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Preparation and in vitro characterization of HSA-mPEG nanoparticles

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Abstract

Surface modified human serum albumin (HSA) nanoparticles with a size of approximately 150 nm in diameter were prepared from a PEG-HSA conjugate, methoxy-polyethylene glycol modified human serum albumin (HSA-mPEG) using a coacervation method and crosslinked with glutaraldehyde. The ζ-potential of the surface modified nanoparticles was significantly lower than that of unmodified HSA nanoparticles. The existence of a hydrated steric barrier surrounding the nanoparticles was confirmed by electrolyte and pH induced flocculation tests. The surface modified nanoparticles showed a reduced plasma protein adsorption on the particle surface compared with unmodified particles.

Keywords: Human serum albumin; PEG-albumin conjugates; Nanoparticles; Surface characterization; Protein adsorption

1. Introduction

The biodistribution of colloidal drug delivery systems after intravenous administration is mainly determined by their physicochemical properties such as size and surface characteristics. This is achieved through recognition or non-recognition of the colloidal system by the body's defence system (Davis and Illum, 1986; Moghimi et al., 1991). Particles that are small enough to escape the capillary beds of the lungs are normally sequestered rapidly by the cells of the reticuloendothelial system (RES), particularly the Kupffer cells of the liver (Illum et al., 1982). This sequestration has been identified as a major obstacle to targeting cells or tissues elsewhere in the body such as the bone marrow and solid tumours (Artursson, 1987). On intravenous administration, particles are normally rapidly coated by the adsorption of specific blood components known as opsonins and then recognized and taken up by the RES. The adsorption of amphipathic copolymers, such as polyoxyethylene–polyoxypropylene (PEO-PPO) block copolymers (commercially available as poloxamer and poloxamine surfactants), onto polystyrene latex can form a hy-

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drophilic steric barrier. This hydrophilic layer can dramatically affect the opsonization of particles by plasma components (Moghimi et al., 1993a; Moghimi, 1995). The uptake of the particles by the RES is consequently reduced and provides particles with a significantly longer circulation half-life (Illum and Davis, 1984). In some cases such particles can deposit preferentially in a specific organ site such as the bone marrow (Porter et al., 1992) and spleen (Moghimi et al., 1993b).

Human serum albumin (HSA) is widely used as a material for microsphere preparation since it is considered to be non-antigenic and biodegradable, and is readily available (Arshady, 1990; Bogdansky, 1990). HSA nanoparticles with a size less than 150 nm in diameter can be prepared using a pH-coacervation method (Lin et al., 1993). Particles of this size have a good chance to escape from the vascular system to target to sites outside the circulation, provided they have not been sequestered previously by the RES (Artursson, 1987). However, since poloxamer or poloxamine surfactants are poorly adsorbed on the hydrophilic surface of albumin particles, the required steric PEO barrier on albumin nanoparticles cannot be created simply by adsorption of block copolymer surfactants. We previously reported the preparation and characterization of dextran-PEG, poly(amidoamine)-PEG and poly(thioeramido acid)-PEG modified HSA nanoparticles (Lin, et al., 1994; Lin et al., 1997). This paper describes the surface modification of HSA nanoparticles by PEG using a PEG-HSA conjugate: methoxy-polyethylene glycol modified human serum albumin (HSA-mPEG). This material offers the possibility of producing HSA nanoparticles with a sole PEO surface layer which will be similar to those created on polystyrene or PLGA nanoparticles by coating with poloxamer and poloxamine surfactants and, therefore, nanoparticles prepared from this conjugate may show a similar in vivo behaviour to that of PEO-PPO surfactant coated polystyrene or PLGA nanoparticles (Illum and Davis, 1984; Porter et al., 1992; Stolnik et al., 1994). The PEG-HSA conjugates used in the present study were synthesised using mPEG with a molecular weight of 2000 and 5000 Da and with different HAS:mPEG ratios. HSA-mPEG nanoparticles were prepared using modified coacervation methods. The surface characteristics of the nanoparticles produced using these new conjugates have been investigated and the adsorption of plasma proteins on the HSA-mPEG nanoparticles compared with unmodified HSA nanoparticles was also studied.

2. Experimental

2.1. Materials

HSA (Albutein, 20% albumin solution, BP) was supplied by Alpha (Thetford, UK). Methoxy-polyethylene glycol 2000 modified human serum albumin (mol. wt. 115 000 Da, 40% lysine modified, HSA content 60% w/w, HSA_{60}-$mPEG_{2000}$), methoxy-polyethylene glycol 5000 modified human serum albumin (mol. wt. 81 500 Da, 5% lysine modified, HSA content 80% w/w, HSA_{80}-$mPEG_{5000}$) and methoxy-polyethylene glycol 5000 modified human serum albumin (mol. wt. 173 700 Da, 35% lysine modified, HSA content 50% w/w, HSA_{50}-$mPEG_{5000}$) were synthesized and purified by the Biomaterial Group, Department of Organic Chemistry, University of Gent, Belgium. Guanidine hydrochloride (GuHCl) was purchased from Aldrich (Gillingham, UK). Glutaraldehyde (50% solution) and other chemicals was purchased from BDH (Poole, UK).

2.2. Nanoparticle production

2.2.1. Preparation of HSA_{60}-$mPEG_{2000}$ nanoparticles

Two ml 2% (w/v) HSA_{60}-$mPEG_{2000}$ aqueous solution was mixed with 5 ml acetone under magnetic stirring. Approximately 1.4 ml ethyl acetate was added dropwise through a syringe until the system became turbid. The resulting particles were crosslinked with glutaraldehyde (50 μl 4% glutaraldehyde ethanol solution) and stirred continuously at room temperature for 16 h. The particle suspension was heated at 60°C for 15 min and cleaned by centrifuging three times at 35 000 × g for 30 min (Beckman J2-21, USA). The cleaned

nanoparticles were redispersed in distilled water for further study. The influence of glutaraldehyde on the degree of crosslinking and ζ-potential of the nanoparticles was investigated by adding 25, 50 or 100 μl of a 4% glutaraldehyde ethanol solution.

2.2.2. Preparation of HSA-mPEG$_{5000}$ nanoparticles

Two ml 2% HSA_{50}-$mPEG_{5000}$ or HSA_{80}-$mPEG_{5000}$ aqueous solution was mixed with 5 ml acetone and 60 μl 0.5 M NaOH solution under magnetic stirring. Approximately 1 ml acetone was added dropwise through a syringe until the system was turbid. Four percent glutaraldehyde ethanol solution (50 μl) was added followed by immediate addition of 60 μl 0.5 M HCl solution to neutralize the alkaline suspension. After crosslinking (room temperature, 16 h, then 60°C for 15 min), the nanoparticles were cleaned as described above. The influence of the addition of glutaraldehyde on particle crosslinking efficiency was also investigated under the conditions as described above.

2.2.3. Preparation of HSA nanoparticles

As a control unmodified HSA nanoparticles were prepared using a pH-coacervation method (Lin et al., 1993). Briefly, approximately 5 ml acetone was added dropwise into 4 ml 2% HSA aqueous solution (pH to 9.0) under magnetic stirring until the solution became just turbid. The HSA nanoparticles so formed were crosslinked by adding 50 μl of a 4% glutaraldehyde ethanol solution and stirring continuously at room temperature for 4 h. After crosslinking the HSA nanoparticles were cleaned by centrifuging as described above.

2.3. The influence of pH on the preparation of HSA-mPEG$_{5000}$ nanoparticles

Two ml 2% HSA_{80}-$mPEG_{5000}$ solution was adjusted to a selected pH value between 8.0–12.5 using 0.5 M NaOH solution. The conjugate solution was mixed with 5.0 ml acetone and ethyl acetate was added dropwise under magnetic stirring till the system became turbid (when the $pH \geq 12$, the turbidity appeared during the addition of acetone and no ethyl acetate was used). The particles were crosslinked with glutaraldehyde and cleaned by centrifugation as described above. Size and ζ-potential of the resulting nanoparticles were compared. The morphology of the particles was investigated by transmission electron microscopy (TEM, Jeol 1200 EX12, Japan).

2.4. The influence of pH on the stability of HSA-mPEG$_{5000}$ conjugates

The influence of pH on the stability of the HSA-$mPEG_{5000}$ solution was also investigated by a gel permeation chromatography (GPC) method. HSA_{80}-$mPEG_{5000}$ aqueous solution (5 ml of 0.5%) was adjusted to pH 7 and 12, respectively, using 0.5 M NaOH solution and incubated at room temperature for 30 and 60 min. After incubation, 0.5 ml of the sample was passed down a mini-GPC column (1.0×20 cm), packed with Sephadex G200 gel (Sigma, USA), using distilled water as an eluent with a flow rate of 0.5 ml/min. The eluent fraction was monitored using a refractive index detector (Gilson, model 131, France).

2.5. Particle size and ζ-potential of the nanoparticles

The size of the albumin nanoparticles was determined by photon correlation spectroscopy (PCS) with a Malvern 4700 submicron particle analyzer system (Malvern Instruments, UK). The size distribution was characterized by a polydispersity index (PI). TEM was also used to investigate particle size and provide information on nanosphere morphology. The ζ-potential of the albumin nanoparticles in a phosphate buffer (pH 7.0, ionic strength 0.05 M) was measured with the technique of electrophoretic laser Doppler anemometry by using a Malvern Zetasizer 4 (Malvern Instruments, UK).

2.6. Turbidity ratio test

A turbidity ratio test that measures the stability of albumin nanoparticles in a concentrated

guanidine solution was used to investigate the crosslinking efficiency obtained in the nanoparticles (Rubino et al., 1993; Lin et al., 1994). Briefly, 2 mg albumin nanoparticles was incubated in 1 ml 6 M GuHCl solution and distilled water at room temperature for 1 h and the resulting turbidity of the samples were measured using a spectrophotometer (Uvikon 860, Switzerland) at 540 nm. The turbidity ratio, i.e. the turbidity of the nanoparticles in GuHCl solution compared to that in water, was used as an indicator of the degree of crosslinking of the albumin particles.

2.7. Electrolyte and pH induced flocculation of albumin nanoparticles

Albumin nanoparticles (1 mg) were incubated in 1.0 ml sodium sulphate solution of different concentrations (0–0.8 M) to assess their electrolyte induced flocculation. The optical turbidity of the resultant suspensions was measured at 600 nm using a spectrophotometer.

Albumin nanoparticles (1 mg) were incubated in 1.0 ml of a constant ionic strength (0.01 M) acetate buffer of different pH values (pH 3.5–6.0) to assess pH induced flocculation. The optical turbidity at 600 nm of the resultant suspensions was measured. The ζ-potentials of the nanoparticles at the different pH values were also determined.

Table 1
Particle size and ζ-potential of HAS-mPEG nanaoparticles

Nanoparticles	Size (nm)	Polydispersity index	ζ-potential (mV)
HSA_{60}-$mPEG_{2000}$	153.7 ± 2.7	0.098 ± 0.015	-8.1 ± 0.8
HSA_{80}-$mPEG_{5000}$	154.3 ± 30.6	0.104 ± 0.023	-4.3 ± 1.2
HSA_{50}-$mPEG_{5000}$	157.6 ± 30.1	0.117 ± 0.027	-4.8 ± 0.7
HSA (control)	119.3 ± 4.1	0.092 ± 0.019	-18.9 ± 0.6

2.8. Plasma protein adsorption on the albumin nanoparticles

Albumin nanoparticles (2 mg) were incubated in 1.5 ml 5 mM McIlvaine buffer (pH 7.4) containing 0, 2, 5 and 10% rat serum. The samples were shaken on a vibratory shaker (IKA-VIBRAX-VXR, IKA-Labortechnik, Germany) at room temperature for 2 h. The adsorption of the proteins onto the nanoparticle surface was investigated by comparing the hydrodynamic diameter and ζ-potential of the particles after incubation with different concentrations of rat serum measured as described above.

3. Results and discussion

HSA is characterized as having a high content of charged amino acids and is insoluble in organic solvents. HSA nanoparticles may be prepared by adding water miscible organic solvents such as alcohol or acetone to HSA aqueous solution (Lin et al., 1993; Chen et al., 1994). However, when HSA is modified with mPEG, the amphipathic nature of the PEG molecule confers a higher solubility of HSA-mPEG in hydrophobic solvents. This makes it difficult for HSA-mPEG to coacervate and thereby form nanoparticles by using water miscible organic solvents. A more hydrophobic solvent, ethyl acetate, was, therefore, introduced into the coacervation method for the purpose of preparing HSA-$mPEG_{2000}$ nanoparticles. Since ethyl acetate is not water miscible, a suitable amount of acetone was employed as a co-solvent. HSA-$mPEG_{2000}$ nanoparticles of approximately 150 nm in diameter were produced using this method (Table 1) and the TEM micrograph in Fig. 1A shows that spherical particles were obtained.

However, HSA-$mPEG_{5000}$ nanoparticles could not be successfully produced by the same technique. Adding ethyl acetate to the HSA-$mPEG_{5000}$ water–acetone solution, particles with a ζ-potential around -3 mV and a size in excess of 230 nm were produced (Fig. 2). The TEM study showed that the particles so formed were a mixture of nanoparticles and entwined conjugate

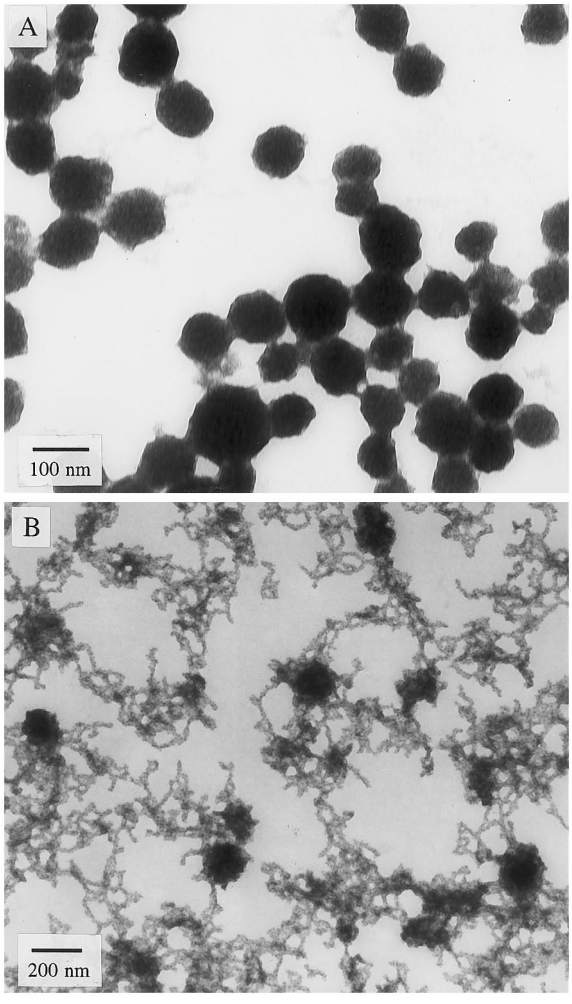

Fig. 1. TEM micrographs of HAS-mPEG nanoparticles prepared by adding ethyl acetate to the conjugate acetone–water solution. A: HSA_{60}-$mPEG_{2000}$; B: HSA_{80}-$mPEG_{5000}$.

chains (Fig. 1B). Increasing the pH of the HSA-$mPEG_{5000}$ solution above 12 led to a decrease in the solubility of the conjugate and the nanoparticles prepared at this pH exhibited a ζ-potential and size which were very similar to those of the HSA nanoparticles prepared from HSA solution by the pH-coacervation method. These results indicated that mPEG may detach from the HSA due to the hydrolysis of the conjugate during the preparation of the HSA-$mPEG_{5000}$ nanoparticles at high pH.

The results of the stability of HSA-$mPEG_{5000}$ from GPC analysis confirmed that the conjugate was stable under neutral conditions but unstable at higher pH (Fig. 3). The conjugate was hydrolysed at pH 12 releasing mPEG from the conjugate. It was also found that the extent of hydrolysis of HSA-mPEG is time dependent. The longer the sample was incubated, the more the conjugate hydrolysed. Thus, during the preparation of the nanoparticles at a high pH, the hydrolysis of the HSA-mPEG conjugate could be divided into two stages: mPEG cleaved from HAS-mPEG molecules during the formation of nanoparticles and mPEG cleaved from the

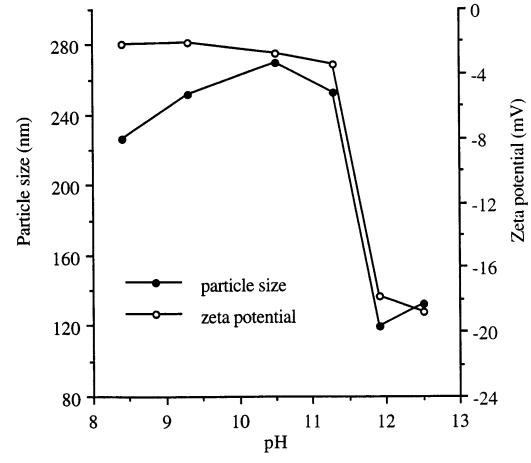

Fig. 2. Influence of pH on the formation of HSA_{80}-$mPEG_{5000}$ nanoparticles (2 ml 2% HAS-mPEG, 5 ml acetone, ethyl acetate added dropwise, $n = 2$).

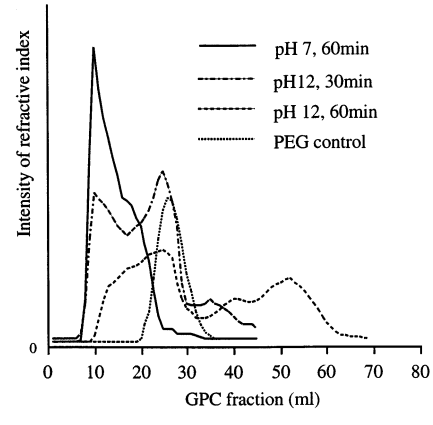

Fig. 3. Stability of HSA_{80}-$mPEG_{5000}$ in pH 7 and 12 solutions.

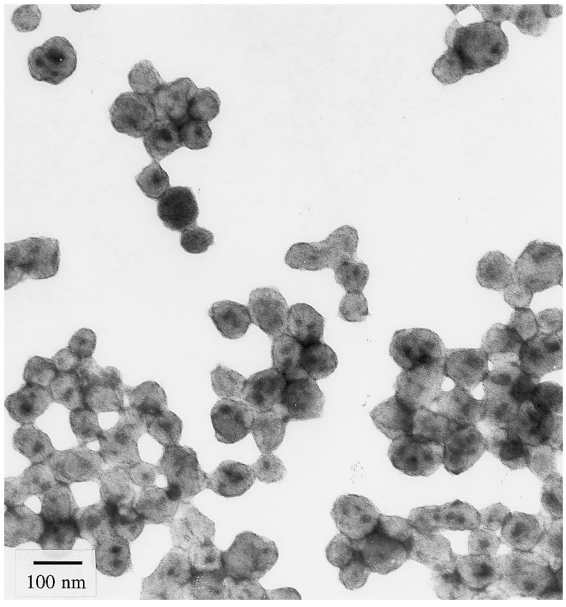

Fig. 4. TEM micrograph of HSA_{80}-$mPEG_{5000}$ nanoparticles prepared at high pH. The alkaline suspension was neutralized as soon as the crosslinking reaction of the nanoparticles started.

nanoparticles during the closslinking of nanoparticles. At the first stage only a very small fraction of mPEG was expected to separate from the HSA-mPEG. The resulting free HSA molecules would coacervate to form nanoparticles in the unfavoured environment. During the formation of nanoparticles the HAS fragment in the non-hydrolysed HSA-mPEG conjugate would also be captured into particles leaving the mPEG fragment on the particle surface. During the second stage where the formed nanoparticles are crosslinked, the mPEG fragment could gradually be further cleaved from the particles.

To prevent the further hydrolysis of mPEG from the particles, the HSA-$mPEG_{5000}$ nanoparticles were produced by neutralizing the alkaline suspension as soon as the nanoparticles were formed and the crosslinking reaction started. HSA-$mPEG_{5000}$ nanoparticles with a size approximately 150 nm were prepared by using this modified coacervation method (Table 1). TEM micrograph in Fig. 4 shows that spherical nanoparticles were obtained. It should be kept in mind that a certain unknown amount of mPEG will be lost from the surface of the nanoparticles using this method. However, the differences in the ζ-potentials between HSA and HSA-$mPEG_{5000}$ nanoparticles indicate that most of mPEG segments were present at the nanoparticle surface.

HSA-mPEG nanoparticles have a significantly lower ζ-potential value than that of the HSA nanoparticles (Table 1). The ζ-potentials of unmodified HSA nanoparticles at pH 7.0 is about -19 mV whereas those of HSA-$mPEG_{2000}$ and the HSA-$mPEG_{5000}$ nanoparticles are -8 and -4 mV, respectively. Interestingly, no significant difference in ζ-potential was observed between the particles prepared from HSA_{50}-$mPEG_{5000}$ and HSA_{80}-$mPEG_{5000}$ even though the two types of HSA-mPEG conjugates have different degrees of modification (20 and 50% of $mPEG_{5000}$ by weight) and hence a different surface density of PEG would be expected. It is not clear whether this is caused by a part hydrolysis which may lead to a degree of modification to the same level, or on the other hand, the relative low degree of the modification, such as 20% of $mPEG_{5000}$, may provide enough PEG surface density to move the shearing plane of the electric double layer outside the nanoparticles towards the surrounding medium. This will lead to a lower ζ-potential for the particles, and the higher degree of the modification may not further influence the ζ-potential. The fact that the HSA_{80}-$mPEG_{5000}$ nanoparticles have a less negative surface charge (-4.3 mV) than the HSA_{60}-$mPEG_{2000}$ nanoparticles (-8.1 mV) indicates that the molecular weight of the PEG is more important on the influence of the ζ-potential of the particles than the degree of the PEG modification due to the highly efficient movement of the shearing plane of the electric double layer by the higher molecular weight of PEG.

During preparation of HSA-mPEG nanoparticles, by the pH-coacervation method, higher concentrations of organic solvent were used than that for preparation of HSA nanoparticles. Since the samples containing a high concentration of organic solvent are not recommended for high speed centrifugation, the particle suspension was heated at 60°C for 15 min to remove the organic solvent. This stage, of course, could also contribute to the

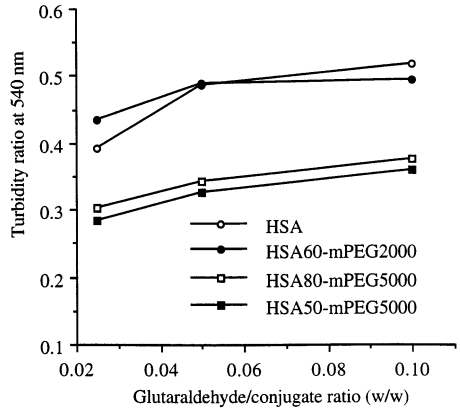

Fig. 5. Influence of glutaraldehyde concentration on the degree of crosslinking of HAS-mPEG nanoparticles ($n = 2$).

degree of crosslinking of the nanoparticles. However, when HSA was modified with mPEG, which is connected to the residual amino groups in lysine, the number of free amino groups available for crosslinking are decreased. In addition, when HSA is modified with mPEG, a steric obstacle may exist between the free amino groups in the newly formed nanoparticles and the aldehyde groups in the crosslinking agent, which may repress the crosslinking interaction. Moreover, a large amount of organic solvent and a relative low pH, compared with the conditions of the pH-coacervation method, were employed in the preparation of HSA-mPEG nanoparticles, which

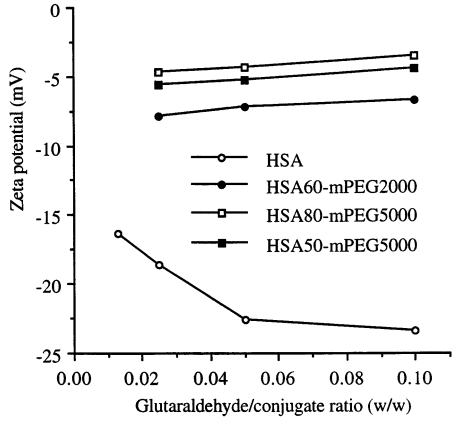

Fig. 6. Influence of glutaraldehyde concentration on ζ-potential of HAS-mPEG nanoparticles ($n = 2$).

is not favourable for the crosslinking reaction. Thus, the degree of crosslinking in the HSA-mPEG nanoparticles was expected to be lower than that of the HSA nanoparticles. To increase the degree of crosslinking of the HSA-mPEG nanoparticles, higher concentrations of glutaraldehyde and longer crosslinking time were used.

The results from the turbidity ratio test shows that the degree of crosslinking in the HSA-mPEG nanoparticles generally increased with an increase in the concentration of glutaraldehyde (Fig. 5). The degree of crosslinking obtained in HSA-$mPEG_{2000}$ nanoparticles is similar to that in HSA nanoparticles prepared by the pH-coacervation method, whereas that of the HSA-$mPEG_{5000}$ nanoparticles crosslinked under the same condition as used for the HSA-$mPEG_{2000}$ nanoparticles was much lower. The lower degree of crosslinking in the HSA-$mPEG_{5000}$ nanoparticles may be caused by the different technique used for particle preparation and the relative long PEG chain in the steric barrier introduced from $mPEG_{5000}$ compared with $mPEG_{2000}$ which may interfere with the crosslinking reaction.

Fig. 6 shows that an increase in the concentration of the glutaraldehyde led to a higher negative value of the ζ-potential for HSA nanoparticles. However, only a slight change in the ζ-potential was measured when the HSA-mPEG nanoparticles were crosslinked with higher concentrations of glutaraldehyde due to the presence of the steric mPEG layer on the particle surface.

In the present study, the existence of a hydrated mPEG barrier was investigated by electrolyte and pH induced flocculation of the nanoparticles. This is based on the fact that the physical stability of a colloidal system is mainly dependent upon the competitive processes of attraction (van der Waals forces) and repulsion (either electrostatic repulsive force or steric stabilizing barrier or both) (Florence and Attwood, 1988). If particles are mainly stabilized electrostatically, destruction of the electrostatic double layer surrounding the particles will result in aggregation of the particles into clusters with a corresponding increase in optical turbidity. However, if the particles are mainly stabilized by a hydrated steric stabilizing barrier,

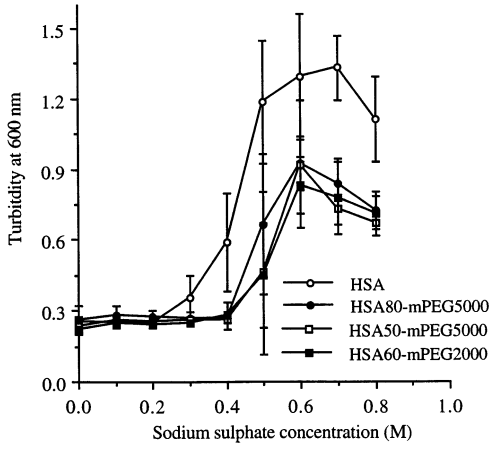

Fig. 7. Influence of sodium sulphate on the flocculation of HAS-mPEG nanoparticles ($n = 3$).

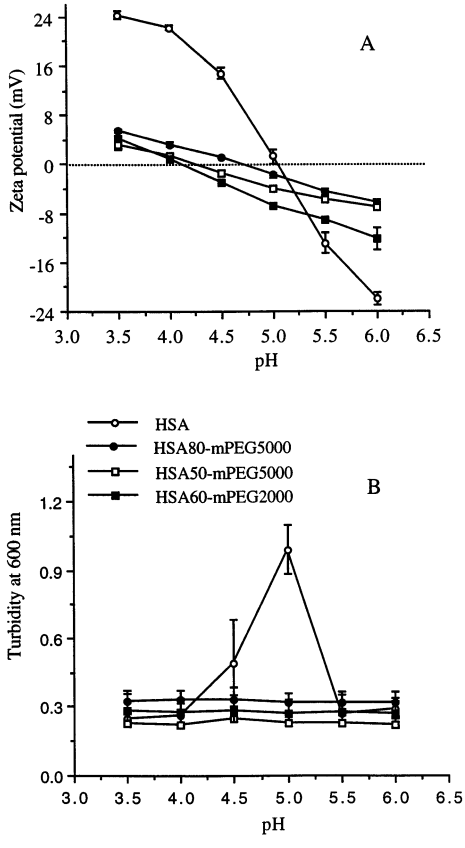

Fig. 8. Influence of pH on the ζ-potential (A) and flocculation (B) of HAS-mPEG nanoparticles (in 0.01M acetate buffer; $n = 3$).

the colloidal system should be stable even if the electrostatic double layers have been destroyed. The results of Na_2SO_4 induced flocculation of HSA-mPEG nanoparticles are shown in Fig. 7. When HSA nanoparticles were modified with mPEG, the critical flocculation concentration of the nanoparticles in Na_2SO_4 solution increased from less than 0.3 M to about 0.5 M. It was reported that when HSA nanoparticles were modified by dextran-mPEG, the particle were stable in Na_2SO_4 solution until the concentration was higher than 1.2 M (Lin et al., 1994). The relatively small increase in the stability of HSA-mPEG nanoparticles in Na_2SO_4 solution was thought to be due to the fact that Na_2SO_4 can not only compress the electrostatic double layer of HSA nanoparticles but also dehydrate the mPEG layer on the particle surface. A similar result was reported by Stolnik et al. (1994). They found that when PLGA and polystyrene nanoparticles were coated with poloxamine 908 or PLA-PEG copolymer, which creates a steric PEO barrier on the particle surface, the stability of the resulting colloidal systems in Na_2SO_4 solutions was increased but they still flocculated at a critical concentration around 0.5 M.

The results of pH induced flocculation of the HSA-mPEG nanoparticles at different pH values are shown in Fig. 8. The ζ-potential *o*f HSA nanoparticles was almost zero when the pH was at 5, whereas the surface charge on HSA-mPEG nanoparticles was neutralized at relatively lower pH values. This phenomenon may be explained by the fact that an increase in PEG modification leads to less free amino groups in the conjugates and results in a relatively lower isoelectric point of the formed nanoparticles. Since a steric hydrated PEO layer, which exists on the surface of HSA-mPEG nanoparticles, play an important role in the stability of the nanoparticles, no flocculation of the HSA-mPEG nanoparticles occurred even when the ζ-potential was near zero. However, HSA nanoparticles, only stabilized by surface charge, were unstable when the ζ-potential reached zero.

The rapid and efficient uptake of colloids by the RES is considered to be a consequence of the opsonization which is basically the adsorption of

plasma proteins on the surface of particles (Senior, 1992). In general, particles which can prevent or modify the adsorption of plasma proteins on the surface, may be able to avoid being cleared by the RES to a certain extent. In the present study rat serum was used as model to investigate the opsonization of nanospheres in vitro and the adsorption of proteins onto the nanoparticle surface was investigated by comparing the hydrodynamic diameter and ζ-potential of the particles after incubation with different concentrations of rat serum. The results listed in Table 2 show that when unmodified HSA nanoparticles were incubated in pH 7.4 buffer containing rat serum, the particle size was significantly increased with an increase of serum concentration. The ζ-potential of the HSA nanoparticles was also significantly changed. In contrast, changes in size and ζ-potential were less pronounced when HSA-mPEG nanoparticles were incubated in different concentrations of serum because of the effect of the hydrated steric barriers on the surface of HSA-mPEG nanoparticles. However, an unexpected finding in the serum adsorption on the HSA-mPEG nanoparticles is that HSA-$mPEG_{5000}$ nanoparticles, having a longer mPEG chain on the particle surface and a lower ζ-potential than HSA-$mPEG_{2000}$ nanoparticles, did not show any advantage in resistance to protein adsorption as revealed by the particle size and ζ-potential measurements (Table 2). Moreover, a higher protein adsorption is found on the nanoparticles prepared from HSA_{50}-$mPEG_{5000}$, even though this material has a higher percentage (compared with HSA_{80}-$mPEG_{5000}$) and a higher molecular weight (compared with HSA_{60}-$mPEG_{2000}$) of mPEG. A possible explanation for this phenomenon is that different particle preparation methods lead to a lower PEO density on the surface of HSA-$mPEG_{5000}$ nanoparticles due to the hydrolysis of the conjugates.

Table 2
Adsorption of rat serum protein on HAS-mPEG nanoparticles

Nanoparticles[a]	Serum (%)	ΔSize (nm)[b]	$\Delta\zeta$ (mV)[c]
HAS (control)	2	5.6 ± 2.7	2.4 ± 0.7
	5	12.1 ± 5.1	4.5 ± 1.3
	10	26.8 ± 4.8	6.0 ± 0.4
HSA_{60}-$mPEG_{2000}$	2	2.2 ± 0.9	0.6 ± 0.4
	5	3.0 ± 1.2	0.9 ± 0.4
	10	3.6 ± 1.3	1.4 ± 0.7
HSA_{80}-$mPEG_{5000}$	2	4.0 ± 1.8	-0.9 ± 0.3
	5	6.9 ± 2.2	-1.3 ± 0.5
	10	8.5 ± 2.6	-1.9 ± 0.7
HSA_{50}-$mPEG_{5000}$	2	6.5 ± 2.0	-1.0 ± 0.6
	5	9.5 ± 2.5	-1.7 ± 0.7
	10	14.0 ± 1.7	-2.3 ± 0.7

[a] Glutatraldehyde:HAS (or HAS-mPEG) ratio: 0.05.
[b] Change of particle size.
[c] Change of ζ-potential.

4. Conclusion

Surface modified albumin nanoparticles with a size approximately 150 nm in diameter were prepared from HSA-mPEG conjugates using modified coacervation methods. The ζ-potential of the surface modified nanoparticles was significantly lower than that of the unmodified HSA nanoparticles. The molecular weight of the PEG component of the conjugate seems to be a more important factor for the ζ-potential than the degree of PEG modification. The existence of a hydrated steric barrier surrounding the nanoparticles was confirmed by electrolyte and pH induced flocculation tests. The surface modified nanoparticles showed a reduced adsorption of plasma protein on the particle surface compared with the unmodified HSA particles. However, an increase of mPEG molecular weight or the degree of the PEG modification does not lead to a lower protein adsorption on the HSA-mPEG nanoparticles. The results indicated that the surface modified HSA nanoparticles prepared from HSA_{60}-$mPEG_{2000}$ offer a higher ζ-potential of being able to avoid uptake by the RES than the other two HSA-mPEG nanoparticles. A study to investigate the biodistribution of the HAS-mPEG and other PEG modified nanoparticles has been carried out. Interesting results have been found for some of the systems. The results will be published in a future publication.

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