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Note

Influence of liposomal formulation parameters on the in vitro absorption of methyl nicotinate

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Abstract

In the present study the influence of liposomal formulations on the in vitro absorption of methyl nicotinate (MN) taken as model drug was investigated. Special attention was paid to the possible correlations between the characteristics of the liposome formulation and its 'in vitro' performance. The preparation of a set of MN-containing liposomal formulations is described; these differ in soybean phosphatidylcholine (PC) concentration, lipid charge (by using either cationic or anionic surfactants), vesicle size and viscosity. Liposomes were prepared as aqueous suspensions, or as viscous gels using the acrylic polymer carbomer. The in vitro determination of MN permeability when released from the different liposomal formulations was performed using a Franz cell assembled with a synthetic silastic/cellulose/silastic multimembrane system. The results indicated that MN permeability was directly related to PC concentration and inversely related to liposome size and to vehicle viscosity; the liposome charge only slightly influenced the in vitro absorption of MN.

Keywords: Liposomal formulation; Methyl nicotinate; Soybean phosphatidylcholine

It is well known that liposomes represent an interesting approach to the administration of active substances to the skin. This feature arises from at least two contradictory properties of liposomes. Firstly, liposomes can act as permeability enhancers, increasing the percutaneous absorption of drugs administered to obtain systemic effects (Foldvari et al., 1990). On the other hand, in the case of drugs that should act topically (i.e. at the epidermal or dermal level), liposome-based formulations are known to localize the active substance at skin level, acting as a drug reservoir (Rougier et al., 1985, Du Plessis et al., 1992, 1994, Gabrijelcic et al., 1994).

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This contrasting behaviour could be accounted on the basis of differences in the formulations such as liposome size, lipid charge, lipid concentration and viscosity.

In a previously published paper we evaluated the influence of liposomes and phospholipid-based microemulsion on the in vitro and in vivo skin absorption of methyl nicotinate (MN) chosen as model drug (Bonina et al., 1995).

In order to evaluate better the performance of liposomes as topical forms, in this paper we focus our attention on the possible correlations between the characteristics of the liposome formulation and its 'in vitro' performance.

For the design of a liposome-based formulation, the selection of lipid composition and additives must be made carefully to guarantee liposome integrity in the final product. In particular, the presence of surfactants in emulsion formulations can be a considerable problem for the stability of liposomes with respect to their molecular assembly structure (Nastruzzi et al., 1993a); nevertheless, the liposomal suspension should have a viscosity enabling its permanence on the skin surface. For these reasons we designed and produced surfactant-free formulations based on carbomer hydrophilic gel, thus enabling the viscosity of the liposomal suspensions to be adjusted adequately.

Summarizing, in the present report are described: (1) the preparation of a set of MN containing liposomal formulations differing for soybean phosphatidylcholine (PC) concentration, lipid charge (by the presence of cationic or anionic surfactants), vesicle size and viscosity; (2) the 'in vitro' determination of MN absorption from the different liposomal formulations.

Liposomes were prepared using the reverse-phase evaporation method (Szoka and Papahadjopoulos, 1978). Briefly, soybean lecithin

Table 1
In vitro permeability coefficients of methyl nicotinate incorporated into different liposomal formulations

Liposomal formulation	PC (mg/ml)	Extrusion size (nm)	Carbomer (%, w/w)	J_n (cm/h $\times 10^3$)	S.D.
Aqueous formulations					
Neutral liposomes	25	–	–	6.6	1.2
	50	–	–	7.7	0.9
	75	–	–	10.1	1.6
Negative liposomes	50	–	–	9.8	1.4
Positive liposomes	50	–	–	9.3	1.2
Neutral liposomes	50	400	–	8.0	0.7
	50	200	–	9.7	1.3
	50	100	–	15.3	0.8
	50	50	–	13.7	1.2
Viscosized formulations					
Neutral liposomes	25	–	0.75	6.1	0.7
	50	–	0.75	9.2	1.9
	75	–	0.75	10.2	1.5
Negative liposomes	50	–	0.75	7.2	0.8
Positive liposomes	50	–	0.75	7.9	1.8
Neutral liposomes	50	400	0.75	5.8	0.5
Neutral liposomes	50	200	1	4.8	0.6
	50	200	0.75	5.3	0.5
	50	200	0.5	7.3	0.4
Neutral liposomes	50	100	0.75	8.7	0.8
	50	50	0.75	8.2	0.5

The determinations were performed using a multimembrane system composed of a cellulose mixed-ester membrane sandwiched between two silastic membranes. The reported results represent the average of six independent experiments.
S.D., standard deviation.

(Epikuron 200; Lucas Meyer, Hamburg, Germany) and cholesterol (8:2, mol/mol) were solubilized in methanol–methylene chloride (1:1, v/v), then the organic phase was dried to form a lipid film. The film was dissolved in 40 ml of diethyl ether; to this solution 10 mg of MN were added, then the two-phase system was sonicated at 0°C for 10 min and the ether was removed at room temperature by rotary evaporation.

The MN concentration in the liposome suspension was 1% (w/w). Liposomes were then diluted 1:1 (w/w) with water or carbomer gels, resulting in a final MN concentration of 0.5% (w/w), while the lipid concentration was 25, 50 or 75 mg/ml.

Positively and negatively charged liposomes were obtained by using dioctadecyldimethyl ammonium bromide and dicetyl phosphate, respectively.

In order to obtain homogeneously sized vesicles, liposomal suspensions were subjected to five extrusion cycles through polycarbonate filters. The extruded liposomes were named VET_n, according to Mayer et al. (1986), where VET indicates vesicles produced by extrusion techniques and the subscript number the pore size of the membrane used for the extrusion.

The gels used for viscosizing liposomes were prepared by placing carbomer (Carbopol 934P®; BF Goodrich, Cleveland, OH) in water and leaving it to swell at room temperature. After an overnight incubation, triethanolamine was added to neutralize the solution. After dilution with liposome suspensions, the use of carbomer resulted in the formation of a transparent jellied liposomal form.

The in vitro permeation analyses were carried out using a Franz-type glass diffusion cell assembled with a synthetic silastic/cellulose/silastic multimembrane system as previously reported (Nastruzzi et al., 1993b, Bonina et al., 1995). The multimembrane system consists of a hydrophilic cellulose ester membrane sandwiched between two lipophylic silastic membranes which satisfactorily reproduces the lipophilic–hydrophilic structure of human skin, mimicking the stratum corneum barrier properties.

The system was found to be appropriate for simulating the dermal absorption of MN

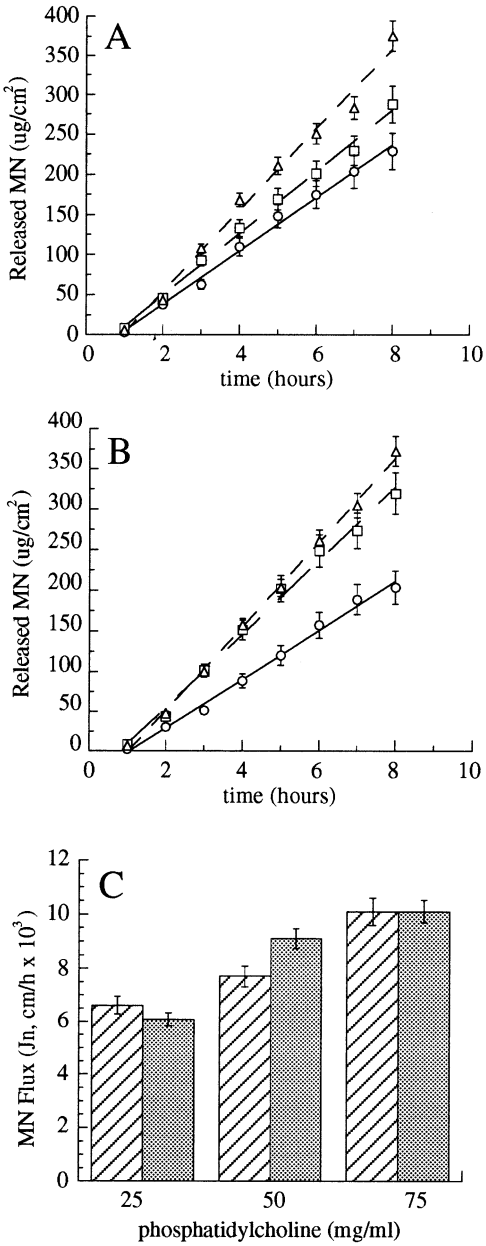

Fig. 1. In vitro release kinetics of MN from liposome suspensions (A) or in liposome suspensions viscosized by Carbomer (B). PC concentrations were 25 (○), 50 (□) or 75 (△) mg/ml. (C) PC effect on J_n values for MN incorporated into aqueous liposomes (square with right diagonal lines) or into viscosized liposomes (square with criss-cross lines). All the data represent six independent experiments; the mean ± standard deviation values were calculated.

(Nastruzzi et al., 1993b, Bonina et al., 1995), enabling differentiation of the physicochemical-dependent properties of permeation from the permeation processes that depend on the biological properties of the skin, and providing a quality control tool to ascertain batch-to-batch uniformity; in this respect, it represents a reliable method for studying the influence of liposomal formulation on the 'in vitro' release of MN.

A 1-g amount of liposomal formulation was placed into the donor cell compartment, and the MN concentration in samples of receptor phase was measured using a high-performance liquid chromatographic analytical procedure (Nastruzzi et al., 1993b). To calculate the permeability coefficients, the amount of MN that penetrated through the membrane(s) per unit area was plotted against time and the slopes were calculated by linear regression. It should be emphasized that the calculated regression coefficients were never less than 0.97. The slopes were then substituted into the equation $J_n = J_o/C$, where J_n is the permeability coefficient (normalized flux), J_o is the observed flux and C is the MN concentration (mg/ml). In Table 1 are reported the J_n values for all the in vitro experiments performed.

The influence of lipid concentration on MN in vitro absorption was determined testing liposomes of 25, 50 and 75 mg/ml PC. From the analyses of MN diffusion kinetics (reported in Fig. 1A) it is evident that J_n increases with increasing PC concentration. This trend was confirmed when viscosized forms were analysed (Fig. 1B). Fig. 1C summarizes the data obtained, showing that the viscosity does not influence MN permeability to a great extent. In order to explain this behaviour, it can be hypothesized that, in the case of non-extruded vesicles, the limiting step to MN permeability is vesicle size rather than the viscosity of the formulation.

The influence of lipid charge on MN permeability was tested on positively and negatively charged liposomes. Both positive and negative liposomes displayed slightly higher J_n values (9.76 and 9.26 cm/h $\times 10^3$, respectively) when compared with the neutral ones (see Fig. 2A). Again the viscosized forms showed minimal variations in J_n values (Fig. 2B). In this case, the situation is

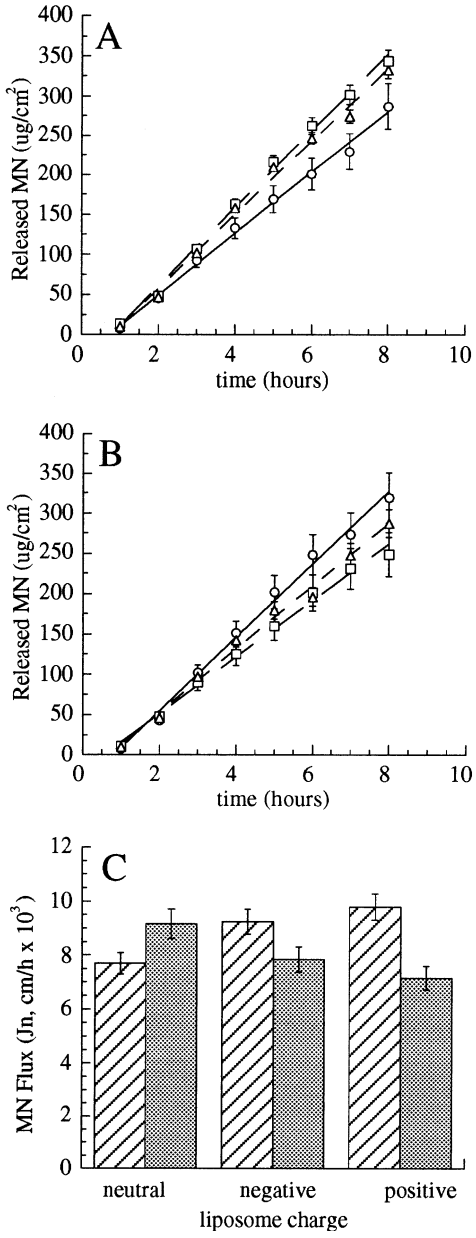

Fig. 2. In vitro release kinetics of MN from liposome suspensions (A) or in liposome suspensions viscosized by Carbomer (B). PC concentration was always 50 mg/ml. Liposomes were neutral (○), positively (□) or negatively (△) charged. (C) Effect of liposome charge on J_n values for MN incorporated into aqueous liposomes (square with right diagonal lines) or viscosized liposomes (square with criss-cross lines). All the data represent six independent experiments; the mean ± standard deviation values were calculated.

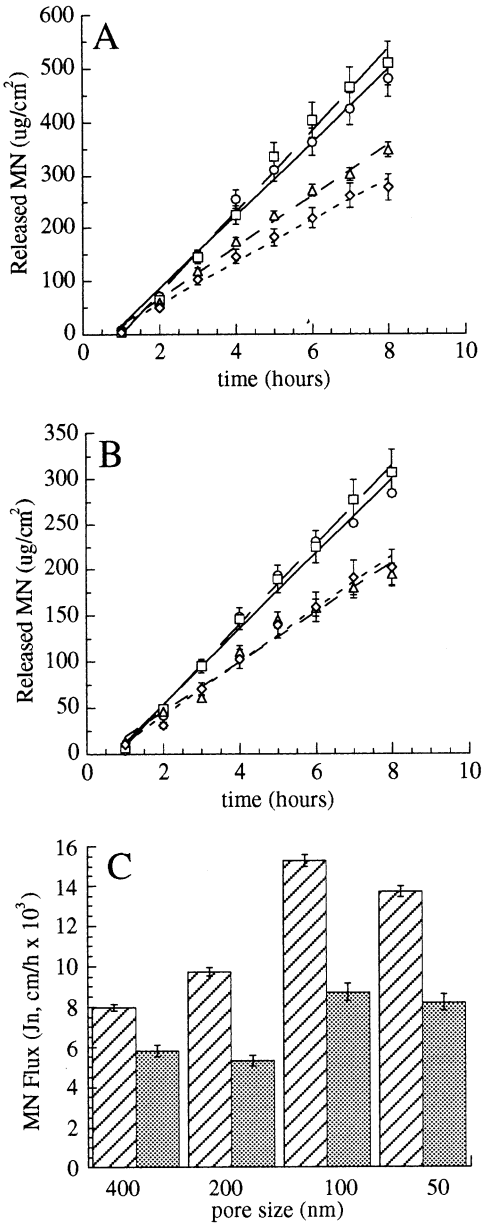

Fig. 3. In vitro release kinetics of MN from liposome suspensions (A) or in liposome suspensions viscosized by Carbomer (B). PC concentration was always 50 mg/ml. Liposomal suspensions were subjected to five extrusion cycles through polycarbonate filters with 50 (○), 100 (□), 200 (△) or 400 (◇) nm pore size. (C) Effect of liposome size on J_n values for MN incorporated into aqueous liposomes (square with right diagonal lines) or viscosized liposomes (square with criss-cross lines). All the data represent six independent experiments; the mean ± standard deviation values were calculated.

reversed, the neutral liposomes having the higher Jn, whilst charged forms displayed lower J_n values (see Table 1) with minimal variations.

The effect of liposome size on MN in vitro permeability was studied using liposome extruded through polycarbonate filters with pores of 400, 200, 100 and 50 nm (namely, VET_{400}, VET_{200}, VET_{100} and VET_{50}).

From the analysis of the data reported in Table 1 and Fig. 3A two major conclusions can be reached: (1) J_n values for extruded vesicles are higher than those of non-extruded vesicles, and (2) J_n is inversely related to liposome size. The lower J_n value found for VET_{50} (versus VET_{100}) was tentatively attributed to possible aggregation phenomena between vesicles. In Fig. 3B are reported the data for viscosized formulations, confirming the above described trend, even though the J_n values for viscosized forms are smaller than those of aqueous suspensions. Here, in fact, the limiting step to the MN permeability is no longer represented by the vesicle size but by the viscosity of the formulation.

The influence of gel viscosity on MN permeation was studied in more detail by using carbomer gels at different concentrations. As is clearly evident from the results reported in Fig. 4, the J_n of MN is inversely related to the vehicle viscosity. In fact, J_n values range from 9.73 cm/h $\times 10^3$ for the 0% carbomer gel to 4.75 cm/h $\times 10^3$ for the 1% (w/w) carbomer gel.

To summarize, the results reported in the present paper indicate that (1) the permeability of MN from liposomal formulations increases with increasing PC concentration, (2) liposome charge only slightly influences the in vitro permeability of MN, and (3) the permeability of MN is inversely related to vesicle size and to vehicle viscosity.

In addition, we have demonstrated that, in the case of non-extruded vesicles, the limiting step to MN permeability was related to vesicle size rather than to vehicle viscosity, while in the case of extruded vesicles the limiting step was the viscosity of the formulation.

In conclusion, the utilization of liposomal formulations with different characteristics could

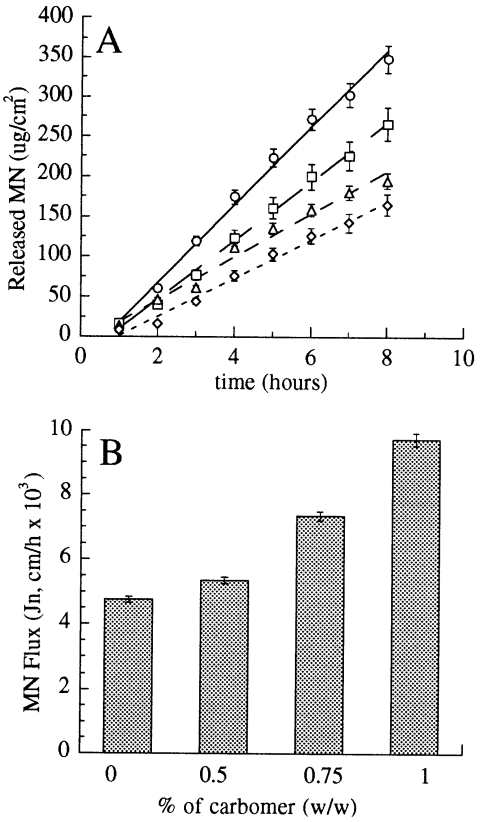

Fig. 4. (A) In vitro release kinetics of MN from liposome suspensions incorporated into 0% (○), 0.5% (□), 0.75% (△) or 1% (◇) (w/w) Carbomer gels. PC concentration was always 50 mg/ml. Liposomes were extruded through polycarbonate filters with 200 nm pore size. (B) Effect of gel viscosity on J_n values for MN incorporated into Carbomer gels. All the data represent six independent experiments; the mean value ± standard deviation values were calculated.

modulate drug permeability, allowing different therapeutic strategies involving either systemic absorption or skin localization of the administered drug.

Acknowledgements

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