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Biodegradable Surfactants for Advanced Drug Delivery Strategies

C. Hönnscheidt and R. Krull

***Abstract*—**Oxidative stress makes up common incidents in eukaryotic metabolism. The presence of diverse components disturbing the equilibrium during oxygen metabolism increases oxidative damage unspecifically in living cells. Body´s own ubiquinone (Q_{10}) seems to be a promising drug in defending the heightened appearance of reactive oxygen species (ROS). Though, its lipophilic properties require a new strategy in drug formulation to overcome their low bioavailability. Consequently, the manufacture of heterogeneous nanodispersions is in focus for medical applications. The composition of conventional nanodispersions is made up of a drug-consisting core and a surfactive agent, also named as surfactant. Long-termed encapsulation of the surfactive components into tissues might be the consequence of the use during medical therapeutics. The potential of provoking side-effects is given by their non-biodegradable properties. Further improvements during fabrication process use the incorporation of biodegradable components such as modified γ-polyglutamic acid which decreases the potential of prospective side-effects.

***Keywords*—**Biopolymers, γ-Polyglutamic acid, Oxidative stress, Ubiquinone.

I. Introduction

SINCE the early stages of nanotechnology there is special interest in medical therapeutics by forming nanosized drug delivery systems (DDS). This new strategy is not only in focus by its perspective of specific drug transport to the center of inflammation but it is also based on limited reasons by forthcoming active pharmaceutical drugs (APIs). The further development of specific APIs leads to decreased side-effects onto human organisms but often implicates as well poor water solubility of the drug substance itself. Paclitaxel and doxorubicin make up as representatives for natural products which became of increased interest in cancer therapy during the last few years [1]–[3]. Alongside, modern strategies discuss moreover combination therapies with tailor-made drugs synthesized to oligo-chained molecule clusters. These include for example the drug formulations of Gefitinib or Lapatinib [4], [5]. Special interest discussed in this script has the application of the body´s own drug substance ubiquinone, also known as coenzyme Q_{10}. Ubiquinone acts efficiently as an anti-oxidative agent against ROS [6]–[9]. Oxidative stress is noted for the initiation of diverse diseases. Linked into the electron transport chain as membrane bounded component ubiquinone is characteristic for its poor water solubility.

The manufacture of nanosized ubiquinone dispersion was improved by Bunjes et al. [10], [11]. Formulations are generated by mechanical high-pressure homogenization processes. A special characteristic of ubiquinone during process is observed by forming liquid crystals in dispersion so that it can be defined as an emulsion [12]–[14]. Besides the construction of nanoparticulate colloids long-termed stability of nanodispersions/-emulsions in general hardly depends on the surfactant used for formulations. Conventional polymeric surfactants like polyvinyl alcohol (PVA) [15], polysorbate (PS) [16], [17] or polyethylene glycol (PEG) [18]–[20] are established as surfactive agents in many disperse formulations. A main disadvantage next to their efficient stabilization is their non-biodegradability. Due to long-termed encapsulation into tissues these components own the potential to induce non-predictable side-effects beyond therapeutic applications [21]–[23]. To circumvent the potential risk of toxic side-effects by chemical surfactants there is a chance in reducing by replacement with biopolymeric surfactants. Thus, an optimized process of biotechnologically γ-polyglutamic acid (γ-PGA) production by *Bacillus licheniformis* seems to be a promising treatment in generating biopolymeric surfactants [24]–[31]. Additional modifications generate in first step an amphiphilic character of the polymer that makes it accessible for the use as surfactant. In few further steps steric stabilization and targeting for specific drug delivery can be obtained [32]–[33].

Stability of nanoparticulate dispersions and emulsions play a key role for therapeutic applications. Next to their electrostatic surface character steric shielding makes up the most important property under physiological conditions. In complex media electrostatic stabilization of colloidal forms suspends caused by salts and mediators. Under these conditions primary particles are favored to agglomerate what makes the therapeutic impact inefficient. Polymeric components such as PVA and modified γ-PGA often combine these characteristics that make them to ideal surfactants for stabilizing ubiquinone emulsions long-termed [34]–[36].

Saccharomyces cerevisiae is an ideal organism for the investigation of therapeutic effects caused by ubiquinone nanoemulsions during oxidative stress. Its composition and eukaryotic metabolism are similar those of humans. The strain used for investigation contains a GFP-expressing gene at the beginning of the OXR1 gene promoter. OXR1 proteins are expressed during oxidative stress so that enhanced GFP-fluorescence is directly linked to the intensity of cellular stress [37], [38].

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Intracellular oxidative stress is specifically induced by the stress mediator diamide. Its effect is based on disturbing the constitutive acting glutathione-glutathione disulfide (GSH/GSSG) buffer inside of cells [39]–[41]. ROS produced during aerobic respiration raises immediately and causes GFP-expression. Comprehensive attempts clarify the maximum of stress reducing effects generated by nanoemulsions [7], [42–45]. Beneath quantitative fluorescence determination there is special interest in the consequences of ubiquinone nanoemulsions onto genetic expression clusters. Along with quantitative results a holistic pharmacodynamic effect of nanoemulsions can be generated to assess their application for prospective therapeutics.

II. Experimental

A. Materials

The strain *S. cerevisiae* N34 was provided by Prof. Volkert, University of Massachusetts Medical School, Worcester MA, for disposal.

γ-PGA (M_n = 265 kDa) was produced by the strain *B. licheniformis* ATCC 9945A in high titers. In next step L-Tryptophan ethyl ester (L-TrpE) was coupled by EDC-coupling to generate an amphiphilic surfactant. The coupling degree is about 30%.

Ubiquinone/PVA nanoemulsions were provided by Prof. Bunjes, Technische Universität Braunschweig, Germany, for disposal. The compositions imply 6.5g L^{-1} ubiquinone, 7.5 wt% PVA (M_w = 30 kDa) and 5 wt% glycerol, 85% (v/v).

Ubiquinone/γ-PGA-L-TrpE nanoemulsions were manufactured with a solvent evaporation process. The compositions imply 1.0g L^{-1} ubiquinone and 2.0g L^{-1} P(γ-GA-*r*-L-TrpE). A Tetrahydrofuran (THF) water mixture was initially used for the implementation.

Diamide is commercially available at Sigma-Aldrich (Germany).

B. Nanoparticle Characterization

Scanning electron microscopy (SEM) was used for visual characterization of the liquid crystalline ubiquinone nanoemulsions. The preparation was implemented by freeze-dried samples.

Time-dependent mean size distributions of nanoemulsions in complex media were determined by dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS). Mean size distributions and ζ-potential could be obtained over the time.

Increased salt concentrations in nanodispersion/-emulsion hold the induction of agglomeration or further aggregations of the primary particles. Focused beam reflectance measurement (FBRM, Lasentec, Mettler-Toledo, USA) was used to detect raising chord length distributions with sizes greater than 1μm.

C. Yeast Cultivation

S. cerevisiae was cultivated under fed-batch conditions. Complex media contained 20g L^{-1} glucose monohydrate (100 mOsM), 5g L^{-1} of bactopeptone and of yeast extract (50 mOsM) and a Sörensen buffer at pH 5.5 (150 mOsM). Total osmolality of 300 mOsM was defined for growth as an isotonic solution (0.9 wt% NaCl solution). In order to that yeast cells are harvested at the end of the exponential phase with at a cell dry weight of 1.2g L^{-1}.

D. Yeast-Nanoparticle Interactions

Investigation of stress induced GFP-fluorescence was determined by Fluoroskan Ascent Microtiter Fluorometer.

III. Results and Discussion

A. Nanoparticle Characterization

Ubiquinone/PVA nanoemulsions were formed during high-pressure homogenization [9], [10]. With PVA as surfactive agent mean size distributions of 130nm ± 5nm could be obtained. As shown in Fig. 1 disperse ubiquinone is present as liquid crystals. During freeze-drying process emulsive spheres converted partially into platelets. The surfactive acting PVA surrounds fiber-like the lipophilic ubiquinone cores to form stable emulsion.

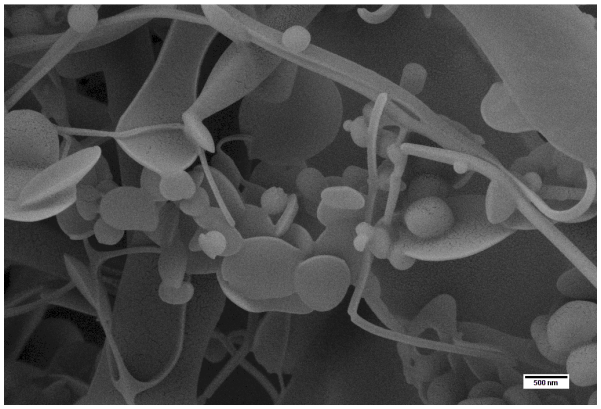

Fig. 1 Scanning electron micrograph of ubiquinone/PVA nanoemulsion after freeze-dried preparation. Magnification is of ×50.0K

In a different procedure ubiquinone/P(γ-GA-*r*-L-TrpE) nanoemulsions could be generated, as shown in Fig. 2.

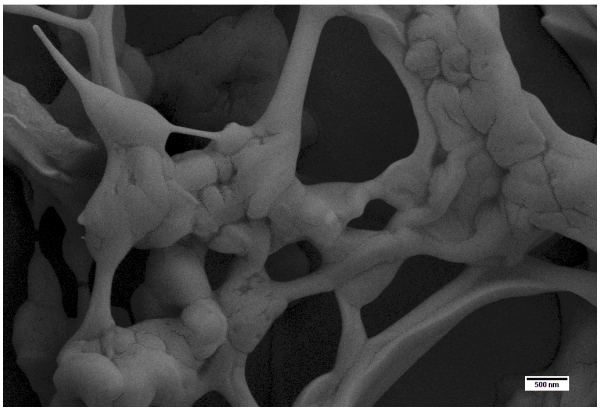

Fig. 2 Scanning electron micrograph of ubiquinone/P(γ-GA-*r*-L-TrpE) nanoemulsion after freeze-dried preparation. Magnification is of ×50.0K

By solvent evaporation technique nanoemulsions were established with a maximum of 1g L^{-1} drug content. Their appearance is very similar to those of ubiquinone/PVA nanoemulsions. Here, ubiquinone emulsions with mean size distribution of 750nm ±150nm could be achieved. The biopolymeric surfactant P(γ-GA-*r*-L-TrpE) surrounds identically as the PVA the heterogeneous lipophilic core made up of ubiquinone.

To investigate the behavior of the nanoemulsions under physiological conditions ζ-potential of ubiquinone/PVA nanoemulsion was measured with increasing osmolalities. Fig. 3 shows the course of ζ-potential dissolved in pure water up to dissolved in complex media solution with an osmolality of 100 mOsM.

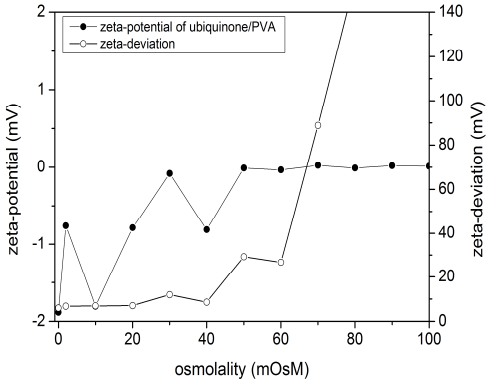

Fig. 3 ζ-potential versus osmolality made up by complex media (pH 5.5)

Stabilization depends on surface properties of the disperse material. In the ubiquinone/PVA nanoemulsion the surfactive PVA conveys more steric than electrostatic stabilizing properties what can be attached by ζ-potential of -1.8mV in pure water. Nevertheless increased salt concentrations lead to ζ-potentials of approximately 0.0mV as well as to a high slope of ζ-deviation beyond 60mOsM. In consequence, electrostatic stabilization suspends completely under physiological conditions at 300mOsM. Only steric stabilizing components, such as PVA, can maintain a time-dependent stability of nanodispersions/-emulsions during applications. Ubiquinone/P(γ-GA-*r*-L-TrpE) nanoemulsions offer different requirements based on their nature. Negatively charged carboxyl groups on the surface of the disperse phase led to ζ-potentials of about -50mV in pure water.

By suspending electrostatic stabilization of the nanoemulsions it is necessary to investigate potential effects caused by agglomeration or further aggregation under physiological conditions. Mean size distributions were determined over a period of 5h via DLS in pure water as well as in 300 mOsM complex media with pH = 5.5, as shown in Fig 4. The scatter plot illustrates that ubiquinone/PVA nanoemulsion is nearly stable in pure water. The polydispersity index PDI is arranged in a low value range between 0.01 and 0.12. In addition the mean size distribution is tight fluctuating between 119 and 140nm. On the opposite nanoemulsion in complex media shows very broad distributions of PDI in the range of 0.19 to 0.58 and mean size between 90 and 170nm. The results are in good agreement with the previous discussed effect by suspending electrostatic stabilization under physiological conditions. A metastable state of the nanoemulsion is induced by ambient salts and other mediators. Time-dependent collapse caused by agglomeration or further aggregation is the consequence after about 1h. The preferential aim is to form nanoemulsions with an equal behavior under physiological conditions as in pure water. The substitution and further modifications of ubiquinone/P(γ-GA-*r*-L-TrpE) nanoemulsions seems to be a promising treatment to induce distinct steric stabilization into disperse phases.

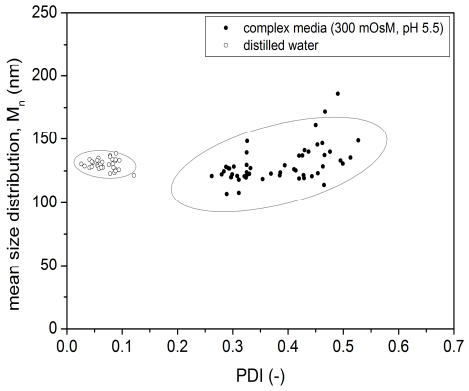

Fig. 4 Scatter plot of mean size distribution M_n versus polydispersity index (PDI) with their confidence ellipses, 95%, via DLS. Ubiquinone/PVA nanoemulsion was dissolved in water as well as in complex media. The graph shows fluctuating results over a time period of 5h

An identical course of the behavior of ubiquinone/PVA nanoemulsion is observed by FBRM measurement, shown in Fig. 5.

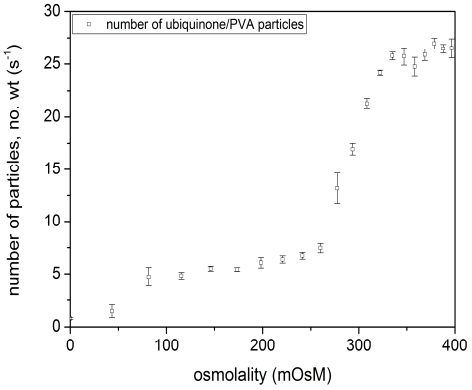

Fig. 5 FBRM measurement of ubiquinone/PVA nanoemulsion by increasing salt concentration in complex media (pH = 5.5) up to 400 mOsM. Heterogeneous particles with chord lengths larger than 1µm s^{-1} are determined

The nanoemulsion starts to collapse at osmolalities of about 100 mOsM. Particle detection gains in the range between 275 and 330 mOsM of the complex media significantly to achieve the detection of 28 particles per second at maximum. Next to the detection via DLS it could be demonstrated that the method of FBRM is a meaningful treatment to characterize the potential agglomerating behavior of nanodisperse phases under physiological conditions.

B. Yeast Cultivation

Fed-batch process was used for cultivation of *S. cerevisiae* strain to obtain cultures in the end of their exponential growth phases with a cell dry weight (cdw) of 1.2g L^{-1}. Fig. 6 illustrates the characteristic appearances of yeast. Growing cells as well as budding cells with its mother/daughter clusters can be identified.

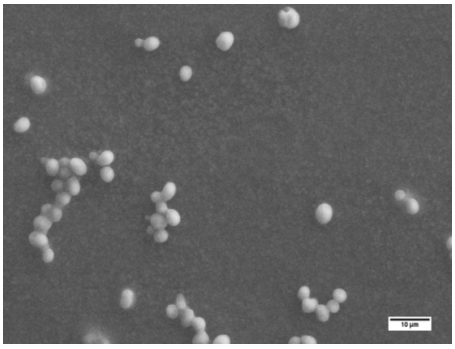

Fig. 6 Scanning electron micrograph of *S. cerevisiae* N34. Magnification is of ×1.0K

C. Yeast-Nanoparticle Interactions

S. cerevisiae N34 is able to express GFP by induction of oxidative stress. Fig. 7 shows a typical course of fluorescence intensities caused by varying conditions of yeast culture. The nearly stable fluorescence intensity over time represents the control of the undisturbed yeast culture during fluorescence detection. No further component was added. On the opposite the highest fluorescence deflection is caused by the addition of the stress mediator diamide. As a typical sigmoidal course fluorescence rises after a period of adaptation to an asymptotic boundary value. During the alignment toward the stress mediator yeast turns on the expression of SOD and catalases. The process takes about 15min. The subsequent linear phase is affected by the reduction of ROS inside yeast cells. In the meantime reactive diamide is dissipated by glutathione buffer as well as by other metabolic components. At the beginning of about 3h the linear increase of fluorescence reduces until it is stable over time after about 5.5h. Now diamide is completely metabolized to an unreactive component and glutathione buffer system recovers to its operating level. SOD and catalases production stops slowly. The incubation of ubiquinone/PVA nanoemulsion before stress induction leads to a reduced increase of fluorescence over time. Probably nanoemulsive ubiquinone is able to support the disturbed glutathione buffer system until stress mediator is completely metabolized so that total stress of the cell is reduced. As discussed before induced agglomerations of the nanoemulsions under physiological conditions seem to have a minor influence onto their anti-oxidizing effect.

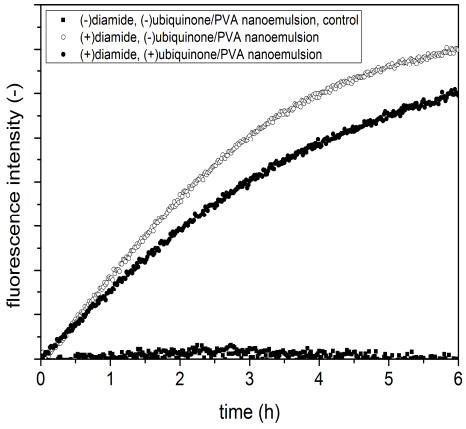

Fig. 7 Development of fluorescence intensity over an exposure time of 6h (■) control without any addition of a component, (○) yeast culture with the addition of diamide, (●) yeast culture with the addition of ubiquinone/PVA nanoemulsion and subsequent addition of diamide

IV. Conclusion

The present work demonstrates the advanced development of nanotechnology based on pharmaceutical applications. Despite of the current successes in manufacture and stability of nanodispersions/emulsions there are still a couple of barriers to cross by generating safe nanoformulations. As intensively discussed salts and metabolic components in complex media have an immense influence onto particle-agglomeration equilibrium. The introduction of modified biopolymeric γ-PGA followed by further modifications offer a promising treatment by preventing agglomeration under physiological conditions as well as specific transport to the center of inflammation. Even the reducing stress effect induced by non-biodegradable ubiquinone/PVA nanoemulsions hypothesizes the potential of more efficient stress reduction. Summarized, nanoformulated ubiquinone as applied drug substance against oxidative stress is an efficient component to reduce ROS overproduction induced by environmental incidences. In future, the focus has to be put on the surface modifications which have large influence onto disperse phase behavior.

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