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Development of hemoglobin aquasomes from spherical hydroxyapatite cores precipitated in the presence of half-generation poly(amidoamine) dendrimer

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Abstract

Spherical hydroxyapatite cores were prepared by using carboxylic acid terminated half-generation poly(amidoamine) (PAMAM) dendrimer as templates or crystal modifiers. The hydroxyapatite cores were characterized by infrared spectroscopy (IR), X-ray diffraction (XRD) and transmission electron microscopy (TEM). The spherical core formation depended on phosphate saturation, pH of the simulated body fluid (SBF) and rate of crystal growth. Hydroxyapatite so formed was amorphous and a mixture of various calcium phosphates. Ca/P ratio determination which showed phosphate rich apatite formation. Hydroxyapatite ores were coated with a sugar layer followed by hemoglobin to obtain aquasomes. Aquasomes were characterized for size, hemoglobin loading, oxygen-binding characteristics and storage stability. The nanometric sized aquasome formulation could load approximately 13.7 mg hemoglobin per g of core and retained oxygen-affinity and cooperativity and stability for at least 30 days. Formulation efficacy was tested in albino rats and indicated its potential utility as blood-substitute.

Keywords: Dendrimer; Crystal modifiers; Hydroxyapatite; Self-precipitation; Aquasomes; Hemoglobin

1. Introduction

During the last decade a radically new class of carrier has emerged comprised of surface modified nanocrystalline ceramics named as aquasomes. Aquasomes consist of a ceramic core whose surface is non-covalently modified with carbohydrates to obtain a sugar ball, which is then adsorbed with therapeutic proteins. The cores provide structural stability to largely immutable solid. The surface modification creates a glassy molecular stabilization film that adsorbs protein pharmaceuticals with minimal structural denaturation. Kossovsky and coworkers have successfully developed surface-modified nanocrystalline ceramics to deliver viral antigens for the purpose

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of evoking an immune response, oxygenated hemoglobin for cell respiration and insulin for carbohydrate metabolism (Kossovsky et al., 1994, 1996).

Three types of core materials are mainly used for producing aquasomes: tin oxide, nanocrystalline carbon ceramics (diamonds) and brushite (calcium phosphate dihydrate) (Kossovsky et al., 1994, 1996). Owing to its natural presence in the body, calcium phosphate is the core of interest. The brushite, however, is unstable and converts to hydroxyapatite upon prolong storage. Hydroxyapatite seems, therefore, a better core for the preparation of aquasomes. It is widely used for the preparation of implants for drug delivery. They are particularly suitable for protein delivery because of their high adsorption capability of proteins (Barroug et al., 1998). Hydroxyapatite can be prepared by direct precipitation–calcination technique or by slow self-precipitation from simulated body fluids (SBFs). Template-directed self-precipitation or co-precipitation of hydroxyapatite to obtain ordered structural organization is interesting and widely applicable method. Templates like chitosan (Yamaguchi et al., 2001), bile salts (Koutsopoulos and Dalas, 2001), poly(L-lactic acid) foams (Zhang and Ma, 1999), niobium oxide gels (Miyazaki et al., 2001), cellulose cloth, bioinert collagen membranes with the aid of citric acid (Rhee and Tanaka, 2000) and many others have been reported. The reports show that the functional groups that bind calcium induce the apatite nucleation in body environment. These methods are important to understand template directed biomineralization process occurring in nature but most of them are not suitable for preparing aquasomes either because of the large size or irregular shape. Therefore, the process to produce uniform spherical hydroxyapatite particles would be of interest to both biomaterial and pharmaceutical scientists.

Poly(amidoamine) (PAMAM) dendrimers have recently attracted attention for studying drug delivery (Esfand and Tomalia, 2001). Dendrimers with carboxylate terminals i.e. half-generation dendrimers were used to study crystallization of calcium carbonate in aqueous solution (Naka et al., 1999). The report shows that it produces spherical calcium carbonate composed mainly of valerite crystals. The calcium ion binding ability, monodispersity and spherical shape suggests the possibility of the use of carboxylic acid terminated dendrimers to produce spherical hydroxyapatite. Therefore, an attempt was made to produce spherical nanometric hydroxyapatite particles by using carboxylic acid terminated PAMAM dendrimers as templates or crystal modifiers. Besides studying the template directed biomineralization, our aim was to use these particles for preparing aquasomes containing adsorbed hemoglobin.

Blood have numerous functions, one of the important functions being oxygen transporter to the tissues. In many conditions of excessive blood loss such as accident and surgery, an immediate resuscitative solution is to deliver the patient with oxygen carrying fluid. Other potential applications include: to prevent of reverse hypovolemia and subsequent organ failure, as a part of hemodilution in patients undergoing elective surgery, to reduce ischemia after myocardial infarction or stroke, and to improve radiation therapy by supplying oxygen to the tumor tissues. Hemoglobin-based oxygen carriers are being developed as resuscitative agents and many of them are in final phase of clinical trials (Gulati, 2000). A very exhaustive review by Riess (Riess, 2001) provides details of chemistry and physiology of hemoglobin based oxygen carriers.

We are reporting here the preparation and characterization of hemoglobin aquasomes prepared using calcium hydroxyapatite particles as cores, which were produced by self-precipitation in the presence of half-generation PAMAM dendrimers from SBF. In our knowledge, this is the first report on the preparation of spherical hydroxyapatite particles using carboxylic acid terminated PAMAM dendrimers.

2. Materials and methods

2.1. Materials

Trehalose, bovine serum albumin, concanavalin A (Sigma, USA), 3.5, 4.0 and 4.5 generation PAMAM dendrimer (Aldrich), salts and buffers

components were of A.R. grade obtained from E Merck (Germany). Fresh blood was obtained from sacrificed sheep from local slaughterhouse, which was stored in heparinized vials. Hemoglobin solution was prepared by technique reported by Winslow and Chapman (1994). Distilled deionized water was used for all experiments. The equipments were depyrogenated and the solutions were bacteria and pyrogen free. The experiments were carried out in sterile conditions.

2.2. Preparation of hydroxyapatite core

The SBF was prepared as reported (Kim et al., 2001) except that no divalent salts except calcium were used. 3.5 4.0 and 4.5 generation PAMAM dendrimer (1.0 ml, 10% w/v methanolic solution) were dissolved in 30 ml SBF (pH 7.4). The solutions were kept in properly sealed glass vials at 37 °C for 7 days with mild stirring. The vials were observed visually everyday for precipitate formation. Alternatively, hydroxyapatite was allowed to self-precipitate from SBF solutions containing dendrimer after adjusting pH of SBF by dropwise addition of 0.1 M sodium hydroxide solution. All the solutions were adjusted to uniform phosphate levels by addition of 0.01 M Na_2PO_4 solution such that in no case more than 0.2 ml was required to induce direct precipitation of calcium phosphate. The directly precipitated calcium phosphate crystals were treated as control (Fig. 1a). Then, the vials were kept for 7 days at 37 °C to allow nucleation and crystal growth. The precipitate so formed was washed with deionized water multiple times by centrifugation at 7000 rpm for 10 min. The powder obtained was characterized by infrared spectroscopy (IR) (Equinox 55/S, Bruker, Germany), X-ray diffraction (XRD) (D8 advance, Bruker AXS, Germany) and transmission electron microscopy (TEM) (Zeiss EM 912, Germany).

2.3. Preparation of aquasomes

The powder (500 mg) obtained was dispersed in trehalose solution (10 mg ml^{-1}) and lyophilized in a round bottom flask. The lyophilized powder was dialyzed against 1 l deionized water for 12 h with three intermittent changes. The adsorption of sugar was confirmed by studying zeta-potential using Malverns Zetasizer 4 (Malvern Instruments, U.K.) and concanavalin-induced aggregation of particles on UV-Vis spectrophotometer (Shimadzu 1601, Japan) (Merz et al., 1999). The aggregation was measured as a function of absorbance at 450 nm. The particles had the tendency to aggregate, therefore, appropriate blank experiments were also conducted and subtracted from the data. The sugar-coated particles were dispersed in 1% w/v hemoglobin solution (Winslow and Chapman, 1994), lyophilized and washed three times with pure water by centrifugation at 7000 rpm for 10 min. The coating of hemoglobin on the particles was confirmed by change in color of the particles and zeta-potential measurement. The hemoglobin-loaded particles were observed by scanning electron microscopy. Hemoglobin loading and met-hemoglobin content was determined by reported method (Benesch et al., 1973) after dissolving the particles in HCl. The hemoglobin-loaded particles were then dispersed in 7.5% w/v bovine serum albumin/phosphate buffer solution for further characterization.

2.4. Oxygen-binding characteristics

The oxygen dissociation curve was determined by exposing 1.0 ml of the hemoglobin containing samples to an increasing partial pressure of oxygen and deoxygenating it with nitrogen gas. A Clark oxygen electrode (Radiometer A/S, Denmark) was used to detect the change in oxygen tension (pO_2) that was plotted on x-axis. The resulting increase in oxyhemoglobin fraction (SO_2) is simultaneously monitored spectrophotometrically (Shimadzu 1601 spectrophotometer) at 560 and 576 nm and plotted on y-axis. The value of $[\log(SO_2/(100 - SO_2)]$ was plotted against $[\log pO_2]$. The Hill's coefficient, n, was calculated from the slope of the line.

2.5. Efficacy testing

Albino rats (Sprague–Dawley) weighing between 200 and 240 g were anesthesized with ketamine (80 mg kg^{-1}) and acepromazine (1 mg

kg^{-1}). Surgical implantation of silastic canulae (0.025 in i.d. × 0.047 in o.d.) was done in jugular vein. The femoral artery was canulated with 0.01 in i.d. × 0.02 in o.d. canula. The animals were allowed to recover overnight and the next day isovolemic exchange transfusion experiments were performed. The isovolemic blood exchange rate of 0.2 ml min^{-1} was maintained using a peristaltic pump. The blood was removed from femoral artery and simultaneously replaced with 5% dextran-40 in PBS or the aquasome formulation. The hematocrit was determined using microhematocrit. In another experiment, a third canula was implanted in rats in carotid artery to monitor blood pressure, heart rate and respiration rate. The 50% exchange transfusion was conducted with test formulations and changes in blood pressure, heart rate and respiration rate were monitored.

2.6. Stability studies

The formulation was kept in glass vials sealed under nitrogen for the period of 1 month in a refrigerator. The oxygen binding characteristics, hemoglobin and met-hemoglobin content were determined. The formulation was exposed to five freeze-thaw cycles between 4 and 25 °C for 24 h at each condition and the above characteristics were measured.

3. Results and discussion

3.1. Hydroxyapatite core preparation and characterization

Calcium phosphates were self-precipitated from SBF in presence and absence of half-generation PAMAM dendrimer. Upon direct precipitation in the absence of dendrimer at pH > 9.0, needle or flake like crystals were immediately obtained (Fig. 1a) which served as control. The size of these crystals was approximately 100–200 nm. This is due to phosphate saturation at higher pH. The phosphate saturation was independently studied by addition of 0.01 M Na_2PO_4 solution to SBF of different pH values. The volume of Na_2PO_4 solution required was less for a higher pH than the lower pH SBF solutions. The phosphate concentration was adjusted to saturation before addition of dendrimer to SBF solutions. Calcium phosphates were self-precipitated only in presence of 4.5 generation PAMAM dendrimer in about 1–2 days from SBF of pH 7.4. The precipitate was elliptical (Fig. 1b) consisting of needle shaped whiskers arranged in an elliptical shape (Fig. 1b inset). The size of elliptical particles was approximately 200 and 100 nm in major and minor diameter, respectively. The calcium phosphate was also not self-precipitated in presence of 3.5 generation PAMAM dendrimer in 7 days. However, SBF of pH > 8.0 could induce self-precipitation. This indicates that –COOH groups are essential for nucleation and self-precipitation and $–COO^-$ charge density is an important prerequisite for calcium binding and nucleation. Direct precipitation by the addition of sodium phosphate in presence of 3.5 generation dendrimer lead to formation of similar elliptical precipitate (Figure not shown) of slightly bigger size than obtained by self-precipitation in presence of 4.5 generation PAMAM dendrimer. Adjustment of SBF pH to 8.5, transition from elliptical to spherical calcium phosphate particles with protruding whiskers (Fig. 1c) was observed. The precipitation could be visually observed within 24 h indicating fast crystal growth. The size was increased to approximately 400–500 nm (Fig. 1c inset). The particles obtained by self-precipitation at pH 8.0 were spherical sub-micrometer sized hydroxyapatite particles (Fig. 1d) without whiskers. The turbidity was first observed in about 3–4 days indicating quite slow crystal growth. The size ranged from less than 50 to approximately 150 nm as shown in TEM (Fig. 1d). The particle surface was grainy instead of needle shaped protrusions due to relatively slow and controlled crystal growth (Fig. 1d inset). The particles appear hollow under TEM as observed by distinct electron contrast at the surface of each spherical particle. This was however not confirmed by other methods. The average size of the particles was 72 nm and the polydispersity was 0.16. The pH of the mother liquor (SBF) was found to increase as a result of precipitation in all cases and was in the range of 8.4–8.8 after 7 days.

In the presence of 4.0 generation PAMAM dendrimer, no self-precipitation occurred in 7 days from SBF at all pH values hence, studies were not conducted further. This finding also suggests the importance of –COOH groups for inducing self-precipitation.

Thus, it can be concluded that phosphate saturation and presence of ionized carboxylic acid groups are important prerequisites to induce self-precipitation of hydroxyapatite from SBF. The overall size and shape of the particles obtained depends upon the conditions that allow slow or fast crystallization process. The influence of dendrimer is less when the crystallization process is fast and the deviations from spherical shapes (whiskered spherical or elliptical) are observed. Maintaining conditions for slow controlled crystallization, the spherical shape is obtained. Two mechanisms are proposed for the growth of spherical particles from slow self-precipitation in pres-

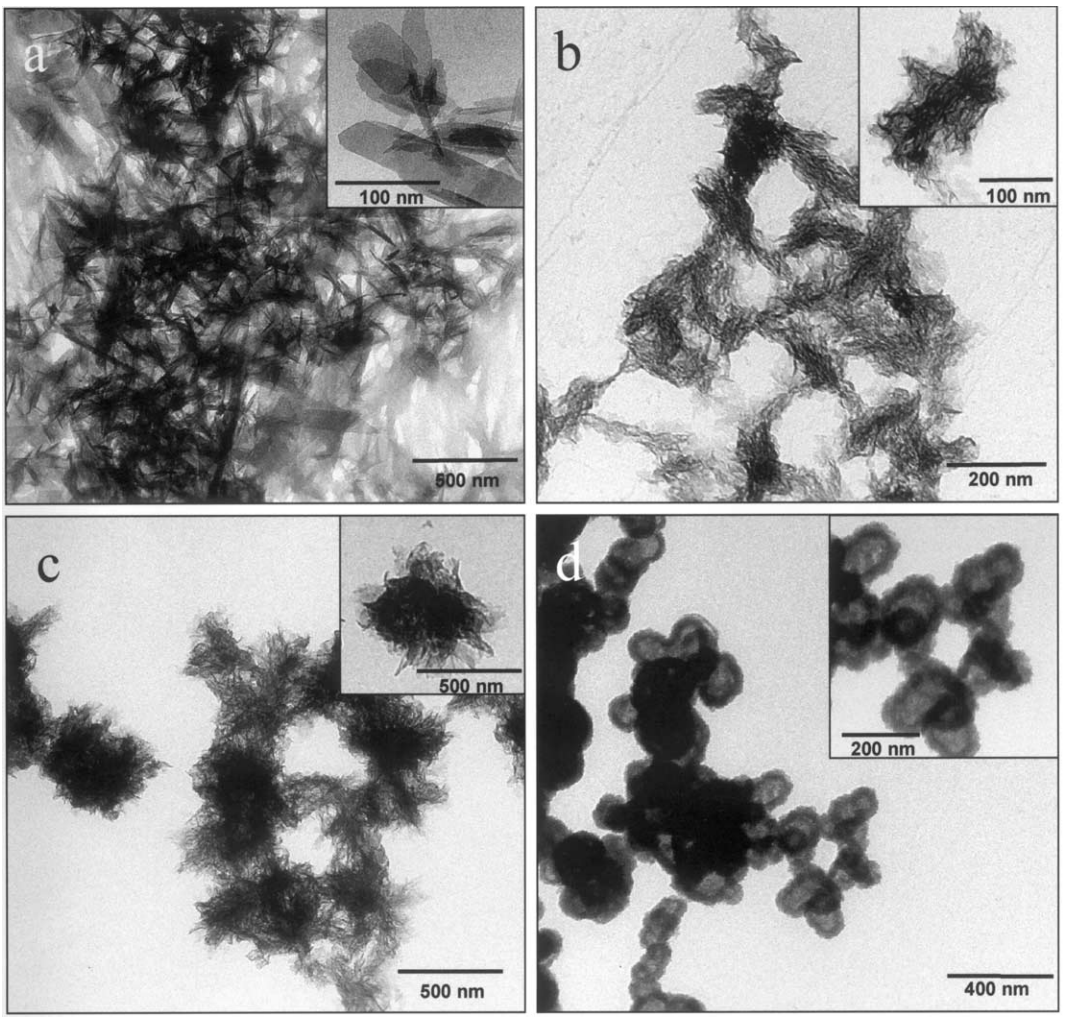

Fig. 1. TEM image of hydroxyapatite prepared by self-precipitation from SBF. (a) Direct precipitation from SBF at pH 9.0 in absence of dendrimer. (b) Self-precipitation in the presence of 4.5 generation PAMAM dendrimer from SBF at pH 7.4. (c) Self-precipitation in the presence of 3.5 generation PAMAM dendrimer from SBF at pH 8.5. (d) Self-precipitation in the presence of 3.5 generation PAMAM dendrimer from SBF at pH 8.0. Inset in all figures show typical hydroxyapatite particle shape and morphology under magnification.

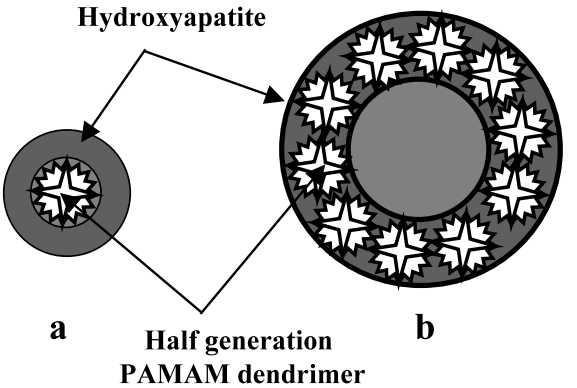

Fig. 2. Possible mechanisms of slow hydroxyapatite growth. (a) Dendrimer as a nucleus for hydroxyapatite precipitation and growth. (b) Dendrimer supramolecular aggregate as a nucleus for hydroxyapatite precipitation and growth.

ence of 3.5 generation PAMAM dendrimer (Fig. 2): (a) the spherical dendrimer might serve as nucleation site due to calcium binding on the carboxylic acid groups present on the surface and, (b) the amphipathic half-generation dendrimer (Bosman et al., 1999) act as supramolecular (micellar) aggregate which might serve as nucleation site. The occurrence of these mechanisms together is also a possibility.

The spherical particles produced from 3.5 generation PAMAM dendrimer were further characterized by IR, XRD and Ca/P analysis. IR spectra showed strong $–PO_4$ frequency bands (stretching modes) at 960–1090 and 570–605 cm^{-1}, which are characteristics of hydroxyapatite (Fig. 3). A small amount of $–CO_3$ is also recorded around 1430 cm^{-1} but this is also weakened by H–O–H interference at 1635 cm^{-1}. The typical amide II bands of PAMAM dendrimer, expected at 1550, 1640 cm^{-1} are also overlapped by H–O–H interference. The $–NH_2–$ stretching at 3300–3400 cm^{-1} overlaps –OH and H–O–H peaks. The peaks at 2850, 2920 cm^{-1}, typical for $–CH_2$ stretching, however, reflect presence of dendrimer because they are not observed for pure crystalline hydroxyapatite samples. The lower intensity and sharpness of these peaks suggest that the hydroxyapatite obtained is of low crystallanity. The XRD was measured to confirm crystallanity of the hydroxyapatite particles. The precipitate obtained from solution without dendrimer shows

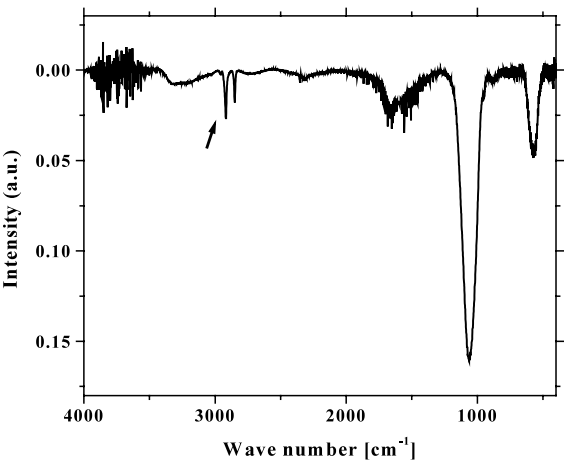

Fig. 3. IR spectrum of hydroxyapatite obtained by self-precipitation in presence of 3.5 generation PAMAM dendrimer from SBF at pH 8.0. An IR spectrum is obtained by KBr pellet method. The arrow shows typical $–CH_2$ stretching peaks indicating presence of PAMAM dendrimer in hydroxyapatite precipitate. Such peaks are not found in pure hydroxyapatite samples.

poorly resolved diffraction peaks indicating low crystallanity (Fig. 4a) while the submicron-sized spherical particles obtained in the dendrimer system exihibited a broad bump and no peaks indicating that the precipitate was amorphous (Fig. 4b). The lower Ca/P ratio (1.35) than stoichiomet-

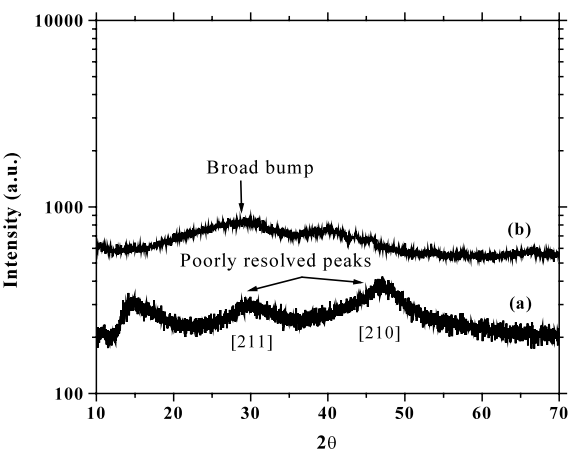

Fig. 4. XRD spectra of hydroxyapatite obtained from: (a) direct precipitation from SBF at pH 9.0 in absence of dendrimer and (b) self-precipitation in the presence of 3.5 generation PAMAM dendrimer from SBF at pH 8.0.

ric ratio (1.67) shows that particles are calcium deficient hydroxyapatite. The low calcium phosphate ratio may also be due to their precipitation from SBF with pH greater than 7.4, which resulted in more phosphate being bound to calcium (acidic calcium phosphate are formed). Li et al. (1997) have studied the calcium phosphate formed from SBF and reported formation of acidic calcium phosphate with increase in pH of SBF. Although the hydroxyapatite is thermodynamically the most stable species of calcium phosphate, presence of other species such as dicalcium phosphate dihydrate, amorphous calcium phosphates and octacalcium phosphate incorporating combination lattice defects cannot be ignored. The lattice defects due to $-CO_3$ is yet another possibility. The characterization results are consistent with other reports on calcium deficient amorphous apatite (Hirai et al., 2000).

3.2. Aquasome preparation and characterization

The sugar provides structural stability to the protein against conformational change including hemoglobin whose cooperativity is conformationally sensitive. Trehalose is a nonreducing, biologically inert disaccharide that is abundant in living organisms adapted to survive extreme desiccation conditions was adsorbed over the hydroxyapatite cores. The zeta potential of the particles was $+4.6 \pm 1.4$ that was reversed to -2.0 ± 1.1 after coating of trehalose, which suggests the presence of sugar layer on hydroxyapatite cores. The variations in the reading were large, therefore, it was further proved by concanavalin A induced aggregation studies. Concanavalin A binds to the sugar adsorbed over the particles and aggregate them causing increase in optical density (measured as a function of absorbance at 450 nm). The increase in absorbance at 450 nm over the time period confirms the sugar layer over the hydroxyapatite core (Fig. 5). The particles turn light reddish-brown after hemoglobin coating providing visual proof of hemoglobin adsorption. The zeta potential was -33.5 after coating of hemoglobin. The negative zeta potential of hemoglobin-coated particles is due to the neutral pH (6.9 ± 0.3) of the suspension, which is greater than pK_a of

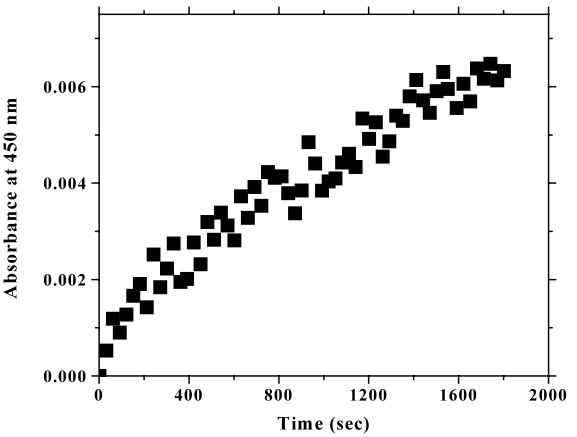

Fig. 5. Concanavalin A induced aggregation of sugar coated hydroxyapatite cores. Concanavalin A solution (100 μg ml^{-1}) was added to sugar-coated hydroxyapatite core suspension (10 μg ml^{-1}) in quartz cuvette and absorbance at 450 nm was measured as a function of time. The data was subtracted from blank experiment conducted without addition of concanavalin A.

hemoglobin. The particles retained their spherical shape and size (Fig. 6). The hemoglobin loading was 13.7 mg g^{-1} of hydroxyapatite, which is greater than our studies with hemoglobin adsorption on hydroxyapatite produced by direct precip-

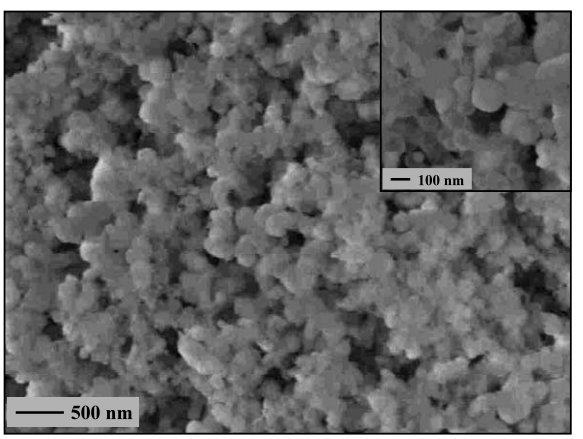

Fig. 6. Scanning electron microscopy image of hemoglobin adsorbed, sugar-coated hydroxyapatite cores before dispersing in albumin/buffer. The particles tend to aggregate as a result of drying but spherical nature of particles is clearly seen in magnification (inset).

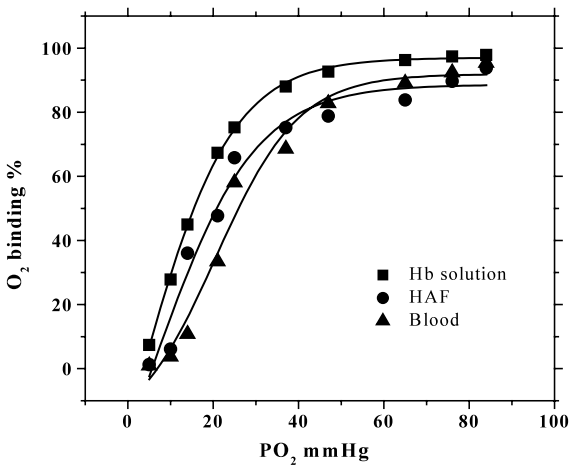

Fig. 7. Oxygen binding characteristics (oxygen saturation curve) of fresh blood, hemoglobin solution and hemoglobin aquasome formulation as indicated in figure.

itation (Patil et al., 1999) perhaps as a result of differences in surface area of the particles formed by two different methods. This was suspended in water containing 7.5% w/v bovine serum albumin to make 14.0 g dl^{-1} hemoglobin i.e. within the range of theoretical value for human blood. The osmolarity was adjusted to 295 ± 5 m Osm using osmometer (Precision Systems Inc.). This hemoglobin aquasome formulation, (HAF) was used for further studies.

3.3. In-vitro and in-vivo oxygen carrying characteristics

To study the activity of hemoglobin adsorbed over the sugar-coated hydroxyapatite cores, oxygen-binding characteristics of the aquasome formulations were studied. Fig. 7 shows the oxygen binding characteristics of fresh blood, hemoglobin solution and HAF. The P_{50} (oxygen partial pressure for which oxy-hemoglobin was 50% saturated) was 29.1, 15.2 and 28.4 mm Hg, respectively, for fresh blood, hemoglobin solution and HAF that accounts for good oxygen-affinity. The Hill coefficient values were 2.44, 2.25, and 2.69, respectively, for fresh blood, hemoglobin solution and HAF that accounts for oxygen-cooperativity characteristics of hemoglobin. This shows that the properties of hemoglobin and its ability to carry oxygen are retained by the HAF. It is recently reported that calcium causes transition in hemoglobin molecule due to dehydration effect (Kelemen et al., 2001) and therefore, may affect oxygen-binding characterisitics of hemoglobin. The formation of trehalose/hemoglobin glass on the surface of hydroxyapatite core during lyophilization seems to provide stability against calcium induced dehydration effect (Crowe et al., 1987). The detailed studies are, however, underway.

Efficacy studies of the formulation was conducted in rats to demonstrate the acute life support provided to rodents whose hematocrit is reduced to levels, which result in their death. In control rats 100% mortality was seen at 4.5–5.0% hematocrit that corresponds to approximately > 90% exchange. The rats transfused with HAF survived at this value. Isovolemic exchange transfusions with HAF to hematocrit values of 50% had no significant effect ($P = 0.01$) on blood pressure and heart rate of animals (Table 1). Although not significant, but slight increase (average values are greater than control) in heart rate with

Table 1
The effect of 50% isovolemic exchange transfusion on arterial systolic blood pressure and heart rate

Hematocrit	Blood pressure (mm Hg)		Heart rate (min^{-1})	
	HAF	Dextran	HAF	Dextran
46	117.5 ± 5.0	115.6 ± 2.2	324 ± 8.0	318 ± 2.5
40	115.0 ± 4.2	114.3 ± 3.7	320 ± 5.0	317 ± 3.0
34	115.4 ± 4.0	113.7 ± 4.5	321 ± 9.5	310 ± 6.0
28	114.0 ± 5.0	111.9 ± 4.6	320 ± 6.0	313 ± 7.5
23	116.3 ± 4.2	111.7 ± 4.9	319 ± 7.5	304 ± 7.5

Table 2
Stability studies

Days	Total Hb (g dl^{-1})	Hb desorbed (%)	met-Hb (%)	P_{50} (mm Hg)	Hill's coefficient, n
1	14.32	0.56	3.61	28.4	2.65
7	14.24	0.73	8.34	28.3	2.54
15	14.15	1.02	9.50	27.7	2.50
30	14.06	2.34	10.40	27.0	2.32

HAF formulation was observed. This data, however, is of less significance, as the dextran solution, which was used as control, did not show negative effect either. It can nevertheless be concluded from the two studies that the aquasome formulation has potential for use as oxygen-carrying system.

3.4. Stability studies

The data demonstrating stability of the formulation is shown in Table 2. There was almost no desorption of hemoglobin from the formulation. The met-hemoglobin content was increased to $\sim 10\%$. The freeze-thaw (4–25 °C) five 24 h cycles show less than $\sim 3\%$ met-hemoglobin formation, which suggests refrigerated storage condition for the formulation. The P_{50} and Hill coefficient values were also almost constant over the period of 30 days indicating the oxygen-binding characteristics of the aquasome formulation were retained. The suggested mechanism for improved stability is that the carbohydrate (trehalose) form a pseudo-hydrated layer with surface water surrounding protein and stabilize the tertiary structure of hemeprotein molecule (Masatoshi and Yongning, 1998).

4. Conclusion

Therefore, it can be concluded that half generation PAMAM dendrimer modifies crystallization of calcium phosphate from SBF as a function of pH and phosphate saturation. Highly amorphous and spherical calcium phosphates are obtained in which the presence of dendrimer was detected. The characteristic shapes and morphology of hydroxyapatite precipitates reported here are quite different from particles obtained from other templates reported in literature and may be of interest to biomaterial scientists. The hemoglobin aquasomes prepared using hydroxyapatite cores are submicron sized, have good hemoglobin loading capacity, stable in terms of hemoglobin desorption, met-hemoglobin formation and oxygen-binding characteristics both in-vitro and in-vivo, thus may be of interest to pharmaceutical scientist as potential artificial oxygen carrying system.

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