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Challenging the hydrophilicity of natural bacterial nanocellulose for dermal applications

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Introduction

The current advanced research demonstrated a high potential of the biopolymer bacterial nanocellulose (BNC) in a wide range of applications in medical and pharmaceutical fields due to its outstanding physiochemical and biological properties. BNC consist of a unique structure of a three-dimensional network of nanostructured cellulose fibres, with over 90% water, a high purity, excellent biocompatibility and unique mechanical stability that provides an excellent basis as a biomaterial for dermal applications [1]. Although many attempts were made using BNC as drug carrier material, incorporation of lipophilic substances is still considered as an unsolved task. In this study, the encapsulation of the lipophilic coenzyme Q10 (Q10) into the hydrophilic BNC utilizing dermal friendly and flexible colloidal carrier systems was investigated.

Materials and Methods

As carrier systems to encapsulate Q10 Hydro-Tops (w/o/w nanoemulsion), Lipo-Tops (o/w emulsion) and liposomes were produced using a high pressure homogenizer. Stability of the carrier systems, hydrodynamic diameter and zeta potential were investigated over 90 days. BNC fleeces were produced by strains of Komagataeibacter xylinus (DSM 14666) in Hestrin-Schramm medium in 24-well plates, harvested and alkaline purified [2]. Standard sorption method [3] and other post synthetic loading techniques were used. Release was studied at 32 °C using the Franz cell diffusion system. Penetration studies using porcine skin samples were preformed.

Results and Discussion

Production of different carrier systems containing 0.5% Q10 was successfully performed with negative zeta potentials and hydrodynamic diameters of about 65-130 nm with an excellent stability over 90 days. BNC fleeces were efficiently loaded with these systems by different post synthesis loading techniques. Q10 release could be controlled depending on the type of carrier system, the BNC condition (native or freeze dried), and the loading technique.

Conclusion

Successful loading of lipophilic substances into the hydropolymer BNC was achieved which opens a variety of applications in the pharmaceutical field. Moreover, drug release for custom-designed applications could be adjusted by variation of different parameters.

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SmartCrystals® – development of commercial concentrates for cosmetic industry

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SmartCrystals are a technology for efficient penetration enhancement of poorly soluble cosmetic and pharma actives [1]. Using this technology, molecules which are not active at all or much too low in activity can be made sufficiently bioavailable, e.g. anti-oxidants such as rutin. The trick is to reduce the insoluble micrometer crystals in size to the nanodimension (< 1000 nm = SmartCrystals) by a combination of bead milling and high pressure homogenization. By entering the nanodimension, the saturation solubility Cs increases strongly (e.g. factor of 10-100), and thus the diffusive flux from the dermal formulation with SmartCrystals into the skin. For example, the anti-oxidant activity of rutin could be increased in-vivo by a factor of 1,000 (human study).

To use this technology in products, SmartCrystals need to be available for purchase by companies. Apart from being IP-protected, the production process is too complex and thus to costly to be established for products in one cosmetic company. A number of years ago, large scale production was established for formulations using highly accepted pharmaceutical stabilizers (e.g. Tween 80, Poloxamer 188, even being i.v. injectable). However, nowadays in cosmetics PEG containing molecules are not preferred any more – one looks for skin-friendly, PEG-free alternatives. Thus a study was performed, screening of 10 different stabilizers with preferred application onto the skin, e.g. alkyl polyglycosides such as Plantacare®. A few stabilizers were identified which stabilized the produced crystals in a size range of about 400 nm, e.g. Plantacare® 810 UP, 1200 Up and Eumulgin® SG. From the short term stability by now (increase in size below 4% over 1 month) a long-term stability as SmartCrystal suspension of at least 1 year can be predicted (data by now).

The size of the SmartCrystals can be adjusted by the input of milling energy and milling time. In general, the size is in the submicron range, that means distinctly above 100 nm. The crystals are no nanoparticles (EU, US definition), but possess the properties of nanoparticles (e.g. saturation solubility increase adhesiveness onto the skin).

Rutin formulations are planned to be launched as SmartCrystal concentrates (5% active) in April this year. They can be simply admixed to any cosmetic product in the last step of production with a dilution factor of typically 50. The rutin concentrates will be available in the original composition (used in the human study) but also with other novel stabilizers. Also tailor-made products are possible with exclusive molecules of the cutomer.

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How to improve dermal penetration of poorly soluble actives?

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Introduction: Human skin is an important drug target, but not every drug is able to overcome the intact skin barrier. Various factors can hamper the successful topically application of pharmaceutical and cosmeceutical actives, for example their poor solubility due to the lipophilic character and associated poor bioavailability. Formulating poorly soluble drugs as nanocrystals (smartCrystals®) is an established strategy to increase their dermal availability [1]. A reduction in size leads to an increase in solubility and increased bioavailability [2]. To further promote the penetration efficacy of dermally applied drugs, penetration enhancers can be used. However, to date no systematic study has been conducted to demonstrate the influence of the size of nanocrystals and of penetration enhancers on the penetration efficacy of poorly soluble actives.

Material and Methods: The plant active hesperetin was used as model drug. Nanocrystals of different sizes (200 nm, 400 nm, 600 nm, 800 nm) were produced by high pressure homogenization, bead milling and combinations of these methods [3]. Skin penetration was studied by tape stripping: the formulation was applied on the skin of fresh porcine ears and after 30 min penetration time the tape stripping procedure was performed. Stratum corneum was removed layer by layer using 30 tape strips. The concentration of hesperetin on each tape was determined by using HPLC analysis. In the next step of this study the influence of penetration enhancers on the penetration efficacy was determined. Different actives were used to increase the penetration of hesperetin – urea (5%, 10% and 15% solution) as a moisturizer and hydrophilic active and olive oil as lipophilic excipient. The penetration enhancer was applied to the skin prior to the hesperetin nanosuspension. Skin bio-physical parameters, e.g. transepidermal water loss (TEWL), pH and skin hydration were also determined.

Results and Discussion: Results proved that both, the size of nanocrystals and the type of the penetration enhancer, have a tremendous influence on the penetration efficacy of the active. Penetration studies showed an increase in the penetration efficacy with decreasing size of nanocrystals, i.e. the highest amount of hesperetin penetrated into the stratum corneum by using nanocrystals with a size of about 200 nm. Results for the penetration enhancers were unexpected. In general urea is used as moisturizer and is known to enhance the penetration of many actives due to the improved hydration of the stratum corneum. However, in this study, instead of promoting the penetration of hesperetin, data show that urea even impairs the penetration efficacy of hesperetin. Results can be explained by the lipophilic nature of the model drug and by the theory of Neubert et al. [4], who suggested that urea can only enhance skin penetration for polar actives via improving the polar route of penetration. In contrast, olive oil – a lipophilic excipient - promoted the penetration of hesperetin, however only to a limited extent, when compared to the smartCrystals® in pure water. TEWL and skin hydration measurements confirmed

this theory.

Conclusion: Nanocrystals improve passive dermal penetration of poorly soluble actives in a size dependent manner - for effective dermal drug delivery small sized nanocrystals, i.e. sizes of about 200 nm should be preferred. Urea did not enhance the penetration efficacy of the lipophilic active hesperetin from nanocrystals. To further improve the dermal penetration of nanocrystals consisting of lipophilic actives, excipients and vehicles that improve the nonpolar penetration pathway, e.g. oils, should be used.

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Correlation between free aqueous preservative concentration in emulsion gels measured by equilibrium dialysis and antimicrobial efficacy

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Introduction: Antimicrobial testing is a time consuming and cost-intensive but essential method for evaluation of newly developed pharmaceutical formulations for topical use. Partition between oil and aqueous phase of semisolid formulations and the lipophilicity of the preservative are key factors for effective antimicrobial efficacy [1,2]. In this study the correlation between free preservative concentration measured by equilibrium dialysis and the successful preservative effectiveness testing (PET) for Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Aspergillus brasiliensis (analysed according to Ph. Eur. and USP [3,4]) was investigated.

Methods: Equilibrium dialysis was performed to calculate the free preservative concentration in the aqueous phase. Emulsion gels with varying oil phases (liquid paraffin/ medium chain triglycerides) and emulsifier types (PEG-21-stearylether, polysorbate 80) were prepared. PEG 400 was added to further formulations. Donor media contained 0.5% phenoxyethanol in the emulsion gels. Donor and acceptor medium (citric buffer) were filled in PTFE-cells (n=5) separated by a 5 kDa hydrophilic cellulose ester membrane and equilibrated at 25 °C for 24 h. The membranes were extracted in methanol. Additionally, partition coefficients between the oil phases and citric buffer were measured. Samples were analysed via UPLC with PDA detector. The phenoxyethanol concentration in the aqueous phase of the donor was calculated according to [1]. A two-sided t-test was used for statistical analysis (α=0.05). PET-test was performed according to Ph. Eur. 5.1.3.

Results: Medium chain triglycerides showed a significantly ($p<0.010$) higher partition coefficient for phenoxyethanol (log P 0.709 ± 0.070) than liquid paraffin (log P -0.746 ± 0.052) as it was a H-bond acceptor and therefore, increased the solubility in the oil phase.

The 5 kDa membrane allowed the transport of un-bound phenoxyethanol. Increased emulsifier concentrations reduced the free amount of the preservative due to micellar interactions. The addition of PEG 400 led to significantly increased preservative concentrations in the aqueous phase ($p<0.02$). The higher the lipophilicity of the oil phase and the lower the content of the aqueous phase with regard to dissolved ingredients, the more preferably phenoxyethanol is distributed to the water phase and, consequently, the higher was the efficacy against the microbes. Required phenoxyethanol concentrations in the aqueous phase for a successful PET-test

were: Ph. Eur.: 1.085±0.315%; USP: 0.640±0.407%. High standard deviations underlined the variability in the response of microorganisms. Aspergillus brasiliensis was the most resistant and Staphylococcus aureus the most sensitive microorganism for emulsion gels preserved with phenoxyethanol. The criteria of the pharmacopeias showed significant differences in the PET-test acceptance of the emulsion gels for the fungi as the USP had less stricter requirements.

Conclusion: Free preservative concentration can be measured with equilibrium dialysis. This method might be used as a predictive tool for estimation of the required preservative concentration for antimicrobial stability. Emulsifier concentrations and oil phase composition influenced the partition of preservative into the aqueous phase. For successful PET-testing Aspergillus brasiliensis should be used as the key factor as it is the most resistant microorganism for topical formulations.

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Glabridin smartPearls – selection of appropriate mesoporous particles & optimization of production conditions

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Glabridin can be extracted from the roots of Glycyrrhiza glabra. It efficiently inhibits the tyrosinase activity [1], therefore, can be used either in cosmetics against pigment disorders as a whitening agent with natural origins or in pharmaceutics as an API against melanoma. The obstacle for practical use of glabridin is its insufficient dermal bioavailability due to its poor solubility, e.g. in water. Attempts have already been made to increase its water solubility, for example chemical modifications [2]. However, the tyrosinase inhibiting properties drastically decreased. Also using another solvent, e.g. alcohols or high surfactant concentrations are not suitable for skin application. Therefore, up to now no satisfying solution were found.
One approach to increase the glabridin solubility in water and thus its dermal bioavailability without reducing its efficacy is the use of smartPearls as dermal delivery system. smartPearls are mesoporous silica particles in which an active can be loaded and long-term stabilized in amorphous state. Compared to the crystalline active, the solubility is pronouncedly increased [3]. In addition, pore sizes are in nm range. Consequently, the active is nano-dispersed, showing the same solubility increasing effects as for nanocrystals.
Therefore, aim of this study was to find appropriate mesoporous silica particles as well as suitable conditions to produce stable glabridin loaded smartPearls.

Silica particles with different pore sizes (3, 6, 10, 17 nm) were loaded with glabridin by the solvent evaporation method. Means, defined amounts of ethanolic glabridin solutions (ratio 9:1) were added to respective silica particles followed by controlled ethanol evaporation at 150 mbar and 40 °C for at least one hour. The so obtained loaded silica particles were further dried for 12 h under vacuum to obtain the final glabridin smartPearls. Amorphous state of loaded glabridin was confirmed by dynamic scanning calorimetry (DSC), showing the absence of crystallinity peaks in respective thermograms. Localization of glabridin in the different sized pores was verified by nitrogen ad- and desorption, relating the BET surface, pore volume and pore size. Difference of theoretical and real loading was investigated by high pressure liquid chromatography (HPLC). For storage stability investigations, all measurements were repeated after one week and one month.

HPLC showed that both theoretical and real loading of 36% are congruent with each other, proving that the solvent evaporation method is gentle and even suitable for loading chemically highly sensitive actives e.g. glabridin. However, DSC thermograms revealed that not all silica were suitable for the production of glabridin smartPearls. Silica with pore sizes of 3 and 17 nm had crystallinity peaks, indicating the presence of a non-amorphous glabridin fraction. In contrast, silica with pore sizes of 6 and 10 nm were indeed able to form crystalline free smartPearls and

stabilize this state for 1 month up to now. Therefore, it can be concluded, that the successful loading is much affected by the ratio of pore size to molecule size and should be considered beforehand to select the most suitable silica. Nitrogen ad- and desorption revealed that the pores of the smartPearls were evenly filled from bottom to top, having a concave shape. Due to the nano-sized pores the surface area of glabridin is increased. Following the Noyes Whitney equation, an increased saturation solubility as well as dissolution velocity can be assumed. Further, the strongly curved surface of the concave shaped interface will lead to an increased dissolution pressure according to the Kelvin equation. Altogether, a supersaturated state of glabridin will be reached, finally increasing the dermal bioavailability.

Summarizing, the solvent evaporation method is suitable for smartPearls production even for the chemically labile active glabridin. A high loading of 36% can be achieved in one loading step. Silica for loading should be selected carefully beforehand in dependence of the pore size and the to be loaded active. Further investigations will follow to confirm the long-term storage stability of amorphous state as well as the resulting increased saturation solubility and dermal bioavailability of glabridin smartPearls.

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