

## Determination of the Surface Tension of Block Copolymer Micelles by Phagocytosis

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**Purpose.** This work was carried out to determine the surface tension of block copolymer micelles of <sup>14</sup>C labelled ABA poly (oxyethylene-bi-isoprene-b-oxyethylene) which have a long circulating half life in animals.

**Methods.** The method used was that of phagocytosis. The percentage of micelles phagocytosed by human mononuclear cells was determined in solutions of different surface tension.

**Results.** The values obtained were 72 mN/m which may be predicted for a particle with a long circulating half life in animals. The method also gave an estimate of the surface tension for the mononuclear cells.

**Conclusions.** This technique has the advantage of determining the surface tension of highly hydrated small particles including stable micelles in an environment similar to that in which they normally exist.

**KEY WORDS:** surface tension; micelles; block copolymers; phagocytosis.

### INTRODUCTION

A considerable amount of work has been carried out in recent years on coating potential drug delivery particulate systems with polyoxyethylene compounds (PEO). This has increased the *in vivo* circulation of the original particulates by reducing uptake due to the presence of cells of the mononuclear phagocytic system (MPS) (1). A PEO exterior can also be produced in discrete micelles formed from poly (oxyethylene-b-isoprene-b-oxyethylene) (e.g. block copolymers) (2). These have also been found to have long circulating blood half lives in experimental animals, with various values in excess of fifty hours being reported (2).

For these systems to have increased blood circulating times, one or more of the principle mechanisms responsible for removal of these particles would have to be reduced. The MPS is the main route of removal and opsonisation of the particle by protein adsorption prior to phagocytosis by the MPS being the most important mechanism, although non opsonophagocytosis can also occur (3).

Adsorption, normally a prerequisite for phagocytosis, is controlled by a range of forces including dispersion, electrostatic, steric, hydration and chain elasticity (4). The relative importance of these factors has been considered by many workers in various fields of research (1,5). For PEO coated surfaces both interfacial surface tension and steric repulsion

have been indicated to be playing important roles in the extent of adsorption of proteins etc. and the resulting biocompatibility (3,6,7).

The interfacial surface tension for a particulate in solution ( $\gamma_{SL}$ ) may be related to the surface tension of the particle ( $\gamma_{SV}$ ) and that of the liquid ( $\gamma_{LV}$ ) using the equation of state (8).

$$\gamma_{SL} = \gamma_{LV} + \gamma_{SV} - 2(\gamma_{LV}\gamma_{SV})^{1/2}e - \beta(\gamma_{LV} - \gamma_{SV})^2$$

where the parameter  $\beta = 0.0001247 \text{ (m}^2/\text{mJ)}^2$ .

From this equation the interfacial surface tension will be zero when the surface tension of the particulate equals that of the solution. It has also been shown (9) that the free energy of phagocytosis has a maximum value, that is minimum extent of phagocytosis occurs, under these conditions. Thus knowledge of the surface tension of particulates used for drug delivery may give information on their behaviour *in vivo*.

Many methods have been developed to measure the surface tension of solids ( $\gamma_{SV}$ ), contact angle determinations being the most common. However, for small particulates with highly hydrated surfaces the number of suitable techniques is limited (10). One which is suitable for very small particles is that based on phagocytosis. Neumann et al (9) showed that when the surface tension of bacterium is equal to that of the suspending solution, phagocytotic uptake of the bacteria is minimal. The same argument can also be applied to drug carrier particulates phagocytosed by cells of the MPS. This procedure will also mimic the *in vivo* situation more closely than other methods and is therefore particularly suitable for monitoring drug delivery particulate systems. To monitor the phagocytosis of particles, several methods are available (11). These include the incorporation of compounds into the phagocytic cells which produce fluorescent degradation products when the phagocytic process occurs (12) and tagging the particles with radiolabel or fluorescent molecules.

The purpose of the present work was to determine the surface tension of the block copolymer micelles of poly (oxyethylene-b-isoprene-b-oxyethylene) by phagocytosis. The method adopted was based on that of measuring the effect of varying the surface tension of Hank's balanced salt medium on the interactions of <sup>14</sup>C labelled micelles with human macrophage mononuclear cells. The same procedure was adopted for determining the surface tension of rhodium labelled polystyrene latex particles using a fluorescent activated cell sorter to monitor the uptake. The viability of the macrophages in solutions with surface tension modifiers present was also monitored.

### MATERIALS AND METHODS

<sup>14</sup>C labelled poly (oxyethylene-b-isoprene-b-oxyethylene) was prepared as described by Rolland et al (2). The two polymers used in this study were copolymer 9 and 11 which have relative molecular masses of 24,800 and 13,900 respectively. Micelles were prepared by dissolving 0.1% w/v of the copolymers in distilled water with stirring for 24 h at room temperature. These solutions were then dialysed against Hank's medium (Gibco Ltd) in Visking Tubing with a

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11,000 molecular weight cut off for one week. Characteristics of the micelles are given by Rolland et al (2). Rhodamine labelled polystyrene microspheres, diameter 260nm, (Polyscience Inc.) were also dialysed against Hank's buffer for a week.

Human mononuclear cells (MNC) were isolated from blood using Histopaque 1077 (Sigma Ltd). Basically the blood (5cm<sup>3</sup>) was diluted one in one with Hank's medium balanced salt without phenol red, layered carefully onto the Histopaque 1077 in centrifuge tubes, centrifuged at 900g for 30 mins. The top layer containing the mononuclear cells was diluted with 10cm<sup>3</sup> of Hank's medium, centrifuged at 400g, then washed again with medium and pelleted. The pellet was resuspended in 0.25cm<sup>3</sup> of Hank's medium and all samples pooled.

The cell number was assessed using a Coulter Channelyser 256 and the cell suspension diluted with Hank's medium to give a total cell count of  $2 \times 10^6$  cells cm<sup>-3</sup>.

Viability of cells in Hank's medium containing DMSO or n propanol was monitored after 90 mins, using a mixture of fluorescein diacetate (1  $\mu\text{g cm}^{-3}$ ) and propidium iodide (50  $\mu\text{g cm}^{-3}$ ). Experiments were also carried out incubating the cells with 10  $\mu\text{M}$  lucifer yellow. The fluorescence of the cells was measured with a fluorescence activated cell sorter (FACS), (Beckton Dickenson 440) using excitations of 488nm for fluorescein diacetate and propidium iodide and 465nm for lucifer yellow and the fluorescence measured at 520, 620 and 600 nm respectively.

The surface tensions  $\gamma_{LV}$  of the dimethylsulphoxide Hank's solutions and those with n propanol present were determined by Wilhelmy plate using a microforce balance (C I Electronics) coupled to a recorder. The surface tension of the solutions were also measured after dilution with an equal volume of Hank's medium containing the copolymer micelles.

#### Mononuclear Cell Micelle Interactions

Hank's medium (0.5cm<sup>3</sup>) containing either n propanol or DMSO at various concentrations was mixed with 0.5cm<sup>3</sup> of mononuclear cell suspension ( $10^6$  cells) containing 10 $\mu\text{l}$  of <sup>14</sup>C copolymer micelles to give a count of at least  $10^5$ dpm in the final incubation solution. After 2hrs at 37°C the cells were analysed to give total counts present and counts associated with the mononuclear cells only.

The method adopted was to add to 100 $\mu\text{l}$  aliquots of the solution taken after incubation, 600  $\mu\text{l}$  of Protosol (NEN Research Products Ltd) which digested the cellular material when allowed to stand for 30 minutes at 60°C. Then 10cm<sup>3</sup> of scintillation fluid, Biofluor (NEN Research Products Ltd) was added, shaken and the vials were left to stand for 24 hours before counting on a LS1801 Beckman scintillation counter for ten minutes. This gave the total count. The count associated with the cells was obtained by washing a known volume of the incubated solution with 1cm<sup>3</sup> of Hank's medium, centrifuged and then washed twice more and finally suspended in 400  $\mu\text{l}$  of Hank's medium. 200  $\mu\text{l}$  of this solution was taken for digestion and scintillation counting as described above. Blanks were carried out and subtracted from the counts obtained for the samples. The results are expressed as a % radioactivity associated with the cells compared with total activity.

Surface tension measurements at the air water interface for solutions of block copolymer 9 and 11, concentration range,  $10^{-9}$  M to  $10^{-4}$  M, were determined by the Wilhelmy plate method. Solutions were allowed to equilibrate at room temperature (23°C) with readings taken until a constant value was obtained.

#### RESULTS AND DISCUSSION

The surface tension values of the DMSO Hanks and propanol Hanks solution gave values similar to those in the literature (14). On dilution by copolymer solution, the surface tension value was altered as expected for a 50% v/v change.

The viability of MNC was unaffected by DMSO up to 20%v/v. However, for propanol at 2.0% v/v, after 90 minutes incubation, there was a decrease in fluorescence intensity when using fluorescein diacetate (Figure 1) indicating a change in cell metabolism. With propidium iodide and lucifer yellow, increases in fluorescence intensity (Figure 1) occurred for 9% v/v and 5% v/v of propanol respectively, indicating changes in membrane permeability for these compounds. These results show that DMSO is the preferred surface tension modifier.

A typical plot (n=8) of the % radioactivity taken up by the MNC as a function of surface tension of the solution used for the incubation is given in Figure II. The general shape of

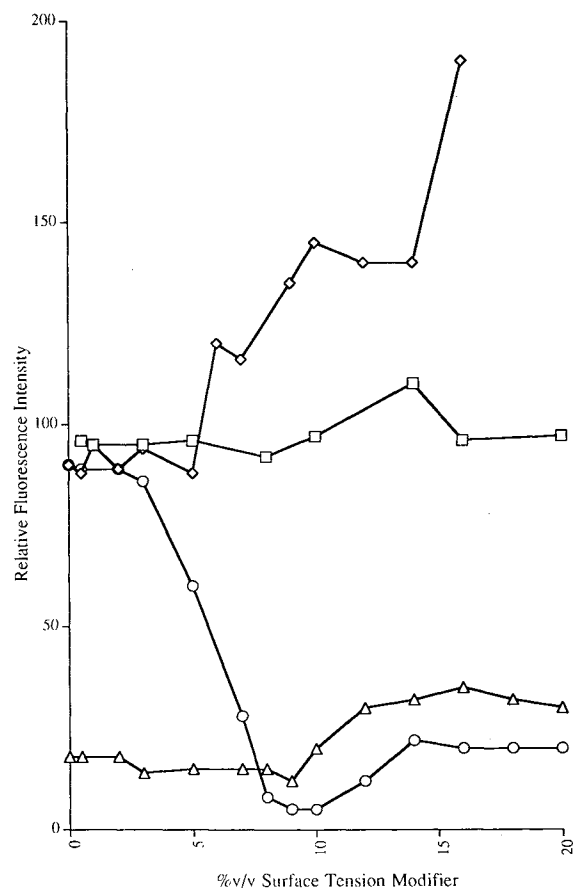


Fig. 1. Viability of human mononuclear cells in the presence of propanol using fluorescein diacetate, ○, propidium iodide, △ lucifer yellow ◇ and in the presence of DMSO using lucifer yellow □.

the plot is similar for both copolymer 9 and 11 with either DMSO or propanol as surface tension modifiers. Two minima are found in the concentration range of the surface tension modifiers where the cells are functioning. The theory of Neumann (10) predicts that these minima occur at surface tensions of the solutions equal to that of the particles in the solution. The main minimum at  $72 \text{ mNm}^{-1}$  was at zero concentration of DMSO or propanol with a smaller minima at between  $68$  and  $69 \text{ mNm}^{-1}$ . The former value is similar to that found for PEO copolymer poloxamers 188, 407 and 908, coated polystyrene latex particles (13) and is the surface tension of the copolymer micelles. The latter value could be for an alternative surface structure of the micelles due to the surface tension modifiers or possibly the value of the surface tension for the mononuclear cells since this corresponds to the literature values for these cells (9)

For copolymers 9 and 11, the CMC's were found to be  $0.005$  and  $0.08\%$  w/v and the surface tensions of  $0.1\%$  w/v solutions were  $54$  and  $48 \text{ mNm}^{-1}$  respectively. When using the Wilhelmy plate method, these values are of the same order as those found for block copolymers using polyoxypropylene instead of polyisoprene (7).

The structure of these copolymer micelles (2) would produce flexible polyoxyethylene chains as the outer layer, which would be highly hydrated. This would be expected to produce a surface tension similar to that of water, as was found in this work for the phagocytosis method. The resulting interfacial tension with the aqueous media would be very

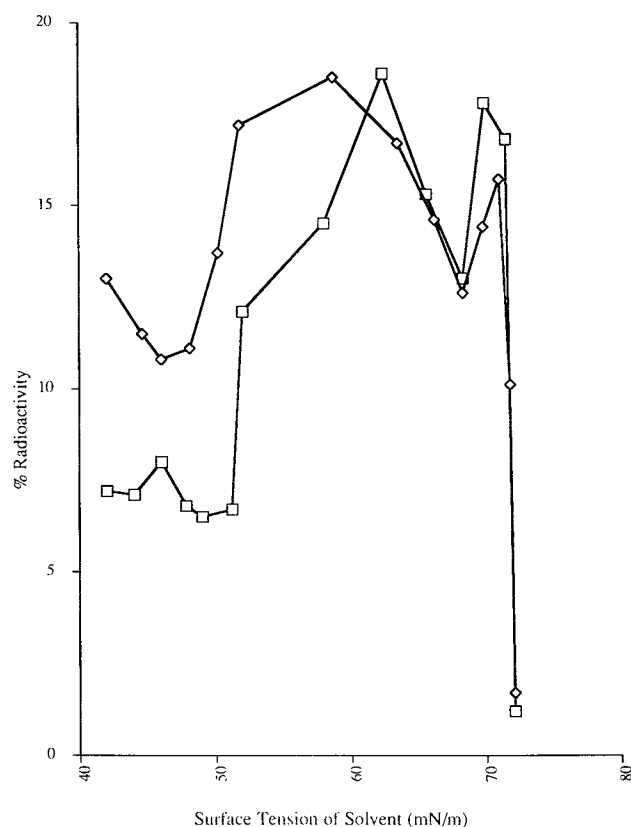


Fig. 2. Effect of surface tension of the solution on the uptake of  $^{14}\text{C}$  labelled copolymers 9,  $\square$ , and 11,  $\diamond$ , micelles by human mononuclear cells using n propanol as surface tension modifier.

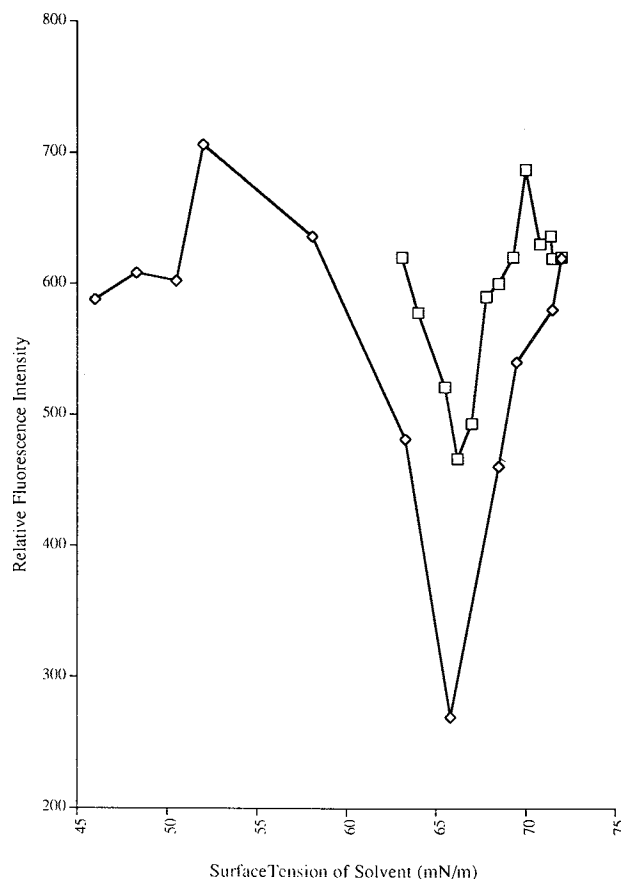


Fig. 3. Effect of surface tension of the solution on the uptake by human mononuclear cells of rhodamine sulphonated polystyrene particles using DMSO,  $\square$ , and n propanol,  $\diamond$ , as surface tension modifiers. Fluorescent activated cell sorter was used for the analysis.

small or zero, resulting in minimal interaction with the MPS and a long half life for the micelle. The difference between this surface tension value of  $72 \text{ mN m}^{-1}$  and the values found in this work for the block copolymers at the air solution interface could be a result of the differences in extent of hydration of the surfaces due to the techniques used. Other possibilities are the surface density of the PEO chains (7) or simply that in the micellar structure the PEO chains can readily arrange to minimise the interfacial free energy. This minimal interfacial surface tension for PEO micelles along with steric stabilisation may be the reason these micelles have such long circulating half lives (3). This argument would also apply to particulates coated with poloxamers which have also shown increased circulating half lives (1, 15). Further work is being carried out on these and other systems which avoid the MPS.

## CONCLUSION

Phagocytosis has been used to determine the surface tension of micelles of block copolymers, the values found being similar to that of water. This may be one property of the surface of particulates that is important to attain, in order to reduce interactions with biological systems. Phagocytosis can readily be used for the determination of surface tension

of small particles and this technique mimics the *in vivo* situation more closely than many other methods.

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