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Chemical stability and phase distribution of all-*trans*-retinol in nanoparticle-coated emulsions

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ABSTRACT

The influence of silica nanoparticle coating on the chemical stability and phase distribution of all-*trans*-retinol in submicron oil-in-water emulsions is reported. The chemical stability was studied as a function of UVA + UVB irradiation, and storage temperature (4 °C, ambient temperature, and 40 °C) for emulsions stabilised with lecithin and oleylamine as the initial emulsifier with and without silica nanoparticle layers. The chemical stability of all-*trans*-retinol was highly dependent on the emulsifier type and charge, with negligible influence of the initial loading phase of silica nanoparticles. A significant stability improvement (~2-fold increase in the half-life of the drug) was observed by nanoparticle incorporation into oleylamine-stabilised droplets (i.e. electrostatically coated), with no considerable effect for partially coated lecithin-stabilised droplets. The chemical stability of all-*trans*-retinol incorporated into nanoparticle-coated emulsions was well-correlated to the phase distribution of the active agent, and the interfacial structure of emulsions as determined by freeze fracture-SEM. Specifically engineered nanoparticle layers can be used to enhance the chemical stability of active ingredients in emulsion carriers.

1. Introduction

Retinoids (retinol, retinal, and retinoic acid) are a homologous series of lipid soluble molecules (general structure is shown in Fig. 1) which have been of great interest in pharmaceutical, nutritional, and cosmetic preparations (Asai and Watanabe, 2000). All-*trans*-retinol, with *trans* double bonds in the isoprenoid side chain, undergoes degradative reactions characteristics of conjugated double bonds which result in the partial or total loss of vitamin A bioactivity. These reactions include isomerisation to *cis* isomers, molecular fragmentation and chemical oxidation (Lee et al., 2002). Given the many beneficial effects of retinol, formulation strategies have been used to overcome the decomposition problem.

In conventional emulsions, vitamin A mainly converts to anhydro-vitamin A via proton mediated hydrolysis (Nam et al., 2003). Vitamin A encapsulated within hydrophobic poly (methyl methacrylate)-g-polyethyleneimine (PMMA-g-PEI) microspheres showed improved chemical stability compared to plain PMMA microspheres. The proton buffering capacity of polyethylenimine (PEI) moiety dramatically increased the chemical stability of vitamin A. The combined polymer-based microencapsulation and chemical conjugation is a useful approach for stabilisation of vitamin A due to the decreased dehydration of the terminal hydroxyl group of all-*trans*-retinol. However, the properties of microspheres are highly dependent on the process parameters including polymer molecular weight and solubility, volume ratio of organic/aqueous phases, rate of solvent evaporation, and drug loading efficiency (Lee et al., 2004).

Degradation kinetics of all-*trans*-retinol incorporated into soybean phosphatidylcholine multilamellar liposomes was investigated as a function of α-tocopherol as an antioxidant, pH, temperature, as well as ambient and UV light (Lee et al., 2002). Under neutral or alkaline pH and low temperature, the level of vitamin A stabilisation was higher compared to the protective effect of α-tocopherol, a well-known stabiliser of vitamin A. Lower molecular oxygen permeability in the solid gel phase compared to the fluid liquid crystalline phase of lipids also explains the slower degradation of retinol at 4 °C and alkaline pH. At temperatures higher than the transition temperature of the phospholipid and at low pH, the lipids are destabilised as a result of the change from gel phase to liquid crystalline phase and electrostatic repulsion due to the protonation of phosphate head groups of the phospholipid respectively (Lee et al., 2002).

The use of vitamin A in the form of fatty acid esters instead of free retinol also increases its stability against oxidation; vitamin A palmitate, propionate, and linoleate were used as active agents in therapeutic and dermatological formulations and to study stability improvements (Halbaut et al., 1997; Arsic et al., 1999; Asai and Watanabe, 2000; Gatti et al., 2000; Carlotti et al., 2002; Shefer and Shefer, 2003; Scalzo et al., 2004). However it has been

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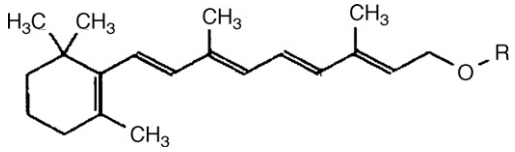

Fig. 1. Chemical structure of retinoids.

shown that retinol or short chain esters of retinol are more efficient than long chain esters, e.g. retinyl palmitate (Shefer and Shefer, 2003). Tsunoda and Takabayashi studied the stability of all-*trans*-retinol in cream formulations with different oil phases as a function of oil/water percentage, surfactants, and antioxidants at high temperature by reverse phase HPLC (Tsunoda and Takabayashi, 1995). Thermal isomerisation from all-*trans*-retinol to 13-*cis*-retinol increased as a function of the oil weight percentage in the cream. On the other hand the remaining concentration of all-*trans*-retinol in the formulation decreased as a function of water percentage in the cream. The use of antioxidants especially oil soluble agents, e.g. BHT, BHA, vitamin E was considered invaluable due to the prevention of thermal isomerisation as well as decomposition by lipid peroxides. The balance between the ratio of oil and water in the cream formulation was found to be essential for the stability of all-*trans*-retinol.

Spectroscopic and chromatographic analysis showed an improvement in the solubility and stability of vitamin A alcohol in aqueous media by complexation with sulfobutyl ether-7-β-cyclodextrin (Captisol®). At an optimum Captisol to retinol ratio of 50:1, the degradation of vitamin A was minimised up to 72 h compared to complete degradation of vitamin A in buffer within 30 min (Semenova et al., 2002).

Other approaches used to improve the stability of retinol include the use of solid water-insoluble organic polymer microspheres in oil-in-water emulsion templates (Froix et al., 1998), the application of w/o/w multiple emulsions (Isabelle et al., 2000).

In the current research, nanoparticle-coating of emulsion droplets has been investigated as a novel approach to improve the chemical stability of all-*trans*-retinol incorporated into medium chain triglyceride oil-in-water emulsions. Our specific focus is on the correlation between the stabilising effect of hydrophilic silica nanoparticles on the active agent and the interfacial characteristics of nanoparticle-coated emulsions. Nanoparticle-coated emulsions are formed by incorporation of hydrophilic silica nanoparticles (Aerosil®380) from either the oil or water phases of emulsions initially stabilised with lecithin or oleylamine. Hydrophilic nanoparticles, although an attractive encapsulating material and biocompatible (Vallet-Regi et al., 2007), are generally only weakly adsorbed at emulsion droplet surfaces and consequently do not stabilise emulsions unless an additional driving force for attachment is provided (Binks and Lumsdon, 1999; Binks and Whitby, 2005). The rationale for the choice of lecithin and oleylamine lies in the fact that instead of using hydrophobically modified Aerosil®, which has been recently included into the European Pharmacopoeia, we concentrated on proven biocompatible emulsifiers with GRAS (generally recognised as safe) status. Additional motivation was to understand nanoparticle interfacial adsorption and stability of the emulsions with the oppositely charged droplets and nanoparticles. These two surfactants are also well documented as biocompatible skin penetration enhancers (Benita, 1998), and this is desirable for potential application as dermal delivery vehicles. In our previous studies, nanoparticle layers have proved to reduce the coalescence kinetics and enhance the emulsification efficiency and physical stability of drug-free oil-in-water emulsions (Prestidge and Simovic, 2006; Ghouchi Eskandar et al., 2007), and influence the release kinetics of the lipophilic model drugs from oil-in-water emulsions depending on the nanoparticle layer structure and drug loading level (Simovic and Prestidge, 2007). This was a motivation for the current study and the degradation kinetics of all-*trans*-retinol has been investigated for drug-loaded emulsions through UV irradiation and long-term stability tests and correlated to the interfacial structure and physical stability of the emulsion carrier, emulsifier/nanoparticle/drug interactions, and the interfacial activity and phase distribution of all-*trans*-retinol.

2. Materials and methods

2.1. Materials

High-purity (Milli-Q) water (pH = 6.5 ± 0.5) was used trough the study. Caprylic/capric triglyceride (Miglyol®812) from Hamilton Laboratories (Australia); Soybean Lecithin (>94% phosphatidylcholine and less than 2% triglycerides) from BDH; oleylamine (primary amine, >98%) from Aldrich were used as received. Fumed silica particles (Aerosil®380) from Degussa are reported to have a primary average diameter of 7 nm, BET surface area of 380 ± 30 $m^2 g^{-1}$, and 2.5 Si–OH groups/nm^2 (determined from Li–Al hydride method) (Degussa, 1994). Contact angles estimated from enthalpy of immersion data are reported to be 14° (water/air) and 0° (toluene/water) (Yan et al., 2000). All-*trans*-retinol (synthetic, ≥95% HPLC, crystalline) was purchased from Sigma. The solvents acetonitrile (LiChrosolv®), acetone (LiChrosolv®) were purchased from Merck and orthophosphoric acid (HiPerSolv®) from BDH.

2.2. Methods

2.2.1. Preparation of oil-in-water emulsions

The initial emulsifier (either lecithin or oleylamine) was added to the oil phase of emulsions (Miglyol®812) in concentrations of 0.6 and 1.0 wt% with regard to whole emulsion respectively and stirred for 2 h for complete mixing. Then all-*trans*-retinol was added and the oil phase was stirred until complete dissolution of drug and formation of a clear phase. The control lecithin or oleylamine emulsions (L, O) were prepared by addition of the oil phase containing the emulsifiers and drug to MilliQ water to form a premix and then homogenised by high pressure homogeniser (EmulsiFlex-C5, Avestin® Inc., Canada) at 500–1000 bar for five cycles. To prepare silica-coated emulsions from oil phase (LSO, OSO), silica nanoparticles are added to the oil phase and the mixture was sonicated (Branson 2510, 100 W, USA) for 60 min to achieve a reproducible level of dispersion (50–100 nm). Alternatively, in the case of nanoparticle incorporation from the water phase (LSA, OSA), a 1.0 wt% aqueous silica dispersion (size: 50 ± 5 nm, zeta potential: −25 ± 2.5 mV, pH = 4.5 ± 0.5) was prepared by addition of silica mass to MilliQ water and sonication for 1 h which was later used in various volume fractions to achieve the desired concentration of silica in the water phase of the emulsion. After addition of the aqueous phase to the oil phase, the premix was formed and was homogenised using equivalent conditions to the control emulsions. With the aforementioned method, oil-in-water emulsions with 10% oil phase volume fraction and submicron size droplets were prepared. To avoid the effect of UV light, high temperature and oxygen during the preparation process, the samples were prepared in amber glass vials under nitrogen gas and ice bath.

2.2.2. Characterisation of oil-in-water emulsions

Size, polydispersity, and zeta potential of the control and silica-coated emulsions were measured with Zetasizer Nano Series ZS (Malvern Instruments, Ltd.). Emulsions were diluted 100 times with MilliQ water (pH: 6.5 ± 0.5) and measured at 25 ± 0.1 °C. The type of

emulsion (o/w in all cases) was confirmed by observing the dispersion of a drop of the emulsion in MilliQ water (continuous phase). A Philips XL 30 FEG scanning electron microscope with an Oxford CT1500 cryo transfer system was used to characterize the interfacial structure of the emulsions. The process involved emulsion cryofixation, fracturing, etching, platinum coating, imaging, and EDX (Energy-Dispersive X-Ray) analysis. Emulsion samples were injected into a "split" brass tube mounted on the cryo transfer specimen holder. The "split" brass tube consisted of two brass cylinders (ID 1 mm, OD 1.5 mm, L 3.5 mm) lightly glued together with superglue (cyanoacrylate). The emulsions were cryofixed by plunging the "split" brass cylinder and cryo transfer specimen holder into a liquid nitrogen–solid nitrogen slush (−196 °C). After cryofixation, the specimen was transferred under vacuum to the specimen exchange chamber of the cryo transfer system (−150 °C and 10^{-6} Torr) and the "split" brass tube was broken with a single scalpel blade precooled to −150 °C. The freshly exposed surface of the emulsion was etched by increasing the temperature to −95 °C for 2 min taking care to avoid droplet disintegration. After etching, the fractured sample was cooled to below −110 °C and then sputter-coated with platinum (∼1.5 nm) for conductivity prior to SEM imaging. Standardless semi-quantitative X-ray microanalysis (EDAX Genesis V5.21) with ZAF corrections was used for elemental analysis of the selected areas on the emulsion droplet surface and bulk.

2.2.3. Quantitative assay of all-trans-retinol

Pure authentic all-*trans*-retinol standard showed a maximum absorption at 325 nm in UV spectra (Moren et al., 2005). High performance liquid chromatography (HPLC) analysis was conducted under isocratic conditions at ambient temperature on a reversed phase column (LiChrospher RP Select B 5μ, 250 mm × 4.6 mm ID, Alltech); the method was adopted from (Jenning and Gohla, 2001). The system consisted of an isocratic pump (Shimadzu LC-6A), autoinjector (Shimadzu SIL-10A), a UV spectrophotometric detector (Shimadzu SPD-6A). The data were processed and analysed using a chromatopac (Shimadzu C-R3A). Acetonitrile/water, 80:20 plus 0.1 vol.% orthophosphoric acid constituted the mobile phase and the isocratic flow rate was adjusted to 1 ml min^{-1}. Detection was performed at 325 nm at ambient temperature for a run time of 15 min and retention time of all-*trans*-retinol was ∼12 min. Calibration graphs of all-*trans*-retinol were constructed in acetone using the external standard method. The stock solution of external standard in acetone with concentration of 25 μg ml^{-1} was prepared in an amber glass volumetric flask and diluted with acetone to achieve calibration solutions. The standard curve was linear ($r^2 > 0.99$) for all-*trans*-retinol concentrations ranging from 0.25 to 1.5 μg ml^{-1}. The limit of detection (LOD: signal-to-noise ratio of 3) and limit of quantification (LOQ: signal-to-noise ratio of 10) of all-*trans*-retinol in acetone was determined to be 100 and 250 ng ml^{-1} respectively. The intra-day precision was expressed by relative standard deviation (RSD) of six replicate injections on the same day and was 0.47%, 0.66%, and 0.69% for the lowest, middle and highest calibration points. Due to the instability of all-*trans*-retinol, all samples were prepared fresh and the inter-day precision was not performed. The accuracy for the aforementioned samples was 100.3%, 96.6%, and 107.4%, respectively.

2.2.4. Interfacial activity of all-trans-retinol

Three-phase contact angles and equilibrium oil–water interfacial tensions were measured to assess the interfacial activity of all-*trans*-retinol in the medium chain triglyceride oil–water system. A video-based high-speed contact angle measuring device (OCAH 200, DataPhysics, Germany) was used in the sessile drop mode and static contact angle measurements were performed in a rectangular cell with optical glass sides using the method described by Binks and Whitby (2005) with some modifications. The three-phase contact angle was measured through water at oil/water/hydrophilic glass substrate after equilibrium of the substrate in the oil phase; the equilibrium time was established by measurements at different time intervals between time zero and 45 min until values became stable. The mean values of three measurements (average of six values for left and right sides of the drop) were reported for advancing and receding contact angles. Interfacial tension measurements were carried out using the pendant drop mode; dynamic tracking of the interfacial tension as a function of time was employed until equilibrium, after which the value was recorded. Oil–water interfacial tensions were measured at 20 °C by forming a drop of the aqueous phase (water or silica dispersion) in the ambient oil phase. The ambient phase contained the oil phase of emulsion with different concentrations of the emulsifier and silica nanoparticles. Interfacial tension was calculated using the SCA20 software by fitting to the Young–Laplace equation. Measurements were performed at constant drop formation rate and the mean value of three repeats was reported for each sample. The phases were pre-equilibrated before the measurements and equilibrium times were determined to be in the range between 15 and 45 min.

2.2.5. Distribution of all-trans-retinol between emulsion phases

The partitioning of all-*trans*-retinol was determined at room temperature in medium chain triglyceride oil–MilliQ water system in the absence and presence of the emulsifiers (lecithin and oleylamine) and hydrophilic silica nanoparticles. 500 μl of 500 μg ml^{-1} solution of all-*trans*-retinol in the oil (presaturated with MilliQ water) was added to 500 μl of MilliQ water (presaturated with the oil) and mixed for 6 h in a rotary mixer (RSM6, RATEK Instruments, Victoria, Australia). Then, the mixtures were centrifuged (Hermle Z36HK Refrigerated High Speed Centrifuge, Germany) at 25,000 rpm for 30 min and the concentration of all-*trans*-retinol was quantified in both the oil and aqueous phases using HPLC.

2.2.6. UV irradiation test

Three samples from each control and silica-coated emulsion were located in a UV chamber (Ultra Violet Products, UVP, Australia) at an equal distance of ∼60 cm from the lamps and exposed to UVA (320–400 nm) and UVB (290–320 nm) irradiation for up to 6 h and the remaining concentration of all-*trans*-retinol in the emulsion was determined with HPLC.

2.2.7. Long-term stability tests

Long-term stability studies have been carried out at three different storage temperatures; emulsions are divided into three samples and transferred into amber glass vials immediately after preparation and stored at 4 °C, ambient temperature, and 40 ± 0.5 °C. The chemical stability (drug content) and physical stability (organoleptic visualisation for signs of creaming and coalescence) was monitored up to 6 months. Periodically, samples were taken, extracted and diluted (if necessary) with acetone and analysed with HPLC for residual all-*trans*-retinol. In contrast to some studies that used samples sealed with nitrogen gas and discarded the samples after analysis at each time point, in this study the initial samples were stored during the analysis period without topping up with nitrogen gas to mimic the real storage and consumption conditions of the formulation.

2.2.8. Statistical analysis

The test of normality using Shapiro–Wilk test with 5% confidence level showed the normal distribution of UV stability data. The statistical differences between the control and silica-coated emulsions were determined using One-Way Analysis of Variance (ANOVA) test followed by post hoc Bonferroni's test. p values ≤ 0.05 were considered as significant.

Table 1
Properties of drug-loaded oil-in-water emulsions.

Emulsion	Mean diameter (nm)	PI[a]	ζ potential (mV)
Oil-in-water (O/W)	350.3 ± 10.3	0.47 ± 0.02	−0.9 ± 0.09
Control lecithin (L)	213.3 ± 3.2	0.23 ± 0.01	−53.2 ± 0.6
Lecithin + silica in oil phase (LSO)	171.4 ± 2.6	0.08 ± 0.01	−49.9 ± 1.9
Lecithin + silica in water phase (LSA)	166.5 ± 2.1	0.13 ± 0.02	−52.0 ± 1.6
Control oleylamine (O)	317.2 ± 7.8	0.51 ± 0.04	+41.8 ± 2.7
Oleylamine + silica in oil phase (OSO)	248.0 ± 5.4	0.32 ± 0.03	+35.6 ± 1.6
Oleylamine + silica in water phase (OSA)	261.6 ± 6.9	0.39 ± 0.01	+32.0 ± 3.9

Mean ± SD, $n = 3$.
[a] Polydispersity index or size span which is a measure of the width of size distribution.

3. Results

3.1. Characterisation of emulsions

The size distribution and zeta potential of vitamin A-loaded lecithin and oleylamine-stabilised emulsions in the absence and presence of silica nanoparticles are presented in Table 1. All-*trans*-retinol containing o/w emulsion in the absence of any emulsifier showed a mean droplet size of 350.3 nm with a small population of 5 μm droplets. The mean droplet size of the control emulsion stabilised solely by lecithin was 213.3 nm with a size span of 0.23. Nanoparticle coating of the control lecithin emulsion from either oil or water phase reduced the droplet size (171.4 and 166.5 nm versus 213.3 nm). The control oleylamine emulsion showed larger droplet size (317.2 nm) compared to the control lecithin emulsion, but the droplet size has decreased significantly by inclusion of nanoparticles from the oil (248.0 nm) and water (261.6 nm) phases. Considering the chemical structure of lecithin (mainly phosphatidylcholine) and oleylamine and the arrangement of these emulsifiers at the oil–water interface (Rabinovich-Guilatt et al., 2004), they confer negative and positive charge to emulsion oil droplets respectively. The positive charge of oleylamine-stabilised droplets (+41.8 ± 2.7 mV) decreased as a result of adsorption of negatively charged silica nanoparticles from either oil (+35.6 ± 1.6 mV) or water (+32.0 ± 3.9 mV) phase. Rabinovich-Guilatt et al. conducted comprehensive investigations on the zeta potential of oleylamine-stabilised o/w emulsions and found that almost all oleylamine is localised and fully ionised at the surface of emulsion droplets (Rabinovich-Guilatt et al., 2004). The observed decrease in the zeta potential is due to the depletion of oleylamine from the oil–water interface and adsorption on silica and consequently the adsorption of the oleylamine–silica complexes at the droplet surface. This was confirmed by the increase in the oil–water interfacial tension value, and up to 52° increase in the static three-phase contact angle (measured through water) at hydrophilic silica surfaces–oil–water interfaces as a function of the increase in oleylamine concentration in the oil phase (0.0–1.0 wt%) (Ghouchi Eskandar et al., 2007).

Pickering emulsions stabilised solely by hydrophilic silica nanoparticles in the oil phase, showed poor stability, i.e. phase separation immediately after homogenisation**,** and limited concentration of nanoparticles that could be added to the emulsion oil phase. No emulsion could be formed when the initial loading phase of silica nanoparticles was the aqueous phase.

Freeze fracture-SEM imaging of the control lecithin and oleylamine emulsions showed smooth emulsion droplets in the submicron size range. Semiquantitave EDX analysis showed partial coating (2.35 at% silicon on the droplet surface) of lecithin-stabilised emulsions (Fig. 2, middle and lower left). The majority of nanoparticles when included from the water phase were localised in the external aqueous phase and formed networks or aggregates in the emulsion continuous phase (Fig. 2, lower left); up to 0.65 at% silicon was detected in the background in the EDX analysis. On the other hand, greater nanoparticle coverage (up to 3.01 at% silicon) was detected on the droplet surface of silica-coated oleylamine emulsions. Atomic % silicon is based on a semi-quantitative elemental analysis of a small area/line/spot on the droplet surface; therefore it cannot be connected to the amount of nanoparticles at the interface. However, our theoretical calculations in the previous work (Simovic and Prestidge, 2008), showed that 22 and 40 wt% silica relative to droplets is required for hexagonal monolayer coverage of 0.5 μm droplets with 20 and 50 nm nanoparticles, respectively.

3.2. Interfacial activity of all-trans-retinol

All-*trans*-retinol may potentially adsorb to the oil–water interface due to the hydroxyl group in the isoprenoid side chain (Washington, 1996); therefore, oil–water interfacial tension (Table 2) and three-phase contact angle (Fig. 3) measurements were performed to test the interfacial activity. The advancing contact angle (measured through water) at hydrophilic silica substrate–oil–water boundary is equal to 107.6 ± 2.4° and decreased to 99.5 ± 2.2° with addition of 0.05 wt% all-*trans*-retinol to the oil phase. The contact angle hysteresis varied between almost 0° and 5° and was not considered significant. By addition of lecithin and oleylamine to the oil phase, the advancing contact angle value increased to 165.5 ± 0.5° and 161.6 ± 2.2°, respectively. No significant change in the value of the advancing contact angle or contact angle hysteresis was observed due to the addition of all-*trans*-retinol into the oil phase in the presence of the emulsifiers.

The equilibrium oil–water interfacial tension was determined to be 13.36 ± 0.63 mN m^{-1} (Table 2) and increased to 17.31 ± 0.08 mN m^{-1} by addition of all-*trans*-retinol to the oil phase. The interfacial tension decreased to 9.69 ± 0.5 mN m^{-1} in the presence of 0.6 wt% lecithin in the oil phase and further decreased to 8.65 ± 0.21 and 8.24 ± 0.1 by addition of 0.5 wt% silica nanoparticles to the oil and water phases, respectively. We presume that lecithin will be mostly located in the oil phase with minimal partitioning in the water phase at the concentration used (Benita, 1998). On the other hand, the oil–water interfacial tension in the presence of oleylamine in the oil phase (9.54 ± 0.08 mN m^{-1}) increased by

Table 2
The influence of addition of all-*trans*-retinol to the oil phase on the equilibrium oil–water interfacial tension in the presence of lecithin and oleylamine in the oil phase and silica nanoparticles in both oil and water phases.

Oil–water system	Oil–water interfacial tension (mN m^{-1})	
	Without all-*trans*-retinol	With all-*trans*-retinol
MCT oil–water	13.36 ± 0.63	17.31 ± 0.08
MCT oil + lecithin–water	9.69 ± 0.50	8.82 ± 0.74
MCT oil + lecithin + silica–water	8.65 ± 0.21	8.68 ± 0.44
MCT oil + lecithin–silica disp.	8.24 ± 0.10	8.08 ± 0.13
MCT oil + oleylamine–water	9.54 ± 0.08	9.99 ± 0.03
MCT oil + oleylamine + silica–water	13.22 ± 0.11	13.15 ± 0.08
MCT oil + oleylamine–silica disp.	8.48 ± 0.03	8.62 ± 0.14

Mean ± SD, $n = 3$.

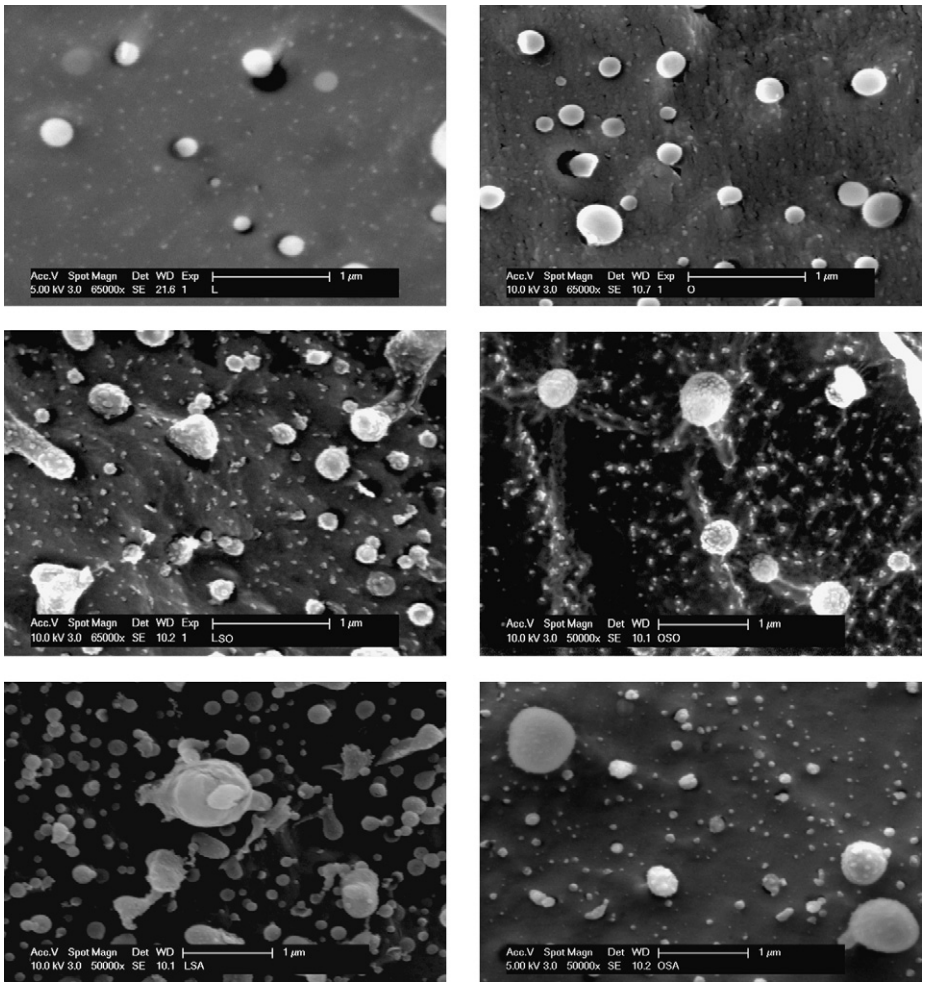

Fig. 2. Freeze fracture-SEM images of control (upper) and silica-coated (silica included from oil phase: middle; silica included from water phase: lower) lecithin (left) and oleylamine (right) emulsions.

addition of nanoparticles to the oil phase (13.22 ± 0.11 mN m^{-1}), but decreased by addition to the water phase (8.48 ± 0.03 mN m^{-1}). The detail explanations regarding the observed phenomenon is reported previously for drug-free droplets (Ghouchi Eskandar et al., 2007); the increase in the oil–water interfacial tension in the presence of oleylamine and silica nanoparticles in the oil phase is due to the strong electrostatic interactions between positively charged oleylamine and negatively charged nanoparticles which causes the depletion of the surfactant from the oil–water interface and adsorption on the silica surface. No significant change was detected in the oil–water interfacial tension value due to all-*trans*-retinol in the presence of both the emulsifiers and silica nanoparticles.

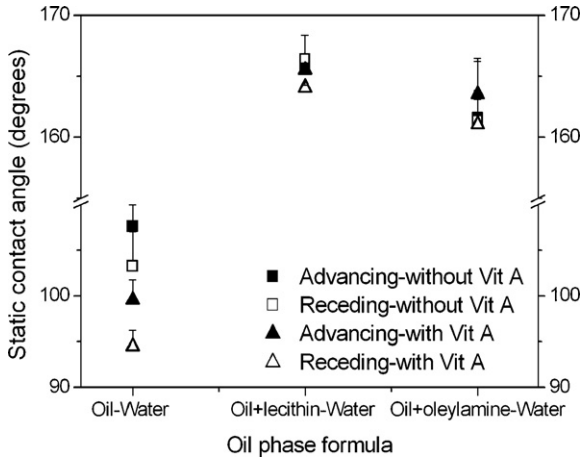

Fig. 3. The advancing and receding contact angle in the absence and presence of the emulsifiers (lecithin/oleylamine) and all-*trans*-retinol in the oil phase measured through water on hydrophilic silica substrate.

3.3. Distribution of all-trans-retinol between emulsion phases

The distribution of all-*trans*-retinol between oil and water in the presence of the emulsifiers and silica nanoparticles has been depicted in Table 3. In a pure triglyceride oil and water mixture, 13.01± 2.42% of the initial all-*trans*-retinol in the oil phase partitioned to the water phase due to the lack of an interfacial barrier. Upon addition of lecithin, the phase distribution of all-*trans*-retinol

Table 3
Distribution of all-*trans*-retinol between emulsion oil and aqueous phases.

Oil phase–water phase	Oil phase (%)	Water phase (%)
MCT oil–MilliQ water	86.38 ± 7.32	13.01 ± 2.42
MCT oil + lecithin–MilliQ water	93.64 ± 2.64	2.06 ± 1.07
MCT oil + lecithin + silica–MilliQ water	95.21 ± 3.78	2.73 ± 0.64
MCT oil + lecithin–silica disp. 0.5 wt%	95.98 ± 2.59	2.07 ± 0.85
MCT oil + oleylamine–MilliQ water	93.80 ± 0.91	8.52 ± 1.79
MCT oil + oleylamine + silica– MilliQ water	97.20 ± 2.86	2.39 ± 1.34
MCT oil + oleylamine–silica disp. 0.5 wt%	97.27 ± 2.67	2.91 ± 0.86

MCT oil: medium chain triglycerides.

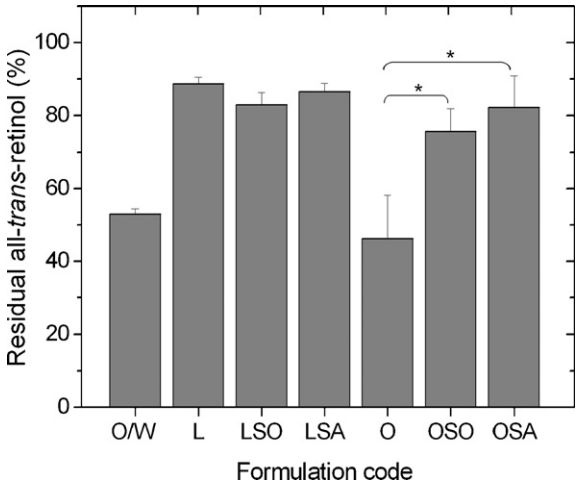

Fig. 4. Residual all-*trans*-retinol after 6 h UV exposure in oil-in-water emulsion without emulsifier (O/W); stabilised solely with lecithin (L); lecithin and silica in oil phase (LSO); lecithin and silica in aqueous phase (LSA); and emulsion stabilised solely with oleylamine (O); oleylamine and silica in oil phase (OSO); oleylamine and silica in aqueous phase (OSA).

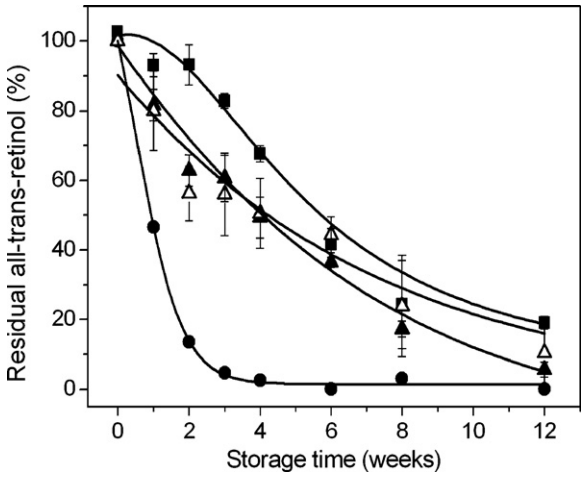

Fig. 5. Long-term chemical stability at 4 °C of all-*trans*-retinol incorporated into emulsions stabilised with no stabiliser (solid circles), only lecithin (solid squares), lecithin and silica nanoparticles in the oil phase (solid triangles), lecithin and silica nanoparticles in the aqueous phase (open triangles).

reduced, i.e. 2.06 ± 1.07% in water phase. An intermediate emulsion phase was formed between oil and water by addition of lecithin and some drug distributed to this phase. This intermediate phase in the presence of lecithin is an emulsion which is formed at the interface between oil and water as a result of energy input by agitation and due to the high stability, this emulsion phase was not broken after ultracentrifugation. In the presence of lecithin in the oil phase, the interfacial transfer of all-*trans*-retinol was not significantly changed by incorporation of silica nanoparticles to either oil or water phases. In the presence of oleylamine in the oil phase, no intermediate phase was formed and 8.52 ± 1.79% of all-*trans*-retinol was partitioned into the water phase. Less partitioning of all-*trans*-retinol to the water phase was observed by addition of nanoparticles to the oil (2.39 ± 1.34%) and water (2.91 ± 0.86%) phases in the presence of oleylamine.

3.4. UV irradiation studies

The residual percentage of all-*trans*-retinol after 6 h UV exposure is plotted for the control and silica-coated emulsions in Fig. 4. All-*trans*-retinol incorporated into the oil-in-water emulsion without stabiliser showed an extensive degradation with more than 50% of the drug decomposed within 6 h. The chemical stability of all-*trans*-retinol significantly improved in the control emulsion solely stabilised with lecithin; 88.77 ± 1.7% remained after a 6-h UV exposure period. No additional stabilising effect was observed as a result of inclusion of silica nanoparticles from the oil or water phase of the control lecithin emulsion. In the presence of oleylamine as the initial emulsifier of the oil-in-water emulsion, ~50% of all-*trans*-retinol decomposed within 6 h. However, nanoparticle coating of emulsion droplets from either oil or water phases significantly ($p \leq 0.05$) improved the chemical stability of all-*trans*-retinol. The residual concentration of the active ingredient after 6-h UV exposure increased to ~71% and 92% by inclusion of silica nanoparticles from the oil and water phases, respectively.

3.5. Chemical stability of all-trans-retinol as a function of temperature

3.5.1. Lecithin-stabilised emulsions

The chemical stability at a storage temperature of 4 °C for all-*trans*-retinol incorporated into the emulsions without any stabiliser and emulsions initially stabilised with lecithin in the absence and presence of silica nanoparticles is reported in Fig. 5. The oil–water emulsion without any emulsifier showed an exponential degradation pattern with degradation of ~50% of all-*trans*-retinol in less than a week. Stabilisation of the emulsions with lecithin showed a significant improvement in the chemical stability of the active agent at 4 °C. Despite the fact that significant improvement was observed in the long-term physical stability of drug-free lecithin-stabilised emulsions by nanoparticle encapsulation (Ghouchi Eskandar et al., 2007); no additional stabilising effect on all-*trans*-retinol was observed when silica nanoparticles were added from either the oil or aqueous phase. Accordingly, no significant improvement in the chemical stability of all-*trans*-retinol was observed at ambient temperature and 40 °C when it is incorporated into silica-coated emulsions compared to the control emulsion solely stabilised with lecithin. Despite the lack of chemical stability at high temperatures, both the control and silica-coated emulsions showed very high physical stability even at elevated temperature (40 °C) with no signs of creaming and coalescence upon storage up to 6 months.

3.5.2. Oleylamine-stabilised emulsions

The chemical stability of all-*trans*-retinol at storage temperature of 4 °C is shown in Fig. 6 for the o/w emulsion without stabiliser, the control emulsion solely stabilised with oleylamine, and silica-coated emulsions. No all-*trans*-retinol was quantified in the oil-in-water emulsion without stabiliser after 3 weeks storage at 4 °C. In agreement with lecithin-stabilised emulsions, the addition of oleylamine considerably improved the chemical stability of all-*trans*-retinol compared to the oil–water mixture. Silica nanoparticle coating of oleylamine-stabilised oil droplets further improved the chemical stability of all-*trans*-retinol due to the decreased partitioning of drug between emulsion phases (Table 4). All-*trans*-retinol showed the highest stability in silica-coated oleylamine emulsions with ~70% of the active agent remained after 3 months. The degradation kinetics of all-*trans*-retinol incorporated into oleylamine emulsions was best fitted to a first-order model (Fig. 7) and the degradation rate constants were calculated for the control and silica-coated emulsions. The emulsion solely stabilised with oleylamine showed a biphasic degradation pattern with fast degradation of all-*trans*-retinol in the first phase (30% of the initial drug loading) followed by a slower degradation rate in the second phase. Silica nanoparticle coating of emulsion droplets inhibited the first degradation phase of all-*trans*-retinol through the control of drug

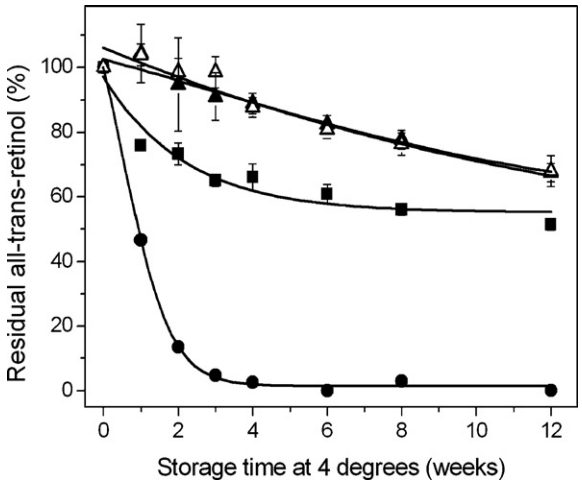

Fig. 6. Long-term chemical stability at 4 °C of all-*trans*-retinol incorporated into emulsions stabilised with no stabiliser (solid circles), solely oleylamine (solid squares), oleylamine and silica nanoparticles in the oil phase (solid triangles), oleylamine and silica nanoparticles in the aqueous phase (open triangles).

Table 4
The first-order degradation rate constant for the control and silica-coated oleylamine-stabilised emulsions at three different storage temperatures.

Storage temperature/formula	k (1/day)	r	$T_{1/2}$ (weeks)
4 °C			
Oil-in-water emulsion without stabiliser	0.439	0.98	1.58
Oleylamine-stabilised emulsion			
Phase I	0.176	0.90	3.93
Phase II	0.067	0.99	10.27
Oleylamine-stabilised emulsion + silica in oil	0.027	0.99	25.66
Oleylamine-stabilised emulsion + silica in water	0.031	0.97	22.08
Ambient temperature			
Oleylamine-stabilised emulsion	0.102	0.92	6.75
Oleylamine-stabilised emulsion + silica in oil	0.092	0.96	7.56
Oleylamine-stabilised emulsion + silica in water	0.084	0.92	8.21
40 °C			
Oleylamine-stabilised emulsion	0.117	0.98	0.84
Oleylamine-stabilised emulsion + silica in oil	0.147	0.92	0.67
Oleylamine-stabilised emulsion + silica in water	0.120	0.97	0.82

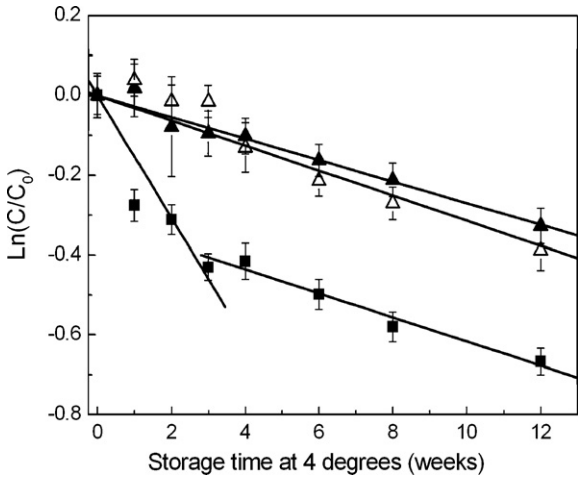

Fig. 7. Degradation kinetics of all-*trans*-retinol at 4 °C in the control (solid squares), and silica-coated oleylamine emulsions (solid triangles: incorporation from the oil phase and open triangles: incorporation from the aqueous phase).

partitioning between the emulsion phases. As can be seen in Fig. 7 and Table 4, silica incorporation from either phase of the emulsion resulted in a significant improvement in the chemical stability and accordingly half-life of all-*trans*-retinol (~25 weeks by incorporation from the oil phase and ~22 weeks by incorporation from the water phase); the effect of the initial location of nanoparticles in the emulsion was negligible. With respect to physical stability, the control oleylamine emulsion showed slight creaming after 6 months storage at 4 °C, but silica-coated emulsions were completely homogeneous and stable. Both the control and silica-coated emulsions stored at ambient temperature showed obvious signs of creaming. At 40 °C, a high level of physical instability was observed including the release of pure oil on the emulsion surface and phase separation after 8 weeks.

4. Discussion

The chemical stability and phase distribution of all-*trans*-retinol has been investigated and correlated to the physical stability and interfacial structure of silica nanoparticle-coated submicron oil-in-water emulsions. The larger droplet size of emulsions stabilised with oleylamine as an initial emulsifier compared to lecithin can be attributed to the lower surfactant activity at the MCT oil–water interface (CMC: 0.1 g cm^{-3} for oleylamine vs. 0.01 g cm^{-3} for lecithin) (Ghouchi Eskandar et al., 2007). Freeze fracture-SEM (Fig. 2) showed that virtually all nanoparticles are located at the surface of oleylamine-stabilised emulsion droplets, as evidenced by decreased zeta potential (Table 1). Negatively charged lecithin-stabilised emulsion droplets are less coated due to the lack of electrostatic attraction. Simovic and Prestidge also noted that lecithin-stabilised submicron droplets are only partially coated even at silica nanoparticle levels well above the multilayer formation (Simovic and Prestidge, 2008). The partial coating of lecithin-stabilised droplets was attributed to the weak interactions between charged nanoparticles and P–N dipole of phospholipid head group (Zhang and Granick, 2006); decrease in the oil–water interfacial tension (Bian and Roberts, 1992; Martinez-Landeira et al., 2003) and accordingly decreased attachment energy of nanoparticles (Binks and Lumsdon, 2000); and pronounced hydration forces (Washington, 1990; Binks and Lumsdon, 1999). The strong electrostatic interaction between negatively charged silica nanoparticles ($pH_{i.e.}$: 2–3) (Hassander et al., 1989) and positively charged oleylamine resulted in the strong attachment of silica nanoparticles at the oil–water interface in the presence of oleylamine. These interactions are further confirmed by equilibrium oil–water interfacial tension measurements; the addition of silica nanoparticles to the oil phase in the presence of oleylamine resulted in an increased interfacial tension value due to the depletion of oleylamine from the interface and adsorption on the silica surface (Table 2). The presence of all-*trans*-retinol at the pure oil–water interface increased the oil–water interfacial tension and decreased the three-phase contact angle as a result of increased hydrophilicity due to the hydroxyl group.

Although the experimental water solubility of retinol at room temperature and pH = 7.3 is relatively low (0.06 μM), it is significant and accounts for the movement of retinol through the aqueous phase (Szuts and Harosi, 1991). This can result in the fast partitioning of all-*trans*-retinol from the oil phase of the emulsion to the aqueous phase which causes the degradation of drug (Dingler et al., 1999). Due to the lipophilic nature of all-*trans*-retinol ($\log PC_{oct.}$: 6.8 ± 0.3), it is mainly located in the oil phase (~86%), but also partitioned to the water phase (~14%) in the pure MCT oil–water system. In the presence of lecithin in the oil phase, all-*trans*-retinol was mainly determined either in the oil phase or the interfacial layer formed between oil and water. The effect of addition of silica nanoparticles to both oil and water phases on the drug partition-

ing was negligible in the presence of lecithin. On the other hand, in the presence of oleylamine in the oil phase, the distribution of all-*trans*-retinol was limited as a result of nanoparticle presence in both oil and water phases which is due to the additional interfacial barrier property of silica-oleylamine complexes at the oil–water interface.

The enhancement in the chemical stability of vitamin A derivatives due to phospholipids has been previously reported. The stabilising mechanism of soybean phosphatidylcholine liposomes on retinol was reported to be the reduction in oxygen permeability of the liposomes at low temperatures due to the more tightly packed structure of the solid gel phase. Considering the length of retinol (15.5 Å) and the thickness of lipid bilayers (40 Å), it is suggested that retinol can distribute in both the planner interface between the hydrophobic acyl chains and within each acyl chain with its long axis parallel to the phospholipids acyl chain (Carlotti et al., 2002). The coexistence of emulsion droplets (with surface monolayers of phosphatidylcholine and a core of retinyl palmitate or retinal) with vesicular particles (bilayers) of phosphatidylcholine was reported to be critical for the stability of dispersions of the drug and lipid (Asai and Watanabe, 1999; Asai and Watanabe, 2000). It is also reported that lecithin has an antioxidative property which highly depends on the phospholipids content or purity. The higher stability of γ-linoleic acid, D,L-α-tocopherol and ascorbyl acetate against oxidation was achieved by addition of phospholipids especially a purified fraction (with 80% PC) (Arsic et al., 1999). In the presence of lecithin as the initial emulsifier, no additional stabilising effect was observed due to nanoparticles; the residual concentration of all-*trans*-retinol after 6 h UV exposure was ~84% and ~86% for silica-coated emulsions compared to ~89% for the control lecithin emulsion. This may be due to the partial coating and low density of silica nanoparticles at the droplet surface of lecithin-stabilised emulsions which was evident from the localisation of silica aggregates/networks in the external water phase. The slight destabilising effect due to nanoparticles (Fig. 5) can also be due to the effect of negatively charged silica particles on the charge of phospholipid head groups and the enhanced electrostatic repulsion between the phospholipid molecules which prevents the formation of organized structures in bilayers or around emulsion droplets. Lee et al. also related the more rapid degradation of retinol incorporated into phosphsatidylcholine liposomes at low pH due to the destabilisation of lipids as a result of electrostatic repulsion due to the protonation of head groups (Lee et al., 2002). The chemical stability of all-*trans*-retinol was improved towards UV exposure (up to 45% increase in residual all-*trans*-retinol after a 6-h UV exposure period) and long-term storage (up to 25% increase in the residual all-*trans*-retinol after 3 months storage at 4 °C) due to the silica nanoparticle coverage of the emulsion droplet surface as a result of favorable electrostatic interactions between the oppositely charged emulsifier (oleylamine) and particles at the oil–water interface. It is also interesting to note that the chemical stability of the active agent in the submicron oil-in-water emulsions was not correlated to the physical stability of the emulsions. This is due to the fact that, in the presence of nanoparticles, the drug stability and release is mainly controlled by particle/emulsifier/drug interactions and these interactions in turn control the interfacial structure and phase distribution of the active agent. Despite improved physical properties of silica-coated compared to the control lecithin-stabilised emulsions, the chemical stability of all-*trans*-retinol was not affected by nanoparticle-coating. In contrast, oleylamine-stabilised emulsions showed poor physical stability but the chemical stability of the active agent was higher due to the nanoparticle coverage of the emulsion droplets as well as the decrease in the drug partitioning between emulsion phases. The results of this study stress the importance of the physicochemical and interfacial characteristics of oil-in-water emulsions as drug delivery systems. It is noted that further work is required to fully optimize the nanoparticle coating process for optimal formulation properties.

5. Conclusions

The chemical stability and phase distribution of all-*trans*-retinol in nanoparticle-coated submicron oil-in-water emulsions is highly dependent on the emulsifier type that in turn controls droplet coating by nanoparticles, with negligible influence of the initial loading phase for nanoparticles. No significant effect was observed due to the nanoparticle coating of lecithin-stabilised droplets on the chemical stability of all-*trans*-retinol towards UV irradiation and long-term storage. Nanoparticle layers controlled the drug partitioning and improved the long-term stability of all-*trans*-retinol in oleylamine-stabilised emulsions as a result of strong electrostatic coverage of emulsion droplets. Equilibrium oil–water interfacial tension and contact angle values in conjunction with interfacial characterisation of emulsions using freeze fracture-SEM confirmed the correlation between the physicochemical and interfacial structure with drug delivery characteristics of nanoparticle-coated emulsions.

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