Biocompatible polymeric nanocapsules by miniemulsion technique

A. Musyanovych*, E.-M. Rosenbauer and K. Landfester[*]
Institute of Organic Chemistry III / Macromolecular Chemistry and Organic Materials, University of Ulm – 89081 Ulm, Germany
(anna.musyanovych@uni-ulm.de)

Introduction

Core-shell particles consisting of a liquid cavity surrounded by a unique polymeric membrane have received considerable attention owing to their application as sub-micron containers for microencapsulation. In the fields of bio-nanotechnology, fabrication of biocompatible/biodegradable capsules with controlled and adjustable characteristics is of high advantage.
Significant benefits of miniemulsion technique offer the ability to obtain polymeric nanocapsules in a controlled way with different shell thickness and versatility in the polymeric materials. Generally, miniemulsion allows one to create small stable droplets in a continuous phase by applying high shear stress. Under high shear, e.g. ultrasonication, the broadly distributed macrodroplets are broken into narrowly distributed and defined nanodroplets in the size range between 50 and 500 nm[1]. The stability of the system is achieved by using an efficient composition of surfactant and an osmotic pressure agent (or: co-stabilizer). The main role of the osmotic pressure agent is to suppress the Ostwald ripening by creating the osmotic pressure inside each droplet. The high stability of the droplets gives an opportunity to perform the reactions within the droplets or at their interface.
In the current paper, we describe the formation of biodegradable poly(n-butylcyanoacrylate) (PBCA) capsules via interfacial anionic polymerization and synthesis of polyurea nanocapsules by polyaddition reaction between the water-soluble diamine and oil-soluble diisocyanate at the water-oil interface; and the preparation of biodegradable poly(L-lactide) (PLLA), poly(ε-caprolactone) (PCL), and poly(lactide-co-glycolide) (PLGA) particles from the preformed polymer by the combination of miniemulsion and solvent evaporation techniques. The influence of several factors, such as the amount of surfactant, the amount and type of monomer/polymer, phase ratio, etc. on the final particle size and wall thickness has been studied. The cellular uptake studies were carried out on the example of PLLA particles.

Materials and methods

Chemicals

1,6-diamonohexane (HMDA) and tolylene-2,4-diisocyanate (TDI, 98%) were purchased from Fluka. n-butyl cyanoacrylate (BCA), was sponsored by Henkel, Germany. The polymers used were PLLA (M_w = 101,700 g/mol) supplied from Fluka, PCL (M_w = 115,000 g/mol) from Aldrich and PLGA (50:50) was sponsored by Boehringer Ingelheim (Ingelheim, Germany). Sodium dodecyl sulfate (SDS, Merck), Span®80 (Aldrich), and Lubrizol U (polyisobutylen-succinimide pentamine) were used as surfactants. Carboxyfluorescein (Fluka) and N-(2,6-Diisopropylphenyl)-perylene-3,4-dicarbon-acidimide (PMI) (BASF) were used as hydrophilic and hydrophobic fluorescent markers, respectively. Miglyol 812 N (caprylic/capric triglycerides), cyclohexane and chloroform (HPLC-grade) were used without further purification as an organic phase.

Preparation of PBCA capsules

7.6 g of Mygliol 812 N and different amounts of Span®80 were mixed with 1 g of 5 μM Cy3-ssDNA in phosphate saline buffer (pH 7.2) solution. The mixture was vigorous stirred using a

magnetic stirrer (15 min, 1200 rpm) at a room temperature and then sonified for 60 s at 70% amplitude (Branson sonifier W450 Digital, tip size 6.5 mm). A monomer, n-butylcyanoacrylate was drop-wise added to the miniemulsion. After 4 h of polymerization, the PBCA capsules were separated from the oil phase by centrifugation at 14 000 rpm for 20 min and pellet was resuspended under sonication in 0.5 wt% aqueous solution of SDS.

Preparation of polyurea capsules

0.825 g of the disperse phase consisting of 75 mg HMDA and 15 μM carboxyfluorescein solution in PBS-buffer were added to a 6 g of cyclohexane containing defined amount of Lubrizol U. After stirring about 1 h for pre-emulsification, the miniemulsion was prepared by ultrasonicating the mixture for 180 s at 70% amplitude under ice cooling. Then a mixture of 4 g cyclohexane and defined amounts of TDI was drop-wise added to the miniemulsion. The reaction was carried out for 3 h at 25 °C.

Preparation of PLLA, PCL and PLGA particles

Polymer particles were obtained by the solvent/evaporation method combined with the miniemulsion technique. A given amount of polymer and PMI was dissolved in 10 g of chloroform. The macroemulsion was prepared by adding the aqueous phase consisting of dissolved SDS in 24 g water to the organic phase, and subsequent magnetic stirring of the mixture at a high speed for 60 min. Afterwards, the macroemulsion was subjected to ultrasonication under ice cooling for 180 s at 70% amplitude in a pulse regime (30 s sonication, 10 s pause). The obtained miniemulsion was transferred into the reaction flask and left at 40 °C for a complete evaporation of the organic solvent.

Characterization of samples

The average size and size distribution were determined by dynamic light scattering (DLS) using a Zeta Nanosizer (Malvern Instruments). Transmission electron microscopy (TEM) (Philips EM400) and high resolution scanning electron microscopy (SEM) (Hitachi S-5200) were used to study the morphology of polymer particles. The amount of entrapped carboxyfluorescein and Cy3-ssDNA was determined from the fluorescence measurements performed on FluoroMax-3, HORIBA Jobin Yvon, GmbH. The amount of entrapped hydrophobic fluorescent marker was determined from UV/VIS absorption spectra of the particles. The absorbance of the solution was measured at 479 nm using UV/VIS spectrometer Lambda 16, Perkin Elmer. For the cell studies, 5 x 10^5 HeLa cells were allowed to attach over night in 6-well plates. The nanoparticles were added at a concentration of 75 μg/ml. For more details see Ref. [2].

Results and Discussion

PBCA and polyurea nanocapsules with entrapped hydrophilic compounds were prepared by interfacial polymerization from water-in-oil droplets employing the miniemulsion technique. The formulation process and obtained nanocapsules are presented in Figs. 1 and 2, respectively.
Generally for the inverse systems, non-ionic surfactants with a low HLB values are of the best choice. Therefore, Span®80 and Lubrizol U were chosen as stabilizers for the synthesis of PBCA and polyurea capsules, respectively. The mean sizes of the obtained capsules were in the range between 100 and 350 nm, and mainly depend on the monomer system and the amount of stabilizing agent. Higher surfactant concentrations usually result in the smaller size droplets and subsequently smaller capsules. This trend was observed in the both prepared capsules (see Fig. 3). The PBCA

capsules were slightly bigger in size compare to the polyurea ones, which can be explained by the fact that the viscosity of the continuous phase (Miglyol 812 N vs cyclohexane) is nearly 20 times higher.

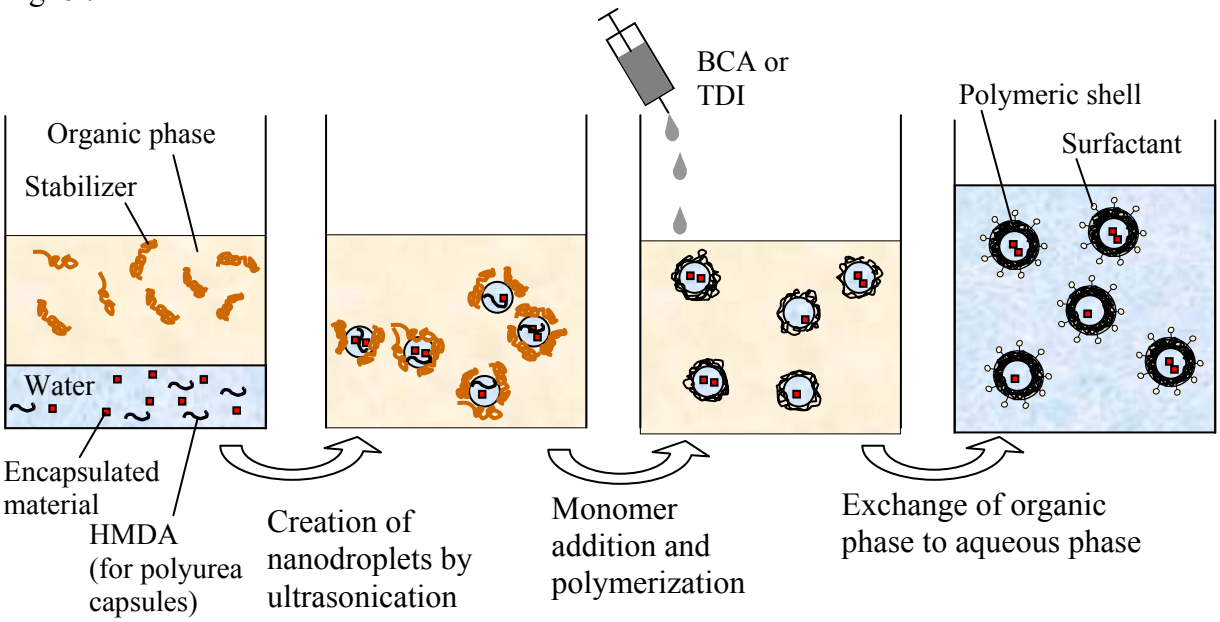

Fig. 1. Synthesis of polymeric capsules through reaction at the miniemulsion droplets' interface.

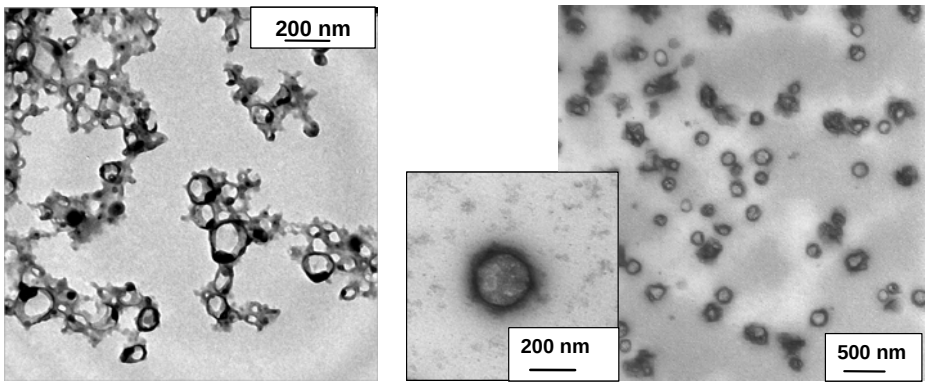

Fig. 2.
TEM images of polyurea (left) and PBCA (right) capsules.

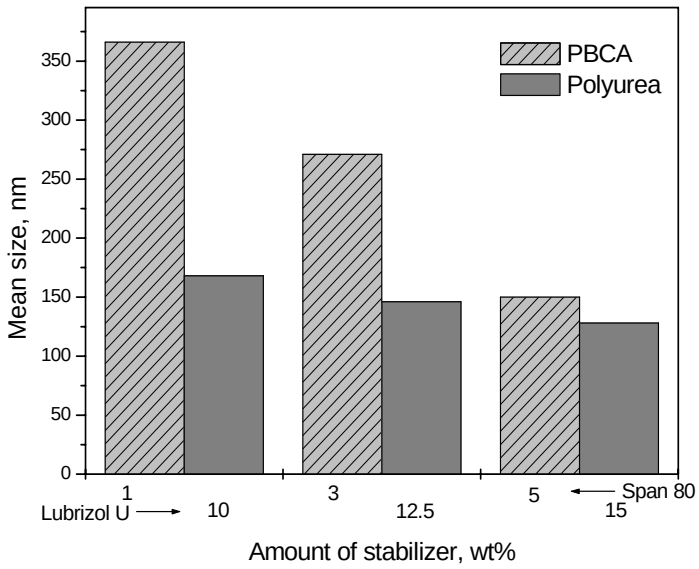

Fig. 3. Effect of the stabilizer concentration on the nanocapsule size. Amount of BCA was 0.1 g. Weight ratio HMDA:TDI = 1:2

By varying the amount of the added BCA or HMDA to TDI ratio, the thickness of the capsules' wall can be adjusted in a controlled way. The increase in the amount of BCA from 0.07 g to 0.4 g results in the increase of wall thickness from 5 to 50 nm. In the case of polyurea capsules, the increase in HMDA to TDI ratio from 0.5 to 1 leads to the capsule with a thinner polymeric shell (from 22 to 15 nm). Independent from the capsule size and material, the loading capacity was around 94±1% of the initial hydrophilic substance`s concentration.

The emulsion/solvent evaporation method and miniemulsion technique were combined and applied in the formulation of biodegradable monodisperse nanoparticles in a size range between 80 and 200 nm using different biocompatible and biodegradable polymers such as poly(L-lactide) (PLLA), poly(D,L-lactide-co-glycolide) 50:50 (PLGA), and poly(ε-caprolactone) (PCL) [3]. The amount of polymer used for the formulation of particles in the direct (oil-in-water) miniemulsions is an important factor in determining the mean droplets/particles size. The particles` size increases with an increase of the polymer amount that is used during the preparation. On the other hand, the size of the droplets shows the opposite trend, i.e. smaller droplets were obtained with the higher polymer amounts (> 0.3 g). These results are consistent with the principle of miniemulsion formulation, whereby the presence of higher amounts of hydrophobic compounds can effectively suppress the Oswald ripening and resulting in a more homogeneous and stable droplets. Depending on the type of polymer, the size of particles decreases in the following order: PCL>PLLA>PLGA. An encapsulated hydrophobic fluorescent dye PMI was used as a model marker in order to study the entrapment efficiency, which was found to be 70±1% of the initial concentration.

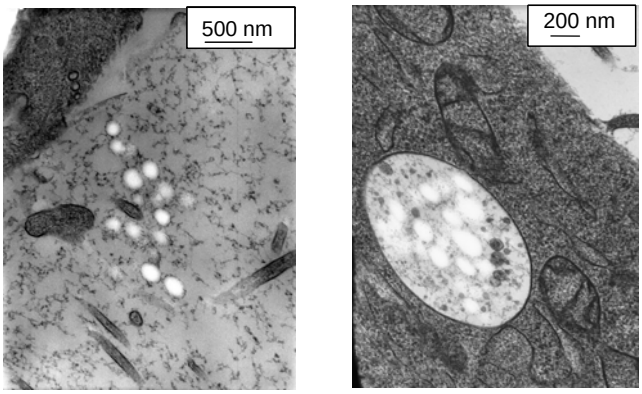

Fig. 4. TEM images of PLLA particles before (left) and after (right) uptake into HeLa cells.

Cellular uptake of the obtained particles was observed in Jurkat and HeLa cells (Fig. 4) Uptake kinetics reveals that the PLLA and PCL particles are much faster endocytosed compared to the non-biodegradable polystyrene nanoparticles.

Conclusions

One-step encapsulation of hydrophilic and hydrophobic compounds into biocompatible polymeric matrix was successfully achieved by the miniemulsion technique. Controlled release of the encapsulated material can be adjusted by choosing the appropriate synthesis conditions (e.g. surfactant concentration, monomer/polymer amount, ratio of the monomers, etc.)

Bibliography

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