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NOTE

Proteinated Surfaces at Solid–Water–Octane Interface

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The surface energy and interfacial parameters of synthetic polymers have been of interest, since Lyman[1] indicated their relevence with blood compatibility. Such concepts were further extended by Kaelble and Moacanin[2] and Sharma,[3] however the inter-relation of polar contribution of the surface interactions has been questioned, since either (Lewis) acidic or basic interactions[4] may be dominent. But the interesting feature of *in vivo* data (regardless of the contact angle system employed) as mentioned by Bagnall *et al.*[5] that stationary values fell into two apparent classes $132 \pm 4°$ and $160 \pm 7°$ indicated that such studies might be quite informative in order to understand the varied nature of bound protein layer at equilibrium onto polymer surfaces. Therefore, in order to develop an understanding of protein–polymer interactions at the solid/liquid interface, which is closer to *in vivo* conditions, we have attempted to investigate the contact angle of 99.99% pure *n*-octane (Aldrich Chemicals, Gold Label) on different polymer surfaces, before and after their exposure to protein adsorbed layer onto the surface as described elsewhere.[6] The proteins (Nutritional Biochemical Corporation) used, were albumin bovine crystalline and α-globulin bovine FR IV 1, β-globulin bovine FR III and γ-globulin bovine cohn FR II, Fibrinogen bovine FR I. The method of contact angle measurements at 24°C using a Rame–Hart goniometer is already described by Andrade *et al.*[7] The proteinated polymer surfaces facing downward on microscopic slides were positioned and finaly held in place with adequate support in a container. The container was filled with doubly distilled water carefully until the microscopic slide was completely immersed. The goniometer was aligned and focussed on the polymer–water interface. At this point microsyringe containing 99.99% pure *n*-octane was lowered into the water. A drop of ~0.1—0.2 μl was formed on the syringe tip, positioned underneath the polymer surface, "snapped" from the tip, and allowed to rise to the polymer–water interface. The apparent octane–polymer contact angle was then immediately measured. Angles on both sides of each bubble were measured to assure symmetry, and generally five or more contact angles were measured on each surface, followed by five or more octane bubbles. The estimated experimental error was ±10%. The polymers studied were polyether–urethane–ureas (PEUU) based on polypropylene glycol (PPG) having molecular weights of 710, 1025, and 2025. These polymers were synthesized and characterized in our laboratory.[8] Biomer (Ethicon Inc., NJ, U.S.A.) was also chosen for relative evaluation of our data. Film of these polymers was casted on microscopic plates from 10% solution in *N,N*-dimethylformamide.

For all proteins the contact angle appeared to be higher in case of Biomer than in case of our laboratory PEUU polymers, as shown in Table I. This may be due to the differences in domain-matrix structure as is obvious from the domain size in case of our laboratory polymers.[9] So the distribution of polar–nonpolar forces at the interface may be different. Further processing parameters also play an important role towards the change of surface properties,[9] which are not known to us in case of Biomer. It seems, therefore, that the changes

Table I. Equilibrium contact angles in degrees on bare and proteinated polymer surfaces

Polymer	Domain size[9] Å	Average contact angle					
		Bare	Albumin	α-Globulin	β-Globulin	γ-Globulin	Fibrinogen
PEUU-2025	150—200	90.0	104.0	95.0	122.0	132.0	135.0
PEUU-1025	80—120	112.0	130.0	130.0	140.0	139.5	136.5
PEUU-710	70—100	101.0	147.0	142.0	139.0	151.0	152.0
Biomer	—	139.0	157.5	149.5	160.5	152.5	166.0

Table II. Equilibrium contact angle in degree on polymer PEUU-1025 surface exposed to protein solutions for different length of time

Exposure time min	Average contact angle		Amount adsorbed[10] μgm cm^{-2}	
	Albuminated surface	Fibrinogenated surface	Albumin	Fibrinogen
5	117.0	112.0	—	—
10	123.0	117.0	1.65	0.64
20	125.0	128.0	—	—
40	128.0	130.0	—	—
60	—	—	4.99	1.7
120	—	—	7.28	2.23
150	128.0	135.0	—	—
180	129.0	137.0	9.07	2.96

due to different polymer surfaces even of the same type are equally significant as the changes due to adsorbed layer of any particular protein on contact angle values at equilibrium. Further, after exposing the polymer surfaces to proteins for different length of times, the fibrinogenated surfaces in the beginning showed slightly lower equilibrium contact angle than the albuminated surfaces. But as the time of exposure of polymer surfaces to fibrinogen solution was increased the equilibrium contact angles observed, were higher than the albuminated surfaces, as shown in Table II. It might be because of incomplete coverage of the surface with fibrinogen initially; so complete association might not be there. On the other hand, more stable coverage of albumin might be taking place at a shorter time on this particular polymer surface of PEUU-1025, as is obvious from the adsorbed amount of proteins[10] with time in each case, however then surface was exposed to fibrinogen for longer time, the higher equilibrium contact angles were observed relatively. In case of our polymer PEUU-1025, the amount of albumin adsorbed is higher than that of fibrinogen. The exact reason for such preferential adsorption of albumin is not yet known except considering the variations of intermolecular forces at the interface.

Further depending upon the heterogeneities of the polymer substrate, *e.g.*, domain–matrix structure etc. as indicated in Table I, protein molecules may form clusters, which may cause conformational changes. Such conformational changes may further vary with time of exposure due to increased density of protein molecules on substrate causing changes in contact angle. Long-range Van der Waals' interactions between substrate and the organic liquid (n-octane) used may also affect contact angle, particularly, when the protein layer is relatively thin. Since fibrinogen molecule denatures easier than albumin so the changes of variations in the cluster formation and/or incomplete coverage (stripping mechanism[11]) will be more in case of fibrinogen, when exposure time is short, therefore in the be-

ginning when long-range Van der Waals' interactions may also be playing a role, contact angle is close to Bare surfaces, which increases with time and more or less stabilizes (after about half an hour), when protein layer may be more uniform on the substrate.

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