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Measurement of the surface tension of mucin solutions

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Summary

The surface tension of various mucin solutions at constant or varying pH was measured using the pendent drop method The surface tension was independent of added electrolyte concentration, pH and mucin concentration. It only depended on the source of mucin. The results are related to the mechanism of bioadhesion between polymers and mucus.

Introduction

In previous work on the bioadhesive characteristics of polymer microparticles, especially in contact with nasal or sublingual mucus, it was pointed out that the surface tension of the mucus or mucin solution may be important in the development of the adhesive bond (Peppas and Buri, 1985). Some energetic characteristics of mucin solutions have been discussed by Baszkin and Lyman (1980), Proust et al. (1984), and Levine et al. (1987). More recently, Bloomfield (1983) has also examined some of the hydrodynamic characteristics of mucus. Yet, systematic studies of the effect of pH electrolyte and mucin concentrations on the surface tension are not available.

The experimental methods that can be used to measure the surface tension of protein and polymer solutions include the pendent drop method, the sessile bubble (or drop) method, the rotating bubble method, and the Wilhelmy plate method. The suitability of these methods and the limitations of other less popular techniques were discussed by Wu (1974). The first 3 methods which are based on drop (or bubble) profiles are the most accurate. They are independent of contact angle but require knowledge of the density of the system. Density measurements are not needed for the Wilhelmy plate method which presumes a zero contact angle of the liquid on the plate. Such a condition is not always the case, which restricts the applicability of the method. In addition to density, the value of the refractive index of a liquid is necessary in order to calculate its surface tension with the rotating bubble method.

The pendent drop method, which is equivalent to the sessile bubble (or drop) method, was selected to measure the surface tension of various mucin solutions. The difficulty in isolating pure mucins renders this method more advantageous than others since it only requires small samples.

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Materials and Methods

Aqueous and buffered mucin solutions were prepared with crude mucin from porcine stomach (PSM) (Sigma Chemical Co., St. Louis, MO) and mucin from bovine submaxillary glands (BSM) (Sigma Chemical Co.), which was purified by the Nisizawa and Pigman (1959) technique. The aqueous solutions were prepared with different concentrations of NaCl.

The effects of NaCl concentration, mucin concentration and solution pH on the mucin solution surface tension were investigated for values of these parameters within the physiological range. All studies were performed at 37 °C. The densities of the mucin solutions were measured with a digital densitometer (model DMA 40, Mettler/Paar, Graz, Austria) with an accuracy of ±0.1 kg/m^3.

A contact angle goniometer (model 100-00, Ramé-Hart, Mountain Lakes, NJ) equipped with an environmental chamber (model 100-07) and a 35 mm photomicrographic camera (model C-35DA-2, Olympus) was employed in the surface tension experiments. A pendent drop was formed in the environmental chamber with a microsyringe. A photomicrograph of the pendent drop profile was taken once equilibrium was established. A typical photomicrograph is show in Fig. 1. The drop age at equilibrium was approximately 30 min. The equatorial and selected plane diameters were measured from the photomicrograph.

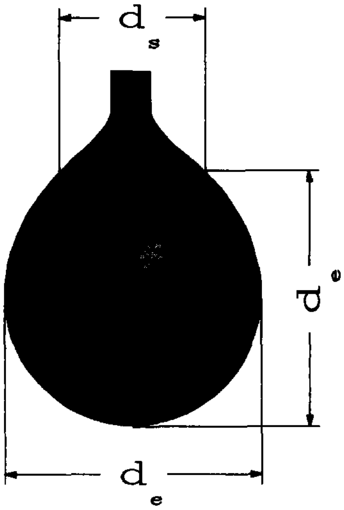

Fig 1. Photomicrograph of the pendent drop profile of an aqueous porcine stomach mucin (PSM) solution (5%wt PSM) showing the definition of the equatorial diameter, d_e, and the selected plane diameter, d_s, for the surface tension calculation.

Theoretical analysis

The mathematical framework for the surface tension calculation from the pendent drop profile has been discussed before (e.g., Ambwani and Fort, 1979). The analysis is based on the equation of capillarity (Laplace equation), which relates the pressure difference, Δp, across a curved surface (interface) to the surface (interfacial) tension, γ.

$$\Delta p = \gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{1}$$

Here R_1 and R_2 are the two principal radii of curvature of the surface.

The surface tension is a function of the geometry of the pendent drop profile at equilibrium. In the simplest case two dimensions of the drop shape are needed to calculate the surface tension, (i) the maximum or equatorial diameter, d_e, and (ii) the selected plane diameter, d_s, at a distance d_e from the apex of the drop (see Fig. 1). Recently, digital video image processing techniques were developed to calculate the surface tension from the entire drop profile (Qutubuddin et al., 1984; Rotenberg et al., 1983).

The surface tension is obtained as

$$\gamma = \frac{\Delta\rho g d_e^2}{H} \tag{2}$$

where g is the gravitational acceleration and $\Delta\rho$ is the density difference between the liquid and its vapor (for surface tension measurements).

$$\Delta\rho = \rho_l - \rho_v \tag{3}$$

The parameter H is a correction factor and is a function of the shape parameter, S, defined as

$$S = d_s/d_e \tag{4}$$

The values of H for different shape parameters were obtained by numerically solving the Laplace equation and were tabulated by Adamson (1982). By best fitting these values, Misak (1968) derived the following equations for the variation of H with S:

$$1/H = 0.32720S^{-2\,56651} - 0.97553S^2 + 0.84059S - 0.18069 \quad (0.401 \leq S \leq 0.46) \tag{5}$$

$$1/H = 0.31968S^{-2\,59725} - 0.46898S^2 + 0.50059S - 0.13261 \quad (0.46 < S \leq 0.59) \tag{6}$$

$$1/H = 0.31522S^{-2\,62435} - 0.11714S^2 + 0.15756S - 0.05285 \quad (0.59 < S \leq 0.68) \tag{7}$$

$$1/H = 0.31345S^{-2\,64267} - 0.09155S^2 + 0.14701S - 0.05877 \quad (0.68 < S \leq 0.90) \tag{8}$$

$$1/H = 0.30715S^{-2.84636} - 0.69116S^3 + 1.08315S^2 - 0.18341S - 0.20970 \quad (0.90 < S \leq 1.00) \tag{9}$$

The above equations are sufficiently accurate and were used in our calculations.

Results and Discussion

The correction factor, H, was calculated from the measured values of d_e and d_s (see Fig. 1) by one of the Eqns. 5–9 depending on the value of the shape parameter, S. The surface tension, γ, was derived from Eqns. 2 and 3 by using the measured liquid density, $\rho_l[g = 9.80665\ \mathrm{m/s^2}$, $\rho_v = 1.1\ \mathrm{kg/m^3}$ at 37 °C (Weast, 1985)].

The surface tensions of aqueous porcine stomach mucin (PSM) solutions (5%wt PSM) were measured for the NaCl concentrations of 0.0, 0.1, 0.5, 1.0 and 5.0 wt.%. The experimental findings, which are presented in Table 1, indicate that there is no dependence of the surface tension on the NaCl concentration. The γ values of buffered PSM solutions of pH 7 were measured for various mucin concentrations and are shown in Table 2. It was found that the surface tension was unaffected by the mucin concentration for values in the range from 1.0 to 30.0%wt.

TABLE 1

Dependence of the density and the surface tension on the NaCl concentration for 5 wt % aqueous porcine stomach mucin solutions at 37 °C

NaCl concentration (wt.%)	Density (kg/m^3)	Surface tension (J/m^2)
0.0	1008	0.04619
0.1	1010	0.04694
0.5	1012	0.04574
1 0	1016	0.04597
5 0	1045	0.04604

TABLE 2

Dependence of the density and the surface tension on the mucin concentration for buffered porcine stomach mucin solutions (pH = 7) at 37 °C

Porcine stomach mucin solution (wt.%)	Density (kg/m^3)	Surface tension (J/m^2)
0	1001	0.06972
1	1004	0.04479
2	1007	0.04599
5	1017	0.04648
10	1035	0.04552
15	1050	0.04674
20	1070	0.04507
30	1107	0.04316

TABLE 3

Dependence of the density and the surface tension on the solution pH for buffered 5 wt.% porcine stomach mucin solutions at 37 °C

pH	Density (kg/m^3)	Surface tension (J/m^2)
2	1013	0.04629
3	1014	0.04571
4	1013	0.04688
5	1017	0.04774
6	1016	0.04640
7	1017	0.04648
8	1018	0.04402

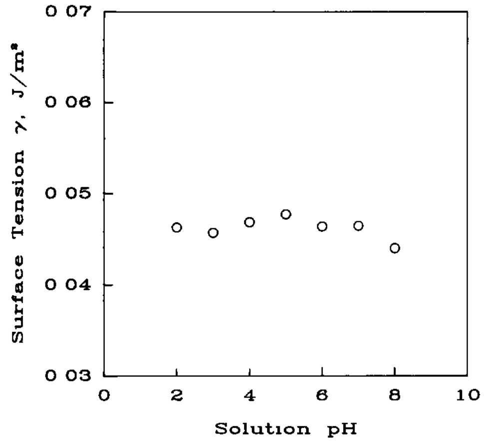

Fig 2. Variation of the surface tension, γ, of buffered porcine stomach mucin (PSM) solutions (5%wt. PSM) with the solution pH at 37 °C.

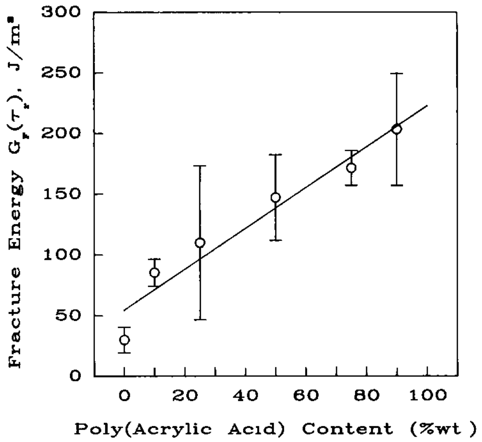

Fig 3. Influence of poly(acrylic acid) concentration on fracture energy for bioadhesive controlled release tablets tested in contact with bovine sublingual mucus (Ponchel et al., 1987).

The dependence of the surface tension of buffered PSM solution (5 wt.% PSM) on the solution pH was examined for the pH values of 2, 3, 4, 5, 6, 7 and 8. From Table 3 it can be deduced that the value of γ is also invariant with the solution pH. As shown in Fig. 2, there is a slight decrease at abnormally high pH values.

The surface tensions of buffered bovine submaxillary mucin (BSM) and PSM solutions of the same mucin concentration and pH are compared in Table 4. The value of γ for BSM is smaller than that for PSM. From these results it was concluded that the mucin surface tension only depends on the mucin type and remains constant with changes in the ionic strength, mucin concentration and solution pH.

These results may have a profound effect on our understanding of the bioadhesion of spherical controlled-release microparticulate systems. First, as mentioned before (Mikos and Peppas, 1986), for a bioadhesion phenomenon to be considered reversible, the measured fracture energy during separation of the polymer surface from mucin must be of the same order as 2γ. However, our recent experimental data of fracture energy as a function of poly(acrylic acid) content in controlled release devices in contact with bovine sublingual mucosal tissue (Ponchel et al., 1987) indicate an energy of the order of 50 to 180 J/m², (see also Fig. 3) which is of course 3 orders of magnitude greater than 2γ based on the values of γ determined here. Thus, the bioadhesion phenomenon is not a reversible thermodynamic process, because the fracture energy is of the order of brittle polymer fracture. Besides, experimental studies indicate that in bioadhesion there is a dependence on pH, ionic strength or mucin concentration.

TABLE 4

Surface tension and density of various buffered 1 wt% mucin solutions (pH = 7) at 37 °C

Mucin type	Density (kg/m³)	Surface tension (J/m²)
Bovine submaxillary	1004	0.03783
Porcine stomach	1004	0.04479

Thus, bioadhesive interactions of a polymer with mucus have much to do with the surface tension of the mucus. However, for the better bioadhesive system the fracture energy (required to initiate surface detachment) will be much larger than the surface tension, indicating a highly irreversible separation process.

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Note added in proof

Recent studies showed that the bioadhesion of PAA-based formulations on mucus strongly depended on the solution pH and ionic strength (Lejoyeux et al , 1988) Consequently, one infers that the bioadhesion of controlled release formulations on soft tissues is a thermodynamic phenomenon only for poor bioadhesives In this case, the mucin solution surface tension determines the surface and interfacial energetics However, for the better bioadhesive systems (e.g. those containing PAA) the fracture of their interfaces with mucus disentanglement, similar to those occurring in polymer fracture (Mikos and Peppas, 1988a and b). Here, the interfacial interactions of polymeric materials and mucus are related to their macromolecular structure, topology and dynamics, much in the same way as in polymer–polymer adhesion (Mikos and Peppas, 1988b)

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