Copolymers Containing Aminohexyl Residues in Side Chains for Attaching Heparin

Polymeric materials are increasingly incorporated in artificial devices and organs. Blood compatibility of these polymers has been the subject of a number of studies in regard to interactions of blood proteins and platelets with these foreign surfaces, and yet the true nature of the blood–polymer interaction is not fully understood.[1] In the meantime, a considerable amount of work has been carried out in order to develop antithrombogenic polymer surfaces by attaching heparin, nature's own anticoagulant by ionic and/or covalent bonding,[2] with limited success. Since heparin is a negatively charged polysaccharide with a molecular weight of about 12,000, a principal means of heparin attachment is via ionic bonding to positively charged, e.g., aminated, surfaces. Usually, the positive charges are produced by chemical modification of polymers.

A major part of the success (or failure) of heparinized surfaces in respect to their performance in contact with blood lies in the uniformity of, and density of placement of, heparin on the surface. The considerations are the rate at which heparin, if bound only via ionic bonds, desorbs into the blood; and, if covalently bound (e.g., via glutaraldehyde), whether antithrombogenic activity is impaired. In any case, it is important to have polymeric surfaces of controlled content of positive charges. One means to this end involves copolymerization of monomers one of which is positively charged. N-Acrylhexamethylenediamine has been chosen as a comonomer which can become positively charged. The rationale of choosing aminohexyl residue lies in the findings by Hoffman and co-workers who reported that the extent of binding increased measurably when the biomolecule was attached through an arm containing six carbon atoms, compared to the direct bonding.[3]

N-acrylhexamethylenediamine has been prepared by the method described by Kojima and co-workers[4] as a monomer which can be positively charged. N-acrylhexamethylenediamine was copolymerized with acrylate and methacrylate monomers by suspension polymerization employing benzoyl peroxide as initiator and gelatin as protective colloid. Table I summarizes the polymerization compositions. The copolymers were obtained in the form of crosslinked beads in sizes of 0.25–0.8 mm in diameter in order to provide sufficient area for platelet activation test. The properties of copolymers which had 5–10% of amine-containing comonomer in the initial feed changed from glassy in the case of isopropyl methacrylate copolymer to rubbery for methyl acrylate, isodecyl methacrylate, and *n*-lauryl methacrylate copolymers.

In order to determine the content of N-acrylhexamethylene residues in copolymers, we employed a fluorescence assay by using fluorescamine, 4-phenylspiro[furan-2(3H),1′-phthalan]-3,3′-dione. Fluorescamine has been used to detect the amount of primary amines in amino acids and peptides as a substitute for ninhydrin and has several advantages over ninhydrin assay such as greater sen-

TABLE I
Copolymerization of N-Acrylhexamethylenediamine with Acrylates and Methacrylates

Organic phase				Aqueous phase
Monomer A[a]	Monomer B[b]	Benzoyl peroxide	Crosslinking agent[c]	Pharmagel R 1%[d]
MA, 30 g	7.4 g	0.30 g	1.3-BDMA, 2 g	300 ml
IPMA, 19 g	2 g	0.25 g	none	300 ml
IDMA, 15 g	2 g	0.25 g	1.3-BDMA, 4 g	300 ml
n-LMA, 15 g	2 g	0.25 g	1.3-BDMA, 4 g	300 ml

[a] MA = methyl acrylate; IPMA = isopropyl methacrylate. IDMA = isodecyl methacrylate; *n*-LMA = *n*-lauryl methacrylate. All are monomers from Polysciences.
[b] Monomer B = N-acrylhexamethylenediamine.
[c] 1.3-BDMA = 1,3-butylene dimethacrylate.
[d] Pharmagel R from Knox Co.

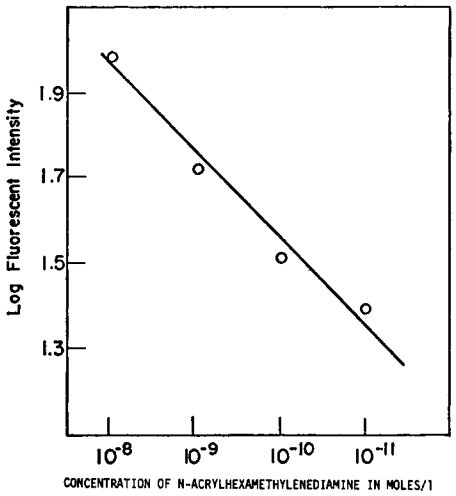

Fig. 1. Fluorescent intensity of N-acrylhexamethylenediamine when reacted with fluorescamine at pH 8.5: fluorescamine concentration $0.5 \times 10^{-6}M$, λ excit. = 390 nm, λ emission, max. = 481 nm, intensity corrected for blank.

sitivity, fast reaction rate, and exclusive reaction with primary amines, thereby eliminating the background intensity from secondary and tertiary amines.[5-8]

Figure 1 shows the calibration result of fluorescamine assay with N-acrylhexamethylenediamine. Within the concentration range of 10^{-11} to $10^{-8}M$, the fluorescent intensity is linearly proportional to the concentration.

Fluorescence assay was employed with copolymers which were uncrosslinked and uncontaminated with the stabilizing agent gelatin. It was found that N-acrylhexamethylenediamine is readily copolymerizable in the range of 5–10 molar per cent composition with acrylates and methacrylates.

The fluorescence test turned out to be also useful for the detection of minute amount of gelatin adsorbed on polymer beads. Before testing for platelet activation, gelatin was extracted in a Soxhlet extractor by monitoring its concentration with the fluorescamine assay.

Platelet activation tests performed in the laboratory of Dr. E. W. Salzman at Beth Israel Hospital of Harvard Medical School indicate that, while methyl acrylate homopolymer beads provide a relatively passive surface, copolymer beads with 10% N-acrylhexamethylenediamine were very reactive toward human blood platelets. The reactivity of platelets with amine groups are well known.[9] This result strongly suggests that we do, indeed, have free amine groups on the surface of our copolymer beads which can be used for attachment of heparin. Several routes of heparin attachment are under study, e.g., via soaking in heparin solution in acidic condition followed by glutaraldehyde fixation.

The prime goal of heparin attachment with aminohexyl residues of copolymers lies in a permanent covalent bonding in such a way that the conformation of heparin remains as an active form.

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