5-Fluorouracil in topical liposome gels for anticancer treatment – Formulation and evaluation

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Liposome gels bearing an antineoplastic agent, 5-fluorouracil, intended for topical application have been prepared and drug release properties *in vitro* have been evaluated. Different formulations of liposomes were prepared by the film hydration method by varying the lipid phase composition (PL 90H/cholesterol mass ratio) and hydration conditions of dry lipid film (drug/aqueous phase mass ratio). Topical liposome gels were prepared by incorporation of lyophilized liposomes into a structured vehicle (1%, *m/m*, chitosan gel base). Also, hydrogels containing different concentrations of 5-fluorouracil were prepared and drug release properties were investigated. The rate of drug release from liposome gels was found to be dependent on the bilayer composition and the dry lipid film hydration conditions. Also, liposomes embedded into a structured vehicle of chitosan showed significantly slower release than hydrogels. The drug release obeyed the Higuchi diffusion model, while liposomes acted as reservoir systems for continuous delivery of the encapsulated drug.

Keywords: liposomes, 5-fluorouracil, chitosan gel, drug release

In the formulation of topical dosage forms, attempts are being made to utilize drug carriers that ensure adequate localization or penetration of the drug within or through the skin in order to enhance the local and minimize the systemic effects, or to ensure adequate percutaneous absorption (1).

The application of liposomes as drug carriers on the skin surface has been proven to be efficient in the delivery of liposome-entrapped drugs to and into the skin (2). Applied on the skin, liposomes may act as a solubilizing matrix for poorly soluble drugs, penetration enhancers, as well as a local depot for sustained drug release, at the same time diminishing the side effects of these drugs (3–5). Summarily, topical liposome formulations could be more effective and less toxic than conventional formulations (6).

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Liposomes are usually applied to the skin as liquids or gels (7–9). For topical application of liposomes in gel form, hydrophilic polymers are considered to be suitable thickening agents. However, the type and concentration of the polymer, which forms the gel matrix, could influence the stability as well as the release rate of the incorporated drug (10).

Chitosan, a well-known natural polyamino saccharide formed through *N*-deacylation of chitin manufactured from shrimp or crab shells (11), may be used as a gelling agent. For pharmaceutical purposes, it has also been used as a direct compression diluent (12), as an enhancer of the dissolution properties of less soluble drugs (13), as well as a drug carrier for sustained release preparations such as microparticles, tablets, beads and gels (14–17). Applied topically, chitosan has the special feature of adhering to the surface and forming elastic, water-absorptive, biocompatible films, useful in burn treatments. Applied to the open wound of a burn patient, the chitosan film provides a cool and pleasant soothing effect and allows excellent oxygen permeability (18). Also, it has an acceleratory effect on the wound healing process. Recent studies show that regenerated chitosan fibres, sponges, and films can increase the wound healing by over 30% (19). These characteristics of chitosan could also be considered as a potentially beneficial approach in the formulation of topical forms of certain groups of drug substances, such as antineoplastic agents. Namely, conventional topical formulations of antineoplastics (ointments, solutions), indicated for example in solar and actinic keratosis and other skin carcinomas, are uncomfortable for the patient, since they often cause crusting, allergic contact dermatitis, erosions, rash, soreness, burning and pain (20–22).

Considering all the above mentioned, liposome vesicles, embedded in a chitosan gel matrix, could be attractive candidates for the use as drug delivery vehicles of antineoplastic agents for topical application.

The aim of our study was to formulate a liposome gel for controlled and localized delivery of the antineoplastic agent 5-fluorouracil (5-FU) *via* the topical route. Optimization of the liposome formulation and preparation conditions, *i.e.*, lipid phase composition and hydration conditions, in terms of the biopharmaceutical properties of the gel *in vitro*, has been carried out.

EXPERIMENTAL

Materials

The following chemicals were used in the study: Phospholipon 90H – gel state (PL 90H, Natterman Phospholipid, Germany), cholesterol (CH, Galenika, Yugoslavia), 5-fluorouracil (Ebewe Arzneimittel, Austria), chitosan (Katakura Chikkarin, Japan) and saccharose (Merck, Germany). All other chemicals used were of analytical grade.

Preparation and characterization of liposomes

Liposomes containing 5-FU as an antineoplastic agent were prepared by the modified lipid film hydration technique (23). The lipid phase was prepared by dissolving different quantities of lipid components, PL 90H and CH, in chloroform (series L1, L2, L3*)*.

Afterwards, the organic solvent was removed by evaporation under vacuum at 65 °C and a dry lipid mixture was obtained. The lipid film was hydrated and gently shaken with different quantities of the aqueous phase bearing total drug, 5-FU, in phosphate buffer pH 7.4 (formulations marked a, b, c; drug/lipid phase mass ratio 1:3.2). The final lipid/aqueous phase mass ratio was 1:31.3, 1:18.8 and 1:12.5, respectively (Table I).

After 24 h, in order to remove the unentrapped portion of the drug, liposome dispersions were washed with phosphate buffer pH 7.4 and centrifuged (20000 rpm, 45 min, 3 times; Ultracentrifuge MLW K24D, Janetski, Germany). The resulting liposome dispersions were lyophilized (temperature – 40 °C, pressure 200 Pa; Crist alpha 2–4, Bioblock, Scientific, France) using saccharose as a cryoprotector, incorporated in the internal and external aqueous phase of liposomes (lipid phase/saccharose mass ratio = 1:1.3).

The mean particle diameter of liposome vesicles was determined by the laser diffraction technique (Particle size analysette D LAB/22, Fritsch, France). The percentage of 5-FU encapsulated into liposomes was quantified by spectrophotometry at 266 nm (Lambda 16, Perkin Elmer, USA) after dissolving the liposomes in a chloroform/methanol mixture.

Preparation of topical gel formulations

Lyophilized liposomes were incorporated into a structured vehicle of chitosan in a 1:3 ratio (Fig. 1) (series LG1, LG2, LG3). The structured vehicle of chitosan (1%, *m/m*) was prepared by continuous mixing of chitosan with a 1% (*m/V*) solution of lactic acid. Also, hydrogels (HG1, HG2, HG3) containing different concentrations of 5-FU (0.2, 0.5 and 0.8%, respectively) in 1% (*m/m*) chitosan gel base were prepared.

In vitro *dissolution studies*

Studies of drug release from liposome gel and hydrogel formulations were performed using the *in vitro* dialyzing method (glass cells with hydrophilic membrane of regenerated cellulose; Dialysis tubing D-0530, Sigma, USA) at 37 °C, 100 rpm, within a period of 8 h. A weighed amount of prepared liposome gel or hydrogel formulation was poured into the glass cell and dialyzed against phosphate buffer pH 7.4 as a dialyzing me-
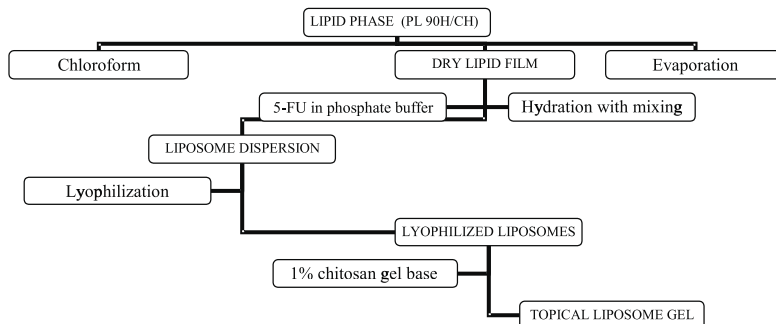

Fig. 1. Schematic presentation of the liposome gel preparation.

dium. Aliquots were taken at regular intervals and analyzed spectrophotometrically, as stated above. All experiments were carried out in triplicate and average values are presented.

Also, kinetic parameters were obtained by mathematical processing of the drug release data. Evaluation of the influence of both formulation variables on the release rate constant k values, obtained for different groups of liposome gel preparations, was supported by statistical analysis using the two-factorial ANOVA without replication (significance level, $p < 0.05$).

RESULTS AND DISCUSSION

Characterization of 5-FU loaded liposomes

The effects of the formulation variables, the lipid phase composition (PL 90H/CH mass ratio) and hydration condition (drug/aqueous phase mass ratio) on the drug entrapment efficiency and mean particle size of liposome vesicles are shown in Table I.

Decreasing the amount of cholesterol in the lipid phase and increasing the drug/ aqueous phase mass ratio, the entrapment efficiency of 5-FU into liposomes increased. Having in mind that the drug substance has a slight affinity for the lipid liposome phase,

Table I. Composition, mean particle size and encapsulation efficiency of the prepared liposome formulations

Series of liposomes	Composition of liposomes		Mean geometric diameter (μm)[a]	Encapsulation efficiency (mean ± SD, %)[b]
	PL 90H/CH mass ratio	5-FU/aqueous phase mass ratio		
L1				
a	9:1	1:100	5.04	3.7 ± 0.4
b	9:1	1:60	5.56	7.7 ± 1.8
c	9:1	1:40	4.61	10.5 ± 2.0
L2				
a	12:1	1:100	5.03	5.2 ± 1.0
b	12:1	1:60	4.53	9.4 ± 1.0
c	12:1	1:40	4.75	15.4 ± 2.0
L3				
a	15:1	1:100	4.89	7.9 ± 1.8
b	15:1	1:60	4.96	12.5 ± 1.6
c	15:1	1:40	4.97	25.4 ± 1.2

PL 90H – phospholipon 90H
CH – cholesterol
5-FU – 5-fluorouracil
[a] $n = 2$
[b] $n = 5$

encapsulation of 5-FU in liposomes is related to the overall volume of aqueous phase encapsulated during liposome formation (24). Thus, since the prepared liposomes are of similar size, with the mean geometric diameter (d_{geo}) from 4.53 to 5.56 µm, higher portion of 5-FU, as a hydrophilic drug substance in the captured aqueous core, could be related to the higher drug concentration in the hydration medium. Also, a linear relationship between the quantity of encapsulated 5-FU and PL90H/CH mass ratio (Fig. 2a), as well as the drug/aqueous phase ratio (Fig. 2b), was observed. Liposome formulations prepared with the lipid phase of PL 90H/CH = 15:1 and a drug/aqueous phase mass ra-
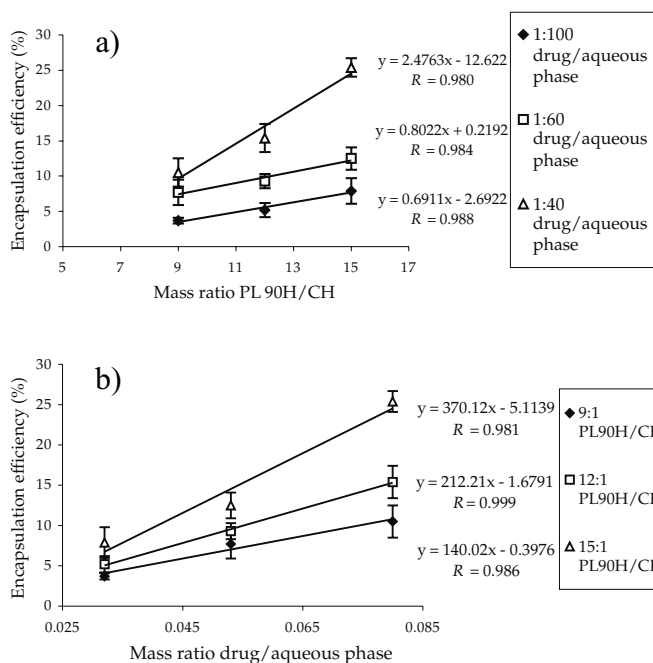

Fig. 2. Linear correlation of the concentration of liposome-entrapped 5-FU and a) PL 90H/CH mass ratio, b) drug/aqueous phase mass ratio (mean ± SD, *n* = 5).

tio = 1:40 showed the highest encapsulation efficiency.

Drug release from liposome gel formulations

The release of 5-FU from liposome gel formulations is presented in Fig. 3. It was found that only about 30–40% of the encapsulated drug was released during a period of 8 h. Incorporation of cholesterol into the phospholipid bilayer affected the release rate of the encapsulated drug. By increasing the amount of cholesterol in the lipid phase (PL 90H/CH mass ratio from 15:1, 12:1, 9:1), the release rate of 5-FU decreased, which could be related to the increased rigidity of the phospholipid bilayer (25), followed by its decreased permeability for the encapsulated drug.

The two-factorial ANOVA without replication confirmed that the lipid phase composition (PL 90H/CH mass ratio) dominantly influenced the release rate constant k at all the hydration ratios investigated. The critical value of F for testing the dominant influence was 6.94, while F_{tested} was 37.506 (for diffusion model) and 18.634 (for zero-order kinetics), which confirms the differences in the release rate constant k due to the variations in the lipid phase composition.

Regarding the hydration conditions, an increase of the drug/aqueous phase ratio (from 1:100, 1:60, 1:40) for each PL 90H/CH ratio led to a slight decrease of the drug release rate. Having in mind that the percentage of entrapped drug increased at the same time (Table I), it could be concluded that the phospholipid bilayer acted as a rate-limiting membrane barrier for the release of encapsulated 5-FU.
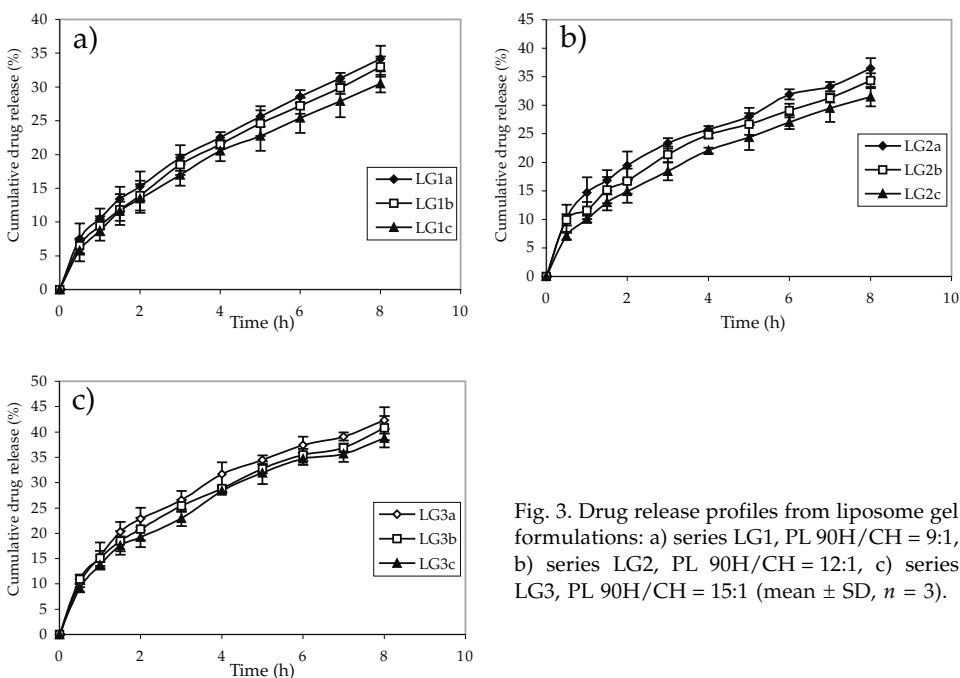

Fig. 3. Drug release profiles from liposome gel formulations: a) series LG1, PL 90H/CH = 9:1, b) series LG2, PL 90H/CH = 12:1, c) series LG3, PL 90H/CH = 15:1 (mean ± SD, n = 3).

Drug release from chitosan-hydrogels and aqueous solutions

In order to evaluate the effect of 5-FU incorporation into liposomes on the release rate, the 5-FU release rate from conventional formulations, the corresponding chitosan hydrogels as well as aqueous solutions of 5-FU were also estimated.

Profiles of drug release from hydrogel formulations (HG1, HG2, HG3) and the corresponding aqueous solutions of 5-FU (0.2, 0.5 and 0.8%, respectively) are presented in Fig. 4. 5-FU was fully released from aqueous solutions within a period of 4 h, while

hydrogel formulations released 60–70% of initially entrapped drug during a period of 8 h. As expected, entrapment of 5-FU into a structured vehicle of chitosan resulted in a prolonged release rate compared to the corresponding aqueous solutions due to the restriction imposed by the polymeric network of hydrogel (10). Moreover, release of 5-FU from liposomes embedded into the chitosan gel base was significantly slower (40% released within 8 h) than the release from chitosan hydrogels (70% released within 8 h), which confirms that encapsulation of 5-FU into liposomes resulted in a prolonged drug release rate. Fig. 5 shows comparatively the release profiles of the liposome gel formulation and the corresponding hydrogel and aqueous solution of 5-FU.

Fig. 4. Release rate of 5-FU from hydrogels (HG1, HG2, HG3) and aqueous solutions (0.2, 0.5 and 0.8%) (mean ± SD, *n* = 3).
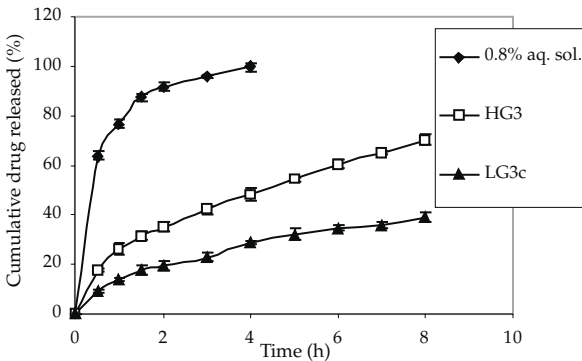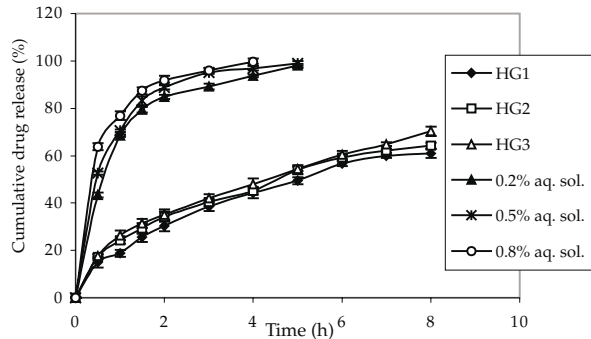

Fig. 5. Comparative release of 5-FU from liposome gel LG3c, hydrogel HG3, and 0.8% aqueous solution (mean ± SD, *n* = 3).

Kinetic analysis

Mathematical processing of the *in vitro* release data showed that the release of 5-FU from liposome gels and hydrogels obeyed the Higuchi release kinetics (square root of time), indicating a diffusion-controlled model (Table II). However, after 1.5 h, high correlation coefficients were obtained for the zero-order drug release kinetics for liposome gels, which suggests that liposomes acted as reservoir systems for a continuous delivery of encapsulated 5-FU.

Table II. Kinetic parameters for the prepared liposome gels and hydrogel formulations

Formulation	Diffusion model		Zero-order kinetics[a]	
	R	$k \pm SD$ (% $h^{-1/2}$)[b]	R	$k \pm SD$ (% h^{-1})[b]
LG1a	0.998	12.53 ± 0.71	0.997	3.17 ± 0.36
LG1b	0.999	12.47 ± 0.46	0.995	3.18 ± 0.29
LG1c	0.999	11.56 ± 1.12	0.996	2.87 ± 0.40
LG2a	0.998	11.90 ± 0.81	0.994	2.89 ± 0.51
LG2b	0.997	11.68 ± 0.46	0.991	2.87 ± 0.36
LG2c	0.999	11.61 ± 0.72	0.995	2.85 ± 0.52
LG3a	0.997	14.73 ± 0.64	0.990	3.35 ± 0.33
LG3b	0.998	13.68 ± 0.80	0.992	3.34 ± 0.38
LG3c	0.993	13.69 ± 0.63	0.986	3.33 ± 0.29
HG1	0.997	22.61 ± 0.29	–	–
HG2	0.997	22.85 ± 0.31	–	–
HG3	0.998	24.10 ± 0.26	–	–

[a] 1.5–8 h
[b] $n = 3$

CONCLUSIONS

Topical formulations containing 5-FU loaded liposomes embedded into a structured vehicle of chitosan have been prepared and evaluated. The release rate of 5-FU from topical liposome gels was affected by the formulation variables. Comparing the liposome gels with hydrogel formulations, the release rate of liposome-entrapped drug was prolonged, while a steady-state release rate, established after 1.5 hours, suggests that the liposomes function as a reservoir system for continuous delivery of the encapsulated drug substance.

Further investigations on the effects of selected formulations *in vivo*, which are in progress, should show the suitability of such delivery systems for prolonged action of topically applied antineoplastic agents, with fewer side effects than conventional formulations.

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S A Ž E T A K

5-Fluorouracil u gelovima s liposomima za topičku primjenu u terapiji karcinoma – Priprava i evaluacija

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Priređeni su i evaluirani gelovi s liposomima za topičku primjenu s citostatikom 5-fluorouracilom. Varirajući sastav lipidne faze (omjer PL 90H i kolesterola) i hidratacijske uvjete suhog lipidnog filma (omjer ljekovite tvari i vodene faze) načinjeni su različiti pripravci liposoma film-metodom (metodom hidratizacije fosfolipidnog filma). Liposomski gelovi za površinsku primjenu priređeni su inkorporacijom liofiliziranih liposoma u podlogu (1%, *m/m*, kitozan baza u obliku gela). Pripravljeni su i hidrogelovi s različitom koncentracijom 5-fluorouracila. Oslobađanje ljekovite tvari ovisilo je o sastavu dvosloja i uvjetima hidratacije suhog lipidnog filma. Oslobađanje ljekovite tvari iz liposoma u kitozanskoj podlozi bilo je značajno sporije nego iz hidrogelova. Proces je slijedio Higuchijev difuzijski model, a liposomi su se ponašali kao spremnici za kontinuiranu isporuku ljekovite tvari.

Ključne riječi: liposomi, 5-fluorouracil, kitozan gel, oslobađanje ljekovite tvari

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