THE HAEMOLYTIC ACTIVITY OF NON-IONIC SURFACTANTS

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There is some concern with the toxicity of surfactants, used ubiquitously e.g. as components of industrial, domestic, cosmetic and pharmaceutical preparations, and a common method of toxicity evaluation is to test them in animals for their irritant potential. Surfactants are of course active against membranes and at the molecular level of organisation, the basic phospholipid bilayer architecture is reason to believe that a surfactant will have essentially the same effect on membranes from diverse organisms. An extension of this concept is that toxic effects might be detected on simple, inexpensive membrane systems, e.g. cultured cells, rather than on a whole animal. We found a positive correlation between the physico-chemical properties of a series of alkylphenyl polyoxyethylene ethers (TRITON surfactants) and their ability to inhibit the motility of the protozoan Tetrahymena elliotti (Baillie et al, 1989). We describe here the haemolytic activity of a series of p-t-octyl phenyl polyoxyethylene ether surfactants, the erythrocyte providing a ready indication of membrane damage by the release of its haemoglobin contents.

Human blood was obtained by venepuncture (anterior antecubital vein) into lithium heparin tubes (Searle) and ovine and porcine blood collected at slaughter, rapidly into heparinised tubes. 0.05 mL of heparinised blood was added to 5 mL surfactant solution then incubated at 37°C for 20 min with gentle agitation. After centrifugation (10 min, 900 g) the absorbance (At) of the supernatant at 540 nm was determined. The absorbance of positive (Ap, 0.05 mL blood in 5 mL water) and negative (An, 0.05 mL blood in 5 mL physiological saline) controls was similarly determined. Haemolysis (%) was quantified as At-An/Ap x 100). The concentration of surfactant (C50) to cause 50% haemolysis was determined for each surfactant in the series. The protein (Lowry method), cholesterol (Cholesterol C-System, Boehringer Mannheim) and the phospholipid (as phosphate) contents of haemoglobin-free erythrocyte ghosts prepared from blood of each species was also determined.

There was an abrupt cut-off in haemolytic activity (1/C50) above 12 ethylene oxide (EO) residues such that only the more hydrophobic members of the series were markedly haemolytic although among these active surfactants EO had little apparent influence on activity. With the lipophilic members of the series, haemolysis was observed below surfactant CMC. Some of the hydrophilic surfactants were not haemolytic even at the highest concentration tested (200 mM) and where haemolysis was observed it occurred at concentrations well above the CMC. The high hydrophilicity apparently inhibited surfactant membrane interaction and in this respect it would seem that for the hydrophobic moiety of these surfactants, EO>12 represents a minimal, protective hydrophilicity. It is not known whether the low activity of the hydrophilic surfactants represents an inability to interact with the erythrocyte membrane or an inability to lyse it after interaction. In spite of species differences in the content of lipid and protein among the erythrocytes used, that is differences in the target membranes, little difference in susceptibility to surfactant induced haemolysis was found.

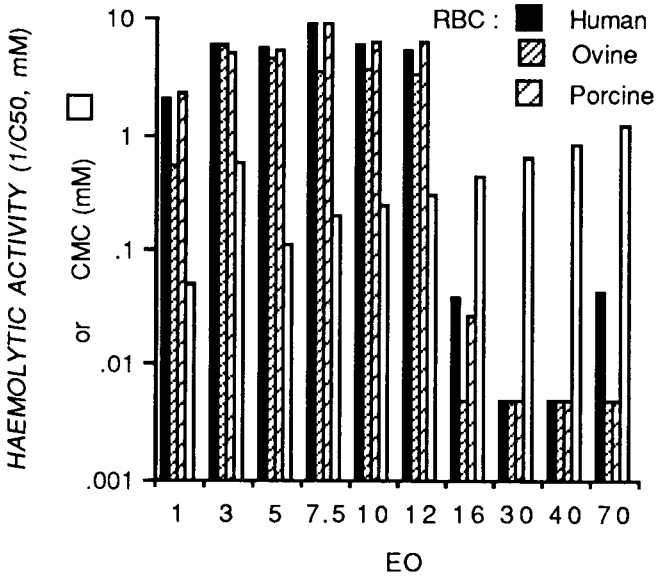

Figure 1. The influence of ethylene oxide number (EO) of p-t-octyl phenyl polyoxyethylene ethers on surfactant CMC and their haemolytic activity (1/C50) against human, ovine and porcine erythrocytes.

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