Synthetic polymeric nanoparticles by nanoprecipitation†‡

Stephanie Hornig,[a] Thomas Heinze,*[a] C. Remzi Becer[bc] and Ulrich S. Schubert*[bc]

Received 1st April 2009, Accepted 6th April 2009
First published as an Advance Article on the web 1st May 2009
DOI: 10.1039/b906556n

Nanoprecipitation is applied for the first time as a general concept for manufacturing nanoparticles of versatile hydrophobic polymer classes. As a result, polymer molecules self-assemble into nanospheres or irregularly shaped nanoparticles during the transition from the dissolved state to the solid state while using different solvents and methods for the conversion.

Nanotechnology has achieved breakthroughs in therapeutics, bioengineering, diagnostics, imaging, and optics.[1] In particular, the development of functional nanosystems is in the focus of the research for combining the unique properties of the nanoscale materials, *i.e.* due to their high surface to volume ratio, with specific features. For this purpose, tailor-made macromolecules represent interesting materials as they possess an enormous range of potential functionalities, and, thus, can be designed to create nanodevices having advanced structures and properties.[2]

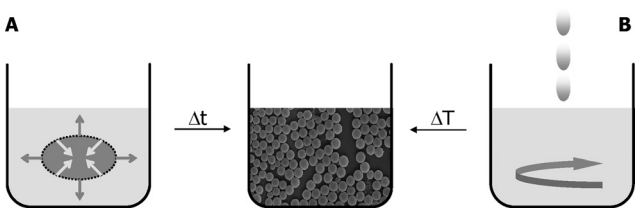
The transformation of the material into a nanoparticular system is just as demanding as the synthesis of well-defined functional polymers. For instance, polymers can be directly prepared in nanoscale templates as commonly applied in radical emulsion polymerizations, *e.g.* of alkylcyano-acrylates.[3] However, the purification of the nanospheres from monomers, oligomers, residual reagents like initiators, and surfactants is a challenging task, as well as achieving the control over the molar mass of the polymers. Moreover, natural polymers or semi-synthetic systems are not accessible for nanoparticle formation *via* this route. An alternative approach for nanoparticle formation is the self-assembly of polymeric materials into nanoscale objects. Therefore, amphiphilic block copolymers are dissolved in a block-selective solvent and subsequently stabilized by covalent interactions, *i.e.*, core- and/or shell-crosslinking.[4] The versatility of the copolymers allows the control of the size, shape, targeting function and encapsulation ability of the polymeric micelles, and can only be achieved with a high level of synthetic expertise.

The emulsion solvent evaporation technique is not only limited to amphiphilic polymers but also applicable for completely hydrophobic polymers.[5] For this purpose, the polymers are dissolved in an organic solvent and emulsified with an aqueous phase with following evaporation of the organic solvent. The particle size and the size distribution strongly depend on the quality of the emulsion. The emulsions need to be homogenized at an ultra-fine level, which is only feasible with the use of surfactants and/or high energy requirements such as sonicators or homogenizers.

The nanoprecipitation technique is a more facile, less complex, less energy consuming as well as widely applicable technique without any additives for the manufacturing of defined nanospheres. This technique is based on the interfacial deposition due to the displacement of a solvent with the non-solvent.[6] Essential prerequisites are the miscibility of the solvents and the existence of dilute polymer solutions. Two different nanoparticle preparation techniques can be applied for the manufacturing of polymeric nanoparticles *via* nanoprecipitation, namely dialysis and the dropping technique, as illustrated in Scheme 1.

Even though nanoprecipation represents an easy and reproducible technique, its application has mainly been limited to the best of our knowledge to the preparation of poly(lactic acid) (PLA) and poly(-lactic-*co*-glycolic acid) nanoparticles since these polymers have been widely used for drug encapsulation studies.[7] As a matter of fact, very few studies describe the formation of polymeric nanospheres *via* nanoprecipitation from other polymers. For instance, cellulose acetate, a widely applied polysaccharide ester, can be manufactured into regularly- or bean-shaped particles by varying the preparation conditions.[8] In this context, viscosity measurements indicate that the concentration for nanoprecipitation should be below the critical overlap concentrations of the polymers in solution. Other polysaccharide derivatives were also precipitated to form nanoparticles like pullulan acetate,[9] alkyl- and phenyl-dextran ethers,[10] and multiple functionalized dextran derivatives (for a very recent example see ref. 12).[11] One specific example for a synthetic polymer that can be formed into nanoparticles from acetone solution is poly(ε-caprolactone) (PCL).[13] The ternary phase diagram of PCL in acetone and water shows a narrow area of mixtures that is usable for the self-assembly of polymers into nanospheres.

The knowledge of the self-assembly of other synthetic polymers requires a clarification to allow a generic application of such a facile



Scheme 1 A schematic representation of the nanoprecipitation methods applying dialysis in a membrane (A) and the dropping technique under stirring (B), respectively.

[a]*Center of Excellence for Polysaccharide Research, Friedrich-Schiller-University Jena, Humboldtstr. 10, 07743 Jena, Germany. E-mail: thomas.heinze@uni-jena.de*
[b]*Laboratory of Organic and Macromolecular Chemistry, Friedrich-Schiller-University Jena, Humboldtstr. 10, 07743 Jena, Germany. E-mail: ulrich.schubert@uni-jena.de*
[c]*Laboratory of Macromolecular Chemistry and Nanoscience, Eindhoven University of Technology, Den Dolech 2, 5600 MB Eindhoven, The Netherlands*

† Dedicated to Prof. Dr Dieter Schubert on the occasion of his 70th birthday.

‡ Electronic supplementary information (ESI) available: Synthesis, materials and methods. See DOI: 10.1039/b906556n

technique to a wide range of polymers. To the best of our knowledge, this contribution represents the first extended study expanding the concept of nanoprecipitation to water insoluble and non-amphiphilic synthetic polymers except the few polymer samples mentioned before. Technical samples are included as well as well-defined polymers that were synthesized by controlled "living" radical polymerization (CRP) methods. In particular, technical grade samples of poly(styrene) (PS), poly(methyl methacrylate) (PMMA), PCL, poly(vinyl carbazole) (PVK), poly(styrene-*co*-acrylic acid) (PS-*co*-AA), poly(vinyl acetate) (PVAc) and poly(carbonate) (PC) were tested regarding their ability to form nanoparticles *via* nanoprecipitation. Furthermore, CRP techniques allow the design of well-defined polymers with controlled molar mass (number average, M_n), low polydispersity indices (PDIs) and functionalities.[14] Atom transfer radical polymerization (ATRP) and the reversible addition–fragmentation chain transfer (RAFT) polymerization were used for the synthesis of the PMMA samples.[15] In addition, nitroxide-mediated radical polymerization (NMP) was utilized for the preparation of specific PS samples with varying molar masses and low PDI values.[16]

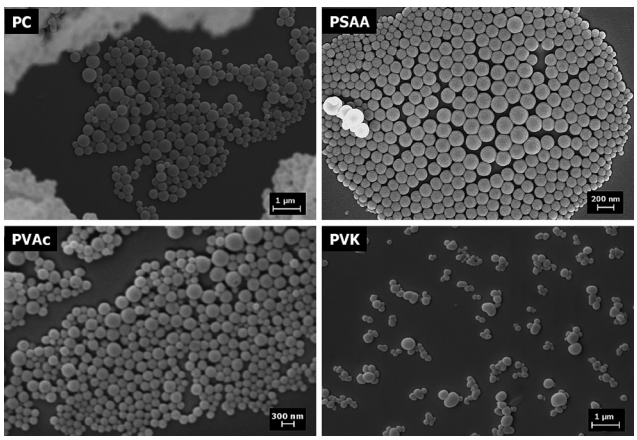


Fig. 1 SEM images of polymeric nanoparticles of poly(carbonate) (PC), poly(styrene-*co*-acrylic acid) (PS-*co*-AA), poly(vinyl acetate) (PVAc) and poly(vinyl carbazole) (PVK) prepared by dialysis of the polymers.

In general, low concentrations are required to ensure that the molecules are in a dispersed state and able to separate into nanodomains following the addition of the non-solvent. Therefore, a concentration of 4 mg/mL was chosen for the formation of small particles. In the first set of experiments, *N*,*N*-dimethylacetamide (DMA) was used as an organic solvent. The dialysis technique represents an efficient tool for the controlled and ideally complete exchange of solvents (in particular with high boiling points) against water. The polymer solutions were transfered into a dialysis membrane and the surrounding distilled water was exchanged several times until the DMA was completely replaced. In this way, low molar mass agents (*i.e.* monomers, additives, and initiators) that might still be present in the bulk material after the polymerization are removed. The resulting nanoparticle suspensions were filtered through paper filter in order to remove small amounts (less than ~10 wt%) of larger aggregates. Single samples were dialyzed several times and show no changes in the size of the resulting nanoparticles. The suspensions were further analyzed for size and shape by dynamic light scattering (DLS) and scanning electron microscopy (SEM). Fig. 1 displays selected SEM images of nanoparticles that were only prepared by dialysis from DMA solution because of their insolubility in acetone.

Table 1 shows the mean diameter and the polydispersity indices of the size distribution (PDI_Ps) of the nanoparticles obtained by dynamic light scattering (DLS). The particles that are formed have particle sizes in the range of 196 to 718 nm, whereas comparatively narrow size distributions were obtained for the samples PS-8, PVK and PS-*co*-AA, indicated by a PDI_P below 0.15. Only PMMA-1 formed large, undefined aggregates during dialysis from DMA. No

Table 1 Overview of selected molecular properties and synthetic methods of the polymer samples and z-average mean diameters of the resulting nanoparticles prepared by nanoprecipitation applying two different methods[f]

				Method **A** Dialysis DMA–H_2O		Method **B** Dropping Ac–H_2O	
Sample	M_n/kg mol^{-1}[a]	PDI[a]	Synthesis/grade	d/nm[b]	PDI_P[b]	d/nm[b]	PDI_P[b]
PS-1	5.2	1.13	NMP	409	0.211	141	0.144
PS-2	7.2	1.10	NMP	422[c]	0.274	107	0.129
PS-3	8.2	1.12	NMP	555[c]	0.375	119	0.118
PS-4	13.6	1.13	NMP	409[c]	0.290	106	0.137
PS-5	14.1	1.14	NMP	349[c]	0.232	107	0.097
PS-6	16.2	1.16	NMP	653[c]	0.248	117[c]	0.132
PS-7	78.6	4.79	TG	718[c]	0.219	—[d]	—[d]
PS-8	147.1	2.79	TG	522[c]	0.091	—[d]	—[d]
PMMA-1	8.0	1.19	RAFT	—[e]	—[e]	116	0.120
PMMA-2	10.6	1.42	ATRP	543	0.165	—[e]	—[e]
PMMA-3	42.6	1.73	TG	341[c]	0.221	207	0.158
PCL-1	42.5	1.53	TG	—[d]	—[d]	271	0.159
PCL-2	84.0	1.79	TG	—[d]	—[d]	313	0.136
PVK	140.1	5.37	TG	267	0.136	—[d]	—[d]
PS-*co*-AA	170.6	2.38	TG	196	0.054	—[d]	—[d]
PVAc	54.0	6.80	TG	258	0.207	—[d]	—[d]
PC	17.9	2.52	TG	335	0.199	—[d]	—[d]

[a] Determined by means of GPC. [b] Average values of three DLS measurements, see the ESI.‡ [c] Also some larger, undefined aggregates. [d] Insoluble in organic solvents. [e] Only large, undefined aggregates. [f] PDI: polydispersity index (M_w/M_n). PDI_P: polydispersity index of the particle size as determined by DLS. NMP: nitroxide-mediated polymerization. RAFT: reversible addition–fragmentation chain transfer. ATRP: atom transfer radical polymerization. TG: technical grade.

correlation of the molar mass with the resulting particle size was found for the PS samples (PS1-PS6) that were all prepared by NMP. Even the technical grade samples with high molar mass, PS-7 and PS-8, form particles in the same range as the low molar mass samples.

In principle, not only DMA but also other water-miscible solvents can be used for dialysis. However, in case of volatile solvents, the dropping technique seems to be more facile, less time consuming and also does not require a dialysis membrane. Therefore, the polymers dissolved in acetone were introduced into water in droplets while stirring. The acetone can subsequently be completely removed from the aqueous suspension by evaporation. This preparation technique results in particles in the range of 106 nm to 313 nm and narrow size distributions indicated by PDI_P values between 0.097 and 0.159 (see Table 1). Consequently, the dropping technique leads to smaller and more uniformly distributed nanoparticles.

A direct comparison of the shapes of the nanoparticles formed can be found in the SEM images in Fig. 2. Particles of PS-1 are ideal globes obtained by both dialysis and the dropping technique. However, PMMA-1 forms irregularly shaped particles in DMA solution whereas preparation in acetone leads to more spherical shaped units. PCA-1, which is not soluble in DMA, appears in uneven spheres. Moreover, up to now, no direct correlation can be identified for the resulting appearance of the particles when applying the different preparation techniques, although a non-spherical shape would be expected applying the dropping technique.

In summary, all presented polymers from technical grade as well as well-defined polymers prepared by utilizing CRP techniques show nanoparticle formation *via* nanoprecipitation either by dialysis from a DMA solution or by dropping of an acetone solution under specific medium conditions, *i.e.*, dilute solutions. The extension of the concept for other polymer classes, encapsulation studies and characterization of the nanoparticles by analytical ultracentrifugation is currently under investigation. By varying the parameters, in particular the choice of the solvent, the concentration of the solution, and the method applied, it should be possible to structure a wide range of versatile functional polymers, *e.g.* glycopolymers, into nanoscale particles; thus, an alternative general path for the well-controlled nanoparticle formation can be provided. This approach opens completely new avenues in nanotechnology for the formulation of drug delivery devices or heterogeneous catalytic systems.

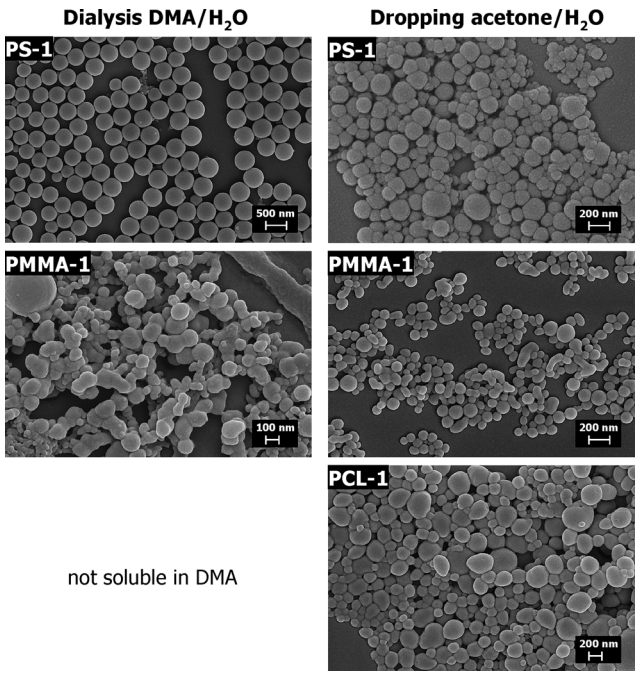


Fig. 2 SEM images of polymeric nanoparticles of poly(styrene) (PS), poly(methyl methacrylate) (PMMA), poly(ε-caprolactone) (PCL) prepared by dialysis of the polymers dissolved in DMA (c = 4 mg/mL) and by dropwise addition of water into an acetone solution of the polymers containing 4 mg/mL.

Financial support from the Dutch Polymer Institute (project number 502) is greatly acknowledged. We also thank F. Steiniger (EMZ Jena) for technical assistance with the SEM equipment and the Department of Pharmaceutical Technology for providing access to the zetasizer.

Notes and references

1 (*a*) S. O. Kim, H. H. Solak, M. P. Stoykovich, N. J. Ferrier, J. J. de Pablo and P. F. Nealey, *Nature*, 2003, **424**, 411–414; (*b*) M. Nahar, T. Dutta, S. Murugesan, A. Asthana, D. Mishra, V. Rajkumar, M. Tare, S. Saraf and N. K. Jain, *Crit. Rev. Ther. Drug Carrier Sys.*, 2006, **23**, 259–318; (*c*) R. Ruiz, H. M. Kang, F. A. Detcheverry, E. Dobisz, D. S. Keercher, T. R. Albrecht, J. J. de Pablo and P. F. Nealey, *Science*, 2008, **321**, 936–939.
2 A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A. K. Patri, T. Thomas, J. Mule and J. R. Baker, *Pharm. Res.*, 2002, **19**, 1310–1316.
3 C. Chauvierre, D. Labarre, P. Couvreur and C. Vauthier, *Macromolecules*, 2003, **36**, 6018–6027.
4 R. K. O'Reilly, C. J. Hawker and K. L. Wooley, *Chem. Soc. Rev.*, 2006, **35**, 1068–1083.
5 S. Desgouilles, C. Vauthier, D. Bazile, J. Vacus, J.-L. Grossiord, M. Veillard and P. Couvreur, *Langmuir*, 2003, **19**, 9504–9510.
6 H. C. Fessi, J.-P. Devissaguet, F. Puisieux and C. Thies, *US Patent*, 5 118 528, 1990; H. Fessi, Puisieuxm J. Devissaguet, N. Ammoury and S. Benita, *Int. J. Pharm.*, 1989, **55**, R1–R4.
7 (*a*) T. Govender, S. Stolnik, M. C. Garnett, L. Illum and S. S. Davis, *J. Control. Release*, 1999, **57**, 171–185; (*b*) R. A. Jain, *Biomaterials*, 2000, **21**, 2475–2490; J. Molpeceres, M. Guzman, M. R. Aberturas, M. Chacon and L. Berges, *J. Pharm. Sci.*, 1996, **85**, 206–213; (*c*) S. Guterres, H. Fessi, G. Barrat, F. Puisieux and J. Devissaguet, *Int. J. Pharm.*, 1995, **113**, 57–63; (*d*) M. Chorny, I. Fishbein, H. D. Danenberg and G. Golomb, *J. Control. Release*, 2002, **83**, 389–400.
8 S. Hornig and T. Heinze, *Biomacromolecules*, 2008, **9**, 1487–1492.
9 Y.-I. Jeong, J.-W. Nah, H.-K. Na, K. Na, I.-S. Kim, C.-S. Cho and S.-H. Kim, *Drug Dev. Ind. Pharm.*, 1999, **25**, 917–927.
10 A. Aumelas, A. Serrero, A. Durand, E. Dellacherie and M. Leonard, *Colloids Surf. B*, 2007, **59**, 74–80.
11 (*a*) S. Hornig and T. Heinze, *Carbohydr. Polym.*, 2007, **68**, 280–286; (*b*) S. Hornig, C. Biskup, A. Gräfe, J. Wotschadlo, T. Liebert, G. J. Mohr and T. Heinze, *Soft Matter*, 2008, **4**, 1169–1172.
12 C. R. Becer, K. Babiuch, D. Pilz, S. Hornig, T. Heinze, M. Gottschaldt and U. S. Schubert, *Macromolecules*, 2009, **42**, 2387–2394.
13 (*a*) S. Stainmesse, A.-M. Orecchioni, E. Nakache, F. Puisieux and H. Fessi, *Colloid Polym. Sci.*, 1995, **273**, 505–511; (*b*) M. Snehalatha, K. Venugopal and R. N. Saha, *Drug Delivery*, 2008, **15**, 267–275; (*c*) F. Lince, D. L. Marchisio and A. A. Barresi, *J. Colloid Interface Sci.*, 2008, **322**, 505–515.
14 J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559–5562; M. Kato, M. Kamigaito, M. Sawamoto and T. Higashimura, *Macromolecules*, 1995, **28**, 1721–1723; J. Wang and K. Matyjaszewski, *J. Am. Chem. Soc.*, 1995, **117**, 5614–5615; C. J. Hawker, G. G. Barclay, A. Orellana, J. Dao and W. Devonport, *Macromolecules*, 1996, **29**, 5245–5254.
15 C. R. Becer, R. Hoogenboom, D. Fournier and U. S. Schubert, *Macromol. Rapid. Commun.*, 2007, **28**, 1161–1166.
16 C. R. Becer, R. M. Paulus, R. Hoogenboom and U. S. Schubert, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 6202–6213.

