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Research paper

Improvement in physicochemical parameters of DPPC liposomes and increase in skin permeation of aciclovir and minoxidil by the addition of cationic polymers

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ABSTRACT

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes were prepared by high-pressure homogeniser and coated with two cationic polymers, chitosan (CS) and for the first time Eudragit EPO (EU), respectively. Compared to the control liposomes, the polymeric liposomes showed greater physicochemical stability in terms of mean particle size and zeta potential at room temperature. In the present study, aciclovir and minoxidil have been used as hydrophilic and hydrophobic candidates. In the presence of the drugs, the polymeric liposomes still showed constant particle size and zeta potential. Influences of polymers and model drugs on thermotropic phase transition of DPPC liposomes were studied by micro-differential scanning calorimetry (microDSC). The influences on configuration of DPPC liposomes were investigated by Fourier transform infrared spectroscopy (FTIR). According to DSC results, cationic polymers had a stabilising effect, whereas aciclovir and minoxidil changed the physical properties of the DPPC bilayers by influencing the main phase transition temperature and erasing the pre-transition. The investigation of C=O stretching bands of DPPC at 1736 cm^{-1} in FTIR spectra showed that aciclovir has strong hydrogen bonding with C=O groups of DPPC, whereas carbonyl groups were free in minoxidil presence. Moreover, the coating of liposomes with CS or EU led to higher skin diffusion for both drugs. This could be explained as an effect of positively charged liposomes to interact stronger with skin negatively charged surface and their possible interactions with structures below the stratum corneum.

1. Introduction

Liposomes are spherical structures consisting of one or more phospholipid bilayers enclosing an aqueous core and are generally produced from highly purified phospholipids extracted from soy oil or egg yolk or synthetic phospholipids what is the case in the current study. They have been claimed to improve transdermal drug delivery and can be used as a model for the skin membrane [1]. Plenty of studies reported that liposomes were able to improve skin permeation of various entrapped drugs through the major barrier stratum corneum [2]. The possible mechanism of liposomes action on distribution of drug through the skin is probably through the fusion of the vesicles either in the channel-like structures between corneocytes or in hair follicles or by their disintegration of extended lipid structures [3,4].

However, liposomes have some limitations. They generally adhere to each other and after certain time fuse to form larger vehicles. As an alternative, many attempts to use biocompatible polymers for the surface modification of liposomes, obtaining a stabile liposomal system for application in different environments [2,5] Polymers are macromolecule constituents of many useful materials that, depending on their molecular structures, can have different properties. At present, polymers have applications in many different areas including their use as chromatographic material, carriers for molecules and cells, for modulation of living processes, changing properties of food products and for the development of many pharmaceutical applications. Moreover, during the last decade, the use of polymers in drug delivery has been extensively studied.

Chitosan (CS), the natural, bioadhesive, biocompatible and biodegradable polymer seems to be an optimal candidate to be combined to liposomes. As a cationic biopolymer, it showed the ability to improve skin compatibility of skin formulations and enhancing effect on the penetration of cosmetic ingredients and drugs [6,7]. Eudragit EPO (EU) was selected for liposome coating as a second cationic polymer. This polycation, insoluble at physiological pH exhibited mucoadhesive characteristics [8]. Moreover, Alasino et al. [9] showed additional membrane-destabilising properties and increase in efflux of doxorubicin from liposomes. In some studies, EU has been regarded as a dissolution modifier. Some other groups used EÚs ability to improve the solubility of drugs with low aqueous solubility [10].

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In the present study, three main goals should be achieved:

(i). To increase physicochemical stability of DPPC liposomes in terms of mean particle size and zeta potential in the presence and in the absence of the drugs by coating with the two cationic polymers, CS and EU, respectively.
(ii). To characterise interactions of the polymers and the drugs with DPPC by microDSC and FTIR.
(iii). To improve skin permeation of aciclovir and minoxidil by coating with CS and EU, respectively.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine was kindly donated by Lipoid (Switzerland). The product was synthetic Lipoid PC 16:0/16:0. The content of phosphatidylcholine was at least 99% related to the dry weight. Eudragit EPO (EU, <150 kDa, structure is presented in Fig. 1) was kindly provided by Evonik (Darmstadt, Germany). Chitosan (CS, <500 kDa) in the powder form was a gift from Syntapharm (Mülheim, Germany). The degree of deacetylation was determined by NMR with 95% [11]. Aciclovir was purchased from Fagron GmbH (Barsbüttel, Germany) and minoxidil from Kwizda (Vienna, Austria). All other chemicals used in this study were of analytical reagent grade and were used as received, without any further purification.

2.2. Liposomes preparation

DPPC liposomes were prepared according to a modified method of Brandl et al. [12] as described recently [13]. Briefly, the phospholipid powder was dispersed in distilled water at 60 °C in the concentration of 5% (w/w) and magnetically stirred until the powder was completely dissolved and thoroughly mixed using an ultraturax (Omni 500, Glendale, Canada). Afterwards, the dispersion was homogenised with a high-pressure homogeniser (EmulsiFlex-C3, Avestin, Canada) for 16 times at approximately 1100 bar. The liposomes were kept at 4 °C over night and diluted with purified water 1:1 the day after and characterised in terms of mean particle size and PDI.

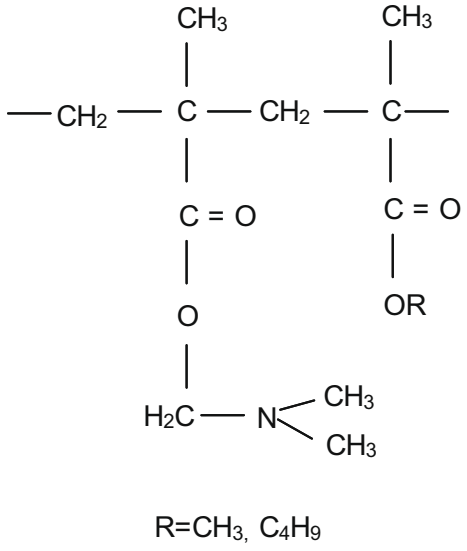

Fig. 1. Structure of Eudragit EPO.

An aqueous dispersion of 2% (w/w) of aciclovir and minoxidil, respectively, was added to already formed liposomes in the volume ratio 1:1 and afterwards mechanically stirred over night at 50 °C.

2.3. Chitosan and Eudragit EPO (EU) coated liposomes

The already prepared liposomes described above coated with CS and EU by adding a polymer solution in volume ratio 1:1 and mechanically stirred for 30 min. First, different polymer concentrations in 0.01 M acetate buffer ranging between 0.125% and 1% (w/w) were used. The final products were determined in terms of mean particle size, zeta potential and polydispersity index (PDI). For incorporation of aciclovir and minoxidil, the liposomes with the lowest polymer concentration of 0.125% were used. The drugs were suspended in this polymer solution at a concentration of 2% (w/w) and further diluted with aqueous liposomes 1:1 (v/v) and stirred over night at 50 °C.

2.4. Analysis of size and zeta potential of DPPC liposomes

The particle size and polydispersity index (PDI) of liposomes were analysed by photon correlation spectroscopy, and the zeta potential was determined by electrophoretic mobility using a Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom).

To observe changes in the particle size (expressed in *Z*-average size) and zeta potential, the batches of pure and coated liposomes with and without drugs were stored at room temperature over a period of 20 weeks. Samples were diluted 1:30 with filtered 0.1 mM acetate buffer obtaining conductivity of <0.05 ms/cm and monitored weekly ($n = 5$). Each sample was measured three times in 13 runs (sampling time was 5 and 15 min).

2.5. Micro-differential scanning calorimetry (microDSC) studies

Differential thermal analysis was performed using a Setaram III micro-calorimeter as previously reported [13]. Samples of pure and polymer-coated liposomes, prepared as described above, were scanned. We also performed consecutive runs for each sample [14]. The measurement conditions were 1.00 °C/min for the scanning rate over the temperature range of 15–65 °C, using purified water as reference.

Thermal transitions were calculated using Setsoft 2000 Setaram software. After baseline subtraction, raw power data were converted to molar heat capacity data. Baselines were fitted to the pre-transition and main transition regions using a linear baseline function, so that transition temperatures and enthalpies of reaction could be calculated for each lipid concentration.

2.6. Fourier transform infrared (FTIR) spectroscopy

Spectra were recorded on a FTIR spectrophotometer (model: Tensor 27, Bruker Optics, Ettlingen, Germany) with a photovoltaic MCT detector at a temperature of 25 °C. The samples of pure and coated DPPC liposomes with and without aciclovir or minoxidil were scanned on Bio-ATR II tool against demineralised water as a reference ($n = 5$).

2.7. In vitro aciclovir and minoxidil permeation

In vitro permeation studies with porcine abdominal skin were carried out as previously reported [15]. Briefly, about 0.5 ml of aciclovir and minoxidil-loaded liposomes with and without coating was respectively applied to the skin surface. The diffusion studies were performed for 10 h with a phosphate buffer (pH 7.4) as an acceptor medium, where the samples were taken every 2 h and measured by HPLC. The HPLC analysis of permeated drug ($n = 4$)

was carried out on a C-18 RP column (Nucleosil 100-5, 250 mm × 4 mm, Macherey–Nagel, Düren, Germany) at 40 °C. The mobile phase consisted of methanol/water 10/90 for aciclovir and methanol/water/acetic acid 75/25/1 for minoxidil [16,17]. The aciclovir and minoxidil concentrations used to construct the calibration curve were in the range from 0.005 to 0.25 mg/ml ($n = 6$).The flow rate was maintained at 1.00 ml/min, and the injection volume was 20 μl.

Furthermore, the flux J ($\mu g\ cm^{-2}h^{-1}$) of both drugs in each preparation was calculated from the slope of the linear portion of the cumulative amount permeated through the porcine skin per unit area versus time plot.

2.8. Statistical data analyses

Results of current studies are expressed as the means ± S.D. of four (skin diffusion studies) and of five experiments (particle size, zeta potential, microDSC, FTIR). Data were exported to the GraphPad Prism statistics software package (GraphPad Prism Software, USA). Analysis groups consisted of independent mean values and the Gaussian distribution of the data was verified using the Kolmogorov–Smirnov test. Statistics were performed using one-way ANOVA with post hoc Dunnet's test (95% confidence interval). The values of diffusion studies were analysed with nonparametric t-test. P-values of <0.05 were considered significant.

3. Results and discussion

Different preliminary experiments were necessary to optimize the preparation process with ultra-turax and following high-pressure homogenisation. On the one hand, we figured out that the temperature of 60 °C during the suspension of DPPC was very important to obtain reproducible liposomes. On the other hand, the ultra-turax speed and mixing time had only a small effect on particle size. According to a previous investigation, 16 cycles in homogeniser at 1100 bar were suitable for liposomes production [12,13].

By monitoring the particle size, zeta potential and PDI using light scattering the unloaded liposomes at room temperature have shown constant mean particle size, PDI and zeta potential for 7 weeks (Table 1, $P > 0.05$). After this time period, the size and PDI started to increase significantly ($P < 0.05$), indicating merging of liposomes that could lead to phase separation [18]. Therefore, physical stability is a general problem with liposomes. As an alternative CS and EU, two cationic polymers were employed to improve the stability. As seen in Tables 2a and 2b, the higher the polymer content the larger the particle size, for both polymers ($P < 0.05$). That was the result of the chemical interaction that probably involves hydrogen bonding between the polymer and the phospholipid head groups on the vehicles surface [7]. The charge of liposomes increased from approximately 0.50–40 mV with the addition of CS or EU. The values of zeta potential were almost in the same range independent of the amount of polymer (Tables 2a and 2b). Finally, both polymers caused stable particle size and uniformly PDI values for longer time that could be explained by the positive charge of the particles. Immediately after production, the coated liposomes had a zeta potential between 30 and 40 mV and stayed significantly longer unaltered than control liposomes (Table 1). The EU-coated liposomes have not shown any changes within 20 weeks ($P < 0.05$), while the zeta potential of

Table 2a
Mean particle size as Z-average (MPS), poly dispersity index (PDI) and zeta potential (ZP) of CS-DPPC liposomes.

CS (%)	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.
0	88.15 ± 1.00	0.18 ± 0.01	0.51 ± 0.79
0.125	97.11 ± 0.48[a]	0.23 ± 0.01	40.72 ± 0.79
0.25	108.67 ± 0.64[a]	0.25 ± 0.01	42.09 ± 0.75
0.5	139.87 ± 0.51[a]	0.28 ± 0.01	40.81 ± 1.38
1.0	157.99 ± 2.70[a]	0.32 ± 0.04	41.41 ± 2.26

Values are means ± S.D. of five experiments.
[a] Significantly larger particles compared with the 0% (Dunneťs test).

Table 2b
Mean particle size as Z-average (MPS), poly dispersity index (PDI) and zeta potential (ZP) of EU-DPPC liposomes.

EU (%)	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.
0	88.15 ± 1.00	0.18 ± 0.01	0.51 ± 0.79
0.125	95.66 ± 1.54[a]	0.20 ± 0.01	36.64 ± 0.60
0.25	98.42 ± 1.00[a]	0.18 ± 0.01	38.07 ± 2.13
0.5	100.51 ± 1.30[a]	0.16 ± 0.01	35.98 ± 0.96
1.0	105.08 ± 1.31[a]	0.14 ± 0.01	32.00 ± 2.92

Values are means ± S.D. of five experiments.
[a] Significantly larger particles compared with the 0% (Dunneťs test).

Table 1
Physicochemical stability of DPPC, CS DPPC and EU-DPPC liposomes expressed in mean particle size as Z-average (MPS), poly dispersity index (PDI) and zeta potential (ZP).

Week	DPPC			CS DPPC[b]			EU DPPC[b]		
	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.
Start	88.15 ± 1.00	0.18 ± 0.01	0.51 ± 0.79	97.11 ± 1.80	0.27 ± 0.02	40.72 ± 0.79	95.66 ± 1.54	0.20 ± 0.01	36.64 ± 0.60
1	87.22 ± 0.71	0.17 ± 0.01	0.50 ± 0.85	97.64 ± 0.48	0.23 ± 0.01	40.72 ± 0.80	95.99 ± 0.52	0.20 ± 0,01	31.58 ± 1.56
2	89.03 ± 1.29	0.16 ± 0.01	1.02 ± 0.26	102.80 ± 1.3	0.25 ± 0.01	47.84 ± 1.18	94.53 ± 0.68	0.19 ± 0.01	32.69 ± 0.39
3	85.97 ± 0.42	0.16 ± 0.01	2.00 ± 0.80	101.72 ± 1.6	0.24 ± 0.01	40.76 ± 1.28	99.38 ± 1.40	0.21 ± 0.01	34.9 ± 0.84
4	86.04 ± 0.44	0.16 ± 0.01	3.23 ± 0.91	102.58 ± 0.9	0.24 ± 0.01	40.7 ± 1.99	99.42 ± 1.02	0.20 ± 0.01	38.94 ± 1.50
5	84.51 ± 0.48	0.13 ± 0.01	2.12 ± 0.89	98.65 ± 0.75	0.23 ± 0.01	36.19 ± 1.11	97.95 ± 1.43	0.20 ± 0.01	38.11 ± 1.31
6	88.89 ± 2.07	0.15 ± 0.01	1.15 ± 0.79	98.55 ± 0.62	0.23 ± 0.01	40.72 ± 1.03	97.97 ± 1.30	0.21 ± 0.01	40.86 ± 4.37
7	90.82 ± 1.28	0.17 ± 0.01	2.18 ± 0.59	97.73 ± 0.39	0.22 ± 0.01	41.99 ± 3.06	98.54 ± 1.39	0.21 ± 0.01	39.26 ± 0.54
8	100.72 ± 1.9[a]	0.21 ± 0.02	1.12 ± 0.36	95.21 ± 1.22	0.22 ± 0.01	28.29 ± 0.25	98.29 ± 1.50	0.21 ± 0.01	37.94 ± 1.05
9	173.29 ± 2.0[a]	0.43 ± 0.01	0.13 ± 0.19	95.25 ± 1.3	0.21 ± 0.01	28.29 ± 0.22	94.94 ± 0.81	0.21 ± 0.01	35.66 ± 0.93
10	–	–	–	96.58 ± 0.99	0.22 ± 0.01	23.00 ± 1.16	95.51 ± 0.53	0.21 ± 0.01	36.42 ± 1.16
12	–	–	–	97.65 ± 0.75	0.23 ± 0.01	23.50 ± 3.05	94.78 ± 0.68	0.22 ± 0.01	34.19 ± 0.65
14	–	–	–	98.55 ± 0.62	0.23 ± 0.01	24.10 ± 3.12	95.84 ± 0.34	0.22 ± 0.01	33.63 ± 0.74
16	–	–	–	120.05 ± 0.4[a]	0.41 ± 0.05	10.00 ± 0.50	93.70 ± 0.94	0.22 ± 0.01	31.44 ± 1,67
18	–	–	–	–	–	–	94.05 ± 1.50	0.24 ± 0.02	31.96 ± 0.66
20	–	–	–	–	–	–	95.51 ± 0.53	0.21 ± 0.01	36.42 ± 1.16

Values are means ± S.D. of five experiments.
[a] Significantly larger particles compared with the start (Dunneťs test).
[b] Amount of polymer is 0.125%.

CS-coated liposomes decreased after 8 weeks and after 16 weeks liposomes started to aggregate resulting in larger particle size and PDI values (Table 1). Microbial growth could not be detected in the polymer containing preparation compared to the control DPPC liposomes, which were microbial affected after 2 weeks.

As model substances, aciclovir and minoxidil were suspended into the described DPPC liposomes and into the coated DPPC liposomes without influencing significantly ($P > 0.05$) their particle size, immediately after production (comparing start values of Table 1 with Tables 3 and 4). However, after 2 weeks of storage, aciclovir caused the significant growth of particles and PDI values. In contrast to this, minoxidil preparations were stabile for 8 weeks (Table 4). Moreover, an addition of the polymers CS or EU induced a higher stabilization effect in all liposome preparations, independent of the model drug (Tables 3 and 4). Furthermore, in Tables 3 and 4, zeta potential increased in DPPC liposomes by the addition of the drugs, while in coated liposomes with minoxidil this was significantly lowered. In the case of aciclovir, the zeta potential decreased after one week. These results might be suggesting drugs association with the lipid bilayer [19].

Further investigations to clarify and explain the stabilizing effect of polymers and possible association of the drugs with the lipid bilayer should be performed. Two well-established biophysical techniques, microDSC and FTIR, were chosen for this purpose.

DPPC liposomes display typical thermotropic phase behaviour with a pre-transition endotherm near 38.4 ± 0.25 °C and the main endotherm near 41.4 ± 0.05 °C, which corresponds to the gel-to-liquid crystalline ($P\beta' \rightarrow L\alpha$) phase transition (Table 5). The smaller pre-transition temperature, reflecting the lamellar to rippled gel ($L\beta' \rightarrow P\beta'$) transition, has been generally attributed to the surface structure of the vehicle and is related to rotations of the phospholipid head groups or transformation in the lamellar structure and changes in the hydrocarbon chain packing [13]. In the present study, as seen in Table 5, an addition of CS and EU could not induce any significant changes in the pre and main transition temperature. However, comparing CS and EU-DPPC liposomes with DPPC liposomes heating curves, the pre-transition onset temperature shifted significantly to a higher temperature, pointing to the interaction with the polar region of the phospholipid bilayer (Table 5). This could mean that DPPC molecules get anchored to polymeric chains through interaction between their head groups, and the specific functional groups of polymer by electrostatic interaction. Moreover, the hydroxyl groups of DPPC may interact with the hydroxyl groups of polymers through hydrogen bonding, stabilizing the lamellar structure of the liposomes [20,21] what is in coherence with the increased physicochemical stability of coated liposomes. Furthermore, the pre-transition enthalpy is reduced with increased CS or EU content, confirming the possibility of their interaction with the liposomes polar domain and the interfering with tilting of phospholipid acyl chains (Table 5) [22,23]. Additionally, the enthalpy values of main transition temperature increased significantly in the presence of polymers (Table 5), what may be also approving an electrostatic interaction between DPPC and polymers. de Oliveira Tiera et al. also suggested that hydrophobic interactions may induce the CS incorporation into the lipid bilayer [24].

Interestingly, the addition of the drug resulted in disappearance of the pre-transition temperature, whereas the main phase transition temperature was significantly raised by aciclovir and significantly lowered by minoxidil, ($P < 0.05$), (Table 5). This could be described as the substances ability to interact with the surface and the phospholipid bilayer [22]. However, the addition of the drugs increases the enthalpy main transition significantly confirming the interaction with liposomes membrane. In the second and third subsequent scans, main transition peak and enthalpy remain constant. This means that there are no further lipid interactions with the drugs [14].

An additional biophysical method is FTIR spectroscopy in aqueous systems that can be used to analyze interactions on the molecular level. With this technique, it is possible to see the subtle changes in the configuration of lipid assemblies by analyzing the

Table 3
Mean particle size as *Z*-average (MPS), poly dispersity index (PDI) and zeta potential (ZP) of DPPC formulations with 1% (w/w) aciclovir.

Aciclovir			
Week	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.
DPPC			
0	84.48 ± 5.21	0.14 ± 0.02	8.84 ± 2.10
1	81.10 ± 4.49	0.12 ± 0.02	5.04 ± 0.29
2	82.54 ± 4.64	0.13 ± 0.02	6.25 ± 2.30
3	127.53 ± 3.80[a]	0.36 ± 0.10	5.21 ± 1.02
CS DPPC			
0	99.78 ± 0.28	0.19 ± 0.01	38.00 ± 2.79
1	90.06 ± 4.86	0.19 ± 0.01	23.80 ± 4.49
2	97.81 ± 4.65	0.16 ± 0.01	20.11 ± 3.45
5	98.15 ± 4.00	0.17 ± 0.01	23.06 ± 2.62
7	94.85 ± 1.17	0.19 ± 0.01	16.84 ± 1.40
8	126.40 ± 3.84[a]	0.25 ± 0.03	17.46 ± 1.49
10	135.00 ± 2.20[a]	0.27 ± 0.02	17.39 ± 1.09
12	160.00 ± 10.0[a]	0.37 ± 0.03	19.80 ± 1.20
EU DPPC			
0	95.92 ± 4.10	0.15 ± 0.01	37.81 ± 2.81
1	97.00 ± 4.53	0.16 ± 0.02	41.83 ± 3.95
2	97.35 ± 3.83	0.16 ± 0.02	34.76 ± 9.01
5	94.55 ± 4.36	0.17 ± 0.01	28.42 ± 2.08
7	93.57 ± 3.84	0.19 ± 0.02	20.88 ± 3.62
8	130.60 ± 5.20[a]	0.25 ± 0.03	20.10 ± 2.22
10	132.12 ± 3.80[a]	0.24 ± 0.03	20.50 ± 2.52
12	144.60 ± 2.00[a]	0.28 ± 0.03	18.26 ± 1.03

Values are means ± S.D. of five experiments.
[a] $P < 0.05$ vs. week 0 (Dunnetś test).

Table 4
Mean particle size as *Z*-average (MPS), poly dispersity index (PDI) and zeta potential (ZP) of DPPC formulations with 1% (w/w) minoxidil.

Minoxidil			
Week	MPS (nm) ± S.D.	PDI ± S.D.	ZP (mV) ± S.D.
DPPC			
0	84.41 ± 5.95	0.16 ± 0.04	4.58 ± 0.32
1	84.02 ± 5.67	0.14 ± 0.03	3.96 ± 0.97
2	84.28 ± 4.87	0.13 ± 0.03	4.73 ± 0.48
5	84.51 ± 4.48	0.14 ± 0.03	4.10 ± 0.67
8	94.67 ± 4.57	0.22 ± 0.03	4.85 ± 1.66
9	120.50 ± 5.01[a]	0.23 ± 0.04	3.90 ± 1.23
CS DPPC			
0	94.97 ± 4.23	0.16 ± 0.03	10.80 ± 1.37
1	96.73 ± 3.87	0.13 ± 0.02	10.51 ± 0.39
2	96.76 ± 3.96	0.14 ± 0.03	11.41 ± 0.57
5	96.32 ± 3.85	0.14 ± 0.02	15.62 ± 8.43
8	96.18 ± 3.06	0.15 ± 0.02	11.06 ± 0.89
10	94.83 ± 3.51	0.14 ± 0.03	12.87 ± 1.29
12	97.33 ± 2.20	0.12 ± 0.02	11.64 ± 1.07
16	96.53 ± 5.02	0.14 ± 0.05	12.60 ± 1.50
20	95.45 ± 3.02	0.14 ± 0.05	12.35 ± 1.65
EU DPPC			
0	94.65 ± 4.33	0.13 ± 0.02	23.84 ± 5.77
1	93.94 ± 4.89	0.14 ± 0.02	22.80 ± 0.35
2	95.76 ± 4.89	0.13 ± 0.01	23.96 ± 1.42
5	94.60 ± 4.87	0.14 ± 0.02	23.06 ± 2.87
8	94.83 ± 3.91	0.16 ± 0.04	20.29 ± 2.90
10	95.05 ± 2.59	0.14 ± 0.03	20.90 ± 1.51
12	95.50 ± 3.56	0.18 ± 0.02	16.10 ± 0.90
16	101.10 ± 1.26	0.20 ± 0.03	16.30 ± 0.50
18	120.40 ± 2.31[a]	0.26 ± 0.04	15.30 ± 0.52

Values are means ± S.D. of five experiments.
[a] $P < 0.05$ vs. week 0 (Dunnetś test).

Table 5
Transition temperature and enthalpy values of DPPC liposomes, DPPC with 0.125% (w/w) CS or EU and DPPC with 1% (w/w) aciclovir or minoxidil.

	T_{max} (°C)	Linear onset (°C)	Enthalpy (J/M)
DPPC	38.4 ± 0.30 pre-transition	36.5 ± 0.60	1193 ± 121
	41.4 ± 0.10 main transition	40.1 ± 0.10	30600 ± 430
0.125%CS DPPC	38.7 ± 0.01 pre-transition	37.9 ± 0.03[a]	166 ± 10[a]
	41.4 ± 0.03 main transition	40.1 ± 0.20	53000 ± 484[a]
1.00%CS DPPC	38.8 ± 0.01 pre-transition	38.1 ± 0.02	102 ± 6[a]
	41.5 ± 0.01 main transition	40.2 ± 0.01	54202 ± 469
0.125%EU DPPC	38.7 ± 0.06 pre-transition	37.9 ± 0.03[a]	145 ± 30[a]
	41.4 ± 0.01 main transition	40.1 ± 0.02	51766 ± 967[a]
1.00%EU DPPC	38.9 ± 0.05 pre-transition	38.2 ± 0.01[a]	65 ± 10[a]
	41.5 ± 0.02 main transition	40.4 ± 0.04	53254 ± 419
Aciclovir DPPC	41.9 ± 0.10[a] main transition	40.3 ± 0.01	54967 ± 200
Minoxidil DPPC	40.7 ± 0.04[a] main transition	39.4 ± 0.01	54829 ± 832

Values are means ± S.D. of five experiments.
[a] $P < 0.05$ vs. DPPC (Dunnets test).

frequency and the bandwidth changes in the vibrational modes. For this purpose, the position of the symmetric and antisymmetric CH_2 bands at 2800–3000 cm^{-1} and C=O stretching mode at 1736 cm^{-1} of DPPC were chosen to compare different liposomal formulations [25].

In a first set of experiments by the addition of polymers, no shift of the acyl bands at 2800–3000 cm^{-1} could be seen in DPPC liposomes implying a stabilization of configuration and dynamics of the bilayer [26]. In order to analyze the interaction of CS and EU with the glycerol backbone near the head group of the phospholipids, the C=O stretching band was analyzed. In contrast to previous published studies, the C=O band at 1736 cm^{-1} does not shift by the addition of solely CS or EU [5].

In Figs. 2 and 3, it is clearly seen that this characteristic band of DPPC, arising from the ester group vibrations, was shifted significantly to lower wave numbers in the presence of aciclovir and to higher wave numbers in the presence of minoxidil ($P < 0.05$, Dunnets test).

For all formulations with aciclovir, the lower frequency values suggest that hydrogen bonding may occur between the C=O groups of DPPC and either with NH_2 or with OH groups of aciclovir [25–27]. Whereas the higher frequency values for all of minoxidil formulations indicate no evidence of hydrogen bonding, instead there are free carbonyl groups in the system [27]. Interestingly, this negative and positive shifting of the C=O band is more pronounced in the presence of polymers (Figs. 2 and 3).

In the third part of this work, diffusion studies on porcine skin were performed to see whether the confirmed interaction between DPPC liposomes and polymers could influence the skin permeation of model drugs. As seen in Figs. 4 and 5, used polymers increased the skin permeation of both model drugs significantly. The coating of liposomes with CS and EU improved the permeation of aciclovir 1.61-fold and 1.64-fold after 10 h, respectively, in comparison with control. In case of minoxidil, the skin permeation was 1.54 times higher with CS and 1.88 with EU (Table 6). One of the reasons for this enhancement could be the negative charge on the epithelial cells surface, selective to positively charged liposomes [28]. Another reason of this effect might be the tendency of positive polymers to disrupt the tight junctions, which was confirmed in the case of CS [29]. Wydro et al. showed also that CS significantly modifies lipids occurring in the skin [21].

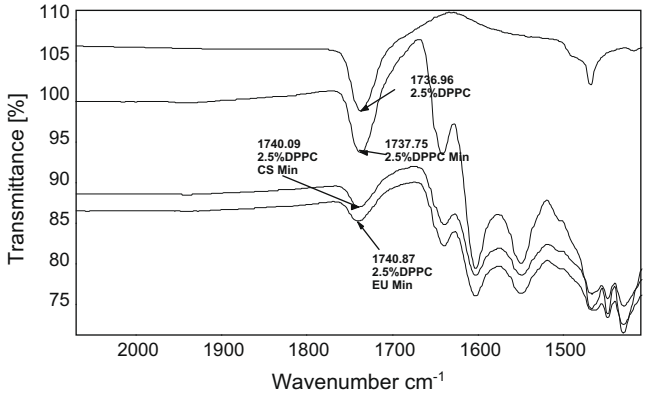

Fig. 3. FTIR transmission spectra of DPPC (control), DPPC with minoxidil (min), CS DPPC and EU DPPC with minoxidil (min) (as indicated with arrows) ($n = 5$).

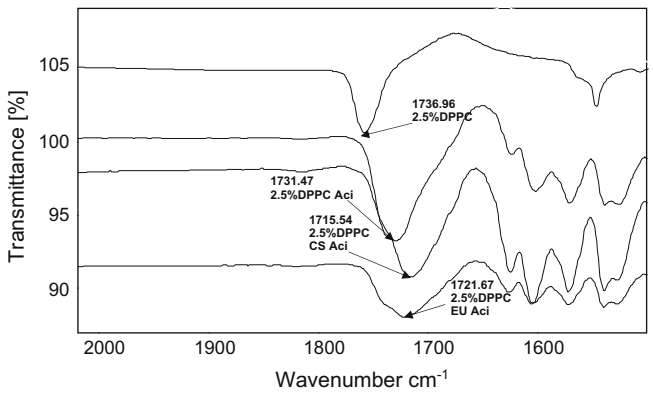

Fig. 2. FTIR transmission spectra of DPPC (control), DPPC with aciclovir (aci), CS DPPC and EU DPPC with aciclovir (aci) (as indicated with arrows) ($n = 5$).

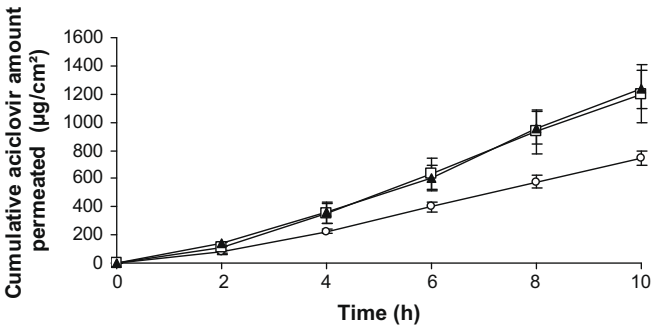

Fig. 4. Comparison of aciclovir permeation through porcine skin from different liposomal formulations (means ± S.D. of four experiments): -▲- EU-coated liposomes; -□- CS-coated liposomes; -○- DPPC liposomes.

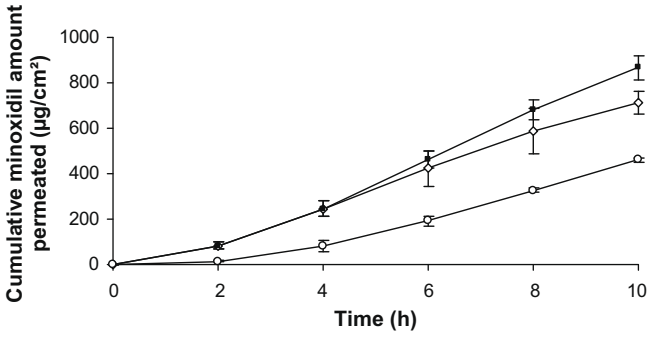

Fig. 5. Comparison of minoxidil permeation through porcine skin from different liposomal formulations (means ± S.D. of four experiments): -■- EU-coated liposomes; -◇- CS-coated liposomes; -○- DPPC liposomes.

Table 6
Skin permeation rates of aciclovir and minoxidil from DPPC, CS DPPC and EU DPPC and influence of polymers on their permeation through porcine skin.

Model drug	Formulation	Cumulative amount permeated after 10 h (µg/cm²)	Enhancement factor of polymers (CS and EU) compared to DPPC	Flux *J* (µg/cm²/h)
Aciclovir	DPPC	744.61 ± 54.51	Control	77.24 ± 5.5
	CS DPPC	1201.36 ± 203.6[a]	1.61	125.11 ± 19.5
	EU DPPC	1223.21 ± 131.9[a]	1.64	126.83 ± 13.9
Minoxidil	DPPC	460.56 ± 10.6	Control	47.96 ± 5.9
	CS DPPC	713.18 ± 50.9[a]	1.54	79.64 ± 13.3
	EU DPPC	866.71 ± 52.6[a]	1.88	90.61 ± 5.9

Values are means ± S.D. of four experiments.
[a] $P < 0.05$ vs. DPPC (*t*-test).

4. Conclusion

It can be concluded that the physicochemical stability in terms of mean particle size and zeta potential of DPPC liposomes with and without aciclovir or minoxidil was significantly increased by the addition of two different cationic polymers, CS and EU. According to microDSC data, this phenomenon can be interpreted as hydrogen bonding between the polymer and liposome surfaces. Furthermore, FTIR spectra showed that aciclovir has strong hydrogen bonding with C=O groups of DPPC, whereas carbonyl groups were free in minoxidil presence. This data might be clarifying the longer stability of liposomes with minoxidil.

The skin permeation efficiency of aciclovir and minoxidil from coated liposomes increased, what could be explained as a tendency of positively charged liposomes to interact stronger with the skin surface. Another possibility to explain this effect is that the CS and EU might be interacting with skin lipids and going deeper disrupting the tight junctions in lower epidermis layers.

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