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Biodistribution of fluoresceinated dextran using novel nanoparticles evading reticuloendothelial system

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Abstract

The rapid clearance of circulating nanoparticles from the blood stream coupled with their high uptake by liver and spleen has thus far been overcome by reducing the particle size, and by making the particle surface hydrophilic with poloxamers and poloxamines. We have prepared hydrogel nanoparticles of polyvinylpyrrolidone of a size less than 100 nm diameter with precise size distribution. Since the inner cores of these particles are also hydrophilic, these particles are capable of encapsulating water-soluble compounds. Biodistribution of these particles shows practically negligible ($<1\%$) uptake by the macrophages in liver and spleen, and ~ 5–10% of these particles remain in circulation even 8 h after i.v. injection. Increasing the surface hydrophobicity as well as particle size can increase the RES uptake of these particles. Because of longer residence in blood, the hydrogel nanoparticles have potential therapeutic applications particularly in cancer: the water-soluble cytotoxic agents encapsulated in these particles can be targeted to tumors while minimizing the likelihood of toxicity to reticuloendothelial system (RES).

Keywords: Hydrogel nanoparticles; Long circulating nanoparticles; Polyvinylpyrrolidone; Drug targeting; RES evasion

1. Introduction

The potential of site specific delivery in optimizing drug targeting has given tremendous advancement in manipulation of novel dosage forms such as nanoparticles which are solid colloidal polymeric carriers in size range of 1–1000 nm. Nanoparticle encapsulation has been proven useful for reducing the toxicity of certain drugs (Doughlas et al., 1987; Kreuter, 1994; Davis, 1997). Nanoparticles, like other colloidal carriers extended for drug delivery such as liposomes, after intravenous administration, are normally retained mainly by the Kupffer cells of liver and macrophages of the spleen (Patel, 1992). There-

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fore, the liver constitutes a major obstacle and sink to the efficient targeting of colloidal carriers other than reticuloendothelial system (RES). Numerous recent investigations have been aimed at reducing RES uptake and to increase the concentration of the particulate carriers at the desired targets. The most promising among the various strategies followed until now is by reducing the particle size and sterically stabilizing the nanoparticles with a layer of amphiphilic polymer chains like polyethylene oxide (PEO), poloxamers, poloxamines etc., on the particle surface (Allemann et al., 1993). The presence of these poloxamers and poloxamines on the surface, decreases protein adsorption (opsonization) and subsequent phagocytosis of the nanoparticles by the non-parenchymal cells of the liver (Illum et al., 1987; Moghimi et al., 1991; Amiji and Park, 1992). This has led to partial avoidance of the RES uptake and longer circulation time of these particles in blood; however, complete evasion of RES by these surfactant coated particles has not been possible (Gref et al., 1995; Stolnik et al., 1995; Torchilin and Trubetskoy, 1995).

Hydrophilic nanoparticles of polymeric micelles were found to have long circulation in blood (Kataoka et al., 1993; Kwon and Okano, 1996). As these particles have inner hydrophobic core, only hydrophobic materials can be encapsulated into these particles.

Our strategy of achieving long circulating, RES evading hydrogel nanoparticles loaded with water-soluble materials was based on the following prior observations. (i) Preparation of hydrophilic nanoparticles is possible in the aqueous core of reverse micellar droplets (Munshi et al., 1995). (ii) The precise size modulation of these particles down to a couple of tens of nanometer diameter can be done by regulating the size of the aqueous core of reverse micelles (Munshi et al., 1997; Maitra et al., 1999). (iii) Since the polymerization reaction and subsequent encapsulation take place in the aqueous core of reverse micelles, it is possible encapsulate water-soluble molecules inside these particles. (iv) Adsorption of serum proteins on the hydrophilic nanoparticles is drastically reduced which is believed to be the main reason for RES evasion (Norman et al., 1992).

In the present study, we report on the biodistribution of the hydrogel nanoparticles and its modulation with the change in particle size and surface hydrophobicity. Polyvinylpyrrolidone (PVP) was selected as a polymer of choice for the preparation of nanoparticles as it is highly biocompatible (Savva et al., 1998) and has long been used as a plasma expander in human beings (Robinson et al., 1990). PVP has recently been used as a coating material for effectively prolonging the circulation time of liposomes in vivo, broadly by reducing the extent of opsonization and consequent RES uptake (Torchilin et al., 1994). Our results revealed a strong correlation between the evasion of RES and particle size as well as the surface hydrophobicity of nanoparticles.

2. Materials and methods

2.1. Materials

Sodium bis-2-ethylhexylsulphosuccinate, i.e., Aerosol OT (AOT), *N,N,N′,N′* tetramethylethylenediamine (TEMED), *N,N′*methylene bis acrylamide (MBA), fluorescein isothiocyanate dextran (FITC-Dx) (mol. wt. 19.3 kDa) and Sephacryl S-200 were products of Sigma and were used directly without further purification. n-Hexane (99%), sodium monohydrogen phosphate, dihydrogen phosphate and ferrous ammonium sulphate (FAS) were procured from SRL (India). *N*-isopropylacrylamide (NIPAAM) was procured from ACROS ORGANICS and was recrystallized in n-hexane before use. Vinylpyrrolidone (VP) was purchased from Fluka and was used freshly distilled, before polymerization. Double distilled water was used.

2.1.1. Experimental animals

The 6–8-week-old male Swiss albino mice were obtained from the National Institute of Nutrition, Hyderabad, India. They were pathogen free and had free access to food and water. Mice were randomized before initiation of experiments.

2.2. Preparation of PVP nanoparticles

The PVP nanoparticles were prepared following the methods developed originally by Birrenbach and Speiser (1976) and modified in our recent patent (Maitra et al., 1997). The outline of the preparative method is as follows. The surfactant, AOT, was dissolved in n-hexane (usually 0.03–0.1 M of AOT solution). Water-soluble monomer, VP was used for polymerization with MBA as a cross-linking agent for nanoparticle preparation. Aqueous solutions of monomer, cross-linking agent, initiator and FITC-Dx were added to AOT solution in hexane and the polymerization was carried out following the standard procedure. Additional amount of buffer may be added in reverse micelles in order to get the host micellar droplets of desired size. In a typical experiment, 40 ml of 0.03-M AOT solution in hexane was used to which 316 μl of freshly distilled VP, 100 μl of MBA (0.049 g/ml), 20 μl of 1% FAS, 20 μl of 11.2% aqueous solution of TMED, 30 μl of 20% ammonium persulphate as initiator and 50 μl of marker compound, FITC-Dx (160 mg/ml), were added. The solution was homogeneous and optically transparent. Polymerization was done in N_2 atmosphere at 35°C for 8 h in a thermostatic bath with continuous stirring. The above method produced PVP nanoparticles cross-linked with MBA and containing FITC-Dx as encapsulated material. To extract the nanoparticles from reverse micelles and to purify them from other unreacted and toxic materials the following procedures were adopted. The organic solvent was evaporated off in a rotary evaporator and the dry mass was resuspended in 5 ml of water by sonication. Then 2 ml of 30% $CaCl_2$ solution was added drop by drop with continuous stirring to precipitate the surfactant as calcium salt of bis(2-ethylhexyl)sulphosuccinate, ($Ca(DEHSS)_2$). The precipitated $Ca(DEHSS)_2$ was separated by centrifugation at 10 000 rpm for 10 min. The supernatant contained nanoparticles, which were homogeneous and transparent. The pellet of $Ca(DEHSS)_2$ after centrifugation containing some nanoparticles absorbed in it was dissolved in 10 ml of n-hexane and the hexane solution was washed two to three times each time with 1 ml of water. The phase separated aqueous layer was added to the previously centrifuged supernatant solution containing nanoparticles. The total aqueous dispersion of nanoparticles was lyophilized immediately to dry powder for subsequent use. Lyophilized nanoparticles are easily redispersable in aqueous buffer, which was subjected to purification by gel filtration (as given in Section 2.5) and the separated nanoparticles free from unencapsulated FITC-Dx and other unreacted compounds were directly used for biodistribution and other studies. The sizes of these particles in buffer were found to be same before and after lyophilization, establishing that aggregation does not take place by lyophilization of these particles.

2.3. Preparation of copolymer nanoparticles with increased surface hydrophobicity

To prove that it is the surface hydrophilicity of nanoparticles which is the primary factor responsible for RES evasion, we prepared nanoparticles composed of copolymer of VP and NIPAAM. Increasing the quantity of NIPAAM in the copolymer increases the surface hydrophobicity of nanoparticles. In the aqueous core of the reverse micelles, a mixture of VP and NIPAAM was dissolved. The isopropyl group of NIPAAM being a hydrophobic entity is projected towards the surfactant monolayer of AOT molecules in the reverse micellar aggregates as shown in Fig. 1. Therefore, in the final form of nanoparticles, the isopropyl groups cover the particle surface. The surface density of isopropyl groups depends upon the amount of NIPAAM added. We increased the amount of NIPAAM up to 50% w/w of the total monomer mixtures.

The nanoparticles of VP and NIPAAM copolymer in the reverse micelles were prepared by the same method as described above. The copolymer of 50% w/w VP + NIPAAM was also prepared using an excess amount of aqueous buffer in AOT solution so that the reaction took place in emulsion medium.

Conventional liposomes consisting of soybean phosphatidylcholine (SPC):cholesterol (Chol):phosphatidic acid (PA) in the molar ratio

of 55:45:5 and stealth liposomes of SPC:Chol:polyethyleneglycol (PEG)-phosphatidyl ethanolamine (PE) in the molar ratio of 55:45:10 were also prepared by hand shake method following a standard procedures.

2.4. Size determination

2.4.1. Quasi-elastic light scattering (QELS) measurements

QELS measurements for determining the size of the nanoparticles were performed using Brookhaven 9000 instrument with BI200SM goniometer. Air cooled argon ion laser was operated at 488 nm as light source. Using 128-channel digital correlator, the time dependence of the intensity auto-correlation function of the scattered intensity was derived. The size of the nanoparticles was determined from diffusion of the particles using Stoke–Einstein equation. A representative size distribution spectrum is shown in Fig. 2(a).

2.4.2. Transmission electron microscopic (TEM) pictures

A total of 200 μl of the original solution containing nanoparticles was diluted to 50 ml in buffer to have a clear solution and the samples for TEM were prepared with this. TEM picture (Fig. 2b) was taken in a JEOL JEM2000 Ex 200-model electron microscope.

2.5. Separation of loaded nanoparticles from free FITC-Dx by gel permeation chromatography

In order to purify and to separate the unentrapped compound from the nanoparticles the sample was passed through Sephacryl S-200 (1 × 34 cm) column pre-equilibrated with 50 mM PBS at a flow rate of 12 ml/h at 25°C. All the fractions collected were analyzed for their fluorescence values at excitation and emission wavelengths of 495 and 520 nm, respectively, with the help of Shimadzu Spectrofluorimeter, RF-540.

2.6. In vitro stability studies of FITC-Dx loaded nanoparticles in serum

The in vitro stability of hydrophilic nanoparticles entrapped with 100 mM FITC-Dx was studied by incubating the particles in fresh serum at 37°C. The ratio of nanoparticle amount to serum was chosen in order to approach conditions of intravenous administration. Thus, 200-μl aliquots of nanoparticle samples were added to 1800 μl of prewarmed (37°C) mouse serum. Similar incubations were made in PBS (50 mM and pH 7.4) as control. At varying time intervals (2–48 h) aliquots of incubated mixtures were withdrawn and were passed through Sephacryl S-200 column to measure the fluorescence values. Results are expressed as the percentage of FITC-Dx released from nanoparticles with respect to the total FITC-Dx encapsulated.

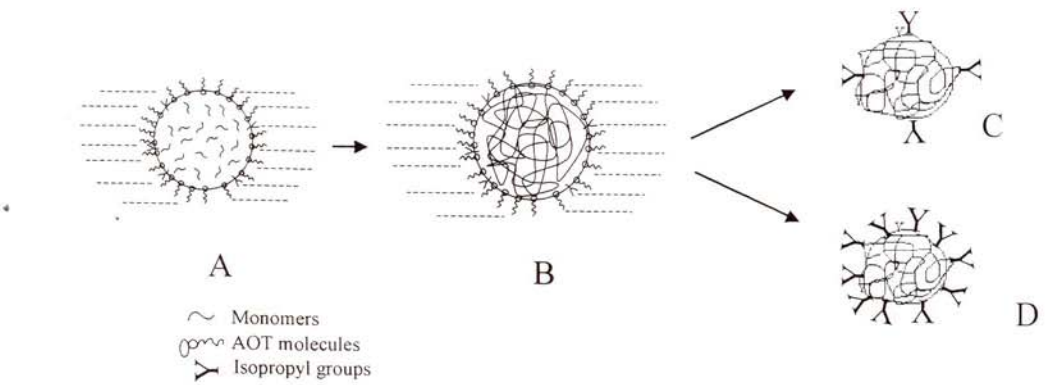

Fig. 1. Schematic diagram of co-polymer nanoparticles. (A) Dissolution of monomers, cross linking agent etc. in AOT reverse micelles. (B) Polymerization of the monomers inside the aqueous core of reverse micelles. (C,D) Polymeric nanoparticles with less and more isopropyl groups respectively, projected towards the particle surface.

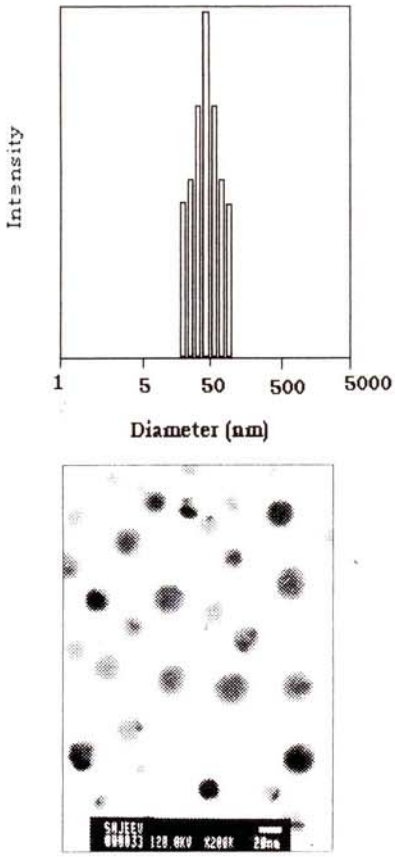

Fig. 2. (a) Representative quasi-elastic laser light scattering data of encapsulated FITC-Dx loaded PVP nanoparticles. (b) A representative TEM micrograph of encapsulated FITC-Dx loaded PVP nanoparticles.

2.7. SDS–polyacrylamide gel electrophoretic (SDS–PAGE) analysis of protein adsorption on nanoparticles

Protein adsorption was performed by SDS–PAGE using the Mini Protean® II electrophoretic apparatus (Bio-Rad) on gradient gel under non-reducing conditions. Nanoparticles and liposomes were opsonised by incubation in 50% v/v fresh mouse serum for 30 min at 37°C followed by centrifugation at 60 000 rpm for 45 min to pellet the opsonised nanoparticles.

Between 5 and 10 μg of protein in 20 μl of each sample was loaded on to an acrylamide gel consisting of 10% separating and 7.5% stacking gel. A standardized staining and destaining schedule was adopted to obtain quantitative data (Neuhoff et al., 1990). The gel was stained with 0.1% w/v Coomassie blue R250 (Sigma, UK). For identification of protein bands on the gel, known standard molecular weight protein markers were loaded (range 14–240 kDa). The unbound protein was quantified in the supernatant spectrophotometrically by Bradford's assay at 595 nm.

2.8. In vivo biodistribution of nanoparticles

All animal experiments were carried out with male Swiss albino mice weighing 26–32 g. Mice (three in each set) were injected intravenously in tail vein with 0.2 ml PBS solution of FITC-Dx entrapped nanoparticles. Similarly, a solution of free FITC-Dx of similar concentration in PBS (pH 7.4) was also injected i.v. At different time intervals, i.e. 0, 30, 60, 120, 240 and 480 min postinjection, mice were sacrificed and blood was withdrawn from the heart. The organs liver, spleen, lung, kidney and heart were excised, thoroughly washed with saline and blotted. The excised organs were then homogenized in PBS containing 0.005% Triton X-100. The homogenate was kept at 4°C for 30 min and centrifuged at 12 000 rpm for 30 min. The supernatant and serum were analyzed for FITC-Dx by monitoring the fluorescence in spectrofluorimeter as described above. Tissue and serum from uninjected animals were used as control. The fluorescence values in the experimental mice were corrected for any endogenous fluorescence. To determine the amount of nanoparticle associated fluorescence in the blood, total blood per mice of 7.5% of body weight was assumed (Patel et al., 1983). The results were presented as percent of fluorescence present in blood at zero hour of administration.

3. Results

3.1. Size of the nanoparticles

The representative size of PVP nanoparticles entrapping FITC-Dx was measured by QELS and TEM and are shown in Fig. 2(a,b). The mean

Table 1
Sizes of various formulations of nanoparticles

Ratio of VP:NIPAAM	Size, nm (mean ± S.D.)
100:00	35 ± 2.08
95:05	38 ± 2.5
50:50	45 ± 1.5
50:50*	126 ± 2.34

* Prepared by emulsion polymerisation method.

diameters of the encapsulated particles were maintained below 100 nm, by maintaining the size of the aqueous core of reverse micellar droplets using nanoreactors (Maitra et al., 1999). The size of these nanoparticles was always found to be larger than that of the aqueous core of the micellar droplets. This was due to the dynamic behaviour of the micellar droplets, which formed transient clusters, leading to the intermixing of the aqueous hydrophilic materials among different droplets. This resulted in secondary growth forming larger nanoparticles (Munshi et al., 1997). The size of nanoparticles was slightly affected by polymer composition, however, all formulations appeared to be very homogenous irrespective of their composition (Table 1). The same conclusion can be drawn from the TEM micrograph of FITC-Dx loaded nanoparticles, which shows their spherical shape as well as their narrow size distribution.

3.2. Entrapment efficiency

The entrapment efficiency of FITC-Dx by various formulations of nanoparticles was found to be between 70 and 80% (Fig. 3). Entrapment was enhanced with the increased percentage of cross-linking agent but it decreased with the decrease in particle size as well as increase of surface hydrophobicity (data not shown).

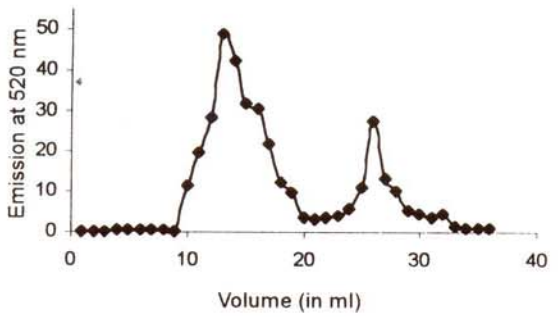

Fig. 3. Separation of PVP nanoparticles from free FITC-Dx by gel permeation chromatograph on Sephacryl S-200 column.

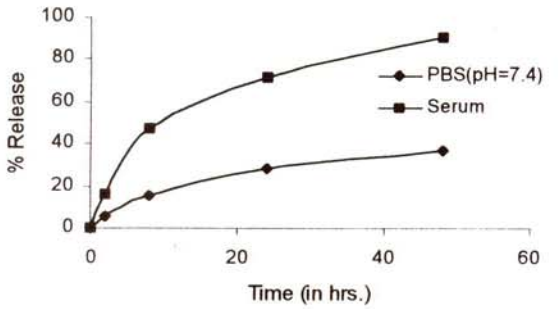

Fig. 4. In vitro release of entrapped FITC-Dx from PVP nanoparticles in presence of serum and buffer as described in Section 2.

3.3. In vitro release of FITC-Dx

The in vitro release of FITC-Dx from the PVP nanoparticles in serum as well as in aqueous buffer (pH 7.4) at different time intervals is shown in Fig. 4. The results show that the release rate of the marker compound from the nanoparticles is much faster in serum as compared to that in aqueous buffer. This may be attributed to the effect of the presence of serum protease, in addition to the swelling of the polymer. This enzyme might decompose the amide bonds of the cross-linked matrix and thereby facilitate the enhanced release of FITC-Dx in serum. The percentage release in serum at 2, 8, 24 and 48 h was found to be 16, 47, 71 and 90%, respectively, as compared to that in buffer in which it was 5, 15, 28 and 36%, respectively, during the same period of time.

3.4. Serum protein adsorption on PVP nanoparticles

Since RES uptake of particulate materials is preceded by the opsonization process, the adsorption of serum proteins bound to the nanoparticles was studied under conditions which mimic physiological environments (pH 7.4, 37°C). Fig. 5 illus-

trates the plasma protein adsorption to PVP nanoparticles, copolymer nanoparticles, conventional liposomes and stealth liposomes at 50% v/v plasma incubation concentration. It can be seen that proteins of 185, 66, 52 and 20 kDa which correspond to complement C3b, albumin, IgG heavy chain and IgG light chain, respectively, bound mostly to the copolymer nanoparticles, stealth liposomes and conventional liposomes. These opsonins bound to the particle surface, form a bridge between the particles and the macrophage. These opsonins bound to the particle surface form a bridge between the particles and the macorphages and thereby macrophages phagocytose the particles (Greenberg and Silverstein, 1993). PVP hydrophilic nanoparticles show least protein adsorption, which is in agreement with the studies of Uniyal and Brash (1982) who reported the adsorption of serum albumin in substantial quantities to hydrophobic surfaces but not to the hydrophilic surfaces. The quantified results for the total amount of serum proteins adsorbed on the particle surface as measured by Bradford's assay are given in Table 2.

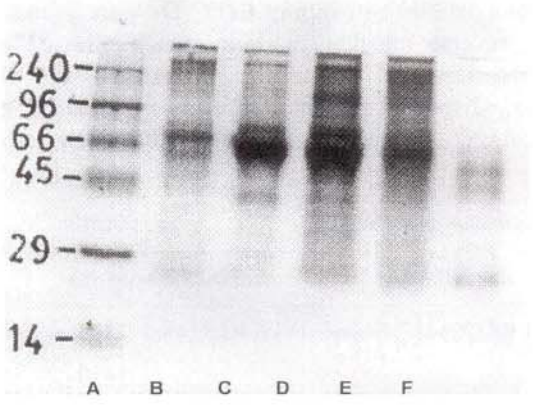

Fig. 5. SDS–PAGE gel showing the adsorption of serum proteins to PVP nanoparticles, co-polymer nanoparticles etc. (as described in Section 2) incubated in 50% v/v serum. Lane A represents standard mol.wt. protein marker whilst lanes B–F represent serum proteins desorbed from PVP nanoparticles, co-polymer nanoparticles (50:50), conventional liposomes, stealth liposome and serum, respectively.

Table 2
The adsorption of serum proteins to different colloidal carriers incubated in murine serum and quantified by Bradford assay

Colloidal carrier	Total protein present in 50% v/v serum	
	% Of protein bound	% Of unbound protein
PVP nanoparticles	2.4	90
Copolymer nanoparticles	7.6	77
Stealth liposome	8.4	80.2
Conventional liposome	37.7	54.4

3.5. Biodistribution of nanoparticles

To facilitate a comprehensive analysis of nanoparticle distribution in the body system, we have presented in Figs. 6 and 7 the tissue distribution data of (i) blood, (ii) liver, (iii) spleen, and (iv) kidney. The data for lung and heart are not presented since the fluorescence associated with these organs is found to be negligible. The results of nanoparticle distribution obtained at various time points after i.v. injection are expressed by RES/blood ratio and kidney/blood ratio at different surface hydrophobicity (depending on NIPAAM content) of the polymer. Within 10 min 95% of free FITC-Dx is rapidly cleared from circulation following intravenous injection in mice. In contrast, the rate of decrease of FITC-Dx entrapped in nanoparticles is much lower with

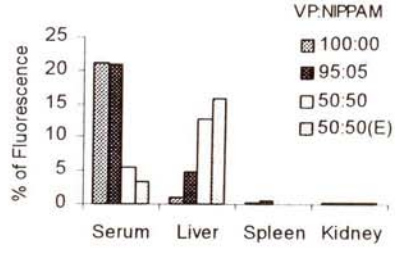

Fig. 6. Biodistribution of FITC-Dx encapsulated PVP and copolymer nanoparticles with different ratios of hydrophilicity to hydrophobicity 2 h post injection as described in Section 2. The values are mean values of three different animals per sample. E, nanoparticles were prepared in emulsion system.

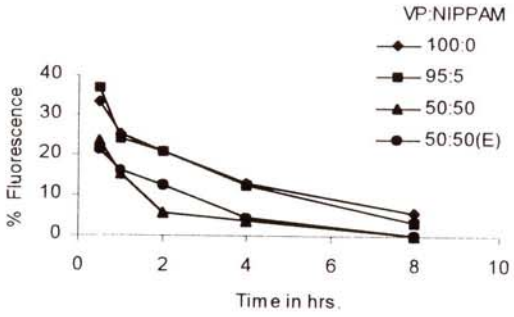

Fig. 7. Plasma clearance studies of FITC-Dx encapsulated PVP and copolymer nanoparticles with different ratios of hydrophilicity to hydrophobicity as described in Section 2. The values are mean values of three different animals per sample. E, nanoparticles were prepared in emulsion system.

~55% of the dose remaining in the circulation even after 15 min post-injection (data not shown). The results of nanoparticle distribution obtained with various copolymer formulations at 2 h post-injection (this is the time of maximum RES uptake) demonstrated a dramatic impact on the in vivo behavior of the particles with the increased NIPAAM content as well as increased particle size. A maximum 40–50-fold increase in RES/blood ratio and 4-fold increase in kidney/blood ratio were observed when the NIPAAM content was increased from 0 to 50% in the polymer. The results as revealed from Figs. 6 and 7 as well as from Table 3, highlight that copolymer modification affected the biodistribution of nanoparticles and the effect was dependent on the extent of the resultant surface hydrophilicity/hydrophobicity of the composite copolymer.

4. Discussion

It has been previously reported that particle size, polymer composition and surface characteristics of nanoparticles govern their in vivo stability, rate of clearance from blood and tissue specificity (Dunn et al., 1994; Gref et al., 1994, 1995). The fate of i.v. injected particulate carrier system is mainly determined by the action of RES. The function of this physiological defense system is the extraction of potentially foreign and antigenic materials from blood stream (Patel, 1992). It has earlier been postulated that the decrease in RES uptake of PEG coated particles is possibly due to the presence of steric barrier which decreases the adsorption of plasma proteins on the surface of these nanoparticles (Allen et al., 1989; Allen and Hansen, 1991). Since these coating materials were usually physically adsorbed on the carrier surface, fraction of these adsorbent get desorbed from the surface by preferential high affinity binding of certain plasma proteins for the surface (Lasic et al., 1991). As a result, opsonization followed by increased RES uptake occurs. Therefore instead of making the particle surfaces hydrophilic by coating them with amphiphilic polymers, we have prepared nanometer size hydrophilic particles of vinyl monomer with successful RES evasion to nearly 100%. These nanoparticles entrapping FITC-Dx were prepared by reverse micellar method, which allowed the formation of smooth spherical ultrafine monodispersed particles of size less than 50 nm diameter with high entrapment efficiencies.

Table 3
Biodistribution of PVP and co-polymer nanoparticles in RES/blood and kidney/blood ratios[a]

Time, min	0% NIPAAM		5% NIPAAM		50% NIPAAM		50% NIPAAM (E)	
	RES/blood	Kidney/blood	RES/blood	Kidney/blood	RES/blood	Kidney/blood	RES/blood	Kidney/blood
30	0.02 ± 0.15	0.05 ± 0.11	0.08 ± 0.16	0.07 ± 0.11	0.28 ± 0.03	0.04 ± 0.012	0.50 ± 0.06	0.02 ± 0.11
60	0.04 ± 0.09	0.13 ± 0.03	0.08 ± 0.24	0.01 ± 0.02	0.52 ± 0.04	0.02 ± 0.03	1.03 ± 0.62	0.01 ± 0.2
120	0.05 ± 0.10	0.06 ± 0.13	0.22 ± 0.32	0.09 ± 0.14	2.26 ± 0.06	0.01 ± 0.06	2.75 ± 0.41	0.08 ± 0.1
240	0.06 ± 0.2	0.04 ± 0.08	0.40 ± 0.36	0.09 ± 0.12	2.67 ± 0.3	0.07 ± 0.2	3.63 ± 0.32	0.10 ± 0.02
480	0.1 ± 2.6	0.04 ± 1.92	1.3 ± 0.3	0.03 ± 0.18	100 ± 0.5	0.15 ± 0.08	Very large value	Very large value

[a] Mean ± S.D., $n = 3$.

In vitro stability studies revealed that these hydrophilic nanoparticles are stable in buffer as compared to serum. The rapid release of FITC-Dx was assumed to result from two mechanisms: (i) the swelling of polymer in an aqueous medium (Sahoo et al., 1998); and (ii) the degradation of the polymer at their amide linkage by serum protease. However, the in vitro serum stability (90% release after 48 h) does not coincide with in vivo circulation time of nanoparticles which most likely represents the more complex nature of in vivo system.

The pattern of protein adsorption onto colloidal carrier is assumed to control the organ distribution. Indeed immunoglobulins and C3 proteins of complement are recognized by specific receptors on macrophages (Greenberg and Silverstein, 1993). Our SDS–PAGE results suggest that there exists a direct relationship between surface hydrophobicity and opsonization process. As shown in Fig. 5 and Table 2 conventional liposome showed maximum protein adsorption followed by stealth and copolymer nanoparticles.

Our report is the first of its kind to show complete RES evasion combined with long circulation of colloidal carriers. The biodistribution studies of the PVP hydrophilic nanoparticles showed maximum blood circulation time, negligible RES uptake in comparison with our other copolymer formulations having increased surface hydrophobicity. We could account for 50% of the injected dose of FITC-Dx nanoparticles in blood, liver, spleen and kidney. We presume that a large number of these nanoparticles were degraded at their amide cross-linkages by serum proteases. As a result, the FITC-Dx is released more rapidly and the free molecules are immediately cleared up from the body through the kidney, which results in substantial loss of materials.

5. Conclusion

Current literature suggests that nanoparticles are removed rapidly from the circulation by the phagocytic cells of the RES, and coating the particle surface by poloxamers and poloxamines can significantly prolong circulation time. This has led to the view that nanoparticles targeting tissues outside RES can only be possible by PE-Gylating the particle surface so as to make the surface hydrophilic. Our study shows that the uptake of hydrogel nanoparticles by RES can be practically eliminated and therefore, the particles remain in blood for longer period of time. This may, perhaps, facilitate the accessibility of these hydrogel nanoparticles to other organs like implanted tumors, bone marrow, etc. The small size of these particles (< 100-nm diameter) is thought to help enhanced extravasation through the relatively larger fenestration of the endothelium layer of the vasculature passing through the solid tumors.

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