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Protein adsorption characteristics of plasma treated polyurethane surfaces

T. L. STERRETT, R. SACHDEVA
Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, USA
P. JERABEK
Department of Chemistry, University of California, Irvine, CA 92714, USA

The effect of various plasma surface treatments on the protein adsorption characteristics of two polyurethane elastomers (Acushnet E417-0 [ACx] and Texin 480 AR [TN]) were studied. Both substrates are based upon diphenylmethane 4,4′-diisocyanate (MDI) hard segments and polyester soft segments. Adsorption characteristics of the untreated samples were initially established, followed by plasma treated surfaces. Contact angle and 2 h albumin adsorption were determined. (1) Results of this study indicate that the protein adsorption characteristics of crosslinked substrate ACx is more linear than that of non-crosslinked substrate TN. Further, substrate TN adsorbs seven-fold greater protein at a rate four times higher on its surface than ACx. *N,N*-Ethylene bis (stearamide), a processing aid used in substrate TN, may encourage greater protein adsorption on substrate TN and variation in the soft segment mobility between the substrates also may affect their adsorption characteristics. (2) Plasma treatments using CH_4 and/or C_xF_y chemistries increased the contact angle for both substrates while those with O_2 and O_2/CF_4 decreased the contact angle for the substrates considered. In general, the contact angle of the substrates exhibiting greater protein adsorption was smaller.

1. Introduction

The surface interactions of biomaterials with tissue and bodily fluids have been investigated extensively. Of particular interest are those interactions with proteins. It has been established that the types of protein adsorbed, as well as the nature of such adsorption, dictates the biocompatibility of the material under some conditions. For example, surfaces which preferentially adsorb albumin have been shown to be less thrombogenic than those which adsorb fibrinogen [1–5]. The existence of such phenomena has led to the establishment of the concept of improved material biocompatibility via surface passivation through the preferential adsorption of proteins such as albumin [6, 8].

Numerous studies have been performed which attempt to optimize the adsorption of such beneficial proteins. Methods used for the promotion of the preferential adsorption of proteins are based mainly on surface modification techniques [7–22]. Of interest to the present study are those techniques which are based upon plasma polymerization, and coronal discharge. Such techniques have the advantage of providing the substrate with a strongly adhered thin film having minimal effect on appearance and bulk properties. Furthermore, these methods have been shown to favourably alter the surface of various biomaterials with respect to biocompatibility and presumably protein adsorption characteristics [7, 11–19].

While the literature has several examples of surface modification via plasma polymerization techniques, very little exists in which surface modification is achieved strictly through exposure to a plasma. Besides surface polymerization, plasma surface treatments are known to affect polymer surfaces through crosslinking ablation and cleaning [7, 23]. Given that the potential biocompatibility of materials has been related to such factors as surface roughness and cleanliness [24, 26], it is naturally of interest to establish the effects various plasma treatments have on these parameters as well. That is, if one accepts that the initially adsorbed monolayer of protein dictates a material's subsequent biocompatibility, then an understanding of the factors affecting protein adsorption and its modification is needed.

The purpose of the present study was to investigate the effects different plasma surface treatments had on the protein adsorption characteristics of selected polyurethane elastomers. The plasma treatments investigated were chosen to represent different potential outcomes based upon plasma chemistry. The study was structured to establish the protein adsorption characteristics of the materials by using radio-labelled albumin. Once the adsorption characteristics of the materials in the untreated state were established, each was subjected to a particular plasma treatment, after which contact angle and 2 h albumin adsorption were determined.

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TABLE I Process parameters for plasma treatment of poly(ester)urethane substrates. Samples treated at a radio frequency of 6.78 MHz for 5 min

Plasma	Gas mixture (0/0)	Classification of plasma treatment	Temperature (°C)	Pressure (Pa)
CF_4/CH_4	77/23	Fluorine deposition/plasma polymerization	30.0	18.0
A_5	100	Ablation/surface cleaning	30.4	42.0
O_2/CF_4	50/50	Surface oxidation/fluorine deposition	29.4	21.3
O_2	100	Surface oxidation	29.4	21.7
CF_4	100	Fluorine deposition	30.5	24.0
C_2F_6/CH_4	77/23	Fluorine deposition/plasma polymerization	33.5	23.3
CH_4	100	Plasma polymerization	30.0	16.9

2. Methods and materials

2.1. Surface treatments

Test substrates were subjected to plasma surface treatments using a model PS 0500 plasma treatment system (Plasma Science, Inc., Belmont, CA). The apparatus was equipped with a mass flow-controlled gas blending system which allowed for the introduction of gases and gas mixtures into the reactor (33 cm × 40 cm × 50 cm). The urethane substrates were suspended in the reactor so as to be symmetrically placed between electrodes. All plasma treatments were conducted at a frequency of 6.78 MHz, for 5 min, at a power of 300 W. Further detail on plasma chemistry and process parameters is given in Table I.

Using a sessile water drop, the room temperature contact angle of the substrates was determined subsequent to plasma treatment. Further details of contact angle measurements are given in the literature [7, 27, 28].

2.2. Substrates

The substrates used for the investigation were commercially available poly(ester)urethane elastomers exhibiting shore A hardnesses in the range of 85 to 93. Both substrates are based upon diphenylmethane 4,4′-diisocyanate (MDI) hard segment. Substrate ACx is crosslinked with 1,4-bis(hydroxyethoxy) benzene. Polycaprolactone was used to form the soft segment for substrate ACx, while polyethylene adipate was used to form the soft segment for substrate TN. Since substrate ACx is a crosslinked material, specimens were formed by casting sheets, from which samples were cut (sample ACx: Acushnet E417-0, Acushnet Co., New Bedford, MA). Test substrates were prepared from material TN by cutting specimens from extruded sheets (sample TN: Texin 480, Mobay Corp. Plastics and Rubber Div., Pittsburgh, PA). A standard rectangular specimen configuration (19.05 mm × 5.00 mm × 0.76 mm) was used for all testing. Before testing, control test substrates were wiped clean with ethanol. Test substrates that were subjected to plasma surface treatments were tested without any further preparation.

2.3. Protein adsorption

The protein adsorption characteristics of each substrate was determined by using a modified method after Lee *et al.* [3]. The protein used was albumin (Bovine, Fraction V, Sigma Chemical, and ^{125}I-albumin, ICN Radiochemicals). For each experiment, a stock protein solution containing 2.37 mg ml^{-1} albumin in phosphate buffered saline (PBS, pH 7.4) was prepared (25 ml). The PBS was then spiked with 10 µl of ^{125}I-albumin, specific activity 0.757 µCi ml^{-1}. Samples were then individually submersed in test solution thus denoting time zero. After the desired submersion time, samples were removed from the test solution and rinsed with distilled water for 30 s. Each test substrate was then tamped dry and allowed to further dry for an additional 30 min, after which the sample was placed in a vial. Radioactivity was assayed by using a sodium iodide, thallium activated solid scintillation detector. A total of three specimens were used for each experiment.

3. Results and discussion

The concentration of adsorbed protein as a function of time for untreated substrate surfaces is plotted in Fig. 1. Examination of the plots indicates that the magnitude of protein adsorption of substrate TN is greater than that measured for ACx. The 5, 120 and 480 min adsorption for substrate TN was found to be 22.9 ± 0.9, 26.0 ± 2.1, and 73.0 ± 3.3 µg cm^{-2} respectively. By using the same increments of time, the level of protein adsorption measured for substrate ACx was 2.3 ± 0.1, 5.0 ± 0.2, and 10.0 ± 0.6 µg cm^{-2}. The data indicate that throughout all time intervals investigated, substrate TN had a much greater affinity for albumin than did substrate ACx.

At time intervals less than 120 min, protein adsorption appeared to be oscillatory for both substrates. The result is indicative of the adsorption/desorption of protein and is most likely associated with equilibrium phenomenon. It should be noted, however, that neither substrate exhibited an equilibrium surface concentration of protein for the time intervals studied. When the time of submersion exceeded 120 min both materials exhibited a continuing linear increase in the adsorption of protein. In this region of the adsorption versus time curve (i.e. $t > 120$ min), it was found that the rate of adsorption for substrate TN was nearly 10 times greater than that measured for ACx.

A linear least-squares analysis of the data plotted in Fig. 1, indicates that the protein adsorption characteristics of substrate ACx tend to be fairly linear ($r^2 = 0.94$), while those exhibited by TN were found to be highly non-linear ($r^2 = 0.62$). The observed dissimilarity in the protein adsorption characteristics of the

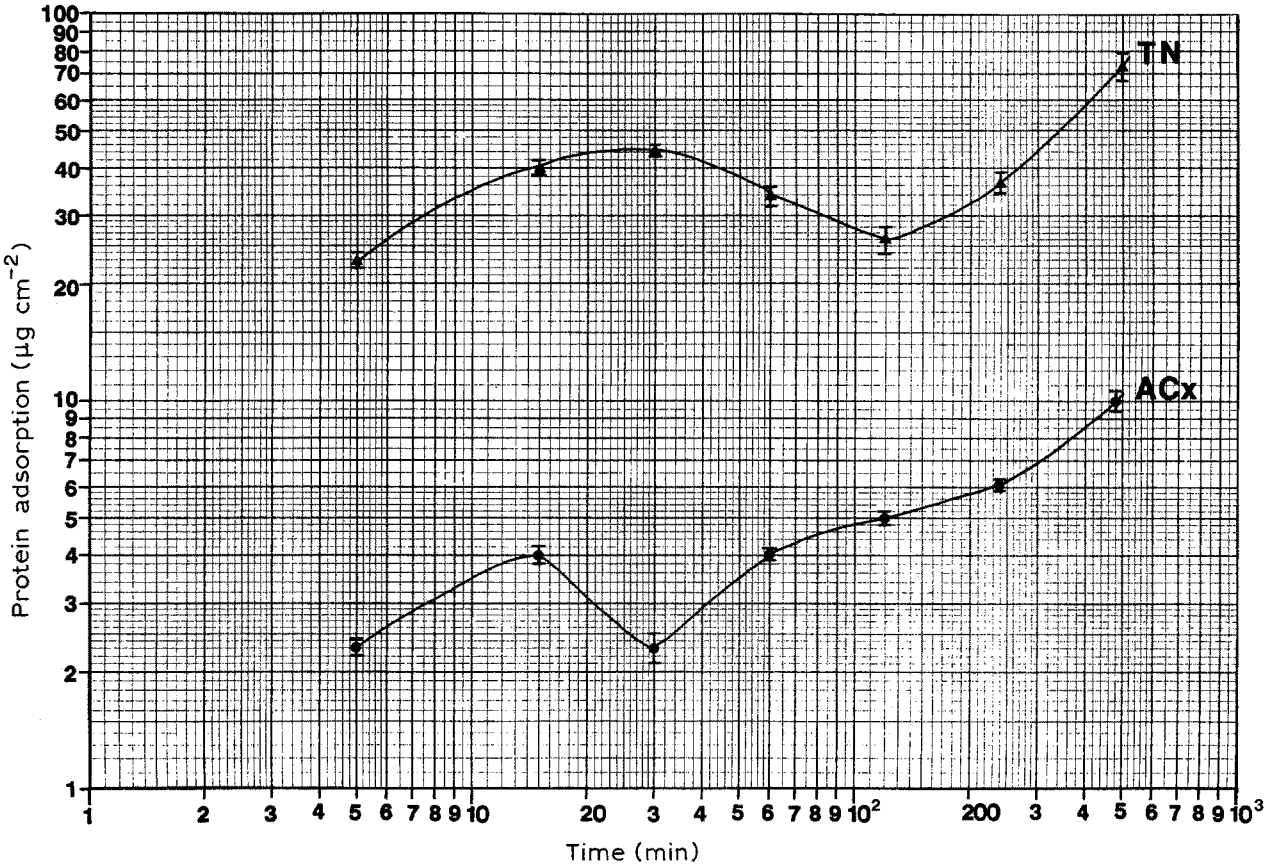


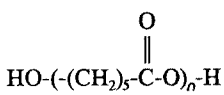
Figure 1 Protein adsorption versus time for materials ACx and TN.

untreated materials is believed to be the result of basic differences in the chemistry and bulk composition of each polymer, as well as variations in the method of fabricating articles from each material. The chemical nature of the materials differs due to selection of the polyol used to form the soft segment of each substrate, as well as the presence or absence of crosslinking. These differences are further exacerbated by the necessity to utilize dissimilar processing techniques to fabricate articles from each material. Such differences lead to varying degrees of hydrophobicity, soft segment mobility, and extent of surface contamination of each material.

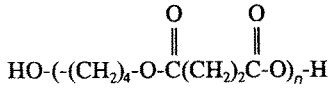
Polyurethane elastomers are block copolymers in which the hard and soft segments of the polymer are separated into different domains due to their incompatibility. The macroglycol soft segment is generally amorphous or semi-crystalline. In this phase the rigid isocyanate hard segment is dispersed at low to moderate concentrations. This particular domain is generally more hydrophobic than the soft segment and acts as a crosslinking site thus yielding the unique properties of high elasticity and strength. Interfacial properties of polyurethane elastomers are dictated to varying degrees by the soft segment. Factors such as segment mobility and hydrophobicity affect how the material will interact with its surrounding environment.

Polycaprolactone is used to form the soft segment of substrate ACx, whereas polyethylene adipate is used for material TN. The chemical structure of each polyol is given in Fig. 2. Given that polyester polyols tend to be more hydrophilic than alternate polyol systems used to form urethanes (i.e. polyethers), there still exists a varying degree of hydrophobicity for each polyol based upon chemistry. Generally, polycaprolactone polyols tend to be more hydrophobic than polyethylene adipate polyols since for a given molecular mass it will possess approximately half the number of ester groups than will the adipate. The literature generally indicates that increased surface hydrophobicity leads to higher levels of protein adsorption [3, 4, 29]. One must note, however, that in a study in which hydrophilic groups were grafted on to the surface of a poly(ether)urethane, it was found that as the

Polycaprolatone



Polyethylene adipate



N,N'-Ethylene bis-stearamide

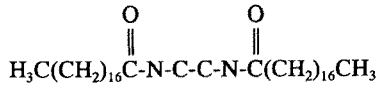


Figure 2 The chemical structure of each polyol and amide processing aid.

hydrophilic character of the surface increased (i.e. increasing grafting yield) so did the adsorption of albumin [15]. In particular it was found that the affinity for albumin increased for grafts with sulphate and diol groups. Protein adsorption was found to decrease for grafts yielding carboxyl function. It therefore appears that both surface hydrophobicity and functionality affect protein adsorption.

ACx is crosslinked with 1,4-bis(hydroxyethoxy) benzene. The molecules which comprise the material are therefore interconnected by covalent bonds. Such intermolecular covalent bonding provides the material with increased strength and elasticity. Substrate TN is not crosslinked and is therefore devoid of intermolecular covalent bonding. Materials such as TN derive their strength and elasticity through physical interaction of the molecules, i.e. hydrogen bonding and intermolecular entanglement. Consequently, the methods used to form articles from each material are quite dissimilar. Since material TN is not crosslinked, manufacturing techniques based upon melt processing such as extrusion and injection moulding are used. These manufacturing techniques, however, cannot be used with ACx since they do not possess the appropriate physicochemical properties for melt processing. Typically, articles that are manufactured from crosslinked materials are made by first reacting the liquid components of the urethane system and then moulding or casting the material in the shape of the part to be formed.

It has been suggested in the literature that the method of fabrication of articles made from a poly(ether)urethane elastomer, affected blood compatibility due to differences in the surface concentration of polyether soft segment [29, 30]. It was found that soft segment surface concentration was greatest for extruded material and lowest for solution cast material. An interesting conclusion from the study was that the surface rich in polyether soft segment was more thromboresistant than the same material when solution cast. The results suggest greater plasma protein adsorption for soft segment enriched surfaces.

Due to the differences in manufacturing techniques required for each material as based upon polyurethane chemistry, the compounded nature of each material also varies. Materials that are processed by melt processing techniques such as injection moulding or extrusion require processing aids which act as both internal lubricants and mould release agents. Material TN is such a material. Fatty acid esters such as *N,N'*-ethylene bis (stearamide), see Fig. 2, are used as processing aids for thermoplastic or melt processable materials. These materials are absent from substrate ACx.

Processing aids function by migrating to the surface of the polymer upon thermal processing. Removal of processing aid from the surface with organic solvents has been demonstrated [31, 34]. However, the same study also found that extraction of the processing aid with soap solution was not possible, thus demonstrating the resistance of the processing aid to removal in aqueous media.

The effect of the presence of such materials on the protein adsorption characteristics of polyurethane elastomers has been investigated by others [33, 34]. Through electron spectroscopy for chemical analysis (ESCA) and ATR-IR surface analysis, it was found that increased surface hydrocarbon content due to the migration of processing aids could be correlated with improved thromboresistance [34]. Surfaces with high hydrocarbon content have been shown to preferentially adsorb proteins such as albumin [35]. Presumably both the surface energy and chemistry of the substrate is changed by the presence of these materials. That is, as the hydrocarbon character of the surface increases it will in turn become more hydrophobic thus decreasing wetting by polar materials.

Dissimilarities in the protein adsorption characteristics of the two materials could include differences in soft segment mobility. In an investigation concerned with the protein adsorption characteristics of polyurethane elastomers as related to soft segment mobility, it was demonstrated by Takahara *et al.* that the soft segment of model polyurethanes rearranged themselves for given environmental conditions so as to minimize interfacial surface free energy [29]. It is felt that the presence of crosslinking in material ACx could preclude or significantly reduce the molecular mobility of the soft segment thus eliminating such rearrangement.

The effect plasma surface treatments had on the protein adsorption characteristics of the substrates was investigated. Each plasma chemistry, its 2 h protein adsorption, and contact angle are summarized by Table II. In general, treatments based upon CH_4 and/or C_xF_y chemistries were found to increase the contact angle measured for both substrates. The corresponding protein adsorption measured for these chemistries was found to be lower than that of the control. The effect was found to be greatest for substrate TN. Plasma chemistries based upon O_2 and O_2/CF_4 were found to yield increased protein adsorption while decreasing contact angle. The results indicate that as the surface becomes more hydrophilic (lower contact angle as measured by the present investigation) the degree to which the surface adsorbs protein increases. Such results appear to be contrary to those typically found in the literature, where it is generally maintained that more hydrophobic surfaces tend to adsorb protein to a greater extent than those which exhibit hydrophilic character. This, however, assumes the absence of surface functionality. The surface treatments presently investigated are believed to impart varying degrees of functionality to the surfaces of the materials studied.

Normalized protein adsorption and contact angle (θ) are plotted in Figs 3 and 4. Examination of the plots indicates that relative to the control surface treatment, the relationship of each plasma to one another was similar for the two materials studied. However, the curve for substrate TN was shifted in the direction of lower protein adsorption and higher contact angle. Another difference observed when comparing the plots is that unlike material ACx, the Ar surface treatment resulted in decreased protein adsorption for substrate TN relative to its control.

TABLE II Room temperature 2 h protein adsorption for poly(ester)urethane elastomers subjected to various plasma surface treatments (2.37 mg ml^{-1} bovine serum albumin in PBS pH 7.4) (n = 3) (NS, not significant)

Plasma	Substrate ACx		Substrate TN	
	Albumin adsorption (μg cm^{-2})	Contact angle (deg)	Albumin adsorption (μg cm^{-2})	Contact angle (deg)
CF_4/CH_4	5.1 ± 0.3 NS	97 ± 3[c]	15.3 ± 1.1[b]	107 ± 3[c]
Ar	8.6 ± 0.6[c]	47 ± 3[b]	17.6 ± 1.6[b]	77 ± 2[c]
O_2/CF_4	11.5 ± 0.3[c]	42 ± 6[b]	24.2 ± 1.2 NS	43 ± 3[a]
O_2	10.8 ± 1.0[c]	31 ± 1[c]	38.5 ± 2.3[b]	46 ± 7 NS
CF_4	4.3 ± 0.1[b]	112 ± 5[c]	12.5 ± 0.9[c]	132 ± 4[c]
C_2F_6/CH_4	3.5 ± 0.2[b]	108 ± 4[c]	6.7 ± 0.3[c]	120 ± 5[c]
CH_4	4.6 ± 0.1 NS	111 ± 3[c]	9.2 ± 0.7[c]	100 ± 0[c]
Control	4.9 ± 0.2	68 ± 3	26.0 ± 2.1	53 ± 3

[a] Significant at the 5% level.
[b] Significant at the 1% level.
[c] Significant at the 0.1% level.

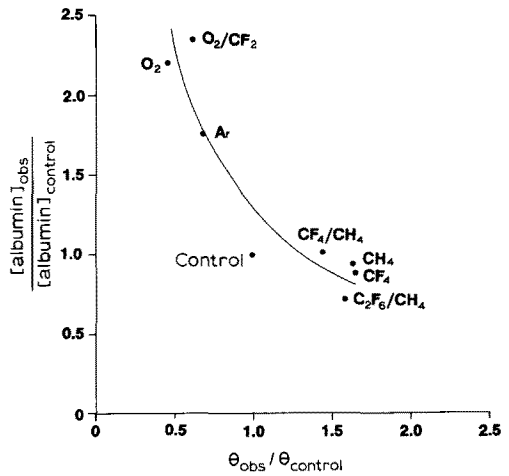


Figure 3 Normalized protein adsorption versus normalized contact angle (θ) for material ACx.

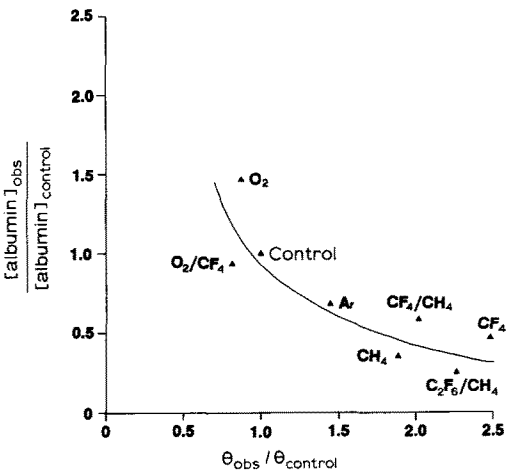


Figure 4 Normalized protein adsorption versus normalized contact angle (θ) for material TN.

As previously mentioned, the two materials investigated possess dissimilar bulk composition. Material TN was compounded with an amide wax that served as an internal lubricant and mould release. The presence of such processing aids masks the surface chemistry of the underlying material and possibly caused the material to exhibit the characteristic biocompatibility of the amide wax instead of the polyurethane [31–34]. Surface analysis of urethane elastomers which were contaminated by such processing aids, typically yielded ESCA spectra which showed no evidence of urethane functionality [31, 34]. Such analysis has also revealed that the thickness of the amide wax can be greater than 10 nm [34]. In studies performed by Larsson *et al.* [34] the ESCA spectra of a poly(ether)urethane was compared before and after toluene extraction. The results indicated that the surface concentration of oxygen was substantially increased subsequent to extraction, thus increasing the degree of surface hydrophilicity. Larsson *et al.* then related platelet adhesion to surface hydrophilicity and found that as the hydrophilic character of the surface decreased platelet adhesion increased. Since it is known that protein adsorption precedes platelet adhesion [6], the results seem to indicate that more hydrophilic surfaces tend to adsorb proteins which are associated with thromboresistance; while more hydrophobic surfaces tend to adsorb proteins that are associated with thrombogenesis.

With respect to the present study, it appears that the surface enrichment of materials ACx and TN with oxygen leads to increased albumin adsorption for the reasons previously discussed. The effect was more pronounced for material ACx, presumably due to the absence of surface contamination in the form of amide wax processing aid. It appears that the desired effect of imparting surface functionality by surface oxidation is negated when materials such as processing aids are present on the surface since the net effect of the treatment is most likely that of cleaning instead of surface functionalization. It is believed that the lack of processing aid from the surface of ACx allowed the oxygen surface treatment to react more efficiently with the material, thus yielding a greater change in contact angle and protein adsorption characteristics.

Plasma surface treatments based upon chemistries that are thought to yield surface fluoridation tended to yield decreased protein adsorption with increased contact angle. The result was more pronounced for substrate TN. The result indicates that the fluorocarbon plasma caused the material to exhibit an increased hydrophobic character when compared to the

untreated control surface. Unlike the oxygen surface treatments, which function by reacting with the substrate surface, the intended function of the fluorocarbon, CH_4 and Ar plasmas are believed to be that of film deposition, surface crosslinking, and/or ablation. These treatments are then expected to act mainly upon the physical characteristics of the surface rather than its chemistry. Therefore their effects would be expected to be greatest for material TN due to the varied interactions the plasmas may have with the processing aid present on its surface, as well as the potential for a greater degree of surface crosslinking.

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