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Gel-like microemulsion for simultaneous delivery of vitamins C and E for skin antioxidant protection

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Introduction

Microemulsions (ME) – nanostructured systems composed of water, oil and surfactants – have frequently been used to increase cutaneous drug delivery. They are superior to conventional vehicles like emulsions or hydrogels for dermal delivery of hydrophilic and especially lipophilic drugs. Since ME are usually low viscosity liquids, it is important to ensure that they are easy aplicable and adhere to skin sufficiently (1). Optimizing rheological behaviour is therefore one of the crucial steps in development of topical ME. It is known that from o/w ME under certain conditions upon the addition of specific amounts of water transparent microemulsion gels can be formed (2,3). However, the most usual way to optimise the rheological behaviour is addition of thickening agent that increases the viscosity of the system without affecting its stability and spontaneous formation (4).

The aim of this work was to develop and evaluate microemulsion gel (gel – like ME) – a system with higher viscosity which is only due to the high proportion of aqueous phase - as an effective and safe carrier system enabling simultaneous delivery of hydrophilic vitamin C and lipophilic vitamin E into the skin. Gel-like ME was compared to liquid o/w ME and o/w ME conventionally thickened with addition of polymer (o/w ME carbomer).

Results

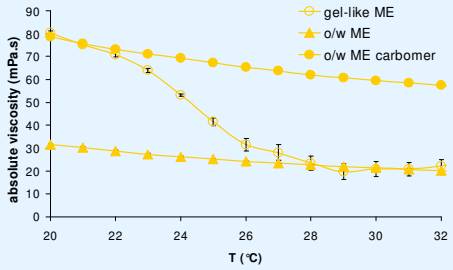

Fig.1. Temperature dependence of absolute viscosity for gel-like ME, o/w ME and o/w ME thickened with carbomer.

Gel-like ME exhibited at 20ºC 3-fold higher viscosity than liquid o/w ME while the viscosity of carbomer thickened o/w ME was appr. the same and was estimated as appropriate for topical application. The viscosity of gel-like ME decreased drastically with T; at 32 ºC it was the same as that of o/w ME, confirming T driven changes in rheological behavior of gel-like ME.

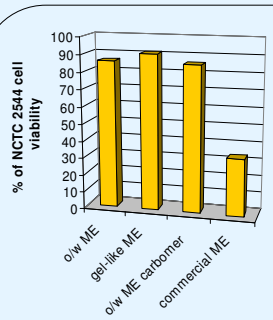

Fig. 3. Cell viability of keratinocyte cell line after 4 hours of contact with ME determined by MTT test.

MTT test on NCTC 2544 keratinocyte cell line showed that all tested ME are considerably less toxic than commercially available ME. No difference in cytotoxicity of tested ME was observed.

ME gel with temperature sensitive rheological behavior has been proven an effective and non irritant vehicle with functionally suitable consistency for simultaneous delivery of vitamins C and E into skin to provide antioxidant protection.

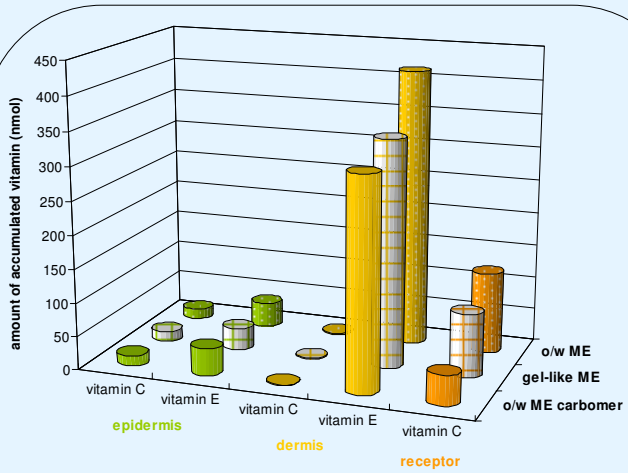

Fig. 2. The amounts of vitamins C and E accumulated in epidermis, dermis and receptor fluid after 6h of contact.

Permeation behaviour was evaluated by two parameters: vitamins concentration in skin layers (epidermis, dermis) and their permeation into receptor fluid. Gel-like ME delivered in epidermis app. the same amounts of both vitamins as other two tested ME. Concerning delivery into dermis and receptor fluid gel-like ME delivered fewer vitamins than o/w ME which can be explained by lower content of oily phase, a well-known permeation enhancer. However, it delivered more vitamins than o/w ME thickened with carbomer. Vitamin E was not found in receptor fluid.

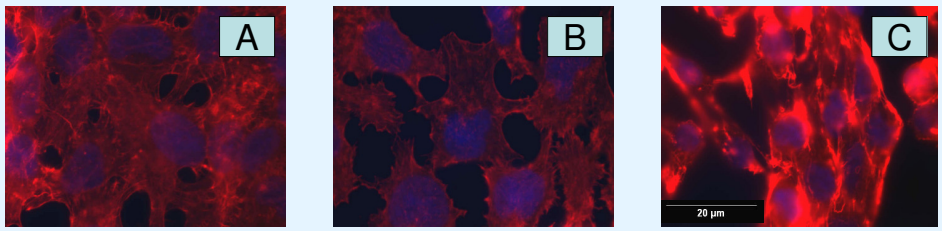

Fig. 4. Fluorescent micrographs of NCTC 2544 cells grown on a cover slip: A – treated with PBS (control), B – treated with gel-like ME, C – treated with commercial product.

As in MTT test no difference between control cells (Fig.4A) and cells treated with tested ME were observed. On Fig. 4B representative micrograph of NCTC 2544 cells treated with gel-like ME is presented. A striking difference was seen on cells, treated with commercial ME (Fig.4C).

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Experiments

Vitamins C and E (purchased from Sigma Aldrich) were incorporated in different ME in 0.4% and 1% concentration, respectively. o/w ME consisted of 30% of surfactant mixture (Imwitor 308: Tween 40= 1:1), 45% water and 25% isopropyl myristate (Fluka) as oily phase. It was thickened by adding 2.5% of carbomer (Carbopol 974 PNF, BF Goodrich). Gel-like ME contained 30% of surfactant mixture, 60% of water and 10% of isopropyl myristate.
The absolute dynamic viscosity of ME was determined in triplicate in a temperature range 20-40 °C using a SV-10 Vibro Viscosimeter, A&D Company, Japan.
In vitro permeation studies were performed on pig ear skin. The amounts of vitamins accumulated in epidermis and dermis and passed into receptor solution were determined using Franz diffusion cells and infinite dosing after 6 hours of contact. All samples were analyzed by HPLC.
Human keratinocyte cell line (NCTC 2544), cultured in supplemented EMEM, were treated with ME diluted in PBS (900 µg/ml) for 4h. Control cells were incubated with PBS. The effect of ME on the metabolic activity of cells was evaluated in vitro using the MTT test. The results were expressed as a fraction of the absorbance of untreated cells. Cell growth was determined using an inverted phase-contrast microscope (Olympus CKX41, Tokyo, Japan)

References. *1.* K. Welin-Berger et al. Eur J Pharm Sci. (2001). *2.* D. Libster et al. J Colloid Interface Sci. (2006). *3.* G. M. Eccleston. Microemulsions. In Encyclopedia of Pharmaceutical Technology (1994). *4.* P. Spiclin et al. Int J Pharm. (2003).