**Development, Characterization and Stability Evaluation of Ciprofloxacin-Loaded Parenteral Nutrition Nanoemulsions**

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Physicochemical Characterisation and Stability Evaluation of Ciprofloxacin-Loaded Parenteral Nutrition Nanoemulsions

In this study, two licensed total parenteral nanoemulsion formulations (Clinoleic® and Intralipid®) were loaded with ciprofloxacin (CP). The physicochemical characteristics and stability profiles of the formulations were investigated using a range of drug concentrations. Furthermore, formulation stability was evaluated over a period of six months at room temperature or 4°C. Loading CP into nanoemulsions resulted in no significant differences in their measured droplet size, polydispersity index (PI), zeta potential and pH. Drug entrapment efficiency (EE) was relatively high for all formulations, regardless of nanoemulsion type, and the drug release was sustained over 24 h. Stability studies of all formulations were performed at 4oC and room temperature (RT) for 180 and 60 days, respectively. At 4oC for 180 days, both Clinoleic and Intralipid formulations at a range of drug concentrations (1-10 mg/ml) showed high stabilities measured periodically by the average droplet sizes, PI, pH and zeta potential values. Similar results, but pH values, were shown when the formulations for both nanoemulsion stored at RT for 60 days. Overall, this study has shown that CP was successfully loaded into clinically licensed TPN lipid nanoemulsions. The resultant CP-loaded nanoemulsion formulations demonstrated desirable physicochemical properties and were stable upon storage at 4°C for up to six months.

Keywords: droplet size; nanoemulsion; ciprofloxacin; stability; zeta potential; polydispersity index.

# Introduction

Ciprofloxacin (CP), belonging to the second generation antibacterial quinolones, has broad bactericidal activities, making it appropriate for the treatment of respiratory tract infections, anthrax, gonorrhea, chronic prostatitis, urinary tract infections and the prophylaxis against meningitis (1–3). The superior activity of CP is attributed to its structure, specifically the fluorine group located at position 6 of the quinolone rings, and the cyclic amine at position 7, in addition to carboxylic acid and carbonyl groups at positions 3 and 4, respectively (Figure 1) (4). These features enable CP to selectively bind to DNA-gyrase, the enzyme responsible for DNA replication in prokaryotic cells, preventing bacterial replication and resulting in death of prokaryotic cell (5).

The relatively high bioavailability of CP (up to 77%) (6) and its accumulation in macrophages (7) and neutrophils (8) are responsible for the high systemic biodistribution of the drug in the main organs including lung, liver, spleen and lymph nodes. However, these excellent pharmacokinetic characteristics are detained by its short half-life (3-5 h) and high protein binding (6), limiting the drug’s ability to penetrate into the infected tissues and prohibiting the sustainability of its bactericidal effects. Furthermore, the poor aqueous solubility of CP is a major challenge (9). The drug is classified as practically insoluble in water, since its intrinsic aqueous solubility is as low as 67 μg/mL (10). Thus, designing a delivery system that can incorporate CP, enhance its aqueous solubility and penetratability into prokaryotic cells would be a desirable achievement.

Amongst many nanotechnology-based delivery systems, nanoemulsions, consisting of oil, water, and an emulsifier, are colloidal formulations made of nano-sized oil droplets homogeneously dispersed in an aqueous phase. The presence of an emulsifier is essential for the stability of nanoemulsion by reducing the interfacial tension between oil and water, hence preventing droplet-droplet interaction and subsequent oil phase separation (11). Nanoemulsions have been used to enhance the absorption of many hydrophobic drugs and improve their bioavailability (12). For example, paclitaxel can be dissolved in the lipid phase of nanoemulsion, promoting their potential for intravenous administration (13,14). Similarly, licensed lipid nanoemulsions have been used as vehicles for enhancing the bioavailability (15) and reducing the neurotoxicity of the anticancer drug hexamethyl melamine (16).

Recently, we have successfully loaded paclitaxel into clinically established parenteral nutrition nanoemulsions such as Clinoleic® and Intralipid® (17,18). Clinoleic nanoemulsion formulations were demonstrated to have smaller lipid droplets, and higher drug loading. Furthermore, the short-term stability studies (14 days) showed that paclitaxel-loaded nanoemulsions were more stable at 4°C than at room temperature (17). Both paclitaxel-loaded emulsions demonstrated a concentration-dependent cytotoxicity against glioma cell lines, resulting in a greater selectivity of the Clinoleic nanoemulsion compared to the Intralipid against cancer cells (18). It is hence proposed that exploration of the potential of licensed nanoemulsions in a field alternative to that of anticancer therapy, namely antimicrobial drug delivery, would add another milestone and open a new research venue for further investigations.

In this study, novel CP-loaded Clinoleic and Intralipid nanoemulsions were designed and characterized in terms of droplet size, polydispersity index (PI), zeta potential and pH of the resultant formulations. The drug entrapment efficiency and the in-vitro drug release profile were also studied. Moreover, the stability of the CP-loaded Intralipid® and Clinoleic® nanoemulsions were evaluated periodically for six months at 4°C and two months at room temperature.

# Materials and Methods

## Materials

Dimethyl sulfoxide (DMSO) and phosphate buffered saline (PBS) were purchased from Sigma Aldrich, UK. Absolute ethanol and HPLC-grade water were supplied by Fisher Scientific, UK. Ciprofloxacin (CP) was obtained from Sigma Aldrich, UK and the parenteral nutrition emulsions, Clinoleic® 20% and Intralipid® 20% were supplied by Baxter Healthcare, USA and Fresenius Kabi, Germany, respectively. These nanoemulsions were not prepared in our laboratory, but were rather supplied as final products from the aforementioned companies. Table 1 shows the composition of each nanoemulsion product.

## Methods

### 2.2.1. Solubilization of CP in TPN nanoemulsions

CP was weighed in amounts of 0 (blank), 10, 20, 30, 40 and 50 mg in separate glass vials. Clinoleic® or Intralipid® emulsions (10 ml) were added to each glass vial followed by vortex mixing for 5 min and bath sonication (Fisherbrand™ P-Series Ultrasonic, UK) at frequency 37 khz and 70% power (equivalent to 84 W ultrasonic effective power) for 2 h at 40°C. Preliminary results showed that there was no effect of bath sonication on the stability of emulsions (data not shown). The procedure used was adapted from our previous publication in which paclitaxel was loaded into the nanoemulsions (18).

### 2.2.2. Particle size and zeta potential analysis of nanoemulsions

Photon correlation spectroscopy was used to analyze the size (Zaverage; also referred to as hydrodynamic diameter) and size distribution (polydispersity index; PI) of nanoemulsions by employing the Zetasizer Nanoseries instrument (Malvern Instruments Ltd, UK). Clinoleic or Intralipid nanoemulsions (40 µl) (without filtration) were diluted with 1 ml of HPLC-grade water within a clean Malvern vial, and the hydrodynamic diameter and PI of the emulsion droplets were analyzed. The same instrument was employed to analyze the zeta potential of the emulsions, by laser Doppler velocimetry, by operating the relevant software. The zeta potential cuvette (Malvern Instruments Ltd, UK) was washed several times with HPLC water and sonicated prior to loading the nanoemulsion samples and measuring the zeta potential values of the different formulations.

*2.2.3. pH determination of nanoemulsions*

The pH of emulsion formulations was determined using a Corning 220 pH meter (Cole-Palmer, Teddington, UK) previously calibrated using the provided pH 4 and pH 7 standard solutions. This experiment aimed to investigate the influence of nanoemulsion type and CP concentration on the pH values of the formulations.

*2.2.4. Loading efficiency of CP in nanoemulsion droplets*

The entrapment efficiency of CP was determined by adapting the separation methods previously described (18–20). These methods are because in an emulsion, hydrophobic drug is expected to be entrapped/solubilised in the oil droplets. However, the excess amount normally remains as crystals suspended, aggregated (within the aqueous phase), or sedimented, hence, unentrapped is separated by filtration (18–20). The nanoemulsion formulations containing (10, 30 and 50 mg per 10 ml) were filtered through a 0.4 µm pore-size syringe filter (Fisher Scientific, UK). The filter was washed with HPLC water until the solution ran clear. The filter was then placed in a mixture of ethanol and acetic acid (2%) (50:50), in order to extract the drug and the proportion of the un-entrapped fraction. The entrapped drug was confirmed by diluting 0.1 ml of the filtrate with ethanol and acetic acid 2% (50:50). Un-entrapped and entrapped drug fractions were determined by Dionex Ultimate 3000 UHPLC using methods reported by Wu et al (21). CP was eluted on BetaBasic column 18 particle size 5µm, pore size 150A, 150mm L x 4.6mm (mobile phase: acetic acid 2%, acetonitrile (84:14, V/V), flow rate 1 ml/min, UV wavelength 280 nm, injection volume at 10 µL). The entrapment efficiency (EE %) (LE) of CP in nanoemulsion was calculated using equation 1:

Equation (1)

*2.2.5**. In vitro release study of CP*

The release of CP from formulations was investigated using dialysis. Prior to assay, loaded formulations of 1 mg/ml batches were filtered through 0.4 µm sterile syringe filters to remove unloaded CP, as described earlier in section 2.2.4. Considering the loading efficiency, volumes of each formulation containing 0.5 mg of CP were topped up to 1 ml with HPLC-grade water and placed in a dialysis tube (MWCO 3500) and tightly sealed. For free drug, 0.5 mg CP was dissolved in 1 ml of a mixture constituting ethanol, water and tween 80 (50:49.9:0.1). Then, the dialysis tube was immersed in 50 ml (total volume) release medium (PBS pH 7.4) containing 0.1% (v/v) Tween 80 followed by incubation with stirring for 24 h at 37oC. Samples (0.2 ml) were taken at time intervals (1, 2, 4, 6, 8 and 24 h) from the release medium for 24 h and replaced by similar volumes of fresh medium. The concentration of CP was determined by Dionex Ultimate 3000 UHPLC using the method described earlier.

*2.2.6. Stability studies,*

Products were stored at 4 ± 2 oC using a lab fridge or at 25 ± 1oC/60 %RH ± 2 % RH using Climate Chamber ICH110L (Memmert GmbH, Germany). Samples were collected every 30 days and analyzed as described above.

*2.2.7. Statistical analysis*

Each type of experiment was performed in triplicate using three different batches and the mean and standard deviation values were calculated. Student’s *t*-tests were used to calculate the significance of the difference between two groups. The difference was considered significant when the calculated probability (p) value was below 0.05. The similarity factor (f2) was used as a statistical technique to compare the dissolution profiles:

Equation (2)

In equation 2, is the reference data, is the test data and n is the number of samples. If the percentage is over 50%, this means that the two groups of data are similar (22).

# Results and Discussion

## Size and size distribution of nanoemulsion droplets

Both Clinoleic and Intralipid formulations have average droplet size measurements ranging between 220 and 250 nm (Figure 2a). Although the Intralipid shows a slightly larger droplet size compared with that of Clinoleic, the difference between the two nanoemulsions is less than 30 nm for each drug concentration. This similarity in droplet size for both nanoemulsions is also shown in all formulations even when different concentrations of CP were used. Figure 2a shows that loading the drug into the oil droplets of both formulations has no significant effect on the droplet size; the difference between the blank and that of the highest drug concentrations (10 mg/ml) was less than 5 nm, regardless of nanoemulsion type.

For both nanoemulsions at all drug concentrations, the PI values were less than 0.250 (Figure 2b). The relatively low PI values of both formulations indicate that nanoemulsions are monodisperse, hence, high colloidal stability is expected (23). This may indicate that the loaded drug is dissolved within the core of oil droplets rather than the oil/water interface. In other words, if the drug was at the surface of oil droplets, it would have caused an increment in droplet surface hydrophobicity and hence caused the droplets to agglomerate, potentially leading to larger size and higher polydispersity (24). Similar to size findings, Clinoleic formulations showed slightly lower PI values compared to that of Intralipid formulations. This might be due to the fact that sodium oleate in Clinoleic emulsion plays a role in decreasing the droplet size by decreasing the oil/water interfacial tension (18) (Table 1). Furthermore, the increase in drug concentration did not affect the PI values, showing no positive or negative correlation between the drug concentration and PI measurements, regardless of nanoemulsion type (Figure 2b).

## Zeta potential analysis and pH determination of nanoemulsions

The zeta potential values for all formulations are negative, ranging between -38 and -53.35 mV (Figure 3a). Drug loading into Intralipid nanoemulsions resulted in a trend of increasing the negative zeta potential values, but this was not statistically significant. This trend, however, was not observed for the Clinoleic nanoemulsions. Since progressive loading of CP into the emulsions caused no appreciable changes in size distribution (Figure 2) and zeta potential (Figure 3a), it is suggested that the drug is likely to be located within the core of oil droplets for both types of emulsion. It is worth to note that Clinoleic nanoemulsions showed lower negative zeta potential values compared to those of Intralipid. This might be attributed to the differences in compositions between the two emulsions (Table 1). Similar results were reported earlier by our group; whereby, loading paclitaxel into TPN nanoemulsions had no effect on the zeta potential (17). This supports the hypothesis of using TPN lipid nanoemulsions as vehicles for drugs that have poor water solubility such as paclitaxel in our previous publication (17) , and CP in our present investigation.

According to Elsheikh, et al (2012), nanoemulsions having zeta potential values of -30 mV or lower are not susceptible to coalescence during storage (i.e. electro-statically stable) (25). In the present study, all formulations had negative zeta potential values lower than -30 mV, indicating stability of the nanoemulsions. It is worth to mention that pH might influence zeta potential measurements. Explicitly, the negativity of the surface charge increases in disperse systems having pH values higher than 6, whereas neutral or positive zeta potential values are anticipated in more acidic formulations (26,27). The pH measurements for all Intralipid and Clinoleic formulations were between 6.5 and 7.7 (Figure 3b), coming in correspondence with their negative zeta potential. In this context, CP, a zwitterionic drug (pKa 6.09 and 8.62) (28), is practically insoluble at the physiological environment of pH 7.4 (10,29,30). This consolidates the merit of TPN nanoemulsion as a potential clinically feasible drug delivery system. As expected, CP was dissolved by the oil droplets, hence, no alteration to the acidity of the formulations was observed, regardless of drug concentration. The neutral pH values of the loaded formulations suggested the need for stability studies to confirm the suitability of TPN nanoemulsions loaded with CP for possible future *in vivo* investigations.

## Entrapment efficiency and loaded weight of CP in nanoemulsion droplets

Entrapment efficiency is the percentage of CP entrapped in the oil phase of the nanoemulsion, whereas drug loading represents the proportional amount (mg) of the drug-loaded to the unit of volume (ml) of nanoemulsion. Drug entrapment efficiency and loading were both investigated for a range of drug concentrations as shown in Figure 4. The highest drug entrapment efficiency among all concentrations for both nanoemulsions is shown at 5 mg/mL CP concentration (Figure 4a), reaching 87.4 ± 12% for Clinoleic formulations and 72.3 ± 12.3% for Intralipid formulations. Moreover, for all drug concentrations, CP had similar entrapment in both emulsions, though a trend of higher entrapment efficiencies was observed for the Clinoleic formulations. Nevertheless, for both types of formulations, the loading weight increased significantly (p < 0.05) with increasing the drug concentrations, reaching the maximum weight at 10 mg/mL with 7.73 ± 0.77 mg and 4.95 ± 0.59 mg for Clinoleic and Intralipid systems, respectively (Figure 4b). In comparison to findings from this study, in our previous investigation, EE% of paclitaxel in Clinoleic emulsion was found to be higher than that of Intralipid nanoemulsions (17). In contrast, Intralipid nanoemulsions offered a superior EE over Clinoleic formulations when amphotericin B was the drug of choice (31), suggesting that drug physicochemical properties is the main determinant of the most appropriate nanoemulsion composition. This indicates that the percentage of entrapment efficiency is likely to be dependent on physicochemical properties of the selected drug. Nevertheless, the advantage of using nanoemulsions loaded with hydrophobic drugs, such as CP, was confirmed because of the increase in the intrinsic solubility of the drug (Figure 4b).

## In vitro release of ciprofloxacin

In vitro release profiles of CP from both Clinoleic and Intralipid nanoemulsions were studied over 24 hours in pH 7.4 at 37 oC (Figure 5). Both nanoemulsion formulations showed sustained release profiles compared to the free drug profile (Figure 5). The presence of essential fatty acid, sodium oleate and olive oil in Clinoleic formulation did not cause a significant change in the release profile compared to that of Intralipid formulation (f2 = 73%). This might be due to the similarity in droplet size, PDI and zeta potential for both nanoemulsions. However, significant differences (f2< 50%) between the release profile of free CP compared to those of both Clinoleic and Intralipid formulations are shown, so that over 95% of drug was released from the traditional free drug formulation after 24 hours (Figure 5).

The rapid release of the free drug compared to that of drug-loaded to nanoemulsion formulations might be due to the free diffusion of CP molecules. Loaded drug molecules must leave the oil droplet of the emulsion before reaching the bulk dispersion medium. Similar release results have been reported for the release of CP from lipid-core nanocapsule formulations (32). In that study, CP was loaded into lipid-core nanocapsules composed of a polymeric shell of poly(ε-caprolactone) (PCL) and a lipid core. The time needed 50% of CP to get released from the nanocapsules was relatively short (32) compared to that found for Intralipid and Clinoleic nanoemulsions in this study. In this context, Türeli et al (2017) have reported the development of CP-loaded Poly(Lactide-co-Glycolide) (PLGA) nanoparticles for enhanced permeability (33). Importantly, the nanoemulsion formulations showed a more prolonged drug release compared to that of the PLGA nanoparticle formulations. In both nanocapsule and nanoparticle formulations, the time needed for 50% of the drug to be released did not exceed 3 h, whereas it was approximately 8 h in our nanoemulsion formulations. Therefore, the results of in vitro release studies revealed that nanoemulsions are comparable or superior to those shown with other delivery systems, and owing to the fact that our nanoemulsions are licensed parenteral formulations, their biocompatibility is well established. However, in vivo studies using appropriate animal models are needed in the near future to investigate the potential influence of CP incorporation on formulation biocompatibility.

## Stability studies

The heterogeneous construction of emulsion systems makes them inherently thermodynamically unstable; this is considered one of the major challenges of designing nanoemulsion formulations that are stable during storage. The stability of an emulsion is dependent on its physicochemical properties as well as the storage conditions (34). Therefore, it is important to investigate the effect of loaded CP on the physical stability of Clinoleic and Intralipid nanoemulsions.

As shown in Figure 6, for both Clinoleic and Intralipid formulations, the average droplet size values did not change significantly (p>0.05) following storage for 180 days at 4oC (Figure 6a). These results apply for both the blank (i.e. drug-free) and drug-loaded emulsions at all concentrations. This gives an initial suggestion of the compatibility of CP (at studied concentrations) with the components of both parenteral nanoemulsions.

Similarly, PI values of both nanoemulsion formulations were not affected by increasing the drug concentration up to 10 mg/mL for 180 days at 4oC (Figure 6b). The PI values for all concentrations remained around 0.250 with small variances, indicating that there is a narrow distribution range of droplet size (35).

Zeta potential measurements for all blank and loaded formulations also showed no significant changes during the period of storage at 4oC for CP (Figure 7a). All zeta potential readings were of high negative values (> -30 mV). This means that the surface charges for all droplets remained stable, allowing active repulsion among the droplets during prolonged storage for 6 months. Consequently, the droplets had less tendency to coalesce, constituting a positive indicator for long-term stability of the emulsions.

**(a)**

**(b)**

Clinoleic 20%

Intralipid 20%

Similar to size, size distribution and zeta potential values, pH values of the nanoemulsions loaded with a range of CP concentrations remained stable for the whole period of 180 days at 4 oC temperature (pH measurements were in the neutral region between 6.0 and 8.0 (Figure 7b)). This correlates with the physiological pH (7.4), indicating no undesirable osmotic effects of the nanoemulsion on blood components are likely to happen.

The stability of nanoemulsions were also studied at room temperature (Figures 8 and 9). The average droplet size (Figure 8a) and the PI (Figure 8b) did not change significantly (P>0.05) in both Clinoleic and Intralipid formulations after 60 days of storage at room temperature. These results confirm that these nanoemulsions did not demonstrate coalescence that may lead to heterogeneity during storage at room temperature or at 4oC.

No change in the values of zeta potential for both Clinoleic and Intralipid nanoemulsion formulations were recorded during storage at room temperature for 60 days (Figure 9a). The zeta potential values for all nanoemulsions were not affected by the inclusion of the drug at all concentrations studied at different storage conditions (i.e. at 4oC and room temperature). However, this was not applied for pH values of the formulations during 60 days of storage at room temperature. As shown in Figure 9b, the pH values were constant for the first month. After the second month, pH values dropped from 7.3 and 6.6 in the day of preparation to 4.1 and 3.4 for blank Clinoleic and Intralipid nanoemulsions, respectively. This might be due to the hydrolysis of phospholipid in the emulsion formulations to fatty acids. Similar pH stability results were shown for Clinoleic and Intralipid nanoemulsion loaded with paclitaxel (18). Nevertheless, in the work conducted by Kadam and co-workers (18), the decrease in the pH was accompanied by an increase in droplet size and changes in the zeta potential measurements (17,30). In contrast, this effect was not observed in the case of CP formulations since only pH values were changed during storage at room temperature. This might be explained by the nature of the chemical structure of CP, classified as a zwitterionic drug, moderating the possible changes in the pH values of the formulation. This ’buffering’ effect can be noticed when increasing the drug concentration in both nanoemulsion formulations. While Clinoleic pH increased significantly (p< 0.05) from 4.6 to 6.1 when the concentration was increased from 1 to 10 mg/mL, Intralipid pH increased from 3.9 to 5.9 for the same range of drug concentrations (Figure 9b).

It is worth noting that these changes on pH values were not observed for both nanoemulsions when they were stored in their original sealed bags (data not shown). This might indicate that when more cautious procedures for drug loading are applied (e.g. within sterile area), more stable formulations at room temperature should be expected.

Although both nanoemulsion formulations behaved similarly in terms of in vitro tests, it has been reported that after 24 h of in vivo application, Clinoleic parenteral nanoemulsion may cause fewer side effects than Intralipid parenteral formulations (36). It was found that Intralipid parenteral nanoemulsion might increase blood pressure, endothelial dysfunction and inflammatory reactions, due to the presence of soybean oil. However, these effects were not observed for olive oil (i.e. in Clinoleic formulations). Nevertheless, both nanoemulsion carriers are approved by regulatory authorities in Europe and USA (36).

# Conclusion

Licensed parenteral nutrition nanoemulsions, Clinoleic® and Intralipid® were successfully loaded with ciprofloxacin at a range of concentrations (1-10 mg/ml). The droplet size, Polydispersity Index (PI), pH and zeta potential values of the ciprofloxacin-loaded emulsions were similar to those of drug-free formulations. Both nanoemulsion formulations (Clinoleic and Intralipid) showed similar loading efficiencies with a higher trend (not significant) for Clinoleic over Intralipid formulations. Both formulations demonstrated sustained drug release over 24 hours.

Despite the fact that CP loaded Clinoleic and Intralipid parenteral formulations have shown excellent in vitro stability at 4oC, more studies are needed to explain the drop in the pH values of both formulations after 2 months of storage at room temperature. Specifically, aseptic conditions of loading might be required to produce formulations with long-term stability at room temperature.

The results suggest that parenteral nutrition lipid nanoemulsions have the potential to be used as biocompatible vehicles for poorly water-soluble antibacterial drugs (e.g. ciprofloxacin). In vivo studies are required to validate this hypothesis.

**Conflicts of Interest**

The authors declare no conflict of interest.

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Table 1. Compositions of the Clinoleic and Intralipid emulsions.

|  |  |
| --- | --- |
| **Clinoleic TPN (100 ml)** | **Intralipid TPN (100 ml)** |
| Refined olive oil (approx. 80%) and refined soybean oil (approx. 20%) 20 g | Refined soybean oil 20 g |
| Purified egg phospholipids 1.2 g | Purified egg phosphatides 1.2 g |
| Glycerol 2.25 g | Glycerol anhydrous 2.2 g |
| Essential fatty acids 4 g  Sodium oleate 0.03 g |  |

Figure 1. The chemical structure of ciprofloxacin.

Figure 2. (a) Size (Z average) and (b) PI of Clinoleic and Intralipid nanoemulsion droplets at a range of CP concentrations (n=3 ± SD).

Figure 3. (a) Zeta potential and (b) pH of Clinoleic and Intralipid formulations at a range of CP concentrations (n=3 ± SD).

Figure 4. (a) Loading efficiency of CP in Clinoleic and Intralipid nanoemulsions and (b) CP loaded per ml of the nanoemulsion (n=3 ± SD).

Figure 5. The release profiles of CP (0.5 mg/ml) from Clinoleic and Intralipid nanoemulsion at 37 oC (n=3 ± SD).

Figure 6. (a) Size (Z average) and (b) PI of Clinoleic (left) and Intralipid (right) nanoemulsion droplets at a range of CP concentrations stored at 4 oC (n=3 ± SD).

Figure 7. (a) Zeta potential (b) pH of Clinoleic (left) and Intralipid (right) nanoemulsion droplets at a range of CP concentrations stored at 4 oC (n=3 ± SD).

Figure 8. (a) Size (Z average) and (b) PI of Clinoleic (left) and Intralipid (right) nanoemulsion droplets at a range of CP concentrations stored at 25 oC (n=3 ± SD).

Figure 9. (a) Zeta potential (b) pH of Clinoleic (left) and Intralipid (right) nanoemulsion droplets at a range of CP concentrations stored at 25 oC (n=3 ± SD).