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Gel-embedded niosomes: Preparation, characterization and release studies of a new system for topical drug delivery

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ABSTRACT

In the present paper physical gels, prepared with two polysaccharides, Xanthan and Locust Bean Gum, and loaded with non-ionic surfactant vesicles, are described. The vesicles, composed by Tween20 and cholesterol or by Tween85 and Span20, were loaded with Monoammonium glycyrrhizinate for release experiments. Size and zeta (ζ)-potential of the vesicles were evaluated and the new systems were characterized by rheological and dynamo-mechanical measurements. For an appropriate comparison, a Carbopol gel and a commercial gel for topical applications were also tested. The new formulations showed mechanical properties comparable with those of the commercial product indicating their suitability for topical applications.

In vitro release experiments showed that the polysaccharide network protects the integrity of the vesicles and leads to their slow release without disruption of the aggregated structures. Furthermore, being the vesicles composed of molecules possessing enhancing properties, the permeation of the loaded drugs topically delivered can be improved. Thus, the new systems combine the advantages of matrices for a modified release (polymeric component) and those of an easier permeability across the skin (vesicle components).

Finally, shelf live experiments indicated that the tested gel/vesicle formulations were stable over 1 year with no need of preservatives.

1. Introduction

The major disadvantage of transdermal and dermal drug delivery is the poor penetration of most compounds across human skin. The main barrier of the skin is located within its uppermost layer, the stratum corneum, and several studies have been developed to weaken such skin barrier. One of the possible approaches to achieve increased skin penetration of drugs and/or cosmetic chemicals is based on the use of vesicular systems, such as liposomes and niosomes.

In the present investigation, a binary mixture of polysaccharides was used for the gel formation, while non-ionic surfactant vesicles of different compositions were selected for the preparation of the vesicular systems.

The proposed formulation of gel-embedded vesicles not only aims to improve the penetration and dermal availability of the loaded drug, but also to obtain a more effective formulation with a better patient acceptability.

Actually, some binary mixtures of polysaccharides exhibit synergistic interactions, e.g., the mixture may form gels under conditions where the individual components are non-gelling. In particular, Xanthan and Locust Bean Gum, when mixed together, give a network whose strength depends on the temperature preparation and on the weight ratio between the two components [1]. It is known that the branching degree of the galactomannan are crucial for gel formation (an increase of the number of galactose units hinders gel formation [2]), but the actual mechanism leading to the gel formation is not yet fully elucidated. It is possible to modulate the mechanical properties of the mixed gel by varying the relative amount of the two polymers and taking into account the different conformations assumed by the Xanthan chains in

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distilled water (room temperature: double-helix ordered structure; $T > 45\,^{\circ}C$: random coil conformation) [3,4].

It is known that the strongest gels are obtained when the polymers are mixed at the same concentration and at high temperatures ($T > 45\,^{\circ}C$), i.e., when both polysaccharides assume a disordered conformation [5,6].

The choice of this binary mixture [7] is supported by the fact that these polymers are already widely used in food industry [8–12].

Thus, the biocompatibility of the two polysaccharides, together with their peculiar synergic behaviour, can still offer new interesting applications, especially for topical drug delivery.

One of the approaches to achieve increased skin penetration of drugs and cosmetic chemicals is the use of vesicular systems, such as liposomes and niosomes. These carriers can act as drug reservoirs showing several advantages over conventional dosage forms [13–18].

Niosomes are self assembled vesicular nanocarriers obtained by hydration of synthetic surfactants and appropriate amounts of cholesterol or other amphiphilic molecules. Just like liposomes, niosomes can be unilamellar or multilamellar, are suitable as carriers of both hydrophilic and lipophilic substances and are able to deliver drugs to the target site. Furthermore, niosomal vesicles, that are usually non-toxic, require less production costs and are stable over a longer period of time in different conditions, thus overcoming some drawbacks of liposomes [19].

In the present study a physical gel, prepared with Xanthan and Locust Bean Gum, was loaded with non-ionic surfactant vesicles to evaluate the potential advantages in combining the properties of different delivery systems for drug release modulation. Since research and development for improved drug delivery systems is at the very core of many of the pharmaceutical investigation activities, a better characterization of such systems together with the understanding of the involved delivery mechanisms becomes a fundamental goal. Monoammonium glycyrrhizinate (AG), a natural compound obtained from *Glycyrrhiza glabra*, showing anti-inflammatory activity *in vitro* and *in vivo*, was chosen as a model drug. The vesicles were composed by cholesterol and Tween20 (in equimolar ratio) or by Tween85 and Span20 (in equimolar ratio and without cholesterol). The new gel-embedded vesicle systems were characterized by rheological and dynamo-mechanical measurements. For an appropriate comparison also a Carbopol gel and a commercial gel for topical applications were tested. When needed, the niosomes were loaded with AG and release experiments of the guest molecules from the embedded niosomes were carried out.

The new formulations showed mechanical properties comparable with those of the commercial product, thus indicating their suitability for topical applications. *In vitro* release experiments showed the positive effect of the polysaccharide network that protects the integrity of the vesicles and at the same time leads to their slow release without disruption of the aggregated structures. Furthermore, the surfactant components of the vesicles are well known to act as enhancers in drug permeation process and therefore may improve this critical step in the drug delivery fate. Thus, the proposed new systems combine the advantages of matrices for a modified release (polymeric component) and those of an easier permeability across the skin (vesicle components).

Finally, the systems were periodically monitored, by recording the rheological properties in order to test the mechanical integrity of the samples.

The shelf live experiments indicated that the tested gel/vesicle formulations were stable over 1 year with no need of preservatives.

2. Materials and Methods

2.1. Materials

Locust Bean Gum (LBG), from *Ceratonia Campestris*, was provided by CarboMer (San Diego, USA). The ratio between mannose (M) and galactose (G) was ≈3.4, as estimated by means of ^{1}H NMR (carried out at 70 °C with a Brucker AVANCE AQS 600 spectrometer, operating at 600.13 MHz). The average molecular weight (M_w) of LBG, 5.0×10^5, was estimated by means of static light scattering measurements [7]. Xanthan, from *Xanthomonas campestris*, was provided by Fluka (Milan, Italy). For each repeating unit, 1.6 acetate and 2.7 pyruvate groups were estimated by ^{1}H NMR measurements (carried out at 85 °C with a Brucker AVANCE AQS 600 spectrometer, operating at 600.13 MHz). The average molecular weight of Xanthan ($M_w = 1.25 \times 10^6$), was obtained by means of static light scattering measurements [7].

The two polysaccharides were used after purification. A given amount of Xanthan, sodium salt (polymer concentration, cp = 0.5%, w/v), was dissolved in distilled water, under magnetic and mechanical stirring, at room temperature for 48 h, while LBG (cp = 0.5%, w/v) was dispersed in distilled water, under magnetic and mechanical stirring, at 80 °C for 24 h and at room temperature for 24 additional hours [20]. The solutions were then exhaustively dialyzed at 4 °C against distilled water using dialysis membranes with a cut-off 12,000–14,000 until the water dialysis conductivity reached the value of ~1 μS at 25 °C. In order to convert Xanthan to the sodium form, NaOH 0.2 N was added to the dialyzed solution up to pH = 7.0. Finally, the samples were freeze-dried and stored in a desiccator until use. For the vesicle preparations Tween20, Tween85, Span20, SephadexG-75 and HEPES salt {N-(2-hydroxyethyl) piperazine-N-(2-ethanesulfonicacid)}were purchased from Sigma–Aldrich (Germany) while cholesterol was a Carlo Erba (Italy) product. Ammonium glycyrrhizate (AG), from *G. glabra*, was purchased by Beckett Corp. (USA). Carbopol 940 (hereafter named Cbp940) (a 0.5% polymer solution shows a viscosity of 50,200 mPa × s and a pH = 3.2 according to the manufacturer) was produced by A.C.E.F. s.p.a. (Fiorenzuola D'Arda, Piacenza, Italy). A commercial topical Gel (hereafter named CG), containing 2.5% ketoprofene and Carbomer 940 (another trade name for Carbopol 940) as main excipient, was purchased in a pharmacy.

All other products and reagents were of analytical grade.

2.2. Vesicles preparation

Two types of surfactant vesicles were prepared using equimolar amounts (15 mM) of Tween20 and cholesterol (NioT) or equimolar amounts (15 mM) of Tween85 and Span20 (without cholesterol) (NioTS). The concentrations of Tween20, Tween85 and Span 20 were always remarkably above their critical micellar concentration (CMC) (Tween20: HLB = 16.7, CMC = 0.06 g/L in water and at 20 °C; Span20: HLB = 8.6, insoluble in water; Tween85: HLB = 11, CMC = 0.023 g/L in water and at 20 °C). Unilamellar non-phospholipid vesicles were obtained by means of the "film" method, after sonication at 60 °C by means of a temperature-controlled bath. The dried films were hydrated by addition of HEPES buffer (10 mM, pH 7.4). The dispersion was vortexed for about 20 min and then sonicated for 5 min at 60 °C with a Vibracells VCX400 (Sonics) instrument, equipped with an exponential microprobe operating at 23 kHz and with an amplitude of 6 mm [21]. When needed, the obtained films were preliminarily hydrated with an AG solution (1% w/v); the multilamellar vesicular suspensions were then vortexed and finally sonicated in the conditions above reported in order to obtain unilamellar niosomes loaded with AG (NioTAG and NioTSAG).