

Preparation and Characterization of Poly-(methyl ethyl cyanoacrylate) Particles Containing 5-Aminosalicylic acid

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Abstract

Poly-(alkylcyanoacrylate) ester particles have been used for the preparation of different formulations ranging from ophthalmic delivery systems to cancer chemotherapy carriers in clinic. The aim of this study was to prepare and characterize poly-(methyl ethyl cyanoacrylate) (PMECA) particles containing 5-aminosalicylic acid (5-ASA). PMECA particles were prepared using the inverse emulsification polymerization technique. The effects of various formulation parameters such as dispersed phase volume, polymer to drug weight ratio, and surfactant concentration on loading efficiency and size of the prepared particles were investigated. The amount of drug was determined by UV spectrophotometry at 331 nm. Morphological characteristics of particles and particle size analysis were studied by Scanning Electron Microscopy (SEM) and Laser Light Scattering techniques, respectively. The results showed that there was no linear relationship between the dispersed phase volume (DPV) and loading efficiency of 5-ASA in the range of 20-50%. Increasing polymer/drug weight ratio in the range of 1-15, enhanced the loading efficiency from 11.4 to 78.2% in a linear mode ($r=0.987$). Increasing the surfactant concentration in the range of 1-3% did not increase the loading efficiency of the prepared particles, and increasing the concentration to 5% even decreased the loading efficiency of particles. Laser light scattering and SEM evaluations showed that in all prepared formulations, particles were in a mixture of micro and nanospheres and micro and nanocapsules and 50 percent of particles had mean sizes in the range of 6.4-10.1 micrometer. Although our results showed significant differences in the mean particle size between some of the prepared formulations, this variation was not practically important. It was concluded that by using proper conditions, it is possible to use PMECA as a polymeric matrix for loading of 5-ASA and the other hydrophilic drugs.

Keywords: 5-Aminosalicylic Acid; Poly-(methyl ethyl cyanoacrylate); Inverse emulsification polymerization; Poly-(alkylcyanoacrylate) esters.

Introduction

Targeted drug delivery systems have been used to direct biologically active molecules to various organs. In these systems,

physicochemical properties of the carrier are important in determining the biological fate and activity of the drug within the delivery devices. An important requisite in drug delivery design is an appropriately controlled rate of drug release from the system. Using properly targeted drug delivery systems, it is possible

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to control the release of the active compounds, so instead of letting drug molecules diffuse freely through the body, they can be localized in a specific body site by passive and active mechanisms (1). Different polymeric colloidal drug delivery systems (liposomes, niosomes, nanoparticles, and microemulsions) and techniques have recently been introduced for this purpose. Among the most promising colloidal drug delivery systems to achieve this goal are nanoparticles. Nanoparticles are solid colloidal particles ranging from 10 nm to 1000 nm (1 μ m). They consist of macromolecular materials in which the active compound (drug or biologically active material) is dissolved, entrapped, or encapsulated, and/or to which the active compound is adsorbed or attached (2). Nanoparticles are made of artificial or natural polymers. The use of these polymers is often restricted by their bioacceptability (3). (Poly-(alkylcyanoacrylic acid) esters (PACA) are polymers which have been used for the production of nanoparticles. Safety profile, biodegradability and biocompatibility of PACA particles have been well documented. A major advantage of PACA nanospheres is their exceedingly rapid elimination from the body, within a few days, as a result of their very rapid degradation *in vivo* (4). Their degradation rate can be monitored by combining cyanoacrylates with different side chain esters, simply by mixing the monomers prior to addition to the polymerization medium (5). In contrast to the other polymerization systems requiring energy input that can affect the stability of the incorporated drug, this polymerization method is spontaneously initiated at room temperature by the hydroxide ions resulting from the dissociation of water, but some basic can drugs also act as initiators. This hydroxide ion-induced polymerization is very rapid. For this reason, the pH of the medium determines the polymerization rate. The pH has to be acidic (below 3.5, with some drugs even below 1.0) in order to prevent excessively rapid polymerization and enable the formation of monodispersed (non-agglomerated) nanoparticles (3, 4). In the case of the other polymers such as poly (methyl methacrylate), the polymerization is initiated either by high-

energy radiation (γ -rays) (6) or chemically by the addition of a polymerization initiator such as ammonium or potassium peroxodisulphate and heating to elevated temperatures (3). Different applications for PACA nanoparticles have been proposed ranging from ophthalmic delivery systems to cancer chemotherapeutic carriers in clinic (1). Recently, the ability of PACA nanoparticles to translocation of the intestinal epithelium, protection of the entrapped bioactive compound and their effects on membrane permeability and lymphatic uptake, have been reported. Such properties make the PACA nanoparticles a good site-specific oral delivery system for many drugs such as protein and peptid drugs (7).

Emulsion polymerization (5) and interfacial polymerization (8) techniques are used for the formation of PACA nanospheres and nanocapsules respectively (8, 9). It has been shown that nanoparticles prepared by these two techniques could release their drug contents by different kinetics. In principle, the difference between these two particulate systems (nanospheres and nanocapsules) is their drug release behavior, which is a first-order and a zero-order release profile for nanospheres and nanocapsules, respectively (10). Alkhourifallouh *et al*, reported a new technique of emulsion polymerization for the production of a new type of nanoparticulate formulation with a high entrapment efficiency for lipophilic drugs. The nanocapsular structure consisted of an oily central cavity surrounded by a polymeric envelope (8). Interfacial polymerization technique has also been applied to encapsulate hydrophilic compounds such as doxorubicin and fluorescein (11) or lipophilic drugs, such as triamcinolone acetonide (4, 9), in the presence of cyanoacrylate monomers using an inverse emulsification polymerization procedure. The inverse emulsification polymerization process was rapidly adopted for the production of biodegradable PACA nanospheres.

In these studies, the hydrophilic drug was dissolved in a small volume of water or hydrophilic solvent (methanol) and emulsified in an organic phase (e.g., isooctane, cyclohexane-chloroform, n-hexane) in the presence of surfactants. Alkylcyanoacrylate monomers were

then added directly or dissolved in an organic solvent to the prepared W/O emulsion under stirring (4). It was initially claimed that due to this subsequent addition of the monomers, particles with a shell-like wall (nanocapsules) were formed, resulting from the interfacial polymerization of monomers at the surface of the water-swollen micelles containing the drug. In fact, on the basis of transmission electron microscopy observations, it was later shown that the presence of nanocapsules was only occasional in the final nanoparticulate suspension and that most of the particles formed during this process were true nanospheres (3, 9).

To increase the efficacy of 5-ASA, different formulations have been proposed for oral delivery of 5-ASA. Site-specific oral delivery of this drug may not only increase its effectiveness, but also decrease its side effects. The aim of this project was to prepare poly-(methyl ethyl cyanoacrylate) (PMECA) particles containing 5-aminosalicylic acid (5-ASA) by the inverse emulsification polymerization procedure to characterize them, and investigate the effects of various formulation parameters on loading efficiency and particle size.

Experimental

Materials

Methyl ethyl cyanoacrylate monomers (MECA), 5-aminosalicylic acid (5-ASA), pluronic F-68 and hydroxypropyl betacyclodextrin (HPBCD) were obtained from Sigma-Aldrich Company (USA). Dichloromethane, n-hexane, dimethylformamide (DMF), potassium dihydrogen phosphate and acetonitrile were purchased from Merck Company (Germany).

Methods

Preparation of particles

PMECA particles were prepared using the inverse emulsification polymerization technique as follows: first, different solutions of MECA monomers were prepared by dissolving 50-750 mg MECA in 20 ml dichloromethane. Different 150 ml series of water in oil emulsions were produced by adding and stirring (at 2000 rpm) 30-75 ml of an aqueous phase (pH=2.5)

containing 5-ASA (30-75 mg), pluronic F-68 (1-5% W/V of total emulsion volume), and hydroxypropyl betacyclodextrin (HPBCD), as solubilizer (1% W/V of aqueous phase), in double distilled water, as the dispersed phase, to n-hexane (75-120 ml), as the continuous phase. Then, 20 ml MECA solution was added gradually to 150 ml W/O emulsion, while stirring at 4000 rpm. The mixture was then thoroughly stirred at 4000 rpm at room temperature for 20 minutes to form particles and then stirred at 1000 rpm for another 12 hours in order to evaporate dichloromethane and n-hexane completely. Finally, a milky suspension was obtained and neutralized by 0.1N NaOH, and centrifuged at 20,000 rpm (34880 g) and at 10 °C for 1 hour to precipitate the particles. Particles were then washed in triplicate with cold double distilled water, collected by filtration (0.4 micron filters), dried at room temperature and stored at 4 °C.

Quantitation of 5-ASA

To quantify the amount of 5-ASA loaded in the particles, 20 mg of each sample was dissolved in 5 ml dimethylformamide (DMF). Once complete dissolution was obtained, phosphate buffer solution (pH=8) was added up to 100 ml. The resulted system was stirred and filtered and the amount of drug in the filtrate was determined using a UV spectrophotometric method at 331 nm.

Calculation of 5-ASA loading efficiency

The loading efficiency of PMECA particles was calculated according to the following equation (12):

$$\text{Percentage of loading efficiency (LE \%)} = (\text{ELDS} / \text{TLDS}) \times 100$$

Where ELDS is the empirical loaded drug in sample (the empirical measured amount of 5-ASA in the sample) and TLDS is the theoretical loaded drug in sample (theoretical amount of 5-ASA which should have been loaded in the sample in case of complete loading).

TLDS is calculated according to this equation:
$$\text{TLDS} = \text{WS} / \text{SRS}$$

Where WS is the weight of sample and SRS is the sum of polymer and 5-ASA weight ratios in the sample.

Table 1. The effect of various dispersed phase volume s on loading efficiency

Sample Code	Dispersed Phase Volume (%) (DPV)	Polymer/ Drug Weight Ratio (P/D)	Surfactant Concentration (SC) (W/V %)	Loading Efficiency (LE %) Mean \pm SD (n=3)
DR1	20%	10 to 1	2%	48.3 \pm 2.7
DR2	33%	10 to 1	2%	56.9 \pm 3
DR3	50%	10 to 1	2%	48.8 \pm 2.6

Morphological characterization and particle size analysis

Morphological characteristics of particles were studied by the Scanning Electron Microscopy (SEM) technique. The SEM samples were prepared by dispersing the particles in ethanol and fixing them onto the metal discs. The samples were then covered by gold microfilm and placed in the SEM apparatus. Particle size and size distribution were measured by a laser-based particle size analyzer (Mastersizer, Malvern Instruments, U.K). The particles were dispersed in absolute ethanol prior to analyzing.

Statistical evaluation

The effect of dispersed phase volume, polymer / drug weight ratio and surfactant concentration on loading efficiency and particle size was analysed statistically by one way ANOVA (Tukey and LSD tests) with a level of significance of $p < 0.05$, using the SPSS computer program (version 11.5).

Results and Discussion

In the presence of surfactant and HPBCD, 5-ASA is dissolved completely in water, dispersed entirely in n-hexane and a W/O emulsion is produced. Upon the addition of MECA solution to the emulsion, probably the MECA rapidly diffuses into n-hexane, allowing the interfacial polymerization process to occur around the droplets and therefore, a mixture of micro and nanocapsules and micro and nanospheres is formed in the medium. The composition of

reverse phase emulsion, polymer/drug weight ratio and surfactant concentration are important and could have a significant influence on the loading efficiency, shape, size and distribution of the resulted PMECA particles.

Effect of dispersed phase volume

The compositions and loading efficiencies of DR1, DR2, and DR3 formulations are shown in Table1. The results show that there is no linear relationship between the dispersed phase volume (DPV) and loading efficiencies of 5-ASA in the DPV range of 20- 50%. The loading efficiency of DR2 (DPV = 33%) was significantly higher than the DR1 (DPV = 20%), and DR3 (DPV = 50%) samples ($p < 0.05$). However, there was no significant difference between DR1and DR3 formulations ($p > 0.05$). Usually in an emulsion system, as the proportion of dispersed phase decreases, the stability of emulsion would be increased. Thus, a DPV less than 33%, probably results in the formation of a more stable W/O emulsion. In such an emulsion, the n-hexane layer covering each droplet of the dispersed phase is thicker. Therefore, probably, the free reactive monomers would reach the droplets of the dispersed phase to a lesser extent, preventing a complete pursuit of polymerization within the medium (4). Consequently, the loading efficiency of 5-ASA decreased in DR1, compared to formulation DR2. On the other hand, in the case of formulation DR3, in spite of an increase in the amount of dispersed phase volume in the polymerization medium,

Table 2. The effect of various polymer /drug weight ratios on loading efficiency

Sample Code	Dispersed Phase Volume (%) (DPV)	Polymer/ Drug Weight Ratio (P/D)	Surfactant Concentration (SC) (W/v %)	Loading Efficiency (LE %) Mean \pm SD (n=3)
P/D1	33%	1 to 1	2%	11.6 \pm 3
P/D2	33%	4 to 1	2%	21.8 \pm 4.4
P/D3	33%	7 to 1	2%	30.7 \pm 5.4
P/D4	33%	10 to 1	2%	56.9 \pm 3
P/D5	33%	12 to 1	2%	60.3 \pm 6.7
P/D6	33%	15 to1	2%	78.2 \pm 3.3

Table 3: The effect of various surfactant concentrations on loading efficiency

Sample Code	Dispersed Phase Volume (%) (DPV)	Polymer/ Drug Weight Ratio (P/D)	Surfactant Concentration (SC) (W/V %)	Loading Efficiency (LE %) Mean \pm SD (n=3)
SC1	33%	15 to 1	1%	76.8 \pm 2.7
SC2	33%	15 to 1	2%	78.2 \pm 3.3
SC3	33%	15 to 1	3%	73.6 \pm 4.4
SC4	33%	15 to 1	5%	65.8 \pm 3.8

the loading efficiency was significantly less than that of formulation DR2 ($p < 0.05$). This reduction could probably be not only due to the lack of emulsion stability and/or insufficiency of surface area for polymeric matrix formation, but also due to the decrease in the n-hexane layer thickness. Probably the free reactive monomers could better reach the droplets of dispersed phase by diffusing out of the n-hexane layer, allowing the polymerization to be pursued rapidly within the dispersed phase. Therefore, the possibility of formation of nanospheres was greater than that of nanocapsules in the polymerization medium. As found by Kruse et al. (9), following the reverse emulsification polymerization procedure, a mixture of nanospheres and nanocapsules, with a predominance of nanospheres, could be formed. Since the loading efficiency of nanospheres is usually smaller than nanocapsules, a decrease in loading efficiency of DR3 compared to formulation DR2 was observed.

In contrast to DR1 and DR3, formulation DR2 with a dispersed phase volume of 33%, probably forms the stable emulsion with an optimum thickness of n-hexane layer which allows a suitable polymerization rate around the dispersed phase droplets, leading to the production of micro and nanocapsules greater than those ones produced in the polymerization medium. Thus, an increase in loading efficiency of DR2 was observed, compared to formulations DR1 and DR3.

Effect of polymer/drug weight ratio

Table 2 compares the compositions and loading efficiencies of formulations P/D1 to P/D6. As the results indicate, an increase in polymer/drug weight ratio in the range of 1/1 to 15/1, enhances the loading efficiency from 11.6 to 78.2% in a linear mode ($r = 0.987$). The loading efficiency of P/D6 formulation was significantly higher

than that of the other formulations ($p < 0.05$). In the inverse emulsification polymerization technique, the polymerization reaction initiated by the free reactive monomers diffuses into the dispersed phase droplets of the W/O emulsion and continues until the complete consumption of monomers (4). Therefore, an increase in polymer/drug weight ratio causes the formation of a more effective polymeric matrix and hence, could engage a greater number of drug molecules within the polymeric matrix network.

Effect of surfactant concentration

In the inverse emulsification polymerization technique, the presence of surfactant is an important parameter, influencing the solubility and availability of active compound in the polymerization medium, stability of prepared emulsion, and the possibility of polymeric matrix formation. Table 3 illustrates the differences between the compositions and loading efficiencies of formulations SC1 to SC4. As shown, there is no significant difference between the loading efficiencies of formulations SC1, SC2 and SC3 containing 1%, 2% and 3% of surfactant, respectively ($p > 0.05$). However, the loading efficiency of SC4 sample, with a surfactant concentration of 5%, was significantly less than that of SC1 and SC2 ($p(\text{SC1, SC5}) < 0.02$ and $p(\text{SC2, SC5}) < 0.01$). This is probably due to an appropriate or slight solubility of monomers in the surfactant. Some of the monomers could interact with surfactant molecules, resulting in a decrease in the number of monomers available to penetrate or interact with the dispersed droplets, which in turn prevents the occurrence of interfacial polymerization around the droplets. Consequently, the possibility of formation of micro and nanospheres or micro and nanocapsules in SC4 was less than that of the other SC formulations.

Table 4: The results of particle size analysis of poly-(methyl ethyl cyanoacrylate) particles containing 5 -ASA

Sample Code	Mean particle size (μm) \pm SD (n=3)			Span Mean \pm SD (n=3)
	10%	50%	90%	
DR1	2.4 \pm 0.14	10.1 \pm 0.5	35.1 \pm 3.1	3.2 \pm 0.16
DR2	1.8 \pm 0.11	7.9 \pm 0.17	25 \pm 1.5	2.9 \pm 0.14
DR3	1.5 \pm 0.13	8 \pm 0.83	23.6 \pm 2.23	2.7 \pm .03
PD2	2 \pm 0.2	8.5 \pm 0.55	26 \pm 0.3	2.8 \pm 0.23
PD4	1.9 \pm 0.25	8.5 \pm 0.45	30.4 \pm 1.4	2 \pm 0.04
PD6	2.3 \pm 0.3	8.7 \pm 1.05	22 \pm 3.2	2.3 \pm 0.06
SC1	1.8 \pm 0.14	7.9 \pm 0.9	22.5 \pm 3.24	2.6 \pm 0.09
SC2	2.3 \pm 0.3	8.7 \pm 1.05	22 \pm 3.2	2.3 \pm 0.06
SC3	1.6 \pm 0.13	6.4 \pm 0.5	19.4 \pm 2.45	2.8 \pm 0.15

DR: Dispersed Phase Volume Ratio ; PD: Polymer/ Drug Weight Ratio ; SC: Surfactant Concentration (%W/V)

Morphology and particle size characteristics of the prepared formulations

Table 4 illustrates the particle size and particle size distribution of the prepared formulations. Laser light scattering evaluation showed that in all formulations, 50 percent of particles had mean sizes in the range of 6.4 μm up to 10.1 μm . Our results did not show any significant differences in the mean particle size between the prepared formulations, except between formulations DR1 and DR2 ($p < 0.01$), DR1 and DR3 ($p < 0.02$) and SC2 and SC3 ($p < 0.04$). Particles span mean was in the range of 2 to 3.2. Span is a parameter indicating the size dispersity of particles, defined as:

$$\frac{d(v, 0.9) - d(v, 0.1)}{d(v, 0.5)}$$
 which means: (volume diameter of 90 percentile of particles) - (volume diameter of 10 percentile of particles)/(volume diameter of 50 percentile of particles) (13).

The pH of the polymerization medium has a significant influence on the polymerization rate.

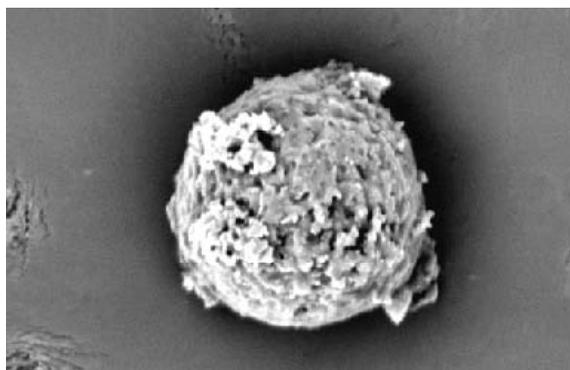


Figure 1. The SEM micrograph of P/D6 (Polymer/Drug: 15/1) sample. The matrix network structure and the porous nature of spherical particle has been shown.

Therefore, using the applied particle preparation method, the pH has to be acidic in order to prevent excessively rapid polymerization and enable the formation of monodispersed (non-agglomerated) particles. Nanospheres with a narrow size distribution could be produced in the polymerization medium, with pH in the range of 1 to 3. The mean span of particles (particles size dispersity) was in the range of 2-3.2 (Table 4). This span value could be diminished by decreasing the pH of the medium to 1 or using sulfur dioxide as the polymerization inhibitor. Increasing the surfactant concentration results in a decrease in the diameter of the produced nanospheres, due to the prevention of agglomerated particles (4). Table 4 shows that the smallest mean particle size (6.4 μm) belonged to formulation SC3 with a pluronic F-68 concentration of 3% W/V. The particle size of formulation SC3 was significantly smaller than the formulation SC2 ($p < 0.04$). On the other hand, probably, because the n-hexane layer in formulation DR1 was thicker than the other DR formulations, the diameter of DR1 particles was significantly greater than formulations DR2 and DR3.

Photographs obtained by scanning electron microscopy (SEM) showed that the prepared particles were a mixture of micro and nanospheres and micro and nanocapsules, with a predominance of micro and nanospheres. A rational explanation for the formation of micro and nanocapsules is that the polymerization process in some cases is so rapid that an impermeable polymer wall may be formed at the interface, preventing the diffusion of further

monomers into the interior of the particles. However, in most cases, the interior of the particles is also polymerized and hence solid monolithic micro and nanospheres are formed (3). It has been proposed that decreasing the pH can decrease the speed of polymerization process in anionic polymerization (4). Therefore, in all formulations by adjusting the pH of the medium to 2.5, immediate polymerization was prevented, and the formation of spherical particles over the capsular particles dominated. The SEM photograph of P/D6 (Polymer/Drug: 15/1) formulation shows the matrix network structure and the porous nature of the spherical particle (Figure1).

A mixture of micro and nanocapsules and micro and nanospheres of 5-aminosalicylic acid (5-ASA) was prepared from poly-(methyl ethyl cyanoacrylate) by the inverse emulsification polymerization procedure. Various parameters including the composition of emulsion (dispersed phase volume), polymer/drug weight ratio and surfactant concentration parameters, showed to have a great influence on the loading efficiency, shape, size and size distribution of the resulted particles. Using proper formulation parameters and conditions, one could increase the loading efficiency of particles up to 78% and decrease the particle size down to 6.4µm.

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References

- (1) Puglisi G, Fresta M, Giammona G and Ventura CA. Influence of the preparation conditions on poly-(ethylcyanoacrylate) nanocapsule formation. *Int. J. Pharm.* (1995) 125: 283-287
- (2) Kreuter J. Evaluation of nanoparticles as drug delivery systems. I. Preparation methods. *Pharm. Acta Helv.* (1993) 58: 196
- (3) Kreuter J. *Colloidal drug delivery systems*. Dekker, New York (1994) 219-235
- (4) Mathiowitz E. *Encyclopedia of controlled drug delivery*. John Wiley and Sons (1999) 644- 648
- (5) Couvreur P, Kante B, Roland M, Guiot P, Baudhuin P and Speiser P. Polyalkylcyanoacrylate nanocapsules as potential lysosomotropic carriers: Preparation, morphological and sorptive properties. *J. Pharm. Pharmacol.* (1979) 31: 331
- (6) Kreuter J and Zehnder H J. The use of Co60-γ-irradiation for the production of vaccines. *Radiat. Effects* (1978) 35: 161
- (7) Watnasirichaikul S, Rades T, Tucker I G and Davies N M. Effects of formulation variables on characteristics of poly-(ethylcyanoacrylate) nanocapsules prepared from W/O microemulsions. *Int. J. Pharm.* (2002) 235: 237-246
- (8) Al Khoury Fallouh N, Roblot Treupel L, Fessi H, Davis Saguet J P and Puisieux F. Development of a new process for the manufacture of polyisobutylcyanoacrylate nanocapsules. *Int. J. Pharm.* (1986) 28: 125-132
- (9) Krause H J, Schwarz A. and Rohdewald P. Interfacial polymerization, a useful method for the preparation of polymethylcyanoacrylate nanoparticles. *Drug Dev. Ind. Pharm.* (1986) 12: 527-552
- (10) Tice T R, Mason D W and Giley R M. Clinical use and future of parenteral microspheres delivery systems. (1989) 232-235
- (11) El-samaligy M S, Rohdewald P and Mahmoud H A. Polyalkyl cyanoacrylate nanocapsules. *J. Pharm. Pharmacol.* (1986) 38: 216-218
- (12) Abu Izza K, Garcia Contreras L and Lu D R. Preparation and evaluation of zidovudine-loaded sustained-release microspheres. Part 2. Optimization of multiple response variables. *J. Pharm. Sci.* (1996) 85: 572-576
- (13) Hamidi M, Tajerzadeh H, Dehpour A R, Rouini M R and Ejtemaee-Mehr Sh. In vitro characterization of human intact erythrocytes loaded by Enalaprilat. *J. Drug Delivery* (2001) 8: 223-230

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