Introduction

In recent years liposomes (phospholipid bilayer vesicles) have attracted increasing attention as a novel drug delivery system for a variety of drugs such as anticancer, antifungal, anaesthetic and antibiotic agents (1-3). With regard to the topical application, liposomes may serve as rate-limiting membrane barriers for modulation of systemic absorption, penetration enhancers or microreservoirs for sustained and controlled release of encapsulated drug (4-8). Topical liposome formulations (as dispersions or hydrogels) could be more effective and less toxic than the conventional topical formulations (9, 10). This potential advantage is especially of great interest in topical application of highly toxic drugs, such as antineoplastics.

5-fluorouracil (5-FU) is one of the most effective antineoplastic agent used for treatment of variety of tumours in many organs. Also, it is used topically in the treatment of different skin carcinomas (11, 12). Having in mind that liposomes as drug carriers may provide controlled drug release, site-directed and site-avoiding delivery (13), the encapsulation of 5-FU in topical liposome formulation is a reasonable approach for improvement of drug effectiveness and reduction of the side effects.

Considering the above-mentioned, the aim of our study was to prepare and evaluate liposome formulations with 5-fluorouracil intended for topical application. The effect of the hydration conditions on the physical and biopharmaceutical properties of the prepared formulations has also been considered. In order to determine physical properties of the prepared liposomes as a function of different hydration conditions, microscopic study, particle size analysis and determination of the encapsulation efficiency were carried out. Also, drug release studies from liposome dispersions and corresponding liposome gel formulations were performed to follow the effect of hydration conditions and the influence of gel matrix over the release profile of 5-fluorouracil.
Materials and methods

Materials

For the preparation of liposomes and topical gel formulations, the following materials were used: Phospholipon 90H - gel state (PL 90H, Natterman Phospholipid, Germany), cholesterol (CHOL, Galenika, Yugoslavia), 5-fluorouracil (5-FU, Ebewe Arzneimittel, Austria), chitosan (Katakura Chikkarin, Japan) and saccharose (Merk, Germany). All other used chemicals were of analytical grade.

Preparation and characterization of liposomes

Liposomes containing 5-FU were prepared by the modified lipid film hydration method (14). The lipid components, PL 90H and CHOL, were dissolved in chloroform and the organic solvent was removed by evaporation under vacuum (65 °C). Afterwards, the dried lipid film was hydrated with different quantities of aqueous phase bearing total drug quantity (5-FU in phosphate buffer pH 7.4), samples LD1, LD2 and LD3, respectively (Table 1).

After 24 h, in order to remove an unentrapped portion of the drug substance, liposomes were washed with phosphate buffer pH 7.4 and ultracentrifuged (20000 rpm, 45 min., 3 times; Ultracentrifuge MLW K24D, Yugoslavia). Liposomes were lyophilized (temperature -40 °C, pressure 200 Pa; Crist alpha 2-4, Bioblock, Scientific, France) using saccharose as a cryoprotector, incorporated on both sides of the phospholipid lamellae (lipid phase:saccharose = 1:1.25).

Liposome gel formulations (LG1, LG2, LG3) intended for topical application, were prepared by incorporation of lyophilized liposomes (series LD1, LD2 and LD3) in structured vehicle of chitosan (1% m/m) in ratio 1:3.

Table 1. Formulations of liposomes

| Samples | LD1 | LD2 | LD3 |
|---------|-----|-----|-----|
| Lipid phase composition | PL 90H : CHOL | 12:1 | 12:1 | 12:1 |
| Hydration conditions | Mass ratio lipid/aqueous phase PL 90H-CHOL/phosphate buffer pH 7.4 | 1:31.3 | 1:18.8 | 1:12.5 |
| | Mass ratio drug/aqueous phase | 1:100 | 1:60 | 1:40 |
| | 1 % Chitosan gel base | LG1 | LG2 | LG3 |

The encapsulation efficiency of 5-FU in liposomes was quantified UV spectrophotometrically (266 nm; Perkin Elmer, Lambda 16, USA) after dissolving of liposomes in chloroform:methanol mixture.

In vitro dissolution studies

Release of the drug substance from freshly prepared liposome dispersions and liposome gel formulations was followed in vitro using dialysis through hydrophilic membrane of regenerated cellulose against phosphate buffer pH 7.4 at 37 °C. Quantity of the released 5-FU in dialyzing medium, within a period of 8 h, was analysed spectrophotometrically. All experiments were carried out in triplicate and average values are presented. To deduce the mechanism of the drug release from the prepared formulations, the release data were mathematically processed.
Results and discussion

Characterization of liposomes

Microscopic observations confirmed formation of spherical vesicles with average size of 5 µm. The size-frequency distribution curves and cumulative distribution plots for the prepared formulations are presented in Fig. 1. By varying the drug/aqueous phase mass ratio during the preparation of liposomes, different efficiency of 5-FU encapsulation has been achieved. Lower concentration of the drug substance in the hydration medium resulted in lower incorporation efficiency. At a concentration of 10 mg/ml, 16.66 mg/ml and 25 mg/ml of 5-FU in hydration medium (samples LD1, LD2, LD3), the efficiency of 5-FU encapsulation was 5.2, 9.4 and 15.4 %, respectively (Fig. 2). The encapsulation of hydrophilic drug substance into liposomes bears relationship to the overall volume of aqueous phase that is encapsulated during liposome formation (15). Having in mind that the prepared vesicles are of similar size (mean geometric diameters were 5.04 (± 2.01), 4.53 (± 2.02) and 4.75 µm (± 1.89) for samples LD1, LD2 and LD3 respectively), higher encapsulation efficiency of 5-FU into liposomes could be related to the higher drug concentration in the hydration medium.

Drug release from liposome dispersions and liposome gel formulations

Drug releases from liposome dispersions are presented in Fig. 3a. As it can be seen, by increasing the drug/aqueous phase mass ratio (from 1:100, 1:60, 1:40), the release rate of 5-FU decreased. Bearing in mind the percentage of liposome-encapsulated 5-FU, it is obvious that formulations with higher percentage of encapsulated drug showed slower release rate. This may suggest that liposome bilayer acts as a rate-limiting membrane barrier for the release of encapsulated drug substance.

The release of 5-FU from liposome gel formulations was found to be a function of liposome characteristics and properties of the gel matrix. Gel liposome formulations (Fig. 3b) showed initially faster release rate (first 1.5 hours) compared to corresponding liposome dispersions, followed by a continuous much slower release rate. The release pattern during the first 1.5 hour could be related to the portion of drug substance that leaked out of liposomes during the process of lyophilization (1, 16). Slower release rate after 1.5 hour correlates to the release of liposome entrapped 5-FU and the influence of the viscosity of the gel matrix.

To examine the mechanism of the release of 5-FU from liposome dispersions and liposome gel formulations, the Higuchi diffusion model and zero order kinetic were applied. Kinetic data indicated that the release of 5-FU followed the diffusion model of Higuchi (rate constant \( k = 8.55 - 14.47 \% h^{-1/2} \)).

| Samples | Diffusion model \( k (% h^{1/2}) \) | Diffusion model \( * k (% h^{1/2}) \) | Zero order kinetic \( ** k (% h^{-1}) \) |
|---------|----------------------------------|----------------------------------|----------------------------------|
| LD1     | 14.47                            | -                                | 3.29                             |
| LD2     | 13.17                            | -                                | 3.22                             |
| LD3     | 8.55                             | -                                | 2.00                             |
| LG1     | 11.90                            | 13.10                            | 2.89                             |
| LG2     | 11.68                            | 9.98                             | 2.87                             |
| LG3     | 11.61                            | 11.39                            | 2.85                             |

* 0 – 1.5 hour
** after 1.5 hour

Fig. 2. Effect of hydration conditions on the percentage of incorporated 5-FU into liposomes

Fig. 3. Release of 5-FU from liposome dispersions and liposome gel formulations, n=3

Table 2. Release rate constants for the prepared formulations (r>0.985)
for liposome dispersions and $k = 11.61 - 11.90 \%h^{1/2}$ for liposome gel formulations), while the release rate after 1.5 hour obeyed the zero order kinetic (rate constant $k = 2 - 3.29 \%h^1$ for liposome dispersions and $k = 2.85 - 2.89 \%h^1$ for liposome gel formulations) (Table 2). This may suggest that liposome vesicles act as a reservoir system for controlled release of encapsulated drug substance 5-FU.

**Conclusion**

As a conclusion, incorporation of aqueous solution of 5-FU into a lipid mixture of phospholipid PL 90H and cholesterol under the proposed hydration conditions, enable formation of liposomes with a potential use as a sustained release depot. Incorporation of liposomes bearing 5-FU into a structured vehicle of chitosan provided formulations suitable for topical application.

**References**

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