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Original Article

Preparation of Stable Plurilamellar Liposomes Dispersed in Two Soluble Types of Collagens and the Effect of Collagens on the Release Rate of Entrapped Sodium Chromate

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Abstract

Combination formulations of liposomes with collagen have been previously introduced as a means of obtaining more stable and less permeable liposomes. In this study, the effect of aqueous solutions of the collagen products COLLAPUR® (COL) and COLLAPURON-DAK® (COLD) from Henkle Co., on the release rate of sodium chromate (CHR) as a water-soluble model drug from stable plurilamellar vesicles (SPLVs) was evaluated. Results showed that dispersing SPLVs in diluted solutions (10%) of the collagens, increased the release rate from liposomes at 32°C. It is speculated that after binding to the hydrophilic surface of liposomes, the structure of the collagen changes and the hydrophobic portions become more exposed. This is likely to cause a penetration of these portions into the bilayer structure (which is fluid at this temperature), thus causing an expansion in the membrane and an increase in permeability. In higher concentrations (30% and 50%), this increasing effect is not observed, which is suggested to be due to the aggregation of collagen fibrils and the resultant higher viscosity. It is concluded that these collagens in optimum concentrations could find a good place in the preparation of topical liposomes, due to their flexible effects on the release rate of liposomes, as well as the dermatological effects of collagen itself.

Keywords: Liposome; SPLV; Encapsulation efficiency; Release rate; Collagen.

Introduction

Liposomes (phospholipids bilayer vesicles) have attracted increasing attention as a novel drug delivery system for controlled and/or targeted release of drugs. They have been used for delivery of various low and high molecular weight drugs, proteins, and genes, and their targeting to cellular and sub-cellular targets (1-3). They have also found a promising place in topical applications with some advantages such as slow release of the active materials as well as localizing the release and effect of the entrapped substance in the skin (4). Investigation of the in-vitro release rate form liposomes is a prerequisite for understanding their in-vivo behavior. This rate depends on the permeability of liposomal membranes, which in turn is affected by temperature, encapsulated substance, the environment of liposomes, and composition of membrane. An approach to the use of liposomes as a sustained release drug delivery system should focus on stabilizing the liposomal membrane, thus decreasing the release rate.

Collagen has various applications as a biomaterial and is widely used as a carrier for delivery of drugs, proteins and genes (5). Among its various applications are sponges for burns/wounds, collagen shields in ophthalmology (5), and also combination formulations with liposomes such as collagen corneal shields impregnated with liposomes (5, 6) and collagen sponge with liposomal

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polymyxin B for wounds (7). In these combination systems, collagen is suggested to make liposomes more stable and less permeable, thus providing a sustained-release liposomal delivery system (5, 6). Various studies have been performed on the effects of collagens on the release rate of encapsulated materials from liposomes, and have suggested the use of gel matrices or solutions of collagen for the stabilization of liposomal formulations and decreasing their permeability (8-10). On the contrary, some studies have reported that hydrophobic collagens at low concentrations could increase the permeability (11).

In this study, the effect of aqueous solutions of collagens, COLLAPUR® (COL) and COLLAPURON-DAK® (COL-D), on the release rate of sodium chromate (CHR) as a water-soluble substance from stable plurilamellar vesicles (SPLVs) was evaluated.

Experimental

Materials

Triton X-100 and chromate (CHR) were from Merck Chemical Company (Darmstadt, Germany), and dialysis bag (D 9777) was from Sigma Chemical Company (St. Louis, MO, U.S.A.). Ovotin® 160 with egg phosphatidylcholine of approximately 60% (O160) was a gift from Faratin Co. Ltd. (Lucas Meyer representative, Tehran, Iran). Collagens, i.e. Collapur® (COL) and Collapuron-Dak® (COL-D) were gifts from Iran Henkle Co. Ltd. (Henkle representative, Tehran, Iran).

Methods

Preparation of collagen solutions

A solution of 10% (v/v) Collapuron-Dak® (COL-D) in distilled water (DW) with a pH of 5.5 was prepared. Three solutions of 10%, 30% and 50% (v/v) of Collapur® (COL) in distilled water (DW) were also prepared.

Preparation of sodium chromate-containing SPLVs

Two liposomal series (A and B) were prepared by a modified reverse-phase evaporation method. In each sample, 46 mg (66 μmole) of O160 was dissolved in 4 ml of a 1:1 v/v mixture of diethyl ether and chloroform (12). Then, 0.5 ml of sodium chromate solution with a concentration of 0.5 M (for A series) and 1.0 M (for B series) in DW was added to the samples. The resulting two-phase systems were purged with nitrogen and sonicated in a bath type sonicator at 2-4°C to produce stable emulsions, which were then rotary evaporated at 30-32°C, under nitrogen at a speed of 200 rpm. Final liposomal dispersions were obtained by breaking the resulting viscous gels through addition of 4 ml of DW. The final volume was adjusted to 5.0 ml by the addition of DW.

Separation of liposomes from unentrapped sodium chromate

One ml of each of the liposomal preparations were placed inside a closed dialysis bag, and dialyzed against six 50-ml portions of DW for 10 minutes. The resulting liposoma preparations, which were free of unentrapped CHR, were used for the following experiments.

Determination of Encapsulation Efficiency (EF) and Captured Volume (CV) of the Liposomes

One ml of each of the dialyzed liposomal preparations obtained from the previous stage was transferred into a vessel, and the dialysis bags were washed with DW in order to obtain the remaining amounts of the samples. Then, 8 ml of a Triton X-100 solution (10% v/v in DW) was added to the samples while shaking at 35-37°C. After disruption of the liposomes, the entrapped CHR was measured spectrophotometrically at 380 nm. By comparing the concentration of CHR in the resulting solution with that in the aqueous solution, used for the preparation of liposomes, the encapsulation efficiency and captured volume were calculated as percentage and L/mole of lipid, respectively.

Suspending the liposomes in collagen solutions

(I) One ml of each of the dialyzed series A liposomes was added to and mixed with 3 ml of DW (A-1), a 10% (v/v) COL solution in DW (A-2), and a 10% (v/v) COL-D solution in DW (A-3). Samples were then refrigerated under nitrogen for 24 h.

(II) One ml of each of the dialyzed seriesB liposomes was added to and mixed with 3 ml of DW (B-1), a 10% (v/v) COL solution in DW (B-2), a 30% (v/v) COL solution in DW (B-3),

and a 50% (v/v) COL solution in DW (B-4). Samples were then refrigerated under nitrogen for 24 h.

Evaluation of the release pattern

Franz diffusion cells containing the dialysis membrane as the semi-permeable membrane was used for studying the release pattern from liposomal samples. Experiments were performed at 32°C with 4 ml of dialyzed samples as donor phase, and 50 ml of DW as acceptor phase. At various time intervals of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes, 4 ml samples of the acceptor phase were taken and the CHR contents determined as mentioned above.

Results and Discussion

As shown in Table 1, the encapsulation efficiency and captured volume for series A liposomes were 28.78±2.29(%) and 21.81±1.74 (l/mole lipid) respectively. For series B liposomes, these values were 33.03±1.75 (%) and 30.03±1.60 (1/ mole lipid) respectively (Table 1). The results were compared to each other and to our previous results for urea-containing SPLVs, by using ANOVA statistical test followed by Scheffe Post-Hoc test. In accordance with previous observations of a decrease in encapsulation parameters on increasing the ionic strength (13), it was observed that the values were significantly ($p<0.001$) lower than our previously reported values for urea-containing SPLVs with the same membrane composition. In that study the encapsulation efficiencies and captured volumes were 51.12±1.13 (%) and 38.63±0.85 (l/mole lipid), respectively (12). This could be due to the nature of the two different entrapped substances, and mostly because of the higher ionic strength of CHR solutions, both in 0.5 M and 1.0 M concentrations. Results obtained from the A and B series of liposomes prepared in present study were not significantly different ($p>0.05$).

The high affinity of collagen to lipids, especially phospholipids, has previously been reported (14, 15). The collagens used in these studies were type I collagen, which are insoluble in water and soluble in diluted acid solutions. It has been suggested that in neutral conditions, collagen fibrils interact with liposome membranes making them more rigid, which in turn decreases the permeability (15). In another study, by using type I collagen a decrease in liposome permeability at 8°C and 20°C was reported, while at 37°C there was no significant difference in permeability compared to liposomes without collagen (9).

It has previously been reported that type I collagen (insoluble form) interacts with liposomes, stabilizes liposomal membrane and reduces the release rate (6), although this decrease in permeability is not as prominent for neutral liposomes as for negative liposomes (16). A recent study has shown that liposomal membranes coated with collagen, while having a decreased permeability, showed no obvious change in fluidity; thus concluding that collagen self-association behavior is closely correlated to its decreasing effect in liposome permeability (10). More hydrophobic derivatives (lauryl- and hexyl- collagens) have been reported to increase the permeability of liposomes to water-soluble entrapped materials, due to surface activity and interaction with phospholipids (11).

In this study, the collagens used were soluble, since their origin was from young animal tissues. Low concentrations of COL and COL-D (i.e. 10% (v/v) in DW), which were used to counteract the effect of high viscosity, increased the release rate from liposomes at 32°C. This increase in release rate in comparison with the plain SPLVs, is shown in Figure 1. Since in these collagens the polar regions are more exposed, they bind to the surface of phospholipid vesicles. It is speculated that as a result the structure changes and hydrophobic portions become more exposed. These portions are likely to penetrate to some extent into the bilayer structure (Which is more fluid at 32°C), causing an expansion in the membrane. This could in turn cause an increase in permeability of the membrane.

Table 1. Encapsulation efficiencies (EF) and captured volumes (CV) of CHR-containging SPLVs (n=3).

SAMPLES	EF (%)	CV(L/mole lipid)
A	28.78± 2.29	21.81 ± 1.74
B	33.03± 1.75*	30.03 ± 1.60*

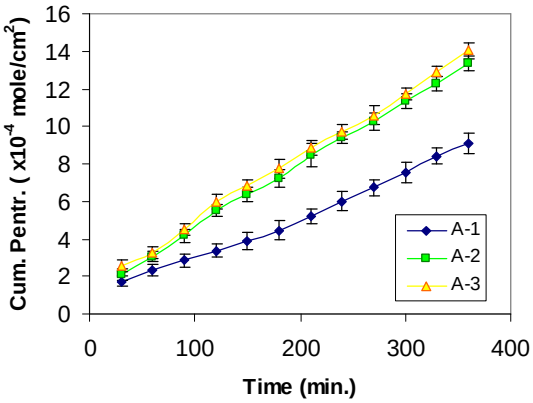

Figure 1: CHR release rate from SPLVs dispersed in DW, COL, and COL-D. Data are mean ± SD of 3 different samples. Cumulative penetration is calculated as the amount of CHR (per mole) in 1 Liter of the samples penetrated through 1 cm^2 of the semi-permeable membrane to the donor phase of Franz diffusion cell.

A-1: "A" liposomal samples dispersed in DW

A-2: "A" liposomal samples dispersed in a 10% solution of COL in DW

A-3: "A" liposomal samples dispersed in a 10% solution of COL-D in DW

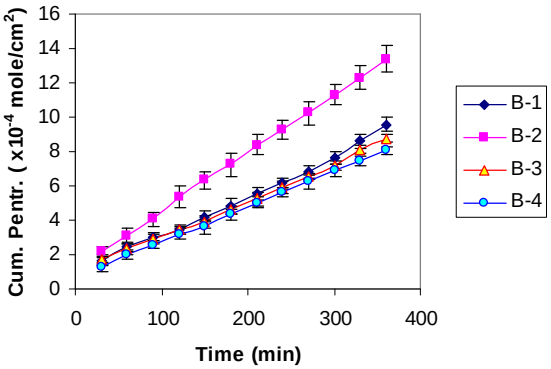

Figure 2: CHR release rate from SPLVs dispersed in DW, and COL solutions of different concentrations. Data are mean±SD of 3 different samples. Cumulative penetration is calculated as the amount of CHR (per mole) in 1 Liter of the samples penetrated through 1 cm^2 of the semi-permeable membrane to the donor phase of Franz diffusion cell.

B-1: "B" liposomal samples dispersed in DW

B-2: "B" liposomal samples dispersed in a 10% (v/v) solution of COL in DW

B-3: "B" liposomal samples dispersed in a 30% (v/v) solution of COL in DW

B-4: "B" liposomal samples dispersed in a 50% (v/v) solution of COL in DW

In higher concentrations of COLLAPUR, i.e. 30% (v/v) and 50% (v/v) in DW, this increasing effect is not observed. It is speculated that the aggregation of collagen fibrils and the resulting higher viscosity of the environment, compensate the increasing effect (11). Therefore, the release rate of CHR from these formulations is slow.

The release rate of CHR from liposomes did not depend on its primary concentration used to produce liposomes, and the release rates in series A and B liposomes were similar (Figures 1 and 2). Also as shown in Figures 1 and 2, the increases in release due to the addition of collagen were similar in series A and B liposomes. This confirms that the passage of drug through liposomal membrane is the rate-limiting step for the process of release. In fact a previous study has also suggested that the rate-limiting step for release from spherical particles less than 1 mm in diameter is the interfacial transfer (17).

It is concluded that the use of soluble types of collagen, Collapur® (COL) and Collapuron-Dak® (COL-D), in low amounts in liposomal formulations could increase the drug release rate. This effect is counterfeited in higher amounts, due to the high viscosity and gel formation of the environment surrounding liposomes. It is suggested that liposomes containing the proper type and amount of collagen, would find a good place in topical application due to the flexible effects of collagen on the drug release rate, as well as the dermatological effects of collagen itself.

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