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(54) Title: POLYMER SUPERSURFACTANTS FOR PROTEIN RESISTANCE AND PROTEIN REMOVAL

(57) Abstract

The invention discloses the processes and materials for treating materials to minimize the deposition of proteins and other molecules, and for removing proteins and other molecules from materials. New copolymers containing polyethylene oxide sidechains called supersurfactants have the ability to bind themselves to interfaces to provide a stable protein-resistant interface. A process is presented to treat and modify interfaces with the new copolymers.

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DESCRIPTION
POLYMER SUPERSURFACTANTS FOR PROTEIN RESISTANCE AND PROTEIN REMOVAL

BACKGROUND OF THE INVENTION

The present invention relates to materials and processes for (1) treating materials to minimize the deposition of proteins and other molecules; and (2) removing proteins and other molecules from materials. We use the word protein because we expect major applications will involve protein solutions. However, we understand that the processes and materials described apply to all interfacial adsorption, deposition and aggregation processes.

There are many applications where materials and devices must contact various aqueous and other solvent media; including biologic and physiologic solutions. Such media often contain proteins or other chemicals or biochemicals which can adsorb and/or aggregate at interfaces. For many applications, it is desirable and often necessary to minimize the deposition and aggregation of such molecules. Examples where protein deposition can be undesirable are:

Cardiovascular devices (activation of coagulation, thrombosis, and/or complement); Ophthalmologic devices (activation of biochemical process, impaired optical properties); Blood bags and related devices for collection and storage of blood and blood components; Food processing and storage, including dairy and meat industries; Pharmaceutical products (adsorption and denaturation of peptides or other active agents); Human hygiene products (such as diapers and sanitary napkins); Membranes (Polarization and fouling); Sensors (non-specific binding); Separation processes, such as chromatography, electrophoresis, and field flow

fractionation; Process biotechnology and biochemical engineering (adsorption and aggregation at reactor interfaces and at bubbles and other gas/liquid or vacuum/liquid interfaces).

In all of the above, and other, applications, it is desirable to have simple materials and processes with which to treat the interfaces in order to minimize protein adsorption, deposition and aggregation. In cases where proteins are adsorbed to untreated interfaces, materials or devices, it is desirable to have simple means of removing the proteins (a good example is the cleaning of contact lenses).

OBJECT

We and others have shown that polyethylene oxide (PEO) (also called polyethylene glycol or PEG), when bound to an interface, results in effective protein resistance in aqueous solutions. Although other neutral, hydrophilic polymers are also effective, PEO is exceptionally effective in promoting protein resistance, due to its unique interactions with water. We and others have shown that non-polymeric and block copolymer surfactants containing PEO can be adsorbed at interfaces to provide protein resistance. Such adsorption is often not very stable, however, and the protein resistance effect is not optimal.

It is the object of the present invention to overcome the mentioned problem by:

1. Describing a novel composition of matter, a copolymer containing PEO sidechains, which is molecularly engineered to optimally bind to interfaces to provide a stable, long-lived, effective protein-resistant interface; and
2. Describing a process for the effective treatment and modification of interfaces by these new polymers.

We call these polymers "supersurfactants". There is provided a polymer surfactant compositions containing PEO chains, spacers, and components to provide strong binding to the interface of interest.

This invention specifically excludes

(1) polyurethanes and other block copolymers containing PEO blocks;

(2) PEO tri-block and related copolymer surfactants of the Pluronic, Tetronic and/or polyalloxomer types;

(3) graft copolymers of PEO-containing monomers with vinylchloride and/or vinyl acetate; and

(4) other processes for grafting PEO onto existing solid materials.

This invention does include those compositions designed to provide binding of the supersurfactant from solution onto suitable interfaces by hydrophobic, ionic, and solvent-phobic interactions. The PEO chain-containing polymers have a minimum PEO chain molecular weight of about 200, below which the protein resistance chemically deteriorates. At 4000 and above, the polymers have approximately the same properties. The PEO chain spacing along the copolymers falls within the range of 4-20 Å. It's chemically very difficult to space any closer than 4 Å; and at larger separations the protein resistance properties deteriorate significantly.

As illustrative of the above, the following examples are cited:

(a) co-monomers containing alkyl chains or groups to provide hydrophobic binding at hydrophobic interfaces, such as air or gas/liquid interfaces and solid/liquid interfaces where the solid/water contact angle is greater than 20°;

(b) co-monomers containing fluoro-alkyl chains or groups to provide hydrophobic or fluorophilic bonding to hydrophobic or fluorocarbon surfaces;

(c) co-monomers containing various siloxane components to provide binding to siloxane and other hydrophobic surfaces;

(d) co-monomers containing negatively-charged groups to provide electrostatic interactions with positively-charged surfaces;

(e) co-monomers containing positively-charged groups to provide electrostatic interaction with negatively-charged surfaces;

(f) co-monomers with an approximate ratio of hydrophobic, fluorophilic, positive or negative character to optimally interact with the multiple binding character of a complex interface.

In all cases, the objective is to produce interfaces containing sufficient PEO to minimize protein deposition.

The monomers used in this process have a carbon-carbon double bond and can be copolymerized by conventional radical initiators such as, for example, benzoyl peroxide, asobisisobutyronitrile, and azobisdimethylvaleronitrile.

After copolymerization, the copolymers are isolated by precipitation into a suitable precipitant. However, it is possible to use the polymerization mixture directly (after adjusting the copolymer concentration) for the deposition on biomaterial surfaces. The unreacted monomers may be rinsed off by proper solvent. These procedures, and other will be explained in further detail below.

THE DRAWINGS

Graphs corresponding to data described in the application are set forth in the following drawings:

Fig. 1 is a graph showing the adsorbed amount of polymers on LDPE surface before and after protein adsorption; and

Figs. 2, 2A and 2B are a comparison of surface properties between the synthesized copolymers and selected commercial surfactants.

Figure 1 Adsorbed amount of polymers on LDPE surface before and after protein adsorption (protein adsorption, human albumin 1 mg/ml, 30 minutes; polymer treatment, 30 minutes desorption in water after 30 minutes adsorption in 1 mg/ml polymer solution; cross-hatched bars, before protein adsorption; diagonal bars, after protein adsorption) (n = 3)

Figures 2, 2A and 2B Comparison of surface properties between the synthesized copolymers and selected commercial surfactants (n = 3-5)

Fig. 2 Adsorption of polymers on LDPE surface

Fig. 2A Protein resistance of polymer-treated LDPE surfaces

Fig. 2B Removal properties of pre-adsorbed protein on LDPE surfaces by polymer solution treatment (cross-hatched bars, albumin 1.0 mg/ml adsorption; diagonal bars, plasma 1.0 % adsorption).

REAGENTS

Monomethoxy poly (ethylene oxide)$_{1900}$methacrylate ($MPEO_{1900}MA$). (1900 is the approximate molecular weight.) To a well-stirred solution of 15.2 g (8 mmol) monomethoxy poly (ethylene glycol) (Polyscience) in 20 ml dry methylene chloride (CH_2Cl_2) cooled to 5° C, 1.67 g (16 mmol) methacryloyl chloride in 2 ml methylene chloride and 1.62 g (16 mmol) triethyl amine was added slowly dropwise. After that the reactants were stirred at room temperature overnight (in the presence of small amount of

inhibitor, tert. octylpyrocatechine). Precipitated triethyl amine hydrochloride was filtered off, macromonomer was isolated by precipitating the solution into cooled diethyl ether and powdered polymer was washed thoroughly with diethyl ether and dried.

Monomethoxy poly (ethylene oxide)$_{4000}$methacrylate ($MPEO_{4000}MA$) was kindly provided by S. Nagaoka (Toray Industries, Inc., Kanagawa, Japan). (We call MPEOMA as "macromonomer" because it is a big molecule with a long PEO chain, while it is a monomer with a species bearing a polymerizable function at the chain end [a methacryloyl end group].)

2.2' -azobisisobutyronitrile (AIBN) (Aldrich) was purified by recrystallization from methanol and used as an initiator for polymerization.

Methyl methacrylate (MMA), hexyl methacrylate (HMA) and lauryl methacrylate (LMA) (Polyscience) were freshly distilled under reduced pressure before use.

EXAMPLES OF PREPARED COPOLYMERS

Example 1:

The copolymers were prepared by tandom polymerization of the monomers in toluene for 45 hours at 50° C.

A polymerization mixture, containing 14.0 wt% of monomers, a 0.6 wt% of AIBN and 85.4 wt% toluene, was bubbled with nitrogen for 15 minutes then sealed in an ampoule. After the polymerization has been finished, the volume of polymer solution was reduced by approximately 50% using a rotary vapor evaporator under reduced pressure. Polymers were precipitated into cooled diethyl ether, washed and dried. To remove non-copolymerized macromonomer (about 20%, determined by gel permeation chromatography [GPC]), the polymers containing PEG_{1900} were dialyzed three days in Visking dialysis tubing (Mol.wt. cut-off, 6,000-8,000). The polymers containing PEG_{4000} were purified by using ultrafiltration (Amicon, membrane PM-30). The polymers were then isolated using

lyophilization. Table 1. shows the list of the synthesized copolymers. Each entry on the Table may be considered a specific example. As most of the polymers were not directly soluble in water or aqueous buffers, a special procedure was used for preparation of aqueous solutions for surface tension or adsorption studies, GPC measurement or purification using dialysis or ultrafiltration. 100 mg of polymer was dissolved in 5 ml warm ethanol (about 50° C), then diluted with 20 ml of water and subsequently dialyzed against water (for surface tension and adsorption studies) or against Tris buffer (for GPC measurement). Then the solutions were diluted to the concentrated needed. The polymers can also be applied in mixed solvents such as a water-ethanol mixture, or can be applied in organic solvents such as ethanol.

Table 1. Composition of PEO-containing surfactants used.

SYNTHESIZED METHACRYLATE COPOLYMERS[α]

Polymer No.	$MPEO_{1900}MA$	$MPEO_{4000}MA$	MMA	HMA	LMA	MA-Tyr-NH_2	Hydrophobic units, WT%
9	20	-	20	60	-	-	22
10	25	-	20	55	-	-	17
11	25	-	40	35	-	-	16
12	25	-	20	0	55	-	24
13	-	20	20	60	-	-	12
14	-	20	20	-	60	-	17
15	-	15	35	-	50	-	16
16	20	-	19	60	-	1	22
17	25	-	19	55	-	1	18
18	25	-	39	35	-	1	16
19	25	-	19	-	55	1	25
20	-	20	19	60	-	1	12
21	-	20	19	-	60	1	17
22	-	15	34	-	50	1	21
23	-	10	19	-	70	1	32

[α]composition, mol%

COMMERCIAL BLOCK COPOLYMER SURFACTANTS

Polymer sample No.	Chain length PEO	PPO	PBO	Wt % PEO
24 (Triblock)	13	30	--	40
25 (Triblock)	13	--	25	40
26 (Star-like block)	26	29	--	40
27 (Alternate block)	13	30	--	40

Example 2: Adsorption of Copolymers onto Hydrophobic Surfaces

Polymer materials to be treated (low density polyethylene film (LDPE, NHLBI DTB Primary Reference Material)) were immersed in the copolymer solutions (1.0 mg/ml) at room temperature for 30 minutes for adsorption (a 30 minute exposure was sufficient to achieve equilibrium). The copolymer-adsorbed films were rinsed in purified water and then immersed again in purified water for 30 minutes for desorption. After rinsing again in purified water, the copolymer-treated surfaces were vacuum dried overnight in an air atmosphere for X-ray photoelectron spectroscopy (XPS) analysis. The oxygen 1S peak from the wide scan was used for the analysis of adsorbed copolymers.

For the quantitation of the copolymer adsorption, small amount of methacryloyl tyrosinamide (MA-Tyr-NHz_2 was introduced in the structure of copolymers during synthesis. The tyrosin content in all copolymers was 16 ± 3 nmol/mg (or about 1.5 mol%). The copolymers were labeled with Iodine-125 using the modified Chloramine-T method. The iodination reaction time was 4 hours and the reaction mixture was continuously shaken during reaction. After iodination, the ^{125}I-labeled copolymer solution was passed twice through the Sephadex G-25 mini-columns prepared separately to remove free ^{125}I. The polymer materials to be treated (LDPE films), whose surface area was predetermined, were immersed in a solution of known ration of ^{125}I-labeled and unlabeled polymer and adsorption was done with the same procedure as in the case

for the samples for XPS analysis. After copolymer adsorption and following rinsing, the copolymer-treated films were directly placed in counting vials and the retained radioactivity was measured in a gamma counter and converted to the values of the adsorbed amount of polymer on the surface.

Table 1 lists the copolymers synthesized for XPS analysis and for Iodination labeling. Figure 1 shows adsorbed amount of copolymers on the LDPE surfaces. Figure 1 also shows that the copolymers are stably adsorbed on the surface, after protein (human serum albumin) exposure.

Example 3: Protein-resistant Properties of the Copolymer-treated Surfaces

The copolymer-treated LDPE films were immersed in protein solutions (human albumin, 1.0 mg/ml or plasma 1.0%) prepared with phosphate buffer saline (PBS) at pH 7.4 for 30 minutes and rinsed in PBS, following by rinsing in purified water and vacuum drying, then the prepared samples were analyzed by XPS. The nitrogen 1S peak was used for the analysis of adsorbed protein. The ^{125}I-labeled copolymer-treated surfaces were also used and radioactivity was counted both before and after protein adsorption on those surfaces, to see the effect of exchange of the protein with the copolymer preadsorbed on the surface (see Figure 1).

Figure 2 compares the surface properties of the copolymers with those of some selected commercial block surfactants containing PEO and polypropylene oxide (PPO) or polybutylene oxide (PBO). Oxygen atomic % from XPS analysis in Figure 2 (A) represents adsorption of the polymers on the LDPE surface, even though exact comparison is not available because the polymers contain different numbers of oxygen. In Figure 2 (B), nitrogen atomic % represents the relative adsorbed amount of protein on the polymer-treated LDPE surfaces. As seen in Figure 2 (B), the synthesized copolymers show much better protein

(albumin and plasma) resistance than the commercial block surfactants, probably due to larger amount of adsorption and longer PEO chains which provide high mobility in water.

Example 4: Removal of Pre-adsorbed Proteins by the Polymer Treatment

For protein adsorption of the LDPE surfaces, LDPE films were immersed in protein solutions (human albumin, 1.0 mg/ml or plasma 1.0 %) prepared with phosphate buffer saline (PBS) at pH 7.4 for 30 minutes and rinsed in PBS, following by rinsing in purified water. After vacuum drying overnight in an air atmosphere, the adsorbed amount of protein on LDPE surfaces was analyzed by XPS. The nitrogen IS peak was used for the adsorbed protein.

The protein pre-adsorbed LDPE films were immersed in the surfactant solution (1.0 mg/ml) for 30 minutes and also rinsed in PBS and purified water, vacuum dried, and then the remaining protein on the surface was also analyzed by XPS.

The effectiveness of the synthesized copolymers for removal of proteins pre-adsorbed on LDPE surface was compared with that of the commercial surfactants in Figure 2 (C). As seen in the Figure, the synthesized copolymers show efficient removal properties of preadsorbed proteins (albumin and plasma), even though the commercial surfactants shows better removal properties. From the studies of XPS analysis, ^{125}I-labeled copolymers, and ^{125}I-labeled protein, we can conclude that the proteins are removed from the surface probably by different mechanisms when we use different kinds of surfactant; synthesized copolymers (possibly acting mainly by an exchange mechanism of the protein with the copolymer) and commercial block surfactants (possibly acting by an elution mechanism of the protein by the surfactant.

CLAIMS

We claim:

1. Soluble polymers and copolymers containing randomly located polyethylene oxide (PEO) chains which can be adsorbed or deposited at interfaces to provide protein resistance properties, such polymers and copolymers having a PEO chain molecular weight greater than 200, and the PEO chain spacing along the copolymers being in the range of 4 to 20 Å.

2. Soluble polymers and copolymers containing randomly located polyethylene oxide PEO chains together with alkyl chains to provide optimum adsorption and binding onto hydrophobic surfaces, including polyolefins, acrylates, and methacrylates, vinyl polymers, hydrophobic block copolymers--including polyurethanes, and all other polymers whose advancing water contact angle is greater than 20°.

3. Soluble polymers and copolymers containing PEO chains together with fluorocarbon chains to provide optimum adsorption and binding onto fluorocarbon surfaces, including per fluorinated polymers and surfaces, and all other polymers whose advancing water contact angle is greater than 70°.

4. Soluble polymers and copolymers containing PEO chains siloxane chains to provide optimum adsorption and binding onto surfaces, and all other polymers whose advancing water contact angle is greater than 60°.

5. Soluble polymers and copolymers containing randomly located polyethylene oxide PEO chains together with components bearing negative or positive charges to provide optimum adsorption and binding onto positive or negative surfaces, respectively.

6. The materials in Claim 1, where the amount of PEO-containing monomer is in the range of 5 to 90% by weight.

7. Soluble polymers and copolymers containing not less than 5% weight of monomer units A, where A can be represented by the formula:

$$CH_2= \underset{\displaystyle R^1}{\overset{|}{C}}-CO-(O-CH_2-CH_2)_X -O-R^2$$

where x is 6 to 200, R^1 is H or CH_3 and R^2 is H Or C_1 to C_8 alkyl and not more than 95% weight of other monomer units, said copolymers being synthesized by copolymerization of A with one or more co-monomers which are themselves copolymerizable with A.

8. A composition as set forth in claim 7, in which one of said co-monomers is of the formula:

$$CH_2= \underset{\displaystyle R^1}{\overset{|}{C}}-CO-O-R^3$$

where R^3 is C_1 to C_{25} alkyl.

9. A composition as set forth in claim 7, in which one of said co-monomers is of the formula:

$$CH_2= \underset{\displaystyle R^1}{\overset{|}{C}}-CO-N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$$

where R^4 and R^5 are H or C_1-C_8 alkyl; other co-monomers may include acrylic acid, methacrylic acid, diacetone acrylamide, N-vinyl pyrrolidone, diethylamineothyl methacrylate, and diemethylaminoethyl methacrylate.

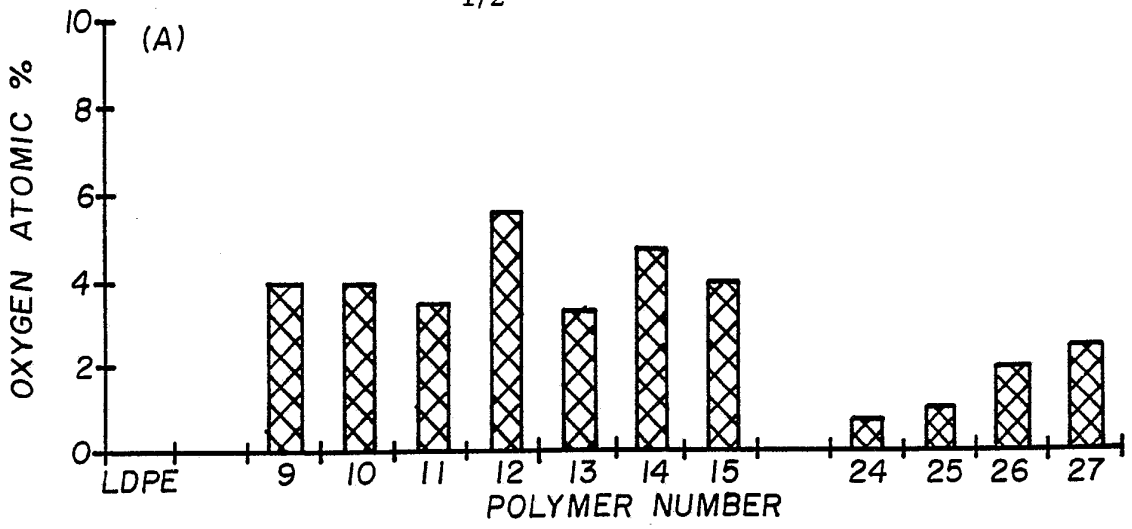

Fig. 2

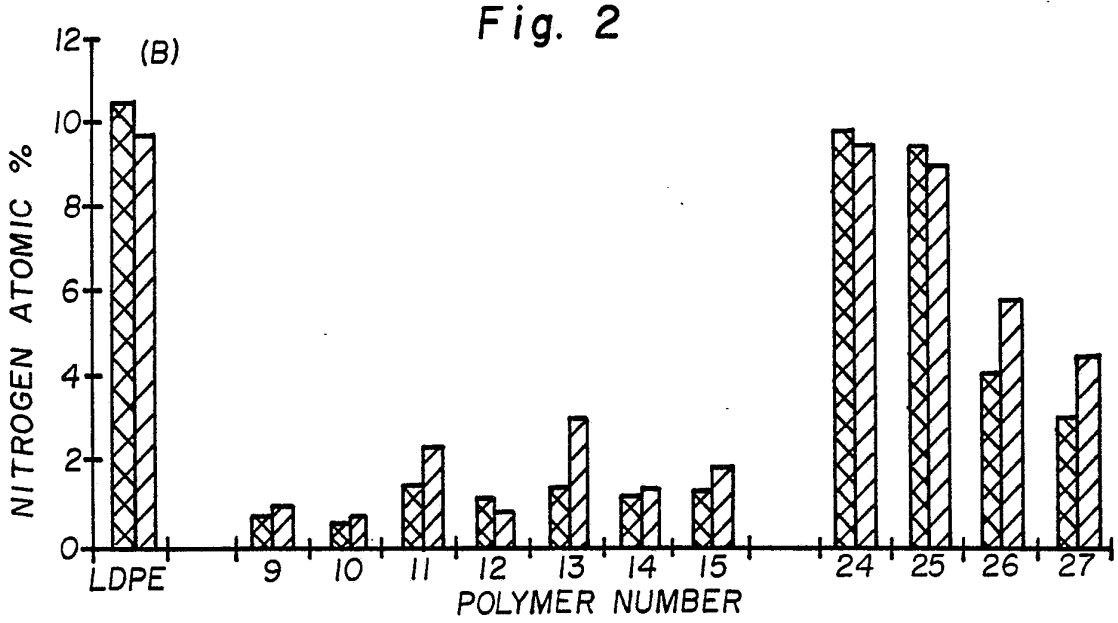

Fig. 2A

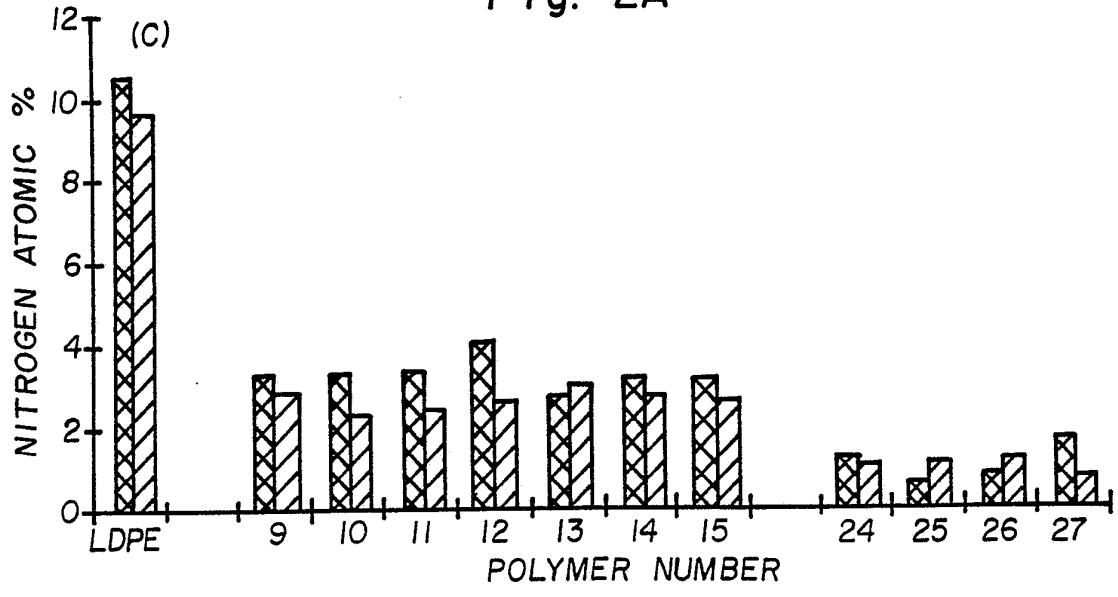

Fig. 2B

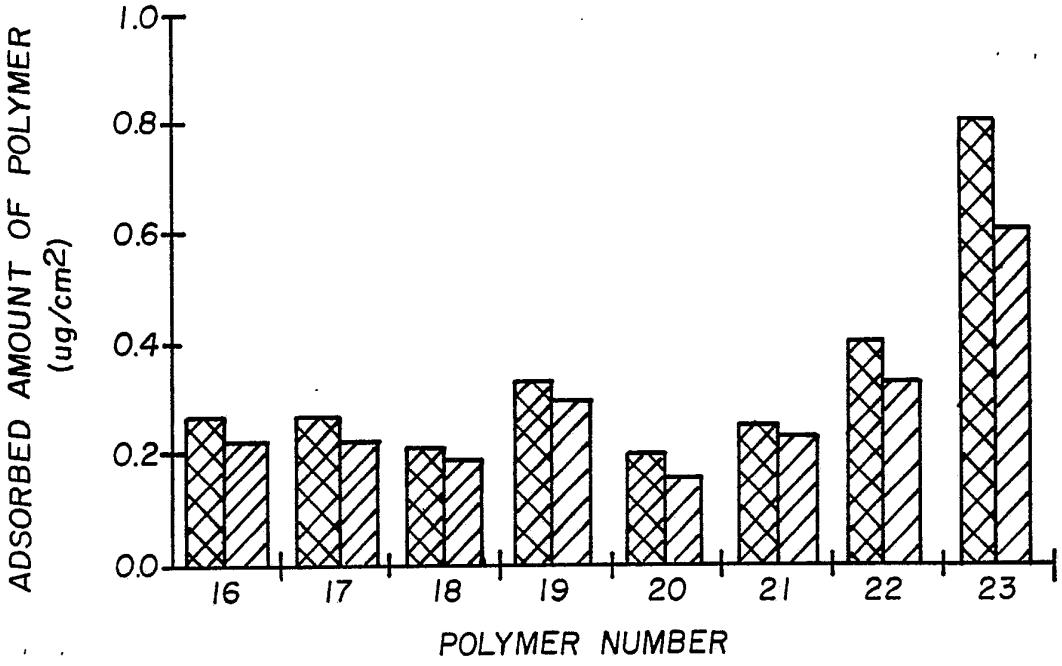
ADSORBED AMOUNT OF POLYMER
(ug/cm2)
1.0
0.8
0.6
0.4
0.2
0.0
16
17
18
19
20
21
22
23
POLYMER NUMBER

Fig. 1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 89/4110

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): C08F 212/00, C08F 20/10, C08F, 126/00, C08F 120/26 , C08L 51/10 *

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

US 525/64, 67, 69, 100, 105; 528/29, 48
526/307.5, 312, 318.41, 320, 279, 242

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,P	US, A 4,424,311 (NAGAOKA) 3 JANUARY 1989, SEE COLUMNS 2-3	1,2,6,7,8,9
Y	US, A 4,200,563 (KOMIYA) 29 APRIL 1980, SEE	1,2,6,7,8,9
A	US, A 4,728,696 (VAN PHUNG) 1 MARCH 1988, SEE ENTIRE DOCUMENT	
Y	US, A 4,429,097 (CHANG) 31 JANUARY 1984, SEE	1,2,6,7,8
X	US, A 4,079,084 (HOUGHTON) 14 MARCH 1978 SEE COLUMNS 2-3	1,3,5-7,9
A	US, A 4,534,799 (AGUIRRE) 13 AUGUST 1985 SEE COLUMNS 4-6	3
Y	US, A 4,600,436 (TRAVER) 15 JULY 1986 SEE COLUMNS 5-7	1,4,6,7,9
P,A	US, A 4,855,379 (BUDNIK) 8, AUGUST 1989 SEE ENTIRE DOCUMENT	4

* Special categories of cited documents: [10]

"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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 DECEMBER 1989	11 JAN 1990
International Searching Authority	**Signature of Authorized Officer**
ISA/US	JEFFREY T. SMITH

International Application No. PCT/US 89/4110

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

*	U.S. CL: 526/307.5, 312, 318.41, 320; 525/64, 67, 69, 100, 105	

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. X OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

GROUP I CLAIMS 2, 8
GROUP II CLAIM 3
GROUP III CLAIM 4
GROUP IV CLAIM 5
CLAIMS 1, 6, 7, 9, REPRESENT LINKING CLAIMS

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.

Groups I, II, III, and IV represent mutually exclusive species. These species contain (I) alkyl chains, (II) fluorocarbon chains, (III) siloxane chains, and (IV) ionic groups. Claims 1, 6, 7 and 9 represent linking claims and are generic to all of the groups. Groups I, II, III, and IV are without a common inventive concept. <Note 37 CFR 1.476(d)>