Synthesis, characterization, and blood compatibility of polyamidoamines copolymers

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Abstract

This work reports the development of new non-thrombogenic polymers based on the linear polymers of polyamidoamines (PAAs), having heparin binding ability, obtained by polyaddition of secondary amines to N,N\textsuperscript{0}-methylene bis-acrylamide. PAAs could not be used directly in the making of blood-contacting materials due to their poor mechanical strength. In order to overcome this lacuna, copolymers of amidoamine with methylmethacrylate (MMA) were prepared. Characterization studies indicated that the PAAs have been suitably incorporated into the MMA matrix. The relative hydrophilic nature of the synthesized copolymers was established from the measurement of water contact angle. The heparinized copolymers showed significant improvement in non-thrombogenic characteristics.

Keywords: Hemocompatibility; Hemolysis; Heparin; Polyamidoamine; Blood compatible polymers

1. Introduction

Blood-contacting biomaterials and artificial organs such as artificial blood vessels, pumps, and artificial heart require improved blood compatibility for clinical uses. At present, the most serious performance problem is surface-induced thrombus formation immediately after implantation of materials in the living system. A substantial part of the current research on biomaterials is focused on the design and preparation of polymers with perfect or near-perfect blood compatibility. It is the surface of a biomaterial, which first comes into contact with the living body when the biomaterial is placed in the body or fresh blood. Therefore, the initial response of the living body to the biomaterial depends on its surface properties [1-3]. Many strategies have been adopted for the synthesis of blood-compatible polymers. One of the methods involves the introduction of highly hydrophobic groups to a hydrophilic polymer [4-6]. Other approaches include use of biological anti-coagulants such as heparin, prostaglandin, urokinase and, chemical modifications [7-9]. Heparin, a heterodisperse mucopolysaccharide, has been extensively used in prophylaxis and treatment of thrombotic disorders [10]. Heparin-immobilized polymers have been shown to improve blood compatibility because of the bioactivity of grafted heparin. To avoid excess heparin administration, many researchers have attached heparin to polymer surface by ionic or covalent bonding [11-13]. It has been shown that polyamidoamines (PAAs) possess the ability to selectively absorb heparin from plasma or blood giving stable complexes without any adverse effect on plasma proteins and blood cells [14,15]. PAAs prepared by nucleophilic addition of diamines to acryloyl piperazine (Pip) had shown little significance in biomedical fields due to their poor mechanical properties [16]. Tanzi et al. [17] synthesized a poly-ether-urea-urethane by copolymerization of 4,4\textsuperscript{th}-diphenylmethanediisocanate (MDI), propanediamine, and poly-oxytetramethylene glycol. Similarly differentially terminated poly(amideamine) (PAA) oligomers were grafted on the surface of poly(ether urethane amide)s (PEUAm) with fumaric or maleic acid moieties by Michael type addition of amino-groups to activated double bonds in the PEUAm backbone and the
materials displayed enhanced heparin adsorption ability [18]. Their heparin adsorption ability, blood compatibility, and cytotoxicity have been evaluated. PAAs were also obtained in a linear form by stepwise polyaddition of primary monoamines or bis(secondary amines), to bis-acrylamides, and endowed with heparin-complexing ability [19]. Azzuoli et al. [20] grafted PAA chains onto the surface of polyurethane. Heparin formed complexes onto the surface with PAA-g-polyurethane, which improved the blood compatibility of polyurethane. Barbucci et al. [21] coated different commercial materials such as polyurethane, polyvinyl chloride, glass, etc., with well-characterized biomaterials PUPA (a copolymer of polyurethane and PAA) to improve hemocompatibility. In vitro results of heparin-coated PUPA devices offered significantly improved blood compatibility [22]. These PUPA surfaces were capable of adsorbing large amounts of heparin due to the basic nitrogens of poly(amidoamine), which once protonated, electrostatically interact with the negative charges carried by the heparin molecules. Yang et al. [23] had grafted dimethyl-aminoethylmethacrylate (DMAEMA) onto styrene-butadiene-styrene (SBS) tri-block copolymer membrane by UV-radiation induced graft copolymerization. The amount of adsorption of albumin and fibrinogen decreased in the graft amount and the heparin content. The blood compatible characteristics of the polyamides had been improved by introducing amidoamine groups in the polymer backbone [24]. Heparinization of the block copolymers had shown a significant improvement in blood compatibility as evident from thrombus formation and hemolysis study. It appears, therefore, that method of heparinization may be one of the best-adopted techniques to prepare the anti-thrombogenic polymer.

In the present study, attempts have been made to synthesize two different PAAs derived from polyaddition reaction of Pip and cyclohexylamine (CHA) separately with N,N0-methylene bis-acrylamide (MBA). Subsequent copolymerization of these PAAs with methylmethacrylate (MMA) along with their physicochemical characterization and thrombogenicity of the polymers are reported.

2. Materials and methods

2.1. Reagents

MBA (E. Merck, Germany), Pip, and CHA (SRL, India) were used as such. MMA (CDH, India) was made inhibitor free using 2% NaOH solution and by subsequent distillation. Glutaraldehyde (25% solution) of Reidel-de-Haen, Germany was used. Sodium salt of heparin (1000 IU) was obtained from Gland Pharma (India). All other chemicals of used in the study such as, acetone, toluene, sodium hydroxide, methanol, benzoyl peroxide, etc., were of laboratory grade.

2.2. Instrumentations

The FTIR spectra were recorded in Perkin-Elmer spectrophotometer model 1800 in the range 4000-500 cm⁻¹, in the KBr phase. The 1H NMR spectra were recorded in CDCl₃, 300 MHz 1H NMR (Bruker DRX 300) instruments. The 13C NMR spectra were obtained from VXR-300-S Varian Supercon NMR spectrophotometer operating at 75 MHz. The DSC spectra were taken in Perkin-Elmer DSC 7 instruments at the heating rate of 10°C/min, under nitrogen atmosphere. Contact angle formed by a drop of distilled water on the surface of copolymers films was determined using Rame goniometer model 100-00-230. The hemolysis study was done using UV-VIS spectrophotometer model UV-160A (Shimadzu).

2.3. Syntheses

2.3.1. Synthesis of poly amido amines and copolymers

In order to obtain PAAs containing vinyl group and high molecular weight, we have attempted to synthesize PAAs by the following procedure. The PAAs were synthesized by taking the monomers in 1:1 ratio, which is described as follows.

Vinyl group containing PAA was prepared by dissolving 7.7 g of MBA and 4.19 g of Pip (1:1 ratio) in 30 ml double distilled water. The reaction mixture was stirred under nitrogen atmosphere for 48 h at 30°C. The viscous solution thus obtained was poured into 50 ml of acetone. The PAA, Pip-MBA, was separated out as a white crystalline product, which was filtered, washed with acetone and dried under vacuum. The product yield was nearly 71%.

Similarly, another vinyl group containing PAA was prepared using 7.7 g of MBA and 3.98 g of CHA (1:1 ratio) in 40 ml double distilled water. The solution was stirred under nitrogen atmosphere for continuously 32 h at 30°C. Pouring the resulting viscous solution to 50 ml of acetone resulted in separation of white crystalline product, CHA-MBA, which was filtered, washed with acetone and dried under vacuum. The yield was nearly 70%.

Both the PAAs obtained above were copolymerized separately with MAA using radical polymerization route. In both the cases, 2.0 g of PAAs were taken in 30 ml of toluene and agitated for 30 min at room temperature for complete dissolution of the PAAs. Then 20 ml of purified MMA (inhibitor free) was added in portion to the reaction flask followed by addition of 0.01 mol of benzoyl peroxide as initiator. The entire mixture was agitated under nitrogen atmosphere for nearly 3 h in water bath carefully monitoring the bath...
temperature to 70 ± 5°C. Then the mixture was poured into cold methanol in which the copolymer of PAA-MMA was precipitated out. It was washed several times with methanol, and collected by scarping with glass rod from the bottom of the reaction vessel. The reaction scheme and the probable structure of the copolymer were shown in Fig. 1. Homopolymers of polymethylmethacrylate (PMMA) was also prepared using similar procedure for comparing the properties of synthesized copolymers with that of control. However, it was observed that the time taken for the formation of the copolymer is less than that of formation of PMMA.

Attempt was also made to synthesize a PAA only from Pip and MBA by taking little excess of MBA monomer (nearly 10% excess in comparison to 1:1 ratio) [18]. Subsequently, it was copolymerized with MMA by following above procedure to get Pip-MBA-MMA-10. Only the contact angle measurement and hemolysis were done on this polymer surface, which could be served as comparative information for future use.

2.4. Viscosity measurement

Viscosity measurements of polymer solutions were carried out with an Ubbelohde viscometer using acetone/toluene as solvents at 26 ± 0.5°C. The flow time was measured for solutions at six different concentrations. The intrinsic viscosity was calculated by plotting specific viscosity, $\eta_{sp}$, at various concentrations versus concentrations (C) of solutions and then extra-plotting to zero concentration.

2.5. Preparation of heparinized copolymers films

Thin films of copolymers were prepared by dissolving copolymers in toluene at room temperature and subsequently spreading the solution over the glass plate. The solvent was then evaporated using vacuum evaporator and the resulting thin film was carefully removed from the glass plate by keeping it in distilled water for 10 h. The thickness of the film varied between 0.2 and 0.3 mm.

Fig. 1. Reaction Scheme for the synthesis of PAA and copolymer.
Films of the copolymers were washed with water and ethanol and equilibrated with saline solution (0.5% NaCl solution in water) for 12 h. Sodium salt of the heparin (1000 IU) was diluted 20 times with 1:1 water/ethanol mixture. Films were treated with dilute heparin sodium solution at room temperature for 12 h. Treated films were immersed in 2% aqueous solution of glutaraldehyde solution for 2 min, washed with water followed by ethanol and dried at room temperature (27 ± 0.5°C) in vacuum desiccator.

2.6. Thrombogenicity assay

Thrombus formation studies were carried out using in vitro 'kinetic method' developed by Imai and Nose [25]. ACD human blood was used for this purpose. ACD blood was prepared by adding 1 ml of acid citrate dextrose (ACD) solution to 9 ml of fresh human blood. ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of trisodium citrate dihydrate and 1.84 g of dextrose monohydrate, to 75 ml of distilled water. Before conducting the test, polymer films were hydrated to equilibrium in saline water (0.5% sodium chloride solution in water) and kept at 37°C in a constant temperature water bath in Petri dishes. ACD human blood (0.2 ml) was placed onto each film. Blood clotting was initiated by adding 0.02 ml of M/10 calcium chloride solution and mixing it properly by teflon stick. Clotting process was stopped by adding distilled water (5 ml) after 15, 30, 45 and 60 min. Clot formed was fixed in 36% formaldehyde solution (5 ml) for 5 min. Fixed clot was washed with distilled water and blotted between tissue paper and weighed.

2.7. Hemolysis assay

Heparinized polymer films were cut into small pieces and the samples were equilibrated in normal saline water for 30 min at room temperature (32°C). Weight of each film was taken after drying it in air after which ACD human blood (0.2 ml) was added to each sample. After a predetermined time period, 4 ml of saline water added to each sample to stop hemolysis and those samples were kept at constant temperature (35°C) for 1 h. Positive and negative controls were produced by adding 0.2 ml of human blood to 4 ml of distilled water and saline water, respectively. All the samples were centrifuged. Optical density of the supernatant was measured at 545 nm. The percent of hemolysis was calculated as follows:

\[ Vo \text{ hemolysis} = \frac{[\text{OD of test sample} - \text{OD(-) control}]}{[\text{OD(+) control} - \text{OD(-) control}]} \times 100. \]

3. Results and discussions

3.1. Solubility and intrinsic viscosity

The freshly prepared polymer (5 mg) was suspended over 10 ml of the chosen solvent and the solubility was checked after 2 h. It was found that (Table 1) PA As were soluble in water almost immediately at room temperature whereas the corresponding copolymers were insoluble in water. Toluene served as an ideal solvent for dissolving both the PAAs and copolymers along with homopolymer PMMA. In acetone, and chloroform, however, a typical behaviour was noticed. In acetone the PAAs were insoluble, whereas, the copolymers along with homopolymer were soluble. In chloroform, however, the PAAs and homopolymer were soluble except the copolymers, which were soluble on heating. These observations conclude the fact that upon copolymerization, the molecular arrangements and the degree of cross-linking within the domain of the polymer chain may varies to certain extent. The low solubility of the copolymers may be expected to display a marked tendency to phase separation depending upon the composition of these materials. It was also physically observed that the films of copolymers casted from acetone were brittle in nature and having less mechanical strength, whereas the same copolymers casted from toluene were soft with improved mechanical strength. The values of intrinsic viscosities in acetone were

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>PMMA (control)</td>
<td>ns</td>
</tr>
<tr>
<td>Pip-MBA</td>
<td>s</td>
</tr>
<tr>
<td>Pip-MBA-MMA</td>
<td>ns</td>
</tr>
<tr>
<td>CHA-MBA</td>
<td>s</td>
</tr>
<tr>
<td>CHA-MBA-MMA</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: s, soluble; ns, not soluble; sh, soluble on heating; PMMA, Polymethylmethacrylate; Pip, Piperazine; MBA, N,N0-methylene bis-acrylamide; CHA, Cyclohexylamine; MMA, methylmethacrylate.
checked for the copolymers and the values have been found to be 0.247 and 0.172 dl/g for Pip-MBA-MMA and CHA-MBA-MMA copolymers, respectively. The intrinsic viscosities data for PAA (Pip-MBA), and PMMA in toluene have been found to be 0.232 and 0.265 dl/g, respectively.

3.2. Spectroscopic characterization

3.2.1. Analysis of IR spectra

The IR spectrum (Fig. 2) of the PAA (Pip-MBA) showed the characteristic amide I and amide II linkages at 1624 and 1537 cm\(^{-1}\), respectively. The two amide bands resulted from the electronic coupling of \(\wedge \text{C}=\text{O}\) and \(\wedge \text{C}-\text{N}\) modes and mechanical coupling of \(\wedge \text{C}-\text{N}\) and in plane \(\text{dNH}\) mode. The peak intensities appear at 1906, 1624, and 951 cm\(^{-1}\) assigned to possible presence of terminal vinyl group unit in the PAA [26], found to be either vanished or diminished in intensities in the copolymer (Pip-MBA-MMA) suggesting that the copolymerization had taken place through the vinyl group units. The Pip-MBA-MMA copolymer showed sharp characteristic peaks at 1733, 1195, and 1148 cm\(^{-1}\) for the presence of COOCH\(_3\) groups from monomer MMA unit [27]. In addition, a small peak of weak intensity appeared at 1340 cm\(^{-1}\), which is assigned to the presence of CH group in the copolymer.

The spectra of CHA-MBA showed the peaks at 3389, 1575, and 1478 cm\(^{-1}\), which were the characteristic peaks for secondary amide group. The amide I and amide II linkages were observed at 1615 and 1545 cm\(^{-1}\), respectively. The presence of vinyl group was characterized by the presence of peaks at 1630, 966, and 925 cm\(^{-1}\). Upon copolymerization, the resulting polymer (CHA-MBA-MMA) showed characteristic peaks at 1743, and broad peak within the range 1200-1100 cm\(^{-1}\) assigned to COOCH\(_3\) groups.

3.2.2. Analysis of \(^1\)H NMR spectra

The \(^1\)H NMR spectrum (Fig. 3) of Pip-MBA showed the peak at 7.26 ppm (d) ascribed to the secondary amide group in the structure. Characteristic higher order pattern was observed for vinylic group protons within the range 5.6-6.3 ppm. The -CH\(_2\)- group bonded to nitrogen observed in the range 4.64-4.78 ppm. Peaks in the range 2.53-2.65 ppm were the characteristic peaks of the -CH\(_2\)- bonded to carbonyl group.

The \(^1\)H NMR spectrum of Pip-MBA-MMA showed reduction of intensity of amide proton peaks in addition to the reduction of intensities of vinyl group protons [28]. Peak at 3.75 ppm showed the presence of CH proton. The methyl ester protons of the monomer MMA unit resonate at 3.60 ppm. The peaks in the range 2.35-1.44 and 1.21-0.84 ppm were ascribed to the presence of methylene and methyl groups, respectively.

Similarly, the \(^1\)H NMR spectrum of CHA-MBA showed the presence of secondary amide group proton at 7.2 ppm. The presence of methylene groups alpha to nitrogen atom were showed as triplet in the range 2.64-2.58 ppm. At high field the methylene groups alpha or beta to carbonyl group overlap to form complex set of multiplet in the range 1.83-1.48 ppm. The cyclohexyl group methylene protons showed at complex set of multiplet in the range 1.8-1.01 ppm.

On the other hand, in the copolymer CHA-MBA-MMA, the secondary amide group protons observed at 7.26 ppm. The methine proton was observed at 3.70 ppm overlapping with OCH\(_3\)- group protons at 3.60 ppm. Other characteristic peaks of MMA and cyclohexyl group protons appeared in the range 2.35-0.84 ppm as complex set of multiplets.
3.2.3. Analysis of $^{13}$C NMR spectra

The $^{13}$C NMR spectra (Fig. 4) of Pip-MBA showed peak at 165.1 ppm ascribed to the -CONH carbon atom. The vinyl group protons present in the PAA's were observed at 130.9 and 125.2 ppm as sharp peaks. Peak at 78.0 ppm corresponds to the carbon of the deuterated chloroform used for dissolving the sample. Peak at 51.7 ppm corresponds to the carbons of the Pip ring. Other peaks within the range 43.1-32.2 ppm referred to various aliphatic group carbons present in the PAA moiety.

On the other hand, the $^{13}$C NMR spectra of Pip-MBA-MMA showed peaks within the range 178.2—176.0 ppm corresponds to the presence of -CONH group and the carbonyl carbon atoms of the ester groups present in MMA. The reduction of intensities of vinyl group carbon observed within the range 128.9-125.1 ppm indicating the fact of participation of vinyl group in the copolymerization process [28]. The presence of methine carbon was observed at 21.3 ppm as small peak. Hill et al. [29] while studying the copolymerization of MMA and diethylene glycol bis-(allyl carbonate) observed the -CH peak at around 30 ppm. Peak observed at 16.3 ppm corresponds to the carbon of the methyl group present in the MMA matrix [30].

3.3. DSC analysis

The DSC spectra were run at a heating rate of 10°C/min (60-360°C) under nitrogen atmosphere for the PAA, Pip-MBA, and its corresponding copolymer, Pip-MBA-MMA, to study their thermal behaviour. For the PAA Pip-MBA, a transition was observed in the range 180-200°C, which could be attributed to the melting of microcrystallites. Beyond 210°C, the material continued to absorb heat and decomposed. The $T_g$ value for the Pip-MBA could not be detected within the range of study. For the copolymer Pip-MBA-MMA, the glass transition temperature was observed as a small endotherm at 116°C. No well-defined melting peaks could be observed in the copolymer.

3.4. Evaluation of surface properties

Interaction of implant materials with tissues and physiological fluids stimulates body's defense mechanism.
The surface of implant material plays a key role in determining the nature of immunological reaction. Surfaces of copolymer films were modified by heparinization. Surface characterization evaluated by contact angle measurements and thrombogenicity assay studies.

3.4.1. Measurement of contact angle

The contact angle measurements data used to evaluate the relative hydrophilicity and hydrophobicity of the synthesized polymers in contact with water. The water contact angle was measured by putting a sessile drop of distilled water on the surface of the polymer. Measurements were made after an equilibration time of 2 min. The water contact angle measurement data was summarized in Table 2. The data showed that copolymers of Pip-MBA and CHA-MBA possess relative hydrophilic characteristics with respect to PMMA surface. Upon heparinization, the surface acquired a hydrophobic characteristic as evident from the increase in contact angle by 10°. The hydrophobic characteristic of the copolymers may be attributed to the presence of hydrophobic aliphatic groups of the glutaraldehyde moiety, which was used during the process of heparinization of the copolymers.

Also, the contact angle measurement data for the copolymer Pip-MBA-MMA-10 also showed relative hydrophilic characteristics with respect to corresponding heparinized copolymer.

In addition, the contact angle measurement data also serve as a strong evidence for the presence of PAA s in the matrix of PMMA through covalent bonding process. The water contact angle measurement was done in control PMMA, and in a separate experiment, PAA was mixed physically with PMMA matrix and the film was casted. In clear contrast to the double bond containing copolymer, PMMA and physical mixture of PMMA and PAA retained the same water contact angle. Characteristic of these findings clearly indicated that PAA chains were covalently bound to the MMA matrix through reacting double bond backbone.

3.4.2. Thrombogenicity assay

The weight of the blood clot formed at intervals of 15, 30, 45, and 60 min with various heparinized copolymer films along with homopolymer PMMA were shown in Table 3. It was observed that the heparinized PAA-MMA copolymer films showed decrease in the weight of the clot. No blood clotting was observed up to 30 min time period. Even after 60 min time period, the weight of the clot formed was very less. It is known that surface properties play an important role at molecular level in surface-induced thrombosis. The inhibition of blood clotting properties of the heparinized copolymer may be due to the adverse interaction of blood components via protein denaturation. Ishihara et al. [31] have recently showed that increase in hydrophilicity does not have similar effect on blood compatibility. Similarly, adsorption of plasma proteins on hydrophobic polymeric surfaces had shown a more relevance to the protein layer/polymer interface than to protein layer/solution interface [32]. In our case, the improvement in blood compatibility of the PAA copolymers might be due to the presence of amine groups, which absorbs heparin, thus inhibiting the clotting process. These results were in agreement with the findings of earlier workers for various PAA s [20,33]. Similar effect had not been found on heparin treatment of homopolymer, i.e., PMAA film. A very small decrease in blood clotting observed in

Table 2
Determination of contact angle of the materials

<table>
<thead>
<tr>
<th>Material surface</th>
<th>Contact angle (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth glass</td>
<td>50-52</td>
</tr>
<tr>
<td>PMMA</td>
<td>55-58</td>
</tr>
<tr>
<td>Pip-MBA-MMA</td>
<td>52-54</td>
</tr>
<tr>
<td>Pip-MBA-MMA (heparinized)</td>
<td>63-65</td>
</tr>
<tr>
<td>CHA-MBA-MMA</td>
<td>53-54</td>
</tr>
<tr>
<td>CHA-MBA-MMA (heparinized)</td>
<td>64-66</td>
</tr>
<tr>
<td>PMMA + Pip-MBA (physical mixture)</td>
<td>56-58</td>
</tr>
<tr>
<td>Pip-MBA-MMA-10 (10% extra MBA)</td>
<td>50-52</td>
</tr>
<tr>
<td>Pip-MBA-MMA-10 (heparinized)</td>
<td>57-58</td>
</tr>
</tbody>
</table>

Note: PMMA, polymethylmethacrylate; Pip, piperazine; MBA, N,N0-Methylene bis-acrylamide; CHA, cyclohexylamine; MMA, methylmethacrylate.

Table 3
Determination of relative thrombogenicity of the materials by measuring weight of the thrombus formed

<table>
<thead>
<tr>
<th>Polymer samples</th>
<th>Weight of the thrombus formed (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>PMMA film</td>
<td>1.0 7 0.02</td>
</tr>
<tr>
<td>PMMA-HEP (control)</td>
<td>0.6 7 0.1</td>
</tr>
<tr>
<td>Pip-MBA-MMA</td>
<td>0.2 7 0.05</td>
</tr>
<tr>
<td>Pip-MBA-MMA-HEP</td>
<td>0</td>
</tr>
<tr>
<td>CHA-MBA-MMA</td>
<td>0.16</td>
</tr>
<tr>
<td>CHA-MBA-MMA-HEP</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: PMMA, polymethylmethacrylate; Pip, piperazine; MBA, N,N0-methylene bis-acrylamide; CHA, cyclohexylamine; MMA, methylmethacrylate.
Table 4  
<table>
<thead>
<tr>
<th>Sample</th>
<th>Optical density at 545 nm</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>2.24</td>
<td>+ve control</td>
</tr>
<tr>
<td>Saline</td>
<td>0.01</td>
<td>-ve control</td>
</tr>
<tr>
<td>Pip-MBA-MMA</td>
<td>0.24</td>
<td>10.3</td>
</tr>
<tr>
<td>Pip-MBA-MMA (heparinized)</td>
<td>0.09</td>
<td>3.58</td>
</tr>
<tr>
<td>CHA-MBA-MMA</td>
<td>0.29</td>
<td>12.55</td>
</tr>
<tr>
<td>CHA-MBA-MMA (heparinized)</td>
<td>0.10</td>
<td>4.12</td>
</tr>
<tr>
<td>Pip-MBA-MMA-10</td>
<td>0.20</td>
<td>8.40</td>
</tr>
<tr>
<td>Pip-MBA-MMA-10 (heparinized)</td>
<td>0.08</td>
<td>3.56</td>
</tr>
</tbody>
</table>

Note: PMMA, polymethylmethacrylate; Pip, piperazine; MBA, N,N'-methylene bis-acrylamide; CHA, cyclohexylamine; MMA, methylmethacrylate.

helin treated PMMA film can be attributed to loosely held heparin, which remains on the films after washing.

3.4.3. Hemolysis  
Hemolysis of the blood is the problem associated with bio-incompatibility [34]. Red blood cells hemolysed when come in contact with water. This problem may be aggravated in the presence of an implant material. Results obtained for hemolysis of ACD blood with heparinized copolymers were shown in Table 4. It was observed that hemolysis was less than 5% in all the cases, which comes well within permissible limit [35]. Hence, it may be conclude that the synthesized copolymers may be suitable as biomaterials for specific application purpose. However, attempt is in progress to further improve the incorporation of PAAs in suitable moieties so as to improve its hemolysis values in comparison to other reported values [24,36].

4. Conclusion  
Two new polyamidoamines consisting of piperazine-N,N0-methylene bis-acrylamide (Pip-MBA) and cyclohexylamine-N,N0-methylene bis-acrylamide (CHA-MBA) having heparin binding capability were synthesized and subsequently copolymerized with methyl methacrylate (MMA) under suitable reaction conditions to yield two copolymers (Pip-MBA-MMA and CHA-MBA-MMA). The solubility behaviour of the synthesized polymers showed marked difference in their properties with respect to their compositions. Observation of increase in water contact angle data of the materials established the relative hydrophilic nature of the copolymers with respect to PMMA surface. The heparinized copolymer films had shown a little thrombus formation (0.22mg) within 60min time period and the percentage of hemolysis in heparinized copolymer showed a value less than 5.0% when compared. Use of 10% excess MBA in the synthesis of polyamidoamine, and hence the copolymer (Pip-MBA-MMA-10) for comparison, showed no difference in surface characteristics as well as hemolysis value as evident from contact angle measurement and evaluation of hemolysis percentage.

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