA-loaded polymeric micelles at 2/10 CA/polymer ratio, particle size distributions were measured by DLS under three conditions: (i) within 1 h after thin-film hydration, (ii) after 48 h of storage of the micellar solution at room temperature, and (iii) after reconstitution of lyophilized micelles obtained by freeze-drying the micellar solution and storing the sealed powder at room temperature. Under all three conditions, the micellar solutions exhibited narrow size distributions and consistently small particle sizes of approximately 24–27 nm across two different CA batches (**Figure 3**).

In contrast, HPLC analysis of drug content revealed a substantial loss of solubilized CA after storage of the micellar solution, with CA levels decreasing to 25% or 64% of the initial concentration, depending on the CA batch (**Table 4**). Significantly improved stability of CA was observed when the micellar solutions were first lyophilized, stored at room temperature for 48 h, and then reconstituted. In this case, 67% or 89% of the initial CA content was recovered in the reconstituted solutions depending on the batch used. These results indicate that although CA stability may vary depending on the source of the compound, lyophilizing the formulation and storing it as ASD, with subsequent reconstitution, provides a superior storage strategy for POx micelle formulations containing CA, when properly optimized.

**Effect of pH on the size of CA-loaded micelles.** CA is a monocarboxylic acid bearing an additional acidic hydroxyl at the C12 position of the C-ring. With  $pK_a$  values of 3.8 for the carboxyl group, 3.8–4.3 for the C12 phenolic hydroxyl, and 14.1 for the second C11 phenolic hydroxyl, CA exists predominantly in its deprotonated (negatively charged) form at physiological pH, but becomes largely protonated under acidic conditions<sup>18</sup>. This pH-dependent ionization can modify CA's localization within polymeric micelles by altering coulombic interactions and may also influence hydrogen bonding between the hydroxyl group of the protonated carboxylic acid (–COOH) and the C11 hydroxyl of CA and the carbonyl (C=O) groups in the POx backbone. Together, these effects may influence micelle size. To investigate the effect of pH on CA-loaded micelle size, particle size was measured by DLS over a pH range from 3 to 10. pH was adjusted using a series of buffers listed in Supplementary **Table S5**. As a comparator, POx micelles loaded with PTX, a diterpene lacking ionizable groups within this pH range, were evaluated at the same drug/polymer ratio (2/10).

CA/POx and PTX/POx micelles (terpene/polymer ratio 2/10) were prepared using a large-batch thin-film method. To evaluate the effect of ionic strength, the thin films were hydrated either with distilled water or with saline, aliquoted, and lyophilized. The resulting micelles were reconstituted in the same respective media and analyzed by DLS (**Figures 4–5**). For both CA and PTX, micelles prepared in distilled water showed higher polydispersity than those prepared in saline. This effect was most pronounced for PTX: PTX/POx micelles formed in distilled water exhibited two distinct size populations (25.9 nm and 203.4 nm, with peak areas of 52.8% and 47.2%), which persisted across the entire pH range, yielding consistently high PDI (**Figures 4b** and **5a**). In contrast, PTX micelles prepared in saline remained small and uniformly sized throughout (**Figures 4c,d** and **5c**).

For CA, micelles formed without added salt also showed distinct size populations, particularly at neutral and alkaline pH; however, micelle uniformity improved and PDI decreased as pH was lowered (**Figures 4b** and **5b**). Similar to PTX, CA micelles prepared in saline remained small and uniform across the entire pH range, including at pH 3, where CA is expected to be negatively charged (**Figures 4c,d** and **5d**). Therefore, these results show that the presence of NaCl during formulation CA and PTX, into POx micelles enhances micellar stability and improves their resilience to pH variations.

## Discussion

This study examined the ability of an amphiphilic POx block copolymer to form micelles capable of solubilizing mono-, di-, and triterpenes. Among all compounds tested, the most stable and uniform micelles were obtained with diterpenes such as CA, CARN, and the taxane PTX. We propose that the cyclized isoprene framework and the presence of multiple hydroxyl groups in these diterpenes are key structural features that facilitate their incorporation into POx micelles. Specifically, the ortho-phenolic hydroxyls at the C11 and C12 positions in CA and CARN likely enable strong hydrogen bonding with the carbonyl (C=O) groups of the POx backbone. These interactions are expected to enhance micelle stabilization and promote the formation of a more rigid lamellar structure as CA and CARN concentrations increase.

Our pH-effect studies showed a linear increase in micelle size with rising pH for CA/POx micelles hydrated with water prior to freeze-drying. Notably, this trend disappeared when CA/POx micelles were hydrated with 0.9% NaCl. A salt-dependent effect was observed for PTX/POx micelles, where acid–base interactions are not expected: hydration with water produced polydisperse micelles, whereas hydration with 0.9% NaCl yielded micelles with a narrow size distribution. These results suggest that Na<sup>+</sup> and Cl<sup>−</sup> ions, by modifying the local hydrogen-bonding network of water and forming structured ion–water hydration shells<sup>19</sup>, may stabilize drug-loaded micelles and shield drug molecules from pH-dependent chemical interactions. It is long known that salts such as NaCl can influence micelle size, stability, and micellization of poloxamers through salting-out effects, including polymer dehydration - an effect comparable to elevating temperature<sup>20</sup>. However, analogous salt effects in POx systems remain far less explored. In our view, more detailed studies on ionic-strength effects in POx micelles are warranted, both for optimizing POx-based drug formulations and for advancing the broader understanding of this block-copolymer class.

CA demonstrated reproducible and stable encapsulation in POx micelles at a CA/POx ratio of 2/10. However, CA remains chemically reactive, particularly toward cations, even after micellization. The well-defined physicochemical properties of CA/POx micelles, together with the enhanced stability of CA in its ASD form, provide a strong platform for further investigation of its antioxidant and free-radical–scavenging activities in aqueous media. These properties may also support studies evaluating CA’s ability to modulate oxidative signaling and mitigate oxidative damage through metal-ion chelation.

In addition, the observed tendency of CA to form aggregated structures with POx, structures that resist hydration and simultaneously prevent the release of precipitated free CA, could be exploited in the development of pH-responsive, co-loaded drug delivery systems. Improved aqueous solubility of CA in these POx micelles may further enable advanced *in vivo* studies of CA-mediated inhibition of globus pallidus externus parvalbumin-positive (GPePV) neurons and its effects on cocaine and opioid reward, sensitization, and self-administration<sup>21</sup>. Finally, CA/POx formulations may find utility in protective coatings for biomedical devices or biomaterials, where the antioxidant activity of CA could help attenuate oxidative stress and enhance biocompatibility<sup>22</sup>.

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**Table 1.** DLS characterization of POx (JR12) micelles loaded with different terpenes.

| Terpene class     | Chemical compound | Z average diameter (nm)* | PDI*          |
|-------------------|-------------------|--------------------------|---------------|
| Monoterpene (C10) | Alpha-pinene      | 84.0 ± 7.7               | 0.64 ± 0.03   |
| Diterpenes (C20)  | CARN              | 23 ± 0.1                 | 0.03 ± 0.01   |
|                   | CA                | 23.6 ± 2.92***           | 0.1 ± 0.1***  |
| Triterpenes (C30) | Betulin           | 1183.0 ± 11.5**          | 0.28 ± 0.04** |
|                   | Lupeol            | 1004.3 ± 389.9**         | 0.76 ± 0.04** |
|                   | Ursolic Acid      | 2377.0 ± 602.4**         | n/a**         |
|                   | Squalene          | 431.8 ± 22.3             | 0.42 ± 0.03   |

\* Unless stated differently data are mean ± SD values of n = 3.

\*\* The particle size exceeded the reliable detection range of the DLS instrument (1–1000 nm).

\*\*\* The mean ± SD values were determined using five independent P2/CA (10/2) batches (Table 2).

**Table 2.** DLS characterization of CA-loaded POx micelles across CA sources and copolymer batches.

| POx batch code | CA vendor code | CA/P2 wt. ratio | Z average diameter (nm)* | PDI*        |
|----------------|----------------|-----------------|--------------------------|-------------|
| JR12           | V1             | 8/10            | 690.1 ± 446.3            | 0.76 ± 0.23 |
|                |                | 4/10            | 292.3 ± 65.7             | 0.36 ± 0.09 |
|                |                | 2/10            | 22.5 ± 0.7               | 0.06 ± 0.03 |
|                | V2             | 8/10            | 156.7 ± 50.0             | 0.57 ± 0.38 |
|                |                | 4/10            | 163.6 ± 14.5             | 0.52 ± 0.06 |
|                |                | 2/10            | 22.1 ± 0.1               | 0.05 ± 0.01 |
|                | V3             | 8/10            | 548.6 ± 427.3            | 0.52 ± 0.38 |
|                |                | 4/10            | 153.5 ± 65.7             | 0.55 ± 0.18 |
|                |                | 2/10            | 28.8 ± 0.7               | 0.27 ± 0.01 |
| 1021           | V1             | 8/10            | 181.4 ± 5.1              | 0.28 ± 0.03 |
|                |                | 4/10            | 288.0 ± 32.6             | 0.64 ± 0.09 |
|                |                | 2/10            | 22.6 ± 0.5               | 0.06 ± 0.04 |

|    |      |               |             |
|----|------|---------------|-------------|
| V2 | 8/10 | 504.8 ± 302.0 | 0.48 ± 0.20 |
|    | 4/10 | 169.9 ± 105.1 | 0.35 ± 0.08 |
|    | 2/10 | 22.0 ± 1.0    | 0.06 ± 0.01 |

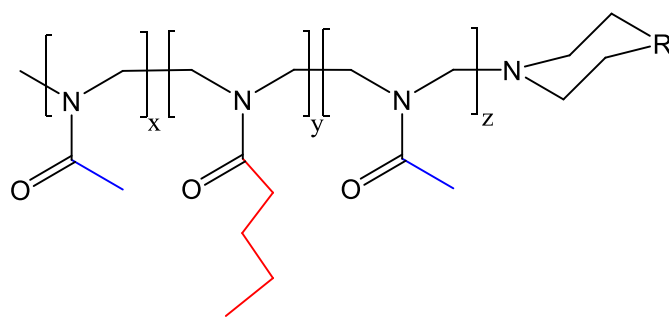
\* Unless stated differently data are mean ± SD values of n = 3

**Table 3.** Characteristics of polymeric micelles, loaded with CA.

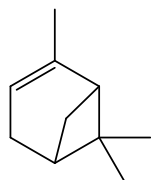
| Feeding ratio CA/POx (g/L) | LC (%) | LE (%) |
|----------------------------|--------|--------|
| 2/10                       | 17.5   | 106    |
| 4/10                       | n.a.   | 9.5    |
| 8/10                       | n.a.   | 0.8    |

**Table 4.** CA concentration (mg/mL) measured in micellar solutions: within 1 hour after thin-film hydration, after 48 hours of storage at room temperature, and after reconstitution of the lyophilized powder stored for 48 hours at room temperature.

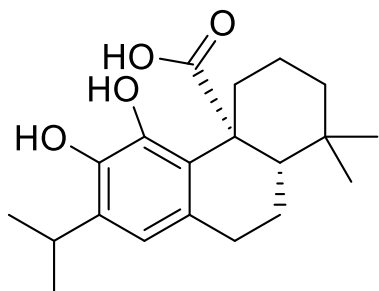
| CA vendor code | After film hydration | After 48h in solution | After reconstitution |
|----------------|----------------------|-----------------------|----------------------|
| V1             | 2.12                 | 0.55                  | 1.42                 |
| V2             | 1.75                 | 1.12                  | 1.56                 |



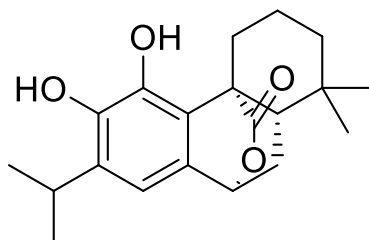
Me-Bu-Me oxazoline



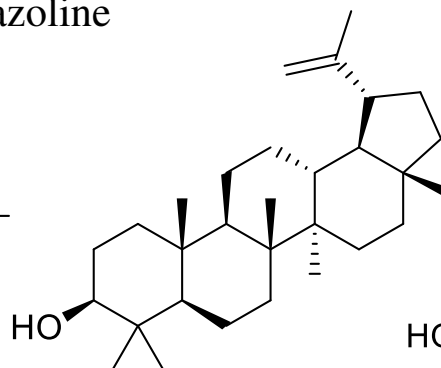
Alpha pinene



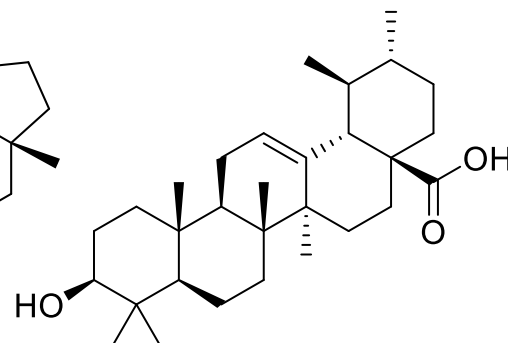
Carnosic acid (CA)



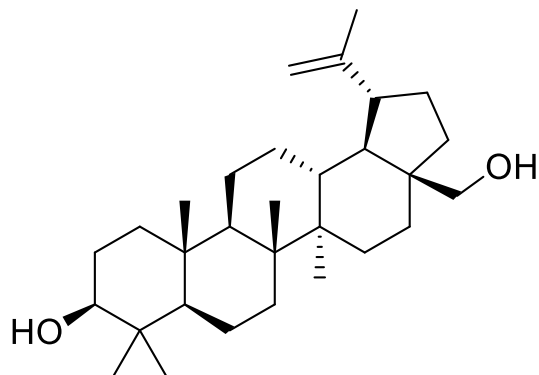
Carnosol (CARN)



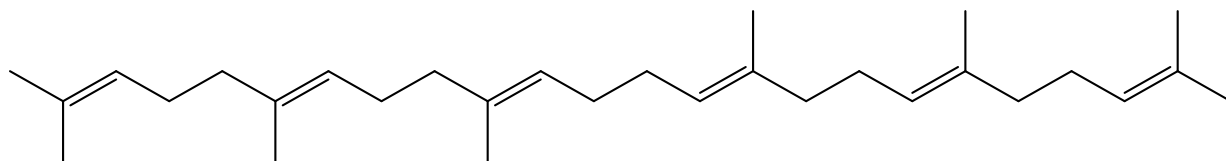
Lupeol



Ursolic acid



Betulin



Squalene

**Figure 1.** Chemical structure of the POx micellized agent and tested terpenes

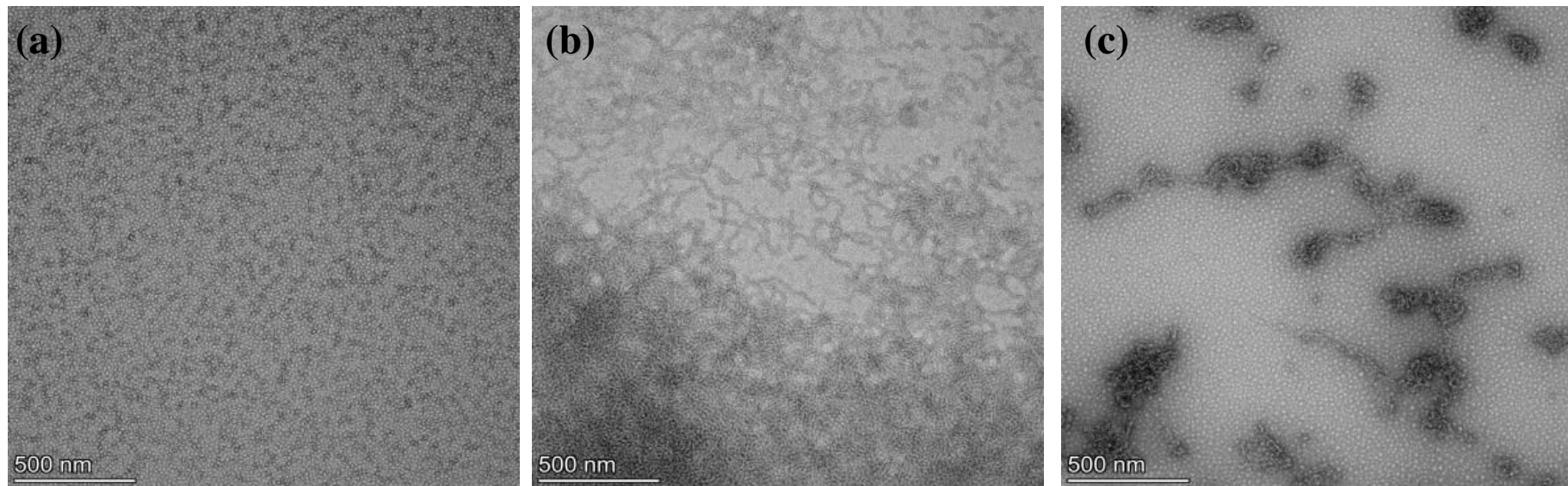


**Figure 2.** TEM images of CA-loaded polymeric micelles prepared at different CA/polymer weight ratios : **(a)** 2/10, **(b)** 4/10, and **(c)** 8/10. Scale bar: 500 nm.

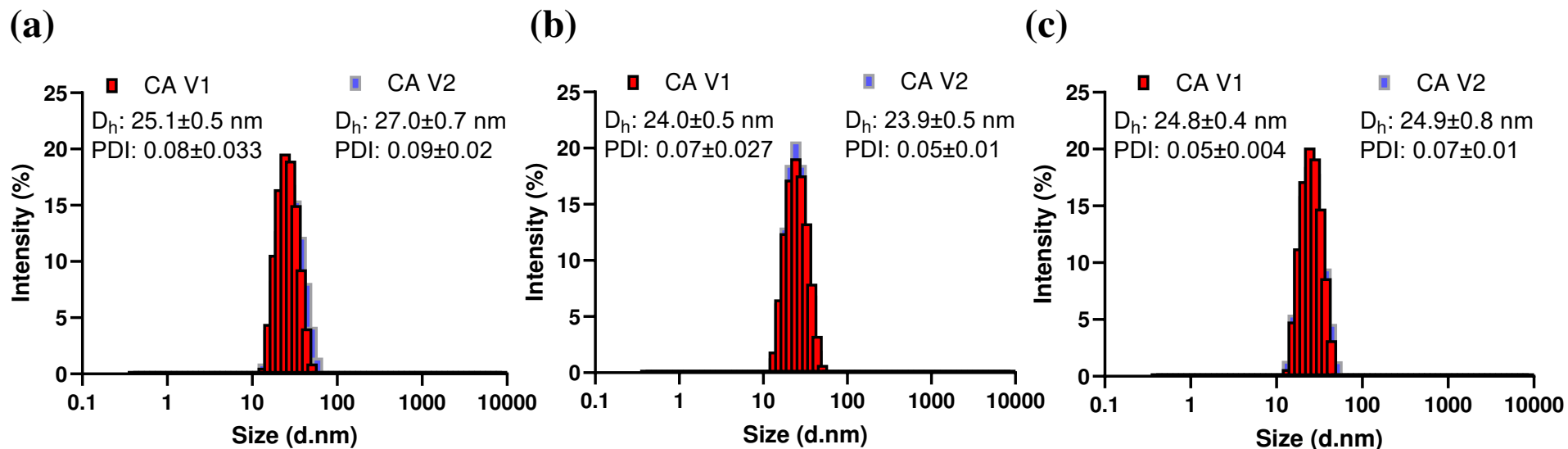


**Figure 3.** DLS intensity weighted size distributions of P2 POx micelles loaded with CA from different sources at a 2/10 CA/polymer weight ratio. Particle sizes were measured at room temperature under three conditions: **(a)** within 1 h after thin film hydration with saline to a polymer concentration of 10 mg/mL (large batch preparation); **(b)** after 2 days of storage of the micellar solution at room temperature (aliquots protected from light by foil wrapping); and **(c)** within 1 h after reconstitution in DI water of the lyophilized powder, which was produced by freeze drying the micellar solution (a), hermetically sealed, and stored for 1 day at room temperature.

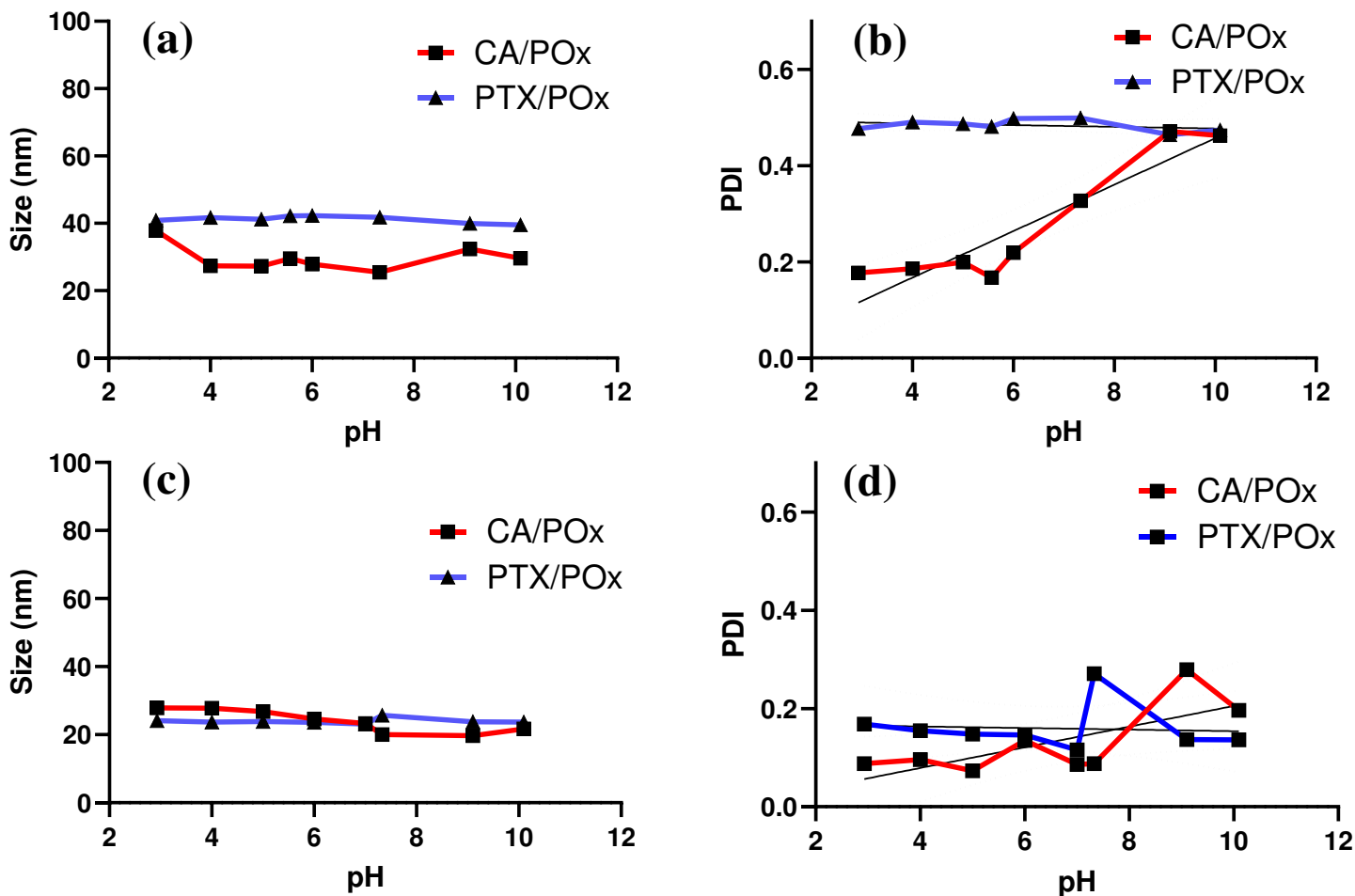


**Figure 4.** Dependence of the (a,c) DLS effective hydrodynamic diameter and (b,d) intensity weighted PDI of CA or PTX loaded polymeric micelles on pH after reconstitution, with formulations prepared using a thin film method and thin film hydrated in (a,b) deionized (DI) water or (c,d) 0.9% NaCl solution at a 2/10 compound/polymer feed ratio (w/w). Linear regression analysis was used to evaluate the relationship between PDI and pH: in DI water (b), CA/POx exhibited  $Y = 0.048 \cdot X - 0.026$  ( $R^2 = 0.864$ ) and PTX/POx exhibited  $Y = -0.002 \cdot X + 0.495$  ( $R^2 = 0.1292$ ); in 0.9% NaCl (d), CA/POx exhibited  $Y = 0.021 \cdot X - 0.005$  ( $R^2 = 0.512$ ) and PTX/POx exhibited  $Y = -0.002 \cdot X + 0.17$  ( $R^2 = 0.13$ ).

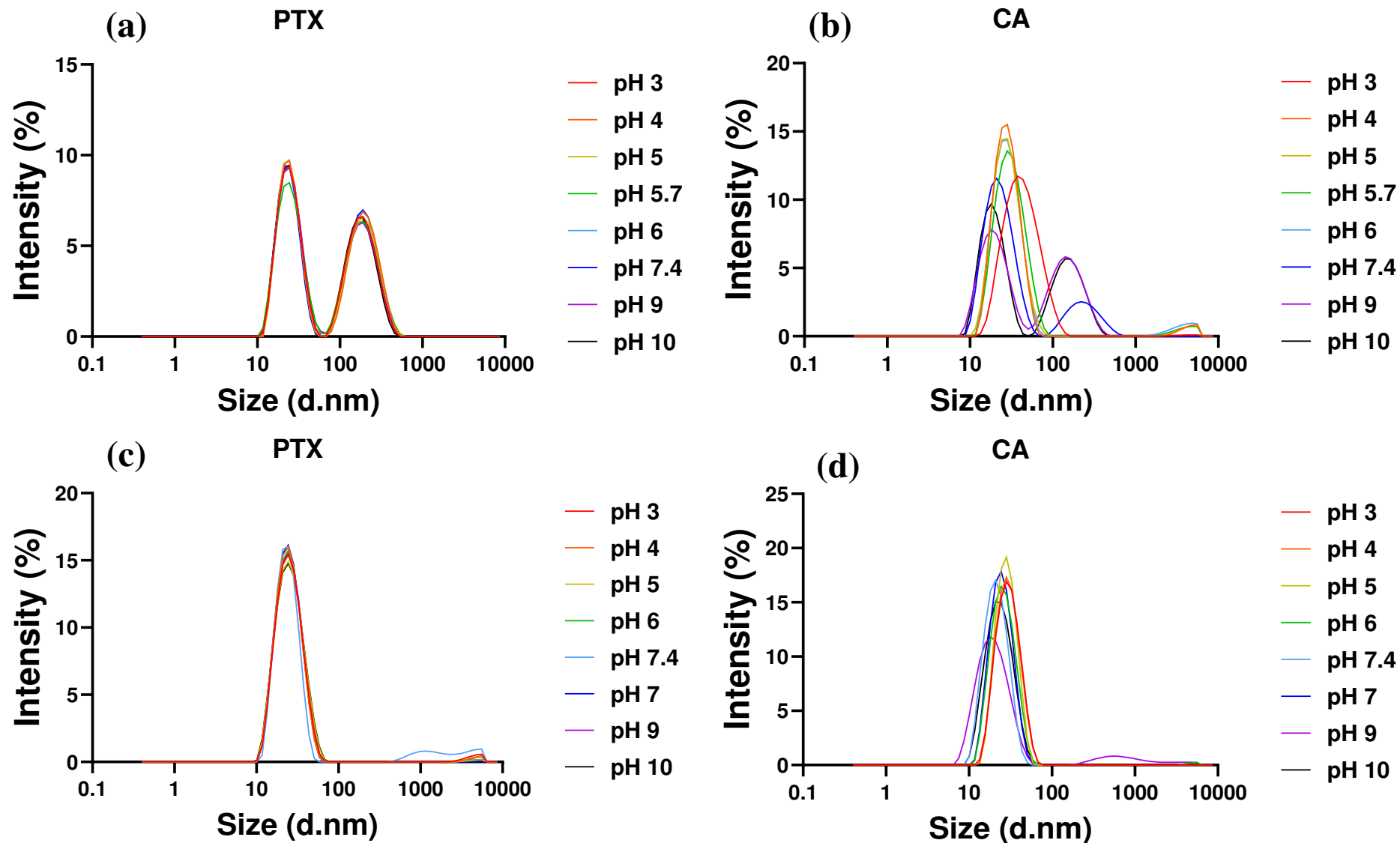


**Figure 5.** DLS intensity weighted size distributions of POx micelles at a 2/10 compound to polymer feed ratio (w/w): **(a, b)** micelles hydrated with DI H<sub>2</sub>O, and **(c, d)** micelles hydrated with 0.9% NaCl solution. Micelles were loaded with **(a, c)** PTX and **(b, d)** CA and analyzed as a function of pH after reconstitution.

# Figures



Figure 2

## Supplementary Files

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