

**BLOOD FLOW ENHANCEMENT IN SKIN OR ORAL MUCOSA AFTER
THE TOPICAL APPLICATION OF LIPOSOME ENTRAPPED
RUBIFACIENT AS MEASURED BY EPR OXIMETRY *IN VIVO*: THE
INFLUENCE OF SIZE AND LIPOSOME COMPOSITION**

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Many studies performed in the last decade showed significantly higher absorption rates and a greater local pharmacological effect for drugs applied to the skin entrapped in liposomes as compared to conventional topical formulation [1]. It is also well established that liposome bilayer properties play an important role in transport. But there is a lack of methods by which it is possible to directly measure the response of the organism to the topically applied drug *in vivo*. One such method is electron paramagnetic resonance (EPR) oximetry *in vivo*. In this study, EPR oximetry was used to measure the difference in the response of the body to a vasodilator drug, which was applied to the skin or oral mucosa entrapped in different types of liposomes.

By EPR oximetry, the partial pressure of oxygen (pO_2) in tissues *in vivo* is measured. For this purpose, a paramagnetic probe has to be introduced into the tissue and its EPR spectra measured. This method is based on the fact that molecular oxygen is paramagnetic and due to its interaction with the paramagnetic probe, it broadens the EPR spectra line-width [2].

In our experiment, two different types of liposomes were used: Hydrogenated soy lecithin (Emulmetec 320) cholesterol (70:30 wt%, total weight 48 mg/ml) (HSL), which, according to *in vitro* EPR measurements, facilitates transport into the skin in the first 30 min after application. The other one had the same composition but instead of hydrogenated SL, natural soy lecithin (phospholipon 80) (NSL) was used, which, according to *in vitro* studies, does not facilitate transport in such a short time [3]. MLV and LUVET obtained by extrusion of MLV through polycarbonate filters with a pore diameter from 800 to 100 nm were used. A vasodilator benzyl nicotinate (BN, 0.83 wt%) was entrapped into the liposomes as a lipophilic drug that can serve as a marker by which the difference in the response of the organism due to different formulations can be followed. Liposomes were mixed with hydrogel hydroxyethylcellulose.

As a paramagnetic probe, lithium phthalocyanine was introduced into the skin of anaesthetised mice or the oral mucosa of rats, and its EPR spectra were measured over time after the topical application of the liposomal preparation with the entrapped vasodilator BN, and after repeated application of the preparations. The measurements *in vivo* were performed with a low frequency EPR spectrometer (1.1 GHz) using surface coil detectors.

The typical time course of benzylnicotinate action on the oxygenation of the skin after the topical application of BN mixed in hydrogel to a mouse is presented in the Figure 1.

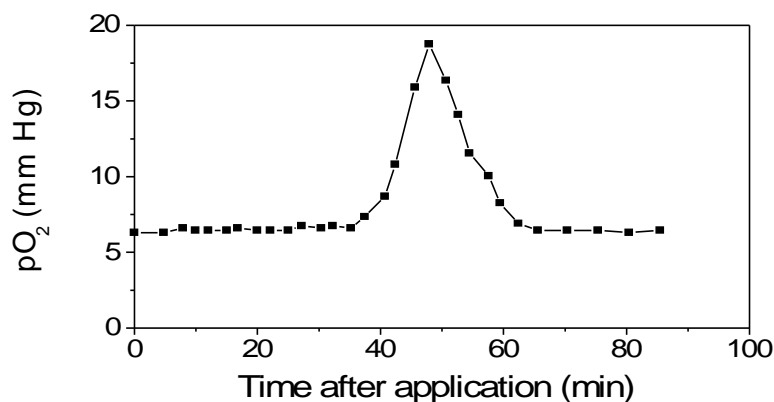


Fig. 1. The time course of pO₂ alteration in mouse skin after the application of 0.83 wt% benzylnicotinate mixed in hydrogel.

From the individual time dependence curves, several parameters were determined, which characterize the time response of an individual animal to a topical application of BN in different formulations. These are: the maximal relative increase of pO₂ due to the action of BN (ΔpO_{2max}), the time expired from the application of the formulation and the time when pO₂ starts to increase (lag time t_{lag}), the time when maximal increase in pO₂ is achieved (t_{max}), and the area under the curve (AUC) which measures the efficacy of BN action.

It was found that the type of formulation strongly influences the action of the active compound. The fastest response is observed with liposomes from hydrogenated soy lecithin ($t_{lag} = 9$ min). It is slower for liposomes from non-hydrogenated soy lecithin ($t_{lag} = 17$ min) and is the slowest for free BN ($t_{lag} = 39$ min). This concurs well with our previous *in vitro* measurements, which have shown rapid transport of the entrapped substance into the deeper skin layer within 30 min of the application of the drug entrapped in HSL, while for NSL, only transport into the stratum corneum was detected in this time period [3]. Correspondingly, the time when the maximal effect is achieved also changes. Maximal pO₂ is within experimental error for all three formulations, showing that liposomes do not influence the vasodilatory action of BN, only the kinetics, while the efficacy of BN action (AUC) is slightly greater when applied in liposomes, especially in HSL, than in hydrogel. The size of liposomes also influences the lag time and especially the duration of the application. Some differences are observed between the reaction of the skin and of the oral mucosa

to the action of BN, but the general trend concerning the influence of liposomal preparations is the same.

The action of BN incorporated in HSL on pO_2 was also followed during its daily application to mouse skin for a period of 5 days. A systematic increase in pO_2 in the skin tissue base line, measured before each day's application, was observed with successive applications. The total increase in the base line was 6.9 mmHg. The increase in pO_2 after the application of HSL is greater in the first three days than in the following days. With repeated application of HSL, the response of the organism becomes faster and t_{lag} and t_{max} decrease with time. The third day after stopping the application, the pO_2 in the skin was the same as before the first application.

By EPR oximetry *in vivo*, we were able to directly follow the time course of drug action on skin oxygenation when applied to the skin in different topical formulations non-invasively for a longer period of time at the same site. Exact information about the dose dependence of the drug and about the influence of subsequent applications over several days on the oxygen level in skin can be determined. From these observations the semi-solid dosage form can be formulated in such a way that the best efficiency of the drug is achieved.

In our opinion this is the first method which directly shows the efficacy of topical application via liposomes *in vivo*, and which can also be used generally for other types of carriers used in the topical application of drugs and cosmetic products. Benzyl nicotinate in such studies could be used as a lipophilic marker for the investigation of the effectiveness of different carriers.

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