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Influence of surfactants on depsipeptide submicron particle formation.

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Abstract:

Surfactants are required for the formation and stabilization of hydrophobic polymeric particles in aqueous environment. In order to form submicron particles of varying sizes from oligo[3-(S)-*sec*-butylmorpholine-2,5-dione]diols ((OBMD)-diol), different surfactants were investigated. As new surfactants, four-armed star-shaped oligo(ethylene glycol)s of molecular weights of 5-20 kDa functionalized with desamino-tyrosine (sOEG-DAT) resulted in smaller particles with lower PDI than with desaminotyrosyl tyrosine (sOEG-DATT) in an emulsion solvent evaporation method. In a second set of experiments, sOEG-DAT of M_n = 10 kDa was compared with the commonly employed emulsifiers polyvinylalcohol (PVA), polyoxyethylene (20) sorbitan monolaurate (Tween 20), and D-α-tocopherol polyethylene glycol succinate (VIT E-TPGS) for OBMD particle preparation. sOEG-DAT allowed to systematically change sizes in a range of 300 up to 900 nm with narrow polydispersity, while in the other cases, a lower size range (250-

400 nm, PVA; ~300 nm, Tween 20) or no effective particle formation was observed. The ability of tailoring particle size in a broad range makes sOEG-DAT of particular interest for the formation of oligodepsipeptide particles, which can further be investigated as drug carriers for controlled delivery.

Introduction:

The size of particles is of high relevance for transport and uptake in biological systems, which is important for drug carriers so that the cargo can reach the site of action. For example, the particle size has been shown to affect the lung deposition, with particles between 0.5 and 5 µm preferably used for inhalation [1]. Similarly, skin penetration is affected by particle size [2], with the deepest hair follicle penetration being observed for particles of approximately 640 nm [3]. Furthermore, particle size influences the cellular uptake efficiency and mechanism [4], which can be decisive to subsequent biological effects of particulate drug carriers.

The size of polymeric particles can be tailored by the particle preparation process. It is sensitive e.g. to the solvents employed in oil-in-water emulsion based dispersion techniques, the method of organic solvent removal, the polymer concentration as well as the type and concentration of surfactant used. The surfactant plays an important role in stabilizing an emulsion in the first step of particle preparation by reducing the interfacial tension between the two immiscible liquids [5]. Moreover, surfactant molecules absorbed on the particle surface act as electrostatic or steric barriers against particle aggregation. The ability of the surfactant to locate at the interface of nascent

droplets and eventually its interaction with the solidified particle surface is of primary importance to obtain a stable particle suspension.

Here, we targeted the formation of sub-micron-sized particles from oligo[3-(S)-*sec*-butylmorpholine-2,5-dione]diols, which may serve as vehicle for drug transportation e.g. into skin in the future. Such oligodepsipeptides (ODPs) are promising candidate materials for pharmaceutical applications due to their biocompatibility and degradability as well as the possibility to introduce different functionalities in the side chain, which could tailor the properties of the material [6, 7]. ODP containing copolymers with e.g. ε-caprolactone, *L*-lactide or *meso*-lactide were investigated e.g. as porous sponges enabling cell growth [7]. Nanoparticulate drug carrier formation from such copolymers was reported, though restricted to a small size range of 150-200 nm [8]. To explore the options to generate particles with a larger variety of mean sizes, the influence of surfactants was here systematically explored.

In previous studies, amphiphilic, four-arm star-shaped oligo(ethylene glycol)s functionalized with desaminotyrosine (sOEG-DAT) or desaminotyrosyl tyrosine (sOEG-DATT) have been shown to act as surfactants [9] and did not induce cytotoxic or immunological adverse effects [10]. As the aromatic DAT/DATT groups should allow good interaction with hydrophobic moieties as present in ODPs, we hypothesized that sOEG-DAT(T)s are suitable for the formation and stabilization of ODP particles in an aqueous phase. Moreover, the degree of functionalization of the end groups of 56-82% present in the sOEG-DAT(T)s should facilitate the required asymmetric orientation with the hydrophobically modified arms directed to the particle surface and the hydrophilic, non-functionalized OEG segment directed to the water phase. In the following, the initial

evaluation of sOEG-DATs and sOEG-DATTs of different molecular weights as emulsifiers for ODP submicron particle formation is presented. In a second step, sOEG-DAT of M_n = 10 kDa has been compared with other stabilizers often employed in particle formation, namely polyvinylalcohol (PVA; partially deacetylated polyvinylacetate), polyoxyethylene (20) sorbitan monolaurate (Tween 20), and D-α-tocopherol polyethylene glycol succinate (VIT E-TPGS) [11]. Formulation parameters, such as surfactant concentration and amount of used polymer were changed in order to tailor the particle dimension and obtain suspensions of a wide range of particle sizes.

The most promising surfactant will be employed in future studies, which are intended to explore OBMD-diol particles as drug delivery systems. Different biomedical applications ranging from ocular to skin delivery are planned to be investigated by using drug-loaded OBMD-diol carriers.

Material and methods:

Materials:

Oligo[3-(S)-*sec*-butylmorpholine-2,5-dione]diol (OBMD-diol) ($M_{n\ (NMR)}$ = 4.5 kDa, PDI = 1.19) was synthesized via ring opening polymerization according to ref. [12]. Four-armed star-shaped oligo(ethylene glycol)s of different molecular weights ($M_{n(Maldi\text{-}TOF)}$ = 5,300, 10,200, and 20,400 Da, polydispersity of 1.0 for all three oligomers) functionalized with desaminotyrosine (sOEG-DAT) (degree of functionalization (d.f.) = 56, 67, and 65% for sOEG of 5, 10, and 20 kDa, respectively) and desaminotyrosyl tyrosine (sOEG-DATT) (d.f. = 82, 77, and 77% for sOEG of 5, 10, and 20 kDa, respectively) were synthesized according to ref. [9]. The commercially available

surfactants were bought from Sigma-Aldrich: Polyvinyl alcohol (PVA, Mowiol 8-88), which is partially hydrolyzed poly(vinyl acetate) (Schnelldorf, Germany), Polyoxyethylene (20) sorbitan monolaurate (Tween 20) (Saint-Quentin-Fallavier, France) and D-α-Tocopherol polyethylene glycol 1000 succinate (VIT E-TPGS) (Buchs, Switzerland).

Methods:

Particle preparation

OBMD-diol particles were prepared by a single oil-in-water emulsion process followed by solvent evaporation. Briefly, OBMD-diol (10-40 mg) was dissolved in 1 mL of $CHCl_3$. The solution was then added dropwise into 5 mL of surfactant solution in water (0.1-1 wt.%). The two phases were mixed at 25000 rpm for 90 seconds using a T 25 digital ultra-turrax (IKA GmbH, Königswinter, Germany). Afterwards, the emulsion was sonicated for 2 minutes with intensity of 52% using a Bandelin Sonoplus sonicator HD 3200 (Bandelin electronic GmbH & Co. KG, Berlin, Germany). The emulsion was poured in a beaker containing 5 mL of surfactant solution of the same concentration initially used. The organic solvent was evaporated at room temperature by stirring the solution at 300 rpm for 5 hours. The suspension was filtrated with a 70 µm nylon cell strainer (neoLab GmbH, Heidelberg, Germany) to remove aggregates formed during solvent evaporation. All experiments were repeated in triplicate.

Particle characterization

The particle size and the polydispersity index (PDI) were determined by Dynamic Light Scattering (DLS) using ZetasizerNano SZ instruments (Malvern, Worcestershire, UK). The measurements were performed at 25 °C with a He-Ne laser operating at 633 nm, detecting the scattered light at an angle of 173°. Each sample was measured three times with 10 sub-runs each time. The hydrodynamic diameter (D_h), given as Z-Average, was calculated by using the Stokes-Einstein equation: $R = (6\pi\eta D)/(kT)$ where R is the hydrodynamic radius of the particle, η is the viscosity of the solvent, D is the diffusion coefficient for a particle in a free volume, k is the Boltzmann constant and T is the absolute temperature. The particle sizes from the different procedures were compared and statistical significance determined by two-tail t-test or ANOVA analysis. The zeta potential was determined using the same instrument measuring at 25°C. Each sample was analyzed in triplicate with manifold runs every time: from 10 to 100 depending on the ability of the instrument to determine the charge. The zeta potential value was calculated by using the Smoluchowsky equation: $\zeta=(4\pi\eta\mu)/D$ where η is the viscosity of the solvent, μ is the electrophoretic mobility of the particles analyzed and D is the electric constant of the solvent.

The particle morphology was analyzed by Scanning Electron Microscopy (SEM) using a Zeiss Gemini Supra 40 VP microscope with a Scottky emitter at an acceleration voltage of 3 kV. The samples were spattered with Iridium (4 nm layer) with a Polaron SC7640 sputter coater (Quorum Technologies Ltd, UK).

Results and discussion:

sOEG-DAT(T)s were synthesized by EDC/NHS mediated acylation of amino-sOEG of molecular weights of 5, 10, or 20 kDa. The degree of functionalization as calculated from integrals in the ^{1}H NMR spectra was higher in case of sOEG-DATT (77-82%) compared to sOEG-DAT (56-67%).

Particles were prepared by emulsification of OBMD-diol solutions in an aqueous phase supplemented by surfactants, followed by solvent extraction/evaporation, and particle hardening. Only formulations that led to particles with hydrodynamic diameters (D_h) below 950 nm and polydispersity index (PDI) up to 0.25 have been considered acceptable, as particles with a broader size distribution would likely result in non-homogeneous biological distribution in a potential later application.

In the formulations carried out to compare sOEG-DAT(T) of different molecular weights (Fig.1a), a 2 wt.% solution of OMBD-diol (Fig.1b) in chloroform and a surfactant concentration of 0.5 wt.% were used. The selected surfactant concentration was above the critical micellar concentrations (CMC) that have been detected for sOEG-DATs [9] in order to allow a good stabilization of the emulsion.

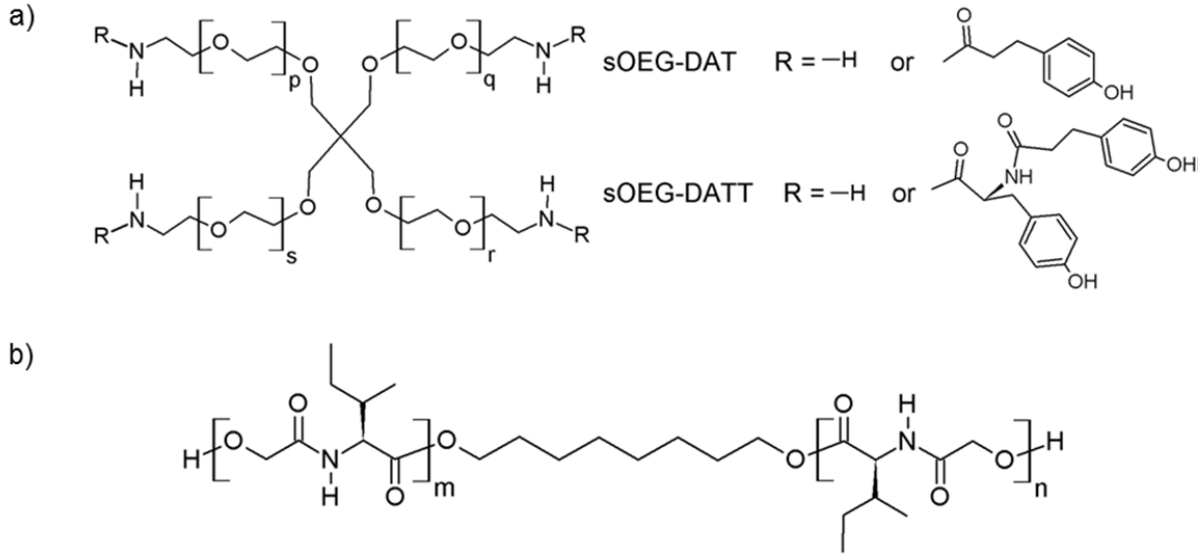

Fig. 1: a) Chemical structures of sOEG-DAT and sOEG-DATT. The residue R can be either hydrogen or the aromatic compound, depending on the degree of functionalization of the sOEG. b) Chemical structure of OBMD-diol.

The suspensions obtained were characterized by DLS (Fig. 2). Using sOEG-DATs, it was possible to prepare OBMD-diol particles with D_h of 300-500 nm and PDI below 0.25. The PDI decreased to values lower than 0.2 in case of sOEG-DAT of 10 and 20 kDa. On the contrary, the use of sOEG-DATT led to the formation of big aggregates in case of sOEG-DATT of 5 kDa and to PDIs higher than 0.25 in case of sOEG-DATT of 10 and 20 kDa. These results indicated that sOEG-DATTs provided a poorer stabilization of the emulsion than sOEG-DAT, which could indicate a potential steric crowding of the more spacious DATT groups. Alternatively, the higher degree of functionalization of the sOEG-DATTs compared to the sOEG-DAT used may not allow an optimal asymmetric organization of the amphiphilic sOEG-DATT at the interface. Further investigations will be performed to clarify how these surfactants interact with the particle surface.

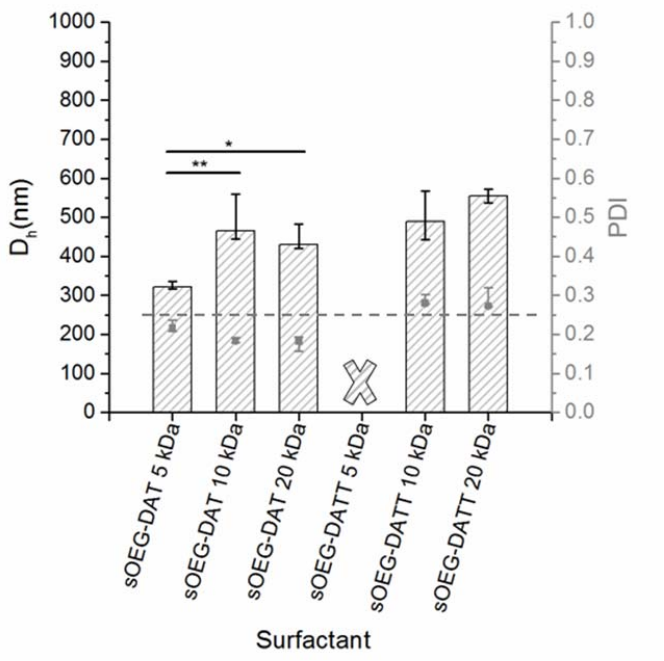

Fig. 2: DLS data of OMBD-diol particles obtained using sOEG-DAT(T) with sOEG of 5,10, and 20 kDa as supernatant. The value shown is the mean value of three formulations. The error bars indicate the value range of three formulations. ⛒ indicates unstable formulation. Bars indicate statistically significant different values with $P < 0.05$ *, $P < 0.01$ **

As sOEG-DATs showed to give particle suspensions with an acceptable polydispersity, sOEG-DAT of 10 kDa has been chosen to be compared with the commercially available surfactants PVA, Tween 20 and VIT E-TPGS. In order to vary the particle size, different surfactant concentrations in aqueous solution (0.1, 0.5, and 1 wt.%) and varying ODP concentrations in chloroform (1, 2, and 4 wt.%) were investigated, and the particle suspensions were analyzed by DLS (Fig. 3). In particle preparation studies, e.g. PLGA particles preparation, VIT E-TPGS has been shown to lead to stable particle suspensions at concentration up to 60 times lower compared to PVA [11]. However, the same tendency was not experienced in case of OBMD-diol particles. VIT E-TPGS was not able to stabilize the emulsion, but resulted in the formation of a precipitate.

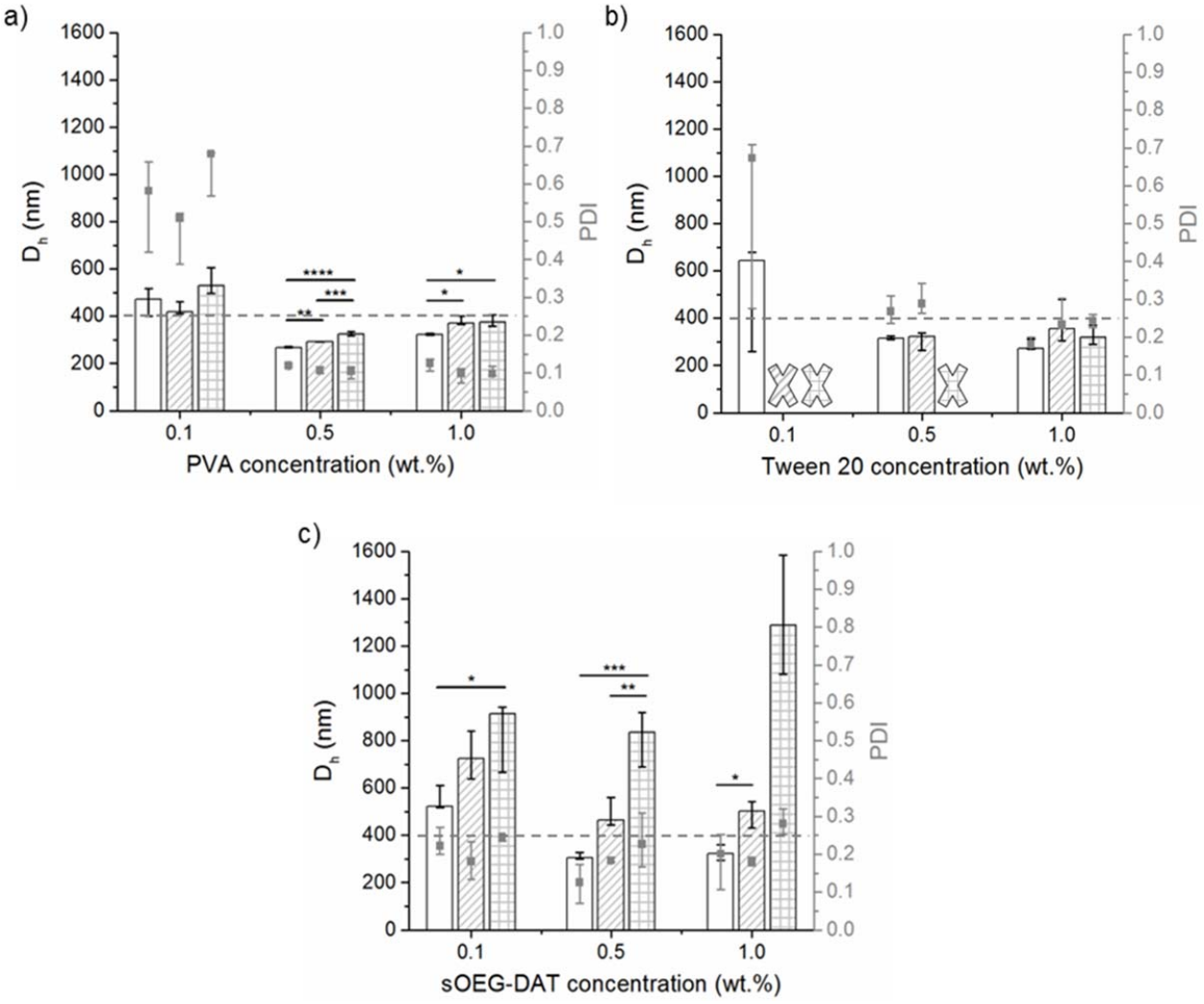

Fig. 3: DLS data of OMBD-diol particles obtained using sOEG-DAT of 10 kDa (a), PVA (b) and Tween 20 (c) as supernatant. The value shown is the mean value of three formulations. The error bars indicate the value range of three formulations. Three different amounts of OBMD are used in the formulations: 10 mg (white), 20 mg (strips), and 40 mg (squares). ⛒ indicates unstable formulation. Bars indicate statistically significant different values with $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***, and $P < 0.0001$ ****

Stable emulsions and the formation of submicron particles were observed when sOEG-DAT of 10 kDa, PVA and Tween 20 were used as surfactants. However, in some formulations prepared with Tween 20, the emulsions were unstable and, therefore, the

formation of a precipitate was observed. For suspensions with an acceptable polydispersity obtained with sOEG-DAT or PVA, it has been observed that the D_h increased significantly by increasing the oligomer concentrations at constant surfactant concentration. In case of PVA, the slight size increment resulted in the formation of particles with D_h that ranged from 250 up to about 400 nm. In contrast, by using sOEG-DAT of 10 kDa, it was possible to obtain submicron particles with D_h that extended from 300 up to 900 nm. Moreover, PVA and Tween 20 could form submicron particle suspensions with an acceptable polydispersity only at concentrations higher than 0.1 or 0.5 wt.%, respectively. On the contrary, sOEG-DAT of 10 kDa led to suspensions with PDI values below 0.25 even at a concentration of 0.1 wt.%.

The zeta potentials of the particles obtained using different concentration of sOEG-DAT or PVA, which were the two surfactants giving the most promising results, showed to be close to neutrality with values between -1.2 and -2.2 mV in case of PVA and between -0.1 and -0.9 mV in case of sOEG-DAT. Such slightly negative values have often been detected for particles prepared using polyesters and uncharged surfactants [13]. The zeta potential is known to influence particle features such as cytotoxicity and stability of the suspension: a zeta potential close to neutrality might be related to a fast particle aggregation, however, this is not the case for large molecular weight surfactants, which provide steric stabilization [14]. Therefore, in case of PVA and sOEG-DAT of 10 kDa, the particle aggregation is most likely to be prevented although the zeta potential has values close to neutrality.

SEM images of OMBD-diol particles obtained using 20 mg of OBMD-diol and a surfactant concentration of 0.5 wt.% PVA or sOEG-DAT (10 kDa) showed that the

particles were round shaped and that the particle size was similar to the one detected by DLS (Fig. 4). The particle aggregates visible by SEM can be attributed to the drying process required for the SEM analysis. When determining the number average diameter from SEM images, particles prepared using PVA exhibited sizes of 270 ± 90 nm, while particles prepared using sOEG-DAT were larger and had an average SEM diameter of 430 ± 150 nm. Since there was no apparent difference between the z-average, which was 490 nm for the particles prepared using sOEG-DAT and 290 nm using PVA, and the mean SEM diameter for both the formulations analyzed, the DLS data have been assumed to show reliable size distributions also for all the other samples without further SEM analyses.

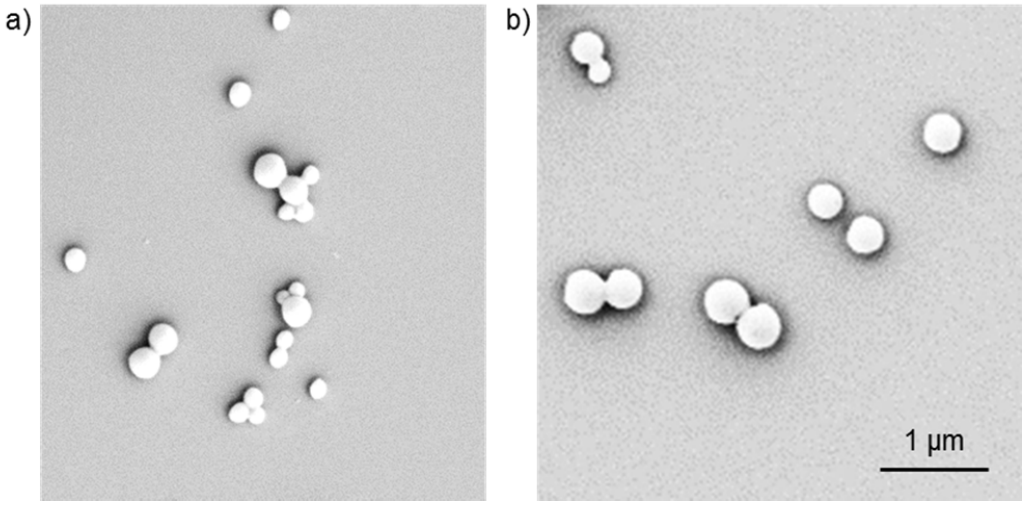

Fig. 4: Representative SEM images of OMBD-diol particles obtained using 20 mg of OBMD-diol and a concentration of 0.5 wt.% of PVA (a) and sOEG-DAT of 10 kDa (b).

In conclusion, it has been demonstrated that sOEG-DATs of 5, 10, and 20 kDa can be used to prepare submicron OBMD-diol particles. The formation of submicron particles

with narrow polydispersity can be achieved by using sOEG-DAT solution of low concentration; moreover, OBMD-diol particles in a wide size range are accessible with this stabilizer. This leads to the conclusion that sOEG-DAT of 10 kDa has proven to be the best choice for OBMD-diol particles preparation in comparison to VIT E-TPGS, PVA or Tween 20. The ability of tailoring particle size in such a broad range makes sOEG-DAT of particular interest for the formation of ODP particles of different dimensions, which might reach specific biological sites.

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Keywords: depsipeptide, particle size, surfactants, submicron particles.

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