A Route to Nonfunctionalized and Functionalized Poly(*n*-butylcyanoacrylate) Nanoparticles: Preparation in Miniemulsion

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ABSTRACT: The miniemulsion process has been applied for the preparation of poly(*n*-butylcyanoacrylate) (PBCA) nanoparticles. In the first step a miniemulsion is prepared from *n*-butylcyanoacrylate in hydrochloric acid solution using sodium dodecyl sulfate as surfactant. In the second step, a base solution is added to initiate polymerization, and the polymeric particles are formed. Using amines or amino acids as initiators allowed the convenient functionalization of the polymer particles' surface. The influence of surfactant concentration and sonication time on particle size and size distribution has been studied as well as the influence of pH, concentration, and amount of initiator on the particle size and the distribution of the molar mass of the polymer. The detected pH dependence of the particles' ζ potential shows the presence of carboxyl groups on the particles' surface after the initiation with amino acids. GPC and NMR measurements indicate a covalent bonding of the amino acid to the polymer.

Introduction

Alkylcyanoacrylates (ACA) have been proven to be valuable monomers for several applications. Besides the broadly known use as "super glue", they are employed in surgery for wound closure (e.g., Indermil, *n*-butylcyanoacrylate BCA). Both applications are based on the fact that the anionic polymerization is easily initiated by traces of nucleophiles like water (humidity, water present on skin), amines (e.g., present in proteins), alcohols, or phosphines.

In the past years, considerable efforts have been put in the synthesis of poly(alkylcyanoacrylate) nanoparticles. Poly(alkylcyanoacrylate) nanoparticles are biocompatible and biodegradable and are reported to show a distinct tendency for the adsorption or respectively entrapment of bioactive compounds, making them promising candidates for the use as drug carrier systems. A large number of different compounds have been used as "payload", ranging from inorganic crystallites, e.g., magnetite,[1] to various drugs (methotrexate,[2] doxorubicin[3−5]) and even oligopeptides (dalargin[6,7]) or proteins (insulin[8−10]).

One of the first methods for the preparation of poly(alkylcyanoacrylate) (nano)particles has been developed by Couvreur[11] in the late 1970s employing a HCl solution with a concentration of 10^{-2}−10^{-3} mol L^{-1} containing a polymeric, nonionic surfactant as steric stabilizer, to which the alkylcyanoacrylate monomer is added dropwise. Since then, a large number of studies (see ref 12) have reported the application of dispersion and emulsion techniques, with or without surfactant. The described particles show a broad distribution of sizes ranging from under 100 nm to more than 1 μm. Particle size, stability of the dispersion, and the molar masses of the polymer depend largely on the pH of the continuous phase[13−16] and on the type and concentration of the surfactant.[17,18]

Despite the extensive application of the emulsion polymerization with nonionic or polymeric surfactants for the preparation of poly(alkylcyanoacrylate) nanoparticles, there are several limitations, especially the low polymer content of the dispersions of about 1 wt % and the high amount of surfactant compared to the monomer with a ratio surfactant to monomer of 1:1 or even more (see e.g. refs 6 and 19). The problem is the fast polymerization rate of the monomer combined with the comparably slow diffusion rate of the monomer through the water phase which causes instability of the system. Additionally, the stabilizer present in the commercially available monomer causes severe problems. Since the applied stabilizers are Lewis acids in an unknown concentration ($MeSO_3H$, SO_2), an influence on the particle properties can be expected and cannot easily be controlled with the techniques applied so far. It has been reported that the amount of SO_2 affects the particle size[14,20] and therefore leads to a lack of reproducibility if using different batches of the alkylcyanoacrylate monomer.

Up to now, modification of the particle surface is achieved by the choice of surfactant, which is physically adsorbed like, e.g., polysorbates[6,7] or chemically bonded via, e.g., a hydroxy group of dextran[17,21] or poly(ethylene glycol) (PEG)[22] to the particles' surface. The modification with polysorbates allows the particles to permeate through the blood brain barrier, while PEGylated particles show long persistence in the circulatory system.

Nevertheless, further chemical functionalization of the polymer particles to specifically designed surface characteristics is difficult to achieve because of the occupation of the particle surface by surfactant molecules due to high amount of surfactant used and the absence of anchor groups like COO^-. These groups are needed in order to conjugate (bio)molecules[23−25] like proteins (e.g., antibodies) for addressing specific receptors for cellular response. Therefore, it would be desirable to considerably lower the amount of surfactant and at the same time be able to introduce functional groups.

Here we report on the preparation of reproducibly functionalizable and loadable PBCA nanoparticles with a narrow size distribution which are stable in a dispersion with a solid content higher than 10 wt % using a minimum of the anionic surfactant sodium dodecyl sulfate (SDS).

A very convenient way to meet these requirements is the application of the miniemulsion technique in which the polymerization is initiated after a stable *n*-butylcyanoacrylate miniemulsion in water has been formed as it was already shown in

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Table 1. Characteristics of PBCA Nanoparticles Obtained with Varying SDS Amounts and Sonication Times[a]

amount of surfactant [wt % with respect to BCA]	sonication time [s]	particle size (*z*-average) [nm]	PDI	M_w[b] [g mol^{-1}]	$D = M_w/M_n$
1	90	292	0.433	5500	2.6
1	120	358	0.308	5200	2.5
1	150	326	0.383	5000	2.5
1	180	302	0.338	6600	2.5
2	90	295	0.289	4500	2.4
2	120	263	0.297	5000	2.5
2	150	331	0.357	4500	2.4
2	180	292	0.297	5300	2.5
4	90	217	0.272	5200	2.6
4	120	284	0.432	4800	2.5
4	150	228	0.346	4000	2.3
4	180	224	0.242	3900	2.4
10	90	161	0.221	5300	2.4
10	120	161	0.250	4500	2.2
10	150	154	0.222	4000	2.1
10	180	158	0.151	3700	2.0
20	90	155	0.272	4600	2.4
20	120	190	0.391	3600	2.1
20	150	107	0.274	3900	2.1
20	180	183	0.205	6500	2.1

[a] NaOH was used as initiating agent for the polymerization. [b] Given to PS standard.

one single model experiment using the miniemulsion technique in order to create a poly(alkylcyanoacrylate) nanoparticle dispersion with a solid content of 5%.[12]

Taking advantage of the high stability of miniemulsions obtained by a hydrophobic agent in order to prevent Ostwald ripening, it will be shown that it is possible to increase the amount of dispersed BCA monomer even further and therefore the solid content of the final dispersion to more than 10%. The polymerization is initiated in the simplest case by the addition of a hydroxide solution. The influence of surfactant concentration and sonication time on particle size and size distribution has been studied as well as the influence of pH, concentration, and amount of initiator solutions on the particle size and the distribution of the molar mass of the polymer. It will be shown that the application of mono- or multifunctional amines as initiator allows the introduction of functional groups to the polymer[26−28] and thus to the particle. The detected pH dependence of the particle's ζ potential indicates the presence of carboxyl groups on the particles' surface after the initiation with amino acids.

Experimental Part

Materials. *n*-Butylcyanoacrylate (BCA, Indermil, Henkel Loctite) was used as received. Hydrochloric acid (0.1 mol L^{-1}), sodium hydroxide solution (0.1 mol L^{-1}), tris-base (tris(hydroxymethyl)-aminomethane), ammonia solution (25%), sodium dodecyl sulfate (SDS), 6-aminohexanoic acid (6AHex), arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), cysteine (Cys), glycine (Gly), and lysine (Lys) were purchased from Merck; phenylalanine (Phe) and hexadecane (HD) were purchased from Aldrich. Tween 20 was purchased from Sigma-Aldrich. Lutensol AT50, a poly(ethylene oxide)−hexadecyl ether with an EO block length of about 50 units, was received as a gift from BASF AG. All chemicals were used as received.

Synthesis of Nanoparticles. Standard procedure for the preparation of a *n*-butylcyanoacrylate miniemulsion and subsequent initiation of the polymerization:

A solution of 0.3 g of SDS in 12.0 g of hydrochloric acid (0.1 mol L^{-1}) was added just prior to ultrasonication to a solution of 0.125 g of hexadecane in 3.0 g of BCA. The two-phase mixture was sonicated with a Branson sonifier W450 (90% amplitude, 0.5 in. tip) for 2.5 min under ice cooling. After sonication a milky white emulsion is obtained. The total amounts used in the standard procedure can be easily increased or decreased as long as the ratio of the reactants used is maintained. The polymerization was initiated by pouring the miniemulsion into a sodium hydroxide solution (0.1 mol L^{-1}) while stirring on a magnetic stirrer.

Variation of Surfactant. In order to determine the influence of the type of surfactant, the miniemulsion was prepared in the way described above, but with different surfactants. Besides SDS, Lutensol AT 50 and Tween 20 were used. The polymerization was initiated by pouring the miniemulsion into 12.0 g of sodium hydroxide solution (0.1 mol L^{-1}) under stirring.

Variation of Sonication Time and Surfactant Amount. The miniemulsion was prepared as described above with SDS as surfactant (for quantities see Table 1). After a sonication time of 90, 120, 150, and 180 s, 500 μL of the miniemulsion was withdrawn and injected into 375 μL of sodium hydroxide solution (0.1 mol L^{-1}). The small volumes were chosen to be able to prepare all the compared samples from one miniemulsion batch. Larger quantities can easily be obtained by scaling up the recipe.

Time Dependence of Molar Mass. A miniemulsion was prepared in the way described above (BCA 9.0 g, HD 0.375 g, HCl 36.0 g; SDS 0.9 g). A 500 μL sample was pipetted and injected into a vessel immersed in liquid nitrogen just prior to pouring the miniemulsion in 36.0 g of sodium hydroxide solution (0.1 mol L^{-1}) under stirring. During the first 120 s, every 10 s a 500 μL sample has been taken and treated the same way as described above. This procedure, with longer intervals between the sampling, has been carried out over 2 weeks. The frozen samples were freeze-dried. The molar masses of the resulting polymer powder were determined by GPC.

Variation of Type of Initiator and Amount of Initiator. In order to initiate the polymerization, 500 μL of the freshly prepared miniemulsion was injected in one shot into various amounts of sodium hydroxide solution (0.1 mol L^{-1}, see Table 2), tris-base solution (0.1 mol L^{-1}), ammonia solution (0.1 mol L^{-1}) (for both see Table 3), and various solutions of amino acids (see Table 4).

Dialysis. The dispersions have been dialyzed against water using Amicon Ultra centrifuge filters (30 000 MWCO membrane, Millipore).

Characterization. The particle size and the ζ potential were determined with a Malvern Zetasizer Nano ZS. For the photon correlation spectroscopy (PCS) measurements 35 μL of the dispersion was pipetted into a single use polystyrene cuvette and diluted with 1.5 mL of distilled water.

DLS measurements give the *z*-average size (or cumulant mean), which is an intensity mean, and the polydispersity index (PDI). The standard cumulant analysis is the fit of a polynomial to the log of the *G1* correlation function (eq 1).

$$\ln(G1) = a + bt + ct^2 + dt^3 + ... \quad (1)$$

Table 2. Characteristics of the PBCA Dispersions Initiated with Different Amounts of NaOH as Initiating Agent[a]

vol of NaOH [μL]	resulting pH (calcd)	pH measured after 7 days	particle size (*z*-average) after 10 min of preparation [nm]	particle size (*z*-average) measured after 7 days [nm]
0	1.00	0.94	139	199
100	1.22	1.41	186	149
200	1.48	1.62	214	144
300	1.85	1.97	203	188
400	7.00	2.71	212	213
500	12.05	4.66	214	207
600	12.30	6.44	188	178
700	12.44	6.57	195	194
800	12.52	6.85	172	173

[a] 500 μL of a 20 wt % BCA miniemulsion (stabilized with 10% of SDS based on monomer) is used.

Table 3. PBCA Particle Sizes of Dispersions Initiated with Ammonia and Tris-base Solution (Measured after Polymerization)[a]

	ammonia solution (0.1 mol L^{-1})		Tris-base (0.1 mol L^{-1})	
vol of initiator solution [μL]	particle size (*z*-average) [nm]	PDI	particle size (*z*-average) [nm]	PDI
100	233	0.236	244	0.168
200	86	0.234	164	0.196
300	64	0.268	104	0.261
400	77	0.243	99	0.228
500	84	0.237	115	0.184
600	97	0.192	125	0.152
700	96	0.203	132	0.132
800	95	0.184	137	0.109
900	98	0.191	141	0.074
1000	111	0.152	145	0.101

[a] The volume of the used BCA miniemulsion (25 wt % monomer) was 500 μL.

The value of second-order cumulant *b* is converted to a size using the dispersant viscosity and some instrumental constants. The coefficient of the squared term *c*, when scaled as $2c/b^2$, is known as the polydispersity or polydispersity index (PDI). The calculations for these parameters are defined in the ISO standard document 13321:1996 E.

For ζ-potential measurements, 50 μL of the dispersion was diluted to a total volume of 5 mL and the desired pH. The pH was adjusted with 0.1 mol L^{-1} NaOH and HCl solutions.

Gel permeation chromatography (GPC) was used to determine the molecular weight of the poly(*n*-butylcyanoacrylate) nanoparticles. After the polymerization had been completed, the samples were frozen at −22 °C and subsequently freeze-dried. The resulting powders were dissolved in 1 mL of THF, and the solution was filtered through a 0.45 μm syringe filter. The setup consisted of a Thermal Separations Products P2000 pump with Waters Styragel 5 μm particles, 100 nm pore size, PSS SDV 5 μm particles, 1 μm pore size, PSS SDV 10 μm pore size columns, and a Thermal Separations Products AS100 autosampler. The eluent was THF p.a. with a flow rate of 1 mL min^{-1}. The signal was detected with a Waters 2410 RI detector and with a Knauer Variable Wavelength Monitor UV detector. The molar masses were calculated with respect to a polystyrene (PS) standard and can therefore not reflect the exact values. In order to visualize the tendencies, the elugrams are additionally provided.

The TEM images were obtained using a Philips TEM 400 with an acceleration current of 80 kV. 5 μL of the dispersion was diluted with 5 mL of demineralized water; a 4 μL drop was put on a carbon-coated copper grid (200 mesh) and air-dried. No further staining has been applied.

NMR spectra have been obtained using a Bruker Avance 400 operating at 400 MHz.

Results and Discussion

Up to now, PBCA nanoparticles were mostly prepared by typical emulsion processes. Now, we were able to prepare stable monomer droplets of the hydrophobic BCA by miniemulsification in 0.1 mol L^{-1} hydrochloric acid as continuous phase since strong acids are known to inhibit polymerization of ACAs efficiently.[26,29,30] Then, after creation of the droplets, the anionic polymerization was initiated by pouring the miniemulsion into a NaOH solution. Evaporation of the monomer during the miniemulsion preparation and heating of the dispersion while polymerization were minimized by using pulsed ultrasound and cooling the system.

The anionic surfactant SDS has been the first choice for the stabilization of the BCA miniemulsion and the subsequently formed PBCA dispersion. The results are summarized in Table 1. The use of SDS allowed the formulation of PBCA dispersions with a solid content of 10% using 1% of surfactant with respect to BCA, leading to stable particles of about 300 nm in diameter. With increasing SDS concentration, the particle size decreases to about 100 nm (see also Figure 1) and the size distribution (polydispersity index, PDI) narrows.

The equilibrium size of the droplets in miniemulsions is determined by the amount of SDS with respect to the amount

Table 4. Characteristics of Dispersions Initiated with 0.5 mol L^{-1} 6AHex Solution and 0.1, 0.5, and 2.0 mol L^{-1} Glycine Solutions (First Value: *z*-Averaged Diameter; Second Value: PDI)

	6AHex (0.5 mol L^{-1})		Gly (0.1 mol L^{-1})				Gly (0.5 mol L^{-1})			Gly (2.0 mol L^{-1})		
vol of initiator [μL]	pH 4.4	pH 5.4	pH 2.4	pH 3.4	pH 4.4	pH 5.4	pH 3.4	pH 4.4	pH 5.4	pH 3.4	pH 4.4	pH 5.4
100	coagulation	129	360	336	302	297	184	204	232	165	121	108
		0.089	0.134	0.163	0.127	0.109	0.056	0.075	0.108	0.016	0.042	0.050
200	238	100	270	223	202	198	146	170	156	144	117	107
	0.107	0.141	0.151	0.084	0.035	0.043	0.019	0.002	0.082	0.018	0.049	0.018
300	192	93	223	189	164	162	137	158	127	139	115	103
	0.010	0.161	0.062	0.052	0.055	0.062	0.045	0.010	0.047	0.030	0.040	0.041
400	195	89	204	159	142	146	136	149	124	134	111	97
	0.141	0.167	0.094	0.037	0.069	0.057	0.035	0.013	0.026	0.020	0.057	0.059
500	185	87	187	139	132	141	137	144	122	129	107	92
	0.042	0.190	0.043	0.074	0.074	0.032	0.085	0.040	0.022	0.070	0.071	0.089
600	178	86	176	126	130	139	129	145	120	126	106	88
	0.065	0.182	0.045	0.063	0.052	0.006	0.045	0.035	0.003	0.029	0.062	0.093
700	187	85	166	125	128	138	128	142	118	124	105	87
	0.105	0.174	0.052	0.088	0.086	0.059	0.048	0.010	0.013	0.063	0.043	0.110
800	182	86	161	118	130	139	127	137	116	121	104	85
	0.209	0.183	0.054	0.058	0.080	0.065	0.030	0.035	0.048	0.087	0.044	0.121
900	202	84	154	122	135	154	127	135	118	120	104	83
	0.197	0.180	0.064	0.045	0.014	0.080	0.016	0.016	0.040	0.058	0.066	0.110
1000	173	85	152	121	126	137	125	132	119	117	102	85
	0.044	0.172	0.029	0.044	0.061	0.064	0.025	0.046	0.022	0.082	0.060	0.092

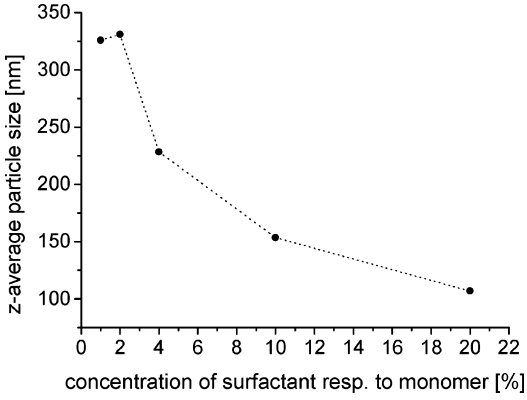


Figure 1. Evolution of the PBCA particle size with concentration of surfactant SDS for a sonication time of 150 s (from Table 1; dotted lines are guides for the eye).

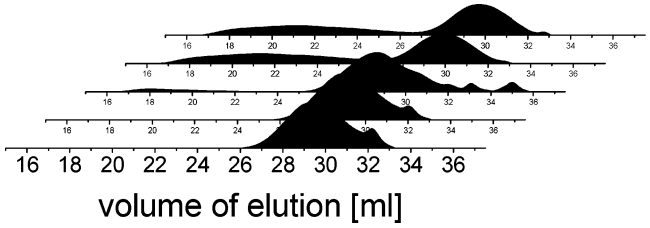


Figure 2. Evolution of elution volume obtained from a PBCA dispersion prepared according to the standard procedure (calculated pH = 7) during the course of 4 weeks (samples taken after 10 s, 600 s, 2 days, 1 week, and 1 month (front to back) after preparation).

of the dispersed phase. This equilibrium is reached by the application of strong shear forces like ultrasound. After a characteristic sonication time, the droplet size cannot be reduced any further. The size distribution still shows a slight narrowing applying a longer sonication time. Figure 1 shows the evolution of the PBCA particle size for the sonication time of 150 s.

The weight-average molecular weight M_w of the polymer obtained from a dispersion prepared with 1% of SDS measured 2 days after mixing with the NaOH solution shows a monomodal distribution at about 5000 g mol^{-1}. (Please note that all molecular weights are given relative to PS standards.) With increasing amount of surfactant, the values for M_w decrease. This means that after 2 days the smaller particles are composed of polymer with shorter chains than the larger particles. Further experiments show that the molar masses of the polymers change over the course of days until an equilibrium distribution is reached. Samples taken and analyzed after 1 week and 1 month show no longer monomodal mass distribution, but a bimodal distribution with the appearance of a long chain polymer fraction (see Figure 2 and also Figure 6 including discussion below).

All polymer dispersions prepared with SDS showed long-term stability. Even 2 months after the preparation, no phase separation could be observed. The application of cationic surfactants, namely quaternary amines, led to immediate polymerization when the monomer and the aqueous phase were mixed. Residual primary and secondary amines may be the cause for this.

As nonionic surfactants, Lutensol AT50 and Tween 20 were chosen. Applying these, dispersions with large particles and high sedimentation tendency were obtained (samples L-10 (sonication time = 120 s, d = 908 nm) and T-10 (sonication time = 120 s, d = 769 nm)) at the desired high solid contents of 10%. Even higher amounts of these surfactants of more than 10% were not able to decrease the particle size and to stabilize the latex dispersions efficiently. Already 1 h after preparation, all dispersions showed phase separation.

On the basis of these data, SDS in a concentration of 10% and a sonication time of 150 s had been chosen for the subsequent experiments.

Figure 3 shows TEM micrographs from three selected dispersions. S-10 was prepared according to the standard procedure with 10 wt % SDS (150 s sonication, see Table 1), L-10 with 10 wt % Lutensol AT50, and T-10 with 10 wt % Tween 20. The two latter samples could only be prepared for TEM analysis after the sedimented dispersions had been shaken in order to redisperse the precipitate. The smaller size and greater uniformity of the particles prepared with SDS are clearly visible.

Influence of the Amount of Initiator NaOH on Particle Size and Molecular Weight. It is known from various BCA emulsion polymerization experiments that the pH of the dispersion media affects the particle size and molecular weight.[13,14]

The influence of the pH on the characteristics of PBCA particles, obtained in miniemulsion, is studied in detail. The pH of the polymerization medium was adjusted by providing different amounts of NaOH solution to initiate the polymerization (see Table 2). It has been assumed that the neutralization reaction between the NaOH solution and the HCl of the miniemulsion is fast compared to the initiation and the growth steps of the polymerization.

In the case of miniemulsion, the polymerization pH could be increased to a pH of 7. With the conventional emulsion polymerization technique, the pH of the polymerization could not be carried out at pH values higher than 4−5, since at higher values the polymerization is too fast and the diffusion of the monomer through the water phase, which is a key step in the emulsion polymerization technique, too slow. Therefore, coagulum is formed.[13] Compared to the conventional emulsion method, the surfactant applied in the miniemulsion process is far more effective in stabilizing the monomer droplets. The polymerization is therefore restricted to each monomer droplet, which is behaving as an independent nanoreactor. Here a fast polymerization can take place without influencing the stability of the dispersion.

Regarding the pH of the dispersion obtained in miniemulsion, it can be noticed that, especially considering the calculated high pH values, the values are constantly decreasing with time, reaching a stable value after 1 day, which is below the expected value (Figure 4). This means OH^- is consumed (about 10^{-2} mol L^{-1}) in an unexpectedly high amount during the reaction, which supports the assumption that OH^- is the initiating molecule (and at least some of the chains end with a salt group), although it cannot be completely ruled out that Cl^- also acts as initiator.

The particle size for all pH can be found in a narrow range at around 200 nm, not considering the small volumes between 0 and 200 μL of added initiator, which will be discussed separately, where no clear dependence can be observed. Since the values do not change significantly during the 7 days, it can be assumed that all of the dispersions are stable toward coagulation.

The values for the miniemulsion without the addition of a NaOH solution (0 μL) show the evolution of the particle size in the "unperturbed" (and unpolymerized) miniemulsion with an extremely slow polymerization. Directly after the preparation, when the first DLS measurement has been performed, it is reasonable to assume that the miniemulsion is still an emulsion and no polymer dispersion, since the pH of the miniemulsion has an unaltered value of pH 1.0. With the dilution of the miniemulsion with water for the DLS measurement, polymerization will be initiated, so the actual droplet size may not be

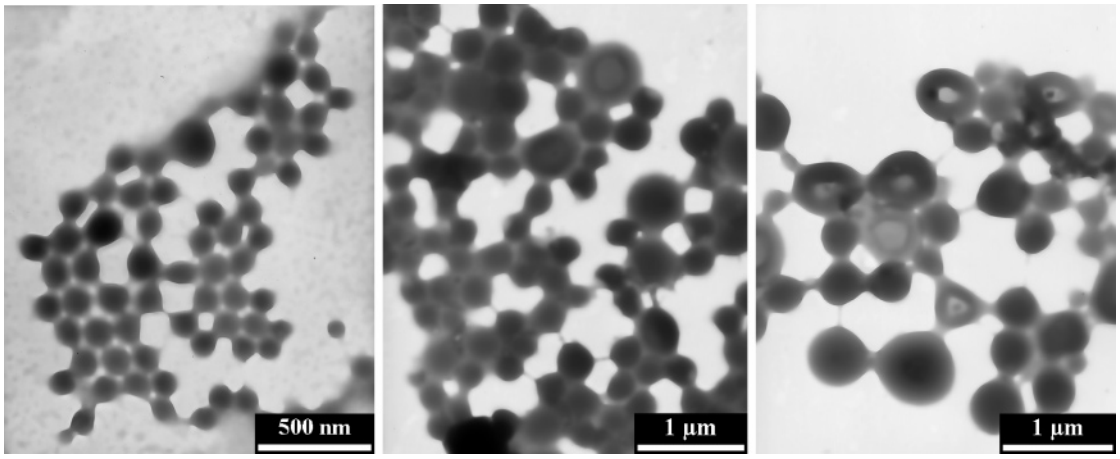


Figure 3. TEM images of dispersions prepared (according to the standard procedure) with SDS (sample S-10), Lutensol AT50 (sample L-10), and Tween 20 (sample T-10) (pictures left to right); the images of the dispersions prepared with Lutensol AT50 and Tween 20 could only be obtained after redispersing the particles for TEM analysis.

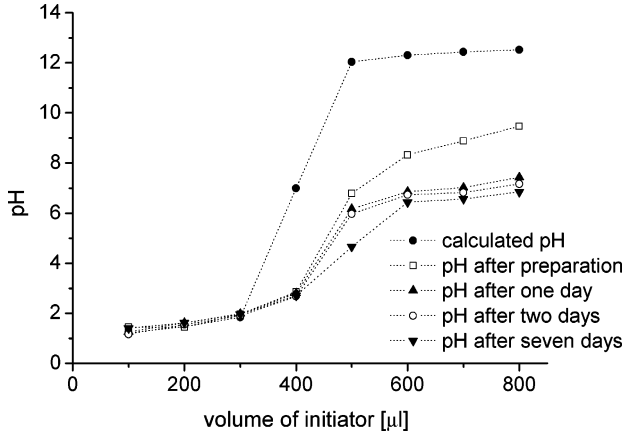


Figure 4. Evolution of pH of the dispersions compared with the calculated pH value. The volumes of the respective NaOH solutions were chosen to yield the calculated pH for the final dispersions. The consumption of OH^- during the reaction has been neglected in the calculation (dotted lines are guides for the eye).

displayed correctly. Since the polymerization rate of the nBCA in the miniemulsion system at the given pH (1.0) is unknown, it is impossible to determine the time of the solidification by polymerization of the droplets. With the obvious growth of the droplets during the first day and the constant size between the first and the second day, the formation of size-stable polymer particles can be regarded as completed after this time. The heterophase initiation reaction leads to an increase in hydrophilicity of the oligomers by the attachment of OH^- to the monomer, which in consequence allows the oligomers to diffuse through the continuous phase and can cause Ostwald ripening of the droplets explaining the growth of the droplets. After 7 days, sedimentation is visible in the dispersion. This leads to a smaller detected particle size (and a narrower distribution) since the large particles are no longer included in the measurement. The same effect, but less pronounced, can be observed for the values up to 200 μL of NaOH solution.

Since the particles, prepared at higher initial pH, do not show this decrease in particle size as distinctively, it can be assumed that the conversion from droplet to particle had been completed after a few minutes.

To summarize, for pH > 2, the particle size seems to be largely unaffected by the pH of the continuous phase during initiation and polymerization. Below this pH the time for the conversion from droplet to particle is lower than the time of droplet growth (Ostwald ripening).

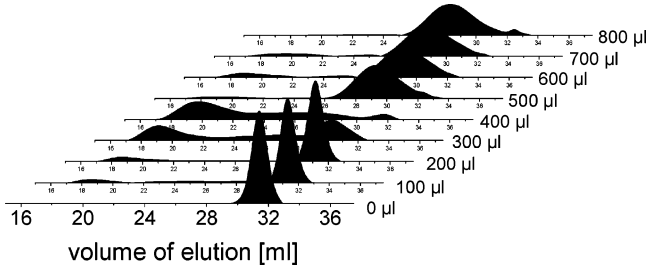


Figure 5. Molar mass evolution of the polymers obtained from PBCA dispersions prepared with increasing initiator (NaOH solution) volume (added volumes given on the right side of each slice); samples taken 7 days after starting the polymerization.

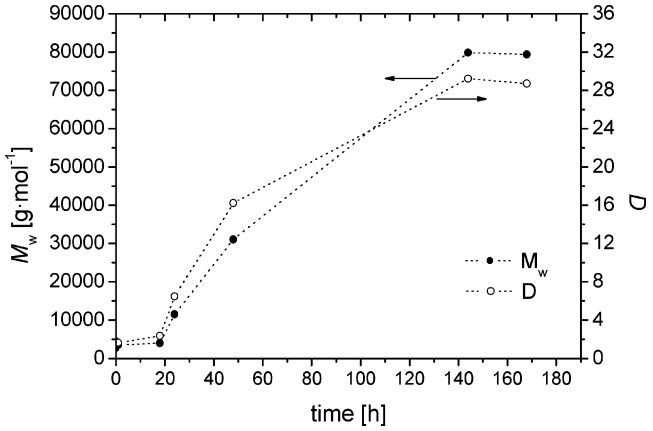
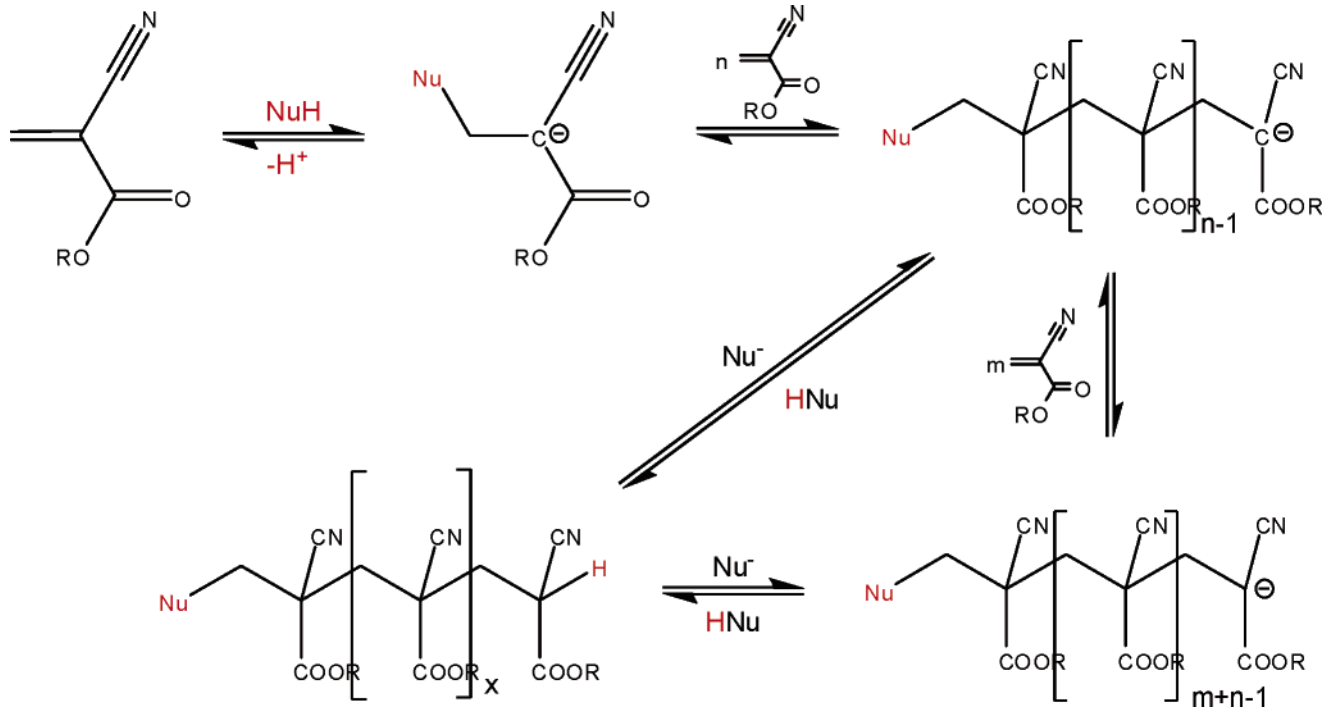


Figure 6. Evolution of M_w and polydispersity D obtained from a PBCA dispersion prepared according to the standard procedure (calculated pH = 7) during the course of 7 days (dotted lines are guides for the eye).

The GPC traces of the samples obtained with the addition of different NaOH amounts and measured 7 days after preparation are presented in Figure 5. The molar masses of the polymers resulting from 0, 100, and 200 μL initiator solution (M at 1100 g mol^{-1}) show a narrow mass distribution with a very low amount of high molecular polymer. The polymers obtained with a volume of initiator of 300 and 400 μL (calculated pH 1.9 and 7.0, measured pH after 7 days 2.0 and 2.7, respectively) show a bimodal mass distribution with one maximum at about 2000 g mol^{-1} and one at 350 000 g mol^{-1} (given to PS standard). A polymer fraction with masses between the extremes is only present in low amounts. The rest of the samples correspond to dispersions with higher pH values, which are significantly lower than the calculated values (calculated pH > 12, corresponding

Scheme 1. Proposed Mechanism of De/repolymerization[31]



to the "plateau" in the pH diagram). The polymers formed under these conditions show monomodal mass distributions with a maximum at about 5000 g mol^{-1}.

These results are comparable to those of Behan et al.,[13] who obtained particles prepared by the conventional emulsion polymerization using dextran as steric stabilizer. As long as the polymerization is carried out in an initially acidic medium (pH $\sim$ 2), polymer with a relatively low molecular weight is obtained, whereas long chains additionally appear as soon as the polymerization medium has an initially higher pH.

It has to be emphasized that the freezing of the dispersions has been performed 7 days after preparation. This is of great importance, since it could be shown for one pH (initially 7) that the initial mass distribution changes during the days after preparation (see Figures 2 and 6). Figure 2 visualizes the elugrams and Figure 6 the weight-average molar mass and the polydispersity. The polydispersity D of the polymer as well as M_w remains nearly constant during the first 18 h. The values obtained after 24 h are higher. This coincides with the appearance of a small visible fraction of high molecular weight polymer (Figure 2). The growth of this fraction can be seen in a significant increase of M_w and polydispersity D of the polymer. After 6 days the values remain nearly constant.

More detailed information can be gained by regarding the elugrams (Figure 2). The elugram of the freeze-dried miniemulsion (before adding the initiator) shows only traces of polymer. This means that even during ultrasonication the acidic continuous phase effectively inhibits polymerization. Immediately after adding the miniemulsion to the NaOH solution, a polymer with a molecular weight maximum at about 3000 g mol^{-1} is formed. A shift toward a slightly higher molecular weight of 4000 g mol^{-1} is visible during the course of 48 h. After this time high and low molecular weight polymer is beginning to be formed. The oligomers formed are signs of an ongoing depolymerization process. These oligomers disappear, whereas the amount of the long chain polymer is increasing until the final distribution is reached after $\sim$1 week. The maximum of the low molecular weight fraction shifts to a value of 2100 g mol^{-1}. The polymer initially formed with a molar mass of $\sim$3000 g mol^{-1} splits in two fractions: one with a high molecular mass (100 000 g mol^{-1}) and a broad distribution and a second with a lower molecular mass (2100 g mol^{-1}) and a comparably narrow distribution.

The observed change in chain length can be explained with the pH-dependent depolymerization/repolymerization/reinitiation mechanism proposed by Ryan, as shown in Scheme 1.[31]

The appearance of two distinct molar mass populations is more difficult to explain. For the appearance of two distinctly different molecular weight species, two different reaction conditions are likely. Considering the fact that the dispersion itself is homogeneous, there are the following two possibilities of realizing such conditions: (1) at least two distinct particle populations with different sizes, each of them with a narrow size distribution; (2) spatially separated reaction conditions within one particle.

Given the fact that two or more distinct particle populations are present after the miniemulsification process and provided that the same amount of initiator per surface area is present on both types, the initiator to monomer ratio will be higher for the smaller particles than for the larger particles, since the surface to volume ratio is larger for the smaller fraction. After approximately 1−2 days (see Figure 2) the smaller particles are completely polymerized, and the larger ones are still monomer swollen. Further chain growth in the larger ones is therefore still possible. After completion of the polymerization the larger particles will consist of longer polymer than the smaller ones. This effect has been observed (see Table 1), but the differences in M_w are small ($M_w \sim 3500-6000$ g mol^{-1}) compared to the changes appearing after longer equilibration times ($M_w > 100\,000$).

The formation of the two different species might also be spatially separated in one particle, which is more likely to be the case in the miniemulsions. First, particles of uniform molar mass polymer are formed. During the polymerization process, the pH of the dispersion is changed. The outer layer of the particle is in direct contact to the continuous phase and therefore

directly affected by changes of the pH. This means, according to Scheme 1, depolymerization to the equilibrium chain length might occur. The liberated monomer units can be used for the growth of the polymer in the core region of the particle. The core of the particle is largely unaffected by changes of the reaction conditions in the aqueous phase.

On the basis of the available data and especially the fact that the prepared dispersions show a narrow particle size distribution and the large differences in molecular weight, mechanism 2 is more likely. Still it cannot explain the occurrence of this remarkable mass distribution only at intermediate polymerization pH.

Amine Initiators. The anionic polymerization of ACAs is initiated by nucleophiles. Even "weak" nucleophiles like acetate ions possess the ability of initiating the polymerization of ACAs.[32−35] As mentioned briefly above, the growing polymer is functionalized by the initiator molecule. If a nanoparticle is formed from such a functionalized polymer and it can be ensured that the functional group (due to its hydrophilicity) will be at the particles' surface, this approach presents a convenient way to prepare PACA (nano)particles with functionalized surfaces.

The surface tailoring of nanoparticles greatly enhances their potential for biomedical applications. The presence of functional groups on the surface is required for further chemical modification with bioactive ligands like proteins or nucleotides. Besides the potential for further chemical reactions, the introduction of charged groups, like amino or carboxylic acid groups, influences the particles' surface charge. This in consequence can affect the particles' stability in dispersion[36] and the uptake behavior in cells.[37,38]

With the application of polar, hydrophilic amines, the resulting oligomers and the polymer will have a surfactant-like amphiphilic structure, with a hydrophilic head, originating from the initiating amine and a hydrophobic tail—the (growing) polymer. Because of this structure, it is very likely that the hydrophilic functionalized end of the polymer can be found on the aqueous side of the interface between monomer and water.

Bifunctional amines allow the further introduction of functional groups to the particles' surface. With the scope of biomedical application and the potential conjugation of proteins to the particles, amino acids are the appropriate candidates as initiators,[27,28] since they incorporate a "strong" nucleophile ($-NH_2$) which is likely to act as the initiating part of the molecule and the "weakly" nucleophilic part ($-COOH$), which at least in its protonated form is not likely to initiate polymerization and is therefore available for subsequent chemical reactions.

As shown above, the amount of initiator OH^- is crucial for the molar mass of the polymer, whereas the particle size is in a close range over all applied pH values. The influence of the various amounts of amine initiator on these parameters will be discussed in this part.

Ammonia and Tris-Base. As model amines, ammonia and tris-base (tris(hydroxyethyl)aminoethane) have been chosen. Solutions with a concentration of 0.1 mol L^{-1} each have been prepared without adjusting their pH values (pH > 9). This means that besides the amine in solution, there is also OH^- present. Thus, there will be competition between the amine and the hydroxyl ions for the initiation of the polymerization. The particle sizes and PDIs are summarized in Table 3. Some of the samples prepared with ammonia (600−1000 μL) show a slight yellow coloring after preparation. This has been observed by Leonard[28] and has been interpreted as reaction products after hydrolysis of the butyl ester group.

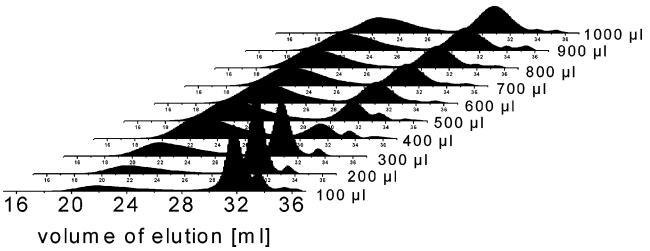


Figure 7. Evolution of elution volume of polymer obtained from dispersions prepared from 500 μL of a 25 wt % BCA miniemulsions with increasing volume of ammonia solution (given on the right side of each slice). The samples were taken 2 days after preparation.

In contrast to the sizes of the particles prepared with NaOH solution, which are more or less unaffected by the concentration of the initiator, a clear dependence on the concentration of the amine initiator can be observed. The sizes of the polymer particles obtained with both amine solutions follow the same pattern; the particles prepared with ammonia are smaller than the particles prepared with tris-base solution. The samples, where 100 μL of the initiator solution is added, exhibit particle sizes significantly larger than the rest of the samples. After a steep decrease the values reach a minimum at about 400 μL initiator solution and increase again.

The comparably large particles obtained with initiator volumes of 100 μL and for tris-base also 200 μL can be explained, as for OH^-, with the longer solidification time of the particles. Here, the droplets are not stabilized enough for the course of polymerization and grow during the solidification process.

The diameters of the particles prepared with amines are significantly smaller than those of the particles prepared with NaOH solution. Instead of values around 200 nm, the particles range from 60 to 100 nm and from 100 to 140 nm for ammonia and tris-base, respectively. This might be a consequence of the additional stabilization due to the surfactant-like structure of the formed polymer.

Molar mass distributions for the ammonia-initiated polymers (see Figure 7) differ from the values obtained from the OH^--initiated polymers. The main difference is the appearance of high molar mass polymers in all samples. The amount of this high molecular weight fraction is increasing from 100 to 400 μL of added initiator solution. The distribution is broad and shows only minor changes throughout the samples. The low molar mass fraction ($M \sim 1000$ g mol^{-1}) is decreasing constantly from 100 to 400 μL with a shift of the maximum of the elution volume to a lower volume ($M \sim 2500$ g mol^{-1}). With the application of more initiator solution, the amount of low molecular weight fraction increases again and shows its maximum at an elution volume of ~31 mL, which corresponds to a molecular weight of 1500 g mol^{-1}. The fraction with the high molecular weight polymer is present in all the samples. The amount increases from 100 to 400 μL of applied initiator solution to remain constant throughout the rest of the samples. The distribution is broad; the maximum of the molecular weight is detected at ~40 000 g mol^{-1}.

The molar mass distributions of the polymers initiated with tris-base solution (see Figure 8) resemble the pattern of the OH^--initiated polymer samples as well as the ammonia-initiated ones. Large amounts of long chain polymers with a molecular weight of 20 000 g mol^{-1} can only be found at intermediate amounts of initiators, showing a broad distribution with values lower than those obtained in the other sets. The maxima of the low molar mass fraction shifts from molecular weights of 1000 to 2500 g mol^{-1}. The relative amount of this fraction drops

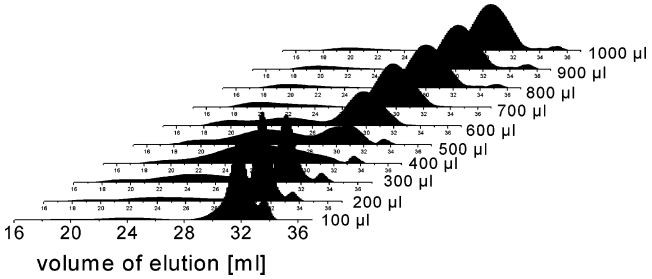


Figure 8. Evolution of elution volume of polymer obtained from dispersions prepared from 500 μL of a 25 wt % BCA miniemulsion with increasing volume of tris-base solution (given on the right side of each slice). The samples were taken 2 days after preparation.

constantly from 100 to 400 μL and then increases again to reach a maximum at 1000 μL of added initiator solution.

The differences in the patterns obtained from the reactions with ammonia and tris-base solutions can be attributed to structural differences of the two molecules and the pH of the respective solutions. Since ammonia and tris-base do not share the same pK_b values, the applied solutions do not exhibit the same pH value. As seen above, the pH of the reaction medium has a distinct effect on molecular weight distribution. Tris-base has also three additional hydroxy groups which can potentially act as initiator in contrast to ammonia, which only possesses one nucleophilic center.

Amino Acids. For a functionalization of the polymer with amino acids, it has to be assured that the amino acid is the sole or at least the main initiating molecule. Thus, the pH of the amino acid solution has to be low in order to minimize the initiation of the polymerization by the hydroxy ions. This also protonates the amino group to a certain extent according to the pK_b of the amino acid. Therefore, the amount of "active" initiator, in this case an amino acid molecule with deprotonated amino group, is decreased. The previous results have shown that these parameters affect the polymerization time, the particle size, and the molar mass distribution.

Stable dispersions could be created using phenylalanine in acidic solution, glycine, and 6-aminohexanoic acid in acidic as well as in basic solutions. Lysine, cysteine, arginine, glutamic, and aspartic acid solutions with pH values lower than 7 led always to coagulation and precipitation of the miniemulsion. In basic solutions no coagulum was formed, but the formation of clear yellow or orange colored solutions could be observed. The dissolution and the coloring is a clear sign for hydrolysis of the butyl ester group and the formation of water-soluble poly(cyanoacrylic acid). The coloring is according to Leonard[28] also a sign for degradation of the PACA.

The data available in Table 4 and visualized in Figure 9 show that the particle sizes cover the range from more than 350 nm to values as low as 70 nm. Nearly all of the dispersions show an extremely narrow distribution, expressed by the PDI smaller than 0.1 or even far below.

In contrast to the experiments with NaOH solution as initiator and in accordance to the results of the particles prepared with ammonia and tris-base, a clear dependence of the particle size on the amount of "active" initiator can be observed. Higher concentration, higher pH, and greater amount of the initiator solution lead to smaller particles in almost any of the series.

Regarding the sizes of the particles prepared with the 6-aminohexanoic acid solutions, a dependence on the pH of the initiator and to some extent on the amount of initiator is visible (see Figure 9a). The particle size increases with increasing pH of the initiator solution, with a large effect between pH 4.4 and 5.4. The particles prepared with the 6-aminohexanoic acid solutions also show a decrease in size from the initial to the following volume of initiator solution. The subsequent values remain almost constant.

The values for the particles prepared with the glycine solutions follow in most cases the pattern mentioned above (see Figure 9b). The particles prepared with the 2 mol L^{-1} glycine solution appear as the smallest, whereas the particles prepared with 0.1 mol L^{-1} solution exhibit the largest sizes. Within one concentration, the particle size decreases from the lowest to the highest applied pH. The same tendency can be observed from 100 to 1000 μL of added initiator solution. The slope of the curves becomes less steep with increasing pH and concentration of glycine solution.

The oligomers resulting from the reaction of the amino acid and few monomer units are expected to be water-soluble because of the high hydrophilicity of the amino acid. Therefore, the polymerization is not restricted to one droplet and the droplets undergo Ostwald ripening as long as the solidification has not started or the hydrophobicity of the PBCA chain dominates, and the molecules are no longer soluble in the aqueous phase. With a low amount of initiated polymer chains, the solidification will take a longer time than in the case with more growing chains present. During this time the particles can increase their size. Despite this effect, a narrow particle size distribution can be observed at all samples. The possible occurrence of micelles formed of surfactant-like oligomers might even complicate the process of particle formation.

If the polymerization time is excessively high, the droplets can exceed a critical size, which leads to coagulation and after polymerization to precipitation of the particles. Thus, dispersions only with large particles and low stability were obtained using 100 μL of 6-aminohexanoic acid solution at pH 4.4 and glycine solutions with concentrations of 0.5 and 2.0 mol L^{-1} at pH 2.4.

Despite the wide range of particle sizes, the molar mass distributions of all the samples can be found at remarkably similar values. For the samples prepared with 6-aminohexanoic acid, the maxima of the molar masses can be found to be around 1500 g mol^{-1} (see Figure 10) and for glycine to be around 1000 g mol^{-1} (see Figure 11). The mass distribution of the polymer prepared with the ω-amino acid is somewhat broader than the mass distribution of the polymer prepared with glycine. In contrast to the particles prepared with basic initiators (NaOH, NH_3, and tris-base), no significant variation in the mass distribution among the samples can be observed with the variation of the initiator volume. This implicates an independence of pH, concentration, and volume of initiator at least in the examined ranges. There is even only minor deviation between the two amino acids used for the experiments.

As shown in Figure 12, a pH dependence of the ζ potential is clearly visible for the samples prepared with amino acids. The ζ potential of the particles prepared with amino acids is about 10 mV higher in acidic medium (pH 3) than in basic medium (pH 10). The particles prepared with NaOH solution also show a ζ potential with a slight pH dependence. This is not surprising, since in basic medium hydrolysis of the butyl ester groups is likely to occur. Compared with the particles prepared with amino acid solutions, this effect is not as pronounced.

The increase of about 50 mV of the ζ potential is due to the removal of SDS (by dialysis), which is adsorbed on the particles. A more thorough dialysis leads to a further removal of the adsorbed SDS, which destabilizes the dispersion and leads to the formation of precipitate. This effect cannot be observed on

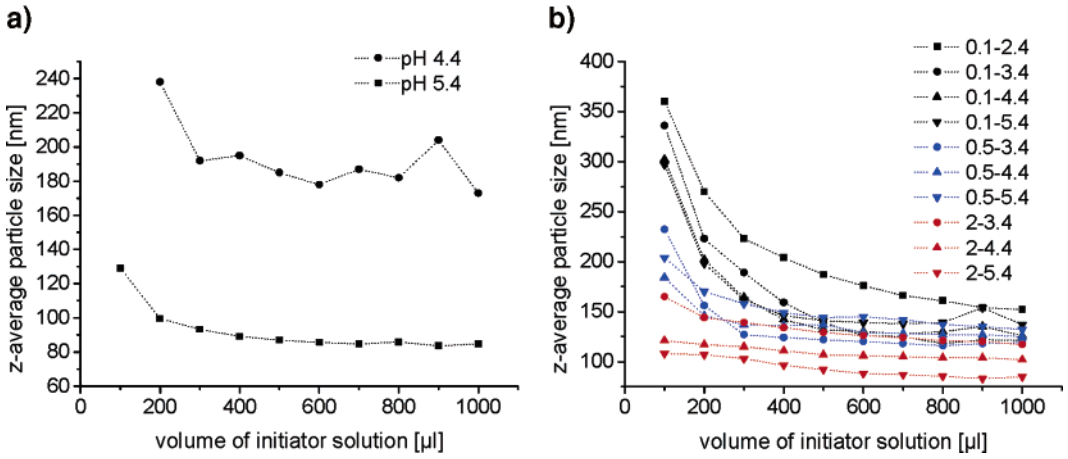


Figure 9. Evolution of particle size obtained from dispersions prepared (a) with 0.5 mol L^{-1} 6-aminohexanoic acid and (b) with glycine solutions (dotted lines are guides for the eye); the numbers in the legend indicate the molar concentration (before dash) and the pH of the solution (after dash).

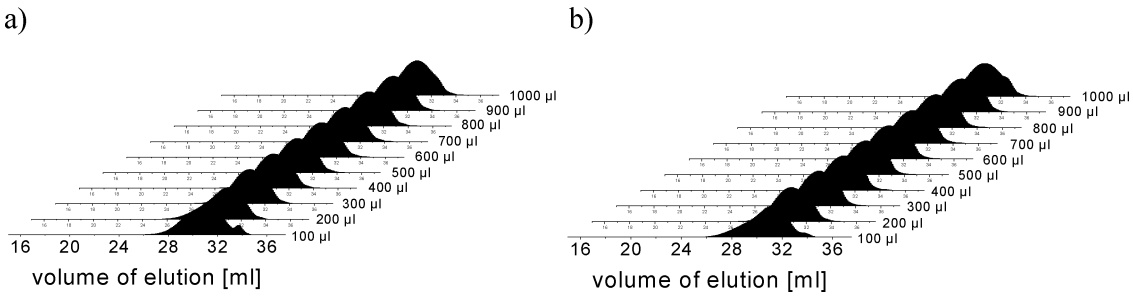


Figure 10. GPC elugrams obtained of polymer samples from dispersions prepared with various amounts of 6-aminohexanoic acid solutions with different pH values: (a) pH 4.4 and (b) pH 5.5.

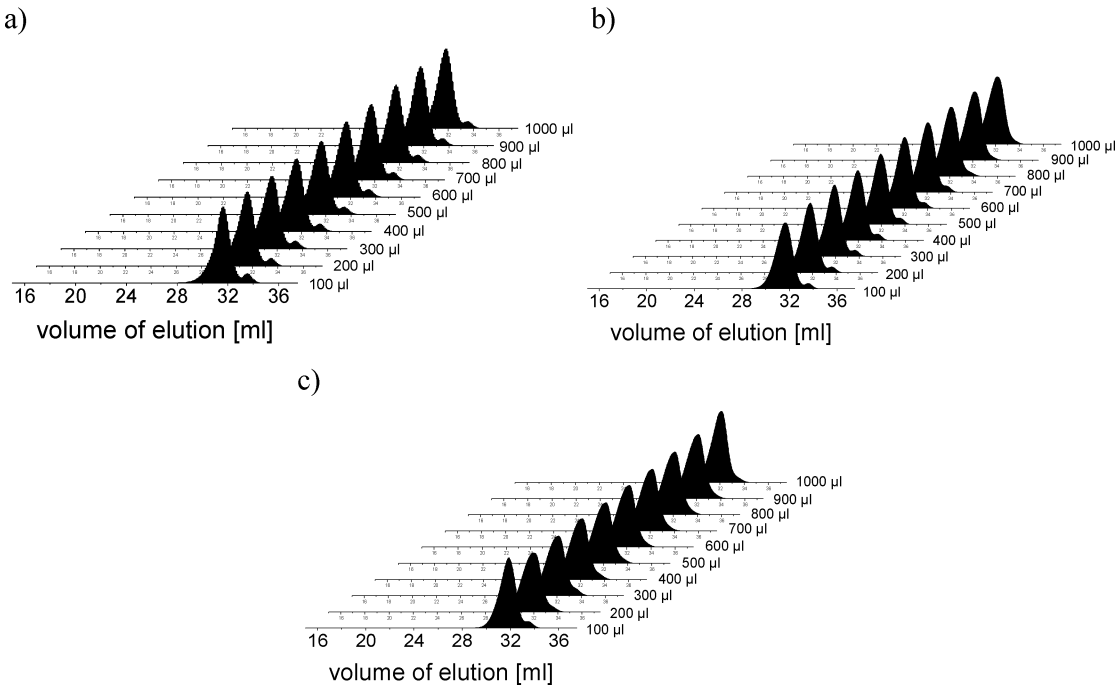


Figure 11. GPC elugrams obtained of polymer samples from dispersions prepared with various amounts of glycine solutions with different pH values: (a) Gly 0.1 M, pH 3.4; (b) Gly 0.5 M, pH 3.4; (c) Gly 2.0 M, pH 3.4.

the samples prepared with amino acid solutions. In contrast, a slight decrease of the ζ potential occurs after dialysis.

As soon as there are carboxy groups present on the particle surface, a pH dependence of the ζ potential should be observable. In basic medium, the acid group is deprotonated leaving a negative charge on the particle; in acidic medium this negative charge is no longer present, since the acid group is protonated. Therefore, ζ-potential measurements have been performed after

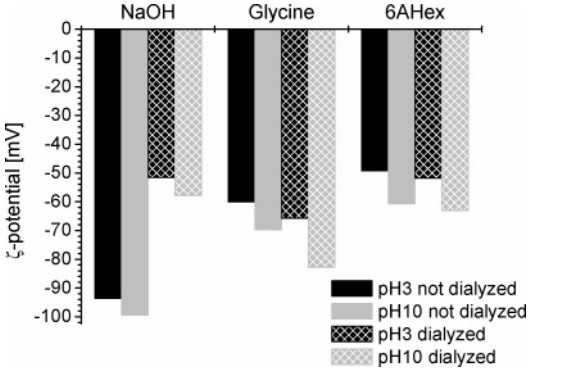


Figure 12. ζ potential of PBCA dispersions prepared with sodium hydroxide solution (NaOH), glycine solution (Glycine), and 6-amino-hexanoic acid solution (6AHex) measured at pH 3 and 10 before and after dialysis.

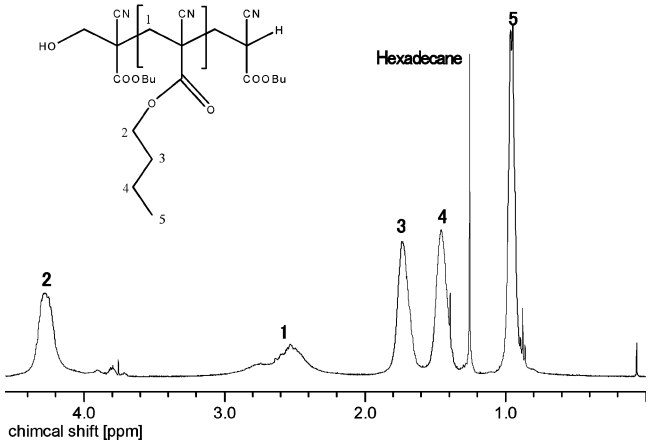


Figure 13. ^{1}H NMR spectrum of polymer obtained from a dispersion prepared with NaOH solution (0.1 mol L^{-1}, calculated pH 7, solvent $CDCl_3$).

adjusting the pH of the diluted dispersion to values of 3 and 10. Still, there is the possibility that the amino acids are only adsorbed to the particles' surface. To exclude this possibility, the dispersions have been dialyzed in order to remove (physically) adsorbed molecules and to some extent also the SDS.

Two further experiments were made in order to confirm the initiation of the polymerization by amino acids. Therefore, phenylalanine was used as initiating amino acid. Since PBCA shows no UV activity, it can only be detected by the RI detector of the GPC setup, but not by the UV detector. The introduction of the UV-active initiator phenylalanine labels the polymer chain, so that it can be observed by the UV detector. Since the signals of both detectors are nearly congruent for the phenylalanine-initiated polymer and it can be assumed that the UV signal originates from the phenylalanine, the amino acid has to be attached to the polymer.

Figures 13 and 14 show ^{1}H NMR spectra of the polymer obtained from a dispersion initiated with NaOH solution (0.1 mol L^{-1}) and with phenylalanine solution (pH 3.4, 0.1 mol L^{-1}). The peaks of the spectrum in Figure 13 can be identified and assigned to the polymer and hexadecane. The spectrum presented in Figure 14 shows additional peaks, which can be assigned to pheylalanine (PA) and to SDS. The amino acid-initiated polymer shows a reduced solubility in chloroform compared to the OH-initiated PBCA; therefore, DMSO has been chosen as solvent. The altered solubility behavior indicates an increase in hydrophilicity caused by the polar amino acid

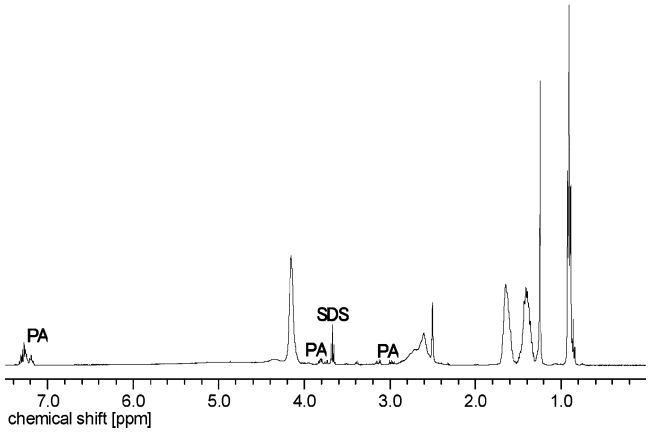


Figure 14. ^{1}H NMR spectrum of polymer obtained from a dispersion prepared with phenylalanine solution (pH 3.4, 0.1 mol L^{-1}, solvent DMSO-d_6).

attached to the polymer. The ratio of the integrals of the phenyl residue and the protons responsible for peak 2 (Figure 13) is ~1:6. Out of this ratio a molar mass of $M \sim 1000$ g mol^{-1} can be calculated, which is in good accordance with the values obtained from GPC experiments. All of the experiments presented confirm the presence of amino acid covalently bonded to the polymer chains.

These results suggest that the amino acid is present on the polymer. Combined with the ζ potential measurements, showing a pH dependence of surface charge, and considering the fact that the hydrophilic part of the polymer, the former initiating amino acid, will be found in the aqueous phase, it seems very likely that the amino acid is finally present on the particle's surface.

It was not possible to observe these particles directly via electron microscopy. The particles tend to form a film during drying, which makes it impossible to prepare TEM samples. Even during freeze drying the film formation process is occurring. Instead of obtaining a fine powder, as is possible with the dispersions prepared with NaOH solution, an off-white gumlike mass is the result of the attempted freeze-drying of the dispersion prepared with amino acid solutions. Low molecular weight, strong adsorption of water by the functionalized polymer, or residual monomer[13] might be the reasons for this effect.

Conclusion

It could be shown that the miniemulsion approach provides a very powerful and convenient tool to prepare PBCA nanoparticles with functionalized surfaces. The application of the anionic surfactant SDS allows the preparation of long-term stable dispersions with solid contents of 10% or more and leads to PBCA particles without covalently bound dextran or other steric stabilizers on the surface. The two-step process extends the pH of polymerization and therefore allows obtaining PBCA of comparably high molecular weight. The particle sizes obtained are largely unaffected by the pH and can be found between 150 and 200 nm.

Initiation with amine solutions provides an easy way of introducing functional groups to the particles' surface. The application of amino acid solutions as initiators gives rise to the possibility to functionalize the particles and tune the particle size in the range between 80 and 350 nm. The presence of amino acids on the particles' surface has been shown by ζ potential measurements; the covalent attachment to the polymer chain has been confirmed by NMR and GPC.

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