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Long-term stability of PBCA nanoparticle suspensions suggests clinical usefulness

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Abstract

The stability of polybutylcyanoacrylate (PBCA) nanoparticle suspensions was examined by measuring particle sizes and size distributions over a period of 2 months in different solutions (hydrochloric acid, phosphate buffered saline). When stored in acidic medium, nanoparticles were found to be stable for at least 2 months. In this case, degradation of the polymer is decelerated and particle agglomeration is reduced. When added to human blood serum, nanoparticles were found not to agglomerate, remaining stable in size for at least five days. Previous stabilization protocols required lyophilization and resuspension by ultrasonification; however, the technique presented here using acidic storage solutions proves to be superior for clinical applications. © 1997 Elsevier Science B.V.

Keywords: Nanoparticles; Polybutylcyanoacrylate; Stability; Agglomeration; Degradation

1. Introduction

Polybutylcyanoacrylate (PBCA) nanoparticles are used in a variety of approaches for drug targeting. Depending on their chemical characteristics drugs, can be incorporated into the nanoparticle core (Beck et al., 1993, 1994) or they can be adsorbed onto the nanoparticle surface (Losa et al., 1991; Beck et al., 1993; Schroeder and Sabel, 1996). The benefits of binding drugs to nanoparticles are, for example, a changed body distribution after administration (Grislain et al., 1983; Verdun et al., 1986; Beck et al., 1994; Kreuter et al., 1995; Schroeder and Sabel, 1996), lower toxicity (Couvreur et al., 1982, 1986; Verdun et al., 1986), and protection of the drug against enzymatic degradation (Lowe and Temple, 1994; Nakada et al., 1996). Concerning a changed body distribution, especially a higher concentration of drugs in the brain is of clinical interest, and nanoparticles are now developed as a carrier system to deliver drugs across the blood-brain barrier (Kreuter et al., 1995; Schroeder and Sabel, 1996).

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The preparation of nanoparticles is not complicated (Kreuter, 1994) and storage in a lyophilized form normally ensures long durability (Verdun et al., 1986). However, the resuspension of these lyophilized nanoparticles is an unpractical and unreliable step when intravenous administration of the nanoparticle suspension is desired (Beck et al., 1990). Due to the danger of particle agglomeration, insufficient resuspension could result in microembolisms (Beck et al., 1993) which have to be avoided. Consequently, resuspension usually requires a powerful ultrasonification bath and, to be certain of sufficient resuspension, it is necessary to determine particle size. These circumstances severely limit the clinical use of nanoparticles by creating instrumental problems for the clinician. To overcome these difficulties, we have now developed new non-perishable forms of PBCA nanoparticles with long-term stability which may be adequate for the clinical setting.

2. Materials and methods

2.1. Materials

n-Butylcyanoacrylate (BCA) was obtained from Sichelwerke (Hannover, Germany), dextran 70 000 from Sigma-Aldrich Chemie (Deisenhofen, Germany) and mannitol from J. Baker (Deventer, Netherlands). Filter paper was obtained from Schleicher and Schuell (Dassel, Germany): No. 595 (used for filtering the suspensions), and No. 402106 (0.05 μm; used for filtering the water before particle size determination). Human blood serum was kindly supplied by the Institute of Clinical Chemistry at the Otto-v.-Guericke University (Magdeburg). All other reagents were of analytical grade.

2.2. Nanoparticle preparation process

Nanoparticles were prepared by a standard fabrication procedure (Kreuter, 1983a) using an acidic polymerization medium (0.01 N HCl) containing 1% dextran 70 000 as stabilizer. Then, 1% of butylcyanoacrylate was added under constant magnetic stirring (500–700 rpm) with a glass coated stirring bar. After 4 h of polymerization, the nanoparticle suspension was first neutralized with 0.1 N NaOH to complete the polymerization, then filtered through filter paper and ultracentrifuged twice (20 000 rpm, 1 h, 4°C; L7-55 Ultracentrifuge, Beckman Instruments Inc., USA). After each centrifugation step the, supernatant was removed and the nanoparticles were resuspended in the same amount of water using ultrasonification (Bandelin, Sonorex Digital 10 P and Sonoplus HD 60, Germany). They were subsequently lyophilized in the presence of 4% mannitol as cryoprotector (Alpha 1-4, Martin Christ Gefriertrocknungsanlagen, Osterode, Germany). Particle size determination was achieved by means of photon correlation spectroscopy (PCS, AutoSizer Lo-C, Malvern Instruments Ltd., UK). The particle size and the polydispersity were calculated based on the average values of three or four measurements.

2.3. Long-term experiments

For long-term experiments, we used one sample of non-centrifuged nanoparticles directly after filtration (batch A) and one sample of lyophilized nanoparticles resuspended in deionized water (H_2O) by sonification (batch B). The samples were diluted with an equal amount of 0.1 N or 0.01 N hydrochloric acid (HCl) or with phosphate buffered (10 mM) saline (pH 7.4; PBS). Particle size and polydispersity in the suspensions were determined repeatedly during the following 2 months by taking small samples and diluting them with filtered water. During this period, the suspensions were stored either at room temperature (ca. 25°C; RT) or at 42°C.

In another series of experiments, we used a non-artificial medium (human blood serum), in order to better simulate the in vivo conditions of nanoparticles administered into blood vessels. This serum was diluted (1:1) with a sample of lyophilized and resuspended nanoparticles (batch C). These suspensions were kept at room temperature for 9 days and were examined repeatedly.

Table 1
Average diameter (nm) and size distributions (polydispersity) of nanoparticle samples

Sample	After preparation		After second centrifugation		After lyophilization	
	nm	Polyd.	nm	Polyd.	nm	Polyd.
A	326	0.44	—	—	—	—
B	164	0.29	345	0.32	485	0.37
C	185	0.39	243	0.32	388	0.41

Average diameter (nm), average values from three or four measurements.
Polyd., polydispersity.

3. Results

3.1. Preparation and purification

As depicted in Table 1 the particle size of the PBCA nanoparticles varied from 164 to 326 nm immediately after preparation. This size differences resulted from the slightly variable preparation conditions such as type of reaction vessel, amount of solution, stirring speed and quality, velocity and place of adding the monomer BCA. That the particles tend to agglomerate after the purification steps by centrifugation and after lyophilization as mentioned in the literature (for example by Kreuter (1983b)) was seen clearly by the increased particle size in our experiments as well. For example, the nanoparticles in batch B had an average particle diameter of 164 nm immediately following filtration, a diameter of 345 nm after resuspension in water following the second centrifugation step, and a diameter of 485 nm after resuspension (20-min ultrasonification) of the lyophilized particles in water (Fig. 1, Table 1). The nanoparticles in batch C, used for the experiments with human blood serum, behaved more regularly: their sizes were 185 nm after preparation, 243 nm after the second centrifugation, and 388 nm after lyophilization (Table 1). These observations are often made during preparation and handling of injectable nanoparticle suspensions, and the final suspensions of purified nanoparticles often contain particles bigger than 1 μm which could cause problems after administration into the blood circulation.

3.2. Long-term stability of nanoparticles in different suspensions

The data in Tables 2–4 clearly demonstrate that the previously mentioned particle sizes are not the final values. The characteristics of the different nanoparticle suspensions are strongly dependent on processing method and storage solution.

3.2.1. Batch A: freshly prepared nanoparticles

Directly after preparation, nanoparticles in the neutralized and filtered suspension A had an average size of 326 nm (Table 2, column 1). After 1 week, nanoparticles sedimented and the superna-

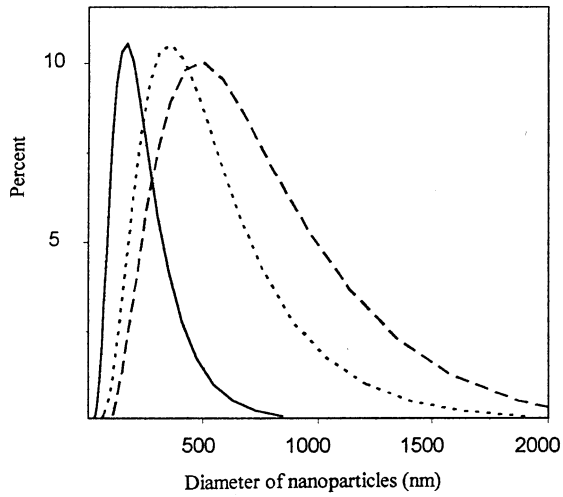

Fig. 1. Nanoparticle distribution curves. (——) Directly after preparation, (· · ·) after second centrifugation step, (- - -) after lyophilization.

Table 2
Average diameter (nm) and size distributions (polydispersity) of nanoparticles in batch A assessed over time

Time (days)	H_2O (RT)		HCl (0.1 N, RT)		HCl (0.1 N, 42°C)		HCl (0.01 N, RT)		HCl (0.01 N, 42°C)		PBS (RT)		PBS (42°C)	
	nm	Polyd.	nm	Polyd.	nm	Polyd.	nm	Polyd.	nm	Polyd.	mn	Polyd.	nm	Polyd.
1	326	0.44	316	0.43	318	0.46	322	0.45	318	0.43	248[a]	0.27	332	0.19
5	307	0.44	325	0.39	334	0.42	326	0.40	317	0.44	487	0.36	398	0.21
9	323	0.45	341	0.42	300	0.40	327	0.42	270	0.9	—[b,c]		970	0.19
18	314[b]	0.47	333	0.39	253	0.42	330	0.44	276	0.27	—[b,c]		1004[b]	0.32
34	286[b]	0.45	342	0.41	238	0.41	315	0.45	284	0.24	—[b,c]		939[b]	0.31
62	274[b]	0.38	343	0.38	222	0.40	303	0.43	302	0.23	—[d]		948[b]	0.43

Particles were suspended in different solutions directly after preparation. Average diameter (nm), average values from three or four measurements.
Polyd., polydispersity. RT, room temperature. [a] After ultrasonification. [b] Beginning of bacterial infection of solution and decay of nanoparticles. [c] Fluffy. [d] Total decay.

Table 3
Average diameter (nm) and size distributions (polydispersity) of nanoparticles in batch B assessed over time

Time (days)	H_2O (RT)		HCl (0.1 N, RT)		HCl (0.1 N, 42°C)		HCl (0.01 N, RT)		HCl (0.01 N, 42°C)		PBS (RT)		PBS (42°C)	
	nm	Polyd.	nm	Polyd.	nm	Polyd.	nm	Polyd.	nm	Polyd.	mn	Polyd.	nm	Polyd.
1	451[a]	0.33	522	0.36	515	0.32	453	0.25	477	0.34	493	0.35	518	0.35
2	490	0.45	544	0.40	558	0.40	552	0.47	708	0.58	532	0.42	745	0.62
3	480[a]	0.39	450[a]	0.34	497[a]	0.32	450[a]	0.30	423[a]	0.26	508[a]	0.29	596[a]	0.41
4	—	—	609[a]	0.46	567[a]	0.39	470[a]	0.27	499[a]	0.37	542[a]	0.40	627[a]	0.36
7	—	—	715[a]	0.52	602[a]	0.40	—	—	—	—	—	—	641[a]	0.35
8	462[a]	0.35	774[a]	0.58	587[a]	0.44	496[a]	0.36	491[a]	0.28	557[a]	0.39	—	—
11	489[b]	0.36	715[a]	0.43	726[a]	0.35	540	0.32	497[a]	0.32	617[a,b]	0.54	478[a,b]	0.34
24	—	—	834[a]	0.57	752[a]	0.32	—	—	554[a]	0.36	1325[a,b]	0.59	337[a,b]	0.19
66	—	—	1384[a]	0.78	1379[a]	0.68	—	—	638[a]	0.45	814[a,b]	0.47	—[d]	—

Particles were resuspended in different solutions after lyophilization. Average diameter (nm), average values from three or four measurements.
Polyd., polydispersity. RT, room temperature. [a] After ultrasonification. [b] Beginning of bacterial infection of solution and decay of nanoparticles. [c] Fluffy. [d] Total decay.

Table 4
Average diameter (nm) and size distributions (polydispersity) of nanoparticles in batch C assessed over time. Particles were resuspended in different solutions after lyophilization and storage at room temperature.

Time	H_2O		PBS		Human serum	
	nm	Polyd.	nm	Polyd.	nm	Polyd.
1 h	364	0.35	515	0.62	373	0.49
3 h	362	0.36	359	0.40	356	0.37
6 h	372	0.41	362	0.40	361	0.37
1 day	335	0.51	406	0.52	378	0.47
2 days	367	0.42	731	0.70	381	0.45
5 days	526	0.49	1601	0.99	383	0.45
8 days	358	0.36	4204	0.70	448	0.51

Human serum, freshly centrifuged.
Average diameter (nm), average values from three or four measurements.
Polyd., polydispersity.

tant appeared clear. It was possible at this point to resuspend the particles by agitation. This solution was agitated and left to sediment again at repeated occasions for a total period of 2 months. The particle size slowly decreased after 2 weeks and with an accelerated decline after 4 weeks. After the 2-month period, particle size was 274 nm and the suspension appeared markedly decomposed. This was not the case for the unpurified nanoparticles which were resuspended in the acidic media and stored at room temperature (Table 2, columm 2 and 4). They sedimented as well, but after agitation showed almost unchanged particle sizes and polydispersity values throughout the two months when measurements were taken. Decomposition seems to be enhanced by higher temperatures (Table 2, column 3 and 5). In contrast, nanoparticle suspensions in PBS at 7.4 pH agglomerated quickly and showed particle sizes of 389 nm after 5 days and between 900 and 1000 nm up to the ninth day (at higher temperature, Table 2, column 7). Polydispersity did not change considerably. It was not possible to reduce the particle size significantly by sonification. For example, the particle size after 62 days was 948 nm (polyd. 0.43) without ultrasonification and 921 nm (polyd. 0.41) after 15 min ultrasonification. The particles in this solution decayed much faster than in the neutralized starting-solution and after 2 months nearly disappeared. Only the remaining particles showed these large diameters. Measurements of particle size from the PBS solution stored at room temperature were not taken since it was not possible to resuspend the agglomerated nanoparticle flakes by sonification (Table 2, column 6). These nanoparticles were completely decayed after 2 months as well.

3.2.2. Batch B: lyophilized nanoparticles

The same measurements were difficult to carry out with nanoparticle batch B which had been lyophilized and resuspended. Even in the acidic medium, they tended to agglomerate very fast to form large particles which were detectable by the unaided eye (Table 3, column 2–5). It was necessary to resuspend them by sonification directly before size determination. However, despite sonification, it was not possible to maintain an injectable suspension over time. The acidic suspensions had a better appearance than the PBS suspensions. Also, this latter suspension started to decompose after only 2 weeks (Table 3, column 6 and 7). The differences between the suspensions stored at room temperature or at 42°C were found to be negligible. The fact that lyophilization changes some characteristics of the nanoparticles was also shown by alterations of adsorption capacity (Harmia et al., 1986).

3.2.3. Batch C: stability of lyophilized nanoparticles in human blood serum

In order to predict the behaviour of nanoparticles after intravenous administration, another sample of nanoparticles which were lyophilized and resuspended in water was added to human blood serum or, for comparison, PBS. The serum-nanoparticle suspension showed much better characteristics (Table 4, column 3) than the PBS suspension (Table 4, column 2). Directly after resuspension, the particles had an average size of 373 nm, only 9 nm more than the same nanoparticles in deionized water (Table 4, column 1; polyd. 0.38 for serum and 0.35 for water). These values were nearly unchanged for 6 days (serum) or 9 days (water) but increased to 448 nm (polydispersity 0.52) in the serum suspension when

tested beyond 8 days. In this series, the PBS nanoparticle suspensions started to agglomerate after several days as well (Table 4, column 2).

4. Conclusions

The results of this study indicate that an acidic medium protects PBCA nanoparticles against decomposition in agreement with earlier observations (Stein and Hamacher, 1992; Scherer et al., 1994), and under these conditions there is only negligible agglomeration for many months. A small temperature dependence was observable; higher temperature seems to promote the degradation of the nanoparticles (Stein and Hamacher, 1992). It is not yet known if this degradation is triggered by esterhydrolysis leading to a more water soluble polymer and butanol (Lenaerts et al., 1984; Stein and Hamacher, 1992). However, at very low pH values and room temperature the production of butanol is negligible (Stein and Hamacher, 1992). The stability of nanoparticles in acidic medium may also explain why they are useful as oral drug delivery systems. Based on our experiments, we believe that it is feasible to consider the clinical use of nanoparticles in the near future. To obtain an injectable solution from the acidic nanoparticle suspension, neutralization should occur immediately prior to administration. Nanoparticles in this form appear particularly useful for drugs which are stable in acid and able to bind onto or into the nanoparticles in a sufficient amount. Additionally, nanoparticles in human blood serum do not show particle agglomeration in vitro, a further reason why they are well tolerated in vivo (Couvreur et al., 1986). Nanoparticles prepared in this fashion appear safe for intravenous administration, and microembolisms due to agglomeration are not to be expected.

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