PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** 6 :
A61M 1/36, C09D 105/00

A1

(11) **International Publication Number:** **WO 98/22162**

(43) **International Publication Date:** 28 May 1998 (28.05.98)

(21) **International Application Number:** PCT/GB97/03191

(22) **International Filing Date:** 20 November 1997 (20.11.97)

(30) **Priority Data:**
9624130.2 20 November 1996 (20.11.96) GB

(71) **Applicant** *(for all designated States except US)*: BIOCOMPATIBLES LIMITED [GB/GB]; Frensham House, Farnham Business Park, Weydon Lane, Farnham, Surrey GU9 8QL (GB).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: STORCH, Joachim [DE/GB]; Biocompatibles Limited, Frensham House, Farnham Business Park, Weydon Lane, Farnham, Surrey GU9 8QL (GB). HANLEY, Robert, Neil [GB/GB]; Biocompatibles Limited, Frensham House, Farnham Business Park, Weydon Lane, Farnham, Surrey GU9 8QL (GB). FREEMAN, Richard, Neil, Templar [IE/GB]; Biocompatibles Limited, Frensham House, Farnham Business Park, Weydon Lane, Farnham, Surrey GU9 8QL (GB).

(74) **Agent:** GILL JENNINGS & EVERY; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

(81) **Designated States:** AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) **Title:** BIOCOMPATIBLE COMPOSITIONS

(57) **Abstract**

Substrate surfaces are provided with zwitterionic and cationic groups, preferably by coating with a polymer formed from monomers including a zwitterionic monomer, a cationic monomer and, optionally, a termonomer providing stable binding at the substrate surface, followed by contacting the coated surface with heparin or another anionic mucopolysaccharide. The double coated substrates have improved heparin activity over extended periods as compared to heparinised surfaces which are commercially available. Gels formed by crosslinking anionically charged mucopolysaccharides crosslinked by compounds including a zwitterionic group and one or, preferably, two or more cationic groups can be used as wound dressings or other absorbent compositions. Preferably the terpolymer is formed from 2–methacryloyloxyethyl–2–(trimethylamonium)phosphate inner salt, choline methacrylate and n–dodecyl methacrylate. The polymers may also scavenge heparin from treated blood to avoid use of potentially toxic heparin inhibitors.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

BIOCOMPATIBLE COMPOSITIONS

The present invention relates to surfaces having improved binding to mucopolysaccharides for improved hemocompatibility. The surfaces have pendant zwitterionic and cationic groups. Compositions containing mucopolysaccharides and compounds (usually polymers) including a zwitterionic moiety and a cationic moiety are also provided, and their use to coat substrates.

In WO-A-93/01221 we describe various polymers and their use to coat surfaces to improve their biocompatibility. The polymers include zwitterionic groups and pendant groups which are capable of providing stable surface binding of the polymer to underlying substrate surfaces. The binding may be by provision of pendant hydrophobic groups which physisorb onto hydrophobic substrates, by counterionic attraction between pendant ionic groups on the polymer and oppositely charged groups at the substrate surface, by providing covalent attachment between coreactive pendant groups on the polymer and groups at the substrate surface or by crosslinking the polymer after coating. Post coating crosslinking may also be used to improve the stability of a polymer which is physisorbed, covalently bonded or counterionically bonded to the surface. The polymers have good hemocompatibility as indicated by the low platelet adhesion values reported in that specification.

It has also been shown that zwitterionic groups at substrate surfaces, for instance of contact lenses, show lower rates of deposition of proteins and lipids from biological liquids such as tear film. In WO-A-92/07885, reduced levels of protein deposition are described for contact lenses formed from a hydrogel of a crosslinked copolymer of copolymerisable zwitterionic monomer and non ionic comonomer.

In WO-A-93/21970 it is disclosed that microorganisms, especially bacteria, adhere to surfaces having pendant

phosphoryl choline groups than to similar surfaces without such groups present.

Another way of reducing the thrombogenicity of surfaces has involved attachment or adsorption of anti-thrombogenic active compounds to substrate surfaces. For instance heparin may be attached through covalent or counterionic bonding to surfaces. In US-A-3,634,123 the binding of heparin to a surface was increased by incorporation of cationic surfactant. A related process is described in EP-A-0350161, in which a surface is first coated with a cationic surfactant and subsequently with heparin. In EP-A-0086187 the surface is first coated with a cationic polymer and subsequently with heparin. In JP-A-53/137268 a cross-linked acrylic copolymer of a cationic monomer and a polyethyleneglycol monomer is blended with polyurethane and made into tubing which can be coated with heparin. In EP-A-0086186 heparin is attached to an underlying surface through a covalent bond via the end carbohydrate unit. In US-A-5,342,621, a complex is formed of heparin with phosphatidyl choline and admixed with a polymer or caprolactone or L-lactic acid (both substantially uncharged overall) and subsequently used to coat medical devices.

The present inventors have discovered that the performance of heparin coated devices which are commercially available, for instance as components of extra corporeal devices, deteriorates after short periods of use, for instance half an hour. It is not known whether this is due to the heparin being removed from the surface or due to the surface becoming fouled by components of blood or other biological liquid in contact with the surface during use such that the heparin is masked. The present invention seeks to provide a substrate surface which is hemocompatible and retains its hemocompatible properties over longer term in use.

Generally patients who are undergoing complex operations requiring that their blood be directed through

extra corporeal circuitry, require administration of heparin into the circulation to prevent the blood clotting. Subsequently the heparin has to be neutralised or removed from the blood stream. In order to remove heparin from the circulation without administering a further active compound to neutralise the heparin, it has been suggested to immobilise protamine, a cationic polypeptide used to neutralise heparin, at the surfaces of a filter used in an extra corporeal blood circuit, to scavenge heparin from a patient systemically heparinised.

In a new process according to the invention a substrate has at its surface zwitterionic pendant groups and cationic pendant groups and is contacted with a solution having suspended or dissolved therein an anionically charged mucopolysaccharide.

The anionically charged mucopolysaccharide may be heparin or a similar anti-thrombogenic compound such as hirudin or chondroitin sulphate, or may be alginate or hyaluronic acid. The provision of cationic pendant groups at the substrate surface provides a charged entity, having the opposite charge to that of the mucopolysaccharide, enabling the mucopolysaccharide to become counterionically bonded to the surface in the second step. The zwitterionic groups seem to minimise adsorption of other components from blood or biological fluids subsequently contacted with the coated surface, thereby preventing fouling of the surface which would mask the mucopolysaccharide's effect.

The mucopolysaccharide coating may be carried out as the second step of a two step process. In the first step a substrate is coated with a liquid composition containing a coating polymer comprising pendant zwitterionic groups and pendant cationic groups, suspended or dissolved in a solvent, preferably followed by removal of the solvent prior to the mucopolysaccharide coating step.

In the two step coating process, the polymer having pendant zwitterionic and cationic groups is generally a copolymer of copolymerisable monomers. Whilst it is most

convenient for the polymer to be formed by addition polymerisation of ethylenically unsaturated cationic and zwitterionic monomers, it may alternatively be a condensation polymer or an addition polymer, for instance formed by ring opening cyclic monomers.

Copolymers of ethylenically unsaturated monomers may be formed from monomers including

a) a zwitterionic monomer of the formula I

YBX I

wherein B is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which optionally include one or more fluorine substituents;

X is an organic group having a zwitterionic moiety; and

Y is an ethylenically unsaturated polymerisable group; and

b) a cationic monomer of the formula II

$Y^1B^1Q^1$ II

wherein B^1 is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which optionally includes one or more fluorine substituents;

Y^1 is an ethylenically unsaturated polymerisable group; and

Q is an organic group having a cationic moiety.

Preferably the copolymer includes additional pendant groups capable of providing stable bonding at the substrate surface. Such groups are generally introduced by incorporation of termonomers into the polymerisation. A termonomer may, for instance, include a hydrophobic group which provides for physisorption at the surface, where the substrate surface is hydrophobic, or may comprise a

covalent reactive group which is capable of forming a covalent bond with coreactive groups at the substrate surface. Alternatively the copolymer may be crosslinked after coating by subjecting a polymer having pendant crosslinkable groups to conditions such that crosslinking takes place.

A termonomer which has a hydrophobic group is generally of the formula III

$$Y^2B^2Q^2 \qquad III$$

wherein B^2 is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which may optionally include one or more fluorine substituents;

Y^2 is an ethylenically unsaturated polymerisable group; and

Q^2 is an organic group having a hydrophobic group selected from alkyl groups having at least six carbon atoms, fluorine substituted alkyl groups and alkyl groups having at least one siloxane substituent.

A covalent reactive termonomer for instance which may be incorporated in the polymer in addition to or instead of the hydrophobic termonomer of the formula III may have the general formula IV:

$$Y^3B^3Q^3 \qquad IV$$

wherein B^3 is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which optionally includes one or more fluorine substituents;

Y^3 is an ethylenically unsaturated polymerisable group; and

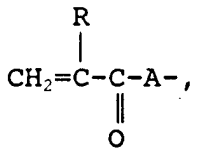
Q^3 is an organic group having a reactive group capable, on imposition of an external stimulus, of reacting

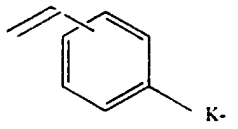
with a coreactive group on the surface of a substrate or which is pendant on the polymer.

Reactive groups Q^3 may also provide crosslinkability on the polymer. For instance such groups may react with each other or may react with different coreactive groups as pendant groups on the copolymer, for instance amine or, more usually, hydroxyl groups. Examples of reactive groups capable of crosslinking with such pendant groups or of reacting to provide covalent binding to a surface, include an aldehyde group or a silane or siloxane group containing one or more reactive substituents such as halogen, for example chlorine, or alkoxy, generally containing from 1 to 4 carbon atoms, for example methoxy or ethoxy, or, more preferably, Q^4 is a hydroxyl, amino, carboxyl, epoxy, -$CHOHCH_2Hal$, (in which Hal is a halogen atom such as chlorine, bromine or iodine) succinimido, tosylate, triflate, imidazole carbonyl-amino or optionally substituted triazine group. A preferred example of a reactive group is a trimethoxy silane group which reacts either with other similar groups or with hydroxyl groups of the polymer or at a surface.

Preferred reactive comonomers IV which are used to crosslink the comonomer, rather than provide covalent binding to the surface, are those Q^3 contains a crosslinkable cinnamyl, epoxy, -$CHOHCH_2Hal$ (in which Hal is a halogen atom), methylol, reactive silyl, an ethylenically unsaturated crosslinkable group, such as an acetylenic, diacetylenic, vinylic or divinylic group, or an acetoacetoxy or chloroalkyl sulfone, preferably chloroethyl sulphone, group. For optimum cross-linking a monomer including a reactive silyl group is used in combination with a further monomer including a hydroxyl group.

In each of the monomers I to IV the ethylenically unsaturated group is preferably selected from





$CH_2=C(R)-CH_2-O-$, $CH_2=C(R)-CH_2OC(O)-$, $CH_2=C(R)OC(O)-$, $CH_2=C(R)O-$, and $CH_2=C(R)CH_2OC(O)N(R^1)-$

wherein:

R is hydrogen or a C_1-C_4 alkyl group;

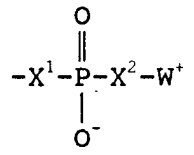
A is -O- or $-NR^1-$ where R^1 is hydrogen or a C_1-C_4 alkyl group or R^1 is -B-X, B^1Q^1, B^2Q^2 or B^3Q^3 where B, B^1, B^2, B^3, Q^1, Q^2 and Q^3 and X are as defined above and

K is a group $-(CH_2)_pOC(O)-$, $-(CH_2)_pC(O)O-$, $-(CH_2)_pOC(O)O-$, $-(CH_2)_pNR^2-$, $-(CH_2)_pNR^2C(O)-$, $-(CH_2)_pC(O)NR^2-$, $-(CH_2)_pNR^2C(O)O-$, $-(CH_2)_pOC(O)NR^2-$, $-(CH_2)_pNR^2C(O)NR^2-$, (in which the groups R^2 are the same or different) $-(CH_2)_pO-$, $-(CH_2)_pSO_3$ -, or, optionally in combination with B, a valence bond and p is from 1 to 12 and R^2 is hydrogen or a C_1-C_4 alkyl group.

Preferably the ethylenically unsaturated groups of all monomers copolymerised together are either the acrylate type or are the styrene type, and, most preferably each has the same formula. Preferably the groups A of acrylate type ethylenically unsaturated groups of the zwitterionic, cationic and termonomer are the same and are most preferably all -O-.

The zwitterionic group X preferably has a phosphate ester group as the anion or a thioester analogue or an amide analogue or phosphonate. The cationic moiety is preferably a quaternary ammonium group, but may be a sulphonium or phosphonium group. Preferably the cationic group is at the end of the group X distant from the group B.

Preferably X is a group of formula



(VI)

in which the moieties X^1 and X^2, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group and a group linking the anionic and cationic moieties which is preferably a C_{1-12}-alkylene group.

Preferably W contains as cationic group an ammonium group, more preferably a quaternary ammonium group.

The group W^+ may for example be a group of formula $-W^1-N^+R^{23}{}_3$, $-W^1-P^+R^{23a}{}_3$, $-W^1-S^+R^{23a}{}_2$ or $-W^1-Het^+$ in which:

W^1 is alkylene of 1 or more, preferably 2-6 carbon atoms optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl, alkylene aryl, aryl alkylene, or alkylene aryl alkylene, disubstituted cycloalkyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, which group W^1 optionally contains one or more fluorine substituents and/or one or more functional groups; and either

the groups R^{23} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, preferably methyl, or aryl, such as phenyl or two of the groups R^{23} together with the nitrogen atom to which they are attached form a heterocyclic ring containing from 5 to 7 atoms or the three groups R^{23} together with the nitrogen atom to which they are attached form a fused ring structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^{23} is substituted by a hydrophilic functional group, and

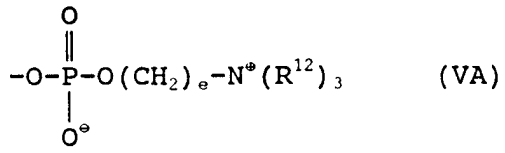
the groups R^{23a} are the same or different and each is R^{23} or a group OR^{23}, where R^{23} is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring, for example pyridine.

Preferably W^1 is a straight-chain alkylene group, most preferably 1,2-ethylene.

Preferred groups X of the formula VI are groups of formula VA.

The groups of formula (VA) are:



(VA)

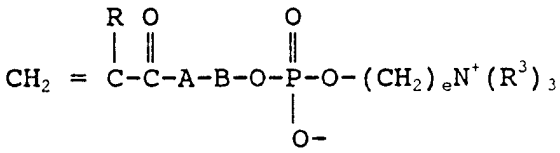
where the groups R^{12} are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 6, preferably 2 to 4.

Preferably the groups R^{12} are the same. It is also preferable that at least one of the groups R^{12} is methyl, and more preferable that the groups R^{12} are all methyl.

Preferably e is 2 or 3, more preferably 2.

When X is a group of formula (VA) preferably B is a group of formula $-(CR^{13}_2)-$ or $-(CR^{13}_2)_2-$, eg. $-(CH_2)-$ or $-(CH_2CH_2)-$.

Preferably the zwitterionic monomer has the general formula VI



VI

wherein R, A and B are defined above,

the groups R^3 are the same or different and each is hydrogen C_{1-1} alkyl, aryl, alkaryl, aralkyl, or two or three of the groups R^1 with the nitrogen atom to which they are attached form a saturated or unsaturated hetero cyclic ring, and e is 1 to 6, preferably 2 to 4.

A cationisable moiety in the group Q^1 is generally a group which can easily be protonated to render it cationic, for instance which is protonated in aqueous environments at pH7.

The group Q^1 of the cationic monomer is preferably a group $N^+R^5_3$, $P^+R^5_3$ or $S^+R^5_2$

in which the groups R^5 are the same or different and are each hydrogen, C_{1-4}-alkyl or aryl (preferably phenyl) or two of the groups R^5 together with the heteroatom to which they are attached from a saturated or unsaturated heterocyclic ring containing from 5 to 7 atoms. Preferably the group Q^1 is permanently cationic, that is each R^5 is other than hydrogen. Preferably Q^1 is $N^+R^5_3$ in which each R^5 is C_{1-4}-alkyl, preferably methyl.

Terpolymers formed from the above mentioned zwitterionic monomer, a cationic comonomer of the formula II and a hydrophobic monomer of the formula III and quaterpolymers of zwitterionic monomer, cationic monomer and trialkoxysilyl and hydroxyl group containing monomers each of formula IV are novel compounds and are claimed in our copending application filed even date herewith (our reference HMJ02820WO).

By incorporating pendant groups to provide stable binding on the surface, the terpolymers can be stably bound to many types of underlying surface, for subsequent provision of a coated substrate for receiving heparin. Alternatively the pendant groups may provide compatibility with other polymers when blended, for instance by solid or liquid blending techniques. Thus pendant hydrophobic groups may interact with hydrophobic blended copolymers whilst reactive groups may be crosslinked, for instance during reactive blending processes or after blending has taken place. Such blends can subsequently be used for form shaped articles which may be coated with heparin in a post shaping step. The novel polymers themselves may have satisfactory properties such that they may be useful to form components of devices which can be treated with heparin to improve their anti-thrombogenicity.

The polymer may be blended with heparin in a pre-blending step and the complex subsequently used to form coatings or be used in a blend with other polymers having desirable mechanical characteristics. A blend may, for instance, be made by dispersing both components in a volent

in which they are both compatible. Alternatively each component is dissolved or dispersed in a solvent which is suitable for the respective component and the two liquid compositions mixed. Other components may be included to stabilise the mixture. Such pre-blended heparin/polymer complexes are primarily of use as coating components, that is for forming deposits from liquid coating compositions onto underlying substrate surfaces. They form a further aspect of the present invention, in liquid form and/or when coated on to a surface. A liquid composition comprises a solvent and, dissolved or dispersed in the solvent, an anionic mucopolysaccharide and a polymer having pendant cationic and pendant zwitterionic groups. Suitable solvents are, for instance, water or alcohols or mixtures thereof. To avoid flocculation or coagulation of the two components, it is preferred for the composition to have an excess of anion over cation or vice versa. The compositions may be made by dissolving or dispersing each component separately in a portion of a solvent and then mixing the solutions together with stirring.

A complex formed by crosslinking anionic mucopolysaccharides by a zwitterionic organic compound having at least one zwitterionic group and at least one cationic group forms a further aspect of the present invention. The zwitterionic organic compound preferably has at least two cationic groups to provide intermolecular or intramolecular crosslinking for the mucopolysaccharide molecules. An example of a zwitterionic organic compound is a compound comprising a basis of a branched alkyl chain having at the ends of two or more of the three or more branches a cationic group and at the end of at least one of the remaining branches a zwitterionic group. For instance such a compound may have the general formula V

$$X^1R^4(Q^4)_n \qquad V$$

in which X^1 is an organic group having a zwitterionic moiety;

n is an integer of 1 or more;

the or each group Q^4 is independently selected from organic groups having a cationic moiety and

R^4 represents an arylene or alkylene group having a functionality of n+1, optionally interrupted by etheric oxygen atoms, or urethane, ester or amide linkages and optionally substituted.

Generally the compound is polymeric, and comprises residues derived from zwitterionic monomers and cationic comonomers. Alternatively the compound may be a relatively small, non-polymeric molecule, for instance in which R^4 has 1 to 12 carbon atoms.

The zwitterionic organic compound (including a polymer as described above) and the anionic mucopolysaccharide are generally used in ratios of equivalent ionic groups in the range 1:10 to 10:1, preferably about 1:2 to 2:1, probably about 1:1. The use of such ratios allows the formation of a crosslinked mucopolysaccharide which may have suitable characteristics such that a gel, comprising a liquid component in which the crosslinked mucopolysaccharide is swellable but not soluble. Such gels may be used for instance as wound dressings, microbial culture media, drug delivery systems, etc. The dry crosslinked materials may be used as absorbent materials for absorbing aqueous or organic solvent based liquids.

Cationic and zwitterionic moieties may alternatively be introduced using reagents having reactive groups capable of reacting with coreactive groups on the underlying surface, simultaneously or sequentially. A sequential reaction process may involve the second reagent reacting with groups present at the surface prior to reaction with the first reagent or with groups introduced at the surface in the first step. For instance, in a first step, cationic groups may be introduced by reaction of a reagent having a cationic or cationisable group. In the second step,

cationic groups may be partially reacted with suitable reagents to convert them to zwitterionic groups. In another process, a first reagent may have two reactive moieties so that in the first step one moiety reacts with the substrate, while in the second step the second reactive moiety (which may be the same or different) reacts with the second reagent which has suitable coreactive groups.

Reagents for attaching cationic groups to surface amine, hydroxyl or carboxylic acid groups are described in our earlier application number WO-A-9106020. Reagents for attaching zwitterionic groups to such surfaces are described in our earlier publications numbers WO-A-9113639 and WO-A-9207858.

The use of a surface which has cationic and zwitterionic groups immobilised in particulate or membrane form to adsorb heparin or other anti-thrombogenic mucopolysaccharides from blood containing heparin or other mucopolysaccharide forms a particularly preferred embodiment of the process of the present invention. In this embodiment, anticoagulant may be scavenged from the blood of a patient to whom the anticoagulant has been administered, for instance, by circulating the patient's blood through an extracorporeal circuit including a filter. Such a filter may, for instance, include membranes having at their surface the cationic and zwitterionic groups. The membranes may be made of regenerated cellulose in hollow fibre form. Such fibres may be provided with the desired pendant groups by coating with a preformed polymer, for instance of one of the types described in the examples herein, or as described in example 16 of WO-A-93/01221 using an excess of the coating polymer or using a similar type of polymer which can be crosslinked after coating using crosslinkable monomers as described above. Alternatively cationic and zwitterionic monomers may be graft polymerised directly onto the surface of the cellulose fibres using the process described in US-A-5,453,467, or onto soluble cellulose which is subsequently

coated onto the fibres as described in WO-A-93/15775. We have found that the terpolymers described in the examples herein can be used successfully to coat various substrates including polyesters, polycarbonates; polypropylene, polyvinyl chloride and steel and filters may include coated surfaces of any of these materials.

Instead of passing anticoagulant-treated blood through an extra corporeal filter, heparin (or other anticoagulant) scavenging may be carried out by implanting, permanently or temporarily, a device into the body in the circulation, which can remove anticoagulant which has been administered systemically. Thus the zwitterion and cation group carrying surface may be the surface of a vascular stent introduced into a blood vessel of a patient. In this embodiment the device may act as a reservoir, formed *in situ*, of active ingredient which may be released slowly into the circulation over an extended period of time. Alternatively a device may be preloaded with counterionically charged mucopolysaccharide prior to implantation, to act as a slow release drug delivery system.

The proportions of zwitterionic and cationic pendant groups in polymers used in the present processes and products depends upon the desired end use. Where high levels of mucopolysaccharide are to be scavenged from a fluid composition and/or it is desired for a high density of anionic mucopolysaccharide to be deposited onto a surface for subsequent use, then the amount of cationic pendant group should be relatively high as compared to the levels of zwitterionic groups. However where lower levels of mucopolysaccharide are required to be adsorbed to achieve anti-thrombogenic performance, whilst minimising deposition of protein and lipid components and platelets forms an important characteristic of the surfaces, then high levels of zwitterionic pendant groups are likely to be desirable. The relative ratios (equivalents) is in the

range 1:100 to 100:1 (zwitterionic to ionic) preferably 1:10 to 10:1, more preferably 1:2 to 20:1.

Termonomer may be present in a monomer mix, for instance to provide the polymer with adsorption properties at a surface or covalent bonding to an underlying substrate. At a molar proportion in the range 0.1 to 75%.

The polymers may include diluent comonomer. Such diluent comonomer may be used in quantities up to 90 mol%, usually less than 50 mol%. Copolymerisable nonionic monomers may be used such as C_{1-24} alkyl(meth)acrylates, -(meth)acrylamides, and hydroxy C_{1-24}alkyl(meth)acrylates and (meth)acrylamides.

The copolymers or terpolymers may include anionic pendant groups, to provide intermolecular crosslinking by counterionic bonding with cationic groups. In such cases, the equivalent level of anionic groups is lower than that of cationic groups in order that the polymer has an overall cationic charge. Anionic copolymerisable monomers may be used, for instance in which the anionic group is derived from carboxylic, sulphonic or phosphonic acid.

It has been found that the binding of heparin and cationic/zwitterionic polymer to a surface provides a coated substrate in which the heparin appears to be in a condition such that good anti-thrombogenic properties are exhibited. Furthermore the coating is very stable and resistant to fouling during use such that the heparin conferred properties are retained even after substantial periods of use. Thus the performance is found to be greatly improved as compared to normal heparin treated surfaces. The binding of heparin to pretreated surfaces which have pendant cationic and zwitterionic groups is achieved merely by contacting the surface with heparin in solution, whereby heparin becomes bound to the cationic groups via counterionic bonding.

The following examples illustrates the inventions.

Performance Tests

Heparin Activity

Loading of samples with heparin

1. Filter strips.

Samples were incubated with 5 ml of a solution of heparin in PBS (usually 50 U/ml. In other experiments, a heparin concentration of 4 or 200 U/ml in saline produced the same heparin surface activity on the cationic polymer) for 30 min on a test tube shaker at room temperature. After 30 min, the samples were rinsed for 10 sec on both sides first with PBS then with deionized water. The samples were dried on tissue paper and in air and stored at room temperature.

2. Whole filters.

Arterial filters were filled with 100 ml of a heparin solution in PBS (50 U/ml) and inlet/outlet sides were closed. The filter was rotated for 30 min, ensuring that all parts of the device were in contact with the heparin loading solution. The filter was then drained and filled/drained 3 times with PBS and then filled/drained 3 times with deionized water. The filter was dried by a stream of air and stored at room temperature.

Preparation of samples for heparin test

Heparin loaded filter strips (dip-coated or removed from whole arterial filters) were usually incubated for 5 hrs at 37°C in PBS/BSA 1%/NaN_3 0.1% to remove unstable bound heparin. The samples were then rinsed with PBS and deionized water as described and dried in air. Samples of 0.2-0.4 x 0.4 cm were cut out and tested as described below.

Heparin test

A chromogenic assay (Heparin CRS106, Sigma). The "Semi-Micro Method" described in the manual was used. Heparin loaded coated samples were placed in polystyrene test tubes. The tubes were placed into a 37°C water bath (5 tubes). 200 µl of bovine factor Xa was added and the tubes were shaken. Following 1 min agitation, 200 µl factor Xa substrate was added to the tubes and they were agitated for 5 min. 200 µl acetic acid (>90%) was added to

the tubes and the tubes were shaken. 200 μl of the solution was removed from the tubes and added to the well of a microplate (2 wells/sample) and measured at 405 nm against wells containing 200 μl of PBS. Previous results had shown that PBS gave the same absorbance reading as a reagent blank. The heparin activity was calculated with the use of a standard curve prepared with soluble heparin.

Platelet adhesion

Heparin loaded and heparin free samples were incubated with human blood (citrate or heparin as anticoagulant) for 2-3 hrs and the degree of platelet adhesion was determined by scanning electron microscopy.

Fibrinogen adsorbance

Samples of heparin loaded or heparin-free coated material were incubated with human plasma for 10 min, washed with PBS/BSA 1%, then incubated for 30 min with an anti-human fibrinogen antibody conjugated to horse radish peroxidase (Dako Code No. A080). The samples were washed and bound antibody was determined by incubating the samples with a substrate for peroxidase (0-phenylenediamine dihydrochloride, 0.4 mg/ml) and a phosphate citrate buffer with urea hydrogen peroxide (Sigma P-9305). After 10 min the absorbance at 450 nm was measured against a reagent blank.

Perfusion with bovine blood

Two arterial filters (a control filter and a coated heparin loaded filter or a coated non-heparin loaded filter) were perfused in parallel for 6 hrs with bovine blood (3.5 L/min) at reduced heparin concentrations and macroscopic blood clots were detected visually and photographs were taken.

Observed Chloride

The counter ion in the polymeric system is chloride ion. Quantification of the chloride ion allows the level of cationic methacrylate to be determined.

Procedure

Add 0.25 g polymer to 25 ml methanol. Once the material has fully dissolved add 75 ml of distilled water to the polymer/methanol mixture. Adjust the pH of the mixture to fall between 8-9. Add 1.0 ml of potassium chromate (5% in w/v distilled water) by pipette to the flask, and titrated to the first brown/red end-point with standardised 0.01 m silver nitrate solution. Repeat the titration, using 75 ml distilled water, but no polymer sample to obtain a blank reading. The level of cationic methacrylate in the polymer is directly proportional to the chloride ion concentration.

Example 1

Preparation of poly (2(methacryloyloxyethyl)-2' trimethylammonium) ethyl phosphate innersalt-co-n-dodecyl methacrylate-co-11 methacryloylundecyl-1-trimethyl ammonium bromide) (40:71:8).

2-(Methacryloyloxyethyl)-2'-(trimethyl ammonium) ethyl phosphate inner salt (2.32g, 0.0079 mole), n-dodecyl methacrylate (3.61g, 0.0142 mole) and 11 methacryloylundecyl-1-trimethyl ammonium bromide (0.59g, 0.0016 mole synthesised according to reference example 1) were dissolved in 43ml of propan-2-ol and 17ml of ethyl acetate.

This monomer solution was thoroughly degassed by bubbling dry nitrogen gas (dried over molecular sieve) through it for 30 minutes. The initiator, AIBN (0.01360g, 0.02 weight % of solution) was then washed into the solution using 3ml of degassed ethanol. The solution was further degassed for five minutes. Maintaining the solution under a slight positive pressure of nitrogen (equivalent to a few ml of mineral oil in a bubbler) the solution was heated to 62°C and stirred vigorously for around 46 hours.

After this time the reaction mixture was allowed to cool to around 40°C before removing all of the solvent using a rotary evaporator under vacuum and at about 40°C giving a solid foam.

This foam was then dissolved in 24ml of dichloromethane and precipitated dropwise into an excess, 200ml, of acetone. The product was collected on a Buchner filter funnel and washed with 3 further 20ml quantities of acetone. The white solid was dried in a vacuum oven for 16 hours at 40°C and weighed.

The resulting polymer, obtained in 83% yield, was a white solid.

^{1}HNMR (400MHz, d, ppm, $CD_3OD/CDCl_3$) 4.31(b), 4.21(b), 4.07(b), 3.98(b), 3.72(b),3.37, 3.33, 3.29(s), 3.22, 3.17, 1.95, 1.84(b), 1.67(b), 1.33(s), 1.06(b), 0.93(s), C_{13} NMR (500MHz, d, ppm, $CD_3OD/CDCI_3$) 176.37, 66.91, 65.90, 63.68, 60.05, 54.50, 53.37, 45.54, 32.69, 30.44, 30.13, 28.92, 26.93, 23.41, 17.31, 14.56.

Example 2

Preparation of poly (2(methacryloyloxyethyl)-2' trimethylammonium) ethyl phosphate innersalt-co-n-dodecyl methacrylate-co-cholinemethacrylate

Using a similar technique to that used in Example 1, but using choline methacrylate (2-methacryloyloxyethyl trimethyl(ammonium chloride) in place of 11-methacryloyl undecyl-1-trimethyl ammonium bromide, various polymerisations were carried out. The zwitterionic monomer, lauryl (dodecyl) methacrylate monomer and choline methacrylate were mixed at the molar ratio shown in Table 1 below and AIBN as initiator was used at the level shown in the table. The total weight percent of solids in the polymerisation solution is also reported in the table, since it was varied between examples.

The polymers were recovered by essentially the same method as in claim 1 although including an extra dissolution and precipitation step to remove lower molecular weight polymer.

The polymer product was subjected to chloride ion determination to establish the rate of inclusion of cationic monomer into the product. Also some rough Molecular weight determinations were carried out.

Example 3

Preparation of Poly(2-(Methacryloyloxyethyl)-2'-(Trimethylammoniumethyl) Phosphate, Inner Salt)-co-(n-Dodecyl methacrylate)-co-(2-(Methacryloyloxy) ethyl trimethyl ammonium chloride)-co-(3-Trimethyoxysilylpropyl methacrylate) 30:60:6:4 polymers

3.1 Monomer Feed Synthesis

Zwitterionic monomer (40.68g, 0.138mole) and cationic monomer (5.73g,0.0275mole) were weighed in a glove box environment dried by P_2O_5. Dodecyl methacrylate (69.45g, 0.273mole), trimethoxysilyl monomer (4.53g,0.0182mole) and α-azo-isobutyronitrile (AIBN) initiator (1.202g, 1%) were weighed in air. A 3 neck reaction flask, fitted with water condenser, nitrogen gas flow and monomer feed tubing, and primed with anhydrous n-propanol (60g) solvent, was immersed in a heated 90°C oil bath. The monomers and initiator were dissolved in 300g of n-propanol solvent and magnetically stirred in a measuring cylinder sealed with parafilm. The reaction mixture was drawn into polypropylene tubing placed inside the measuring cylinder and through silicone tubing via a peristaltic pump to enter the heated reaction vessel in a dropwise process. A complete transfer to the heated vessel took 2.25 hours. The reaction was stirred for another hour. A second charge of AIBN initiator (0.12g), dissolved in 3ml n-propanol, was added and the reaction mixture was stirred for a further 50 min, taking the total reaction time to 4 hours.

Once cooled to room temperature, the reaction mixture was filtered through a sintered glass filter. The solvent was removed at 40°C-50°C by rotary evaporator to give a white foam residue that was later redissolved in 480ml dichloromethane and 40ml methanol solvent mixture and dropwise precipitated into 4000ml acetone. A white solid product settled from the acetone leaving a slightly cloudy supernatant. The product was separated by Buchner flask and 113 Whatman wet strenghtened filter paper, and dried in a room temperature vacuum oven for up to 24 hours prior to

a second workup and precipitation in acetone. The product was weighed (82.9g) to provide a 68.9 wt% yield, bottled in a brown glass vial and refridgerated.

Characterisation of Product

The polymer requires by weight C 63.08%, H 10.13%, P 3.55%, N 1.93%, Si 0.43% Cl 0.81%, found C 58.1%, H 9.98%, P 3.09%, N 1.90%, Si 0.20%, ^{1}Hnmr (400MHz, ppm, CD_3OD:$CDCl_3$ 1:1 v:v) 4.34, 4.30, 3.98, 3.72, 3.38, 3.29, 3.22, 1.67, 1.32, 0.92, 0.10. Specific viscosity of 10mg/ml solution in ethanol:chloroform (1:1 v:v) is 0.13. The polymer product was subjected to the chloride ion assay to establish the rate of inclusion of cationic monomer; required 4.76wt%, found 4.82wt% and 4.94wt%.

3.2 **One Pot Synthesis**

Zwitterionic monomer (4.87g, 1.65 x 10^{-2} mole), dodecyl methacrylate (8.11g, 3.19 x 10^{-2} mole), cationic monomer (0.67g, 0.32 x 10^{-2} mole) and trimethoxysilyl monomer (0.53g, 0.21 x 10^{-2} mole) were rinsed into the reaction vessel with 114 ml solvent mixture of 15:85 v/v% MeOH:EtOH. Anhydrous cationic monomer was predissolved in 3ml pure MeOH before being rinsed into the reaction vessel. Dodecyl methacrylate monomer was pre-columned through activated basic alumina (Brockmann 1 ca.150 mesh, 50g) before use. Dry nitrogen gas was bubbled through for 20 minutes to degas the reaction mixture at room temperature before immersing the reaction vessel in an oil bath heated to 67°C. The vessel was heated for 15 minutes prior to AIBN initiator (0.14g) being rinsed into the reaction mixture with 2ml solvent mixture. The reaction was magnetically stirred and maintained up a positive pressure nitrogen blanket sufficient to bubble through a mineral oil bubbler. The reaction time was 39 hours.

Once cooled to room temperature, the reaction mixture appeared clear with a slight haze. The solvent was removed at room temperature by rotary evaporator to give a white foam residue that was later redissolved in 50ml dichloromethane and added dropwise into vigorously stirred

500ml acetone. A white solid product settled from the acetone leaving a slightly cloudy supernatant. The product was separated by Buchner flask and 113 Whatman wet strengthened filter paper, and dried in a room temperature vacuum oven for up to 72 hours. The product was weighed to provide a 91 wt% yield, bottled in a glass jar and refrigerated.

Characterisation

The polymer requires by weight C 62.93%, H 10.11%, P 3.61%, N 1.95%, Si 0.42% Cl 0.80%, found C 57.88%, H 10.20%, P 3.30%, N 1.84%, Si 0.12% Cl 0.78%; ^{1}Hnmr (400 MHz, ppm, CD_3OD:$CDCl_3$ 1:1 v:v) 4.33, 4.29, 3.97, 3.71, 3.38, 3.34, 3.29, 3.22, 1.67, 1.32, 0.92, 0.09; specific viscosity in a 10mg/ml solution of ethanol:chloroform (1:1) is 0.32.

Example 4

Preparation of Poly(2-Methacryloyloxyethyl)-2'-(Trimethylammoniumethyl) Phosphate, Inner Salt)-co-n-Dodecyl methacrylate)-co-(2-Methacryloyoxy) ethyl trimethyl ammonium chloride)-co-(hydroxy propyl methacrylate)-co-(3-Trimethoxysilylpropyl methacrylate) 23:47:6:20:4 polymers.

4.1 **Monomer Feed Synthesis**

Zwitterionic monomer (34.10g, 0.116 mole) and cationic monomer (6.3g, 0.030 mole) were weighed in a glove box environment dried by P_2O_5. Dodecyl methacrylate (60.01g, 0.236 mole), hydroxypropyl methacrylate monomer (14.51g, 0.101 mole), trimethoxysilyl monomer (5.00g, 0.020 mole) and AIBN initiator (0.2409g, 0.2%) were weighed in air. A 3 neck reaction flask, fitted with water condenser, nitrogen gas flow and monomer feed tubing, and primed with anhydrous n-propanol:isopropyl acetate (60:40 mass ratio) solvent, was immersed in a heated 90°C oil bath. The monomers and initiator were dissolved in n-propanol:iso propyl acetate solvent and magnetically stirred in a measuring cylinder sealed with parafilm. The reaction mixture was drawn into polypropylene tubing placed inside the measuring cylinder and through silicone tubing via a

peristaltic pump to enter the heated reaction vessel in a dropwise process. A complete transfer to the heated vessel took 2 hours. The reaction was stirred for another hour. A second charge of AIBN initiator (0.0241g, 0.02wt%) was added and the reaction mixture was stirred for a further hour, taking the total reaction time to 4 hours. Total solids content was 30 wt% in n-propanol:isopropyl acetate (168.06g:112.08g).

Once cooled to room temperature, the reaction mixture was split into two batches. The first batch of reaction mixture (240ml) was precipitated by dropwise addition to vigorously stirred methyl acetate (2000ml). The product was separated by Buchner flask and 113 Whatman wet strengthened filter paper, and dried in a room temperature vacuum oven for up to 24 hours. The product was rapidly frozen by liquid nitrogen, milled into a fine powder and further dried in a room temperature vacuum for 24 hours. The product (50.67g, 81.8% based on mass recovery) was bottled in a brown glass vial and stored at 4°C.

The polymer requires by weight C 62.4%, H 9.9%, P 3.0%, N 1.9%, Si 0.4% Cl 0.8%, found: C 57.0%, H 9.;4%, N 1.7%, P 2.7%; ^{1}Hnmr (400 MHz, ppm, CD_3OD:$CDCl_3$ 1:1 v:v) 4.41, 4.08, 3.83, 3.46, 3.40, 3.34, 2.07, 1.67, 1.43, 1.18, 1.04.

The product was subjected to chloride ion assay to establish the rate of inclusion of cationic monomer: required 5.23 wt%, found 4.66 and 4.71 wt%.

4.2 **One Pot Synthesis**

Zwitterionic monomer (3.98g, 1.35 x 10^{-2} mole), dodecyl methacrylate monomer (7.009g, 2.76 x 10^{-2} mole), cationic monomer (0.733g, 0.35 x 10^{-2} mole), hydroxypropyl methacrylate monomer (1.691g, 0.67 x 10^{-2} mole) and trimethoxysilyl monomer (0.585g, 0.24 x 10^{-2} mole) were rinsed into the reaction vessel with 98ml solvent mixture of 15:85 v:v% MeOH:EtOH. Anhydrous cationic monomer was predissolved in 3ml pure MeOH before being rinsed into the reaction vessel. Dodecyl methacrylate was pre-columned

through activated basic alumina (Brockmann 1 ca.150 mesh, 50g) before use. Dry nitrogen gas was bubbled through for 20 minutes to degas the reaction mixture at room temperature before immersing the reaction vessel in an oil bath heated to 67°C. The vessel was heated for 15 minutes prior to AIBN initiator (0.14g, 1.1wt%) being rinsed into the reaction mixture with 2ml solvent mixture. The reaction was magnetically stirred and maintained under a positive pressure nitrogen blanket sufficient to bubble through a mineral oil bubbler. The reaction time was 39.5 hours.

Once cooled to room temperature, the reaction mixture was filtered through sintered glass. The solvent was removed at <40°C by rotary evaporator to give a white foam residue that was later redissolved in 58ml dichloromethane and added dropwise into vigorously stirred 600ml acetone. A white solid product settled from the acetone leaving a slightly cloudy supernatant. The product was separated by Buchner flask and 113 Whatman wet strengthened filter paper, and dried in a room temperature vacuum oven for up to 20 hours. The product was milled, further dried in a room temperature vacuum for 24 hours and weighed to provide a 93.2 wt% yield, bottled in a glass jar and refrigerated.

The polymer requires by weight C 62.41%, H9.91%, P 2.99%, N 1.70%, Si 0.47%, Cl 0.89%, found C 58.45%, H 9.45%, P 2.55%, N 1.65% Si 0.34%, Cl 1.06%. ^{1}Hnmr (400 MHz, ppm, CD_3OD:$CD1_3$ 1:1 v:v) 4.33, 4.29, 3.97, 3.71, 3.38, 3.34, 3.29, 3.22, 1.67, 1.32, 0.92, 0.09. Specific viscosity of 10mg/ml solution in ethanol is 0.33. The polymer product was subjected to the chloride ion assay to establish the rate of inclusion of cationic monomer; required 5.24wt%, found 5.16wt% and 5.26 wt%.

Example 5

Preparation of Poly(2Methacryloyloxyethyl) 2'(Trimethylammoniumethyl) Phosphate, Inner Salt)-co-(n Dodecyl methacrylate)-co-(2Methacryloyloxy) ethyl trimethyl ammonium chloride) 33.3:60:6.7 polymers.

Monomer Feed Synthesis

To anhydrous n-propanol:isopropyl acetate (30.0g:8.0g) solvent mixture at room temperature, zwitterionic monomer (13.5g, 4.58 x 10^{-2} mole) dodecyl methacrylate (20.9g, 8.23 x 10^{-2} mole) cationic monomer (2.5g, 1.20 x 10^{-2} mole) were added. To the mixture, AIBN (0.7g, 0.20 wt%), dissolved 4g isopropyl acetate, was added. The stirred mixture was parafilm sealed in a measuring cylinder and dropwise added via a peristaltic pump to stirred anhydrous n-propanol: isopropyl acetate (27g:20g) solvent mixture immersed in a heated 90°C oil bath under N_2 gas flow. Complete transfer took 2 hours. The pump tubing was washed with isopropyl acetate (4g) and n-propanol 4g) into the 90°C reaction mixture. The reaction was stirred for another hour, where upon AIBN, (0.01g, 0.02wt%) dissolved 2ml isopropyl acetate, was added, the pump tubing was washed with isopropyl acetate (2g) and the reaction was stirred for a further hour.

The heating was stopped after 4 hours and the reaction mixture was pumped to ethyl acetate (450g) at room temperature followed by a pump line wash of n-propanol (3g). The product was allowed to settle and the supernatant was decanted. Product was dissolved with isopropanol (47g) solvent, pumped to ethyl acetate (720g) for 45 minutes, the pump line washed with isopropanol (6g) and the product allowed to settle. The supernatant was decanted and the product was washed with acetone (160g) by stirring for 10 minutes. The supernatant was decanted and the product was filtered (Whatman 13 wet strengthened paper) with an acetone wash (80g). The product was dried at room temperature in a vacuum deccicator for up to 16 h, weighed (31.6g, 87% yield based on mass recovery) and stored in a brown glass vial at 4°C.

Characterisation

^{1}Hnmr (400MHz, ppm, CD_3OD:$CDCl_3$ 1:1 v:v) 4.41, 4.08, 3.83, 3.46, 3.40, 3.34, 2.07, 1.67, 1.43, 1.18; Specific viscosity of 10mg/ml solution in ethanol is 0.26.

The polymer was subjected to chloride ion assay to establish the inclusion of cationic monomer, required 5.23 wt%, found 5.28 and 5.36 wt%.

Example 6

Samples of some of the polymers of examples 1 and 2 were tested for their performance in terms of fibrinogen adsorption and heparin activity. A coating solution of the polymer 10mg/ml in isopropyl alcohol, was made up and used to coat the surface of samples of polyethylene terephthalate (p.e.t.). The p.e.t. sample to be subjected to a fibrinogen assay was a 1 x 3 cm sheet, whilst that to be subjected to a heparin assay was 40 micron arterial filter material. The dried coating was subsequently contacted with heparin solution 50 U/ml in PBS, rinsed first with PBS and then with deionised water and dried. The polymer/heparin coated substrate was subjected to the fibrinogen and heparin tests mentioned above. The results for the heparin activity and fibrinogen adsorption for the polymers of example 2 are given in Table 2 below.

Furthermore example 1 polymer/heparin coated materials were subjected to a stability test. For this the polymer(example 1)/heparin coated substrates were immersed in 1% serum albumin in phosphate buffered saline for periods in the range 0.5 to 6 hours at 37°C. The treated samples were removed, rinsed first with PBS and then with deionised water, and the heparin activity measured. The results indicate that there is no significant loss of activity after 6 hours of BSA/PBS incubation, whereas comparative tests carried out on the commercially available Duraflo and Medtronic M-40 surfaces showed very poor stability. The results using the Carmeda Bioactive surface showed equivalent stability.

Example 7

As a further performance test, substrates coated with example 1 polymer, with and without heparin loading, were contacted with heparinised blood 15 U/ml for 60 minutes. The treated samples were removed, rinsed first with PBS and

then with deionised water and the heparin activity measured. The results show that surfaces coated with the polymer with pendant cationic and phosphoryl choline groups attract and bind heparin from blood which contains heparin. The surfaces were also studied under s.e.m. and no biological deposits (e.g. of platelets, blood cells and protein) were observed, for the heparin loaded sample or the non-heparin loaded sample.

As comparisons, tests were also carried out on three commercially available heparinised surfaces. DuraFlo uses ionically bound heparin; Medtronic M-40 is believed to use ionically bound heparin; Medtronic CBM-40 (Carmeda Bioactive) uses end point attached heparin.

For these experiments, filter samples were incubated at room temperature with 5 ml of phosphate buffered saline (PBS) with or without 1% serum albumin (BSA) or fresh heparinized human blood. After 60 min, the samples were rinsed thoroughly with saline and deionised water and heparin activity was measured.

The results are shown in Table 3.

Before incubation with PBS, the heparin activity on the DurafloII sample was 240 mU/cm^2 and 33.5 mU/cm^2 on the Medtronic M40. The Carmeda BioActive Surface heparin appeared to be more stable with BSA, but the initial heparin activity was the lowest of all filters tested. Previous results have shown that another 20 micron Medtronic filter with Carmeda bonded heparin had only 2,3 mU/cm^2.

Table 3 shows that the polymer of the invention attracts and binds heparin from the blood sample which had a heparin concentration of 15 U/ml.

Initial results had shown that the coating not loaded with heparin shows heparin activity following incubation with heparin containing human blood (see Table 3).

Two similar arterial filters were coated with the cationic/zwitterionic heparin binding polymer of example 1. Only one filter was loaded with heparin as described above,

the other filter was only washed with PBS. Both filters were perfused in parallel with bovine blood (3.5 L/min) for 6 hrs. The blood contained 644 U heparin/kg. The activated clotting time (measured by the Hemochron method) of the system was 447 sec after 9 min perfusion and fell to 257 sec after 60 min perfusion. After 306 min perfusion, the activated clotting time was 212 sec. Both filters performed similar and showed significantly less blood clots than uncoated filters in similar previous perfusion experiments.

Example 8

The polymer of example 4 was used to coat arterial filter devices. The filter was air plasma treated for 30s prior to coating. In a separate step two dispersions were made up. The first contained 2500U heparin (bovine lung) in PBS (2.5ml) and water (47.5ml). The second contained 250mg polymer in 50ml isopropylalcohol. The two liquid compositions were mixed together then poured into the plasma treated filter which was shaken vigorously for 15 minutes to ensure contact of all the surfaces of the device with the coating mixture. The mixture was then drained out and the coated device washed three times with water. The rinsed filter was dried and placed in an oven overnight at 50°C to ensure the reactive groups of the polymer had crosslinked.

Example 9

Further samples of polymers of examples 1 and 3 to 5 were coated onto arterial filters using the coating solutions described in example 6. The filters were dip coated with the polymer solutions, which were then dried overnight. The polymers of examples 3 and 4 were kept at 70°C overnight to ensure complete crosslinking. The filters were then tested for their fibrinogen adsorption using the performance test described above. Some samples of filter were, after coating with polymer, were loaded with heparin using the general test described above and then subjected to fibrinogen adsorption and heparin

activity tests. The control was untreated filter. Table 4 shows the results for reduction in fibrinogen adsorption as compared to the control and heparin activity for the heparin loaded devices. Comparisons are quoted for two commercially available heparin coatings Medtronic CB-M40, believed to have covalently (end point attached) heparin and Medtronic M-40 believed to have ionically bound heparin, in terms of fibrinogen adsorption and heparin activity. The results show that heparin is adsorbed onto the polymer, the mechanism assumed to be an ion exchange process. The filters coated with the PC polymer have reduced fouling by fibrinogen.

Example	AIBN	Total	Pm		MI		Cm		Temp	Yield	Cl- Calc	Cl- Obs	MW
	wt%	Solids %	g	Mol %	g	Mol %	g	Mol %	C	%	(mg/l)	(mg/l)	
2.1	2.0	14.7	5.4	36.6	8.4	57.0	0.9	6.4	61	75.6	83.6	76.6	900780
2.2	1.0	14.7	5.4	36.9	8.3	56.8	0.9	6.4	61	74.3	83.0	80.4	678664
2.3	0.2	14.7	5.4	36.6	8.4	56.9	1.0	6.5	61	70.5	84.7	74.7	1597718
2.4	0.1	14.7	5.4	36.7	8.3	56.8	1.0	6.5	61	70.7	84.8	81.4	1850975
2.5	0.2	12.2	5.5	37.4	8.7	59.2	0.5	3.4	61	57.7	44.4	68.0	616454
2.6	0.2	12.2	5.4	37.2	8.7	59.4	0.5	3.4	61	72.6	44.6	37.1	
2.7	0.2	12.3	5.4	37.0	8.3	56.8	0.9	6.2	61	48.3	81.0	84.0	
2.8	0.2	14.7	5.4	36.7	8.4	56.9	0.9	6.4	61	49.9	83.6	71.7	493906
2.90	0.2	14.7	5.4	36.7	8.4	56.9	0.9	6.4	61	67.3	83.6	73.4	
2.10	0.2	12.2	5.4	36.8	8.4	57.1	0.9	6.1	61	73.6	79.8	70.4	395537
2.11	0.2	13.4	5.4	36.6	8.4	57.0	0.9	6.4	61	77.8	83.4	74.3	
2.12	0.2	13.4	5.4	36.6	8.4	57.0	0.9	6.4	61	82.6	83.4	77.6	668891
2.13	0.2	12.4	5.5	37.0	6.9	46.3	2.5	16.7	61	75.4	224.4	226.0	346592
2.14	0.2	14.8	5.4	36.8	6.9	46.4	2.5	16.8	61	78.9	226.1	187.3	
2.15	0.2	12.5	5.5	36.3	4.6	30.4	5.0	33.3	61	68.7	467.9	524.0	685687

Table 1

* The molecular weights are relative values of determinations by gel permeation chromatography (without calibration) but should approximate to Daltons

Example	Heparin Activity mU/cm2	Fibrinogen reduction %
2.5	15.9	79
2.6	11.8	55
2.7	24.3	66
2.8	21.2	74
2.9	36.2	60
2.10	22.1	64
2.11	18.7	67
2.12	35.6	70
2.13	0.9	81
2.14	1.8	57
2.15	0	77

Table 2

	Heparin activity in mU/cm2 following incubation with		
Sample	PBS	PBS/BSA	BLOOD heparinised
DurafloII	25.6	4.8	0
Medtronic M-40	7.1	0.9	0
Medtronic CBM-40	5	4	-
Ex.1/ Heparin	33.1	18.1	16.4
Ex 1	-	0	18.3

Table 3

Polymer of Example	Without Heparin Loading	With Heparin Loading	
	% reduction fibrinogen	% reduction fibrinogen	Heparin activity MU/cm^2
Control	0	100	
1	92	87	42
3	90	82	14
4	91	88	13
5	91	89	39
comparison covalently bound Heparin	N/A	56	9
comparison ionically bound Heparin	N/A	7	<1

Table 4

Reference Example 1

Synthesis of 11-methacryloyl undecyl-1-trimethylammonium bromide.

Step 1

To a solution of 11-bromo-1-undecanol (5.05 g, 0.02 mol), triethylamine (2.86 g, 0.028 mol) in dry ethyl acetate (30 ml), a solution of methacroyl chloride (3.03 g, 0.029 mol) in ethyl acetate (20 ml) was slowly added, and the resulting mixture stirred for 90 min at RT.

The solid was filtered off, and the solvents removed *in vacuo* to afford predominantly 1-bromo, 11-undecylmethacrylate (Yield 6.26 g, 97%). As no starting materials were observed by 1H NMR and TLC Rf 0.69 (chloroform/pet. ether 7:3, v/v), this material was carried through to the second step.

Step 2

The product of step 1 (6.26 g, 0.019 mol) was dissolved in dry acetonitrile (40 ml) and added to a mixture of trimethylamine (2.8 g, 0.047 mol) in acetonitrile (20 ml). The system was purged with nitrogen, and then sealed with a dry ice condenser. The reaction was heated to 50 degrees for 20 hr, and protected from light with aluminium foil.

The remaining trimethylamine was removed on a water pump, and then the solvents removed *in vacuo* to give an off-white powder. This was washed with ether (250 ml) and the white solid collected (5.67 g, 76% yield). The ether was evaporated to dryness, and the residue again treated with ether (100 ml) to yield further white solid (1.02 g, 13%). 1H NMR indicated that the desired product was formed.

CLAIMS

1. A process in which a substrate having at its surface zwitterionic pendant groups and cationic pendant groups is contacted with a solution having suspended or dissolved therein an anionically charged mucopolysaccharide.

2. A process according to claim 1 in which the mucopolysaccharide is heparin.

3. A process according to claim 1 or claim 2 in which the substrate has at its surface a copolymer formed from monomers including

a) a zwitterionic monomer of the formula I

YBX I

wherein B is a bond or a straight or branched alkylene, alkylene-oxa-alkylene, or alkylene oligooxa alkylene group any of which optionally includes one or more fluorine substituents

X is an organic group having a zwitterionic moiety; and

Y is ethylenically unsaturated polymerisable group;

b) a cationic monomer of the formula II

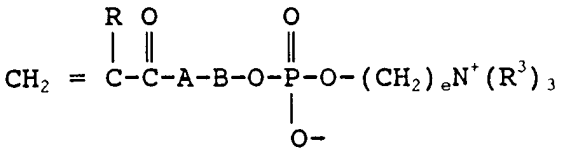
$Y^1B^1Q^1$ II

wherein B^1 is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which optionally includes one or more fluorine substituents;

Y^1 is an ethylenically unsaturated polymerisable group; and

Q is an organic group having a cationic moiety.

4. A terpolymer according to any preceding claim in which X is an ammonium phosphate ester group, the zwitterionic monomer preferably having the formula



VI

wherein R, A and B are as defined in claim 2;

the groups R^3 are the same or different and each is hydrogen C_{1-24} alkyl, aryl, alkaryl, aralkyl, or two or three of the groups R^3 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring; and e is 1 to 6, preferably 2 to 4.

5. A process according to claim 3 or claim 4 in which Q^1 is selected from the group consisting of $N^+R^5_3$, $P^+R^5_3$ and $S^+R^5_2$ in which the groups are the same or different and are selected from hydrogen, C_{1-4} alkyl and aryl, preferably C_{1-4} alkyl.

6. A process according to any of claims 3 to 5 in which the monomers include c) a hydrophobic monomer of the formula III

$$Y^2B^2Q^2 \qquad \text{III}$$

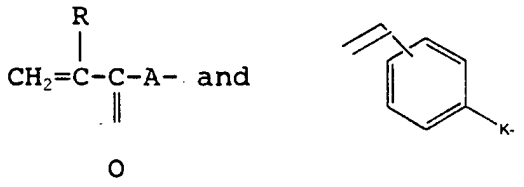
wherein B^2 is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which may optionally include one or more fluorine substituents;

Y^2 is an ethylenically unsaturated polymerisable group; and

Q^2 is an organic group having a hydrophobic group selected from alkyl groups having at least six carbon atoms, fluorine substituted alkyl groups and alkyl groups having at least one siloxane substituent.

7. A process according to claim 6 in which B^2 and Q^2 together represent a C_{6-24}-alkyl group, preferably a straight chain alkyl group, optionally including carbon-carbon unsaturated bonds, preferably being C_{8-16}-alkyl.

8. A process according to any of claims 3 to 7 in which Y, Y^1 and Y^2 are each independently selected from



and

wherein:

R is hydrogen or a C_1-C_4 alkyl group;

A is -O- or $-NR^1-$ where R^1 is hydrogen or a C_1-C_4 alkyl group or R^1 is $-B-X\ B^1Q^1$, B^2Q^2 or B^3Q^3 where B, Q^1, Q^2 and Q^3 and X are as defined above and

K is a group $-(CH_2)_pOC(O)-$, $-(CH_2)_pC(O)O-$, $-(CH_2)_pOC(O)O-$, $-(CH_2)_pNR^2-$, $-(CH_2)_pNR^2C(O)-$, $-(CH_2)_pC(O)NR^2-$, $-(CH_2)_pNR^2C(O)O-$, $-(CH_2)_pOC(O)NR^2-$, $-(CH_2)_pNR^2C(O)NR^2-$, (in which the groups R^2 are the same or different) $-(CH_2)_pO-$, $-(CH_2)_pSO_3-$, or, optionally in combination with B, a valence bond and p is from 1 to 12 and R^2 is hydrogen or a C_1-C_4 alkyl group,

preferably each of Y, Y^1 and Y^2 representing the same group.

9. A process according to any of claims 3 to 8 in which B, B^1 and B^2 each represent a straight or branched C_{1-24} alkylene group.

10. A process according to any of claims 3 to 9 in which the monomers include a crosslinkable comonomer of the formula IV

$Y^3\ B^3\ Q^3$ V

wherein B^3 is a bond or a straight or branched alkylene, alkylene-oxa-alkylene or alkylene-oligooxa-alkylene group, any of which optionally includes one or more fluorine substituents;

Y^3 is an ethylenically unsaturated polymerisable group; and

Q^3 is an organic group having a reactive group capable, on imposition of an external stimulus, of reacting with a coreactive group on the surface of a substrate or which is pendant on the polymer.

11. A process according to any of claims 3 to 10 in which a substrate, is, in a first step, coated with a composition containing the copolymer in a solvent, the solvent is removed and the polymer coated substrate is contacted with the solution containing mucopolysaccharide.

12. A liquid coating composition containing a polymer as defined in any of claims 3 to 10 and an anionic mucopolysaccharide suspended or dissolved in a solvent.

13. A composition comprising an anionic mucopolysaccharide and a zwitterionic organic compound having at least one zwitterionic group and at least one cationic group, preferably having at least two cationic groups.

14. A composition according to claim 13 in the form of a gel of the mucopolysaccharide crosslinked by the zwitterionic organic compound swollen by a liquid, preferably an aqueous liquid.

15. A substrate having a coating comprising a polymer as defined in any of claims 3 to 10 or cross-linked derivative thereof and an anionic mucopolysaccharide.

16. Use of a copolymer having pendant zwitterionic groups and cationic groups to improve the stability of heparin coating on the surface of a substrate by treating the substrate surface with the copolymer prior to heparin coating.

17. A process in which anti-coagulant containing blood is contacted with a surface to reduce the amount of anticoagulant in the blood, characterised in that the surface has pendant zwitterionic groups and pendant cationic groups.

INTERNATIONAL SEARCH REPORT

Inter ..nal Application No
PCT/GB 97/03191

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61M1/36 C09D105/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61M C09D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YOSHIHIRO ITO ET AL.: "Interaction of heparin with amphiphile assemblies and biocompatibility of the heparin complexes" J. BIOMATER. SCI. POLYM. EDN, vol. 6, no. 8, 1994, pages 707-707-714, XP002054989 ---	1
A	GERHARD STEFFAN ET AL.: "Divalent cation-dependent interaction of sulfated polysaccharides with phosphatidylcholine and mixed phosphatidylcholine/phosphatidylglycerol liposomes" CHEMISTRY AND PHYSICS OF LIPIDS, vol. 74, no. 2, 1994, pages 141-150, XP002054990 ---	1

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 1998	20/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Glikman, J-F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/GB 97/03191

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document. with indication,where appropriate. of the relevant passages	Relevant to claim No.
A	WO 93 14127 A (UNIVERSITY OF UTAH (US)) 22 July 1993	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interi ·nal Application No
PCT/GB 97/03191

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9314127 A	22-07-93	AU 3592693 A	03-08-93