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Hemocompatible properties of polymeric derivative of paracetamol

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Abstract—Copolymerization of *N,N*-dimethylacrylamide (DMAA) and *p*-acryloyloxiacetanilide (AOA) was carried out at different mole ratios of the monomers to obtain copolymers of varying compositions. DMAA contents were very near to the corresponding monomer feed and varied between 0.20 and 0.80. Investigation of the protein adsorption of these polymer surfaces showed that copolymers with higher DMAA content adsorbed more albumin than fibrinogen. The scanning electron micrographs of the polymer-coated coverslips after contact with blood showed an antithrombogenic behaviour of these surfaces.

Key words: Antithrombogenic; proteins; compositions; hydrogel; copolymer composition.

1. INTRODUCTION

It is well known that one of the most serious problems usually encountered in the field of cardiovascular prosthetic devices is the thrombogenic activity usually exerted by synthetic materials when they are in contact with blood [1].

The use of synthetic macromolecules in medical applications have attracted considerable interest in recent years [2]. The use of tubes of synthetic polymers for long-term intravenous or intra-arterial catheters have become a key-stone of modern medical investigation and treatment [3]. Thrombosis is the most common complication, followed by infection and bleeding at the site of insertion [4].

It has been reported that polymers with microphase separated stucture or macromolecules with supported antiplatelet agents such as aspirin can supress platelet adhesion [5–6].

One of the most promising approaches concerning the development of new antithrombogenic polymers is the synthesis of polymeric drugs based on well-known pharmacons or drugs with hemocompatible properties bound covalently to a macromolecular support.

Works have recently reported the preparation and study of the pharmacological properties of acrylic formulations based on the synthesis and polymerization of methacrylic esters of typical analgesic and antipyretic drugs like salicyclic acid [7] and of several derivatives of paracetamol [8].

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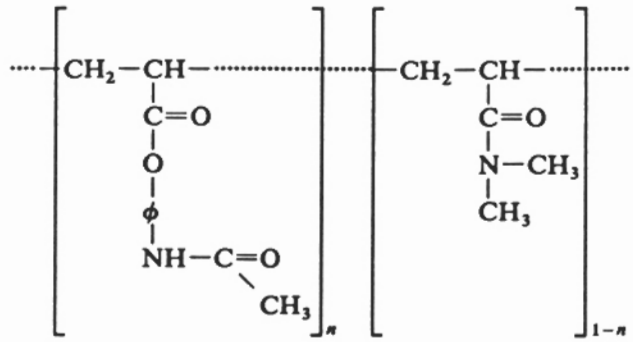
The main aim of this paper is to report the hemocompatible properties of copolymers of *N,N'*-dimethylacrylamide (DMAA) and acryloyloxyacetanilide (AOA):



The monomers DMAA and AOA were chosen because polyDMAA has been assigned as a nonthrombogenic polymer [9] and AOA is a derivative of paracetamol with analgesic and anti-inflammatory properties [10].

EXPERIMENTAL

Copolymerization

Copolymerization between *p*-acryloyloxyacetanilide (AOA) and *N,N'*-dimethylacrylamide (DMAA) monomers were carried out at 50°C in a thermostatic bath using 2,2-azoisobutyronitrile (AIBN) ([I]: 1.5×10^{-2} mol.L^{-1}) and dimethylformamide (DMF) as solvent ([M]: 1.0 mol.L^{-1}). All experiments were carried out in Pyrex glass ampoules sealed off at high vacuum (10^{-4} mmHg). After 90–100% conversion was attained, the reaction was arrested by cooling in ice, followed by precipitation of the polymer in diethyl ether. The solid polymer was isolated by filtration, washed well with the precipitant reagent and dried at reduced pressure until a constant weight was attained.

Substrate preparation

Glass coverslips (1 × 1 cm) were used as support substrates for the test copolymers. The glass coverslips were cleaned thoroughly with chromic acid, followed by water and neutral detergent under sonication, washed with ethanol, and dried under vacuum at room temperature (25°C). These coverslips were then coated with 4% polymeric solutions in DMF by the solvent casting method. The solvent (DMF) was removed by drying in air at room temperature for 48 h. The glass-coated coverslips were placed in a glass ampoule and the residual solvent was removed in high vacuum (10^{-4} mmHg) at room temperature for 48 h. The total removal of the DMF from the films was verified by infrared spectroscopy.

Contact angle

The polymer-coated coverslips were conditioned at the equilibrium humidity of the instrument. The water contact angle was measured by putting a sessile drop of bidistilled and

deionized water on the air-side surface of the polymer-coated coverslip. Each value was taken as the average of five readings.

In vitro *tests*

Protein adsorption. Bovine serum albumin (BSA) and bovine fibrinogen were purchased from Sigma Co. The fibrinogen was 95% clottable and the albumin was 99% pure and were used without further purification. In order to quantify the surface concentrations of albumin (BSA) and fibrinogen adhering to the glass coverslips uncoated and coated with polymeric solutions, the proteins were labelled with ^{125}I by the chloramine *T*-method [11]. The radioactivity of the labeled proteins were 30 μCi mg^{-1}. In order to perform equilibrium experiments, preequilibrated uncoated and coated glass coverslips were introduced into teflon tubes which contained 4 ml sodium phosphate buffer (pH 7.4, ionic strength = 0.01 M) (PBS) at 37°C, before being exposed to the protein solution. Any air bubbles which would adhere to the protein solution. Any air bubles which would adhere to the sample were removed by allowing the samples to cross the air/buffer interface several times. Aliquots (4 ml) of the labeled BSA or fibrinogen solution were then introduced into the tubes.

After the protein solution remained in contact with the samples for 2 h at room temperature, the adsorption was terminated by dilution of the labeled protein into the tubes with PBS (in order to avoid contact of the sample with the protein solution/air interface where some denaturation can take place). The samples were further rinsed gently until the radioactivity of the surface remained constant. The amount of adsorbed proteins was determined by gamma radiation counting, using a Beckman Gamma 4000.

Blood compatibility assessment. Blood compatibility of the polymer-coated coverslips was evaluated by the open-static platelet adhesion test with whole human blood [12]. The tests were performed by depositing 2 ml of fresh blood onto each of the five test surfaces. After contact times of 180 s, the surfaces were washed with saline under carefully controlled conditions to remove all blood components that did not adhere. After fixation with formaldehyde, platelet counts were performed using SEM microphotographs. The average number of adhered platelets was obtained from five photographs of different surface areas (1 cm^2) of the same sample.

RESULTS AND DISCUSSION

Copolymers I–V were synthesized by varying the molar ratio of AOA and DMAA. Feed composition and the copolymer composition data are summarized in Table 1. Copolymer compositions calculated from the ^1H NMR spectroscopy data show that the DMAA and AOA contents are very close to the corresponding monomers in the feed.

Figure 1 shows the infrared spectrum of the polymer-coated and uncoated coverslips. The strong absorption at 1000–2500 cm^{-1} of the uncolated glass coverslip can be assigned to the O—H valence or flexural vibrations resultant of the chemical composition of the glass coverslip (Si—OH) [13]. As could be expected, the infrared spectrum of the glass coverslip coated with DMAA-co-AOA showed absorptions peaks at 1600 cm^{-1} (C=O), 2900 cm^{-1} (CH_2), and 3500 cm^{-1} (NH), characteristics of the copolymer structure and proves that coating of DMAA-co-AOA onto coverslips has taken place.

Hydrophilic polymer gels, called hydrogels, were first introduced as useful biomaterials by Wicherle and Lim in 1960 [14]. Since then, there have been numerous published studies proposing and/or showing these materials to be fairly biocompatible when contacted with

Table 1.
Monomer feed versus copolymer composition

Sample number	Feed		Copolymer	
	F_{DMAA}	F_{AOA}	f_{DMAA}	f_{AOA}
I	0.15	0.85	0.20	0.80
II	0.30	0.70	0.32	0.68
III	0.50	0.50	0.47	0.53
IV	0.70	0.30	0.62	0.38
V	0.85	0.15	0.77	0.23

F and f are the molar fraction of the DMAA and AOA monomers in the monomer feed and in the copolymers samples, respectively. Temperature: 50°C; solvent DMF.

extra-vascular body fluids and tissues [15–19]. Thus, the interface between the water-swollen gel and blood or tissues may have a very low free energy leading to very low adverse interaction of the gel surface with the aqueous biological environment [20].

The hydrophilicity of the coverslips after the coating process were measured by the contact angle. (Fig. 2). The contact angle decreased with the increment of the DMAA content indicating the hydrophilic nature of this monomer in relation to AOA.

When an artificial material is placed in contact with blood the first event to occur is the adsorption of proteins onto the surface [21, 22]. The adsorption of proteins is followed by platelet adhesion and activation [23].

Thus, surface-induced platelet activation is largely dictated by the type and the amount of blood proteins adsorbed at the biomaterial/blood interface [24, 25]. Adsorption of fibrinogen is known to accelerate platelet adhesion and activation [26]. On the other hand, albumin adsorption on the synthetic surfaces can inhibit platelet activation and, therefore, does not promote clot formation.

The albumin adsorption on the polymer-coated coverslips increased with the DMAA content on the DMAA-co-AOA copolymer, which showed an albumin adsorption from 10 to 180 ng cm^{-2} (Fig. 3). This increased amount can be due to the increase in the swollen area caused by the DMAA on copolymers. It has been demonstrated that platelets show little adherence in surfaces precoated with albumin, while fibrinogen-coated surfaces have shown an increased number of adhering and activated platelets [27].

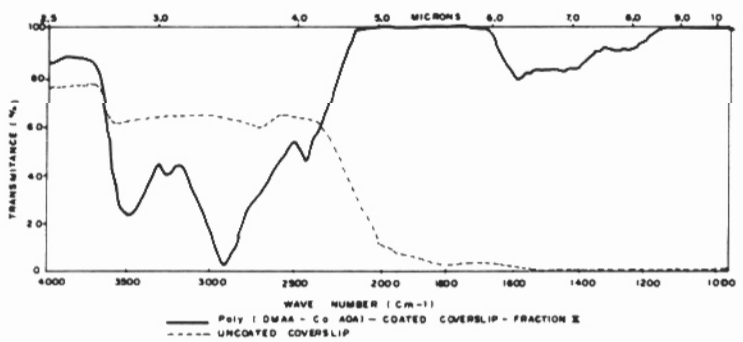


Figure 1. Absorption infrared spectra (IR) of the uncoated coverslips and polymer-coated coverslip.

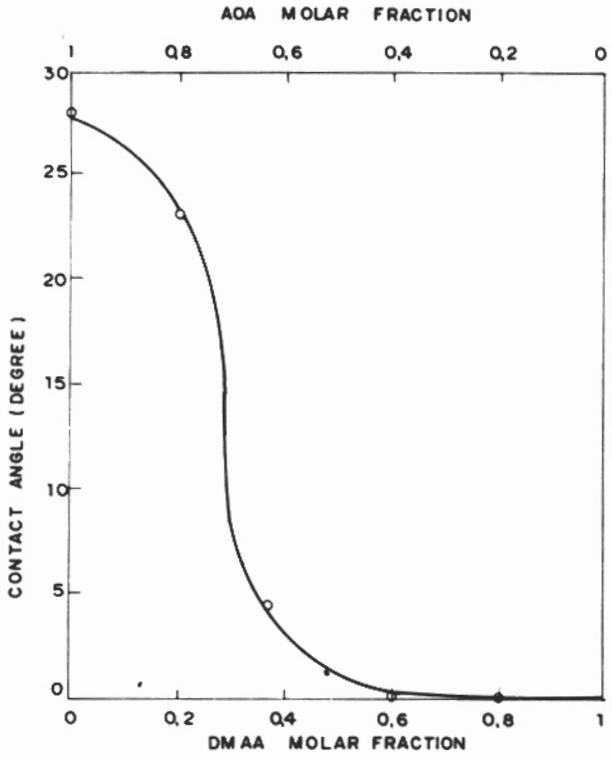


Figure 2. Hydrophilicity of the coverslips after coating with DMAA-co-AOA measured by contact angle.

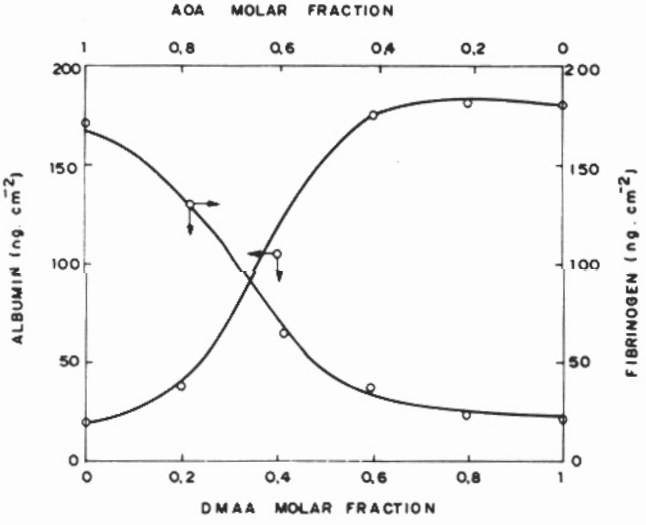


Figure 3. Proteins adsorptions on the polymer-coated coverslips.

Table 2.
Number of adhered and activated platelets per cm^2 (mean ± S.D.)

f_{DMAA}	Copolymer	Contact angle (deg)	Number of platelets
0	—	28	95 ± 10
0.20	I	23	78 ± 6
0.32	II	8	65 ± 7
0.47	III	4	59 ± 6
0.62	IV	0	37 ± 3
0.77	V	0	15 ± 4
1	—	0	30 ± 8

Uncoated coverslips: uncontable platelets adhered.

Thus, the increase of DMAA content on the DMAA-co-AOA copolymers appears to reduce the fibrinogen adsorption onto polymer-coated coverslips surfaces, as shown in Fig. 3.

The results of counting adhered platelets in relation to DMAA content on the copolymeric systems is shown in Table 2. The copolymer with the best blood compatibility, as determined by minimal platelet adhesion and activation, was DMAA-co-AOA with copolymer compositions $f_{AOA} = 0.23$ and $f_{DMAA} = 0.77$, respectively. Suppression of platelet adhesion may be considered to be a common effect of hydrophilic–hydrolphobic microdomains [28]. In fact, this suppression effect does not apear on the AOA and DMAA homopolymers.

Scanning electron micrographs of the polymer surfaces after incubation with human blood for 15 min at 37°C are shown in Fig. 4. Uncoated coverslips are used as reference. As can be seen in the picture, the number of adhered and activated platelets onto polymer-coated coverslips decreased compared to that of the uncoated coverslip.

Taking the above considerations into account, this would tend to further the concept that hemocompatibility is sensitive to the relative hydrophobic/hydrophilic ratio of the polymeric system being evaluated.

However, a more detailed investigation is necessary to make clear the influence of the surface structure of DMAA-co-AOA on their blood compatibility. Additional and more comprehensive studies are under way to speculate upon the nonthrombogenic character of the DMAA-co-AOA systems and the results will be reported elsewhere.

CONCLUSIONS

Thrombus formation on polymer surfaces is a complicated process with multiple factors involved. The nature of the polymer surfaces controls protein adsorption and the adsorbed proteins determine the platelet adhesion via possible enzymatic reactions. The results discussed in this paper indicate the involvement of the hydrophilic and hydrophobic phase-separated structure on the nonthrombogenicity of the DMAA-co-AOA systems. Increasing DMAA content in the copolymers increase the adsorption of albumin and decrease the fibrinogen adsorption. This has been accomplished mainly due to the presence of hydrophilic and hydrophobic domains on the polymer-coated coverslip surfaces. The copolymer DMAA-co-AOA with DMAA content, f_{DMAA}, 0.77 may be able to supress the platelet

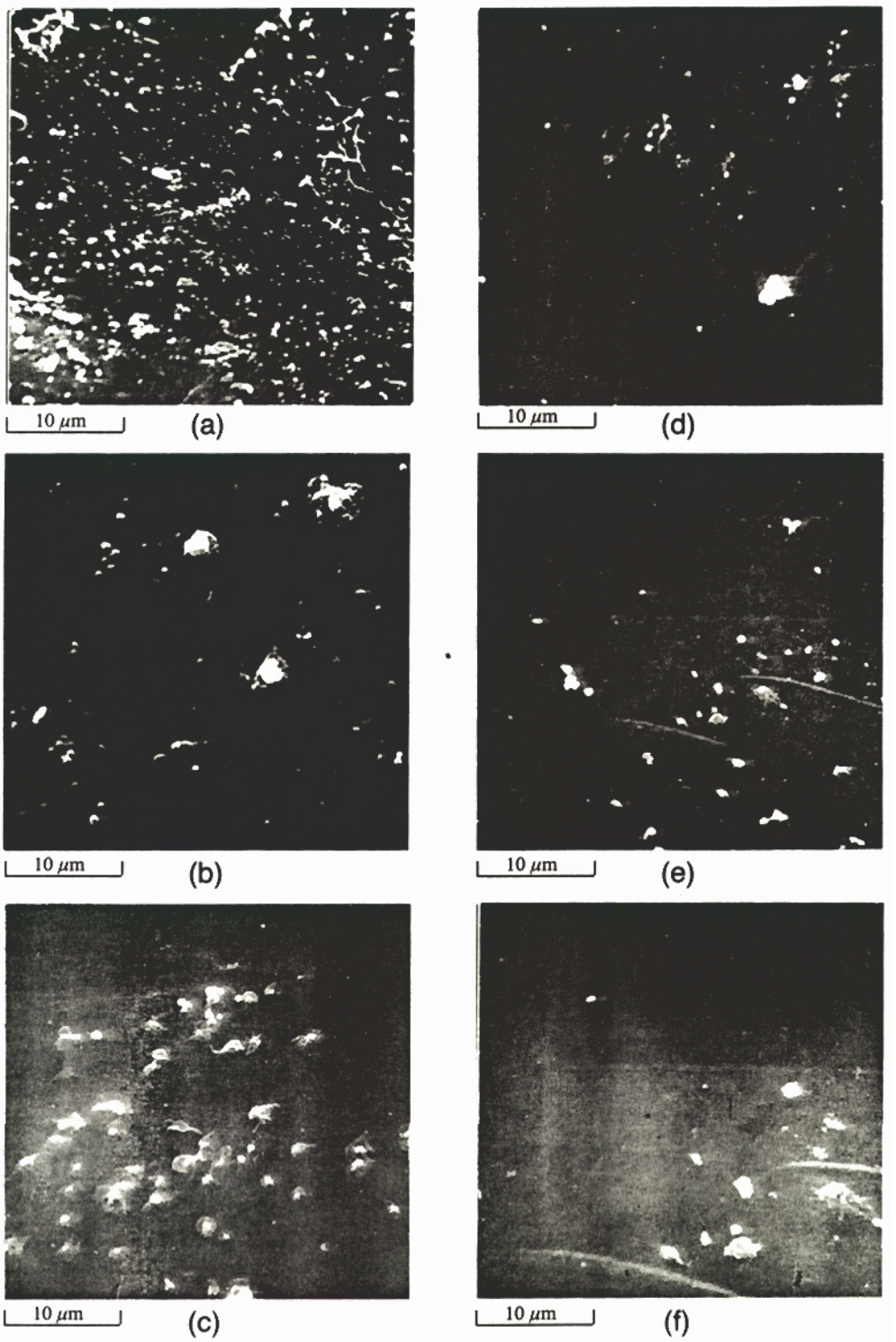


Figure 4. Scanning electron micrographs of polymer surfaces after incubation with human blood at 37°C for 15 min (a) uncoated coverslip, (b) I, (c) II, (d) III, (e) IV and (f) V (×1000).

adhesion. It is then considered that DMAA-co-AOA copolymer may serve as a new type of potential nonthrombogenic material.

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