Novel paclitaxel formulations solubilized by parenteral nutrition nanoemulsions for application against glioma cell lines

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A B S T R A C T

The aim of this study is to investigate using nanoemulsion formulations as drug-delivery vehicles of paclitaxel (PX), a poor water-soluble anticancer drug. Two commercially available nanoemulsion fat formulations (Clinoleic 20% and Intralipid 20%) were loaded with PX and characterised based on their size, zeta potential, pH and loading efficiency. The effect of formulation on the cytotoxicity of PX was also evaluated using MTT assay.

The droplet size of the Clinoleic emulsion increased from 254.1 nm to 264.7 nm when paclitaxel (6 mg/ml) was loaded into the formulation, compared to the drug-free formulation. Similarly, the droplet size of Intralipid increased from 283.3 to 294.6 nm on inclusion of 6 mg/ml paclitaxel. The Polydispersity Indexes (PDIs) of all the nanoemulsion formulations (Clinoleic and Intralipid) were less than 0.2 irrespective of paclitaxel concentration indicating that all nanoemulsion formulations used were homogeneously sized. The pH range for the Clinoleic formulations (7.1–7.5) was slightly higher than that of the Intralipid formulations (6.5–6.9). The zeta potential of linoleic had a greater negative value than that of Intralipid.

Loading efficiencies for paclitaxel were 70.4–80.2% and 44.2–57.4% for Clinoleic and Intralipid formulations, respectively. Clinoleic loaded with paclitaxel decreased the viability of U87-MG cell to $6.4 \pm 2.3\%$, compared to Intralipid loaded with paclitaxel ($21.29 \pm 3.82\%$). Both nanoemulsions were less toxic to the normal glial cells (SVG-P12), decreasing the cell viability to 25–35%. This study suggests that nanoemulsions are useful and potentially applicable vehicles of paclitaxel for treatment of glioma.

1. Introduction

Paclitaxel (PX) is widely used for its anticancer activity against ovarian carcinoma, head and neck cancers, breast cancer, lung cancer and AIDS related Kaposi's sarcoma (Rowinsky and Donehower, 1995). However, the clinical use of paclitaxel is limited because of its poor water-solubility as well as low cellular permeability (Panchagnula, 1998; Singla et al., 2002; Wani et al., 1971; Yoshizawa et al., 2014). For clinical administration, paclitaxel is dissolved in Cremophor® EL (Poly-oxyethylated castor oil) and

ethanol (50:50 v/v) followed by dilution by 5–20 times before parenteral administration. The commercially established formulation of Cremophor® EL-based paclitaxel is "Taxol®". Unfortunately, this formulation causes serious toxic effects such as nephrotoxicity, hypersensitivity reactions, neurotoxicity, laboured breathing, hypotension and lethargy (Singla et al., 2002). Therefore, a biocompatible formulation that can increase aqueous solubility and improve therapeutic efficacy of the drug is needed (Kadam et al., 2014). For example, clinical trials have demosnstrated that Abraxane®, a formulation of paclitaxel loaded onto albumin nanoparticles can provide enhanced penetration of the anticancer drug in the tumour by 33% compared to Taxol® (Garber, 2004). However, as human albumin is used in this formulation, designing alternative nanomedicines that are safe and economically affordable is highly desirable. There has been growing interest in using

liposomes as carriers for paclitaxel; intracranial administration of liposomal paclitaxel in a rat brain tumour model increased the life span of the animals up to 40% compared to cremophor EL and ethanol mixture formulations of paclitaxel (Zhou et al., 2010). Lipusu® (Luye Pharma Group) is a paclitaxel-liposomal formulation that has recently been commercialized and recommended for the treatment of breast, ovarian and non-small cell lung cancer (Koudelka and Turánek, 2012; Wang et al., 2013).

Recently, nanoemulsion formulations have been used as delivery vehicles for paclitaxel and docetaxel. Paclitaxel readily dissolves in the dispersed lipid phase and can easily be administered intravenously due to the continuous aqueous phase (Choudhury et al., 2014a; Pawar et al., 2014). Early reports have shown that lipid nanoemulsions licensed as parenteral nutrition (PN) (e.g. Intralipid®) are potentially applicable solubilizers of lipophilic anticancer drugs. This was achieved by using a cosolvent (e.g. ethanol) to dissolve the anticancer drug followed by addition to the nanoemulsion. Following in vivo studies, these formulations were reported to be suitable for human administration (Fortner et al., 1975; Ames and Kovach, 1982). Recently, we have used licensed nanoemulsionsas delivery vehicles for paclitaxel. However, to avoid the irritant effect of the colsolvent ethanol, loading of the drug into the emulsion droplets was assisted by the use of bath sonication (Kadam et al., 2014).

In this study, two commercially available lipid nanoemulsions that are routinely used in parenteral nutrition (PN)/total parenteral nutrition (TPN) were used to solubilize paclitaxel for application on glioma cell lines. Intralipid (Fresenius Kabi, Germany) and ClinOleic (Baxter, USA) are lipid nanoemulsions having different excipients. Our research group has shown that both these nanoemulsions are suitable for solubilizing the highly hydrophobic antifungal drug amphotericin B and that such formulations can generate inhalable aerosols by air-jet nebulization (Nasr et al., 2012). Intralipid is composed of egg phospholipid, soybean oil and glycerine, while Clinoleic comprises refined olive oil and soybean oil (Table 1). Recently, we reported the short-term stability of paclitaxel-loaded Clinoleic® and Intralipid® nanoemulsions when stored at room temperature and at 4 °C. On storage for 14 days, paclitaxel-loaded nanoemulsions were more stable at 4 °C than at room temperature, and the Clinoleic formulations were more stable than the Intralipid emulsions, having smaller droplet size, and pH values closer to that of blood plasma (Kadam et al., 2014).

In this study, the physicochemical properties of paclitaxel-loaded nanoemulsions of Clinoleic® and Intralipid® such as size distribution and zeta potential of the emulsified droplets, pH of the formulations, and drug entrapment efficiency of paclitaxel in the lipid phase were studied. Moreover, the cytotoxicity of the formulations on grade IV glioma (U87-MG) and normal glial (SVG-P12) cell lines was investigated.

2. Materials and methods

2.1. Materials

Dextran (MW 5000), dimethyl sulfoxide (DMSO), Phosphate buffered saline (PBS), poly-L-lysine (PL; MW 70,000), sodium pyruvate and trypan blue were all purchased from Sigma Aldrich, UK. Trypsin-EDTA (Ethylenediaminetetraacetic acid) solution, absolute ethanol, 70% ethanol and HPLC-grade water were supplied by Fisher Scientific, UK. Eagle's minimum essential media (EMEM), non-essential amino acid solution (100x) and L-glutamine (2 mM) were purchased from Lonza, Switzerland. The anticancer drug paclitaxel 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. The U87-MG (grade IV glioma cell lines) and SVG-P12 (normal glial cell lines) were supplied by the European Collection of Cell Cultures (ECACC).

2.2. Methods

2.2.1. Solubilization of paclitaxel in PN nanoemulsions

Paclitaxel was weighed in amounts of 0 (blank), 10, 20, 30, 40, 50 and 60 mg in separate glass vials. 10 ml of Clinoleic or Intralipid emulsions were added to each glass vial followed by vortex-mixing for 5 min and bath sonication 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).

2.2.2. Particle size and zeta potential analysis of nanoemulsions

Photon correlation spectroscopy (dynamic light scattering) was used to analyse the size distribution of nanoemulsions by employing the Zetasizer Nanoseries instrument (Malvern Instruments Ltd, UK). Clinoleic or Intralipid nanoemulsions (40 μl) (without any filtration) were diluted with 1 ml HPLC-grade water in a clean Malvern sample vial, and the hydrodynamic diameter and polydispersity index (PI) of the emulsion droplets were measured. The same instrument was employed to analyse 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 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 solutions. This experiment aimed to investigate the influence of nanoemulsion type and paclitaxel concentration on the pH, and compare the values with those of blood plasma.

2.2.4. Loading efficiency of paclitaxel in nanoemulsion droplets

Entrapment efficiency of paclitaxel was determined by adapting the separation methods previously described by Kumar et al. (2001) and Gala et al. (2015). The nanoemulsion formulations containing paclitaxel (10, 30 and 60 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 absolute ethanol and paclitaxel was extracted. The extracted fraction was collected to determine the proportion of un-entrapped drug by measuring the absorbance in

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	

ethanol at 227 nm using a UV spectrophotometer (Jenway 7315 Spectrophotometer, UK). This amount was subtracted from the total amount of paclitaxel in the formulation to calculate the amount of entrapped drug. The solubility of paclitaxel in water is less than 0.1 μg/ml (Konno et al., 2003), therefore, the amount of the drug dissolved in water during hydration was negligible. The loading efficiency (LE) of paclitaxel (PX) in nanoemulsion was calculated using the following equation:

$$EE(\%) = \frac{\text{Amount of PX entrapped}}{\text{Total amount of PX in nanoemulsion formulation}} \times 100$$

2.2.5. Cytotoxicity study (MTT assay)

The U87-MG cells (grade IV glioma, passage 28–31) and SVG-P12 (glial cells, passage 15–18) were seeded at 1×10^5 cells/well in 96-well plates and maintained at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in Eagle's minimum essential medium (EMEM) supplemented with 10% FBS, 1 mM sodium pyruvate,

2 mM L-glutamine and 0.1 mM non-essential amino acids. After 24 h incubation, the cells were washed in PBS solution, and 200 μl of tested formulations at a range of concentrations was added. Prior to assay, both loaded nanoemulsions of 1 mg/ml were filtered through a 0.4 μm sterile syringe filters to avoid contamination and to remove unloaded paclitaxel. Resulting filtered nanoemulsions of paclitaxel concentrations of 0.704 and 0.570 mg/ml for Clinoleic and Intralipid, respectively (Fig. 4), were used as stock nanoemulsions diluted by media to 1, 2.5, 5, 25, 50, 250, 500, 1500, 3000 nM of loaded paclitaxel. Additionally, drug-free nanoemulsions and paclitaxel alone (i.e. without emulsions, in similar concentrations range to that of loaded drug) were applied on the cells. Poly-L-lysine (PLL) and dextran were used as positive control and negative controls respectively (Fig. 5). Traces of dimethylsulfoxide (DMSO) (up to 0.3%) were used to solubilise free paclitaxel in cell culture medium. After 72 h incubation at 37 °C, 20 μl of 3-(4,5-dimethythiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added and cells were incubated for a
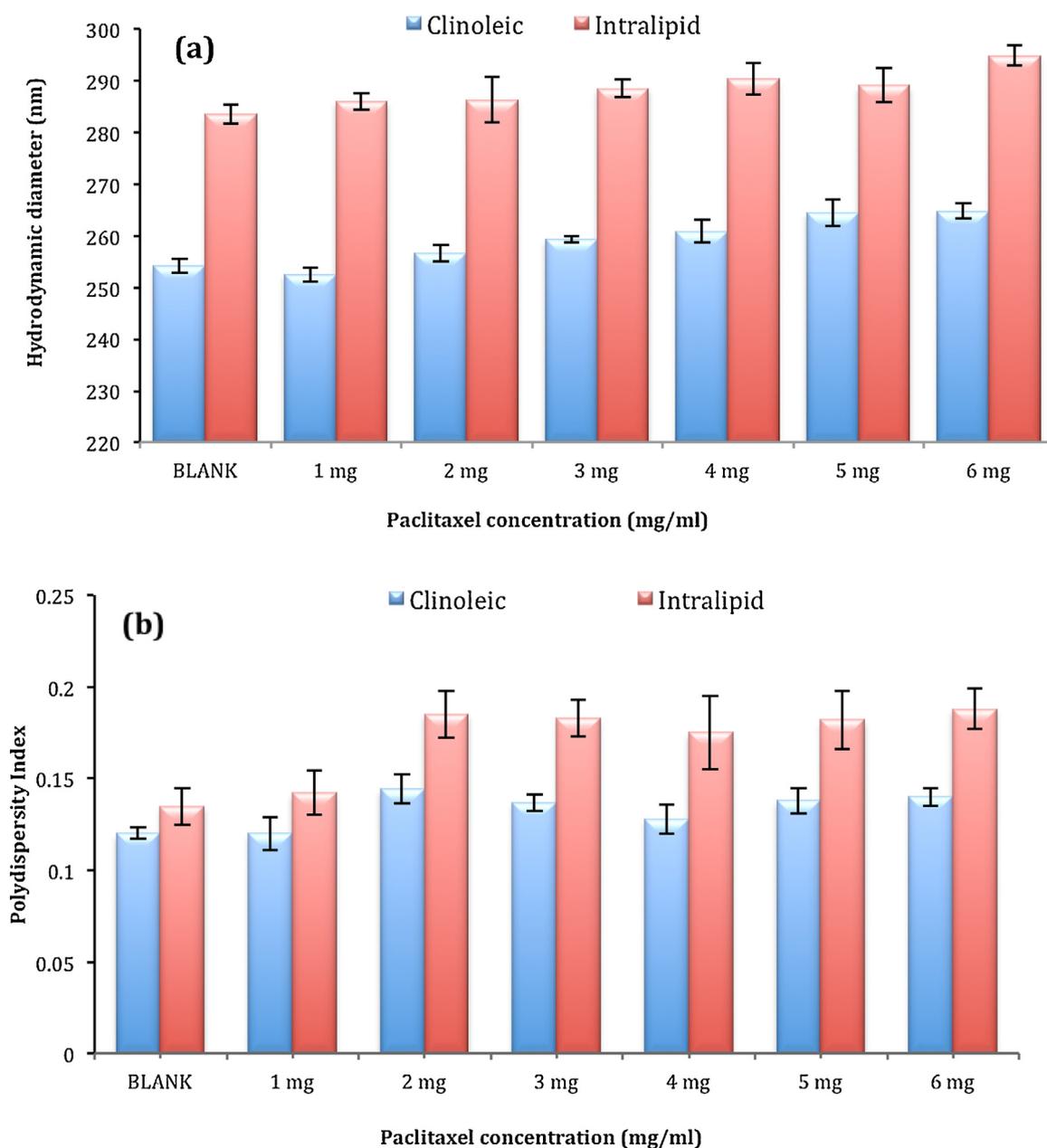

Fig. 1. (a) Size (Zaverage) and (b) PI of Clinoleic and Intralipid nanoemulsion droplets at a range of paclitaxel concentrations (n = 3 ± SD).

further 5 h. Medium was removed, and 100 μl DMSO was added and incubated for 30 min at 37 °C. The optical density at 570 nm was measured (Tecan GENios Plus, Switzerland), and the level of colour development in the control wells (containing medium only) was taken to indicate 100% viability). The IC_{50} values (i.e. concentration resulting in 50% inhibition of cell growth) of the nanoemulsions and paclitaxel were calculated graphically from the cell-viability curves obtained by considering the absorbance of the media containing cells as 100% (Yang et al., 2007).

2.2.6. Statistical analysis

All experiments were performed three times using three different batches and the results are presented as the mean ± SD. The student's *t*-tests and one-way ANOVA tests were performed using SPSS 14.0 software to calculate the significance between the groups. The differences were considered to be statistically significant if the P-value was less than or equal to 0.05.

3. Results and discussion

3.1. Size distribution of nanoemulsion droplets

The droplet size of the nanoemulsions was dependent on both emulsion type and paclitaxel concentration in the formulations (Fig. 1a). The mean size of the Clinoleic emulsion droplets was significantly smaller than Intralipid droplets (P < 0.05) regardless of paclitaxel concentration (Fig. 1a). Moreover, increasing the concentration of paclitaxel caused a significant increase in the mean droplet size (P < 0.05) for both emulsions. Fig. 1a shows that droplet size of the Clinoleic emulsion increased from 254.1 nm when no drug was included to 264.7 nm when the drug concentration was 6 mg/ml, whilst for Intralipid, the mean droplet size was 283.3 nm without drug and 294.6 nm with 6 mg/ml paclitaxel.

All nanoemulsion formulations had polydispersity index (PI) values below 0.2, regardless of emulsion type and paclitaxel concentration (Fig. 1b). Particles may be considered monodispersed if the PI is less than 0.2 (Bernardi et al., 2011). The monodisperse size distribution of paclitaxel nanoemulsions in this study suggests that paclitaxel has been predominantly accommodated in the bulk of the oil droplets.

Similar to droplet size results, for each paclitaxel concentration the PI was significantly lower (P < 0.05) for the Clinoleic nanoemulsion compared to the Intralipid formulation. Furthermore, loading paclitaxel resulted in a significant increase (P < 0.05) of PI for both nanoemulsions and this was greater for the Intralipid emulsion (Fig. 1b). For instance, compared with the drug-free nanoemulsions, inclusion of paclitaxel (6 mg/ml) caused a

significant increase (P < 0.05) in the PI by 16.6% for the Clinoleic droplets and 39.25% for the Intralipid formulation. It is noteworthy that in the preliminary experiments, filtered samples of both nanoemulsions (through 0.4 μM filters) were compared with unfiltered samples in terms of size, pH, and zeta potential. There were no significant differences between filtered and unfiltered nanoemulsions in terms of size, pH and zeta potential (data not shown). This might indicate that unloaded paclitaxel had no impact on the droplets size and polydispersity of both nano-emulsions.

The difference in mean size and polydispersity between the two types of nanoemulsion is attributed to the difference in formulation composition (Table 1), where the additional surfactant in the Clinoleic emulsion (i.e. sodium oleate) seems to be better than phospholipid in the Intralipid formulation at making the droplets smaller even when paclitaxel concentration was as high as 6 mg/ml.

3.2. pH of nanoemulsions

The pH values for the nanoemulsions of Clinoleic formulations were slightly basic, while the Intralipid formulations were slightly acidic, regardless of paclitaxel concentration (Fig. 2). For both nanoemulsion types, the influence of paclitaxel concentrations on the measured pH was not significant (P > 0.05) indicating that these paclitaxel-loaded nanoemulsions would be potentially appropriate for intravenous administration. The combination of a clinically approved anticancer drug namely paclitaxel and clinically established PN nanoemulsions suggests these formulations are particularly appropriate for future *in vivo* investigations for the treatment of cancer.

3.3. Surface charge of nanoemulsion droplets

The zeta potential of the droplets of Clinoleic had higher negative charge than that of the Intralipid emulsion, regardless of paclitaxel concentration (p < 0.05; Fig. 3). The zeta potential of the Intralipid emulsions was almost neutral, particularly at paclitaxel concentrations between 2 and 6 mg/ml (Fig. 3). Overall, the zeta potential was dependent (p<0.05) on emulsion type rather than paclitaxel concentrations (P>0.05 for both emulsions), indicating that the surface charge of these emulsions was determined by emulsion excipients rather than paclitaxel incorporation i.e. paclitaxel is incorporated in the bulk of droplets.

It has been previously reported that zeta potential of nano-emulsion formulations depends on the pH of the preparation. If the pH is high (i.e. basic pH) the zeta potential value is likely to be negative whilst if the pH is low (i.e. acid pH) the zeta potential will
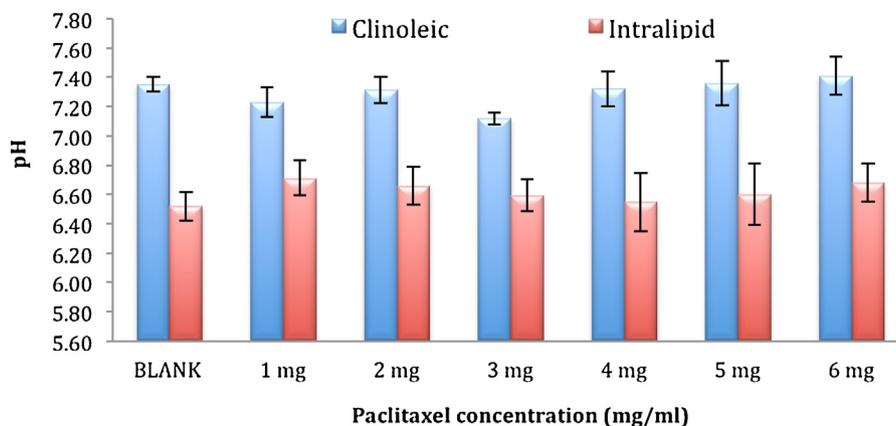

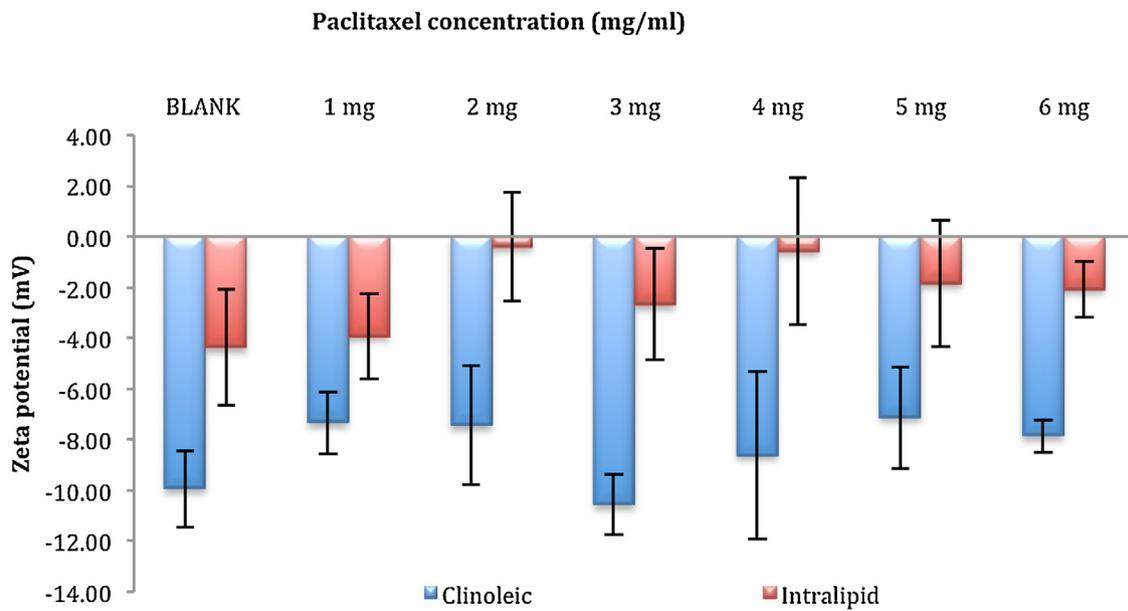**Fig. 2.** pH of Clinoleic and Intralipid formulations at a range of paclitaxel concentrations (n = 3 ± SD).

Paclitaxel concentration (mg/ml)

Fig. 3. Zeta potential of droplets in Clinoleic and Intralipid nanoemulsion formulations at a range of paclitaxel concentrations (n = 3 ± SD).
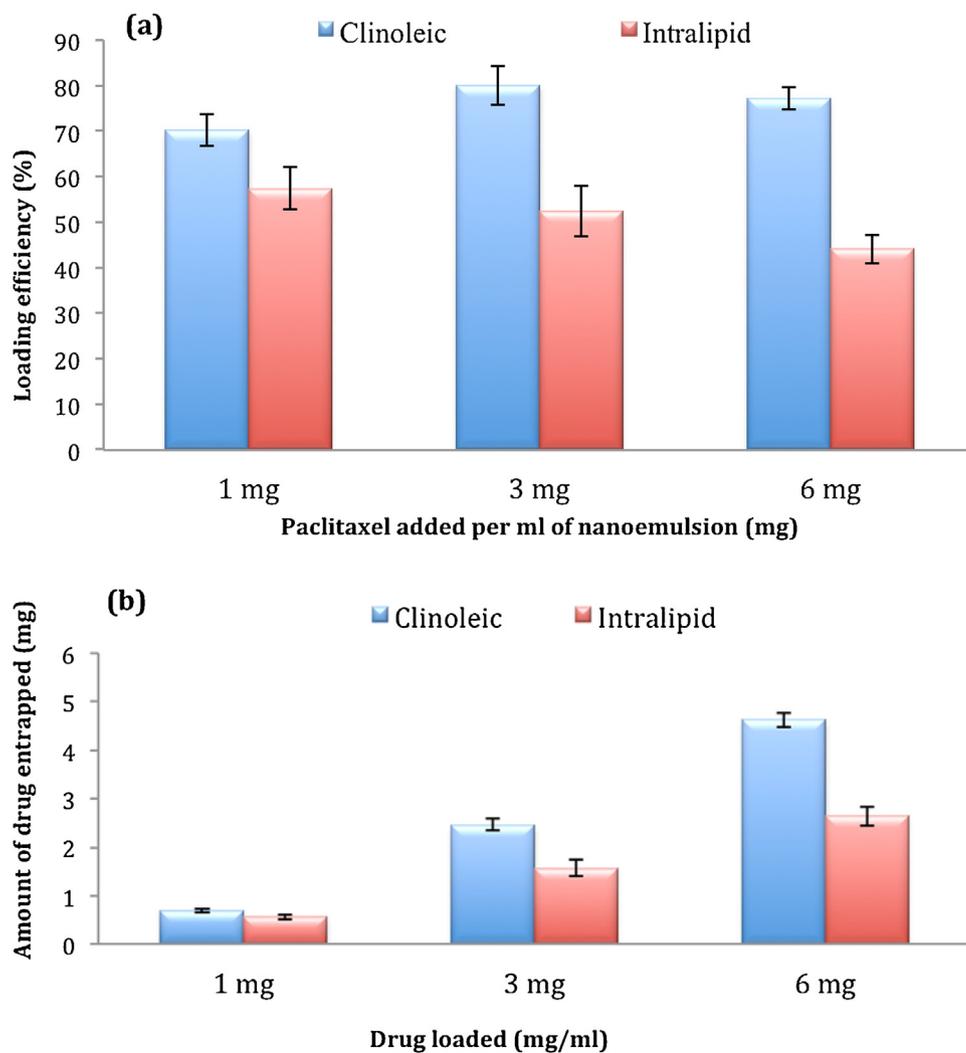

Fig. 4. (a) Loading efficiency of paclitaxel in Clinoleic and Intralipid nanoemulsions and (b) paclitaxel loaded per ml of the nanoemulsion (n = 3 ± SD).

possibly be neutral or positive (Poluri et al., 2011). Thus, the highly negative zeta potential of the Clinoleic emulsion is possibly attributed to its higher pH (Fig. 3). For a nanoemulsion to be electro-statically stable, so that it is not susceptible to coalescence during storage, the zeta potential values should be close to or above ±30 mV (Elsheikh et al., 2012). Short-term (two weeks) stability studies in our laboratory indicated that these paclitaxel-loaded nanoemulsions are stable if stored at 4 °C, but not at room temperature (Kadam et al., 2014). Long-term stability studies are still needed in the future to evaluate the effect of paclitaxel loading on the stability of Clinoleic and Intralipid nanoemulsions.

3.4. Loading efficiency of paclitaxel in nanoemulsion droplets

Loading was calculated as the percentage proportion of paclitaxel loaded into the emulsion droplets (Fig. 4a) and the amount of paclitaxel loaded per unit volume of the emulsion (Fig. 4b). Both nanoemulsions are 20%, thus each ml of the emulsion contains 200 mg oil plus trace amounts of surfactants (Table 1).

Fig. 4a shows that drug loading efficiency was dependent on nanoemulsion composition and drug concentration. In the Clinoleic nanoemulsions, 70.4 ±3.5%, 80.2 ±4.2% and 77.3 ±2.5%
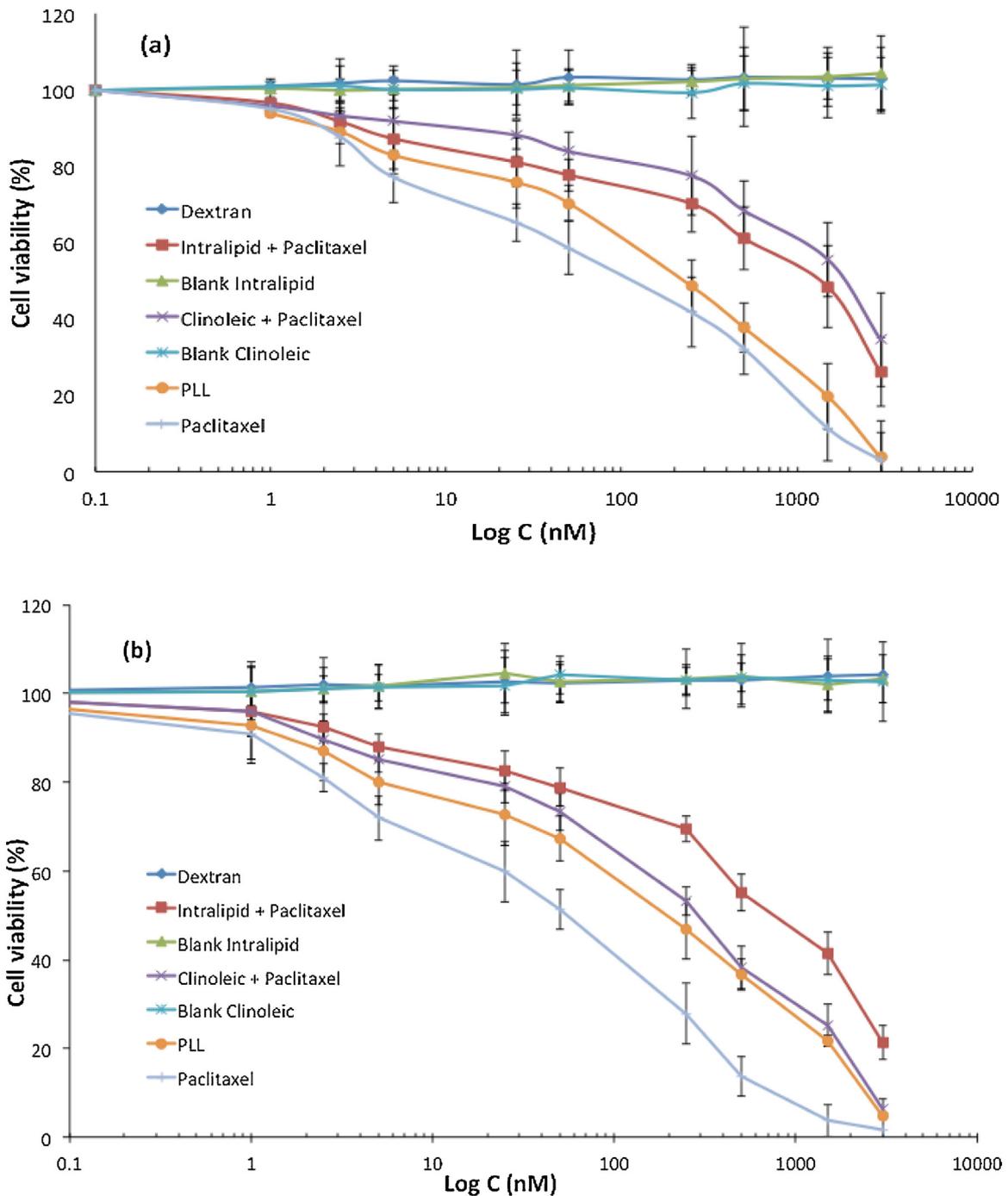

Fig. 5. Viability of (a) SVG-P12 and (b) U87-MG cell lines with increasing concentrations of different formulations in 96-well plates (n = 3 ± SD).

of paclitaxel were successfully loaded into the droplets using drug concentrations of 1 mg/ml, 3 mg/ml and 6 mg/ml respectively (Fig. 4a). This means that loading efficiency of the drug significantly increased ($P < 0.05$) upon increasing the drug concentration from 1 mg/ml to 3 mg/ml, however, no increase in loading efficiency was seen when 6 mg/ ml was compared with 3 mg/ml (Fig. 4a). When compared with the Clinoleic emulsion, the Intralipid nanoemulsion offered lower drug loading efficiencies, regardless of paclitaxel concentration (Fig. 4a). This is contrary to our observation using the antifungal drug amphotericin B loaded into the same emulsions, where the Intralipid accommodated greater drug proportions (approx. 90% of the drug) than the Clinoleic nanoemulsion (around 80% of the drug was successfully loaded) (Nasr et al., 2012), indicating that loading efficiency depends not only on nanoemulsion formulation but also on drug physicochemical properties. Moreover, the loading efficiency in the Intralipid decreased when higher paclitaxel concentrations were used ($P < 0.05$) (Fig. 4a). The loading efficiencies in the Intralipid emulsion were $57.38 \pm 4.7\%$, $52.5 \pm 5.6\%$ and $44.2 \pm 3.1\%$ for the 1 mg/ml, 3 mg/ml and 6 mg/ml formulations respectively (Fig. 4a).

When considering the dose of paclitaxel loaded into the droplets per 1 ml of the nanoemulsion dispersion, using the Clinoleic emulsion, this was 0.704 ± 0.035, 2.46 ± 0.126 and 4.63 ± 0.15 mg/ml for paclitaxel having initial concentrations of 1, 3 and 6 mg/ml respectively (Fig. 4b). For the Intralipid nanoemulsions, the drug loaded was 0.57 ± 0.047, 1.57 ± 0.17 and 2.65 ± 0.19 mg/ml for paclitaxel having initial concentrations of 1, 3 and 6 mg/ml respectively (Fig. 4b). Thus, unlike loading efficiency, the dose loaded per unit volume of the emulsion increased with increasing the paclitaxel concentration; however, higher doses of the drug pre unit volume, were loaded in the Clinoleic nanoemulsions. If no nanoemulsions were used, less than 0.1 μg/ml of paclitaxel can be dissolved in aqueous phase (Konno et al., 2003), indicating that the use of nanoemulsions was highly advantageous at providing paclitaxel formulations that have potential for therapeutic administration.

The solubility of paclitaxel in soybean oil has been reported to be around 0.18 mg/g (Surapaneni et al., 2012). The lipid phase of the Intralipid emulsion comprises mainly soybean oil while the lipid of the Clinoleic emulsion is olive oil (80%) and soybean oil (20%) (Table 1). Also, the Clinoleic emulsion has the surfactant sodium oleate (which is not one of the Intralipid's constituents; Table 1). These differences could explain the higher loading of paclitaxel in the Clinoleic nanoemulsion compared to the Intralipid preparation (Fig. 4). However, recent studies have reported that there was no significant difference in solubility of paclitaxel between both olive oil and soybean oil (Choudhury et al., 2014b), possibly suggesting that sodium oleate might be responsible for enhancing the loading of the drug in the Clinoleic emulsion (Table 1). More investigations are needed in the future to further understand the higher loading efficiency of paclitaxel in the Clinoleic compared to the Intralipid emulsion.

3.5. Tissue culture and cytotoxicity studies

Using a range of seeding densities the percentage growth of SVG-P12 and U87-MG cells were investigated, and the seeding density of 105 was used in tissue culture experiments because this seeding density was demonstrated to cause the highest cell growth rate compared to 103 and 104 cells per well (data not shown). Free paclitaxel was solubilised in cell culture medium using traces of dimethylsulfoxide (DMSO) (up to 0.3%). According to Da Violante et al. (2002) DMSO at concentrations of up to 10% did not produce any significant cytotoxicity.

In the cytotoxicity experiments, dextran was used as a negative control whilst PLL was employed as a positive control. Intralipid and Clinoleic formulations of paclitaxel were used and compared also against other negative controls, namely, drug-free nano-emulsions.

Drug-free Clinoleic and Intralipid nanoemulsions had no toxic effect towards both cell lines (Fig. 5). This might be attributed to the presence of biocompatible materials in the emulsions (Table 1). Dextran had a similar effect to that of the emulsions (Fig. 5). Paclitaxel alone was highly cytotoxic and killed more than 95% of the cells, with no evidence of discrimination between glial and glioma cells, suggesting that the drug in absence of nanoemulsions is not selective in suppressing cellular growth (Fig. 5).

When Intralipid loaded with paclitaxel was applied on the cells, the viability decreased to 26.04% for the SVG-P12 cells and to 21.29% for the U87-MG cells, demonstrating lower but not significant ($P > 0.05$) ability to kill the U87-MG cells than paclitaxel alone (Fig. 5). It is possible that the Intralipid emulsion has formed a barrier between paclitaxel and the cells, resulting in slower release (data not shown), hence; lower cell toxicity as compared to the paclitaxel alone. By contrast, when the Clinoleic loaded with paclitaxel was used, the viability decreased to 34.57% for the SVG-P12 cells (Fig. 5a) and to as low as 6.4% for the U87-MG cells (Fig. 5b), i.e. Clinoleic loaded with paclitaxel has a significantly lower ($P < 0.05$) cytotoxicity towards SVG-P12 than that of free Paclitaxel, indicating a higher level of selectivity of paclitaxel in the Clinoleic emulsion against the malignant cells. Cancerous cells are known to divide faster than normal cells; hence their intake of nutrients is faster (Miller and Perry, 2007); this might explain the reason behind the selectivity of the emulsions towards the cancerous cells (Fig. 5). However, the better selectivity of the Clinoleic nanoemulsion compared with that of the Intralipid suggests that olive oil or sodium oleate might have further enhanced the targeting properties of this formulation towards the U87-MG cells (Table 2). Further studies are required to investigate the uptake of nutritive materials by cancerous cells.

Table 2 shows that the IC_{50} values were highly dependent on formulation for SVG-P12 cells ($P < 0.05$) as the IC50 of Clinoleic with Paclitaxel was significantly higher ($P < 0.05$) than that of Intralipid with Paclitaxel and both were significantly higher ($P < 0.05$) than paclitaxel. Paclitaxel alone had the lowest IC_{50}, indicating that the free drug has higher toxicity than the drug in nanoemulsions since much larger doses of drug in nanoemulsions were needed to kill 50% of the cells (Table 2). The reduced cytotoxicity of paclitaxel loaded to both nanoemulsions as compared to free paclitaxel might be attributed to slow drug release from nanoemulsions (data not shown). The slower release profile of paclitaxel is already established for nanoemulsion (Choudhury et al., 2014a; Ma and Mumper, 2013)

Table 2
IC50 (nM) of paclitaxel-loaded nanoemulsions, paclitaxel and PLL against SVG-P12 and U87-MG cells. ($n = 3 \pm SD$).

Formulation	IC50 Value (nM) $\pm$ SD for SVG-P12 cells	IC50 Value (nM) $\pm$ SD for U87-MG cells
PLL	215 ± 32.4	197 ± 25.6
Paclitaxel	119.5 ± 12.2	65.4 ± 10.1
Intralipid with Paclitaxel	1014.2 ± 132	808.2 ± 151.5
Clinoleic with Paclitaxel	1528.2 ± 180	286.7 ± 65.1

4. Conclusion

Licensed parenteral nutrition nanoemulsions, Clinoleic® and Intralipid® were successfully loaded with the poorly water-soluble anticancer drug paclitaxel. Droplet size of the paclitaxel-loaded emulsions increased compared to drug-free formulations but was still below 300 nm. The Polydispersity Index (PI) results indicated that all the nanoemulsion formulations (Clinoleic and Intralipid) were homogeneous, regardless of paclitaxel concentration in the emulsions. However, the Clinoleic formulations demonstrated slightly higher pH, greater negative zeta potential values and higher loading efficiencies. More studies are necessary to explain the high efficiency of Clinoleic formulations compared to that of Intralipid. Both paclitaxel-loaded emulsions showed concentration-dependent cytotoxicity against both U87-MG cell and SVG-P12 with the greater selectivity of the Clinoleic nanoemulsion compared with the Intralipid towards cancer cells. The results suggest that parenteral nutrition lipid nanoemulsions are able to act as potential nanocarriers of poorly water-soluble anticancer drugs (e.g. paclitaxel). Early studies using alkylating anticancer agents loaded into Intralipid emulsion reported the suitability of the resultant delivery systems for human administration following investigations on around 100 patients (Fortner et al., 1975). Thus, this work has introduced a potentially cost-effective way of delivering paclitaxel, that can be prepared relatively simply from sterile licensed components in a hospital pharmacy close to the patient, with potential for application in the treatment of cancer. Further in vivo studies are required to validate this hypothesis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2016.04.027.

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