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Sterile, injectable cyclodextrin nanoparticles: Effects of gamma irradiation and autoclaving

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Abstract

Sterility is required as stated by compendial requirements and registration authorities worldwide for an injectable drug carrier system. In this study, injectable nanospheres and nanocapsules prepared from amphiphilic β-cyclodextrin, β-CDC6, were assessed for their in vitro properties such as particle size distribution, zeta potential, nanoparticle yield (%), drug entrapment efficiency and in vitro drug release profiles. Different sterilization techniques such as gamma irradiation and autoclaving were evaluated for their feasibility regarding the maintenance of the above mentioned nanoparticle properties after sterilization. It was found that amount these techniques, sterilization with gamma irradiation seemed to be the most appropriate technique with no effect on particle size, drug loading and drug release properties. Gamma irradiation causes some chemical changes on β-CDC6 observed as changes in zeta potential but this does not lead to any significant changes for nanoparticle properties. Autoclaving caused massive aggregation for the nanoparticles followed by precipitation, which led to the conclusion that excessive heat disrupted nanoparticle integrity. Sterile filtration was not feasible since nanoparticle sizes were larger than the filter pore size and the yield after sterilization was very low. Thus, it can be concluded that blank and drug loaded β-CDC6 nanospheres and nanocapsules are capable of being sterilized by gamma irradiation.
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Keywords: Amphiphilic cyclodextrin; Autoclaving; Gamma irradiation; Nanoparticle; Tamoxifen

1. Introduction

Sterility is a crucial factor for drug delivery systems that are to be directly injected into the organism. Injectable nano- or microparticles can be sterilized by a number of techniques all with considerable advantages and drawbacks. Regarding injectable nanoparticles, alternative sterilization techniques include membrane filtration, gamma irradiation, autoclaving, ethylene oxide sterilization and high hydrostatic pressure sterilization.

Membrane filtration is a safe technique based on physical removal of present microorganisms that does not require excessive heat or radiation causing irreversible effects on the nanoparticles or the encapsulated drug. However, it is very much limited to the size of the particles. Nanoparticles with size exceeding 200 nm are not appropriate for this kind of sterilization. Several authors have stated that filtration is not an effective sterilization method since nanoparticles are similar in size to contaminants and also the filter pore size. Moreover, elasticity and size of the nanoparticles could lead to clogging of the filtration membranes (Allemann et al., 1993; Magenheim and Benita, 1991). Adsorption of the nanoparticle material to the filter is another drawback of this technique reducing the yield of the finished product.

Heat sterilization by autoclaving is a highly effective technique involving high temperatures (120 °C), which may influence decomposition or degradation of active ingredient as well as the nanoparticle material, i.e., polymer. An increase in size of nanocapsules from 200 to 500 nm was reported after moist heat sterilization where Miglyol is used as oil phase surrounded by poly(isobutylcyanoacrylate) (Rollot et al., 1986). This increase in size was attributed to either the swelling of polymeric membrane or expansion of oily phase. No change in size was observed for nanospheres.

Sterilization by gamma irradiation is also an effective method accepted by European Pharmacopeia. The main advantage is its high penetration power and the isothermal character of gamma rays that allows suitable treatment for heat-sensitive materials.

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Moreover, gamma irradiation assures homogeneous sterilization and is useful for packaged products, thus avoiding further risk of microbial contamination. However, gamma irradiation also may exert serious effects on the drug delivery system (Sintzel et al., 1997). Energy transfer may induce fragmentation of covalent bonds and production of free radicals that, in turn, are responsible for the majority of the damage that occurs to irradiated materials as a consequence of chemical attack, e.g., radiation could cause alteration of physicochemical properties, decrease of the amount of active ingredient by partial decomposition or create molecular fragments that may result in a toxicological hazard (Boess and Bögl, 1996; Sintzel et al., 1997; Masson et al., 1997).

Amphiphilic cyclodextrins have been widely investigated as excipients for drug delivery systems in the form of nanoparticles since the last decade (Duchene et al., 1999). They have been reported to give stable nanospheres and nanocapsules without the presence of surfactants achieving high encapsulation efficiency and reduction of burst effect in drug release (Memisoglu et al., 2002, 2003). These nanoparticles are designed for injectable carriers mostly for anticancer agents so sterility is an important factor in the manufacturing of cyclodextrin-based nanoparticles.

The objective of this study is to assess the feasibility of sterilization with different techniques employing heat or gamma-irradiation for the first time on amphiphilic β-CD nanoparticles. Effect of sterilization technique on nanoparticle properties such as size, zeta potential, drug loading, in vitro drug release, thermal behavior and nanoparticle yield was assessed for the first time for amphiphilic β-cyclodextrin nanoparticles in a comprehensive approach.

2. Material and methods

2.1. Materials

Amphiphilic β-cyclodextrin modified on the secondary face with 6C aliphatic esters, β-CDC6, was synthesized and purified as described previously. The chemical structure, purity and selective substitution of β-CDC6 were previously described by different techniques such as H NMR spectrometry, Fourier transform infrared spectroscopy, elemental analysis and fast atom bombardment mass spectrometry (Memisoglu et al., 2002) (Fig. 1). Miglyol 812® (Condea Chimie, Germany), trigyceride of capryc/caprylic acid was used as oil in the preparation of nanocapsules, Tamoxifen citrate, model drug, was a kind gift of Teva Pharmaceuticals Inc. (Israel). Acetone was extra pure (Carlo Erba, Italy) for the preparation of nanoparticles. All other reagents were of analytical grade and were used as received.

2.2. Methods

2.2.1. Nanoparticle preparation

2.2.1.1. Preparation of blank nanoparticles. Nanoprecipitation technique was used (Fessi et al., 1998) to prepare the nanoparticles. Briefly, β-CDC6 (1 mg) was dissolved in acetone (1 mL) to give an organic phase, which was added with an

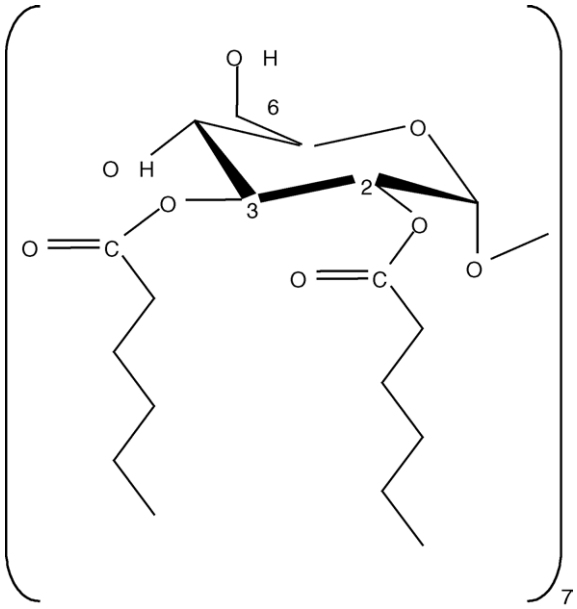

Fig. 1. β-CDC6, selectively substituted and pure amphiphilic β-cyclodextrin per-modified on the secondary face with 6C aliphatic esters.

Eppendorf injector under room temperature at constant stirring to an aqueous phase consisting only of deionized water. Organic solvent was evaporated under vacuum to give nanosphere dispersion of desired volume, which was 2 mL in this study. To obtain nanocapsules, the only difference is the addition of 50 μL of Miglyol 812®, to the organic phase. Organic/aqueous phase ratio was kept at 1:2 (v/v) for nanospheres and nanocapsules.

2.2.1.2. Preparation of drug loaded nanoparticles. Tamoxifen citrate loaded nanoparticles were prepared with a novel technique reported previously (Memisoglu et al., 2003). In this technique, nanoparticles are prepared directly from pre-formed drug:amphiphilic β-CD inclusion complexes. Tamoxifen:β-CDC6 inclusion complexes of 1:1 molar ratio were prepared by co-lyophilization technique and characterized by DSC, FT IR spectroscopy, SEM and MALDI TOF as 1:1 inclusion complexes (unpublished results). Fixed amounts of tamoxifen citrate and β-CDC6 are readily soluble in ethanol were dissolved in 20 mL ethanol. Then 40 mL water is added to obtain a suspension of drug and cyclodextrin. Suspension is left to equilibrate under stirring for 7 days at room temperature and ethanol was evaporated under vacuum to give an aqueous suspension which was then lyophilized (HETO Lyolab Freeze Dryer, UK) yielding the complex in powder form.

Nanoparticles were prepared by weighing and dissolving the TMX:β-CDC6 complex (1 mg) in acetone and further addition of drug solution containing 200 μg TMX in the organic phase during preparation. Organic phase was added to aqueous phase as described in Section 2.2.1.1 and nanoparticles were obtained after evaporation of organic solvent.

2.2.2. Sterilization of nanoparticles

2.2.2.1. Autoclaving (heat sterilization). Samples were divided into two groups of equal volume after preparation. They were put

into glass vials, which were sealed with rubber stoppers and aluminum caps. One group was sterilized at 121 °C for 20 min while the other group (reference) was kept at 8 °C for comparative evaluation of physicochemical properties with their sterilized analogue.

2.2.2.2. Gamma irradiation. Irradiation was performed at room temperature using a ^{60}Co irradiator (Gamma 220) available at Turkish Atomic Energy Agency Facility (Saray, Ankara) at the dose rate of 1.88 kGy/h. Samples in rubber-stopped and aluminum cap-sealed Type I glass vials were irradiated at a dose of 25 kGy. Non-irradiated samples were kept as reference for further comparison.

2.2.3. Evaluation of nanoparticles

2.2.3.1. Nanoparticle yield. Non-sterile and sterile, blank and drug-loaded nanoparticle aqueous dispersions were lyophilized using HETO PowerDry PL3000 Freeze Dryer, Denmark. Resulting powder was weighed. The ratio of sterile nanoparticle sample to its non-sterile analogue was determined as nanoparticle yield indicating nanoparticle loss during sterilization process using the following equation:

$$\text{Nanoparticle yield} = \frac{\text{Weight of sterile nanoparticle}}{\text{Weight of non-sterile nanoparticle}} \times 100.$$

2.2.3.2. Particle size evaluation. Particle size distribution of sterile and non-sterile nanospheres and nanocapsules were measured using a Coulter Nanosizer N4 Submicron Particle Size Analyzer (Coulter Langley Ford Instruments, USA) with mean particle size (diameter, nm ± S.D.) and polydispersity index (PI) determined by Photon Correlation Spectroscopy. Measurements were realized in triplicate at a 90° angle at 20 °C. Nanoparticle dispersions of intensity between 10^4 and 10^6 cps were analyzed for 90 s.

2.2.3.3. Zeta potential analysis. Zeta potential of nanoparticle dispersions was measured by Malvern Zetasizer Nano-ZS (Malvern Instruments, UK) in triplicate among sterile and non-sterile batches to assess the surface charge and thus, the stability of nanosystem and the effect of different sterilization techniques on this parameter. Zeta potential of nanospheres and nanocapsules were measured in aqueous dispersion of 1 mM NaCl.

2.2.3.4. Drug encapsulation efficiency. Loaded drug quantity was determined according to the following procedure: unbound drug was separated by centrifugation at 30,000 × *g* for 10 min and the precipitate was discarded. Supernatant was then ultracentrifuged at 120,000 × *g* at 25 °C for 1 h by a Sorvall RC288 with fix rotor type s20/20 (DuPont, USA) to precipitate the nanoparticles and the encapsulated drug. Precipitate was then lyophilized and resulting powder containing the loaded nanoparticles was dissolved in ethanol to obtain a clear solution and analyzed UV spectrophotometrically (Shimadzu UV160A, Bechman Instruments, Munchen, Germany). To confirm that no unbound TMX is left in the nanosphere dispersion after the initial ultracentifugation, supernatant was subject to ultracentifugation at 120,000 × *g* for 1 h and the resulting supernatant was lyophilized. Powder obtained after lyophilization was dissolved in alcohol and analyzed for free TMX by UV spectrophotometry. Analytical method of tamoxifen citrate quantification by UV spectrophotometry was validated (linearity r^2: 0.9997, repeatability CV: 0.04%, reproducibility: CV: 0.88%, specifity confirmed).

Loading capacity was expressed in terms of entrapment efficiency. Entrapment efficiency is the amount of drug (μg) associated per unit CD (mg) in the nanoparticles.

2.2.3.5. In vitro drug release. Release profiles of tamoxifen citrate from sterile and non-sterile nanosphere and nanocapsule formulations were determined in 20 mL of isotonic phosphate buffer solution pH 7.4 containing 1% polysorbate 80 providing sink conditions in a thermostated bath system (TMX solubility in release medium: 200 μg/mL) (Memmert, Schwabach, Germany) at 37 °C with a nanoparticle/medium ratio of 1/20 with an agitation of 150 rpm. Samples were withdrawn at given time intervals and replaced with fresh buffer solution maintained at the same temperature. Samples were ultracentifuged to precipitate nanoparticles and supernatant was then analyzed for tamoxifen citrate with UV–vis spectrophotometry using an analytically validated method (r^2: 0.9999, CV < 2%) at 299 nm.

3. Results

Sterilization techniques were evaluated for their effect on nanoparticle characteristics. The first evaluated property was nanoparticle yield. As seen in Table 1, gamma irradiation and autoclaving techniques do not have any significant influence on nanoparticle yield. However, filtration directly affects the yield since nanoparticle size is very close to filter pore size. Yield after filtration is very low which makes this sterilization technique inappropriate for amphiphilic cyclodextrin nanoparticles as far as nanoparticle yield is concerned.

Particle size of nanospheres and nanocapsules were determined on sterile and non-sterile samples. Table 2 summarizes the mean diameter and polydispersity index values of nanospheres and nanocapsules after autoclaving and gamma sterilization. As seen in table, gamma irradiation does not seem to have any effect on particle size and polydispersity index. However, alternative technique, autoclaving, has a direct impact on nanoparticle size. Nanoparticles tend to aggregate at high temperature employed in autoclaving which leads to increased particle size and polydispersity index.

Zeta potential values indicate changes in chemical structure of either amphipilic cyclodextrin or active ingredient after

Table 1
Yield (%) of β-CDC6 nanoparticles after different sterilization techniques calculated in reference (100%) to non-sterile samples (n = 6, S.D.)

	Autoclaving	Gamma irradiation
Nanosphere	82 ± 3	98 ± 2
Nanocapsule	74 ± 6	97 ± 1

Table 2
Mean diameter (nm) and polydispersity index (PI) of blank and drug-loaded nanospheres and nanocapsules after different sterilization techniques ($n = 3$, S.D.)

	Non-sterile	Autoclaved	Gamma irradiated
Blank nanosphere	205/0.02	690/0.89	200/0.08
Blank nanocapsule	290/0.1	730/0.76	272/0.12
Tamoxifen citrate loaded nanosphere	235/0.10	805/0.80	240/0.04
Tamoxifen citrate loaded nanocapsule	310/0.09	786/0.66	297/0.03

sterilization. This is particularly important for the gamma irradiation technique since it is known that radiation causes irreversible chemical changes in polymer structure and most active ingredients (Boess and Bögl, 1996; Sintzel et al., 1997). Fig. 2 indicates slight changes in zeta potential of both blank and drug-loaded nanospheres and nanocapsules suggesting chemical changes due to irradiation or heat in the structure of β-CDC6. However, these changes are believed to be indicative of a change in physicochemical properties of β-CDC6 such as increase in aqueous solubility of β-CDC6 due to fragmentation of some of the long aliphatic chains aligning the surface of the molecule. This hypothesis is also confirmed by in vitro release profiles of β-CDC6 nanoparticles.

Fig. 3 demonstrates that gamma irradiation has no significant effect on entrapped drug quantity for nanospheres and for nanocapsules. However, autoclaving causes a significant reduction in drug entrapment since excessive heat disrupts the nanoparticle integrity and causes the loss of considerable amount of active ingredient as observed in Fig. 3.

In vitro release profiles of nanospheres and nanocapsules were determined as shown in Fig. 4. Release profiles are not significantly different than each other indicating that although there may be possible chemical changes in β-CDC6 structure these changes do not alter the release behavior of the nanosphere or nanocapsule for the model drug tamoxifen citrate.

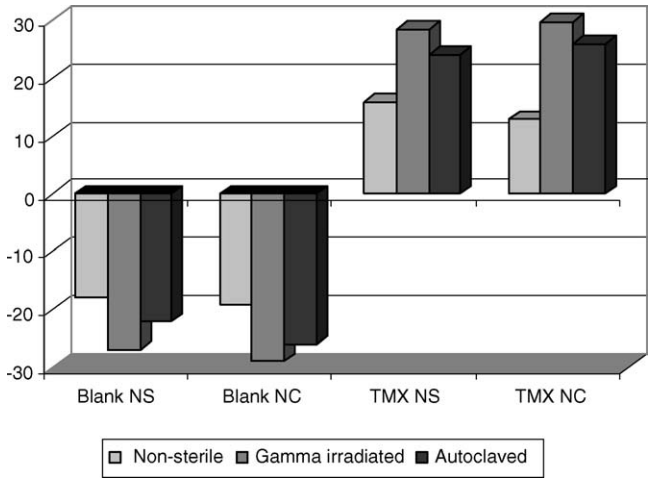

Fig. 2. Zeta potential (mV) values of amphiphilic β-cyclodextrin nanospheres and nanocapsules (NS, nanosphere; NC, nanocapsule; TMX NS, tamoxifen loaded nanosphere; TMX NC, tamoxifen loaded nanocapsule) ($n = 3$).

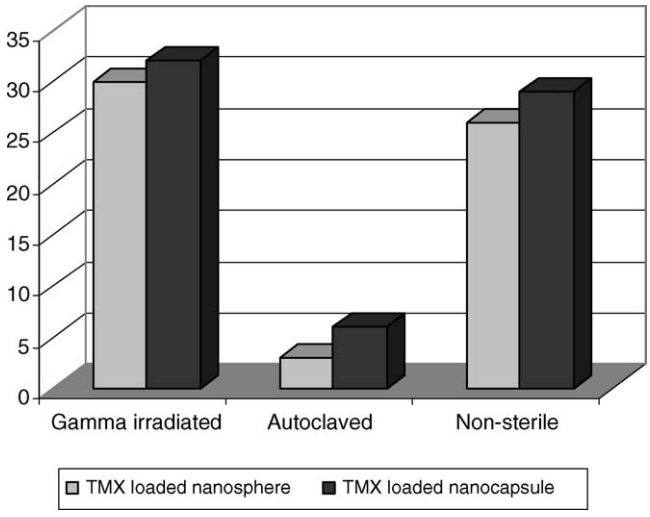

Fig. 3. Entrapped drug quantities (μg/mL) of nanospheres and nanocapsules loaded with tamoxifen citrate after different sterilization techniques ($n = 3$).

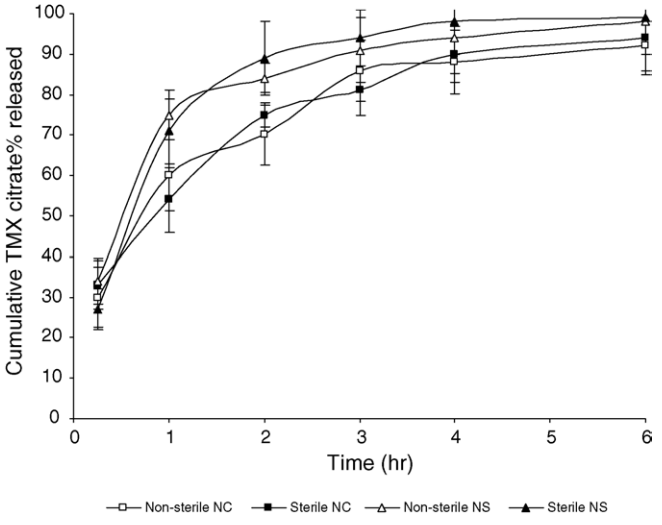

Fig. 4. In vitro drug release profiles of sterile (gamma irradiated) and non-sterile nanoparticles in isotonic PBS pH 7.4 containing 1% polysorbate 80 at 37 °C (NC: nanocapsule, NS: nanosphere) ($n = 6$, S.D.).

4. Discussion

Effects of each sterilization technique will be further discussed in this section in the light of data obtained. Sterile filtration through 0.22 μm membrane filters may be considered as an alternative method for chemically or thermally sensitive material since it has no adverse effect on the polymer or the drug. It is merely a physical removal of microorganisms. In fact, many drug delivery systems in the form of liposomes, microemulsions or nanoparticles below 200 nm size have been successfully sterile filtered (Martin, 1990; Lidgate et al., 1992; Zheng and Bosch, 1997; Konan et al., 2002, 2003). However, this technique is not suitable when the nanoparticle size is larger or close to 220, when the size distribution is not narrow enough, when the drug is adsorbed on the particle surface and when the nanoparticle dispersion is too viscous (Allemann et al., 1993; Magenheim and

Benita, 1991). These factors lead to membrane clogging and make filtration of samples impossible. In the case of β-CDC6 nanoparticles, sterile filtration was not feasible since nanosphere and nanocapsule sizes were around 280–300 nm. Nanoparticle yields were very much reduced (more than 95%) and even after pre-filtering through 0.45 μm, the same effects were observed.

As far as heat sterilization is concerned, it is known that polymeric nanoparticles are prone to degradation at elevated temperatures due to the generally low glass transition temperature of polymers and surface modifiers they contain (Athanasou et al., 1996). Consequently, nanoparticle properties are altered to cause aggregation and flocculation, which makes heat sterilization a difficult approach (Sommerfeld et al., 1998). Although β-CDC6 nanoparticles are not of polymeric nature, hydrolysis reactions induced by heat may have caused chemical modifications leading to aggregation and significant increase in particle size. It has been reported previously that polyesters are degraded by hydrolysis when autoclaved (Athanasou et al., 1996) suggesting a possible degradation of ester structure of β-CDC6 seen in Fig. 1.

On the other hand, surfactants included in nanoparticle formulations were also reported to have an important effect on particle aggregation after autoclaving. In fact, aggregation upon heating is directly related to the precipitation and/or phase separation of the surface modifier at a temperature above its cloud point where this molecule is likely to dissociate from the particle. Several authors have proposed the use of different substances to increase the cloud point of surfactant above the temperature required for sterilization (i.e., PEG400, propylene glycol etc.) (Hollister, 1993; Na, 1993). Amphiphilic β-cyclodextrin nanoparticles do not contain additional surfactants in their formulation but the amphiphilic β-cyclodextrin itself is a proven surface-active agent (Ringard-Lefebvre et al., 2002) with a calculated HLB value of 8.9, which may be acting similar to other surfactants and dissociate to destroy nanoparticle integrity. This may explain the fact that upon autoclaving, massive aggregation is observed in nanospheres and nanocapsules. The active ingredient is not thermolabile, however, autoclaving causes β-CDC6 to behave as a surface-active agent and to dissociate from the nanoparticle at temperatures higher than its cloud point. The molecule then precipitates or forms larger aggregates. Meanwhile, entrapped drug is liberated to a certain extent and precipitates, too. This may be the cause of reduced drug entrapment observed after autoclaving of β-CDC6 nanoparticles. Although heat sterilization may be successfully applied without altering nanoparticle characteristics in some systems (Venkateswarlu and Manjunath, 2004), it does not seem to be an appropriate technique for the sterilization of β-CDC6 nanoparticles.

Gamma irradiation presents an effective alternative as far as nanoparticle characteristics such as entrapment efficiency and in vitro drug release are concerned. Particle size is not affected by radiation, which suggests physical stability of nanoparticles after sterilization. Significant changes are observed for zeta potential values both for blank and drug-loaded nanospheres and nanocapsules. This may be attributed to the fragmentation of covalent bonds and probably partial breaking up of the acyl chains surrounding the β-CDC6. The cross-linking and relative stabilizing effect of irradiation on polysaccharides including starch are known and β-CDC6 nanoparticles may also be subject to these changes which effect mainly the side chains of polysaccharide derivatives (Bartolotta et al., 2005; Yoshii et al., 2003; Wach et al., 2003). Zeta potential values indicate a certain change in overall charge of the system. This change is observed both for blank and drug-loaded nanoparticles. If this is the result of chemical degradation of tamoxifen citrate, drug-loading values should be an indicative for this. However, loading data demonstrate gamma irradiation has no effect on entrapped drug quantity. Tamoxifen citrate UV spectrum also shows no change after irradiation with gamma rays. Gamma irradiation causes an increased release rate in particles due to the radiolytic degradation of the polymer (Faisant et al., 2002). However, amphiphilic β-cyclodextrin nanoparticles do not undergo this effect and maintain their release properties after gamma irradiation. Even though zeta potential measurements indicate chemical changes in β-CDC6 structure, these changes are not sufficient to cause alterations in drug release profiles.

Other complicated or expensive techniques could also be used for nanoparticle sterilization involving high hydrostatic pressure (Brigger et al., 2003) maintaining nanoparticle integrity or chemical sterilization with ethylene oxide which may lead to toxic residues and difficulty in redispersion of nanoparticles after sterilization (Sommerfeld et al., 1998). However, the most appropriate technique for amphiphilic β-cyclodextrin nanoparticles seem to be gamma irradiation considering the maintaining of physicochemical characteristics such as particle size, nanoparticle yield, zeta potential, drug loading and in vitro release.

5. Conclusion

Sterilization of injectable nanoparticles is a major challenge in the designing of appropriate drug delivery systems. Among different alternatives such as sterile filtration, autoclaving and gamma irradiation, the latter seem to be most promising technique which does not alter nanoparticle characteristics ensuring sterility of injectable nanoparticles.

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References

Allemann, E., Gurny, R., Doelker, E., 1993. Drug loaded nanoparticles. Preparation, methods and drug targeting issues. Eur. J. Pharm. Biopharm. 39, 173–191.

Athanasou, K.A., Niederaurer, G.G., Mauli Agrawal, C., 1996. Sterilization, toxicity and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 17, 93–102.

Bartolotta, A., D'Oca, M.C., Campisi, M., De Caro, V., Giandalia, G., Giannola, L.I., Brai, M., Calderaro, E., 2005. Effects of gamma-irradiation on trehalose-hydroxymethylcellulose microspheres loaded with vancomycin. Eur. J. Pharm. Biopharm. 59, 139–146.

Boess, C., Bögl, K.W., 1996. Influence of radiation treatment on pharmaceuticals—a review: alkaloids, morphine derivatives and antibiotics. Drug Dev. Ind. Pharm. 22, 495–529.

Brigger, I., Armand-Lefebvre, L., Chaminade, P., Besnard, M., Rigaldie, Y., Largeteau, A., Andremont, A., Grislain, L., Demazeau, G., Couvreur, P., 2003. The stenlying effect of high hydrostatic pressure on thermally and hydrolytically labile nanosized carriers. Pharm. Res. 20, 674–683.

Duchene, D., Ponchel, G., Wouessidjewe, D., 1999. Cyclodextrins in targeting. Applications to nanoparticles. Adv. Drug Del. Rev. 36, 29–40.

Faisant, N., Siepmann, J., Oury, P., Laffineur, V., Bruna, E., Haffner, J., Benoit, J.P., 2002. The effect of gamma-irradiation on drug release from bioerodible microparticles: a quantitative treatment. Int. J. Pharm. 242, 281–284.

Fessi, H., Devissaguet, J.P., Thies, C., 1998. Process for the preparation of dispersible colloidal systems of a substance in the form of nanospheres. US Patent, 5,118,529.

Hollister, K., 1993. Use of purified surface modifiers to prevent particle aggregation during sterilization, Eur. Patent. Appl. No., EP 605024Az.

Konan, Y.N., Cerny, R., Favet, J., Berton, M., Gurny, R., Allemann, E., 2003. Preparation and characterization of sterile sub-200 nm meso-tetra(4-hydroxyphenyl)porphyrin-loaded nanoparticles for photodynamic therapy. Eur. J. Pharm. Biopharm. 55, 115–124.

Konan, Y.N., Gurny, R., Allemann, E., 2002. Preparation and characterization of sterile and freeze-dried sub-200 nm nanoparticles. Int. J. Pharm. 233, 239–252.

Lidgate, D.M., Trattner, T., Shultz, R.M., Maskiewicz, R., 1992. Sterile filtration of a parenteral emulsion. Pharm. Res. 9, 860–863.

Magenheim, B., Benita, S., 1991. Nanoparticle characterization: a comprehensive physicochemical approach. STP Pharm. Sci. 1, 221–241.

Martin, F., 1990. Pharmaceutical manufacturing of liposomes. In: Tyle, P. (Ed.), Specialized Drug Delivery System-Manufacturing and Production Technology, vol. 41. Marcel Dekker, New York, pp. 267–316.

Masson, V., Maurin, F., Fessi, H., Devissaguet, J.P., 1997. Influence of sterilization process on poly(ε-caprolactone) nanospheres. Biomaterials 18, 327–335.

Memisoglu, E., Bochot, A., Şen, M., Charon, D., Duchene, D., Hıncal, A.A., 2002. Amphiphilic β-cyclodextrins modified on the primary face: synthesis, characterization and evaluation of their potential as novel excipients in the preparation of nanocapsules. J. Pharm. Sci. 91, 1214–1224.

Memisoglu, E., Bochot, A., Şen, M., Duchene, D., Hıncal, A.A., 2003. Non-surfactant nanospheres of progesterone inclusion complexes with amphiphilic β-cyclodextrins. Int. J. Pharm. 251, 143–153.

Na, G., 1993. Use of ionic cloud point modifiers to minimize nanoparticles aggregation during sterilization, Eur. Patent Appl. No., EP601619.

Ringard-Lefebvre, C., Bochot, A., Memisoglu, E., Charon, D., Duchene, D., Baszkin, A., 2002. Effect of spread amphiphilic β-cyclodextrins on interfacial properties of the oil/water system. Colloid. Surf. B. Biointerf. 25, 109–117.

Rollot, J., Couvreur, P., Roblot-Treupel, L., Puisieux, F., 1986. Physicochemical and morphological characterization of polyisobutylcyanoacrylate nanocapsules. J. Pharm. Sci. 75, 361–364.

Sintzel, M.B., Merkli, A., Tabatabay, C., Gurny, R., 1997. Influence of irradiation sterilization on polymers used as drug carriers—a review. Drug Dev. Ind. Pharm. 23, 857–878.

Sommerfeld, P., Schroeder, U., Sabel, B.A., 1998. Sterilization of unloaded polybutylcyanoacrylate nanoparticles. Int. J. Pharm. 164, 113–118.

Venkateswarlu, V., Manjunath, K., 2004. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J. Cont. Rel. 95, 627–638.

Wach, R.A., Mitomo, H., Nagasawa, N., Yoshii, F., 2003. Radiation cross linking of methyl cellulose and hydroxyethyl cellulose in concentrated aqueous solutions. Nucl. Instrum. Meth. B 211, 533–544.

Yoshii, F., Zhao, L., Wach, R.A., Nagasawa, N., Mitomo, H., Kume, T., 2003. Hydrogels of polysaccharide derivatives crosslinked with irradiation at paste-like condition. Nucl. Instrum. Meth. B 208, 320–324.

Zheng, J.Y., Bosch, W., 1997. Sterile filtration of NanoCrystal™ drug formulations. Drug Dev. Ind. Pharm. 23, 239–252.