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Influence of hydrogels on liposome stability and on the transport of liposome entrapped substances into the skin

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Abstract

Different liposome-hydrogel formulations, which are used for topical application of liposomes, have been prepared and the role of hydrophilic polymers on the transport of liposome-entrapped hydrophilic substance into pig ear skin was investigated by one-dimensional electron paramagnetic resonance imaging (1D-EPRI) and reduction kinetic imaging methods. Using conventional EPR the influence of polymers on liposome stability was also determined. Multilamellar liposomes composed basically of hydrogenated soya lecithin and cholesterol, with the entrapped hydrophilic paramagnetic probe ASL (*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-*N*-dimethyl-*N*-hydroxyethylammonium iodide), were prepared in water solution and then mixed into hydrogels with different concentrations of hydrophilic polymers: carboxymethylcellulose or xanthan gum. It was found that the polymers examined do not prevent efficient transport of liposome-entrapped substances into the skin. From the on-going stability study of liposomes in hydrogel formulations, it follows that carboxymethycellulose does not influence the stability of liposomes during the time course of 6 weeks, while in xanthan hydrogels leakage of liposome-entrapped hydrophilic substance was observed already after 10 days. The results support the use of hydrogels in liposome dermatics, but it should be taken into account that some hydrophilic polymers can influence the physical stability of liposomes appreciably.

Keywords: Liposome formulation; Skin; EPR; Topical application; Liposome stability

1. Introduction

The application of liposomes on the skin surface has been proven to be efficient in delivery of liposome-entrapped drugs into the skin (Schäfer-Korting et al., 1989). Liposomes increase the permeability of skin for various entrapped drugs and at the same time diminish the side effects of these drugs (Lasch and Wholrab, 1986; Wholrab et al., 1989). Liposomes have also found an important application in cosmetics for skin care preparations (Wendel and Ghyczy, 1990).

Usually, liposomes are applied to the skin in solution or in hydrogels, since stable liposomal creams are difficult to formulate. For topical application of liposomes hydrophilic polymers are suitable thickening agents, since they make the formulations convenient for application. However, the type and concentration of polymer which

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forms the hydrogel could influence the stability as well as the rate of penetration of liposome-entrapped substances into the skin. As observed by some authors, hydrogels trapped the liposomes into a polymeric network and thus prevented efficient carrier diffusion to and into the skin (Cevc, 1992). On the other hand, we found efficient transport of liposome-entrapped substances into the skin from hydrogels prepared from xanthan gum (Gabrijelčič et al., 1990). In order to elucidate these discrepancies, in this work we investigated the influence of different hydrophilic polymers and their concentrations on the rate of penetration of liposome-entrapped hydrophilic spin probe into the skin as well as the stability of liposomes in hydrogel formulations.

Electron paramagnetic resonance (EPR) together with one-dimensional EPR imaging (1D-EPRI) and EPR kinetic imaging (Gabrijelčič et al., 1994) were used to follow the stability of liposomes in hydrogel formulations and the influence of hydrophilic polymers on the transport of liposome-entrapped substances into the skin.

2. Materials and methods

2.1. Liposomes

Multilamellar liposomes (MLV) were prepared basically from hydrogenated soya lecithin and cholesterol (60:35, wt%) in water by hand shaking. During preparation, the spin probe ASL (*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-*N*-dimethyl-*N*-hydroxyethylammonium iodide), which is readily soluble in water and due to its charge does not penetrate the liposomal membrane easily, was incorporated into the liposomes. The final concentration of ASL entrapped in liposomes was 0.01 mol/l. Uncaptured ASL was removed by dialysis at 4 or 20°C for 24 h. The lipid concentration in the liposome dispersion was 32 mg/ml.

2.2. Liposome-hydrogel formulations

For preparation of hydrogels, two hydrophilic polymers xanthan gum (0.5 and 1.0 wt%) and carboxymethylcellulose (CMC, 1.0 and 2.0 wt%) were used. The gels were prepared with a weighed amount of the polymer, which was swollen in deionised water. The final appropriate polymer concentration was adjusted by the addition of deionised water or liposome dispersion. The final concentration of lipids in liposome-hydrogel formulations was 24 mg/g. The formulations were stored at 4°C.

2.3. Skin

Skin samples were cut from pig ear delivered from a slaughterhouse and used not later than 12 h after killing of the animal. The ear sample was rinsed in water, the apex was cut through and for EPR measurements a slice approx. 1 mm thick, 5 mm wide and 10 mm long was placed in the tissue cell with the narrow surface mounted in contact with a 0.3 mm thick cotton thread soaked with the liposome formulation (Gabrijelčič et al., 1990).

2.4. EPR measurements

2.4.1. Stability study

The liposome formulation was mixed with a 0.1 mol/l sodium ascorbate (NaAsc) water solution in the ratio 1:1 (w/w), kept at room temperature for 30 min and EPR spectral intensity was measured by double integration of the EPR spectra on a Bruker ESP 300 EPR spectrometer, at room temperature.

NaAsc is a reducing agent which reduces the nitroxide spin probe to the EPR-invisible hydroxylamine, as reflected in the EPR spectral intensity decrease (Šentjurc et al., 1990). Neither NaAsc nor ASL penetrate the liposome membrane readily, due to their charges (Marsh and Watts, 1981). Therefore the EPR spectral intensity should be constant as long as the liposomes are stable. However, after liposome disintegration, ASL is released and reduced by NaAsc, as demonstrated by the EPR spectral intensity decrease.

2.4.2. Transport measurements

Transport of liposome-entrapped spin probe (ASL) into pig ear skin was measured by 1D-EPR

and EPR kinetic imaging. 1D-EPRI is based on the use of a conventional EPR spectrometer (Varian E-9, X-band) with an additional magnetic field gradient (0.6 T/m) parallel to the laboratory magnetic field. By this method it was possible to follow continuously the evolution of concentration profiles in skin of the total amount of spin probe (entrapped in liposomes and released from liposomes), monitoring the variations in lineshape of the EPR spectra, measured empirically by parameter l (Fig. 1) which reflects the asymmetry of the first absorption line of the spectra, and to calculate the corresponding penetration rates (D) (Demsar et al., 1988; Gabrijelčič et al., 1990). Using EPR kinetic imaging the rate of reduction by ascorbate or by oxy-redoxy systems in skin of the spin probe molecules, which had been released after the destruction of liposomes, was measured. From the reduction kinetics it was possible to calculate the evolution of concentration profiles in skin, separately for liposome-entrapped substance and for the released, free spin probe molecules, as well as to measure the decay rate of liposomes during their penetration into the skin. Concentration profiles calculated from the reduction kinetic imaging experiment were used to fit the lineshapes of the EPR spectra obtained by 1D-EPRI. This combination of two independent experiments provided a detailed insight into the transport characteristics of liposomes and liposome-entrapped substances (Gabrijelčič et al., 1994).

2.5. Viscosity measurements of hydrogels

Viscosity was measured with a rotational viscometer (Haake VT 500, MV2P system) at 20°C.

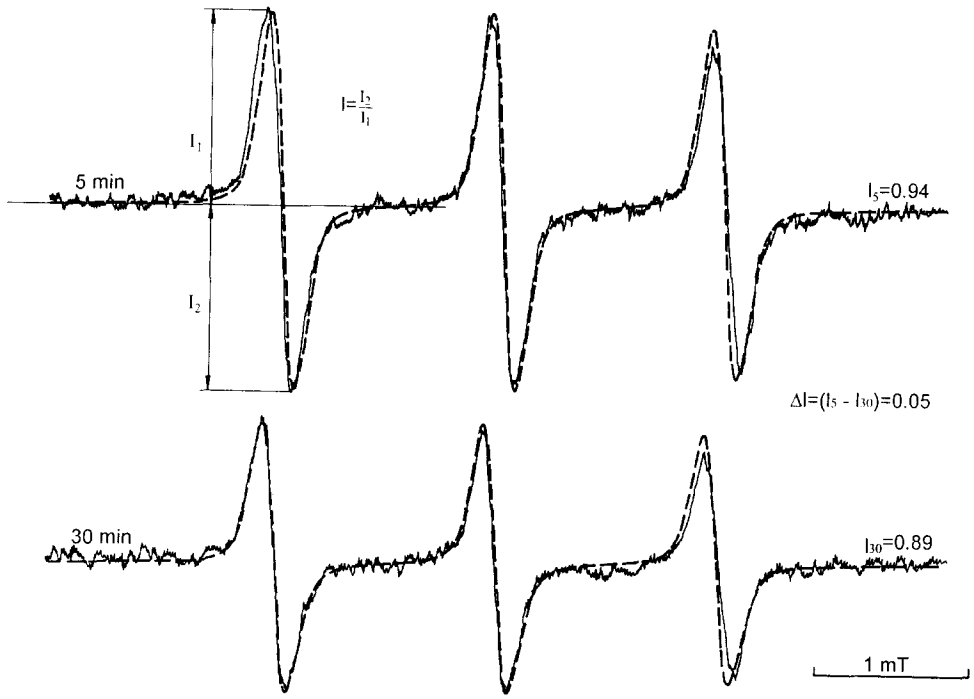

Fig. 1. 1D-EPRI spectra of ASL in the sample of pig ear skin 5 and 30 min after application of MLV from hydrogenated soya lecithin and cholesterol with entrapped 0.01 mol/l ASL. Full line represents the experimental spectra; parameters used: magnetic field gradient, 0.6 T/m; modulation amplitude, 0.1 mT; microwave power, 10 mW; sweep, 10 mT. The spectra were measured at room temperature. Dashed line represents the calculated spectra: concentration distribution function ($C(x,t)$) of ASL obtained from the reduction kinetic experiment was used. Spectra are superimposition of the spectra of released ASL (represented by a composite spectrum, Gaussian lineshape 95%) and the entrapped ASL (Lorentzian lineshape 70%, due to the spin-spin interaction between ASL molecules).

3. Results and discussion

3.1. Physical stability of liposomes in hydrogels

The relative EPR spectral intensity variations with time after preparation of the liposome formulations, i.e., liposomes in water, liposomes in 1% CMC, and liposomes in 0.5% xanthan hydrogel, are presented in Fig. 2. During the time period of 6 weeks, no changes in the EPR spectral intensity were observed for liposomes in water and liposomes in 1% CMC formulations, indicating that in this time period ASL was not released from liposomes. However, for liposomes in xanthan hydrogel the EPR spectral intensity starts to decrease as soon as 10 days after preparation of liposome formulations, indicating that the liposomes in this formulations already begin to disintegrate after such a short period. 50% decrease in ASL concentration ($t_{1/2}$) was reached within only 22 days after preparation of liposome formulations.

In control experiments in which hydrogels with 0.01 mol/l ASL were mixed with 0.1 mol/l NaAsc and incubated for 30 min, no EPR spectrum of ASL was observed, proving that the hydrogel network does not prevent reduction of ASL released from liposomes by NaAsc. Similarly, when

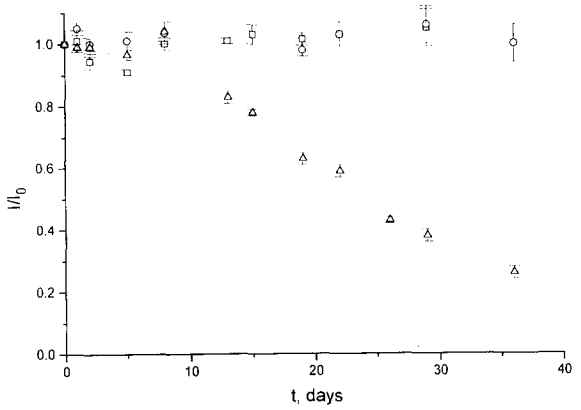

Fig. 2. Relative EPR spectral intensity variations (I/I_0) with time after preparation of liposome formulations: (○) liposomes in water, (△) liposomes in 0.5% xanthan hydrogel and (□) liposomes in 1% CMC hydrogel. I_0 is the intensity measured immediately after preparation of liposome formulation.

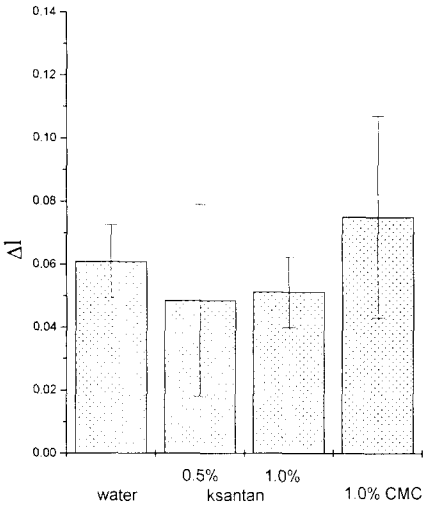

Fig. 3. Difference in parameter l, 5 and 25 min after application of different liposome formulation to the skin surface (Δl). Bars denote standard deviations for three independent measurements. At a shear velocity of 120 s^{-1}, the viscosity of 0.5% xanthan hydrogel is 0.142 Pa s, for 1% xanthan hydrogel, 0.325 Pa s and for 1% CMC, 0.327 Pa s.

the liposomes in hydrogel formulations were intentionally destroyed by freeze-thawing, a significant decrease in EPR spectra was observed after incubation of the formulations with NaAsc. These control experiments demonstrate that the EPR stability assay performed is adequate for the measurement of liposome disintegration. We can conclude that after several weeks hydrogel made from carboxymethylcellulose does not influence the stability of the hydrogenated soya lecithin-cholesterol liposomes investigated and appears to be a convenient vehicle for liposome formulations, while xanthan gum seems to be less suitable for this purpose.

3.2. Transport of liposomes from hydrogels into the skin

Employing 1D-EPRI the parameter l, which indicates the asymmetry of the EPR spectral lines due to the concentration distribution of spin probe in the skin, was measured (Fig. 1). The variation in parameter l with time provides data on the rate of transport of the spin probe into the skin. In Fig. 3, the difference in parameter l (Δl),

5 and 25 min after application of the liposome formulation to the skin, is presented for different liposome formulations. The results show that ASL, applied to the skin entrapped in liposomes, can penetrate into the skin irrespective of the formulation used; the same decrease (within the order of experimental error) in parameter l with time is observed for liposome-entrapped ASL in water, in xanthan (0.5 and 1%) or in CMC (1%) hydrogels regardless of the different viscosities of the formulations (values given in the legend to Fig. 3).

The rates of liposome decay and of penetration of ASL (released from liposomes and entrapped in the liposomes) found by EPR kinetic imaging experiment were calculated and are presented in Table 1 for liposomes in water and xanthan hydrogels (0.5 and 1%). Using the rate constants listed the reduction kinetic curves (Fig. 4) and the corresponding 1D-EPRI spectral lineshapes (Fig. 1) were adequately fitted. Comparing the results obtained with two different concentrations of xanthan gum (0.5 and 1%) it is interesting to note (Fig. 4, + and □) that its concentration does not influence the transport of liposome-entrapped substances into the skin significantly, in spite of increased viscosity at higher concentration of hydrophilic polymer. Moreover, these results demonstrate that the transport of liposome-entrapped hydrophilic molecules into the skin was not prevented by a polymeric network.

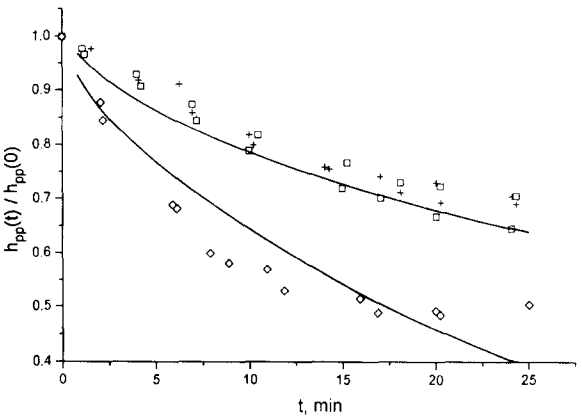

Fig. 4. Kinetics of the EPR spectral amplitude decrease ($h_{pp}(t)/h_{pp}(0)$) of ASL applied on the skin surface of the pig ear skin in: (◇) liposomes in water, (□) liposomes in 0.5% xanthan hydrogel and (+) liposomes in 1% xanthan hydrogel. The marks are the measured amplitudes of the $m = 1$ spectral component. Solid lines are the best fits of the experimental kinetic curves, which also fulfil the condition that the calculated distribution function of ASL ($C(x,t)$), which is used for the EPR spectral lineshape calculation, gives the best fit of the experimental 1D-EPRI spectra. The parameters used in the calculation are presented in Table 1.

When the liposomes were applied to the skin in hydrogels instead of water the penetration rate in thread decreased by a factor of about 2 as is evident from Table 1. This could be expected with respect to the restrictions caused by the polymeric network of hydrogels (Kristl et al., 1992). In skin layers the penetration rates were

Table 1
The parameters used in the calculation of the kinetics of the EPR spectral amplitude decrease (Gabrijelčič et al., 1994), by which the best fits of the experimental curves (in Fig. 1 and 4) were obtained

		ASL in H_2O	MLV with ASL in H_2O	MLV with ASL in xanthan
Rate constant for liposome decay k_2 (s^{-1})	thread	–	0.0	0.0
	stratum corneum	–	0.015	0.0075
	viable skin	–	0.00002	0.00002
Diffusion coefficient of ASL D ($cm^2\ s^{-1}$)	thread	6.0×10^{-7}	2.0×10^{-7}	0.9×10^{-7}
	stratum corneum	0.5×10^{-7}	50.0×10^{-7}	50.0×10^{-7}
	viable skin	0.8×10^{-7}	5.0×10^{-7}	5.0×10^{-7}
Internal volume (ml/ml)		–	0.16	0.16

For calculation the three-compartment model was used: thread, stratum corneum and viable skin. The rate constant of ASL reduction $k_1 = 130$ l mol^{-1} s^{-1}, is taken to be the same in the stratum corneum and in viable skin, while the concentration of reducing agent in stratum corneum is 10^{-2} mol/l and in viable skin 5×10^{-4} mol/l, as calculated from the best fit with the reduction kinetic curve for ASL solution.

not affected by hydrogels. Our results also indicate that hydrogels diminish the rate of liposome decay in stratum corneum. This observation will be examined in more detail in subsequent work from this laboratory.

As a result of the favourable visco-elastic properties of hydrogels, liposome formulations are handy for topical application. Our results, which show that hydrophilic polymers such as xanthan (0.5 and 1% wt:wt) and CMC (1 and 2% wt:wt) do not prevent efficient transport of hydrogenated soya lecithin-cholesterol liposomes into the skin, support their use in liposome dermatics. It should be taken into account that hydrophilic polymers can influence the physical stability of some types of liposomes. Therefore, for each liposome preparation their stability in hydrogels should be controlled.

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