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Impact of electrolytes on double emulsion systems (W/O/W) stabilized by an amphiphilic block copolymer

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ABSTRACT

In this work, the block copolypeptide surfactant, poly(L-lysine·HBr)$_{40}$-b-poly(racemic-leucine)$_{20}$, was synthesized and characterized, then used to build water-in-oil-in-water (W/O/W) double emulsions. Double emulsions are usually prepared by a two-step emulsification process and commonly stabilized using a combination of hydrophilic and hydrophobic surfactants. Herein, we report a one-step phase inversion process to produce water-in-oil-in-water (W/O/W) double emulsions stabilized by a synthetic diblock copolymer and electrolyte. It was found that the O/W ratio and the type of electrolyte had a marked effect on the formation and type of the double emulsions. Moreover, double emulsions containing an NaCl isotonic solution were stable for at least two months, whereas those using glucose as a substitute for NaCl showed a clear compartmental change. The mechanism behind this was related to the electrostatic interaction between the anion of the electrolyte and the cation of the polylysine residues, which affected the HLB value and curvature. This novel finding is very interesting in terms of both scientific research and practical applications.

1. Introduction

Double emulsions are complex liquid-dispersed systems known also as an "emulsion of emulsion" or "emulsions within emulsions", in which the internal droplets are separated from the outer liquid phase by another thin layer of immiscible liquid phase [1]. Thus, double emulsions can be categorized into water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O). Up to now, the most common and the most widely studied double emulsions are W/O/W emulsions since they have wide applications in different fields such as food, cosmetics, encapsulation, separation, targetable drug delivery and material syntheses [2]. In particular, the compartmentalized internal structure of double emulsions can provide advantages over simple oil-in-water emulsions for encapsulation, such as the ability to carry both polar and non-polar cargos, and improve control of the release of therapeutic molecules [3].

Usually, double emulsions are prepared by a two-step emulsification process using two surfactants: a hydrophobic one designed to stabilize the interface of the primary emulsions (W/O) and a hydrophilic one to stabilize the external interface of the reverse emulsions (O/W)[1]. The composition of the internal aqueous phase can be determined at the time of preparation and modified by transport of substances through the oil phase which acts as a liquid membrane between the aqueous phases; whereas the external aqueous phase can be established during its preparation and easily modified by addition of some other component (solute, solvent or ions). Although the two-step emulsification process has been extensively studied, this method is complex and introduces possible destabilization pathways like rupturing of the primary emulsion droplets during the second re-emulsification step [4]. In addition, the two surfactant species have a tendency to diffuse from one interface to the other, which changes the curvatures of both and subsequently destabilizes the structure to give simple O/W emulsions. This is especially true when small-molecule emulsifiers were used, as these have a higher mobility than large molecules such as proteins. Double emulsions therefore tend to be more stable if the surfactants have a large molecular size, as this "anchors" them to the respective interface (the energy required to remove large molecules from an interface is high) and makes them less likely to diffuse between interfaces [5]. Moreover, these destabilization pathways may be improved further when a one-step process using a single-component, synthetic amphiphilic diblock copolypeptide surfactant, is employed. These surfactants are even able to stabilize droplets subjected to extreme flow, leading to direct, mass production of robust double

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nanoemulsions that are amenable to nanostructured encapsulation applications for use in food processing, cosmetics and drug delivery.

The block copolypeptide surfactants designed by Hanson et al. were successfully used to prepare W/O/W double emulsions for the first time. The block copolypeptide surfactants had the general structure of poly(L-lysine·HBr)$_x$-b-poly(racemic-leucine)$_y$, K$_x$(rac-L)$_y$, where x ranged from 20 to 100, and y ranged from 5 to 30 residues (Supplementary Fig. 1). The hydrophilic poly(L-lysine·HBr) segments were highly charged at neutral pH, providing good water solubility and possessed abundant amine groups for chemical functionalization while the hydrophobic poly(rac-leucine) segments with a disordered chain conformation improving the solubility in common organic solvents and helping to promote surface activity compared with poly(L-leucine) present as rod-like a-helical conformations. In addition, the peptidic nature allowed for additional mechanical stabilization of droplet interfaces through interchain hydrogen-bonding in the oil phase [3,6].

In the present work, W/O/W double emulsions were prepared in a direct catastrophic inversion process using single-component, synthetic amphiphilic diblock copolypeptide surfactants, K$_{40}$(rac-L)$_{20}$, with Medium Chain Triglycerides (MCT) as the oil phase. In previous reports, most double emulsions were formed by using a non-injectable oil phase, such as silicone oil, decane, $CHCl_3$, and toluene [1,3,7,8]. However, in our study, MCT was used for double emulsions for the first time, and it has been extensively used for intravenous lipid emulsions [9]. More importantly, we obtained an interesting finding that adding electrolytes to the aqueous phase had a marked effect on the structure of the double emulsions. This novel finding has great implications in terms of both scientific research and practical applications.

2. Experimental

2.1. Materials

Nε-Carbobenzoxyl (CBZ)-L-Lysine, D-Leucine, and L-Leucine (Sichuan Tongsheng Amino Acid Ltd. Co., Sichuan) were dried in nitrogen for 24 h before use; triphosgene (99%; GL Biochem, Shanghai) was recrystallized before use. All organic solvents used in the synthesis experiments were purchased from National Medicine Chemical Reagent Ltd. Co. (Shanghai). Ethyl acetate and dichloromethane (DCM) were dried by refluxing over CaH_2 and distilled prior to use, while tetrahydrofuran (THF) and n-hexane were dried and distilled over sodium immediately before use. The water used was double distilled and all other chemicals were commercially available and of reagent grade.

2.2. Synthesis of K$_{40}$(rac-L)$_{20}$

According to previously published literature protocols with some modification [3], the α-amino acid-N-carboxyanhydride NCA monomers including Nε-CBZ-L-Lysine NCA, D-Leucine NCA and L-leucine NCA were synthesized. Briefly, Nε-CBZ-L-Lysine (10.0 g, 32.7 mmol) was suspended in 100 mL dry THF and heated to 55 °C. After 30 min, a solution of triphosgene (8.0 g, 27.0 mmol) in 50 mL anhydrous THF was added dropwise under stirring, continuing the reaction until the mixture became transparent. Then, the reaction system was purged with nitrogen to eliminate chlorine hydride gas generated by the reaction before totally evaporating the solvent under reduced pressure. The obtained crude crystals of Nε-CBZ-L-Lysine NCA were recrystallized three times using a mixture of THF and n-hexane at −20 °C to obtain white crystalline needles. D-Leucine NCA and L-leucine NCA were synthesized in the similar way except that the recrystallization used a mixture of ethyl acetate and n-hexane. Poly(Nε-CBZ-L-lysine)$_{40}$-b-poly(rac-leucine)$_{20}$ was polymerized via the ring-opening polymerization (ROP) of Nε-CBZ-L-lysine NCA initiated by n-hexylamine in anhydrous DCM with nNCA/n initiator = 40. After being allowed to react for 72 h in an argon atmosphere at room temperature, the product was precipitated in excess diethyl ether and filtered to give a white powder which was washed 2–3 times with diethyl ether and dried in a vacuum oven for 48 h. The prepared Poly(Nε-CBZ-L-lysine)$_{40}$ (PZLL), D-leucine NCA and L-leucine NCA (1:10:10, n/n/n) were dissolved in anhydrous DCM and reacted at room temperature for 72 h in an argon atmosphere. The reaction mixture underwent precipitation in diethyl ether and Poly(Nε-CBZ-L-lysine)$_{40}$-b-poly(rac-leucine)$_{20}$ was obtained by filtration. To synthesize Poly(L-lysine)$_{40}$-b-poly(rac-leucine)$_{20}$, a protective group, the carbobenzoxyl of PZLL, was removed with a trifluoroacetic acid/HBr (33% in acetic acid) mixture in an ice bath followed by precipitation in diethyl ether. After filtering and drying, the isolated polymer was dispersed in water and dialyzed using a 3500 MWCO membrane in deionized water for 7 days. After 2 days of freeze drying, Poly(L-lysine·HBr)$_{40}$-b-poly(rac-leucine)$_{20}$, referred to as K$_{40}$(rac-L)$_{20}$, was obtained.

2.3. Polypeptide characterization

The synthesized polypeptide was characterized by Bruker DMX 300 MHz ^{1}H NMR spectroscopy (Billerica, MA) with CF_3COOD as a solvent. Fourier transform infrared spectra were recorded on a Bruker Tensor 27 spectrometer, and samples were prepared using KBr disks (Scharlau Chemie, Barcelona, Spain). A gel permeation chromatography (GPC) assay was performed on a Waters 1515 GPC instrument (Waters Corp., Milford, MA) equipped with three Styragel columns (Waters Corp; 105, 104, and 103 A) in tandem and a 2414 differential refractive index detector. DMF was selected as the eluent at a flow rate of 1.0 mL/min at 35 °C. The molecular weights were calculated with reference to polystyrene standards. The critical aggregate concentration (CAC) was determined using an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) with pyrene as the hydrophobic dye. The fluorescence intensity ratio at 337 and 334 nm (I337/I334) of pyrene versus the logarithm of the polymer concentration was plotted from the excitation spectra, and the CAC was obtained from the inflection point of this plot.

2.4. Double emulsion preparation

We first dissolved K$_{40}$(rac-L)$_{20}$ copolypeptide in deionized water at the desired concentration. MCT (viscosity 30 mPa S) was added to give the desired volume fraction of oil in the continuous phase. Double emulsions stabilized by the block copolymers, K$_{40}$(rac-L)$_{20}$, were prepared by homogenizing a specific internal fraction of MCT and deionized water using a high speed shear mixer (ULTRA TURRAX®T18 basic, IKA®WORKS, Germany) (10 mm head) at 7000 rpm for 60 s. This emulsion was further dispersed in an ice bath using a probe sonicator for 60 s (Sonics® Vibra-Cell™ at an output of 35–40%).

2.5. Microscopy observations

A Motic DMBA450 upright Digital Biological Microscope (Speed Fair Investment Co. Ltd., Germany) was used to examine the morphology of the double emulsions. Samples were placed on microscope slides and gently covered with a cover slip. Images were captured using an image analyzer (Motic Images Advanced 3.1 Version software, Germany). Each sample was characterized at room temperature (25 °C) in duplicate.