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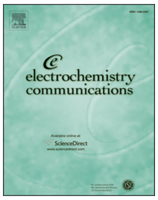

'Soft' electroactive particles and their interaction with lipid membranes

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ABSTRACT

Solid lipid nanoparticles (SLN) are widely used in pharmaceutical and cosmetic applications. SLN, incorporating approximately 10^5 ferrocene units, as core-enriched redox active nanoparticles, were used as a model for probing interactions with solid-supported lipid membranes. Resonance Enhanced Surface Impedance Spectroscopy (RESI) is shown as a sensitive technique for monitoring real time interactions of the soft redox nanoparticles. Cyclic voltammetry is compared as an end of experiment technique.

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1. Introduction

Electroactive nanoparticles combine the unique properties of nanoparticles with an electroactive moiety to facilitate facile electron transport. Nanoparticles (NP) have received attention in various applications due to their high surface area and unique electronic and optical properties [1–7]. Hard nanoparticles are based on materials such as noble metals (e.g. Au, Ag and Pt), quantum dots, magnetic nanoparticles and graphitic materials (e.g. carbon nanotubes and graphene oxide). Soft nanoparticles in contrast utilize materials such as polymers, gels, liposomes, cyclodextrins and dendrimers. Electroactive nanoparticles (eNP) are produced from either hard or soft nanoparticles by incorporating electroactive species such as metal complexes (iron and osmium complexes) [8,9], quinones (hydroquinone, benzoquinone and naphthoquinone) [10,11], organic dyes (methylene blue) [12] and their polymer derivatives and composites [13,14]. We report here a new 'soft' electroactive nanoparticle system comprising of ferrocene in a solid lipid nanoparticle matrix.

Solid lipid nanoparticles (SLN) are widely used in the pharmaceutical, cosmetic and food industries [15,16]. They are comprised of lipid(s), surfactant(s) and water. These soft particles have many characteristics that make them suitable candidates as carrier matrices for biomedical applications; principal among these being biocompatibility and biodegradability [17,18]. Additional lucrative properties of SLN matrices include, compatibility with both hydrophobic and hydrophilic actives [19], the possibility of co-immobilizing more than one active [20], low cost and ease of scaling-up production [15].

In this study, we report the preparation, characterization and initial results of interactions of ferrocene-loaded nanoparticles with lipid membranes using RESI. This work is of potential interest for using electroactive SLN as charge carriers and probing interactions of widely used SLN with lipid membranes.

2. Materials and methods

2.1. Reagents and chemicals

Lauric acid ($C_{12}H_{24}O_2$), ferrocene ($Fe(C_2H_5)_2$), polysorbate 80 ($C_{64}H_{124}O_{26}$), potassium nitrate (KNO_3), hydrogen peroxide (50% wt in water), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were purchased from Sigma-Aldrich, UK. The lipid, 1,2-dipalmitoyl-sn-glycero-phosphothioethanol (DPPTE) (sodium salt) was purchased from Avanti Polar Lipids, USA. Poloxamer 188 ($C_5H_{10}O_2$), HPLC grade n-hexane and laboratory grade chloroform and sulphuric acid were purchased from Fisher Scientific, UK. Solvents were used without further purification. Deionized water from a Sartorius Arium® Comfort ultrapure water system (~18 MΩ) was used in all experiments.

Ferrocene-loaded particles (FcSLN) were prepared using lauric acid (3 g) and ferrocene (0.3 g), which were heated to 55 °C while stirring and n-hexane (2 ml) was added to the lipid melt. The lipid mixture was added to a 100 ml aqueous solution (1.5% w/v poloxamer 188 and 0.75% w/v tween 80) at the same temperature dropwise while stirring continuously at 300 rpm and stirred for a further 60 min. The emulsion was mixed by high shear homogenisation for 20 min at 10,000 rpm and subsequently diluted in 150 ml deionized water at 4 °C for 10 min to precipitate the SLN. The resulting dispersion was centrifuged at 10,000 rpm for 10 min to remove excess surfactant and unincorporated

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ferrocene. The centrifuged particles were further dehydrated in a $CaCl_2$ desiccator until no changes in weight were observed. 75 mg of centrifuged particles were re-dispersed in 330 ml of 0.1 M KNO_3 for RESI measurements; a 10 times dilute solution of this was used for DPV measurements.

2.2. Characterization of SLN

UV/VIS spectroscopy was employed to measure the amount of ferrocene incorporated into the lauric acid matrix using a HR2000 CG UV–NIR (Ocean Optics coupled with a DH2000 UV–VIS–NIR (Mikropack) light source. Morphological studies were carried out using TEM with a 2100F FEG TEM, JEOL. An accelerating voltage of 200 kV was used and the temperature of the stage was set to −50 °C to prevent melting of the solid lipid particles upon exposure to the electron beam. Particle size analysis for the FcSLN system was measured by DLS using a Zetasizer μV (Malvern). The measurements were taken at a 90° angle and the temperature was set to 25 °C. The nanoparticle dispersions were diluted by a factor of 10 in deionized water for measurement. The refractive index of the particles was set to 1.430 (the refractive index of lauric acid) and the absorption value was set to 1.00. The sample holder was a DTS0012 disposable cuvette.

2.3. Electrochemical measurements

All electrochemical measurements (CV, DPV) were carried out using the AUT72109 (Metrohm, Autolab) potentiostat. A thin film integrated electrode system combining a gold working electrode (150 nm thickness and 0.8 mm^2 area) and platinum reference and auxiliary electrodes (MicruX Technologies, Oviedo, Spain) was used. Previously reported procedures [21,22] were adopted for preparation and characterization of SAM and BLM on both Micrux electrodes and for the modification of gold sensor chips for RESI studies. The formation of a compact monolayer (~0.90 ± 0.08 $\mu F\ cm^{-2}$) and bilayer (~0.44 ± 0.03 $\mu F\ cm^{-2}$) on the MicruX electrodes was confirmed using ESI [21]. The redox potential for ferrocene carboxylic acid solution in this cell was measured at 0.59 ± 0.05 V. DPV and CV measurements were performed to test the redox behaviour of the FcSLN on impact with the Micrux electrodes. The potential range was set from −0.4 to 0.8 V. A potential step of 5 mV and modulation amplitude of 25 mV (modulation time 0.05 s) was applied. The interval time was 0.5 s and scan rate was 10 mV/s.

Initial interactions of FcSLN with SAM or BLM modified working electrode surfaces were analysed using CV. Measurements were recorded at time, $t = 0$, 15 and 30 min in 50 μL of the dilute FcSLN dispersion. A potential range of −0.4 to 0.6 V and a scan rate of 50 mV/s was applied.

Real time interaction studies were carried out using RESI with a z-LAB™ instrument (Layerlab AB, Sweden) [23]. The z-LAB™ comprises of an RLC circuit (a resistor (R), an inductor (L), and a capacitor (C), connected in series (Fig. 3a), and a fluidic system with integrated pumps that control the flow rate through the sensor surface. RESI measures changes in impedance at a single frequency in resonance modes. This is accomplished by connecting a pair of electrodes in parallel with an external inductance which becomes part of a resonator with a resonance frequency determined by the interface capacitance via the relationship

$$f_0 = \frac{1}{2\pi\sqrt{LC}}$$

where f_0 is the resonance frequency, L the inductance and C the capacitance. Shifts in the resonance peak are mapped to give a resolution of less than 0.2 pF and a time resolution of 0.25 Hz, providing a highly sensitive, time-resolved and rapid detection system for capacitive changes. Formation of an isolating film on the electrode will lower the interfacial capacitance with a positive shift in f_0.

Additionally, the amplitude of the resonance peak is affected by interfacial resistance and is plotted as V_{peak}. An increase in the magnitude of voltage peak (V_{peak}), is observed when the interfacial resistance increases whereas the presence of a redox species or ions causes a decrease interfacial resistance and consequently a dampening of the peak.

A background solution of 0.1 M KNO_3 was allowed to flow through the SAM and BLM modified sensor chips in the flow cell until a stable response was achieved. A 200 μL aliquot of the FcSLN dispersion in 0.1 M KNO_3 was injected following a stable response with the background solution. The flow rate was 5 μL/min. Injection times lasted approximately 40.0 ± 0.5 min, as shown in Fig. 3. The flow rate and concentration of the dispersions were optimized to achieve reproducible results.

3. Results and discussion

3.1. Characterization of the ferrocene-loaded SLN

3.1.1. Loading capacity and entrapment efficiency

The loading capacity (LC) is defined as the percentage ratio of the amount of ferrocene incorporated to the total amount of lipid. It gives a quantitative measure of the mass of ferrocene incorporated in the solid lipid matrix. The amount of ferrocene loaded onto the matrix and subsequently the LC was calculated from UV/VIS measurements. The UV/VIS spectra of ferrocene in ethanol show a specific peak for ferrocene at 444 nm. The concentration of ferrocene in the FcSLN was found to be 8.6 ± 0.6 mM. The calculated LC was 6.4 ± 0.2%, which gives an entrapment efficiency value (EE) of 64.0 ± 2.2%. All measurements were repeated five times. Assuming the FcSLN is spherical and using the density of lauric acid as the main component of the SLN, an approximate mass for each FcSLN can be calculated. The mass of each particle can be used to calculate the number of particles per ml of sample, which was found to be approximately 6×10^{12}. The calculated charge per particle was 2×10^{-14}C.

3.1.2. Physicochemical characterization

There are three possible models for the partitioning and arrangement of ferrocene in the lipid matrix, these are: solid solution, enriched core and enriched shell model (Fig. 1a) [24]. In order to identify the model that best fits the FcSLN system, a series of experiments were performed. The FcSLN particle size and morphology were investigated using TEM and DLS. DLS was used to measure the hydrodynamic diameter and TEM to give the mean diameter of the electron-rich ferrocene enriched regions within the FcSLN matrix [25]. The TEM image (Fig. 1b inset) shows a ferrocene-enriched core as dark spheres in contrast with the electron transparent lauric acid matrix. The ferrocene-enriched core gives a mean particle size of 37 ± 9 nm. DLS measurements gave an average hydrodynamic diameter of 109 ± 17 nm (Fig. 1c), about 3 times the mean core diameter measured by TEM. These observations suggest a model of incorporation with a ferrocene-enriched core. Chemical analysis carried out using ^{13}C NMR and FTIR (not shown), confirmed the incorporation of ferrocene and the absence of any strong interactions between the lipid matrix and the ferrocene. These observations support the core enriched model. Additionally, thermogravimetric analysis (TGA) and small angle X-ray diffraction (SAXS) (not shown), also confirmed the preservation of the lipid crystalline arrangement and thermal behaviour, which supports a core-shell model of incorporation. The differential scanning calorimetry (DSC) thermograms (not shown) obtained for the non-loaded and the FcSLN showed only slight changes in the melting temperature and enthalpy of fusion ($T_{m(SLN)} = 45.2 \pm 0.3$ °C, $\Delta H_{(SLN)} = 154 \pm 6$ J/g and $T_{m(FcSLN)} = 46.2 \pm 0.1$ °C, $\Delta H_{(SLN)} = 155 \pm 6$, respectively). A sharp melting peak confirmed the solid state of the particles. These observations confirm that the multi-lamellar crystalline arrangement of the lauric acid matrix is maintained and ferrocene is not molecularly dispersed in the matrix to give a solid-solution but is confined to the core.

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