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How hydrophobically modified chitosans are stabilized by biocompatible lipid aggregates

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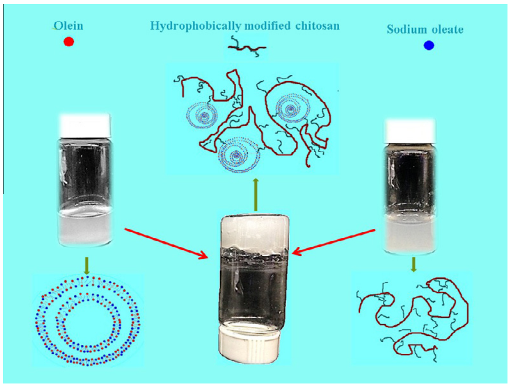
GRAPHICAL ABSTRACT



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ABSTRACT

Nanostructured hydrogels composed by biocompatible molecules are formulated and characterized. They are based on a polymer network formed by hydrophobically modified chitosans (HMCHIT or CnCHIT) in which vesicles of monoolein (MO) and oleic acid or sodium oleate (NaO), depending on *pH*, are embedded. The best conditions for gel formation, in terms of *pH*, length of the hydrophobic moieties of chitosan, and weight proportion among the three components were estimated by visual inspection of a large number of samples. Among all possible combinations, the system C12CHIT–MO–NaO in the weight proportion (1:1:1) is optimal for the formation of a well-structured gel-like system, which is also confirmed by rheological experiments. Electron paramagnetic resonance (EPR) measurements unambiguously show the presence of lipid bilayers in this mixture, indicating that MO–NaO vesicles are stabilized by C12CHIT even at acid *pH*.

A wide small angle neutron scattering investigation performed on several ternary systems of general formula CnCHIT–MO–NaO shows that the length of the hydrophobic tail *Cn* is a crucial parameter in stabilizing the polymer network in which lipid vesicles are embedded. Structural parameters for the vesicles are determined by using a multilamellar model that admits the possibility of displacement of the center of each shell. The number of shells tends to be reduced by increasing the polymer content. The thickness

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and the distance between consecutive lamellae are not influenced by either the polymer or MO–NaO concentration.

The hydrogel presented in this work, being fully biocompatible and nanostructured, is well-suited for possible application in drug delivery.

1. Introduction

In the past and also presently, mixtures of polymers and surfactants are of major interest to both technologists and scientists. The former are interested mainly due to the huge number of combinations that these chemicals form and that can be used in multicomponent formulations such as cosmetics, paints, food products, as well as drug delivery vehicles. The latter are interested in understanding the thermodynamic reasons of their stability or instability and in studying the microscopic structure of their aggregates [1–7]. In recent years, interest has been mainly focused on systems formed by biocompatible polymers and surfactants [8–11]. In this field, charged polysaccharides are of special interest due to their ready availability from renewable sources, their biodegradability, and the absence of toxicity [12,13]. Among these biopolymers, chitosan plays a leading role, not only because it can be easily and plentifully obtained from chitin deacetylation [14] but also for its antinflammatory and antimicotic properties that are of special interest in pharmaceutical applications [15]. Furthermore, chitosan, being a charged polymer, is able to interact with charged surfactant molecules [16,17]. Chitosan is a linear polysaccharide composed of randomly distributed D-glucosamine and N-acetyl-D-glucosamine units. It can be obtained from crustacean shells by deacetylation of chitin under alkaline conditions. This biopolymer can be easily modified [18,19], due to the presence of two types of reactive groups onto which they can be grafted: the free amine group on deacetylated units and the two hydroxyl groups on the gluco ring. The chemical modification of chitosan can be performed in many different ways, allowing this natural polymer to be the basis of applications in very different technological fields, such as drug delivery, tissue engineering, antimicrobial agents, metal ion absorption, dye removal, viscosity control, and coating processes [20–23]. In the present report, we are concerned with chemical modifications of chitosan. This has been obtained by grafting alkyl pendants of different lengths to the free amine group of the saccharide unit, thus preserving its high biocompatibility [20]. These chemical modifications have the aim of changing the hydrophilic–lipophilic balance of this bio-polymer, giving rise to the formation of hydrophobic pools that can interact with bio-compatible surfactant aggregates [17,24]. In this work, we investigate the interaction of these modified chitosans with anionic and non-ionic surfactants, and analyze the microstructure of these mixtures.

Concerning the surfactants, monoolein (MO) is non-toxic, biodegradable, and biocompatible. It has a polar head group and a non-polar hydrocarbon chain, clearly showing its amphiphilic properties. This allows monoolein molecules to self-assemble into different liquid crystalline structures, under varying conditions of temperature and solvent composition [25–27]. On the basis of these properties, monoolein-based nanostructures represent a suitable strategy to formulate useful drug delivery carriers, as well shown in literature [28–32].

Sodium oleate (NaO) is one of the main components (39% as oleic acid) of palm oil and is the principal unsatured fat present in this natural material. As in the case of MO, NaO aggregates in water giving rise to different structures depending on composition and temperature [33]. In summary, both amphiphilic compounds show the tendency to aggregate and are ecologically benign and mild. Taking into account that all compounds are biocompatible, largely available, and affordable in the fields of food preparation and drug delivery, we address the final goal of this paper, which is to determine the composition and ratios among components that give rise to the formation of nanostructured gel-like systems. In the context of their possible use as drug delivery systems, a quantitative analysis of the microstructural characteristics of the CnCHIT–MO–NaO system deals with the aim to obtain a deeper insight on the type of aggregates.

2. Experimental section

2.1. Materials

Chitosan (CHIT), classified by the supplier as medium molecular weight, batch 08028CD, was purchased from Aldrich. Its acetylation degree was determined by NMR with a Varian 300 spectrometer to be 5%, which is in agreement with the value reported by the manufacturer. Pentanal (97%), hexanal (98%), octanal (98%), decanal (99%), dodecanal (92%), sodium cyanoborohydride $NaCNBH_3$ ($\geqslant$95%) and glacial acetic acid ($\sim$99.8%), which were used for the alkylation procedure on the amine group, were all purchased from Aldrich and used as received. We synthesized HMCHIT's alkylating chitosan under mild conditions following a reductive amination procedure suggested by Yalpani [18], and successively modified by Rinaudo [34,35]. The substitution degree of these alkylated chitosans, from here on indicated as CnCHIT, is 10% as determined by nuclear magnetic resonance (NMR), while their molecular weight is $\sim$260 kg mol^{-1}. Monoolein (MO) and sodium oleate (NaO) were provided by Danisco and BioChemica (Fluka), respectively, and were used as received. For simplicity, from here on we will use the acronym NaO also in the case of acidic solutions of sodium oleate in which only oleic acid is present. The EPR probes, 5-doxylstearate (5-DSA) and 16-doxylstearate (16-DSA), were purchased from Sigma–Aldrich. All solutions were prepared by weight using water obtained by inverse osmosis from an Elix Millipore apparatus. Na_2HPO_4, citric acid, $NaHCO_3$, and Na_2CO_3 components were used to prepare buffer solutions with $3 \leqslant pH \leqslant 9$. For SANS measurements all solutions were prepared in heavy water (D_2O) and, if necessary, in deuterated acetic acid (AcD), purchased from Sigma–Aldrich.

2.2. Methods

Our aim is to study of the structures formed by CnCHIT, MO–NaO, and CnCHIT–MO–NaO aqueous systems. The experimental approach follows three lines: (i) visual inspection of binary and ternary mixtures combined with a rheological analysis on the most successful sample, (ii) analysis of the structure of the aggregates by electron paramagnetic resonance (EPR), and (iii) determination of the aggregates structural parameters by small angle neutron scattering (SANS).

2.2.1. Visual and rheological inspection

Several mixtures formed by CnCHIT and MO and NaO in different proportions and at different *pH* conditions, were prepared by weight, left to rest overnight, and then visually analyzed.

The rheological experiments were performed with a rheometer AR-G2, which is an advanced controlled stress instrument. The