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An approach to rheological and electrokinetic behaviour of lipidic vesicles covered with chitosan biopolymer

S. Madrigal-Carballo [b], D. Seyler [a], M. Manconi [c], S. Mura [c], A.O. Vila [a], F. Molina [a,*]

[a] *Unidad de Investigación de Coloides, Dpto. Química Física, Universitat de Valencia, Spain*
[b] *Laboratorio de Polímeros, Escuela de Química, Universidad Nacional, Heredia, Costa Rica*
[c] *Dpt. Farmaco Chimico Tecnologico, Universita'degli Studi di Cagliari, Italy*

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Abstract

The rheological and electrokinetic properties of soybean lecithin vesicles prepared from concentrated soy lecithin dispersions (250 g/L) obtained by slow swelling under shear conditions and mixed with chitosan biopolymer solutions were studied. The rheological behaviour of lecithin–chitosan vesicles was determined by shear stress against shear rate measurements, as well as by the variation on the hysteresis loop area. The results were compared with the rheopectic behaviour of soy lecithin dispersions without chitosan. An important change on the rheological properties of the complex dispersion was observed, depicting in a thixotropic behaviour with a plastic character in the presence of chitosan. This observation indicates that chitosan promotes the transition of planar sheets into closed structures, such as vesicles. The influence on the rheological and the electrokinetic behaviours of several electrolytes, such as NaCl, $CaCl_2$ and $AlCl_3$ with concentrations ranging between 10^{-5} and 10^{-2} mol/L were also studied. In all of the cases, an estimation of the diameters of the closed structures was obtained.

Keywords: Soy lecithin; Vesicles; Chitosan; Rheology; ζ-Potential; Tixotropy

1. Introduction

The closed bilayers are a model system for cell membranes and can be used to study the physical properties of amphiphilic bilayers [1]. Furthermore, the vesicles are able to encapsulate active molecules and therefore are used as drug delivery systems [2,3]. A particular application of this sort that has attracted a lot of attention is the use of liposomes as non-viral carriers [4,5]. Accordingly, they can be applied in a large variety of pharmaceutical and cosmetic applications [6]. Particulate systems, such as liposomes, micro- or nanoparticles have attracted a great deal of attention as possible oral dosage forms [7]. Among these, liposomes have the advantages that they are composed of physiological material (e.g. soy lecithin phospholipids) [8]. However, liposomes are liable to be destructed by pH, bile salts, and pancreatic lipase in the gastrointestinal tract [9]. Previous studies have been analyzing the effects involved in the formation of a polymeric membrane around the liposome in order to minimize these disruptive effects [10]. Since the discovery of polysaccharides on cell surfaces and the high affinity of chitosan to cell membranes, several researchers have been utilized chitosan as coating material for liposomes [11]. Chitosan is a hydrophilic, biocompatible and biodegradable polymer of low toxicity. A lot of studies have shown the potential use of chitosan as an absorption-enhancing agent. Moreover, because of its bioadhesive properties, chitosan has also received substantial attention as novel bioadhesive drug delivery systems [12]. By combining chitosan and liposomal properties, prolonged and controlled release may be achieved [13]. The bioadhesion of the oral dosage forms of poorly absorbable drugs has received much attention in comparison with transdermal and buccal systems. Bioadhesion to mucosa on the gastrointestinal tract can be described in terms of mucoadhesion. Since mucoadhesion can increase the residence time of drug carriers at absorption sites, improved drug absorption is expected by a combination of both, mucoadhesion and controlled drug release, from devices. The delayed gastrointestinal transit, induced by bioadhesive polymers, could lead to an increased on the drug oral bioavailability [14]. Also, numerous studies report that the application of liposomes on the skin surface is able to improve permeability for various entrapped

* Corresponding author. Fax: +34 963544892.
E-mail address: Francisco.Molina@uv.es (F. Molina).

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drugs through the major barrier of the cornea stratum [15–17]. One reason for the liposomes enhancing effect in penetration may be attributed by interactions between intracellular lipids on the skin and the liposome bilayer. The extent of the enhancement depends on the liposome size, lipid composition, liophilic nature of the drug and also skin's nature [18]. Liquid liposomes are applied directly to the skin. Usually, it is impossible to include completely intact liposomes into cream formulations because of the interaction between surfactant's active surface and the liposomal layers [19]. Instead of cream and further cosmetic formulations, polymers can be used to increase the viscosity of liposomal preparations. They are able to modify drug penetration and stability by the formation of a stable layer around the vesicles, which seems to be independent on its concentration and type [20].

In this work, we utilized concentrated soy lecithin dispersions and a chitosan biopolymer gel, in order to obtain wrapped vesicles. Our aim is to improve oral administration of drugs, by studies in long time circulation vesicles used as cream-gel, as well as, apply the versatility of chitosan as drug targeting agent, which allows the incorporation of different chemical/pharmaceutical agents into its core.

Soy lecithin has many important applications for the food industry as well as for the preparation of lipid vesicles [21,22]. The use of phospholipids obtained from natural sources, improves large-scale production, depicting in a costs reduction, when compared with synthetic phospholipids. In general, the build-up of lipid vesicles in water involves the processing of concentrated lecithin dispersions [23].

Nevertheless, soy lecithin dispersions have been interesting, not only from an application point of view, but as they are one of the major structures where amphiphiles can self-assemble. Also, the transition from a lamellar phase of the planar sheets into closed structures is a very important issue during vesicles preparation. This process starts from high concentrated phospholipid dispersions where bilayer sheets (or membranes) can be periodically stacked to form a lamellar phase [24]. This can be done by either swelling-light sonication-freezing-unfreezing procedure [23,25,26], as well as by slow swelling under shear.

The rheological properties of lecithin dispersions show different behaviours, ranging from Newtonian to non-Newtonian fluids [27] For instance, whereas dispersions obtained by simply swelling and sonication showed a Newtonian behaviour (from 60 to 120 g/L) or even a plastic one (from 180 to 240 g/L), those obtained by swelling-sonication followed by a freezing-unfreezing stage showed pseudoplastic and rheopectic behaviour [23]. Rheopectic behaviour is the opposite of tixotropy, which will be characterized by an increase on the viscosity against time and formation of a reverse hysteresis loop when up–down shear sweeps take place [25]. Moreover, previous papers [23,25] have clearly showed a significant influence of the nature, valence and concentration of different cations, as well as the aqueous pH, on the loop hysteresis area. The loop hysteresis area can be correlated to the energy involved on vesicle segregation process under shear, and this energy depends on the geometrical and electrical properties of the vesicles, as well as on the number of segregated vesicles on the shearing process. Previous results [26] confirmed the established hypothesis [23,25] about the rheopectic behaviour of concentrated soy lecithin dispersions exposed to a freezing-unfreezing procedure; where its rheopectic properties were due fundamentally, to a segregation, under shear, of the closed structures (multilamellar vesicles).

In this paper, the rheological and electrokinetic properties of these new mixed dispersions prepared with concentrated soy lecithin and chitosan biopolymer have been studied, emphasizing on the differences showed against the previously reported results for the same system without chitosan [23,25,26]. Moreover, a new and simple preparation method based on the slow swelling of lecithin under shear has been employed.

2. Experimental

2.1. Material and equipment

Soy lecithin (99% purity reagent) was purchased from Guinama S.L. (Valencia, Spain). Chitosan (deacetilation degree of 92% calculated by 1HRMN; mean molecular weight of 1.85×10^6 kDa calculated by specific viscosimetry) was purchased from Microsomas y Biopolímeros S.L. Spain (distributed by Guinama S.L., Spain). Sodium chloride and calcium chloride were purchased from Panreac Química S.A. (Barcelona, Spain); aluminium chloride and glacial acetic acid from Merk (Madrid, Spain). All analytical grades were 99% purity reagent. Water was purified by a Milli-Q-UF unit (Millipore, Bedfore).

A Bohlin Visco 88 viscosimeter with eight-speed settings in geometric progression from 20 to 1000 rpm and parallel plate PP3 0,1 mm was utilized for viscosity determinations. Several Cannon-Fenske viscosimeters of different capillary diameters were also used to measure the electrolyte solutions. The electrophoretic mobility measurements and size vesicle estimation were carried out with a Z-Meter, Zetasizer 2000, from Malvern Instruments. All the ζ-potential values were approximated by the Smoluchowski equation. Temperature of the samples was fixed at 25 ± 1 °C. A Kyowa Microlux-11 microscope with phase contrast and an electron microscope Philips XL 30 ESEM adapted with a microanalysis EDAX PV 9760 were used as tool to observe the morphology changes in the samples.

2.2. Preparation of concentrated lecithin solutions

Soy lecithin dispersions (250 g/L) were prepared by slow swelling under shear force in aqueous medium (10^{-5} M NaCl). Moreover, to investigate the influence of electrolytes, samples were prepared by dispersion of soy lecithin in aqueous solution of an electrolyte (Na^+, Ca^{++}, or Al^{+++}). From each electrolyte, seven different dispersions were prepared with decreasing ion concentration, ranging from 10^{-2} to 10^{-5} M.

Chitosan solutions (0.25, 0.50 and 1.00%, w/v) were prepared in acid acetic 1%. Lecithin–chitosan dispersions were prepared by mixing the previous soy lecithin dispersions with the chitosan biopolymer solutions. The 1.25 and 1.50% chitosan solutions were also studied.