

## EFFECT OF LONGCHAIN HYDROPHILIC POLYMERS ON THE HYDRODYNAMIC BEHAVIOR OF LIPID THIN LIQUID FILMS

*Albena Jordanova\**, *Georgi D. Georgiev*, *Georgi A. Georgiev*,  
*Zdravko Lalchev*

*\*Bulgarian Academy of Sciences, Institute of Biophysics, 1113 Sofia,  
Bulgaria  
Biological Faculty, Sofia University „St. Kliment Ohridski“,  
1164 Sofia, Bulgaria  
Corresponding author: [albena@biofac.uni-sofia.bg](mailto:albena@biofac.uni-sofia.bg)*

**ABSTRACT.** The noncytotoxic biosurfactant Poloxamer 188 (P188) is a three-block copolymer (consist of poly-ethylene oxide–poly-propylene oxide–poly-ethylene oxide /PEO-POP-PEO/) which is known to repair damaged membranes and thus to restore the structural integrity of the cells. P188 can seal membrane pores in skeletal muscle cells after electrical injuries, heat shocks, burns, frostbite, etc. P188 has an average molecular weight of about 8400 and it has amphiphilic structure. The length of the hydrophilic chain might influence the strength of the interaction between the permeabilized membrane and the surfactant and thereby influence the effectiveness of a surfactant in sealing permeabilized membranes.

The aim of the present work was to study the hydrodynamic properties of lipid thin liquid films (formed by the microinterferometric method of Sheludko and Exerowa) from dipalmitoleoylphosphatidylethanolamine (DPoPE) with added P188 and to correlate it with the viscosity (determined with Hoppler falling-ball rheoviscosimeter) of mixed Lipid-P188 dispersions.

The obtained results demonstrate that at both applied pressures in the viscosimetric studies addition of DPoPE leads to decreasing of the reduced viscosity ( $\eta_{red}$ ) values and maintaining of low viscosity for broad P188 concentration range. These results correlate with similar behavior of film thinning time ( $T_{0-1}$ ) values. These effects were explained with adsorption of P188 molecules to the surface of DPoPE vesicles, thus keeping constant low value of the number of “free” P188 monomers for broad range of P188 bulk concentration values.

**KEY WORDS.** poloxamer, lipid thin liquid films (TLFs), viscosity

## INTRODUCTION

The Poloxamers belong to a class of water-soluble multi-block copolymers that have important surface-active properties. The Poloxamer series covers a range of liquids, pastes and solids, with molecular weights varying from 1100 to about 14000. The PEO/POP weight ratios range from about 1:9 to about 8:2. Poloxamer P188 is a three-block copolymer often abbreviated as PEO-POP-PEO with PEO and POP representing poly(oxyethylene) and poly(oxypropylene) respectively. The PEO chains are hydrophilic due to their short carbon unit between the oxygen bridges whereas the POP center is hydrophobic due to the larger propylene unit (Figure 1). P188 has molecular weight of about 8400 and it is prepared from 1750 average molecular weight hydrophobic and its hydrophilic comprises about 80% of the total molecular weight

The possibility of augmenting repairs of cell membrane wounds using synthetic surfactants is now well established although the exact mechanisms of repair (i.e. sealing) is still under investigations (1). Known effective agents include the surfactant class of Poloxamers, representing a group of three-block copolymers. Poloxamer 188 (P188) made by Anatrace (USA) was initially shown to seal cells against loss of carboxy-fluorescein dye after electroporation (2).

In the following years, it has been demonstrated that P188 can also seal membrane pores in skeletal muscle cells after heat shock (3) and enhance the functional recovery of lethally heat-shocked fibroblasts (4) and following high-dose ionizing radiation (5). Very recently, P188 has been shown to protect embryonic hippocampal neurons against death due to neurotoxic induced loss of membrane integrity (6).

Most often P188 is used at a sub-critical micelle concentration (sub-CMC) of 0.1 mM to 1.0 mM for membrane repair *in vitro* (2). Above their CMC surfactants self-aggregate to micelles causing the surfactant monomer concentration to remain constant (=CMC) independently of the total surfactant concentration. The capability of these amphiphilic copolymers to repair cell membranes at millimolar concentrations distinguishes the sealing capability of this copolymer from purely hydrophilic polymers such as PEG poly (ethyleneglycol) which require molar concentrations (7).

Thus the investigation on the interaction between lipids molecules and poloxamer P188 is important for the cell physiology.

The aim of the present work was to study the hydrodynamic properties of Lipid Thin Liquid Films with added poloxamer P188 and to correlate it with the viscosity (determined with Hoppler falling-ball rheoviscosimeter) of Lipid-P188 dispersions.

## MATERIAL AND METHODS

**Preparation of Thin Liquid Films (TLFs):** Black TLFs (Figure 2) were investigated by the microinterferometric method (8) in its currently used version (9). Microscopic horizontal TLFs of radius 0.2 mm were formed in a specially constructed glass cell in the middle of a biconcave drop. Equilibration for about 30 min before film formation was necessary to allow for phospholipid adsorption, vapor

pressure saturation and temperature adjustment. It was equipped with an optical system for observation and investigation of TLFs. The bilayer TLFs consist of two monolayers of amphiphilic molecules in a gaseous phase, which are in contact with each other through their polar head groups (Figure 2C). After sucking the solution from the biconcave drop, the TLF initially forms as a thick film (thickness more than 100 nm) and further spontaneously becomes thinner. During film drainage, black spots may appear on its surface, resulting from local thinning; then these spots fuse and expand to full up the entire film area. Two kinds of black TLFs are known: common black film (CBF) and Newton black film (NBF). The common black films contain a free water layer between the two monolayers and their thickness varies from 10 nm to 20 nm (Figure 2B). The Newton black films contain no free water (Figure 2C). Their thickness is less than 8 nm. The formation of bilayer TLFs depends on the electrolyte conditions. We used 0.5 M NaCl solutions for our measurements, where all the stable DPOPE films we observed were bilayer TLFs.

**Viscosimetric study:** For the viscosity investigations of the pure DPOPE, P188 and lipid+P188 dispersions we used Hoppler falling-ball rheoviscosimeter as previously described (10). Reduced viscosity  $\eta_{red}$  (g/dl) was measured as the ratio among the dispersion specific viscosity ( $\eta_{sp}$ ) and bulk P188 concentration  $\eta_{red} = \eta_{sp}/C$ .

## RESULTS AND DISCUSSION

### Results from formation of TLFs

The probability  $W$  for stable Black TLFs formation ( $W = \Delta N/N$ , where  $\Delta N$  is the number of trials when stable films are formed and  $N$  is the total number of trials; thus the value of  $W$  varies from 0 to 1, reflecting film no formed or formation of a stable film, respectively) as function of DPOPE and P188 concentration in DPOPE/P188 pure and mixed dispersions at room temperature was studied (Table 1,2). The obtained results demonstrated that pure P188 no formed black films at all applied concentrations (from 0.125 mg/ml to 10 mg/ml). But adding different amount of phospholipids (100 or 200  $\mu$ g DPOPE /ml) caused the formation of Newton black film at all mixed P188+DPOPE dispersions. Table 1 shows that the time from the opening of film to the appearance of first black spot  $/T_{0 \rightarrow 1}/$  increased from about 1 minute at lowest P188 concentration (0.125 mg/ml) to about 3 minutes at the highest poloxamer concentrations (7-10 mg/ml).

The obtained results clearly indicate that time of the increase of the black spots  $T_{1 \rightarrow 2}$  grew gradually with increase of concentration of P188 in relation of constant concentration of DPOPE (0.145 M) from 2 seconds at ratio DPOPE/P188=12/1 (molecule/molecule), to 90 seconds at DPOPE/P188= 1/1 (Table 2). We also found that type of the formed films was changed at ratio DPOPE/P188=4/1 to common black film. It should be noted that the presence of large numbers of polymer molecules at the bulk led to transformation of film type from truly bilayer NBFs to thicker CBFs.

### **Results from reduced viscosity ( $\eta_{\text{red}}$ ) measurements**

Results from reduced viscosity measurements at two applied pressures of 0,5 and 2,5 g/cm<sup>2</sup> are shown at Figure 3, panel A and B respectively. It can be seen that at both pressures addition of DPOPE (at concentration  $\geq 100$   $\mu\text{g/ml}$ ) leads to substantial decreasing of  $\eta_{\text{red}}$  value and maintaining of low viscosity for broad P188 concentration range; the data correlate with similar behavior of film thinning time ( $T_{0-1}$ ) value (see tables). These effects were proportional to DPOPE concentration for both experimental techniques - Hoppler rheoviscosimeter and Thin Liquid Films; they could be explained with adsorbition of P188 molecules to the surface of DPOPE vesicles, resulting in constant low value of the number of nonadsorbed "free" P188 monomers for broad range of P188 bulk concentration values. When DPOPE vesicles surface became saturated with adsorbed P188 molecules, at some threshold P188 concentration (3 mg P188/ml for 100  $\mu\text{g}$  DPOPE /ml and 9 mg P188/ml for 200  $\mu\text{g}$  DPOPE /ml)  $\eta_{\text{red}}$  became steeply to increase and finally reaches the value for pure P188 dispersions (3 and 1,8 g/dl at 0,5 and 2,5 g/cm<sup>2</sup>, respectively).

### **CONCLUSION**

The obtained results demonstrate that at both applied pressures (0,5 and 2,5 g/cm<sup>2</sup>) addition of DPOPE leads to decreasing of  $\eta_{\text{red}}$  value and maintaining of low viscosity for broad P188 concentration range. The data correlate with similar behavior of film thinning time ( $T_{0-1}$ ) value (see tables). The effects were proportional to DPOPE concentration for both experimental techniques- Hoppler rheoviscosimeter and Thin Liquid Films; it was explained with adsorbition of P188 molecules to the surface of DPOPE vesicles, thus keeping constant low value of the number of "free" P188 monomers for broad range of P188 bulk concentration value. When DPOPE vesicles surface was saturated with adsorbed P188 molecules, at some threshold P188 concentration  $\eta_{\text{red}}$  became steeply to increase and finally reaches the value for pure P188 dispersions.

On the basis of the obtained results we can conclude that the tri-block PEO-POP-PEO polymer molecules of P188 possess very strong affinity to DPOPE molecules/vesicles. This strong affinity of poloxamer molecules to lipid membranes measured by both used experimental techniques (Hoppler rheoviscosimeter and Thin Liquid Films) could explain the molecular mechanism ensuring the processes of P188 induced sealing of damaged cell membranes observed *in vivo*.

### **ACKNOWLEDGEMENTS**

This work was supported by the Bulgarian Ministry of Education, project MU-K-1301/03-2375.

### REFERENCES:

- LEE, R.C., MYEROV, A., MALONEY, C.P., 1994. Promising Therapy for Cell Membrane Damage in Electrical Injury: A multidisciplinary Approach to Prevention, Therapy & Rehabilitation. Lee R.C., Capelli-Schellpfeffer, M., and Kelley K.M., eds. *Annals of the New York Academy of Science*, Vol. 720, pp. 239-245.
- LEE, R.C., PAN, FU-S., RIVER, L.P., LI Ji and WOLLMANN, R.L., 1992. Surfactant-Induced Sealing of Electroporabi-lized Skeletal Muscle Membranes *In Vivo*. *Proc. Natl. Acad. Sci.* 89(10): 4524-4528.
- PADANILAM, J.T., BISCHOF, J.C., LEE, R.C., CRAVALHO, E.G., TOMPKINS, R.G., 1994. Effectiveness of poloxamer 188 in arresting calcein leakage from thermally damaged isolated skeletal muscle cells. *Ann NY Acad Sci* 720: 111-123.
- MERCHANT, F.A., HOLMES, W.H., CAPELLI-SHELLPFEFFER, M., LEE, R.C., and TONER, M., POLOXAMER, 1998. 188 Enhances Functional Recovery of Lethally Heat-Shocked Fibroblasts. *J Surg Res.* 74: 1031-1040.
- GREENEBAUM, B., CARRILLO, C.S., HANNIG, J., BECKETT, M. A., WEICHSELBAUM, R.R. and LEE, R.C., 2004. Poloxamer 188 Prevents Acute Necrosis of Adult Skeletal Muscle Fibers after High-dose Irradiation. *Burns* Sep;30(6):539-47.
- MARKS, J.D., CROMIE, W., and LEE, R.C., 1998. Nonionic surfactant prevents NMDA-induced death in cultured hippocampal neurons. *Soc Neurosci Abs* 24(1): 462.
- SHI, R., BORGENS, R.B., AND BLIGHT, A.R., 1999. Functional reconnection of severed mammalian spinal cord axons with polyethylene glycol. *J. Neurotrauma* 16(8): 727-738.
- SHELUDKO, A., 1967. Thin liquid films. *Adv Colloid Interface Sci* 1:391-464.
- Exerowa D, Nikolova A Phase transitions in phospholipids foam bilayers. *Langmuir*, 8:3102-3108, 1992.
- KOZAKIEWICZ, J., PENCZEK, P., 2003. Adhesives from polyepichlorohydrin elastomers. I. Studies on the properties of adhesive solutions of polyepichlorohydrin. *Angewandte Makromolekulare Chemie*, 59(1): 67-77.

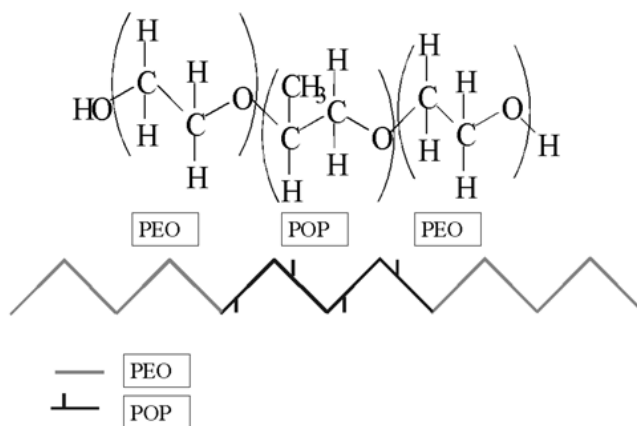
## TABLES AND FIGURES

**Table 1:** Type of the black bilayer film of P188 in 0.5 M NaCl at different concentrations and after adding of different amount of DPoPE at room temperature.

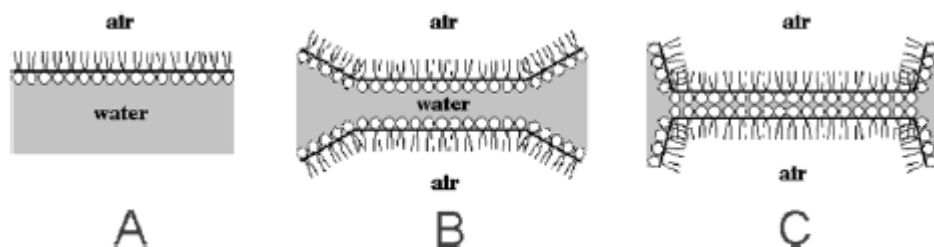
Concentration of P188 in 0.5M NaCl	Amount of added DPoPE	T <sub>0→1</sub> /time from the opening of film to the appearance of black spots/	T <sub>1→2</sub> /time of the increase of black spots/	Type of the black film
0.125 mg/ml	-	Film ruptures	-	Film no formed
	+100 µg/ml	1 min	1-2 sec	NBF
2.5 mg/ml	-	Film ruptures	-	Film no formed
	+100 µg/ml	1.05 min	1-2 sec	NBF
	+150 µg/ml	2.30 min	1-2 sec	NBF
	+200 µg/ml	3 min	2-3 sec	NBF
4 mg/ml	-	Film ruptures	-	Film no formed
	+100 µg/ml	2.30 min	2-3 sec	NBF
	+200 µg/ml	3 min	3 sec	NBF
5.5 mg/ml	-	Film ruptures	-	Film no formed
	+100 µg/ml	2.40 min	2-3 sec	NBF
	+200 µg/ml	3 min	3 sec	NBF
7 mg/ml	-	Film ruptures	-	Film no formed
	+100 µg/ml	2.53 min	3-4 sec	NBF
	+200 µg/ml	3 min	3-4 sec	NBF
10 mg/ml	-	Film ruptures	-	Film no formed
	+100 µg/ml	2.53 min	3-4 sec	NBF
	+200 µg/ml	3 min	3-4 sec	NBF

**Table 2:** Type of the black bilayer film of DPoPE in 0.5 M NaCl at different concentrations and after adding of different amount of P188 at room temperature.

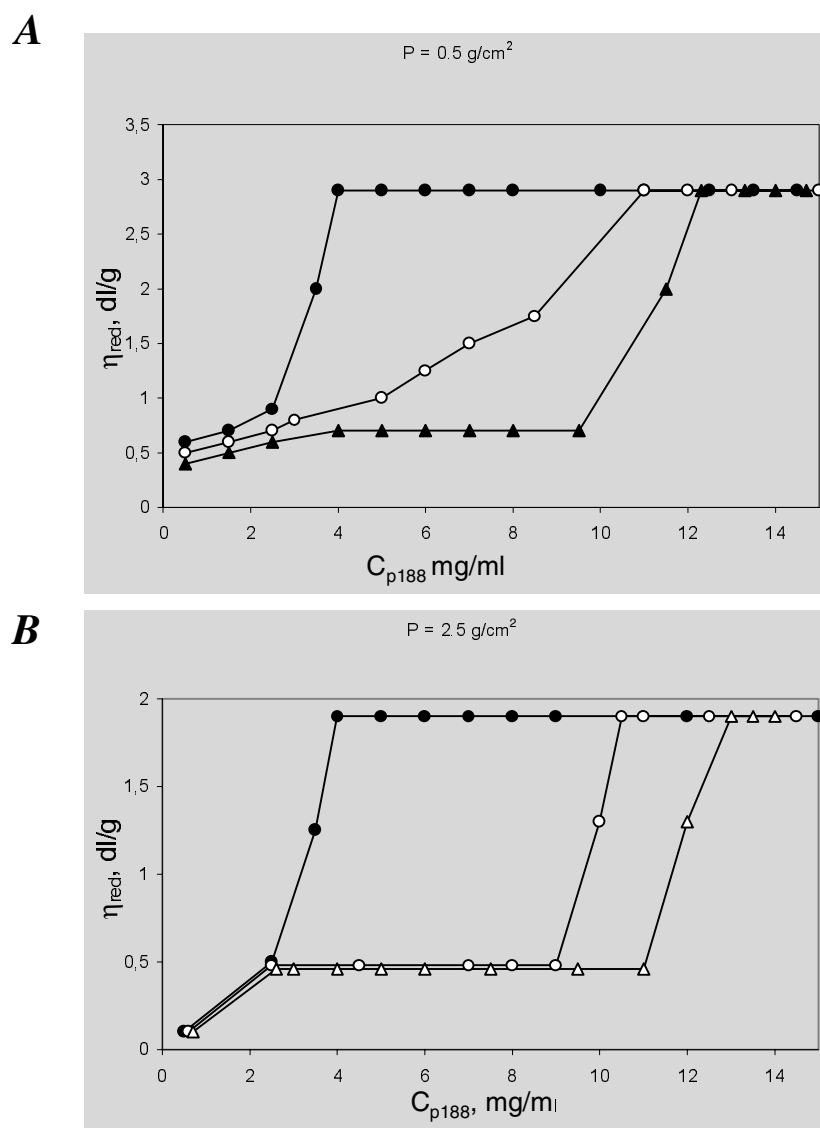
Concentration of DPoPE in 0.5 M NaCl	Amount of added P188 (2.5 mg/ml)	T <sub>0→1</sub> /time from the opening of film to the appearance of black spots/	T <sub>1→2</sub> /time of the increase of black spots/	Type of the black film
100 µg/ml 0.145 M	-	1.30 min	1-2 sec	NBF
	+2 µl /12 numbers PLmolecules:1P188 /	2 min	2 sec	NBF
	+2 µl /6 numbers PLmolecules:1P188 /	2 min	2 sec	NBF
	+2 µl /4 numbers PLmolecules:1P188 /	2.50 min	56 sec	CBF
	+4 µl /2 numbers PLmolecules:1P188 /	2.55 min	60 sec	CBF
	+2 µl /1 number PLmolecules:1P188 /	3 min	90 sec	CBF



**Fig. 1.** Chemical structure of poloxamers. The series of different poloxamers is constituted through varying numbers and ratios for PEO and POP.



**Fig. 2.** Lipid monolayer (A); common black film (B); Newton (bilayer) black film (C)



**Figure 3.** Dependence of P188/P188 + DPOPE dispersions reduced viscosity  $\eta_{red} = \eta_{sp}/C$  on poloxamer P188 concentration at two applied pressures of 0,5 and 2,5  $\text{g/cm}^2$  (panel A and B respectively). Data labels: pure P188-  $\bullet$ ; P188+100mg/ml DPOPE-  $\circ$ ; P188+200mg/ml DPOPE-  $\Delta$ .