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Grafting of Acrylates and Vinyl Chains Onto Collagen with Ceric Initiator

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Synopsis

To determine the scope of the grafting reaction, over 30 monomers were grafted to steer hide collagen and collagen films using ceric ammonium nitrate as initiator. High yields of apparent graft polymer were obtained with most acrylate and methacrylate esters. Yields were not changed greatly by employing the higher homologues. Moreover, monomers containing such diverse substituents as hydroxy, cyano, chloro, trifluoroethyl, or glycidyl groups may be grafted onto collagen. The presence of these functional groups in the products provides potential reaction centers to further modify the collagenous surface. Presence of vinyl polymer was confirmed by IR spectra. The large number of monomers of varying polarity which were found to undergo apparent grafting makes it possible to vary widely the surface properties of collagen. It was shown that certain monomers impart water and oil repellency to collagenous surfaces, whereas others increased the hydrophilicity or oleophilicity of the substrate. Thus, by proper selection of monomers, the desired degree of hydrophilic to hydrophobic or oleophilic to oleophobic balance of the collagen surface to suit specific applications can be obtained.

INTRODUCTION

Grafting of side chains to proteinaceous materials offers an attractive technique of modifying the properties, especially the surface characteristics, of the substrate. Although many investigators have studied grafting to cellulosic materials,[1–3] comparatively little information has been reported regarding the chemically initiated grafting of monomer to collagen.[4–13] Ceric ammonium nitrate (CAN) has been used as an initiator by Rao and co-workers to graft methyl methacrylate, acrylonitrile, and acrylamide to collagen.[4–7] This salt forms an effective redox system with alcohols, aldehydes, amines, and thiols. Mino and Kaizerman[14] showed that alcohols form a ceric ion–alcohol complex and that the dissociation of this complex is the rate-determining step:

$$\text{Ce(IV)} + \text{RCH}_2\text{OH} \rightarrow \text{ceric ion–alcohol complex} \rightarrow \text{Ce(III)} + \text{H}^+ + \text{R}\dot{\text{C}}\text{HOH}$$

Collagen contains alcoholic groups in the hydroxyproline, serine, threonine, and hydroxylysine moieties. Free radicals are probably formed at such sites which in the presence of a vinyl monomer serve to initiate grafted side chains. The free radicals are formed on the side chains of the substrate, and thus a high grafting efficiency compared to other redox systems can be

expected.[15] The object of this study was to determine which acrylic or vinyl monomers containing a variety of functional groups would react with the collagen substrate to yield an apparent graft copolymer. The formation of a true graft has only been established for the collagen–methyl methacrylate copolymer.[7,16] This has been accomplished by hydrolysis of the polymer, dinitrophenylation of the water-insoluble hydrolyzate, and spectrophotometric analysis of this product. For all other reaction products, increase in weight after extraction of soluble homopolymer in excess of the increase in weight obtained in the absence of monomer was used as criterion for successful grafting. It is recognized that some homopolymer, especially homopolymer of the bifunctional methacrylates that crosslink during the reaction, is not removed from the substrate by this treatment. Nevertheless, the interlocking polymeric mixtures should differ in properties from those of the substrate. It was anticipated that by judicious selection of reactive monomers and by employing the most suitable reaction conditions, it may be possible to synthesize tailor-made products with desirable properties. It was hoped that results obtained in this study would be applicable to the modification of collagen-containing surfaces such as soft or hard tissues with the aim of improving adhesion, resistance to environmental conditions, and biocompatibility of skin, tooth, or bone. Thus, rather mild reaction conditions involving aqueous solutions at 37°C were employed which, with suitable modifications, might be tolerated in vivo.

Experimental Materials

The following monomers were used: the methyl, ethyl, isobutyl, lauryl, hydroxyethyl, dimethylaminoethyl, *tert*-butylaminoethyl esters of methacrylic acid, acrylic acid, 1,3-butylene dimethacrylate, butyl- and 2-ethylhexyl acrylate (Rohm and Haas); ethyl acrylate, methacrylic acid, vinyl acetate, acrylonitrile, and styrene (Eastman Organic Chemicals); cyanoethyl acrylate and vinyltoluene (Monomer—Polymer Laboratories); isodecyl and cellusolve acrylate (Union Carbide Corp.); N-vinyl-2-pyrrolidone and butenediol (GAF Corp.); 2,2,2-trifluoroethyl and 1H,1H,5H-octafluoropentyl methacrylate, 2,2,2-trifluoroethyl and 1H,1H,5H-octafluoropentyl acrylate (PCR Inc.); hexafluoroisopropyl acrylate and methacrylate (Columbia Organic Chemicals, Inc.); diallyl phosphite and triallyl phosphate (Weston Chemical, Inc); glycidyl methacrylate (American Aniline and Extract Co.), ethylene glycol dimethacrylate (Sartomer Resins), α-chloroacrylonitrile (Polysciences Inc.), and 4-vinylpyridine (Reilly Tar and Chemical Corp.). All liquid monomers were distilled under vacuum prior to use, with the exception of lauryl methacrylate. This monomer, because of its high boiling point, was shaken with 1% NaOH–25% sodium carbonate solution, washed with water, and dried prior to use. Dioctyl sodium sulfosuccinate was obtained from Fisher Scientific Co. All other chemicals were reagent grade.

Collagen powder had previously been prepared in this laboratory from raw steerhide.[17] The grain and fresh layers of the hide were split off and

discarded. The corium was ground to a powder and freed of extraneous fats, salts, and proteins by a series of aqueous and organic extractions. The final product was ground in a Wiley mill to pass a 40-mesh sieve.

The collagen film (0.005 and 0.0013 cm thick), made up of collagen fibrils of about 99% purity, was an experimental material obtained from Ethicon, Inc.

Methods

Preliminary experiments were conducted to obtain optimum conditions for grafting of methyl methacrylate to steerhide collagen powders. In nearly all these experiments, approximately 1 millimolar concentrations of CAN were used. The yield of the graft product was greatly affected by the completeness of oxygen removal. Various nitrogen flow rates were explored to obtain efficient deaeration. Based on these experiments, the following standardized grafting procedure was developed.

Approximately 1.0 g collagen was stirred in a 100-ml, three-necked flask for 1 hr in 50 ml water containing 2% wetting agent (dioctyl sodium sulfosuccinate). One ml 0.05*M* CAN in 1*N* HNO_3 was added. The mixture was deaerated for 15 min by passing purified nitrogen at the rate of 10 ml/min through the solution before 2.25 ml monomer was added. The mixture through which nitrogen was passed at the rate of 3 ml/min was stirred at 37°C for 3 hr. The solid was recovered, thoroughly washed with water and acetone, and any soluble homopolymer was extracted by stirring for 24 hr at room temperature with 100 ml of an appropriate solvent. Acetone was used for the extraction of most homopolymers. Other solvents employed for the extraction of the homopolymers were: toluene for polystyrene and poly(vinyltoluene); ethanol for calcium polyacrylate, poly(acrylic acid), poly(vinylpyridine), and polybutenediol; dimethylformamide for polyacrylonitrile and poly(α-chloroacrylonitrile ; methyl ethyl ketone for poly(lauryl methacrylate), poly(isodecyl acrylate), and poly(2-ethylhexyl acrylate); chloroform for poly(1,3-butylene dimethacrylate); and water for poly(methacrylic acid). The residues of these extracts were washed with acetone and dried at 1.3 M/m^2 (1×10^{-2} mm/Hg) for 24 hr. Most products were further purified by a 48-hr Soxhlet extraction with methylene chloride and drying in a vacuum oven. They were weighed after storage at room temperature and approximately 50% relative humidity for 24 hr. To determine water content and water uptake, the products equilibrated at 50% R.H. were placed in a vacuum of 6.7 N/m^2 (5×10^{-3} mm) for 48 hr, weighed, and allowed to come to equilibrium at 50% R.H.

To determine the contact angle, the treated collagen specimens were pressed into 1.2-cm-diameter pellets under a pressure of 2.2×10^7 N/m^2. The contact angle was estimated with a commercial contact angle viewer 30 sec after placement of the drop.

The films were conditioned for at least 15 hr in a desiccator over anhydrous $CaSO_4$, clamped in a glass or stainless steel frame (interior opening: 4.8 × 1.9 cm), and stored in a 400-ml beaker containing 50 ml of a 2% dioctyl

sodium sulfosuccinate solution for 1 hr before adding 1 ml CAN. The beaker was covered and the solution was deaerated for 15 min before adding 2.25 ml monomer. The reaction was continued for 3 hr at 37°C in a nitrogen atmosphere with stirring as described for the collagen powder. The films were washed with water and stirred successively for at least 24 hr in 100 ml acetone and ethanol before drying at 37°C and $6.7 \times 10^3 N/m^2$ (50 mm/Hg) for 24 hr.

Infrared spectra of KBr pellets containing from 0.6% to 1.0% of the products were obtained spectrophotometrically.

RESULTS AND DISCUSSION

Preliminary experiments indicated that exposure of the collagen to swelling agents, such as 5% $ZnCl_2$ or 5% KCNS solutions, prior to the addition of initiator and monomer did not result in an increase in weight of the substrate. A marked weight gain was obtained using a 2% concentration of wetting agent. Among these, dioctyl sodium sulfosuccinate resulted in a larger weight increase than nonionic wetting agents (polyoxyethylene lauryl alcohol, sorbitan trioleate, and poly(vinylpyrrolidone–vinyl acetate).

Efficient removal of oxygen is very important to obtain optimum yields. Thus, in initial experiments where nitrogen was not passed through the reaction mixture after initial deaeration, much lower yields of graft product were obtained than in those reported below.

A number of control runs were conducted to establish if the addition of wetting agent or initiator results in polymer formation or in a weight increase of the collagen. In the absence of collagen, only 0.1% to 0.2% (based on initial monomer) of homopolymer is formed when styrene is reacted with the CAN solution and/or wetting agent using the conditions described in the grafting procedure. However, when methyl methacrylate is reacted with CAN under the same conditions, an 8% yield of polymer was obtained.

The weight increase of the collagen substrate resulting from the addition of various reagents used in the grafting procedure to the collagen–water mixture is given in Table I. A considerable weight increase (21–24%) was obtained when only collagen was reacted with nitric acid or CAN solution in the presence of wetting agent. The anionic dioctyl sodium sulfosuccinate wetting agent which is present in a fairly high concentration (2%) is strongly sorbed by the positively charged collagen molecules in the acidic reaction mixture. It is probably insolubilized in the presence of the nitric acid electrolyte and thus is not removed by washing the substrate with water. However, the wetting agent is removed quantitatively by extraction for 24 hr of a CAN-treated control with 3% aq. Na_2CO_3 solution. Even a collagen–methyl methacrylate graft copolymer lost over two thirds of its theoretical amount of residual wetting agent on Na_2CO_3 extraction. The extracted samples after storage in 2% dioctyl sodium sulfosuccinate and thorough washing showed no appreciable weight increase, indicating

TABLE I
Weight Increase of Collagen in the Presence of Reagents Used in the Grafting Procedure[a]

Reagent	Weight increase of collagen, %
1 ml 1*N* HNO_3	0.05
2% Wetting agent[b]	0.9
2.25 ml Methyl methacrylate	0.1
2.25 ml Styrene	0.2
1.0 ml CAN in 1*N* HNO_3	0.04
1.0 ml HNO_3 + 2.25 ml methyl methacrylate	0.5
2% Wetting agent[b] + 1 ml 1*N* HNO_3	23[c]
2% Wetting agent[b] + 1.0 ml 0.05*M* CAN in 1*N* HNO_3	21[c]
2% Wetting agent[b] + 2.25 ml methyl methacrylate	2[c]
1.0 ml 1*N* HNO_3 + 2% wetting agent + 2.25 ml methyl methacrylate	27[c](24)[d]
1.0 ml 1*N* HNO_3 + 2.25 ml styrene	0.6
2.0% Wetting agent + 2.25 ml styrene	0.4
1.0 ml 1*N* HNO_3 + 2% wetting agent + 2.25 ml styrene	24

[a] Procedure: To approximately 1 g collagen in 50 ml water, the various reagents were added as described in the grafting procedure. Reaction time: 3 hr. Precipitate washed with water and dried.

[b] Dioctyl sodium sulfosuccinate.

[c] Duplicate runs.

[d] After extraction with acetone.

that the wetting agent is kept tightly on the substrate only in a strongly acidic medium.

To determine if poly(methyl methacrylate) is sorbed, a 5% acetone solution of the polymer was stirred with collagen powder for five days. After repeated extraction with acetone, the dried collagen powder showed no increase in weight, indicating that sorption of poly(methyl methacrylate) on collagen is negligible.

Results of grafting various monomers to powdered steerhide collagen are given in Table II. As was established in the preliminary experiments, results are not only dependent on the completeness of oxygen removal, but also on the pretreatment of the specimen. Factors such as length of storage in water prior to the reaction, presence and concentration of wetting agent, and purity of the monomer were found to be important.

An increase in weight after extraction of soluble homopolymer (as compared to the control runs in which addition of monomer was omitted) was obtained on reaction of collagen with most monomers. Yields varied widely but were highest for acrylates and methacrylates. Yields did not change greatly with the higher homologues. Based on monomer used, the yields of these two homologous series were generally in the 45% to 90% range. Monomers containing hydroxyl or glycidyl groups such as hydroxy-

TABLE II
Grafting of Polymers to Steer Hide Collagen

Monomer	Increase in weight,[a] %	Apparent yield,[a,b] %	Water sorption at 50% R.H., %
Collagen	—	—	19.2
Control (no monomer, CAN[c])	21[d]	—	11.6
Control (methyl methacrylate, no CAN)	2[d]	—	17.4
Acrylic acid	43	18	9.4
Ethyl acrylate	106	51	4.6
Butyl acrylate	110	55	4.4
Isodecyl acrylate	153	77	4.7
2-Ethylhexyl acrylate	134	67	3.8
2,2,2-Trifluoroethyl acrylate	124	45	3.8
Hexafluoroisopropyl acrylate	120	39	4.3
1H,1H,5H-Octafluoropentyl acrylate	117	35	4.1
Cyanoethyl acrylate	129	54	4.2
Cellusolve acrylate	200	91	5.5
2% Aq. calcium acrylate	11	—	14.2
Methacrylic acid	56[d]	25	10.8
Methyl methacrylate	187	89	5.8
Methyl methacrylate (no wetting agent)	51[d]	24	8.7
Ethyl methacrylate	182	89	4.4
Isobutyl methacrylate	91	46	6.1
Lauryl methacrylate	148	76	4.2
2-Chloroethyl methacrylate	96	39	5.4
2,2,2-Trifluoroethyl methacrylate	118	45	4.3
Hexafluoroisopropyl methacrylate	134	45	6.0
1H,1H,5H-Octafluoropentyl methacrylate	30	9	7.8
Hydroxyethyl methacrylate	226	98	7.4
Glycidyl methacrylate	239	99	4.4
tert-Butylaminoethyl methacrylate	2[d]	1	18.4
Dimethylaminoethyl methacrylate	12	6	15.9
Dimethylaminoethyl methacrylate (acidified to pH 2.5)	34	16	8.1
Ethylene dimethacrylate	104	44	6.9
1,3-Butylene dimethacrylate	58	26	9.0
Acrylonitrile	74	41	9.3
α-Chloroacrylonitrile	77	31	5.3
Vinyl acetate	28	13	7.7
Styrene	33	16	7.8
Vinyltoluene	25	12	9.4
N-Vinyl-2-pyrrolidone	27	12	7.2
4-Vinylpyridine	0	—	16.4
Diallyl phosphite	29	12	8.3
Triallyl phosphate	25	10	10.1
Butenediol	18	7	9.3

[a] After extraction of homopolymer with appropriate solvent.

[b] Based on monomer added and assuming weight increase after extraction is due solely to graft polymer formation.

[c] Ceric ammonium nitrate.

[d] Two or more determinations.

ethyl or glycidyl methacrylate were converted nearly quantitatively to a polymeric form which was not soluble in solvents for the respective homopolymers. With acrylic and methacrylic acid, the yields were considerably lower than those obtained with their esters. The monomers of basic character—calcium acrylate, *tert*-butylaminoethyl methacrylate, dimethylaminoethyl methacrylate, and 4-vinylpyridine—resulted in no apparent grafting. However, when an aqueous solution of dimethylaminoethyl methacrylate was acidified to pH of 2.5 with 2*N* nitric acid prior to the addition of this monomer to the collagen, a considerable weight increase occurred (68%). Although a large amount of the polymer formed was removed by solvent extraction indicating that homopolymer had been formed, some apparent grafting (34% weight increase) had also taken place when this basic monomer had been neutralized and the polymerization was conducted in an acid environment. Fluorinated acrylates and methacrylates also grafted to the collagen substrate. Good yields were obtained with all fluorinated acrylates and methacrylates, with the exception of 1H,1H,5H-octafluoropentyl methacrylate.

The glycidyl methacrylate–collagen product was obtained in good yield and retained the glycidyl groups as evidenced from the infrared spectra. These groups are potential reaction centers; for instance, by converting the glycidyl group to the hydrophilic hydroxyl group by mild alkaline hydrolysis, the collagen can be further modified. Similarly, the side chains containing 2-chloroethyl or hydroxyethyl groups can be readily modified. Ethylene or butylene dimethacrylate also gave a fairly large increase in weight on reacting with collagen. The products are probably crosslinked and should show reduced solubility and increased chemical resistance.

Most vinyl monomers other than those containing acrylic or methacrylic groups are not as readily grafted to collagen in the aqueous environment used in this study. Little or no weight increase compared to the CAN blank took place with 4-vinylpyridine, vinyltoluene, triallyl phosphate, or butenediol. More suitable grafting conditions for these monomers may be obtained by conducting the reaction in appropriate nonaqueous solvents.

The amount of homopolymer formed as determined by weight loss or extraction with suitable solvent was usually less than 10%. Soxhlet extraction with methylene dichloride for 48 hr conducted after the initial extraction of homopolymer with an appropriate solvent did not produce any further removal of side chain polymer. Generally, the reaction with higher homologues of the acrylic and methacrylic esters produced a considerable amount of soluble homopolymer. The dimethylaminoethyl methacrylate polymerized at pH 2.5, and the butylene dimethacrylate-containing product gave the largest weight loss on extraction (24% and 22%, respectively). For the collagen–butylene dimethacrylate product, some of this weight loss may have resulted from the presence of unreacted monomer sorbed on the reaction product.

The presence of polymer on the collagen was confirmed from infrared spectra. After removal of soluble homopolymer, absorption bands char-

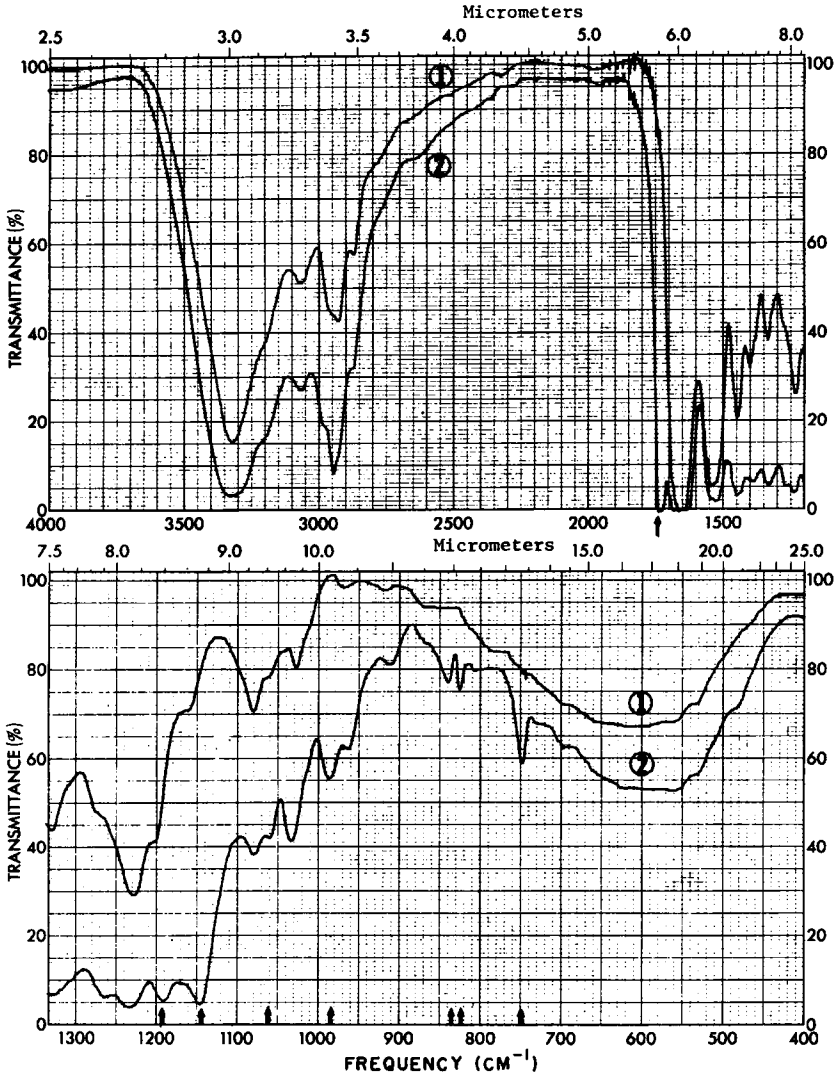

Fig. 1. Infrared absorption spectra: curve 1, collagen film (1.3×10^{-3} cm thick); curve 2, collagen film to which methyl methacrylate had been grafted. Homopolymer was removed by extraction with acetone.

acteristic of the specific synthetic polymer were usually present. Appearance of noncollagenous bands was most pronounced for collagen treated with the lower homologues of the acrylic or methacrylic esters. A typical example of the infrared spectrum of a collagen–methyl methacrylate graft film is shown in Figure 1.

In general, collagenous products having an increase in weight in excess of 25% had more distinctive spectra possessing sharper peaks and additional absorption bands as compared to the original collagen powder. For example, all esters (acrylates, methacrylates, acetate) gave the C=O

stretching band[18] around 1740 cm^{-1} and many had another strong ester C—O stretching band between 1140 and 1160 cm^{-1}. However, collagen blanks run in the presence of CAN without monomer also showed a weak absorption band in the 1740 cm^{-1} region, probably due to the presence of strongly sorbed succinate on the substrate. Thus, only a strong absorption band in this region can be considered as definite evidence of the grafting of monomeric esters to collagen. All grafted methacrylates gave a characteristic band around 750 cm^{-1}, whereas most acrylates had an absorption peak in the neighborhood of 850 cm^{-1}. The presence of grafted polystyrene is indicated by a peak at 700 cm^{-1}. Presence of chlorine in the 2-chloroethyl methacrylate graft was confirmed from the 670 cm^{-1} band. The fluoroacrylate and methacrylate grafts showed strong absorption bands in the 1300 cm^{-1} to 1100 cm^{-1} range, especially around 1280 cm^{-1} and 1130 cm^{-1}. The —CN group of grafted acrylonitrile and cynoethyl acrylate was ascertained from the 2250–2270 cm^{-1} peak. The cellusolve acrylate–collagen graft had a sharp band at 1120 cm^{-1} due to the alkyl ether groups. For the glycidyl methacrylate product, the presence of the oxirane group was confirmed from the absorption peaks around 900 cm^{-1} and 840 cm^{-1}.[19,20]

The appearance of the products varied considerably from the original collagen powder. With some monomers, the grafted materials consisted of powders, while with others, mats or films were formed. The modification of surface properties could often be detected by visual inspection. Thus, with isodecyl, 2-ethylhexyl acrylate, or lauryl methacrylate, rubbery mats were formed.

The water sorption at 50% R.H. (Table II) was always lowered on treatment of the collagen. This even applied for products containing hydrophilic groups in the side chain. Thus the collagen–hydroxyethyl methacrylate product had a water uptake of 7.4%, compared to 19.2% for collagen powder. Water sorption was not only depending on the presence of hydrophilic groups but also on the amount of copolymer incorporated in the product. Lowest water uptake was obtained for products containing fluorinated acrylates or the higher molecular weight esters of acrylic or methacrylic acid.

The orignal collagen powder forms a slurry with water. On filtration a mat is obtained which water wets only slowly (Fig. 2). However, the reaction products of collagen with acrylic acid, methacrylic acid, hydroxyethyl methacrylate, and cyanoethyl acrylate were hydrophilic and gave a zero contact angle within 15 sec. On products derived from the reaction of collagen with cellusolve acrylate or methyl methacrylate, water also wets the surface slowly. The wetting rate decreased with increasing percentage of graft polymer in the products. A majority of the materials, such as those containing grafts of fluorinated acrylates or methacrylates or the higher acrylate or methacrylate homologues, became completely hydrophobic (Fig. 2). Exact contact angles were difficult to determine because of the porous and uneven surface of the specimens. The collagen–lauryl methacrylate and collagen–2-ethylhexyl acrylate had approximate contact

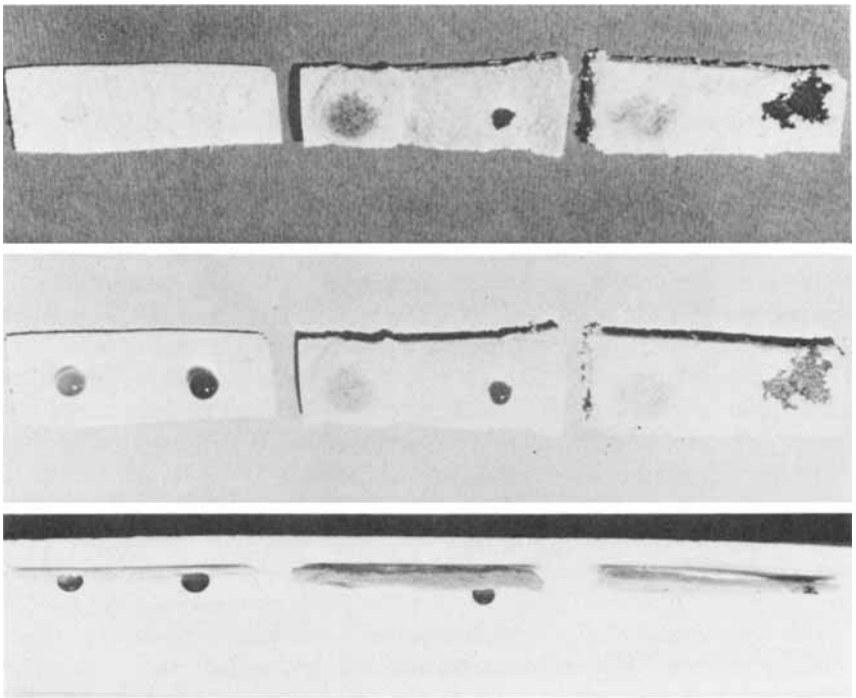

Fig. 2. Water and oil repellency of graft polymers: left, acrylic acid–collagen; center, collagen; right, hexafluoroisopropyl methacrylate–collagen mat; top and center, spreading of water and oil drops placed respectively on left and right of each mat; bottom, mats after blotting off drops with filter paper.

angles of 85°, whereas those of the fluorinated acrylates and methacrylate products had contact angle values over 90°. A mat of 2,2,2-trifluoroethyl methacrylate graft product floated on water and appeared to keep its water repellency indefinitely. On introduction of sufficient fluorine content, the graft polymers also became oil repellent. After removal of drops of water and oil that contained dyes, the mats showed no signs of stains (Fig. 2), thus establishing not only the water and oil repellency of the materials, but also their stain resistance.

To determine if large surface areas, as are present in these collagen powders, are required for successful grafting, collagen films (0.0013 cm and 0.005 cm thick) made up of collagen fibrils of about 99% purity were substituted for the collagen powders in the standard grafting experiment. Although it was not possible to measure the increase in weight of the specimens of 0.0013 cm thickness because of the small quantities of materials used, the infrared spectra of the treated and solvent-extracted films indicated the presence of polymer which apparently had been grafted on the substrate. Thus, a film to which methyl methacrylate had been grafted had absorption bands at 1735 cm^{-1} (C=O stretching band) at 1190 and 1146 cm^{-1} (C—O stretching bands), and at 749 cm^{-1} (characteristic of methacrylates)[18,21] (Fig. 1). The adsorption peaks at 1060, 987, 840, and 827 cm^{-1} that were found in methyl methacylate-grafted collagen but

which were absent in the pure collagen spectrum were also observed in films of poly(methyl methacrylate).

Monomers could be grafted to 0.005-cm collagen films as indicated by an increase in weight relative to that observed for runs conducted in the absence of monomer. Glycidyl methacrylate gave by far the largest weight increase of the monomers studied. Reproducible results were difficult to obtain because the weight of the original samples were small, resulting in small weight increments on grafting. Generally, the grafting of the monomers to the collagen film was considerably lower as compared to the results obtained with collagen powder.

Films were also stored in a 5% solution of poly(methyl methacylate) in acetone for five days. On extraction of soluble homopolymer with acetone and drying, the films increased in weight less than those subjected to "blank" graft runs (no monomer or CAN present). Hence, only small amounts of poly(methyl methacrylate) homopolymer are absorbed by collagen film under the experimental conditions.

Under the experimental conditions employed, surface grafting occurs within less than 30 min after addition of the monomer. Thus, after a 30-min reaction time, an approximate weight increase (after removal of homopolymers) of 100% was obtained on grafting methyl methacrylate and isodecyl acrylate onto collagen powder. Similarly, the increase in weight on grafting glycidyl methacrylate to 0.005-cm collagen films demonstrated conclusively that measurable amounts of surface graft were formed within 30 min. Surface behavior of solid materials and biologic tissues such as wettability, critical surface tension, and adhesion are dependent solely upon the nature and the packing density of the outermost or exposed atoms and functional groups. Grafting yields of even a unimolecular layer grafted to active sites may significantly change the behavior of the proteinaceous surface. Thus, relatively short reaction times are sufficient to modify greatly the chemical and biologic characteristics of the collagenous surfaces.

As was mentioned previously, increase in weight after extraction of soluble homopolymer in excess of the increase in weight obtained in the absence of monomer was used as criterion for successful grafting. It is conceivable that products from a growing polymer chain entwined with macromolecules of the substrate are formed in the reaction. Such interlocking polymeric mixtures may be difficult to separate without degrading or hydrolyzing the collagen backbone. Furthermore, solvent extraction does not remove the insoluble homopolymers of the bifunctional methacrylates that crosslink during the reaction. Nevertheless, copolymers in which the macromolecules of the substrate and the additional polymer chains are not linked by a covalent bond should still exhibit different properties than the original substrate. These modified properties may also be valuable for diversified applications of such products.

The utilization of collagen modified by grafting will depend on specific property changes resulting from this reaction. The broad scope of this reaction is indicated by the large variety in polarity and functional groups possessed by the monomers which were found to undergo apparent grafting.

Thus, using this procedure it is possible to select monomers giving the desired degree of hydrophilic to hydrophobic balance at the collagen surface for specific applications. Desirable properties of modified surfaces may include better adhesion to various substrates, improved dimensional stability, lower water sorption, increased water repellency, better biocompatibility and resistance to fungal and bacterial attack, and increased protection to photochemical reactions.

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Certain commercial materials are identified in this report to adequately specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by the National Bureau of Standards, neither does it imply that the material identified is necessarily the best available for the purpose.

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