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Research paper

Tablets of pre-liposomes govern *in situ* formation of liposomes: Concept and potential of the novel drug delivery system

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ABSTRACT

The purpose of this study was to develop a novel drug delivery system for challenging drugs with potential for scale-up manufacturing and controlled release of incorporated drug. Pre-liposomes powder containing metronidazole, lecithin and mannitol, prepared by spray-drying, was mixed with different tableting excipients (microcrystalline cellulose, lactose monohydrate, mannitol, dibasic calcium phosphate, pregelatinized starch, pectin or chitosan) and compressed into tablets. The delivery system was characterized with respect to (i) dry powder characteristics, (ii) mechanical tablet properties and drug release, and (iii) liposomal characteristics. The pre-liposomes powder was free-flowing, and tablets of similarly high qualities as tablets made of physical mixtures were prepared with all excipients. Liposomes were formed *in situ* upon tablet disintegration, dissolution or erosion depending on the type of tablet excipient used. The liposomal characteristics and drug release were found to depend on the tablet excipient. The new delivery system offers a unique synergy between the ability of liposomes to encapsulate and protect drugs and increased stability provided by compressed formulations. It can be adjusted for drug administration via various routes, e.g. oral, buccal and vaginal.

1. Introduction

In spite of an excessive number of potential drug candidates, the major drawback of many of those molecules remains to be their poor water solubility resulting in poor bioavailability. Several promising formulation strategies have been proposed and developed to improve the delivery of poorly-soluble substances. Most of such formulations can be categorized into those including crystalline solid formulations, amorphous formulations and lipid formulations [1].

Among the lipid formulations, we are particularly interested in liposome-based formulations. The ability of liposomes to solubilize poorly-soluble drugs in the lipid bilayer and incorporate water-soluble drug in the aqueous core is well-established [2]. Due to their biphasic characteristic and diversity in design, composition and construction, liposomes offer a dynamic and adaptable technology for enhancing drug solubility as well as offering protection to drugs that are easily degraded [1,2]. However, aqueous dispersions of liposomes may be subjected to a variety of stability problems associated with aggregation, fusion and phospholipid hydrolysis, which may limit their shelf life. The drying of liposomal dispersions by freeze-drying or spray-drying is an important step resulting in more stable dry powder formulations that can be rehydrated in contact with water with maintaining their structure and functionality [3–5]. Spray-drying is a well-established technique that enables the design of particles with the desired physicochemical properties, e.g. particles size, density, shape and solid form [6]. Additionally, spray-drying has been demonstrated to be a suitable method for preparation of proliposomes [7], where liposomes are formed upon hydration of the dry powder. Preparation of proliposomes by spray-drying should be distinguished from the spray-drying of preformed liposomes, even though both products will form liposomes in contact with water. The drying, as well as hydration, of preformed liposomes is critical steps due to the risk of fusion and/or aggregation and the disruption of the bilayer structure of the membrane [4,8]. The stress that the preformed liposomes are subjected to during the spray-drying can be circumvented by spray-drying the liposomal components, or liposome precursors, since the liposome formation will only take place

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Nomenclature

List of abbreviations

DD	degree of deacetylation
DLS	dynamic light scattering
DM	degree of methoxylation
DSC	differential scanning calorimetry
HR	Hausner ratio
HPLC	high performance liquid chromatography
MWCO	molecular weight cut-off
PI	polydispersity index
PreLipo	pre-liposomes
PreLipo powder	spray-dried pre-liposomes powder
PreLipo tablets	tablets containing spray-dried pre-liposomes powder
SEM	scanning electron microscopy
UV–VIS	ultraviolet and visible light
VFS	vaginal fluid simulant

Annotations

d_h	hydrodynamic diameter
ζ	zeta potential

upon hydration of the dry product. For a precise terminology, and in order to distinguish these types of systems from proliposomes prepared by other methods [9], it is suggested that these systems should be referred to as pre-liposomes.

Up to now, only a few studies have successfully demonstrated formulation of lipid-based nano-/micro-particulate delivery systems that allow compression into tablets [10–14]. These systems were either formulated as emulsions stabilized by polymer [10], sugar [11] and/or porous silica particles [12] and subjected to spray-drying, or the lipids were simply loaded onto porous silica particles by adsorption [13,14]. However, only the silica-based systems resulted in tablets of high mechanical quality.

We propose a novel approach for manufacturing of a stable, spray-dried pre-liposomes (PreLipo) powder that compressed into tablets (PreLipo tablets), combines the solubilizing and protection properties of liposomes with the stability and ease of administration of a solid tablet formulation, as a new delivery system for challenging drugs. The originality of this approach is related to the formation of liposomes *in situ* during disintegration, dissolution or erosion of the tablet. The release behavior of associated drug can be tailored by the optimization of the liposomal composition as well as appropriate selection of the tablet matrix. The new delivery system has high potential for industrial manufacturing since both spray-drying and tableting are already well-established industrial scale manufacturing processes.

The aim of the current paper is to describe and confirm the concept and elucidate the potential of PreLipo tablets as a novel drug delivery system. Metronidazole was chosen as a model drug to illustrate that the concept is applicable for drugs destined to be administered by several routes, e.g. oral, buccal and vaginal, not because of its solubility. Metronidazole is classified as BCS class I drug and is slightly to sparingly soluble in both hydrophilic and lipophilic media (aqueous solubility 10 mg/ml at 20 °C; log$P$ −0.1 in octanol/buffer pH 7.4) [15]. It is a known drug that is still the drug of choice for treatment of several bacterial infections and protozoa [16]. It is associated with numerous side effects when delivered systemically, therefore we were aiming at local therapy, e.g. local treatment of periodontal diseases [17,18], local treatment of bacterial vaginosis [19,20] or local treatment of *Helicobacter pylori* associated peptic ulcer disease [21]. Liposomes have been shown to improve the delivery if antimicrobial drugs to bacteria by allowing better contact with the bacterial membrane [22,23]. Another reason for encapsulating metronidazole in liposomes is to retain the drug at the site of action (class I drugs are highly permeable), avoiding permeation to circulation and consequently reducing systemic side effects and possible toxicity. By using liposomes the localized activity enhanced even at lower doses as compared to the free drug provided by commercially available dosage forms (e.g. immediately release tablets, cream and gel). Soybean lecithin was chosen as the lipid, since it is known that it produces liposomes that are suitable for improved local drug delivery, it is cheap compared to hydrogenated and synthetic lipids, and thus well suited for industrial production. It has been proven suitable for spray-drying [7] and was therefore highly interesting for this concept. To check whether the PreLipo formulations are pressure-sensitive or otherwise challenging in the tablet compression, both conventional tablet excipients and excipient used in "soft tableting" [24] were used as tablet fillers. In respect to buccal and vaginal route of administration, the bioadhesion of the system would be the most advantageous; therefore, mucoadhesive polymers were also studied as possible tablet fillers.

2. Materials and methods

2.1. Materials

Metronidazole was purchased from Fluka Analytical (Steinheim, Switzerland) and D(−) mannitol from Kemika (Zagreb, Croatia). Lecithin (Lipoid S 75; soybean phosphatidylcholine 68–73%, phosphatidylethanolamine 7–10%, lysophosphatidylcholine <3%) was kindly provided by Lipoid (Ludwigshafen, Germany), microcrystalline cellulose (Avicel® PH102) was from FMC biopolymer (Leeds, England), partially pregelatinized maize starch (Starch 1500®) from Colorcon (Dartford, England) and the direct compressible mannitol (Pearlitol® 100SD) from Roquette (Basel, Switzerland). The pectins of different degrees of methoxylation (DM; 5%, 10% and 25%) were donated by Herbstreith & Fox GmbH (Neuenbürg, Germany). Chitosans of different degree of deacetylation (DD; 77%, 82% and 92%) were obtained from Sigma Aldrich (Milwaukee, USA). All other chemicals and solvents used in experiments were of analytical grade.

2.2. Test media

Na_2HPO_4–NaH_2PO_4 buffer solution, in the following referred to as phosphate buffer pH 7.0, was made from 305 ml 0.2 M Na_2HPO_4 and 195 ml 0.2 M NaH_2PO_4 diluted to 1000 ml with distilled water. The pH was adjusted to 7.0 with either HCl or NaOH.

Vaginal fluid simulant (VFS) was prepared by modification of the composition originally reported by Owen and Katz [25]. It contained 3.51 g/l NaCl, 1.40 g/l KOH, 0.222 g/l $Ca(OH)_2$, 2 g/l lactic acid, 1 g/l acetic acid, 0.16 g/l glycerol, 0.4 g/l urea and 5 g/l glucose. The pH of the mixture was adjusted to 4.5 with either HCl or NaOH.

2.3. Methods

2.3.1. Preparation of spray-dried pre-liposomes

Dry PreLipo powder was prepared by the modified spray-drying procedure previously described by Škalko-Basnet et al. [7]. Briefly, empty and metronidazole-containing PreLipo powder was prepared by mixing the ethanolic solution of lecithin and aqueous solution of