Synthesis, Characterization, and Blood Compatibility of Copolymers of Polyamidoamines and *N*-Vinylpyrrolidone

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ABSTRACT: Two new polyamidoamines derived from piperazine (Pip)/cyclohexylamine (CHA) and *N*,*N*′-methylene bisacrylamide (MBA) were synthesized and subsequently copolymerized with *N*-vinylpyrrolidone (NVP) under suitable reaction conditions to yield the respective copolymers (Pip–MBA–NVP and CHA–MBA–NVP). The synthesized materials were characterized by spectroscopic techniques. The material surface characteristics were checked by contact angle measurement, and the data established the relative hydrophilic characteristics of the synthesized copolymers with respect to the control poly(*N*-vinylpyrrolidone). A thrombus-formation study indicated less (<1.2 mg) clot formation on the heparinized material surfaces within a 30-min contact time with the acid citrate dextrose human blood. The percentage of hemolysis of the blood by the materials was also less than 5%. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 4068–4074, 2003

Key words: biomaterials; copolymerization; compatibility; synthesis

INTRODUCTION

Thrombosis is a major complication that occurs when a foreign substance comes in contact with the blood.[1–4] A number of chemicals, bioactive reagents, and different techniques have been investigated to obtain a nonthrombogenic surface.[5–13] A number of polymer surface parameters are responsible for the antithrombogenic properties of synthetic polymer materials, including surface composition and morphology, hydrophilic/hydrophobic character, surface free energy, and surface charges.[14–19] Heparin administration is a quite successful method for preventing blood coagulation. Therefore, another method for making polymers blood compatible is to attach heparin to the polymer surface either by ionic and/or covalent bonding. To avoid excess heparin administration, many researchers have attached heparin to the polymer surface by ionic or covalent bonding.[20–22]

Polyamidoamines (PAAs) possess the ability to selectively adsorb heparin from plasma or blood, giving stable complexes without any adverse effect on plasma proteins and blood cells.[23–24] However, these materials as such have little significance in biomedical fields because of their poor mechanical properties.[25] Azzuoli et al.[26] grafted PAA chains onto the surface of polyurethane, and heparin formed complexes onto the surface with PAA-*g*-polyurethane, which improved the blood compatibility of polyurethane. Similarly Barbucci et al.[27] coated different commercial materials, including polyurethane, poly(vinyl chloride), and glass, with a well-characterized biomaterials copolymer of polyurethane and PAA, to improve hemocompatibility. Differentially terminated PAA oligomers were grafted onto the surface of poly(ether urethane amides) (PEUAm), with fumaric or maleic acid moieties[28] by the Michael-type addition of amino groups to activate double bonds in the PEUAm backbone. These PAA-grafted PEUAm elastomeric biomaterials showed enhanced heparin adsorption capacities. Heparin adsorption capacities were also tested for a segmented polyurethane containing PAA blocks.[29] It appears, therefore, that the method of heparinization may be one of the most suitable techniques for the preparation of antithrombogenic polymers.

In this study, we attempted to synthesize two different PAAs derived from polyaddition reaction of piperazine (Pip) and cyclohexylamine (CHA) separately with *N*,*N*′-methylene bisacrylamide (MBA). The subsequent copolymerizations of these PAAs with *N*-vinylpyrrolidone (NVP), along with their physicochemical characterization and thrombogenicity, are reported.

EXPERIMENTAL

Reagents

MBA (E. Merck, Germany), Pip, and CHA (SRL, India) were used as received. NVP was distilled *in vacuo* to remove the inhibitor. Dimethylformamide (DMF) was distilled before use. Sodium salt of heparin (1000 IU)

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was obtained from Gland Pharma (India). All of the other chemicals used in the study, including acetone, diethyl ether, toluene, methanol, and azobisisobutyronitrile (AIBN), were laboratory grade.

Instrumentations

Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Protege 460 spectrophotometer in the range 4000–500 cm^{-1} (KBr phase). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$, (Bruker Spectrospin 300) instrument. Thermogravimetric analysis (TGA) was carried out in a PerkinElmer thermal analyzer at heating rate of 10°C/min under nitrogen atmosphere. The differential scanning calorimetry (DSC) spectra were recorded in a PerkinElmer DSC 7 instrument at a heating rate of 10°C/min under a nitrogen atmosphere. The contact angle, formed by a drop of distilled water on the surface of the copolymer films, was determined with a Rame goniometer (model 100-00-230). The hemolysis study was done with a PerkinElmer ultraviolet–visible spectrometer (model EZ 201).

Synthesis of PAA

Vinyl-group-containing PAA was prepared by the dissolution of 7.7 g of MBA and 4.59 g of Pip in 30 mL of double distilled water. A little excess (10–15% in excess of the 1:1 ratio) MBA was used for the formation of PAAs. 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 *in vacuo* at 40°C. The product (Pip–MBA) yield was nearly 79%.

Similarly, another vinyl-group-containing PAA was prepared with 7.7 g of MBA and 4.10 g of CHA in 40 mL of double distilled water. Here also, a little excess (~10%) MBA in stoichiometric proportions was used. The solution was stirred under a nitrogen atmosphere continuously for 32 h at 30°C. Pouring the resulting viscous solution to 50 mL of acetone resulted in the separation of the white crystalline product (CHA–MBA). The product was filtered, washed with acetone, and dried *in vacuo* at 40°C. The product yield was nearly 74%.

Synthesis of the copolymers

Both of the PAAs, Pip–MBA–NVP and CHA–MBA–NVP, were copolymerized separately with NVP through the radical polymerization route. In both cases, 3.0 g of the PAA was dissolved in 20 mL of DMF. NVP (10 mL, distilled) was added in portion to the three-necked reaction vessels followed by the addition of 0.5% of initiator (AIBN). The reaction was

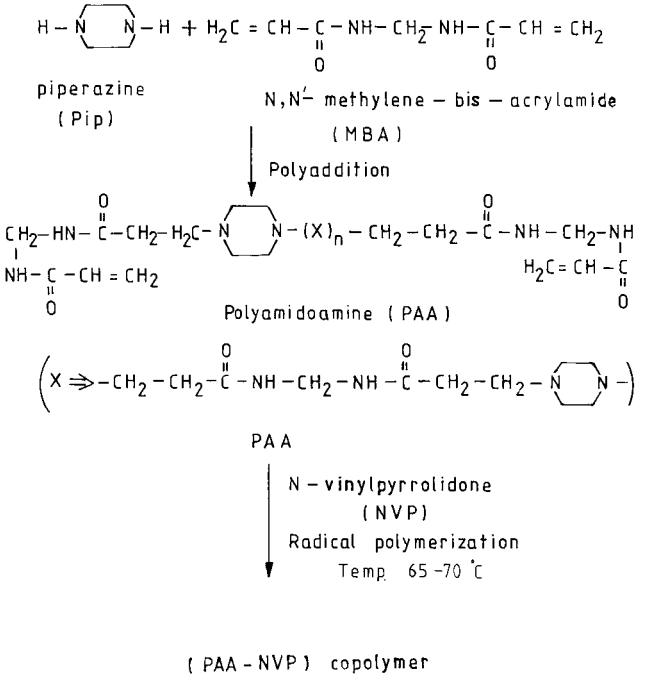

Figure 1 Reaction scheme for the synthesis of PAA and its copolymer.

carried out with the reaction vessel kept in a thermally controlled water bath at 70–75°C for about 45 min. Then, the mixtures were poured into cold diethyl ether, in which the resulting copolymers (Pip–MBA–NVP and CHA–MBA–NVP) were precipitated out. The products were washed thoroughly with acetone to remove any unreacted PAA content and were then washed with diethyl ether. No precipitation of PAAs was observed, whereas washing with acetone indicated the successful copolymerization of the PAAs with NVP, which was later confirmed with spectroscopic observations and contact angle measurement data. The homopolymer of poly(*N*-vinylpyrrolidone) (PNVP) was also prepared with a similar procedure for the comparison of the properties of the synthesized copolymers with that of a control. The schematic diagram of the synthetic procedure is presented in Figure 1.

Viscosity measurements

Viscosity measurements of the polymers solutions were carried out with an Ubbelohde viscometer using methanol as a solvent at 29 ± 0.5°C. The flow time was measured for solutions at six different concentrations. We calculated the intrinsic viscosity by plotting the specific viscosity at various concentrations versus the concentrations of the solutions and then extraplotting to zero concentrations.

Preparation of the heparinized copolymer films

Thin films of the copolymers were prepared by dissolution of the copolymers in methanol at room temper-

TABLE I
Solubilities of the Polymers in Different Solvents

Polymer	Solubility						
	Water	Methanol	Acetone	Chloroform	Tolune	THF	DMF
PNVP (control)	s	s	ps	s	s	s	s
Pip–MBA	s	ns	ns	s	s	ps	ns
Pip–MBA–NVP	s	s	ns	s	ns	ps	s
CHA–MBA	s	s	ns	s	s	ps	s
CHA–MBA–NVP	s	s	ps	s	s	ps	s

THF = tetrahydrofuran; s = soluble; ns = not soluble; ps = partially soluble.

ature and, subsequently, the spreading of the solution over a glass plate. The solvent was then evaporated with a vacuum evaporator, and the resulting thin film was carefully removed from the glass plate. Films were then treated with dilute heparin solution at room temperature for 5–10 min and dried at 30°C in a vacuum desiccator.

Thrombogenicity assay

Thrombus formation studies were carried out with the *in vitro* kinetic method developed by Imai and Nose.[30] Acid citrate dextrose (ACD) human blood was used for this purpose. ACD blood was prepared by adding 1 mL of ACD solution to 9 mL of fresh human blood. ACD solution was prepared by the mixture of 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. ACD human blood (0.2 mL) was placed onto each film. Blood clotting was initiated by the addition of 0.02 mL of M/10 calcium chloride solution and proper mixing by a Teflon stick. The clotting process was stopped by the addition of a drop of distilled water (5 mL) after 10, 20, and 30 min. The clot formed was fixed in 36% formaldehyde solution (5 mL) for 2–3 min. The fixed clot was blotted between tissue paper and weighed.

Hemolysis assay

The polymer films were cut into small pieces, and the weight of each film was taken. ACD human blood (0.2 mL) was added to each sample. After a predetermined time period, 1 mL of saline water was added to each sample to stop hemolysis, and those samples were kept at a constant temperature (35°C) for 1 h. Positive and negative controls were produced by the addition of 0.2 mL of human blood to 4 mL of distilled water and saline water, respectively. All of the samples were centrifuged. The optical density (OD) of the supernatant was measured at 545 nm. The percentage hemolysis was calculated as follows:

$$\% \text{ Hemolysis} = (\text{OD of test sample} - \text{OD}^{-} \text{ control}/\text{OD} + \text{control} - \text{OD}^{-}\text{control}) \times 100$$

RESULTS AND DISCUSSION

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 1 h (Table I). The difference in solubility behavior of the materials was expected to display a difference in molecular arrangements and degree of crosslinking on copolymerization, depending on the composition of these materials. The values of intrinsic viscosities were 0.3625 and 0.3667 dL/g, respectively, for Pip–MBA–NVP and CHA–MBA–NVP.

Analysis of the FTIR spectra

The FTIR 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 the $\mu_{C=O}$ and μ_{C-N} modes and the mechanical coupling of the μ_{C-N} and in-plane δ_{NH} mode. The peak intensities appeared at 1906, 1624, and 951 cm^{-1}, assigned to presence of a terminal vinyl group unit in the PAA, found to be either vanished or diminished in intensities in the copolymer (Pip–MBA–NVP), which suggested that the copolymerization had taken place through the terminal vinyl group units.[31] The Pip–MBA–NVP copolymer showed a broad absorption at 3494 cm^{-1}, assignable to the hydrogen-bonded O—H/N—H stretching frequency. A peak appeared at 2953 cm^{-1} assignable to an sp^3-hybridized —CH_2— group. The shifting of the carbonyl group frequency at 1646 cm^{-1}, along with the strong appearance of C—O and C—N stretching frequencies at 1170 and 1075 cm^{-1}, respectively, suggested the presence of an NVP unit in the polymer structure. The spectra of CHA–MBA showed peaks at 3389, 1575, and 1478 cm^{-1}, which were the characteristic peaks for the secondary amide group. The amide I and amide II linkages were observed at 1615 and 1545 cm^{-1}, respectively. The presence of the terminal vinyl group was characterized by the presence of peaks at 1630, 966, and 925 cm^{-1}. On copolymerization, the resulting polymer (CHA–MBA–NVP) showed characteristic broad peaks at 1739 cm^{-1}

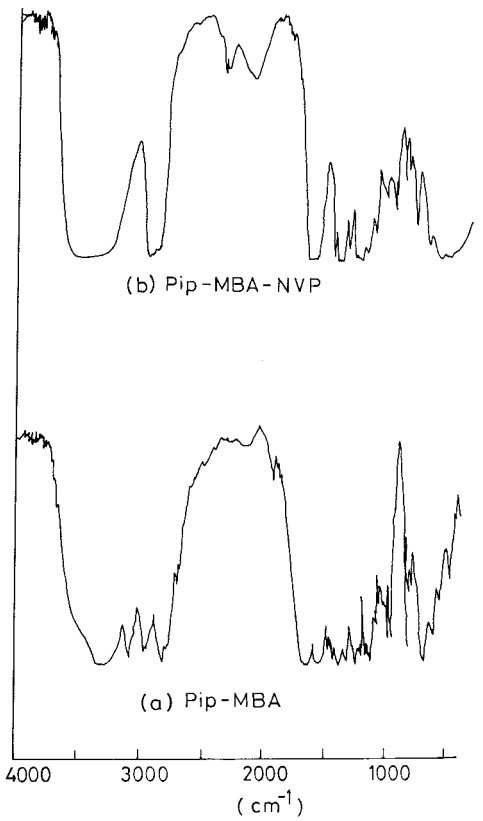

Figure 2 IR spectra of (a) Pip–MBA and (b) Pip–MBA–NVP.

mixed up with amide I and amide II bands at 1644 cm^{-1}. The C—N stretching frequency was observed at 1099 cm^{-1}. The absence of vinyl group frequencies at 990 and 910 cm^{-1} indicated copolymerization through the vinyl group unit.

Analysis of the ^{1}H-NMR spectra

The ^{1}H-NMR spectrum (Fig. 3) of the PAA Pip–MBA showed a peak at 7.26 ppm (d) ascribed to the secondary amide group in the structure. A 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 was 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 a carbonyl group.

The ^{1}H-NMR spectrum of the copolymer (Pip–MBA–NVP) showed a reduction in the intensity of amide proton peaks in addition to the reduction of the intensities of vinyl group protons.[32] A peak at 3.73 ppm represented the chain-methine and ring-methylene groups, which were bonded to the nitrogen atom. The band near 3.22 ppm arose from the ring-methylene group adjacent to the amide carbonyl group. The peaks centered around 2.2 ppm represented the chain-methylene groups and the center methylene group of the pyrrolidone ring. The peaks in the range 2.37–1.42 ppm were ascribed to the presence of methylene groups in the polymer unit.

Similarly, the ^{1}H-NMR spectrum of the PAA CHA–MBA showed the presence of a secondary amide group proton at 7.2 ppm. The presence of methylene groups α to the nitrogen atom were shown as triplet in the range 2.64–2.58 ppm. At high field, the methylene group α or β to the carbonyl group overlapped to form a complex set of multiplets in the range 1.83–1.48 ppm. The cyclohexyl group methylene protons showed a complex set of multiplets in the range 1.8–1.01 ppm.

However, in the copolymer (CHA–MBA–NVP), the secondary amide group protons were observed in the region 7.05–7.14 ppm. A reduction in intensities of the vinyl groups' protons was observed in the region 4.47–4.37 ppm. A peak at 3.73 ppm represented the chain-methine and ring-methylene groups, which were bonded to the nitrogen atom. The peaks near 3.5 ppm arose from the ring-methylene group adjacent to the amide carbonyl group. Other characteristic peaks of NVP and cyclohexyl ring protons appeared in the range 2.96–1.40 ppm as a complex set of multiplets.

Analysis of ^{13}C-NMR spectra

The ^{13}C-NMR spectra (Fig. 4) of Pip–MBA showed a peak at 165.1 ppm, which was ascribed to the

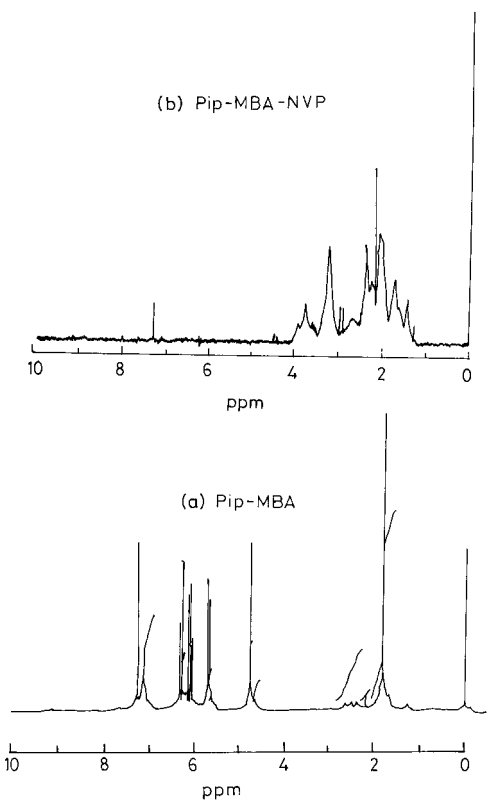

Figure 3 ^{1}H-NMR spectra of (a) Pip–MBA and (b) Pip–MBA–NVP.

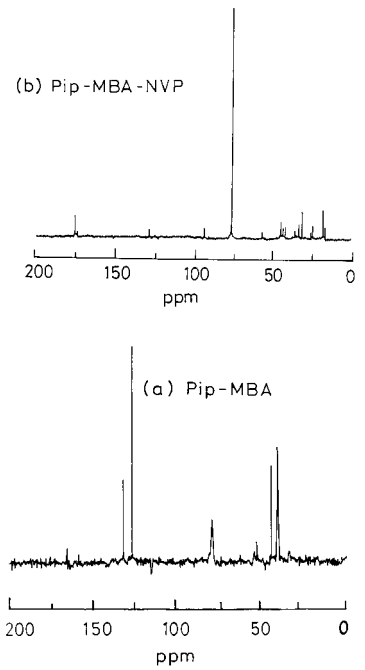

Figure 4 ^{13}C-NMR spectra of (a) Pip–MBA and (b) Pip–MBA–NVP.

—CONH carbon atom. The vinyl group protons present at the two ends of the PAAs were observed at 130.9 and 125.2 ppm as sharp peaks. The peak at 78.0 ppm corresponded to the carbon of the deuterated chloroform used for dissolving the sample. The peak at 51.7 ppm corresponded 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.

However, the ^{13}C-NMR spectra of Pip–MBA–NVP showed a peak at 175.5 ppm, which corresponded to the presence of a —CONH group and the carbonyl carbon atoms of the NVP unit. The intensities of vinyl group carbons were reduced within the range 130–125 ppm, indicating the participation of the vinyl group in the copolymerization process.[32] The peaks observed at 130.0 and 87.0 ppm were assigned to the adjacent methylene carbon nearer to the carbonyl carbon of the NVP unit. The presence of a methine carbon was observed at 32 ppm as a small peak. Hill et al., studying the copolymerization of methylmethacrylate and diethylene glycol bis(allyl carbonate), observed the —CH peak around 30 ppm.[33] Other various aliphatic group carbons were observed in the range 46.0–16.0 ppm.

TGA

The thermograms of the copolymers are shown in Figure 5. Within 120°C, both of the copolymers lost a negligible percentage of their weights, and the weight loss could be attributed to the desorption of solvent molecules from the matrix. However, the percentage of weight loss was faster for Pip–MBA–NVP than for CHA–MBA–NVP. In the range 150–450°C, the percentage of material remained undecomposed, being at 86.91% at 412.4°C and 72.4% at 440.0°C, respectively, for the Pip–MBA–NVP and CHA–MBA–NVP copolymers. The percentage of char yields were 4.717% at 518.9°C and 0.722% at 510.8°C for the Pip–MBA–NVP and CHA–MBA–NVP copolymers, respectively. Up to 450°C, the unfunctionalized free ligands would undergo decomposition and volatilization of the low-molecular-weight fraction would also take place. Beyond 450°C, the loss of complicated anionic fragmentation may have been the predominate cause of rapid degradation. The observation indicated that the thermograms did not provide a clear indication of the relative thermal stabilities of the materials. However, by the employment of various kinetic models, it may be possible to evaluate the relative thermal stabilities of the materials.

DSC analysis

The DSC spectra were run at a heating rate of 10°C/min (in the range 60–360°C) under a nitrogen atmosphere for PAA (Pip–MBA) and its copolymer (Pip–MBA–NVP) to study their thermal behavior. For 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 glass-tran-

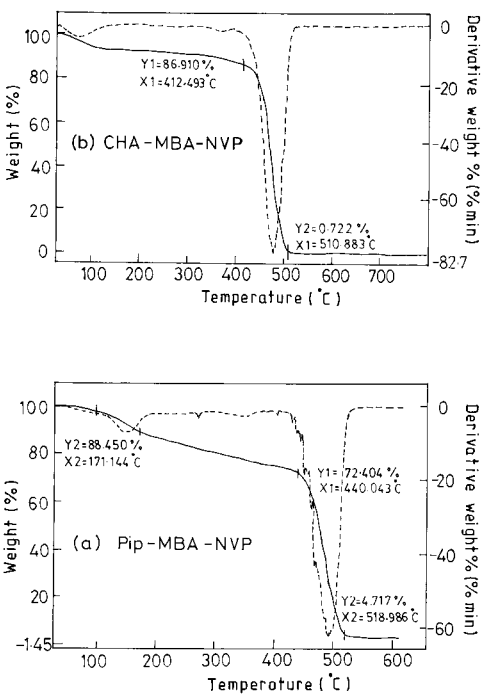

Figure 5 TGA results for (a) Pip–MBA–NVP and (b) CHA–MBA–NVP.

TABLE II
Determination of the Contact Angle of the Materials

Material surface	Contact angle (±2°)
Smooth glass	45
PNVP	39
Pip–MBA–NVP	31
CHA–MBA–NVP	30
PNVP + Pip–MBA (physical mixture)	40

sition temperature for the Pip–MBA was not detected within the range of study. For Pip–MBA–NVP, the glass-transition temperature was also not observed distinctly. However, the exotherm in the range 100–150°C, with an onset at 108.9°C, an end set at 135.7°C, and a peak maximum at 114.4°C, was ascribed to the desorption of solvent molecules along with the onset of molecular motion in the polymer chain. Beyond 135°C, the polymer continued to absorb heat and underwent decomposition.

Evaluation of surface properties

The interaction of implant materials with tissues and physiological fluids stimulates the body's defense mechanisms. The surface of implant material plays a key role in the determination of the nature of immunological reaction.

Measurement of contact angle

The contact angle measurements data were used to evaluate the relative hydrophilicity and hydrophobicity of the synthesized polymers in contact with water. We measured the contact angle by putting a sessile drop of water on the surface of the polymer. Measurements were made after an equilibration time of 2 min. The contact angle measurement data is summarized in Table II. The data showed that Pip–MBA–NVP and CHA–MBA–NVP possessed relatively hydrophilic characteristics with respect to the smooth glass surface.

In addition, the contact angle measurement data also served as a strong evidence for the presence of PAAs in the matrix of NVP through the covalent bonding process. The water contact angle measurement was done in control PNVP, and in a separate experiment, PAA was mixed physically with PNVP matrix, and a film was cast. In clear contrast to the double-bond-containing copolymer, PNVP and the physical mixture of PNVP and PAA retained almost the same water contact angle. The characteristics of these findings clearly indicated that the PAA chains were covalently bound to the NVP matrix through the reaction of the double-bond backbone.

Thrombogenicity assay

The weight of the blood clot formed at intervals of 10, 20, and 30 min with various copolymer films along with the NVP homopolymer are shown in Table III. The heparinized PAA–NVP copolymer films showed a decrease in the weight of the clot. Negligible blood clotting was observed up to 30 min. It is known that surface properties play an important role at the molecular level in surface-induced thrombosis. The inhibition of the blood-clotting properties of the copolymer may have been due to the adverse interaction of blood components via protein denaturation. Ishihara et al.[34] recently showed that an increase in hydrophilicity did not have a similar effect on blood compatibility. In our case, the improvement in the blood compatibility of the PAA copolymers might have been due to the presence of amine groups, which absorbed heparin and thus inhibited the clotting process. A similar effect was not found on the heparin treatment of the PNVP homopolymer film. A very small decrease in blood clotting observed in the heparin-treated PNVP film was attributed to loosely bound heparin. These results were in agreement with the findings of earlier workers for various PAAs.[26,35] However, the slow solubilities of the copolymer films in dilute aqueous solution imposes difficulty to extend the time period of thrombogenic assay measurements. This may be overcome using suitable plasticizers the polymers are synthesized.

Hemolysis

Hemolysis of the blood is a problem associated with bioincompatibility.[36] Red blood cells hemolyse when

TABLE III
Determination of Relative Thrombogenicity of the Materials by the Weight of the Thrombus Formed

Polymer samples	Weight of the thrombus formed (mg)		
	10 min	20 min	30 min
PNVP	1.2 ± 0.01	1.9 ± 0.02	2.4 ± 0.1
PNVP–HEP	1.0 ± 0.02	1.9 ± 0.03	2.2 ± 0.1
Pip–MBA–NVP–HEP	0.5 ± 0.1	0.8	1.0 ± 0.2
CHA–MBA–NVP–HEP	0.3 ± 0.05	0.6 ± 0.04	0.8 ± 0.03

HEP = heparin.

TABLE IV
Hemolysis of Blood by Heparinized Polymer Surfaces

Sample	OD at 545 nm	Hemolysis (%)
Water	2.22	Positive control
Saline	0.01	Negative control
Pip–MBA–NVP–HEP	0.07	2.81
CHA–MBA–NVP–HEP	0.09	3.61

HEP = heparin.

they come in contact with water. This problem may be aggravated in the presence of implant materials. The results obtained for the hemolysis of ACD blood with heparinized copolymers are shown in Table IV. Hemolysis was less than 5% when compared with other reported values, which is well within permissible limit.[37] Hence, we concluded that the synthesized copolymers could be used as biomaterials without hemolysis.

CONCLUSIONS

Two new PAAs consisting of Pip–MBA and CHA–MBA with heparin-binding capabilities were synthesized and subsequently copolymerized with NVP under suitable reaction conditions to yield two copolymers (Pip–MBA–NVP and CHA–MBA–NVP). Our observation of a decrease in the water contact angle of the copolymers established the relative hydrophilic nature of the copolymers with respect to a smooth glass surface. The heparinized copolymer films showed a little thrombus formation (<1.2 mg) within a 30 min time period, and the percentage of hemolysis in the heparinized copolymers showed a value of less than 5.0% when compared with other reported values.

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References

1. Kim, Y. J.; Kang, I. K.; Huh, M. W.; Yoon, S. C. Biomaterials 2000, 21, 121.
2. Silver, J. H.; Lin, J. C.; Lim F.; Tegoulia, V. A.; Chaudhury, M. K.; Cooper, S. L. Biomaterials 1999, 20, 1533.
3. Inoue, H.; Fijimoto, K.; Uyama, Y.; Ikada, Y. J Biomed Mater Res 1997, 35, 255.
4. Kim, Y. H.; Park, K. D.; Han, D. K. In Encyclopedia of Polymeric Materials; Salamone, J. C., Ed.; CRC: Chicago, 1998; p 825.
5. Lee, J. H.; Lee, B.; Andrade, J. D. Prog Polym Sci 1995, 20, 1043.
6. Gorde, G. A.; Anderson, S. J.; Grotta, H. M.; Falb, R. D. Trans Am Soc Artif Intern Organs 1969, 15, 1.
7. Ito, Y.; Lin, L.; Matsuo, R.; Imanishi, Y. J Biomed Mater Res 1992, 26, 1065.
8. Kang, I. K.; Kwon, O. H.; Kim, M. H.; Lee, Y. M.; Sung, Y. K. Biomaterials 1997, 18, 1099.
9. Blezer, R.; Fouache, B.; Willems, G. M.; Lindhout, T. J. J Biomed Mater Res 1997, 37, 108.
10. Ikada, Y. Biomaterials 1994, 15, 725.
11. Gombotz, W. R.; Guanghui, W.; Horbett, T. A.; Hoffman, A. S. J Biomed Mater Res 1991, 25, 1547.
12. Llanos, G.; Sefton, M. V. Biomaterials 1992, 13, 421.
13. Nojiri, C.; Okano, T.; Jacobs, H. A.; Park, K. D.; Mahammad, S. F. J Biomed Mater Res 1990, 24, 1151.
14. Shimada, M.; Miyara Tahara, M. H.; Shinohara Okano, I. T.; Kataoka, K.; Sakurai, Y. Polym J 1983, 15, 649.
15. Ikada, Y. Adv Polym Sci 1984, 57, 104.
16. de Queiroz, A. A. A.; Barrak, E. R.; Castro, S. C. J Mol Struct (Thermochem) 1997, 394, 271.
17. Williams, D. F. Fundamental Aspects of Biocompatibility; CRC: Boca Raton, FL, 1990.
18. Andrade, J. D. Surface and Interfacial Aspects of Biomedical Polymers; Plenum: New York, 1985.
19. Okano, T.; Aoyagi, T.; Kataokak, K.; Abe, K.; Sakurai, Y.; Shimada, M.; Shinohara, I. J Biomed Mater Res 1986, 20, 919.
20. Chen, Z.; Zhang, R.; Kodama, M.; Nakaya, T. J Appl Polym Sci 2000, 76, 382.
21. Young, J. K.; Inn-Kyu, K.; Man, W.; Sung-Chul, Y. Biomaterials 2000, 21, 121.
22. Mollnes, T. E.; Videm, V.; Christiansen, D.; Bergseth, G.; Riesenfeld, J.; Hovig, T. Thromb Haemostasis 1999, 82, 1132.
23. Ferruti, P.; Domini, I.; Barbucci, R.; Beni, M. C.; Dispensa, E.; Sancasciani, S.; Marchisio, M. A.; Tanzi, M. C. Biomaterials 1983, 4, 218.
24. Tanzi, M. C.; Tieghi, G.; Botto, P.; Barozzi, C. Biomaterials 1984, 5, 357.
25. Tanzi, M. C.; Rusconi, L.; Barozzi, C.; Ferruti, P.; Angiolini, L.; Nocentini, M.; Barone, V.; Barbucci, R. Polymer 1984, 25, 863.
26. Azzuoli, G.; Barbucci, R.; Benvenuti, M.; Ferruti, P.; Nocentini, M. Biomaterials 1987, 8, 61.
27. Barbucci, R.; Albanese, A.; Magnani, A.; Tempesti, F. J Biomed Mater Res 1991, 25, 1259.
28. Tanzi, M. C.; Barzaghi, B.; Anouchinsky, R.; Bilenkis, S.; Penhasi, A.; Cohn, D. Biomaterials 1992, 13, 425.
29. Tanzi, M. C.; Levi, M. J Biomed Mater Res 1989, 23, 863.
30. Imai, Y.; Nose, Y. J Biomed Mater Res 1972, 6, 165.
31. Nakanishi, K. In Infrared Absorption Spectroscopy Practical, 4th ed.; Nankodo Company Ltd.: Tokyo, 1966; p 142.
32. Hobson, L. J.; Feast, W. J. Polymer 1999, 40, 1279.
33. Hill, D. J. T.; Perera, M. C. S.; Pomery, P. J.; Joseph, E. Polymer 1997, 38, 659.
34. Ishihara, K.; Iwasaki, Y.; Ebihara, S.; Shindo, Y.; Nakabayashi, N. Colloids Surf B 2000, 18, 325.
35. Tanzi, M. C.; Barozzi, C.; Tieghi, G.; Ferrara, R.; Casini, G.; Tempesti, F. Biomaterials 1985, 6, 273.
36. Shim, D.; Wechsler, D. S.; Lloyd, T. R.; Beekman, R. H. Catheterization Cardiovasc Diagn 1996, 39, 287.
37. Autian, J. In Polymer Science and Technology; Kronenthal, R. L.; Oser, Z.; Martin, E., Eds.; Plenum: New York, 1975; Vol. 8, p 181.