

Physical stability of Pickering nanoemulsions stabilized with chitosan–octenyl succinic anhydride-modified starch polyelectrolyte complex

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ABSTRACT

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This study aimed to investigate the effect of various parameters, including the molar charge ratio of chitosan–OSA-modified starch, starch-to-oil ratio, and preparation methods on the physical stability of Pickering nanoemulsions stabilized with chitosan–octenyl succinic anhydride (OSA)-modified starch polyelectrolyte complex (PEC). The nanoemulsions were prepared via ultrasonication using two different methods, namely, the PEC and layer-by-layer (LBL) methods. The investigated physical properties included appearance, droplet size, size distribution, and zeta potential of the nanoemulsions. The results indicated that at a molar charge ratio of 10:1, the nanoemulsions with chitosan–OSA-modified starch PECs had better physical stability. The droplet size, size distribution, and zeta potential were not statistically changed after 4 weeks of storage at $30 \pm 2^\circ\text{C}$. At a lower chitosan–OSA-modified starch ratio, the LBL method provided better physically stable nanoemulsions than the PEC method. However, at a higher chitosan–OSA-modified starch ratio, both methods gave the nanoemulsions with comparable stability. The higher physical stability was attributable to the PEC of chitosan with OSA-modified starch at the o/w interface. In conclusion, the Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs are a promising emulsion template for the encapsulation of lipophilic drugs.

Keywords: physical stability; Pickering nanoemulsion; polyelectrolyte complex; octenyl succinic anhydride-modified starch; chitosan

1. INTRODUCTION

Recently, Pickering emulsions have been applied to pharmaceutical, cosmetics, and food products because of their low toxicity, high physical stability against coalescence, and high reproducibility (Chevalier and Bolzinger, 2013). It could be used to encapsulate lipophilic compounds to provide greater stability in topical

preparation (Peito et al., 2022) and to enhance the oral bioavailability of poorly water-soluble drugs (Jadhav et al., 2022; Wang et al., 2022). Pickering emulsions are emulsions that are stabilized by solid particles that adsorb onto the interface between two phases (Charoenthai et al., 2022). Examples of the solid particles utilized as stabilizers in Pickering emulsions are cyclodextrin (Mathapa and Paunov, 2013), gliadin (Hu et al., 2016), and starch

nanoparticles (Saari et al., 2017). The partial wettability by water and oil of the solid particles is an important characteristic of stabilizers used in Pickering emulsions (Albert et al., 2019). The surface wettability of the solid particles could be modified using several methods such as polyelectrolyte complex (PEC) formation, chemical grafting, and pH and ionic strength adjustments (Björkegren et al., 2017; Nan et al., 2014; Wang and Heuzey, 2016).

Due to biocompatibility, biodegradability, and nontoxicity, biopolymers such as chitosan and modified starch have gained attraction for use as nonactive ingredients in pharmaceutical preparations. Chitosan is a cationic polymer obtained from partial deacetylation of chitin under alkaline conditions. It is a copolymer of β -(1 \rightarrow 4)-linked 2-acetamido-2-deoxy-D-glucopyranose (acetylated unit) and 2-amino-2-deoxy-D-glucopyranose (deacetylated unit). In acidic conditions, chitosan becomes a positive-charged polyelectrolyte, which could interact with a negative-charged polyelectrolyte to form a PEC (Berger et al., 2004). Octenyl succinic anhydride (OSA)-modified starch is one type of starch modified using an esterification reaction between hydroxyl groups of native starch and OSA starch under alkaline conditions. It is a polyelectrolyte containing a negative-charged carboxyl group. Reportedly, chitosan could form PEC with OSA-modified starch through electrostatic interaction between carboxylate moieties (COO^-) of OSA-substituted groups of OSA-modified starch and protonated amines (NH_3^+) of deacetylated units of chitosan (Shen et al., 2010). The PECs between chitosan and OSA-modified starch were used as a stabilizer for Pickering emulsions (Sodalee et al., 2022; Abbas et al., 2014; Fang et al., 2019; Preetz et al., 2008). The PEC formation could improve the wettability of the solid particles, thus promoting the accumulation and adsorption of the solid particles at the oil/water interfaces to obtain stable emulsions (Yuan et al., 2017). Two different methods were reported for preparing the Pickering emulsions, namely, the PEC method and layer-by-layer (LBL) methods. In the PEC method, the PEC solid particles were prepared and then blended with the oil phase before emulsification (Shen et al., 2010; Sodalee et al., 2022). In the LBL method, the primary emulsion was prepared using one polyelectrolyte, and then the oppositely charged polyelectrolyte was added to the primary emulsion (Abbas et al., 2015; Carvalho et al., 2014; Preetz et al., 2008; Sodalee et al., 2022). Besides the preparation methods, pH, molar charge ratio between two oppositely charged polyelectrolytes, and starch-to-oil ratio could affect the physical and chemical stability of Pickering nanoemulsions. Shen et al. (2010) found that pH had an effect on the electrostatic interaction between chitosan and OSA-modified starch, which consequently affected the stability of fish oil microcapsule powder prepared from the Pickering emulsions. The three-layer polyelectrolyte capsule prepared from the emulsion stabilized with OSA starch and an additional layer of chitosan could improve the stability of a labile substance (Carvalho et al., 2014). Moreover, the adsorption of chitosan on nanoemulsion

stabilized with OSA-modified starch improved the physical stability of curcumin (Abbas et al., 2015).

Recently, chitosan–OSA-modified starch PEC was used as a stabilizer for Pickering nanoemulsion and redispersible dry nanoemulsion (Sodalee et al., 2022). It has been reported that the droplet size, size distribution, and zeta potential of the Pickering nanoemulsions were dependent on the stabilizer amount, starch-to-oil ratio, and preparation method. Additionally, increasing the ratio of starch-to-oil remarkably decreased the droplet size of these nanoemulsions. The physical stability of the Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs was still not reported. In this study, the Pickering nanoemulsions were prepared by two different methods, namely, the PEC method and the LBL method. The effects of the molar charge ratio of chitosan–OSA-modified starch, starch-to-oil ratio, and preparation method on the physical stability of the nanoemulsions were investigated. The appearance, droplet size, size distribution, and zeta potential of the nanoemulsions were characterized to determine the physical properties of Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs.

2. MATERIALS AND METHODS

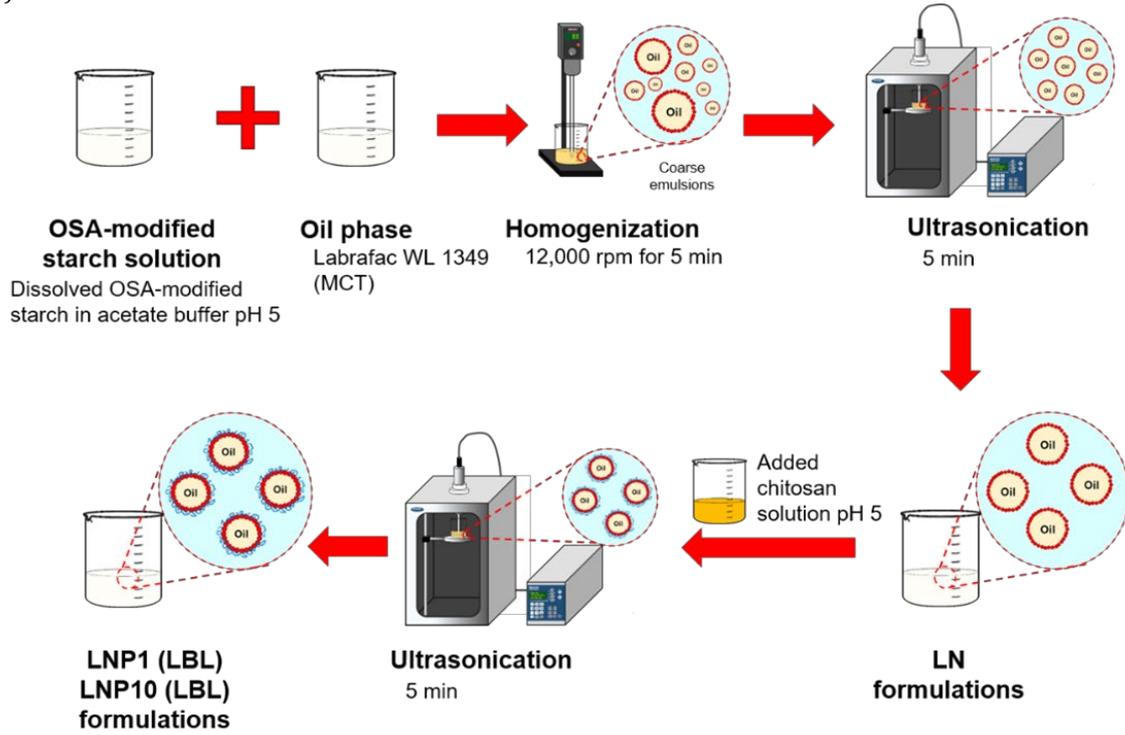
2.1 Materials

Labrafac Lipophile WL 1349, medium-chain triglycerides (MCTs), was purchased from Gattefosse, St. Priest, France, and used as an oil phase. High-deacetylated chitosan with a viscosity of 48 mPas (0.5% w/w chitosan in 0.5% w/w acetic acid aqueous solution at 20°C), deacetylation degree $\geq 98\%$ (Daichitosan 100D, Dainichiseika Colors and Chemicals Manufacturing, Tokyo, Japan), and OSA-modified starch (HI-CAP 100, National Starch and Chemical (Thailand), Bangkok, Thailand) were used to form PECs. The other materials were laboratory-grade chemicals.

2.2 Preparation of Pickering nanoemulsions stabilized with OSA-modified starch

Figure 1a depicts the preparation process of liquid nanoemulsions stabilized by OSA starch (LN). This preparation process was modified from previous publication (Sodalee et al., 2022). First, OSA-modified starch was dissolved in acetate buffer, pH 5. The OSA-modified starch solution (0.08% - 8.00% w/v) was then mixed with an oil phase (MCT) at various starch-to-oil ratios. Table 1 shows each formulation composition. The obtained mixtures were homogenized for 5 min (12,000 rpm) to prepare the coarse emulsions (SilentCrusher M, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Afterward, the coarse emulsions were sonicated for 10 min using a probe sonicator equipped with a disruptor horn (Model 250 Digital Sonifier, Branson, CT, USA). The power was set to pulse mode with an amplitude of 50% (10 s pulse on and 5 s pulse off intervals). An ice bath was used to cool the system. The volume was finally adjusted to 50 mL with pH 5 acetate buffer to get the Pickering nanoemulsions.

(a)



(b)

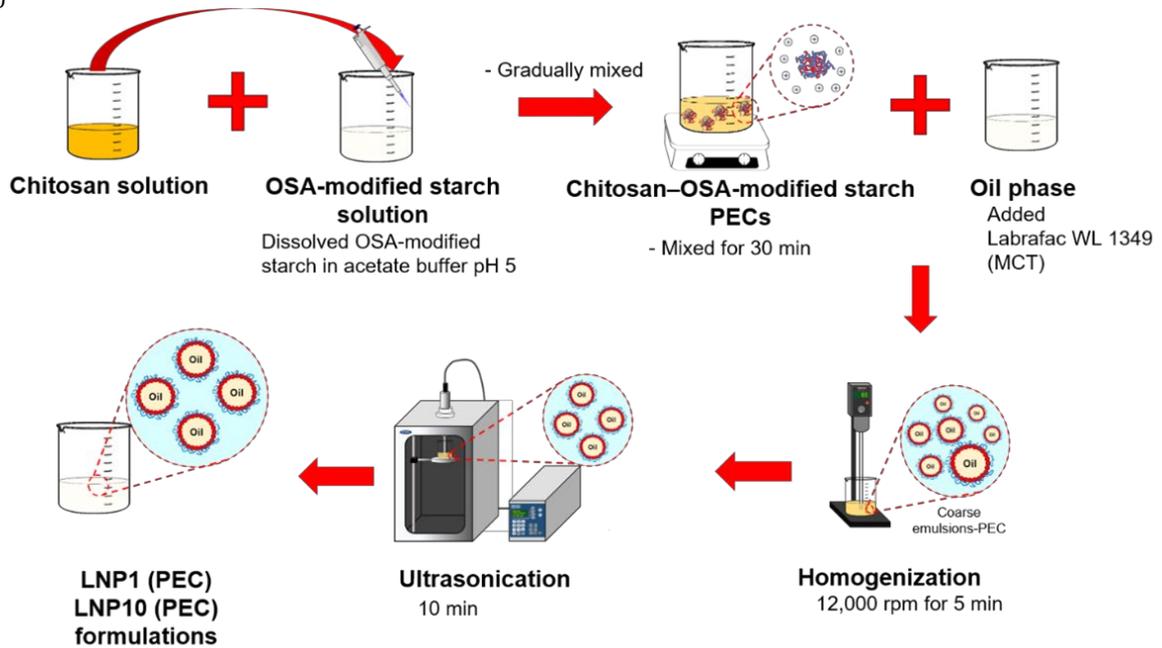


Figure 1. Preparation methods of Pickering nanoemulsions stabilized with OSA-modified starch and chitosan-OSA-modified starch PECs using (a) LBL and (b) PEC methods

Table 1. Composition and emulsion formation of Pickering nanoemulsions stabilized with OSA-modified starch and chitosan–OSA-modified starch PECs

Formulation ^a	Composition (% w/v)			Preparation method	Emulsion formation
	Labrafac WL149	OSA-modified starch	Chitosan		
Liquid nanoemulsions stabilized with OSA-modified starch (LN)					
LN-0.013	5.0	0.0625	0	–	Phase separation
LN-0.025	5.0	0.125	0	–	Phase separation
LN-0.05	5.0	0.25	0	–	Phase separation
LN-0.10	5.0	0.50	0	–	Without phase separation
LN-0.20	5.0	1.0	0	–	Without phase separation
LN-0.40	5.0	2.0	0	–	Without phase separation
LN-0.80	5.0	4.0	0	–	Without phase separation
LN-1.20	5.0	6.0	0	–	Without phase separation
Liquid nanoemulsions stabilized with chitosan–OSA-modified starch PECs at a 1:1 molar charge ratio (LNP1)					
LNP1-0.013	5.0	0.0625	0.0082	PEC	Phase separation
				LBL	Phase separation
LNP1-0.025	5.0	0.125	0.0016	PEC	Phase separation
				LBL	Phase separation
LNP1-0.05	5.0	0.25	0.0033	PEC	Phase separation
				LBL	Phase separation
LNP1-0.10	5.0	0.50	0.0065	PEC	Phase separation
				LBL	Phase separation
LNP1-0.20	5.0	1.0	0.0131	PEC	Phase separation
				LBL	Without phase separation
LNP1-0.40	5.0	2.0	0.0262	PEC	Without phase separation
				LBL	Without phase separation
LNP1-0.80	5.0	4.0	0.0392	PEC	Without phase separation
				LBL	Without phase separation
LNP1-1.20	5.0	6.0	0.0784	PEC	Without phase separation
				LBL	Without phase separation
Liquid nanoemulsions stabilized with chitosan–OSA-modified starch PECs at a 10:1 molar charge ratio (LNP10)					
LNP10-0.013	5.0	0.0625	0.0082	PEC	Phase separation
				LBL	Phase separation
LNP10-0.025	5.0	0.125	0.0164	PEC	Without phase separation
				LBL	Without phase separation
LNP10-0.05	5.0	0.25	0.0327	PEC	Without phase separation
				LBL	Without phase separation
LNP10-0.10	5.0	0.50	0.0654	PEC	Without phase separation
				LBL	Without phase separation
LNP10-0.20	5.0	1.0	0.1307	PEC	Without phase separation
				LBL	Without phase separation
LNP10-0.40	5.0	2.0	0.2615	PEC	Without phase separation
				LBL	Without phase separation
LNP10-0.80	5.0	4.0	0.3922	PEC	Without phase separation
				LBL	Without phase separation
LNP10-1.20	5.0	6.0	0.7844	PEC	Without phase separation
				LBL	Without phase separation

Note: ^aThe values after formulation codes represent the starch-to-oil ratio.

2.3 Preparation of Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs

2.3.1 PEC method

As shown in Figure 1b, the PECs between chitosan and OSA-modified starch were prepared before blending with the oil phase. Table 1 presents the compositions of chitosan–OSA-modified starch PECs. Chitosan and OSA-modified starch were dissolved in pH 5.0 acetate buffer. The chitosan solution was mixed with the OSA-modified starch solution using a magnetic stirrer for 30 min to prepare the PECs. The oil phase was mixed with the PECs solutions and then homogenized for 5 min (12,000 rpm)

to get the coarse emulsions. The obtained emulsions were then ultrasonicated using a probe sonicator for 10 min in a similar manner as presented in section 2.2 to obtain the Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs [LNP1 (PEC) and LNP10 (PEC)].

2.3.2 LBL method

The Pickering nanoemulsion with OSA-modified starch as a stabilizer (LN) (section 2.2) was utilized as a template to fabricate the nanoemulsions via the LBL method. Table 1 shows the compositions of the nanoemulsions. As shown in Figure 1a, chitosan solutions in pH 5 acetate buffer at various concentrations were placed into the nanoemulsion with OSA-modified starch (LN). Then, the mixtures were

sonicated using a probe sonicator for 5 min as previously described in section 2.2 to get the Pickering nanoemulsion stabilized with chitosan–OSA-modified starch PECs [LNP1 (LBL), and LNP10 (LBL)].

2.4 Visual observation

The physical appearance of the nanoemulsions was observed via visual observation. Phase separation and cracking of emulsions were reported.

2.5 Transmission electron microscopy (TEM)

TEM photomicrographs of samples were measured by using the JEM-2100F field emission TEM apparatus (JEOL Co., Ltd., Japan) with an accelerating voltage of 120 kV.

2.6 Dynamic light scattering

The droplet size and size distribution (polydispersity index, PDI) of nanoemulsions were measured via dynamic light scattering using backscattering of 173° at 25°C (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). Each sample was diluted with pH 5 acetate buffer (10 times) and was then gently shaken for 5 min using a vortex mixer. All measurements were repeated in triplicate.

2.7 Zeta potential measurement

The electrophoretic mobilities of the nanoemulsion droplets were measured using Zeta Sizer Nano ZS at the wavelength of 633 nm (Malvern Instruments Ltd., Worcestershire, UK). The same diluted samples prepared in section 2.6 were used. Smoluchowski's equation was used to calculate the apparent zeta potential.

2.8 Physical stability study

Approximately 50 mL of each sample was placed into a glass bottle and stored for 4 weeks at 30 ± 2°C. Cracking of nanoemulsion was identified when the oil and aqueous phases were completely separated and could not be redispersed. The redispersion could be observed by shaking or mixing the nanoemulsions. However,

immediately after redispersion, the nanoemulsions were unstable and phase separation was observed. Moreover, droplet size, size distribution, and zeta potential were determined.

2.9 Statistical analysis

The differences in results were performed using paired and independent t-tests. Data analysis was performed using Microsoft Excel, and the difference was considered significant at a 95% confidence limit ($\alpha = 0.05$).

3. RESULTS AND DISCUSSION

3.1 Effect of type of stabilizer and starch-to-oil ratio

Type of stabilizer and starch-to-oil ratio are considered to be the important factors that affect the properties of Pickering emulsions (Bago Rodriguez et al., 2016). The stabilizers used were OSA-modified starch and chitosan–OSA-modified starch PECs at 1:10 and 10:1 molar charge ratios. Moreover, the ratio of starch-to-oil was varied in the range of 0.013-1.20. The results indicated that phase separation was observed in the formulations containing a low amount of stabilizer (Figure 2). This might be due to an insufficient amount of stabilizer to stabilize the nanoemulsion structure. To obtain the stable nanoemulsions within 2 h without phase separation, the lowest necessary ratio of starch-to-oil required for LN, LNP1 (PEC), LNP1 (LBL), LNP10 (PEC), and LNP10 (LBL) formulations were 0.10, 0.40, 0.20, 0.05, and 0.05 w/w, respectively (Table 1). These nanoemulsions were subjected to a physical stability study at 30 ± 2°C for 4 weeks. Moreover, TEM photomicrographs of the nanoemulsions stabilized with OSA starch (LN-0.40) and chitosan–OSA starch PECs (LNP10-0.40) are depicted in Figures 3a and 3b, respectively. This indicated that the nanoemulsions were formed.

LN



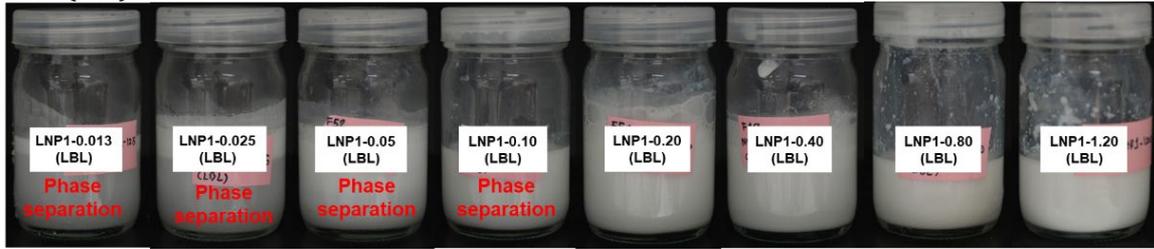
LNP1 (PEC)



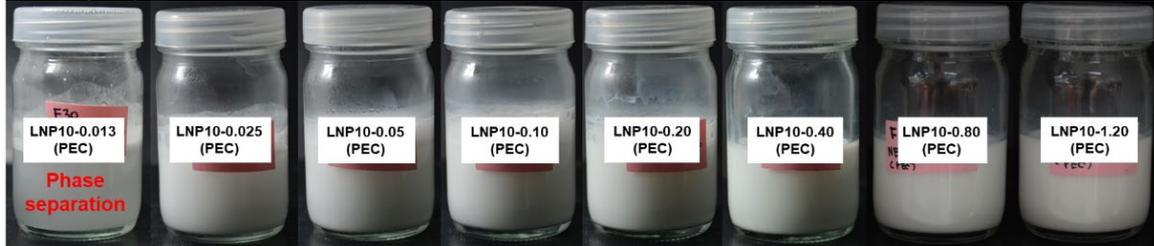
Figure 2. Physical appearance of different Pickering nanoemulsions stabilized with OSA-modified starch and chitosan–OSA-modified starch PECs

Note: The preparation method of each formulation is presented in the bracket.

LNP1 (LBL)



LNP10 (PEC)



LNP10 (LBL)

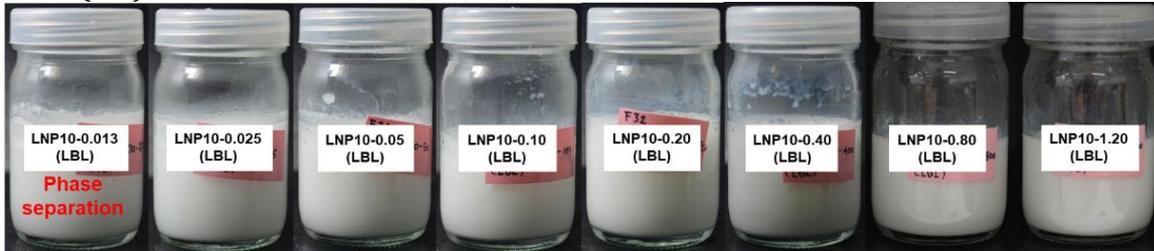


Figure 2. (continued)

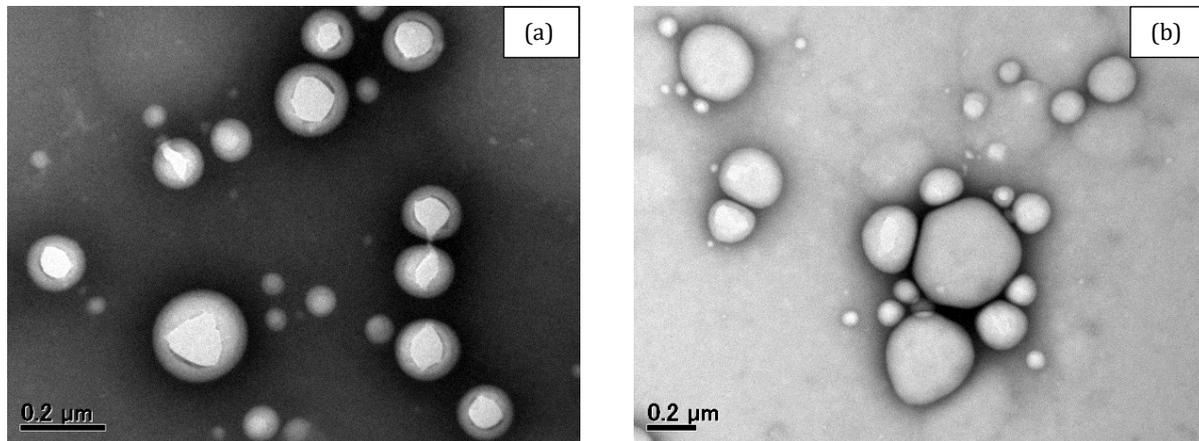


Figure 3. TEM photomicrographs of Pickering nanoemulsions stabilized with (a) OSA-modified starch, LN-0.40, and (b) chitosan–OSA-modified starch PECs, LNP10-0.40

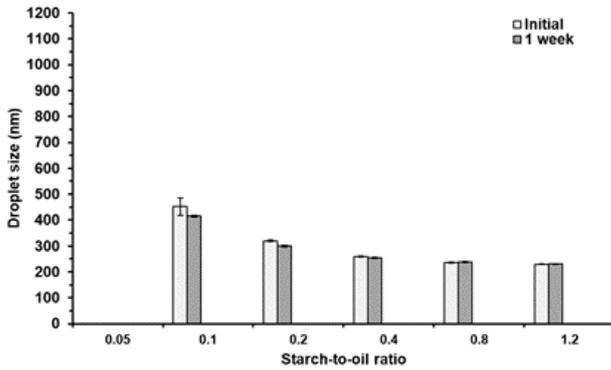
Figures 4-6 show the changes in physical properties of the nanoemulsions, including droplet size, size distribution, and zeta potential after storage for 4 weeks at $30 \pm 2^\circ\text{C}$. The nanoemulsions with OSA-modified starch were stable for only 1 week and showed phase separation after 2 weeks. This result revealed that the level of OSA-modified starch used in these formulations was not sufficient for stabilizing the nanoemulsion structure. Recently, Fang et al. (2019) reported that the amount of OSA-modified starch used as a nanoemulsion stabilizer

was higher than 7% w/w. At a molar charge ratio of 1:1 (LNP1) and a starch-to-oil ratio of less than 0.80, the nanoemulsions with chitosan–OSA-modified starch PECs were also physically stable for only 1 week. Whereas, at the starch-to-oil ratio of 0.8 (LNP1-0.8) and 1.2 (LNP1-1.2), the nanoemulsions prepared by both the PEC and LBL methods were physically stable. However, the droplet size and PDI of LNP1-1.2 dramatically increased ($p < 0.05$) with a slight decrease in zeta potential at 4 weeks of storage. This indicated the tendency of nanoemulsion instability.

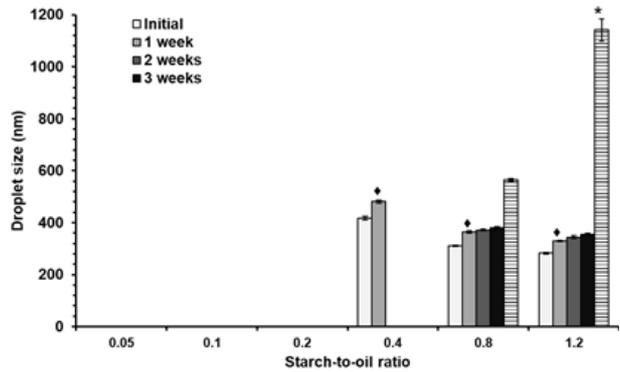
Interestingly, at a molar charge ratio of 10:1, the nanoemulsions with chitosan–OSA-modified starch PECs at the starch-to-oil ratio of 0.2, 0.4, 0.8, and 1.2 were physically stable after storage for 4 weeks. The droplet sizes (Figures 4) were slightly increased after 1 week of storage ($p < 0.05$) but unchanged with time after storing for 2-4 weeks ($p > 0.05$). When comparing LNP1 with LNP10, the 4-week physically stable LNP10 could be obtained at a

lower starch-to-oil ratio (0.2) compared with LNP1 (0.8). It was suggested that the PEC creation of chitosan with OSA-modified starch at the o/w interface was efficient to improve the physical stability of nanoemulsions. Moreover, the lower amount of OSA starch used as a stabilizer in the case of LNP10 could benefit the formulation development.

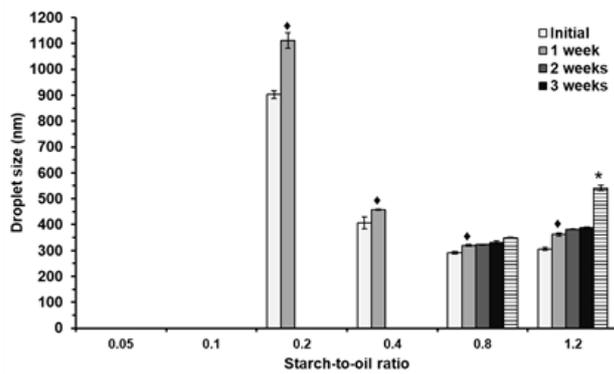
LN



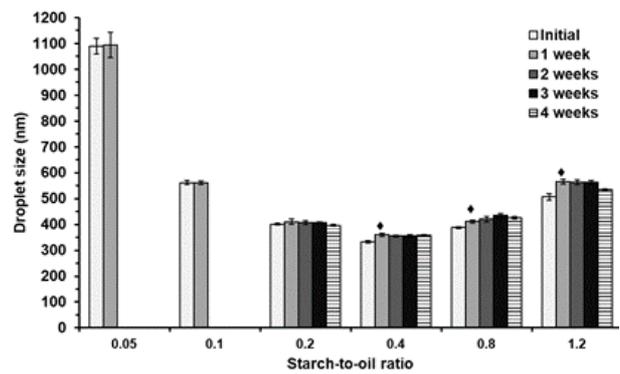
LNP1 (PEC)



LNP1 (LBL)



LNP10 (PEC)



LNP10 (LBL)

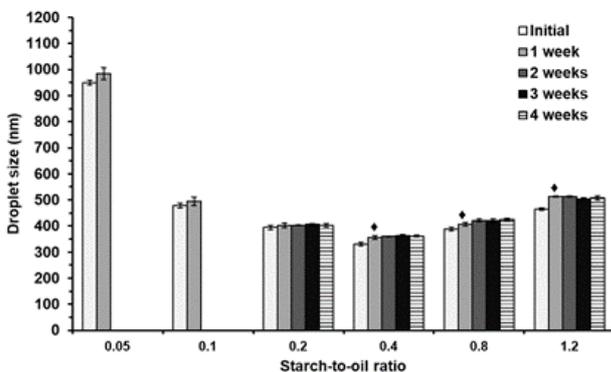
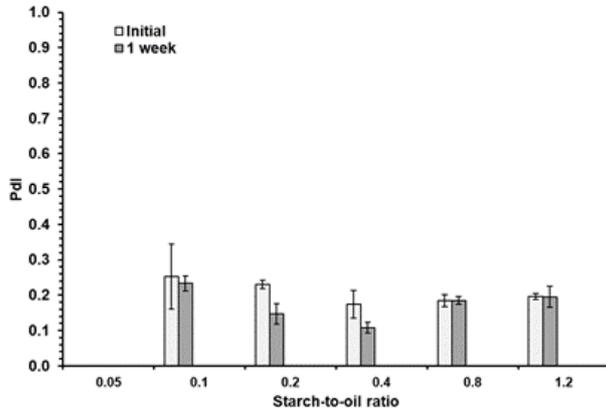
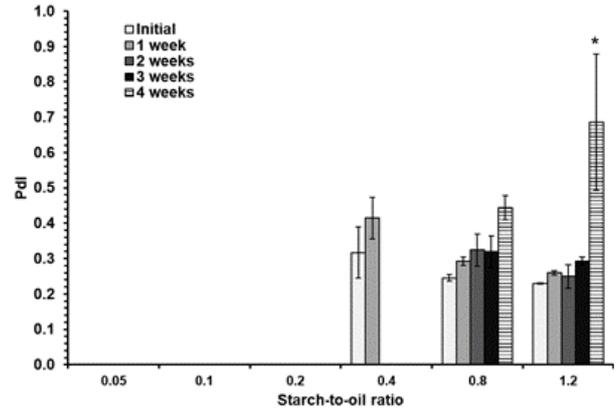


Figure 4. Effect of starch-to-oil ratio on the droplet size of different Pickering nanoemulsions at $30 \pm 2^\circ\text{C}$ for 4 weeks
 Note: *Significant difference ($p < 0.05$) between initial and 1 week storage. *Significant difference ($p < 0.05$) between 3- and 4-week storages.

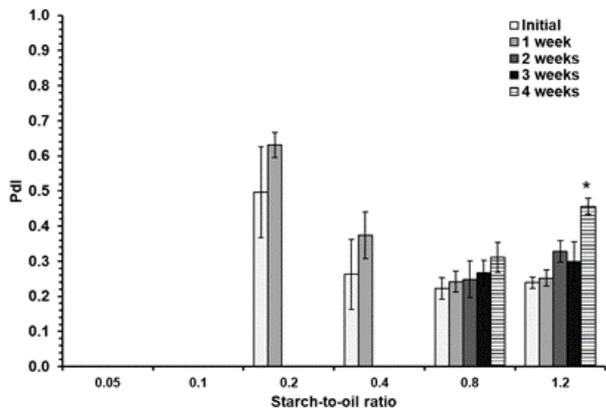
LN



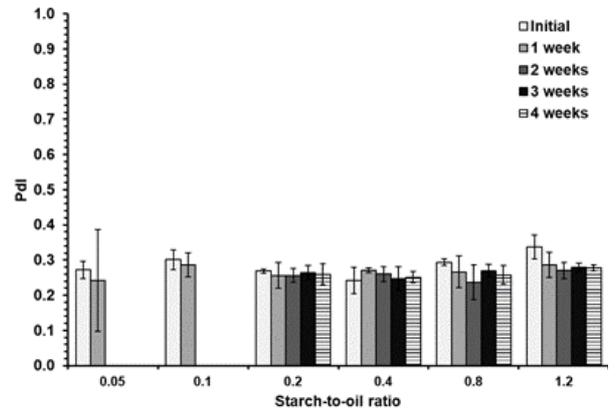
LNP1 (PEC)



LNP1 (LBL)



LNP10 (PEC)



LNP10 (LBL)

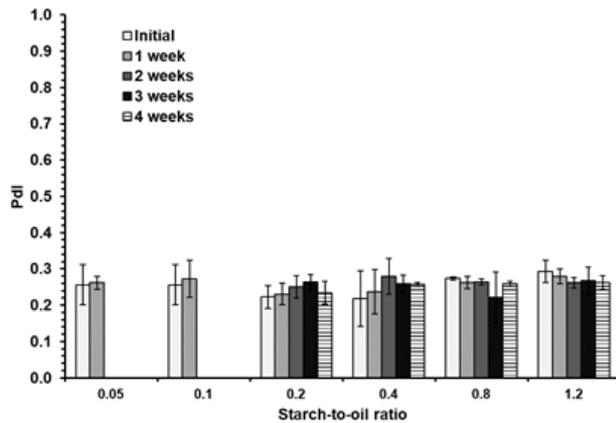


Figure 5. Effect of starch-to-oil ratio on the droplet size distribution (PDI) of different Pickering nanoemulsions at $30 \pm 2^\circ\text{C}$ for 4 weeks

Note: *Significant difference ($p < 0.05$) between 3- and 4-week storages.

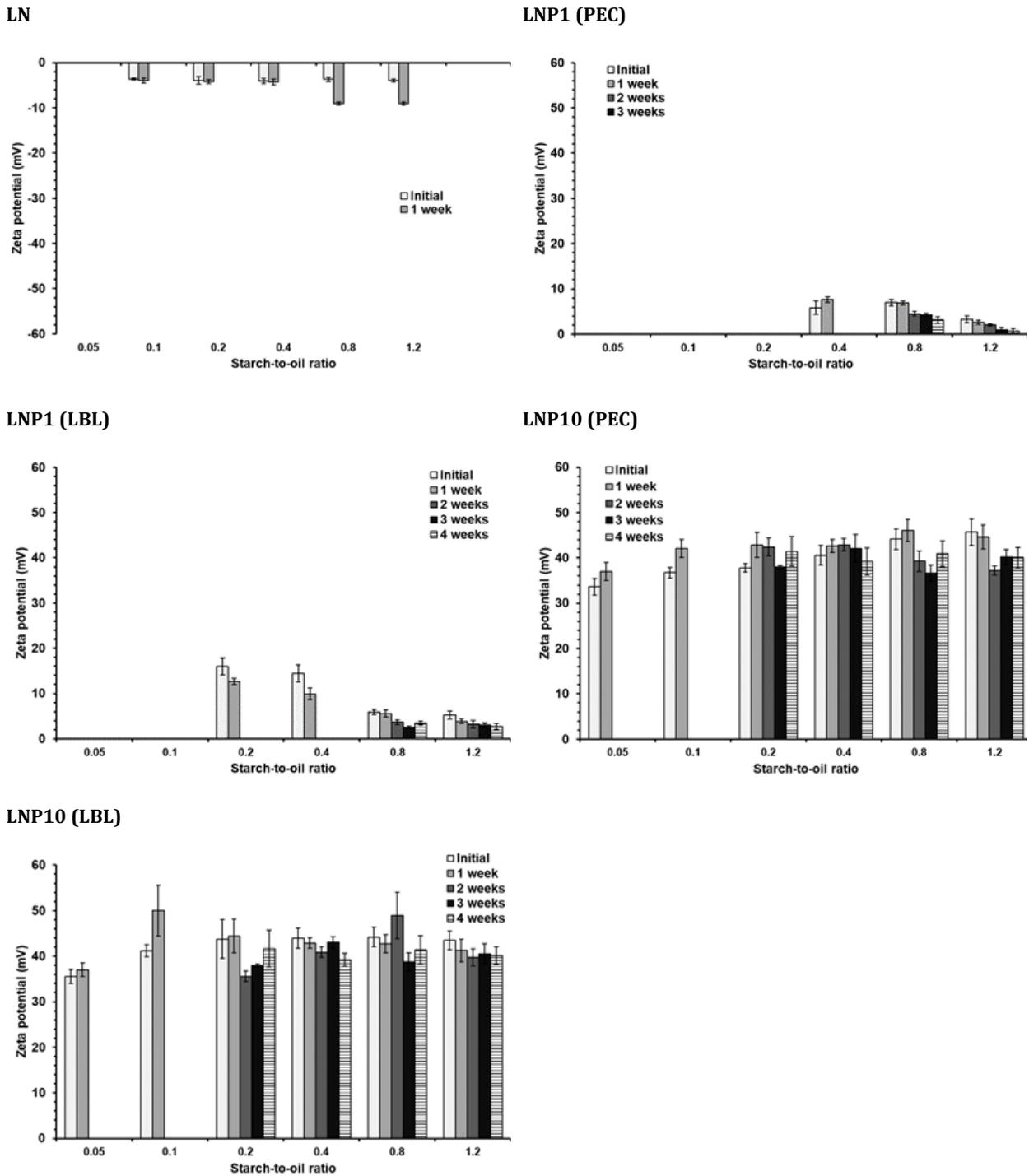


Figure 6. Effect of starch-to-oil ratio on the zeta potential of different Pickering nanoemulsions at $30 \pm 2^\circ\text{C}$ for 4 weeks

3.2 Effect of preparation methods

To determine the suitable method of nanoemulsion preparation, two different methods (PEC and LBL) were compared. In the case of the LNP1 formulations, the LNP1-0.4 (PEC), LNP1-0.2 (LBL), and LNP1-0.4 (LBL) formulations were physically stable at 2 weeks of storage (Figures 4-6). As mentioned above, the LNP1 (PEC) and LNP1 (LBL) formulations at starch-to-oil ratios of 0.8 and 1.2 were more physically stable after 4 weeks of storage. However, the LNP1-1.2 (PEC) formulation exhibited a larger droplet size (1140.3 nm), higher PDI (0.7), and lower zeta potential (0.7

mV), when compared with the LNP1-1.2 (LBL) formulation (541.5 nm, 0.5, and 2.7 mV, respectively) after 4 weeks of storage at $30 \pm 2^\circ\text{C}$.

In the case of the LNP10 (PEC) and LNP10 (LBL) formulations, the droplet size, size distribution, and zeta potential were not significantly altered with time after 4 weeks of storage at $30 \pm 2^\circ\text{C}$ ($p > 0.05$). No differences in droplet size, size distribution, and zeta potential were observed, and the physical stability of the nanoemulsions prepared from both preparation methods was comparable ($p > 0.05$). Moreover, the absolute value of zeta potential of

more than 30 mV indicated that the obtained nanoemulsions were physically stable (Yue et al., 2022).

It was suggested that the addition of a small amount of chitosan onto the nanoemulsion stabilized with OSA-modified starch (LNP1) using the LBL method could better enhance the physical stability of the nanoemulsions compared with the PEC method. However, a higher ratio of chitosan–OSA-modified starch could improve the physical stability of nanoemulsions prepared using both the PEC method and the LBL method (LNP10).

4. CONCLUSION

The effects of the type of stabilizer, starch-to-oil ratio, and preparation method on the physical stability of Pickering nanoemulsions using OSA-modified starch and chitosan–OSA-modified starch PECs as stabilizers were demonstrated. The Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs at a 10:1 molar charge ratio showed better physical stability after storage for 4 weeks at $30 \pm 2^\circ\text{C}$. At a lower starch-to-oil ratio, the droplet sizes of the nanoemulsion decreased when the ratio of starch-to-oil increased. However, when the starch-to-oil ratio was further increased to 0.8 and 1.2, the nanoemulsions with larger droplet sizes were observed. Moreover, at a 10:1 molar charge ratio and the starch-to-oil ratio of 0.2–1.2, both PEC and LBL methods were suitable for the stabilization of the nanoemulsions. In conclusion, the Pickering nanoemulsions stabilized with chitosan–OSA-modified starch PECs could be used as an emulsion template for further drug product development.

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