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Research paper

Interactions between poly(ethylene glycol) and protein in dichloromethane/water emulsions. 2. Conditions required to obtain spontaneous emulsification allowing the formation of bioresorbable poly(D,L lactic acid) microparticles

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ABSTRACT

From microscopic observations, it was established that an oil-in-water emulsion with droplets of a size in the micrometer range can spontaneously form at room temperature without additional external stirring as soon as a solvent that is only partly miscible to water-like dichloromethane (DCM) is put in contact with an aqueous mixture of polyethylene glycol (PEG) and a protein. Experimental results show that emulsification only occurs if the system simultaneously includes PEG with middle chain, an organic solvent partly miscible to water and for which PEG affinity is sufficiently high, and a protein. From adsorption kinetics, it appears that this spontaneous emulsification process is related to the rapid diffusion of DCM towards water through the formation of interfacial turbulences, once the accumulation of PEG close to the DCM/water interface occurs. The oil droplets formed would be then stabilized by adsorbed protein molecules. Since the presence of polylactic acid in the organic phase did not prevent the emulsion formation, we studied the feasibility of formulating microparticles using this polymer. From results, it appears that microcapsules with a polymeric shell, with a homogeneous size of about 50 μm and able to encapsulate a model hydrophobic drug, such as amiodarone, can be obtained by using this spontaneous emulsification method.

1. Introduction

The emulsification solvent evaporation technique is commonly used to encapsulate a wide range of drugs, such as proteins, at high concentration levels in polymer matrices [1,2]. To optimize protein stability within the formulation, polymers, and in particular poly(ethylene glycol) (PEG), can be used to minimize protein adsorption at interfaces created during the emulsification process [3–5]. PEG is a peculiar polymer which is soluble both in water and in organic phases such as dichloromethane (DCM) [6]. From the results of adsorption kinetics and interfacial rheological studies performed on mixed films of PEG and hen egg-white lysozyme (HEWL), a model protein, it appears that the introduction of PEG in water or in DCM influences protein adsorption prevention [7]. Thus, exposure of HEWL at the water/DCM interface is prevented for a longer time if PEG is dissolved in DCM instead of in water. Moreover, it was observed that the simultaneous dissolution of HEWL and PEG in water induces the apparition of a spontaneous emulsion process [7].

Generally speaking, spontaneous emulsification refers to the formation of small droplets having diameters on the order of 1 μm when two immiscible liquids are placed in contact with each other and when high shear rates are not required. Typically, the droplets form spontaneously, without requiring any supply of external energy of agitation, the entire energy required for the emulsification coming from the redistribution of material within the system [8,9].

In 1878, Johannes Gad first observed that a solution of lauric acid in oil would spontaneously form emulsions when placed on top of aqueous alkali [10]. In the past, the self-emulsification process was reported to occur in oil–water–alcohol systems and in various systems containing surfactants [11]. There is considerable literature on the formation of microemulsions using alcohols such as butanol, hexanol, and octanol which can help solubilize large

Abbreviations: BSA, bovine serum albumin; DCM, dichloromethane; HEWL, hen egg-white lysozyme; PEG, poly(ethylene glycol); PLA, poly(lactic acid); SEM, scanning electron microscopy; γ_{eq}, surface tension at equilibrium.

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quantities of oil and water, but these agents are not appropriate pharmaceutical ingredients [12].

In the present paper, we have developed innovative conditions required to obtain a spontaneous oil-in-water emulsion using PEG and a protein. We have tried to explain the mechanism leading to spontaneous emulsification. In order to further analyze this mechanism and to link it with the formulation area, preliminary studies of formulation were carried out by using the spontaneous emulsification method to produce microparticles and to encapsulate a model drug, amiodarone.

2. Materials and methods

2.1. Materials

PEG 2000 was an α-methoxy, ω-hydroxy-PEG 2000. The number-average molecular weight ($\overline{M}n$) of the polymer determined by nuclear magnetic resonance at 360 MHz was 2200. PEG 400, PEG 2000, PEG 5000, PEG 8000, PEG 17,500, HEWL (no. L6876, dialyzed and lyophilized, containing the buffer salts sodium acetate and sodium chloride, 3 × crystallized, protein at approx. 95%), human serum albumin and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (L'Isle d'Abeau, France), and were used without further purification. Amiodarone was obtained from Sanofi AG (Montpellier, France). Poly(lactid acid) (PLA50) was supplied by Phusis (Saint-Ismier, France). According to the Mauduit and Vert classification [13], the gross composition was 50% D-repeating units and 50% L-repeating units. The weight-average molecular weight ($\overline{M}_w$) determined by size exclusion chromatography was 52,000 g/mol. The polydispersity index was 1.7. All the organic solvents were purchased from Prolabo (Paris, France). NaCl (for analysis, ACS, ISO) was supplied by Merck (Nogent-sur-Marne, France). Ultrapure water was obtained from a Millipore® system (Milli-Q Plus 185, Molsheim, France).

2.2. Drop images and interfacial tension measurements

Adsorption kinetics and images of drops were recorded by means of a pendant-drop tensiometer (Tracker®, Teclis, Longessaigne, France) [14]. A drop was formed with an Exmire microsyringe (Prolabo, Paris, France) into an optical glass bowl (Hellma, France) containing the other phase. From the analysis of the numerical image of the drop with the Laplace equation integrating the points of the drop profile, the interfacial tension was recorded in real time (up to 20 measurements per second). The drop surface area was maintained constant during the experiments thanks to a drop volume control system, in such way that the surface tension variation was only related to the adsorption of molecules at the interface.

Images (real size of 5 mm × 5 mm) of the studied drops were recorded in real time. The size of droplets appearing at the surface of the drop in some experiments (see results) was determined by using the software ImageJ on the recorded images.

2.3. Microscopy observations

Spontaneous emulsification was also followed using optical microscopy (Axioskop 2, Zeiss, Le Pecq, France), and images were recorded using a Sony digital still camera supplied by Zeiss (Le Pecq, France). A droplet of the oil was injected using an Exmire microsyringe (Prolabo, Paris, France) into a mixed solution of HEWL (1 mg/mL) and PEG 2000 or PEG 17,500 (10 mg/mL) contained in a rectangular 30 mm × 5 mm glass cell of 0.5 mm thick (VitroCom, New Jersey, USA) directly under the microscope.

2.4. Microparticle preparation

The aqueous phase contained PEG 2000 (10 or 20 mg/mL) and protein (HEWL or BSA at 1 mg/mL), and the organic solution was made of DCM and PLA50 (5 mg/mL). In case of drug-loaded microparticles, amiodarone was solubilized in the organic phase prior to experiments. Using a syringe, 2.5 mL of the organic phase was injected into the aqueous solution (50 mL), and then the mixture was left at room temperature for 90 min, without any external agitation, to allow the spontaneous emulsification to form. The resulting emulsion was poured into deionized water (500 mL) and magnetically stirred for 45 min to extract the DCM. Finally, the formed microparticles were filtered through a 0.45 μm filter (HVLP type, Millipore SA, Saint-Quentin en Yvelines, France), washed with deionized water, freeze-dried (Virtis, France), and stored at 4 °C.

2.5. Microparticle characterization

2.5.1. Morphology and size

The obtained microparticles were observed by optical microscopy (BH2, Olympus, Tokyo, Japan). The surface and the internal morphology of the microparticles were investigated by using scanning electron microscopy (SEM; JSM 6310F, JEOL, Paris, France). Freeze-dried microparticles were mounted onto metal stubs using double-sided adhesive tape, sputter-coated with a fine coat of gold and carbon (JEOL JFC 1100, Paris, France) and examined under SEM. To characterize the internal morphology, the adhesive tape with stuck particles was first folded on itself and secondly roughly unfolded to fracture the microparticles according to Pean et al. [15]. The coating was carried out as previously described. The average particle size and distribution were determined using a Coulter® counter Multisizer (Coultronics, Margency, France).

2.5.2. Drug encapsulation efficiency

Encapsulation tests were performed with amiodarone as a model drug. Amiodarone-loaded microparticles were prepared according to the previously described protocol. Entrapment efficiency was determined by HPLC (Waters 996, Waters, Saint-Quentin en Yvelines, France) based on the method described by Weir and Ueda [16]. Microparticles were dissolved with dimethylsulfoxide (DMSO) prior to injection in the column (RP-18 column, LiChrospher® 100 Merck, Darmstadt, Germany). Samples of 50 μL were injected into the column. Analysis was performed using a mobile phase consisting of methanol, water and ammonium hydroxide (94:5:1 v/v) delivered at a flow rate of 1.5 ml/min. Amiodarone was detected by UV absorbance at 244 nm.

3. Results and discussion

Fig. 1a shows a pendant drop of DCM freshly formed in an aqueous solution of PEG 2000 ($C_{PEG2000}$ = 10 mg/mL) and HEWL (C_{HEWL} = 1 mg/mL). A few seconds after the formation of the drop, a flow of material is observed from the organic phase towards the aqueous phase, and dark spots appear on the surface of the DCM drop (Fig. 1b). With time, more and more of these spots can be distinguished (Fig. 1c). After about 60 min, well-individualized droplets (50 ± 20 μm in diameter) are visible (Fig. 1d). Fig. 2a shows that the formation of a rising drop of the mixed aqueous phase in DCM is not followed by any material flow but by the apparition of the spontaneous formation of droplets in the aqueous drop.

From these results, once a DCM drop is brought into contact with an aqueous mixture of a PEG and a protein, an oil-in-water (o/w)