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Solid-state nanoparticle coated emulsions for encapsulation and improving the chemical stability of all-*trans*-retinol

Nasrin Ghouchi-Eskandar, Spomenka Simovic, Clive A. Prestidge*

Ian Wark Research Institute, The ARC Special Research Centre for Particle and Material Interfaces, Mawson Lakes, Adelaide, SA 5095, Australia

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ABSTRACT

Submicron oil-in-water (o/w) emulsions stabilised with conventional surfactants and silica nanoparticles were prepared and freeze-dried to obtain free-flowing powders with good redispersibility and a three-dimensional porous matrix structure. Solid-state emulsions were characterised for visual appearance, particle size distribution, zeta potential and reconstitution properties after freeze-drying with various sugars and at a range of sugar to oil ratios. Comparative degradation kinetics of all-*trans*-retinol from freeze-dried and liquid emulsions was investigated as a function of storage temperatures. Optimum stability was observed for silica-coated oleylamine emulsions at 4 °C in their wet state. The half-life of all-*trans*-retinol was 25.66 and 22.08 weeks for silica incorporation from the oil and water phases respectively. This was ~4 times higher compared to the equivalent solid-state emulsions with drug half-life of 6.18 and 6.06 weeks at 4 °C. Exceptionally, at a storage temperature of 40 °C, the chemical stability of the drug was 3 times higher in the solid-state compared to the wet emulsions which confirmed that freeze-drying is a promising approach to improve the chemical stability of water-labile compounds provided that the storage conditions are optimised.

1. Introduction

Dry emulsions have several advantages in comparison to liquid emulsions such as reduced susceptibility to physical destabilisation and microbial contamination; enhanced chemical stability towards light and oxidative degradation, controlled release, and improved bioavailability of the incorporated active agents (Hansen et al., 2005; Jang et al., 2006; Welin-Berger and Bergenståhl, 2000). Removal of water from o/w emulsions can be achieved by using rotary evaporation (Myers and Shively, 1992; Porter et al., 1996); freeze-drying (Heinzelmann and Franke, 1999); or spray-drying (Cui et al., 2007; Jang et al., 2006) methods after addition of water-soluble amorphous carriers such as sugars, *e.g.* terahalose (Hansen et al., 2004), maltodextrin (Myers and Shively, 1993) or water-soluble polymers, *e.g.* hydroxypropyl methylcelloluse (HPMC) (Hansen et al., 2005), Eudragit® E100 (a cationic copolymer based on dimethylamino ethylmethacrylate, butyl methacrylate, and methyl methacrylate), and polyvinylpyrrolidone (PVP); or insoluble solid carriers such as colloidal silica (Simovic et al., 2009; Takeuchi et al., 1991; Tan et al., 2009), and magnesium alumino metasilicate (Hansen et al., 2004).

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 (Seung-Cheol et al., 2002). In conventional emulsions, vitamin A mainly converts to anhydro-vitamin A (inactive form of vitamin A) *via* proton mediated hydrolysis, *e.g.* dehydration of the terminal hydroxyl group of all-*trans*-retinol (Yoon Sung et al., 2003). Given the many beneficial pharmaceutical effects of retinol, the decomposition problem has been addressed to some extent by formulating retinol using various stabilising agents, *e.g.* the inclusion of antioxidants especially oil soluble agents such as BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole), vitamin E and chelating agents (Carlotti et al., 2002; Marks, 1981; Yanagida and Sakamoto, 2004); and different strategies, *e.g.* incorporation into liposomes (Seung-Cheol et al., 2002; Singh and Das, 1998); the use of vitamin A in the form of fatty acid esters or retinol-cyclodextrine complexes instead of free retinol (Arsic and Vuleta, 1999; Asai and Watanabe, 2000; Carlotti et al., 2002; Gatti et al., 2000; Halbaut et al., 1997; Scalzo et al., 2004; Semenova et al., 2002; Shefer and Shefer, 2003); or use of stable W/O/W emulsions containing retinol in the oil phase (Afria et al., 2000). In our previous research (Ghouchi-Eskandar et al., 2009), nanoparticle-coating of emulsion droplets was proved as an effective approach to improve the chemical stability of all-*trans*-retinol (up to 2-fold

* Corresponding author. Tel.: +61 8 8302 3569; fax: +61 8 8302 3683.
E-mail address: clive.prestidge@unisa.edu.au (C.A. Prestidge).

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Table 1
The ingredients and their percentage in the control and silica-coated lecithin and oleylamine emulsions.

Ingredients (wt.%)	Formulation code[a]					
	L	LSO	LSA[b]	O	OSO	OSA[b]
Miglyol®812	10	10	10	10	10	10
Lecithin	0.6	0.6	0.6	–	–	–
Oleylamine	–	–	–	1.0	1.0	1.0
Silica nanoparticles (Aerosil®380)	–	0.5	0.5	–	0.5	0.5
MilliQ water	qs 100					

[a] L: The control lecithin-stabilised emulsion, LSO: silica included into the oil phase of lecithin-stabilised emulsion, LSA: silica included into the water phase of lecithin-stabilised emulsion; O: the control oleylamine-stabilised emulsion, OSO: silica included into the oil phase of oleylamine-stabilised emulsion, OSA: silica included into the water phase of oleylamine-stabilised emulsion.
[b] For inclusion of silica nanoparticles from the water phase, 10 ml of a 5 w/v% aqueous solution of silica nanoparticles was added to the emulsion oil phase containing the surfactant.

increase in the half-life of the drug) incorporated into medium chain triglyceride oil-in-water emulsions initially stabilised with conventional surfactants namely lecithin or oleylamine. The chemical stability of all-*trans*-retinol was well-correlated to the phase distribution of the active agent and the interfacial structure of emulsions and was highly dependent on the initial emulsifier type and charge with negligible influence of the loading phase of silica nanoparticles. A significant stability improvement was observed by nanoparticle incorporation into oleylamine-stabilised droplets (*i.e.* electrostatically coated), with no considerable effect for partially coated lecithin-stabilised droplets. In the current research, solid-state nanoparticle-coated emulsions were prepared by freeze-drying and explored as an approach to encapsulate and achieve improved chemical stability of all-*trans*-retinol. Furthermore, results on chemical stability of all-*trans*-retinol obtained for solid state emulsions were comparatively discussed against the previously reported results for equivalent liquid emulsions (Ghouchi-Eskandar et al., 2009). The solid-state emulsions fabricated as macro-porous silica-lipid hybrid (SLH) microcapsules also exhibit improved release and lipolysis kinetics and oral bioavailability in comparison to equivalent liquid formulations (Lim et al., 2011) and may have implications as improved oral delivery systems.

2. Materials and methods

2.1. Materials

Caprylic/capric triglycerides (Miglyol®812) from Hamilton Laboratories (Australia) were used as the oil phase of emulsions. Soybean lecithin with >94% phosphatidylcholine and less than 2% triglycerides (BDH), and oleylamine (primary amine, >98%) from Aldrich were used as emulsifiers. Fumed silica particles (Aerosil®380) from Degussa are reported to have a primary average diameter of 7 nm, Brunauer, Emmett and Teller (BET) surface area of $380 \pm 30\,m^2\,g^{-1}$ (Brunauer et al., 1938), 2.5 Si–OH groups per nm^2 (determined from Li–Al hydride method), and loss on drying ≤2.5 wt.% (1994; Lewis and Harrison, 2010). 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®) and acetone (LiChrosolv®) were purchased from Merck and orthophosphoric acid (HiPerSolv®) from BDH. Freeze-drying protectors D-(+)-Maltose monohydrate, Grade I, minimum purity 98%; Maltodextrine, dextrose equivalent 13.0–17.0; and D-(+)-Terahalose dehydrate ≥99.5% HPLC were purchased from Sigma–Aldrich, Inc., USA. High-purity (Milli-Q) water (pH = 6.5 ± 0.5) was used trough the study.

2.2. Methods

2.2.1. Preparation of oil-in-water emulsions

The emulsifier (lecithin or oleylamine) was added to the oil phase (Miglyol®812) of emulsions at concentrations of 0.6 and 1.0 wt.% and sonicated (Branson 2510, 100W, USA) for 2 h. Then all-*trans*-retinol was dissolved in the oil phase until complete dissolution of drug and formation of a clear phase. When incorporated from the oil phase, silica nanoparticles were added to the oil phase and the mixture was sonicated for 60 min to achieve a reproducible level of dispersion. In case of nanoparticle incorporation from the water phase, 10 ml of the emulsion water phase was replaced with a 5.0 w/v aqueous dispersion of silica nanoparticles, prepared by sonication for 1 h, to achieve the desired concentration (0.5 wt.%) of silica nanoparticles in the total emulsion. After addition of the aqueous phase to the oil phase, the premix was formed which was then homogenised by a high pressure homogeniser (EmulsiFlex-C5, Avestin® Inc., Canada) at 500–1000 bar for 5 cycles to form the final emulsion. Table 1 depicts the formulation ingredients and their concentration in different emulsions. With the aforementioned method, oil-in-water emulsions with 10% volume fraction of the oil phase and submicron size droplets were prepared. To avoid the effect of ultraviolet (UV) light, high temperature and oxygen during the preparation process, the samples were prepared in amber glass vials under nitrogen gas and ice bath. Size distribution and zeta potential of emulsions were fully described in our previous paper (Ghouchi-Eskandar et al., 2009) and summarised in Tables 2 and 3.

2.2.2. Freeze-drying of emulsions

Emulsions were mixed with maltose monohydrate, maltodextrine, or terahalose dehydrate with various sugar to oil ratios and stirred for 2 h. 15 ml of each emulsion was filled in round bottom flasks and frozen using liquid nitrogen before mounting on the sample port of a Labconco Freeze Dryer, Model Lyph-Lock 6 (Labconco Corporation, Kansas, USA). The freeze drying conditions were optimised at a collector temperature of −47 °C to −48 °C and a vacuum pressure of 14–15 ($\times 10^{-3}$) mbar for a drying period of 24 h. Although the type of cryoprotectant had negligible effect on the properties of freeze-dried emulsions, maltose monohydrate with sugar:oil ratio of 1:2 resulted in lyophilised powders which were able to reform the original o/w emulsion with higher long-term stability and smaller size upon reconstitution in water. The freeze-drying process is summarised in Fig. 1.

2.2.3. Characterisation of freeze-dried emulsions

The structure/morphology of freeze-dried emulsions was characterised using field emission gun scanning electron microscopy (FEG-SEM). Freeze-dried powders were deposited on a conductive double-sided carbon tab mounted on an 8-mm aluminium stub and coated with carbon layer (~15 nm). A Field Emission Scanning

Table 2
Characteristics of solid-state lecithin emulsions compared to the initial wet formulations.

	Formulation code		
	L	LSO	LSA
Particle size (d, nm)			
Redispersed dry emulsion	305.9 ± 49.5	382.2 ± 59.0	420.3 ± 25.5*
Initial wet emulsion	213.3 ± 3.2	171.4 ± 2.6*	166.5 ± 2.1*
Polydispersity index			
Redispersed dry emulsion	0.29 ± 0.03	0.47 ± 0.08	0.47 ± 0.05
Initial wet emulsion	0.23 ± 0.01	0.08 ± 0.01	0.13 ± 0.02
Zeta potential (mV)			
Redispersed dry emulsion	−68.1 ± 1.4	−60.4 ± 3.5	−59.4 ± 1.7*
Initial wet emulsion	−53.2 ± 0.6	−49.9 ± 1.9	−52.0 ± 1.6
Moisture content (wt.%)	1.64	1.85	2.17
Process yield (%)	51 ± 2.3	66 ± 2.0	60 ± 2.4

* The statistical differences between the size and zeta potential of the control and silica-coated emulsions were evaluated using *t*-test and two-tail *p* values less than 0.05 was considered significant.

Table 3
Characteristics of solid-state oleylamine emulsions compared to the initial wet formulations.

	Formulation code		
	O	OSO	OSA
Particle size (d, nm)			
Redispersed dry emulsion	459.1 ± 48.5	656.2 ± 58.1*	541.2 ± 25.5
Initial wet emulsion	317.2 ± 7.8	248.0 ± 5.4*	261.6 ± 6.9*
Polydispersity index			
Redispersed dry emulsion	0.49 ± 0.12	0.47 ± 0.08	0.37 ± 0.05
Initial wet emulsion	0.51 ± 0.04	0.32 ± 0.03	0.39 ± 0.01
Zeta potential (mV)			
Redispersed dry emulsion	+53.8 ± 1.8	+46.4 ± 1.0*	+45.9 ± 0.4*
Initial wet emulsion	+41.8 ± 2.7	+35.6 ± 1.6	+32.0 ± 3.9
Moisture content (wt.%)	1.44	2.28	2.75
Process yield (%)	47 ± 8.4	50 ± 2.6	50 ± 6.0

* The statistical differences between the size and zeta potential of the control and silica-coated emulsions were evaluated using *t*-test and two-tail *p* values less than 0.05 was considered significant.

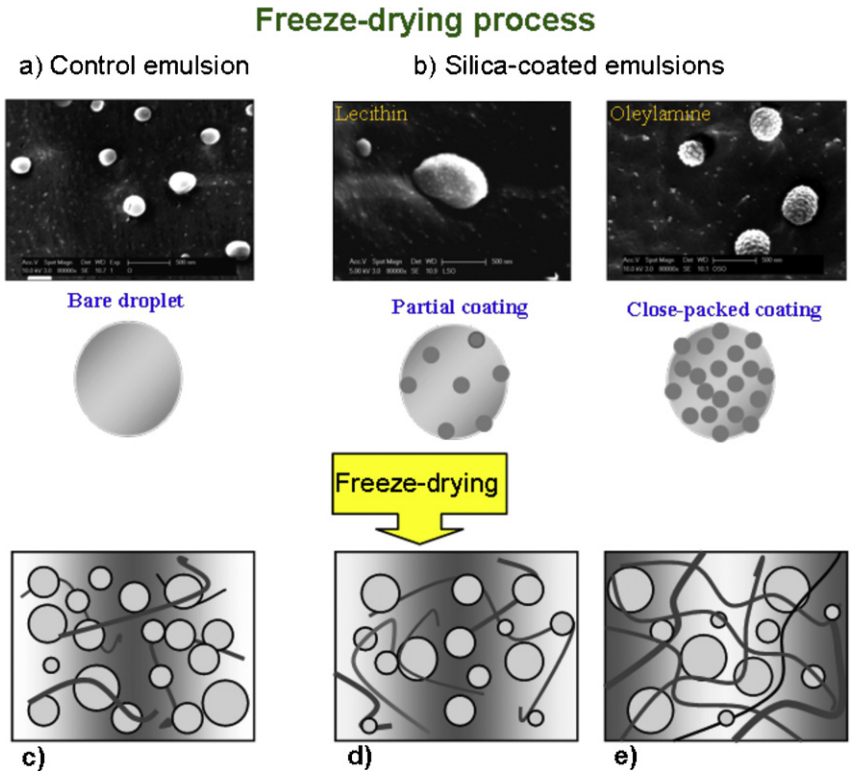

Fig. 1. Schematic of emulsion freeze-drying process showing different structures as a function of the initial surfactant (lecithin *versus* oleylamine) and inclusion of 0.5 wt.% silica nanoparticles; exposed (c and d) *versus* embedded (e) emulsion droplets in a 3-D structure of dried emulsions (Drawing Not To Scale).

Electron Microscope (Philips XL30) coupled with X-ray microanalysis (EDAX Genesis V5.21) was used for imaging the freeze-dried emulsions. Images were taken using a secondary electron detector at an accelerating voltage of 10 kv, 3-μm spot size of electron beam and working distance of 10 mm. Standardless semi-quantitative X-ray analysis with ZAF correction factors (Zi is the atomic number effect, Ai is the absorption of X-rays within the specimen and Fi is the fluorescence effect for each element) was performed on selected areas of the solid mass, *i.e.* droplet surface and background matrix. Particle size distribution and zeta potential of reconstituted emulsions was determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Ltd., UK) at 25 °C after dilution with MilliQ water to the ratio of 1/100. The instrument determined the electrophoretic mobility, the velocity of the dispersed particles in an external electric field, and then applying Smoluchowski or Hückel equation for ζ-potential calculation. Measurements of moisture content were conducted on the freeze-dried powders using thermogravimetric analysis (TGA) (Hi-Res Modulated TGA 2950, TA instruments) which measures the mass change in a sample as a function of temperature. 10 mg of each sample was loaded in the aluminium pan and heated at a rate of 10 °C/min over a temperature range of 25–150 °C, under a flow of dry nitrogen gas (80 ml min^{-1}).

2.2.4. Quantitative assay of all-trans-retinol

High performance liquid chromatography (HPLC) analysis was conducted under isocratic conditions at ambient temperature on a reversed phase column (LiChrospher RP Select B 5 μm, 250 mm × 4.6 mm ID, Alltech); method was adopted from (Jenning and Gohla, 2001). Full details on the procedure adopted for our experiments can be found in Ghouchi-Eskandar et al. (2009). In brief, acetonitrile/water, 80:20 plus 0.1 vol.% orthophosphoric acid constituted the mobile phase and 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 the retention time of all-*trans*-retinol was approximately 12 min. Calibration graphs of all-*trans*-retinol were constructed in acetone using external standard method. High level of linearity ($r^2 > 0.99$) was observed for all-*trans*-retinol concentrations in acetone 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.

2.2.5. Long-term stability test

Long-term stability studies were conducted at three different storage conditions; freeze-dried powders were divided into 3 samples and transferred into amber glass vials (excluding natural light) immediately after preparation and stored at 4 °C, room temperature and a relative humidity of 60 ± 5%, and 40 ± 0.5 °C (inside a storage cabinet). The chemical stability (drug content) was monitored over a period of 12 weeks. At determined time intervals samples were taken, extracted and diluted (if necessary) with acetone and analysed with HPLC for residual all-*trans*-retinol. In contrast to some previous 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 realistic storage and consumption conditions of the formulation.

2.2.6. Statistical analysis

The statistical differences in the size and zeta potential of both wet and freeze-dried emulsions were evaluated using *t*-test and two-tail *p* value less than 0.05 was considered significant.

3. Results and discussion

3.1. Solid-state characteristics and reconstitution properties of freeze-dried emulsions

The structure/morphology of freeze-dried emulsions was characterised using FEG-SEM and presented in Fig. 2. A macroporous sponge-like structure was observed with regions of positive curvature *versus* regions of negative curvature in which the primary oil droplets or larger aggregates were embedded in a three-dimensional matrix structure. The average pore/cavity size was in the micrometer size range and showed a close correlation to the droplet size of the precursor emulsions. In scanning electron micrograph of the freeze-dried control lecithin emulsion (Fig. 2, upper left), a homogeneous network of spherical micron-sized pores with relatively narrow size distribution and smooth walls was observed. The SEM images suggested similar structure of the freeze-dried matrix for the control and silica-coated lecithin emulsions (Fig. 2, middle and lower left). However, the difference in the size and morphology of the freeze-dried control and silica-coated oleylamine emulsions was evident; the three-dimensional matrix structure of the lyophilised control emulsion (Fig. 2, upper right) mainly exhibited positive curvatures with exposed pores/cavities of ~1 μm in diameter. In freeze-dried silica-coated oleylamine emulsions (Fig. 2, middle and lower right), the pores of slightly larger diameter and broad pore size distribution (1–5 μm) and thicker walls were densely branched and interconnected. The inner surfaces of spherical pores/cavities were not exposed due to the negative curvatures in the matrix structure. In general, porous solid phases fabricated from the control emulsions exhibited denser pore distribution and smaller pore size compared to silica-coated emulsion templates. The larger pore sizes in silica coated freeze-dried emulsions can be attributed to the coalescence of oil droplets in the initial emulsion template. In our previous studies of spray-dried nanoparticle-coated emulsions (Simovic et al., 2009), the aggregation patterns of oil droplets at various silica: droplet ratios were established. At 5 wt.% silica nanoparticles relative to the oil droplets (used in this study), the droplets are positively charged but partially neutralised (Table 2), and dispersed (not precipitated) and exhibit typical coalescence pattern, *i.e.* the formation of larger droplets. For positively charged oleylamine emulsion droplets and negatively charged silica nanoparticles, a bridging mechanism can be proposed where a negatively charged nanoparticle patch on one droplet interacts with an uncoated positive patch on the other droplet leading to a limited droplet coalescence and size enlargement. Strong capillary attraction between particles caused by deformed menisci around them is responsible for dense bridging particle monolayer that stabilises emulsion droplets sparsely covered by hydrophobic particles (Horozov and Binks, 2006).

The variation in pore size, shape, and special distribution is important since it may affect the specific surface area and the refractive index of the porous material and accordingly the stability and delivery of incorporated lipophilic compounds (Simovic et al., 2009; Tan et al., 2009, 2010).

Characteristics of initial wet state, solid-state, and reconstituted lecithin and oleylamine emulsions including particle size distribution, zeta potential, residual moisture content (w/w%), and process yield (w/w) are depicted in Tables 2 and 3 respectively. Good redispersibility was observed for the freeze-dried powders, *i.e.* the conversion to the initial emulsion form upon addition of an aqueous medium, and no sedimentation occurred. Takeuchi et al. (1992) have reported that oil droplets in a dry emulsion need to be embedded in a droplet form in the colloidal silica matrix in order to obtain sufficient redispersibility. The results of Simovic et al. (2009) also supported that the oil droplets are equally distributed and adsorbed onto porous silica matrix not as a continuous oil film at the

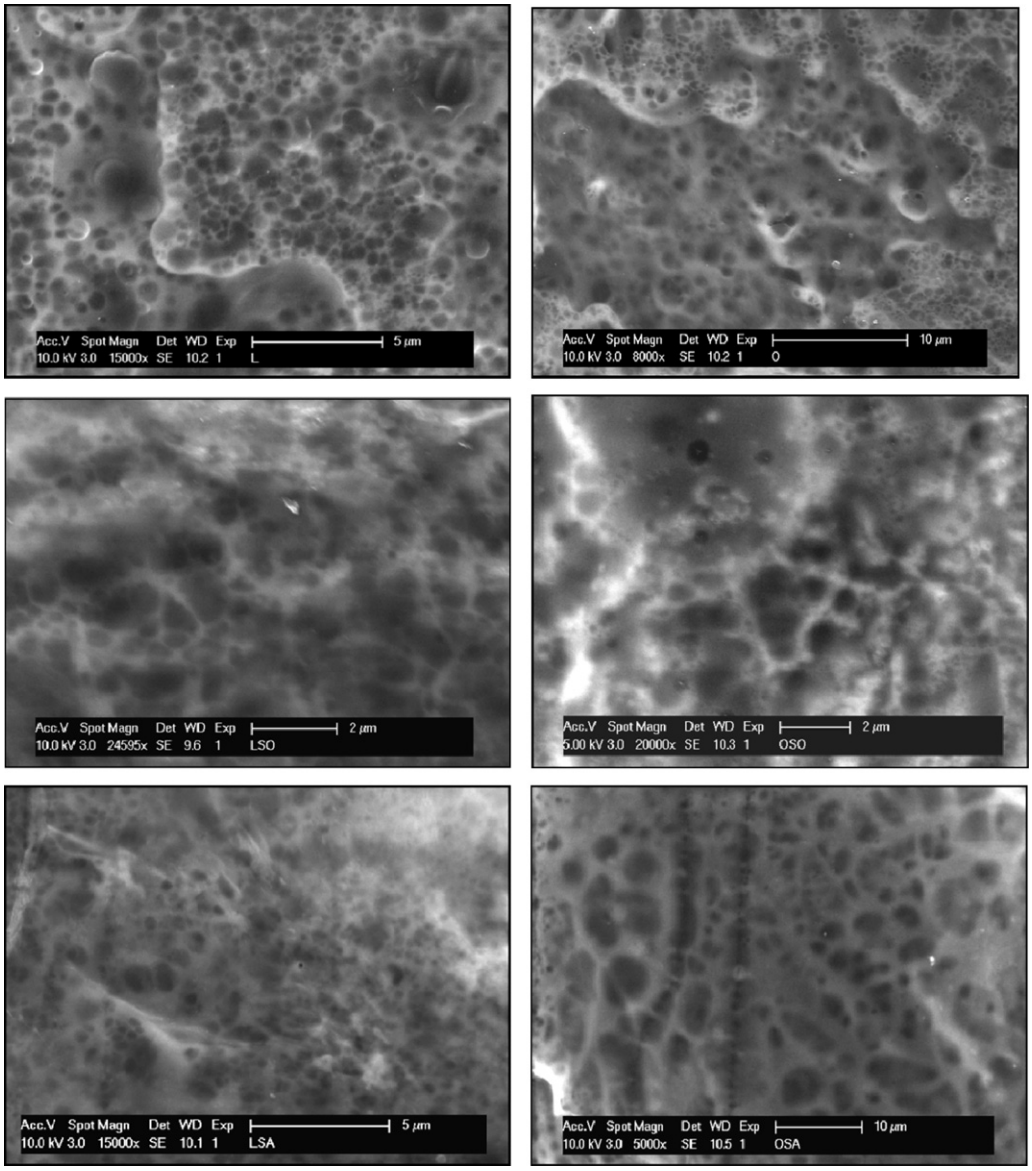

Fig. 2. FEG-SEM images of the control/silica-free (upper), and silica-coated (middle: initial loading phase of oil; Lower: initial loading phase of water) freeze-dried emulsions stabilised with lecithin (left), and oleylamine (right).

surface. Silica nanoparticles form an aggregated network structure into which the lipid droplets are adsorbed. Takeuchi et al. (1992) also showed that redispersibility exponentially increased as a function of increase in the oil phase viscosity from 10 to 100 mPa s, due to greater resistance of the emulsion oil droplets against deformation forces during the drying process. Although from this point of view highly viscous oils seem more suitable for the formulation of dry emulsions, but they posses relatively lower affinity for the silica surface (coated with surfactants) due to higher three phase contact angle, and this may lead to the separation of silica particles from the oil droplets surface and accordingly coagulation. Based on the results of Takeuchi et al. (1992), medium chain triglycerides (C_8–C_{10}): Panastate 810 and Triester F-810 with viscosity of 24.2 mPa s and 28.3 mPa s respectively were recognised to have an appropriate oil viscosity for good redispersibility; this is comparable to the viscosity (27–33 mPa s at 20 °C) of the emulsion oil phase in our study, Miglyol®812.

The mean particle size of redispersed/reconstituted lecithin- and oleylamine-stabilised emulsions increased compared to the mean droplet size of the initial emulsions. In contrast to the precursor emulsions that the droplet size was reduced by inclusion of silica nanoparticles, the average droplet size of solid-state silica-coated emulsions was larger compared to the control emulsions solely stabilised by either lecithin or oleylamine.

In reasonable agreement with the original o/w emulsions, zeta potential values of the rehydrated lecithin emulsions were negative and did not change significantly in the presence of silica nanoparticles. However, partial neutralisation of positive oleylamine emulsion droplets was observed in silica-coated dry emulsions.

The residual moisture content of the freeze-dried emulsions varied between 1.44 to 2.75 wt.%, and was slightly higher for silica-coated emulsions due to the larger particle size and accordingly longer water diffusion path. In addition, evaporation rates of water and oil from emulsions are reported to vary based on the relative magnitudes of oil-water, water-vapour, and oil-vapour interfacial tensions and the strength of repulsive colloidal forces across the water film (Aranberri et al., 2002). In emulsions with an oil droplet volume fraction above the close-packing limit for spheres, evaporation of the aqueous continuous phase results in the

distortion of oil droplets and progressive thinning of the nanometre water films separating them. Such thinning during the drying process is resisted by repulsive colloidal forces at the surface of oil droplets and the water evaporation rate is slowed relative to pure water depending on the magnitude of such forces. The evaporation rate and water content of the control and silica-coated emulsions may vary considering the fact that the values of interfacial tension changes in the presence of silica nanoparticles (Ghouchi Eskandar et al., 2007), and magnitude of repulsive forces varies depending on the level of interfacial assembly of silica nanoparticles at oil droplet surfaces (Simovic et al., 2010).

3.2. Chemical stability of all-trans-retinol at 4 °C, ambient temperature and 40 °C

The chemical stability of all-*trans*-retinol was investigated in freeze-dried emulsion-based powders stored at 4 °C, room temperature, and 40 °C, over a period of 12 weeks. The residual percentage of the active agent after 12 weeks storage at 4 °C was ~12% in the solid-state compared to ~20% in the initial control lecithin-stabilised emulsion. No additional improvement was observed in the chemical stability of all-*trans*-retinol from freeze-dried silica-coated compared to the control lecithin-stabilised emulsions at all storage temperatures. This was in agreement with the results of the precursor lecithin-stabilised emulsions which showed equivalent degradation pattern of all-*trans*-retinol in the control and silica-coated emulsions due to a significant stabilising effect of lecithin on all-*trans*-retinol. The enhancement in the chemical stability of vitamin A derivatives due to phospholipids has been reported and discussed in full detail in our previous paper (Ghouchi-Eskandar et al., 2009). Briefly, the reduction in oxygen permeability of soybean phosphatidylcholine liposomes at low temperatures due to the more tightly packed structure of the solid gel phase; retinol distribution 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); antioxidative properties of lecithin (Arsic and Vuleta, 1999); and the coexistence of emulsion droplets (with surface monolayers of phosphatidylcholine and a core of retinyl palmitate or retinal) with vesicular particles (bilayers) of phosphatidylcholine (Asai and Watanabe, 1999, 2000) were reasons for the observed effect. In addition, lecithin-stabilised droplets were partially coated by silica nanoparticles due 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 (Binks and Lumsdon, 1999; Washington, 1990).

The chemical stability and first order degradation kinetics of all-*trans*-retinol from the freeze-dried control and silica-coated oleylamine emulsions are presented in Figs. 3 and 4 and Table 4 for three different storage temperatures. The residual percentage and half-life of all-*trans*-retinol in the freeze-dried control oleylamine emulsion was ~13% and 3 weeks respectively.

The chemical stability of all-*trans*-retinol at 4 °C significantly improved by inclusion of silica nanoparticles from both the oil and water phases; a highly stable region was observed in chemical stability plots with no loss in the drug content up to 4 weeks storage time after which all-*trans*-retinol decomposition was fitted to the first-order degradation model (Fig. 3b). The half-life of the active agent in the freeze-dried silica-coated emulsions was 6.18 and 6.06 weeks for nanoparticle incorporation from the oil and water phases respectively, *i.e.* negligible influence of the initial loading phase of nanoparticles.

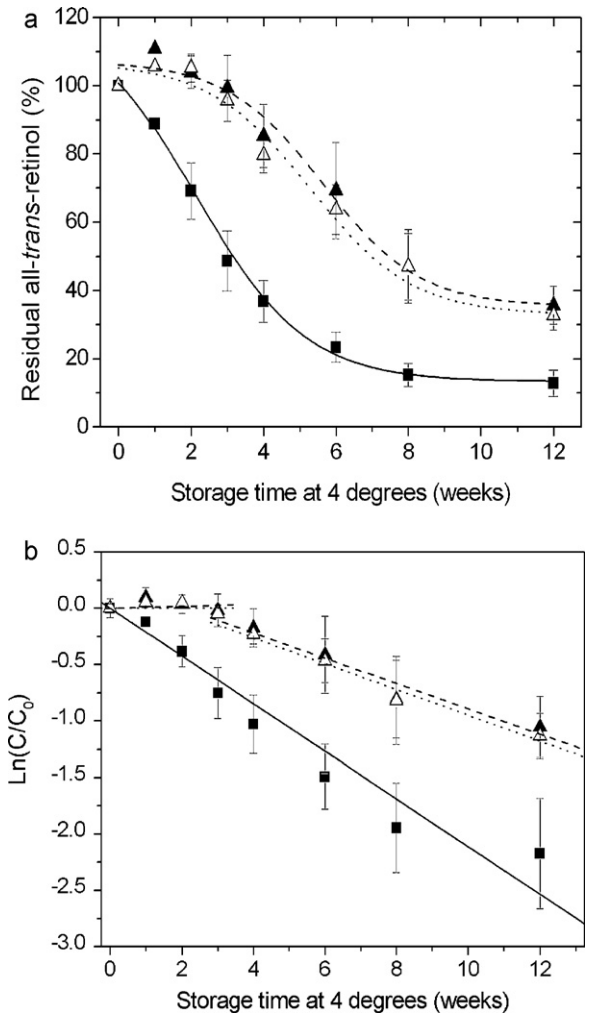

Fig. 3. Chemical stability at 4 °C (a) and first order degradation kinetics (b) of all-*trans*-retinol in the freeze-dried control oleylamine emulsion (solid squares and solid line); silica incorporated from the oil phase (solid triangles and dashed line); and silica incorporated from the aqueous phase (open triangles and dotted line).

In spite of slightly higher residual all-*trans*-retinol in nanoparticle-coated compared to the control freeze-dried powders at room temperature up to 2 weeks (Fig. 4a), no significant difference was observed in the long-term stability and half-life of all-*trans*-retinol in the absence and presence of silica nanoparticles

Table 4
Degradation kinetics of all-*trans*-retinol in the freeze-dried control and silica-coated oleylamine emulsions at different storage temperatures.

Storage temperature	Formula		
	k (1/week)	r	$T_{1/2}$[a] (weeks)
4 °C			
Control oleylamine emulsion	0.216 ± 0.022	0.96	3.20
Silica included from oil phase	0.112 ± 0.019	0.94	6.18
Silica included from water phase	0.114 ± 0.014	0.96	6.06
Room temperature			
Control oleylamine emulsion	0.183 ± 0.011	0.88	3.79
Silica included from oil phase	0.169 ± 0.005	0.94	4.09
Silica included from water phase	0.169 ± 0.007	0.90	4.11
40 °C			
Control oleylamine emulsion	0.36 ± 0.005	0.98	1.92
Silica included from oil phase	0.34 ± 0.002	0.98	2.05
Silica included from water phase	0.37 ± 0.004	0.98	1.88

[a] $T_{1/2}$: all-*trans*-retinol half life.

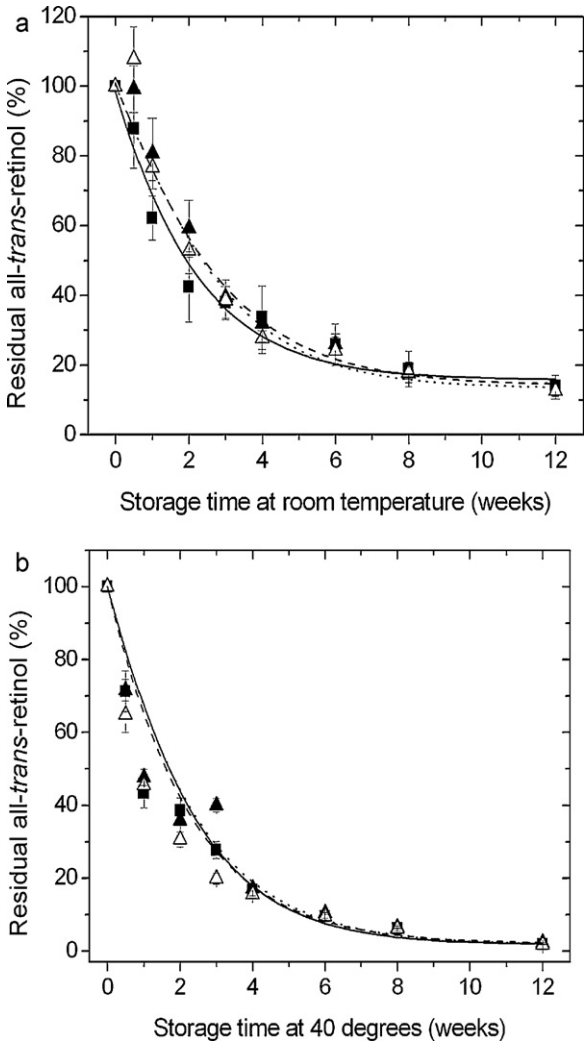

Fig. 4. Chemical stability of all-*trans*-retinol at room temperature (a) and 40 °C (b) of the freeze-dried control oleylamine emulsion (solid squares and solid line); silica incorporated from the oil phase (solid triangles and dashed line); and silica incorporated from the aqueous phase (open triangles and dotted line).

in solid-state oleylamine-stabilised emulsions stored at room temperature and 40 °C (Fig. 4b).

The results showed that the half-life of the active agent at 4 °C and room temperature has not increased as a result of freeze-drying of emulsions. However, up to 3-fold increase in the half-life of all-*trans*-retinol at 40 °C, *e.g.* up to 2.05 weeks was observed as a result of freeze-drying of precursor oleylamine-stabilised emulsions (drug half-life: up to 6 days).

This can be due to the fact that the freeze-dried powders are hygroscopic due to the presence of small carbohydrates, *e.g.* maltose and colloidal silica (Rowe et al., 2003), and may potentially absorb water and this facilitates the degradation of all-*trans*-retinol mainly through promoting sugar crystallisation; lipid oxidation; and structural changes such as plasticization (depending on the glass transition temperature), decreased porosity, coalescence of oil droplets, and powder caking (Ponginebbi et al., 2000). The oil carrier of emulsions (Miglyol®812) has a high stability against oxidation due to the absence of unsaturated fatty acids (peroxide value: max. 1.0 mequiv. O/kg), but lipid oxidation can occur due to the unsaturated fatty acids in the structure of lecithin and oleylamine under certain conditions, *e.g.* high relative humidity. In addition, Ponginebbi et al. (2000) reported changes in the physical structure of freeze-dried emulsions as a function of relative humidity; the porosity decreased by more than 50% at a moisture content of ~6% and powders started to cake over time. Sugar crystallisation in dry emulsions may also potentially promote lipid oxidation due to the release of the internal lipids to the surface (Shimada et al., 1991) and destabilisation of oil-water interface and accordingly oil droplet coalescence (Vincent, 1984).

Simovic et al. prepared dry hybrid lipid-silica microcapsules by spray-drying of submicron lecithin and oleylamine Miglyol 812N oil-in-water emulsions. Surface characterisation using X-ray photoelectron spectroscopy showed that for emulsions containing 5–10 wt.% silica nanoparticles relative to the oil (5 wt.% silica relative to Miglyol®812 was used in current study), microcapsule surfaces were composed of 5.19–11.14% silica (both SiO_2 91.9% and silicate 8.03%) and 56.76–72.69% carbon from either the oil carrier or the surfactant. It is proposed that the porous structures were formed during the drying process as a result of penetration of silica nanoparticles into the emulsion oil droplets or alternatively, lipid adsorption by silica (Simovic et al., 2009). This was in accordance with the kinetic analysis of ubidecarenone photodecomposition by Takeuchi et al. (1992) based on a theoretical model considering both the Aerosil silica layer and oil layer on dry emulsion surface which showed good agreement with the experimental results. Similarly, in our study considering the presence of both silica and oil at the surface of dry emulsions and the fact that all-*trans*-retinol is predominantly located in the oil carrier, the increased decomposition of the active agent can be correlated with more extensive exposure to the environmental factors, *e.g.* UV light, air (oxygen), and humidity. The porous matrix structure of solid-state emulsions and hence high surface area, *e.g.* 184 $m^2\ g^{-1}$ (Simovic et al., 2009) might also contribute to the enhanced exposure of the active ingredient to the aforementioned destabilising factors.

On the other hand, up to 3-fold increase in the half-life of all-*trans*-retinol at 40 °C for both the control and silica-coated freeze-dried compared to liquid oleylamine-stabilised emulsions, is due to the fact that at 40 °C and inside the incubator chamber with low relative humidity, the freeze-dried powders have limited exposure to environmental factors. As a result, the chemical stability of the active agent significantly improved compared to the initial emulsions due to the improved shelf-life of the freeze-dried powders. Furthermore, whilst the silica and sugar mass around oil droplets have a protective effect against high temperatures in freeze-dried powders; the poor physical stability of oleylamine-stabilised emulsions at high temperatures might be responsible for enhanced chemical decomposition of all-*trans*-retinol as a result of emulsion breakdown and phase separation.

4. Conclusions

Lyophilisation of silica-coated submicron emulsions was carried out to achieve solid-state emulsions as potentially improved carriers for model water-sensitive molecules, *e.g.* all-*trans*-retinol. The half-life of all-*trans*-retinol was 3-fold higher in solid-state compared to the original o/w emulsions stored at 40 °C. However, no significant improvement was achieved in the long-term stability of the active agent at 4 °C and room temperature as a result of freeze-drying of emulsions. This can be attributed to the more extensive exposure of active molecule to the environmental factors such as air and structural and chemical changes in the freeze-dried powders over time. Accordingly, the short shelf-life of the freeze-dried powders indicates the necessity of special storage conditions, *e.g.* under inert gas and controlled humidity. Comparing the control to silica-coated freeze-dried emulsions (similar to the initial wet emulsions), the chemical stability of all-*trans*-retinol was significantly improved by nanoparticle inclusion, depending on the initial emulsifier and storage temperature. No degradation of

all-*trans*-retinol was observed in silica-coated oleylamine-stabilised dry emulsions up to 1 month storage at 4 °C compared to the degradation of almost 50% of the active agent in the control emulsion. After this period, drug decomposition showed a first-order degradation pattern in line with the observation of the physical instability in freeze-dried powders.

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