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Sterilization of unloaded polybutylcyanoacrylate nanoparticles

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Abstract

In this study the possibilities of sterilizing polybutylcyanoacrylate nanoparticle suspensions and lyophilized nanoparticle powders by autoclaving or formaldehyde treatment was evaluated. The nanoparticles were fabricated with different stabilizers. In most conditions a significant increase in particle size was determined after autoclaving of the nanoparticle suspensions and the nanoparticle powders were characterized by impaired resuspension characteristics. Even if the sterilization of nanoparticles after fabrication is possible under certain conditions, the preferable way to obtain aseptic material appears to be the production of nanoparticles under aseptic conditions.

Keywords: Nanoparticles; Polybutylcyanoacrylate; Sterilization; Autoclaving; Formaldehyde; Surfactants

1. Introduction

The development of intravenously administered drug carriers for controlled release of drugs and drug targeting has been an ambitious challenge. Polybutylcyanoacrylate nanoparticles (PBCA) play an important role in many of such drug carrier systems (Kreuter, 1994).

For clinical practice parenteral drug delivery systems have to meet the pharmacopoeial requirements of sterility. Therefore, sterilization of the finished formulation is essential but often overlooked. Little is known about the sterilization of nanoparticles (Magenheim and Benita, 1991; Allémann et al., 1993), in particular about PBCA nanoparticles.

Terminal sterilization and aseptic processing are the two major alternatives of obtaining a sterile product. Sterile filtration of nanoparticle suspensions cannot be used to obtain sterility since the nanoparticles, which are similar in size to the contaminants and the pores of many filters, leading to the clogging of sterilization filters. Sterilization by γ-irradiation has the advantage of low cost and microbiological safety, but it suffers the disadvantage of possible chemical and physical stability problems of drugs (Mäder et al., 1997)

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Table 1
Acidic nanoparticle suspensions

Stabilizer	c	T	No sterilization		After sterilization					
					No sonication		2-min sonication		5-min sonication	
			size*	p.d.**	size*	p.d.**	size*	p.d.**	size*	p.d.**
Dextran 70 000	0.5	−	177.3	(0.306)	841.6	(0.198)	817.5	(0.105)	932.7	(0.153)
		+	172.2	(0.055)	217.9	(0.049)	226.5	(0.103)	277.0	(0.049)
	1.0	−	224.0	(0.285)	528.1	(0.111)	582.2	(0.096)	556.4	(0.151)
		+	142.5	(0.106)	163.7	(0.043)	174.4	(0.090)	180.6	(0.020)
Poloxamer 188	0.5	−	350.1	(0.108)	338.5	(0.074)	—	—	—	—
		+	311.7	(0.035)	315.3	(0.041)	325.4	(0.123)	314.6	(0.075)
	1.0	−	274.1	(0.040)	294.9	(0.071)	—	—	—	—
		+	115.7	(0.133)	222.2	(0.191)	225.4	(0.224)	224.2	(0.193)
Polysorbate 85	0.5	−	339.4	(0.346)	241.1[a]	(0.514)	211.8[a]	(0.318)	—	—
		+	378.8	(0.471)	285.4[a]	(0.588)	162.1[a]	(0.400)	600.7	(0.663)
	1.0	−	462.4	(0.434)	260.9[a]	(0.491)	148.4[a]	(0.315)	—	—
		+	524.4	(0.454)	412.3[a]	(0.616)	255.9[a]	(0.437)	393.6	(0.624)

c, concentration of stabilizer in %.
T, autoclaving with (+) or without (−) cooling.
[a]Measured in the supernatant.
*Particle size in nm.
**Polydispersity.

and carriers (Müller, 1991). The major modifications by γ-irradiation—as well as plasma treatment—occur at the surface of the nanoparticles, as it leads to a changed hydrophobicity/hydrophilicity (Müller, 1991). It has been shown that γ-sterilization may increase the drug release from poly(lactic acid) particles (Allémann et al., 1993) or decrease the amount of still intact drugs (Mäder et al., 1997), thus changing the properties of the polymer and the release kinetics (Lewis, 1990; Mehta et al., 1994). Chemical sterilization with ethylene oxide or formaldehyde meets serious toxicological problems due to chemical residues. Dry and moist heat could cause problems due to chemical and physical instabilities as well. For example, Al Khouri Fallouh et al. (1986) did not observe any modifications of PBCA nanocapsules (with an oily core) after autoclaving, whereas Rollot et al. (1986) measured an increased particle size from 200 to 500 nm.

The aim of this study was to clarify in detail the influence of autoclaving nanoparticle suspensions and assess the effects of formaldehyde treatment on lyophilized nanoparticle powders in order to estimate the value of these processes for sterilization of PBCA nanoparticles.

2. Materials and methods

Nanoparticles were prepared by standard fabrication procedure (Kreuter, 1983a; Sommerfeld et al., 1997) using an acidic polymerization medium (0.01 N HCl) containing 0.5 or 1.0% dextran 70 000, poloxamer 188 (Sigma-Aldrich Chemie, Germany), or polysorbate 85 (Tween 85, a gift from ICI Surfactants, Germany) as stabilizer. 1% of butylcyanoacrylate (Sichelwerke, Hannover, Germany) was added under stirring. After 27 h of polymerization the nanoparticle suspension was filtered through filter paper (dextran 70 000 and poloxamer 188) or a glass filter (polysorbate 85). Ten milliliters of the filtrate were transfered into a separate vial. The remaining suspension was neutralized with NaOH and again 10 ml were separated off. The rest of the suspension was ultracentrifuged twice (20 000 rpm, 1 h, L7-55 Ultracentrifuge, Beckman Instruments, Inc.,

Table 2
Neutralized nanoparticle suspensions

Stabilizer	c	T	No sterilization		After sterilization					
					No sonication		2-min sonication		5-min sonication	
			size*	p.d.**	size*	p.d.**	size*	p.d.**	size*	p.d.**
Dextran 70 000	0.5	−	183.1	(0.318)	585.8	(0.233)	642.5	(0.116)	759.9	(0.067)
		+	169.6	(0.103)	1030.5	(0.938)	1066.9	(0.813)	1150.6	(0.627)
	1.0	−	230.3	(0.361)	658.7	(0.074)	651.3	(0.157)	682.3	(0.103)
		+	140.4	(0.089)	1059.4	(0.505)	1073.2	(0.508)	1126.6	(0.759)
Poloxamer 188	0.5	−	342.7	(0.084)	345.5	(0.087)	—	—	—	—
		+	303.9	(0.039)	803.7	(0.378)	738.0	(0.270)	853.2	(0.463)
	1.0	−	283.0	(0.064)	312.0	(0.210)	—	—	—	—
		+	119.2	(0.135)	439.8	(0.136)	432.3	(0.276)	458.1	(0.145)
Polysorbate 85	0.5	−	273.6	(0.219)	597.1[a]	(0.774)	317.3[a]	(0.722)	—	—
		+	286.6	(0.320)	516.0[a]	(0.626)	524.0[a]	(0.716)	286.6	(0.413)
	1.0	−	325.0	(0.323)	327.6[a]	(0.499)	150.3[a]	(0.446)	—	—
		+	328.1	(0.261)	904.7[a]	(0.692)	365.6[a]	(0.554)	531.4	(0.370)

c, concentration of stabilizer in %.
T, autoclaving with (+) or without (−) cooling.
[a]Measured in the supernatant.
*Particle size in nm.
**Polydispersity.

USA). After each centrifugation step the supernatant was removed and the nanoparticles were resuspended in water by ultrasonication (5 min, Bandelin, Sonorex Super RK103H, Germany). Subsequently, the resuspended nanoparticles were divided off, one part was stored in this form, the other part was lyophilized in the presence of mannitol as cryoprotector (Alpha 1–4, Martin Christ Gefriertrocknungsanlagen, Germany).

Particle size determinations were achieved by means of photon correlation spectroscopy (Auto-Sizer Lo-C, Malvern Instruments Ltd., UK). Measurements of the lyophilized samples were carried out after resuspension in water supported by sonication.

The different nanoparticle suspensions were sterilized in the autoclave at 121°C for 20 min with or without cooling to 70°C afterwards. The lyophilized nanoparticle powders were sterilized in a formaldehyde stream (60°C). After sterilization the particle sizes were determined again, sonicated in parts.

3. Results and discussion

Table 1, Table 2, Table 3, and Table 4 show the results of measuring the particle sizes and polydispersity values after different preparation steps and different sterilization treatments. Each experiment was carried out in duplicate, the only difference being cooling conditions after autoclaving.

As depicted in Table 1 (column 1) the particle size of the PBCA nanoparticles varied from 116 to 524 nm and the polydispersity value from 0.04 and 0.45 immediately after preparation. These differences mainly resulted from the different stabilizers and stabilizer concentrations. But the comparison of the equal experiments makes obvious that slightly variable preparation conditions, such as type of reaction vessel, velocity of adding monomer, and stirring speed influenced these values.

The particles prepared with dextran 70 000 and poloxamer 188 as stabilizer tend to agglomerate after the purification steps by centrifugation and

Table 3
Centrifuged and resuspended nanoparticle suspensions

Stabilizer	c	T	No sterilization		After sterilization					
					No sonication		2-min sonication		5-min sonication	
			size*	p.d.**	size*	p.d.**	size*	p.d.**	size*	p.d.**
Dextran 70 000	0.5	−	379.0	(0.271)	2274.0	(0.426)	2198.7	(0.254)	2059.4	(0.370)
		+	377.9	(0.343)	902.9	(0.710)	928.8	(0.477)	969.4	(0.267)
	1.0	−	302.7	(0.104)	852.0	(0.369)	775.8	(0.441)	979.7	(0.463)
		+	301.3	(0.476)	887.4	(0.124)	854.9	(0.175)	836.3	(0.505)
Poloxamer 188	0.5	−	411.0	(0.205)	616.8	(0.471)	619.4	(0.472)	668.6	(0.283)
		+	374.8	(0.228)	396.9	(0.324)	395.8	(0.331)	389.7	(0.212)
	1.0	−	297.6	(0.060)	557.6	(0.446)	481.8	(0.434)	633.0	(0.403)
		+	181.2	(0.096)	273.5	(0.072)	268.9	(0.157)	273.8	(0.172)
Polysorbate 85	0.5	−	304.0	(0.331)	241.9	(0.109)	243.3	(0.103)	—	—
		+	291.7	(0.210)	291.5	(0.276)	278.7	(0.299)	298.3	(0.287)
	1.0	−	293.7	(0.146)	245.6	(0.095)	246.9	(0.124)	—	—
		+	330.1	(0.181)	253.1	(0.257)	257.1	(0.287)	726.6	(0.583)

c, concentration of stabilizer in %.
T, autoclaving with (+) or without (−) cooling.
[a]Measured in the supernatant.
*Particle size in nm.
**Polydispersity.

lyophilization (Table 1, Table 3, Table 4, column 1), a problem often mentioned in the literature (e.g. by Kreuter (1983b)). The particles prepared with polysorbate 85 showed smaller particle sizes after the centrifugation, a circumstance explainable with the removed stabilizer which caused repulsion in case of dextran 70 000 and poloxamer 188 and reversible aggregation in case of polysorbate 85 (own observations). Especially the resuspension of lyophilized nanoparticles caused problems which often can not be overcome by intensified sonication (comparison of column 1 and 2 in Table 4).

The sterilization of the nanoparticle suspensions leads to very different results. For example, autoclaving of acidic poloxamer suspensions seems to be possible without problems (Table 1, column 2), as there was no notable change in particle size. The same procedure with the acidic dextran suspensions resulted in only slightly enhanced particle sizes after autoclaving with cooling but in large size increases after autoclaving without cooling. The values of the acidic polysorbate suspensions have to be considered carefully, because they were measured in the diluted supernatant. The main part of the nanoparticles were agglomerated in a scarcely resuspendable sediment.

After neutralization the heat treatment led to usable nanoparticle suspensions only in the case of poloxamer and autoclaving without cooling (Table 2, column 2). Polysorbate stabilized nanoparticles sedimented again together with the stabilizer.

Removing the stabilizers by centrifugation led to injectable nanoparticle suspensions only in the case of polysorbate and autoclaving without cooling (Table 3, column 2). No sediments were formed in the four polysorbate experiments.

The appearance of all these suspensions was not changed after sterilization except for the precipitated polysorbate nanoparticles (Table 1 and Table 2).

As expected, the different lyophilized-nanoparticle powders showed marked modifications after formaldehyde treatment (60°C). The wetting characteristics were impaired for all the powders after sterilization, indicated by dramatic increases in

Table 4
Lyophilized and resuspended nanoparticle suspensions, formaldehyde treatment

Stabilizer	c	T	No sterilization		After sterilization					
					No sonication		2-min sonication		5-min sonication	
			size*	p.d.**	size*	p.d.**	size*	p.d.**	size*	p.d.**
Dextran 70 000	0.5	−	361.7	(0.185)	381.7	(0.379)	573.6	(0.403)	512.3	(0.407)
		+	391.4	(0.352)	376.3	(0.301)	1075.7	(0.591)	753.5	(0.394)
	1.0	−	287.2	(0.092)	300.3	(0.165)	542.5	(0.269)	495.9	(0.228)
		+	360.4	(0.393)	338.3	(0.380)	692.5	(0.568)	880.5	(0.719)
Poloxamer 188	0.5	−	384.4	(0.060)	357.9	(0.041)	671.7	(0.381)	565.1	(0.241)
		+	397.9	(0.306)	385.7	(0.294)	1426.2	(0.590)	830.4	(0.357)
	1.0	−	297.6	(0.036)	301.7	(0.161)	624.8	(0.349)	531.6	(0.316)
		+	292.3	(0.251)	241.9	(0.191)	592.7	(0.318)	529.5	(0.372)
Polysorbate 85	0.5	−	325.9	(0.222)	330.4	(0.313)	445.4	(0.347)	419.0	(0.279)
		+	443.8	(0.335)	393.9	(0.304)	1364.8	(0.484)	1023.5	(0.402)
	1.0	−	364.9	(0.258)	343.9	(0.277)	508.1	(0.353)	441.0	(0.331)
		+	567.7	(0.372)	541.1	(0.402)	1338.0	(0.232)	1066.4	(0.395)

c, concentration of stabilizer in %.
T, autoclaving with (+) or without (−) cooling.
[a]Measured in the supernatant.
*Particle size in nm.
**Polydispersity.

particle sizes (Table 4, column 3) and clumping. Nevertheless, there was no detectable increase in weight, produced perhaps by adsorption of or the reaction with formaldehyde. No powder was suitable for preparing an injectable suspension.

This study confirms that the sterilization of nanoparticle suspensions or powders by standard procedures (autoclaving or formaldehyde treatment) is not advisable, even if only empty nanoparticles are used. In summarizing the possible problems, it would be preferable to prepare the nanoparticles under aseptic conditions, which was successfully carried out for example by Verdun et al. (1986) and Fouarge et al. (1989). Moreover, butylcyanoacrylate has a strong bactericidal action causing sterility of the polymerization medium (Kreuter, 1985). However, under certain conditions post-fabrication sterilization is also possible.

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