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Notes

Stability and adsorption to polymeric surfaces of rat ANF in poloxamer 407 solutions

Julianna Juhász, Vincent Lenaerts, Monique Bellemare, Clara Pimienta and Huy Ong

Faculté de Pharmacie, Université de Montréal, C.P. 6128, Succursale A, Montréal, Québec H3C 3J7 (Canada)

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Summary

Rat atrial natriuretic factor (ANF) in 15% poloxamer 407 solution was found to remain stable over 3 months (4 °C; pH 4–7) and during 10 freeze-thaw cycles using HPLC and a radioreceptor assay. ANF adsorption to polymeric surfaces was highest for surfaces with lowest free energy and was prevented by poloxamer 407.

Poloxamer block copolymers $(POE)_a(POP)_b(POE)_a$ have been used as surface modifiers for improving the stability of latex particles (Tadros et al., 1980). The enhanced stability was attributed to the adhesion of the central POP block to the latex particles and the repulsion between POE blocks protruding into the water solution (Kayes et al., 1979). It was also observed that poloxamer-coated polystyrene particles were taken up by liver Kupffer cells to a much lower extent following intravenous injection as compared to uncoated particles (Illum et al., 1987) as a result of inhibition of plasma protein adsorption to the particles. On the other hand, concentrated poloxamer 407 solutions were suggested for transnasal delivery of peptide drugs (Juhász et al., 1989a) owing to the low toxicity of poloxamers (BASF Wyandotte, Toxicity and Irritation Data), the possibility of administering the solution in a fluid state, and the good adhesion of the solution to mucus at body temperature (Juhász et al., 1990). As a model of peptide drug, we have chosen rat atrial natriuretic factor (MW 3060), a 28-amino-acid-residue cardiac hormone (ANF) Ser^1–Tyr^{28}. This peptide is characterized by a 17-amino-acid loop, linked by a disulfide bridge between two cysteines (Cys^7–Cys^{23}). ANF was found to be very soluble in methanol, acetonitrile and in water over the pH range 2–8. It is also heat-stable in phosphate-saline buffer pH 7.4 and sensitive to enzymatic degradation (Trippodo et al., 1982). It has been reported that the diffusion coefficient of ANF in poloxamer 407 solutions was reduced as compared to water. This reduction depended upon the poloxamer concentration and temperature (Juhász et al., 1989b). Thus, concentrated poloxamer 407 solutions appear as

Correspondence: H. Ong, Faculté de Pharmacie, Université de Montréal, C.P. 6128, Succursale A, Montréal, Québec H3C 3J7, Canada.

a promising vehicle for the nasal administration of ANF and other peptidic drugs. However, prior to in vivo experiments, it was necessary to evaluate the stability of ANF in poloxamer 407 solutions in comparison with water. For the same reason, it was also important to evaluate ANF adsorption to various materials used in containers and to determine the effect of poloxamer 407 on that adsorption.

The surface free energy of the polymeric sheets was determined by the method of Kaelble et al. (1974) using the contact angles of the different liquids with known surface tensions. The polymeric sheets, polyethylene (PE), polypropylene (PP) and polyester (PET 9921) were a generous gift from Eastman Chemical Products, Inc. (Kingsport, TN). The technical data are available in the Materials Bulletin: for the low-density polyethylene publication no. MB-89A (September 1983), for the PET copolyester 9921 no. DS-212A (April 1988) and for the polypropylene no. PO-16 (February 1990). Prior to contact angle measurements, all polymers were precleaned in filtered distilled deionized water, rinsed twice with filtered absolute ethanol and finally the films were dried in air. A 10 μl drop of each liquid was applied to the surface of the polymers using a microsyringe. The contact angle (θ) was measured using a small telescope equipped with a cross-hair eyepiece coupled to a goniometer. The value was taken every 30 s for 2 min following application of the droplet and the angle at time zero (t_0) was extrapolated. The measurements were performed for several parts of the same film and averaged ($n = 6$). Using the contact angle at t_0 between a liquid and a solid surface and the surface tension of the different liquids, the adhesion work (W_a) was calculated from:

$$W_a = 2(\alpha_s\alpha_l + \beta_s\beta_l); \quad (1)$$

where $\alpha^2 = st^d$ (dispersive component of interfacial tension), $\beta^2 = st^p$ (polar component of interfacial tension) and subscripts s and l solid-air and liquid-air interfaces. The contact angles and W_a are determined for a series of five liquids the α_l and β_l of which are known. Using Eqn 1 rearranged as:

$$W_a(2\alpha_l)^{-1} = \alpha_s + \beta_s\beta_l\alpha_l^{-1}; \quad (2)$$

a straight line is obtained by plotting $W_a(2\alpha_l)^{-1}$ vs $\beta_l\alpha_l^{-1}$, the slope of which is β_s and the intercept α_s. Thus, by using this method, it was possi-

TABLE 1

Contact angles and surface free energies of polyethylene (PE), polypropylene (PP), polyester (PET 9921) and borosilicate glass where γ_p and γ_d are, respectively, the polar and dispersive components of the surface free energy (γ) (measurements were performed at 25 °C)

	Surface tension (mN m $^{-2}$)	PE	PP	PET 9921	Glass [a]
		Contact angles (°)			
Water	72.8	87.0	96.5	76.8	
Glycerine	64.0	89.8	93.5	71.0	
Formamide	58.3	72.8	78.5	51.8	
Ethylene glycol	48.3	70.0	69.3	56.5	
Tricresyl phosphate	40.9	43.8	47.3	16.5	
		Surface free energies (mJ m^{-2})			
γ_p		17.33	22.65	26.03	49.7
γ_d		5.18	3.74	7.16	21.3
γ		22.51	26.39	33.19	71.0

[a] Coleman et al. (1982).

ble to determine the polar and dispersive components of the surface free energy of the different polymeric surfaces used in the adsorption study of ANF in solution (Table 1). Significant differences (ANOVA of triplicate determinations) were observed between all polymers compared in pairs, both for the surface free energy and the dispersive component.

Stability studies were performed as follows: 0.2 mg rat ANF was dissolved in 0.2 N acetic acid in borosilicate glass tubes. After complete dissolution, the pH was adjusted to 7.3 or 4.5 using 0.05 M Trizma buffer containing 0% or 15% poloxamer 407. The tubes were maintained at 4 °C. Samples were withdrawn at t_0 and every week for 3 months and ANF content was determined by HPLC as follows: after a 10-fold dilution in 0.1% TFA, the ANF solution was injected in a Vydac c18 TP 54 column, 250 × 4.6 mm (Separation Group, Hesperia, CA, U.S.A.), and eluted with 0.1% TFA : acetonitrile (77 : 23) at a flow rate of 1 ml/min. The HPLC eluate was collected in fractions corresponding to the main peaks and analyzed by radioreceptor assay (RRA) according to a method reported previously (Ong et al., 1988). Results from HPLC and RRA techniques were always similar, indicating that the assignment of the major HPLC peak to rat ANF 1–28 was

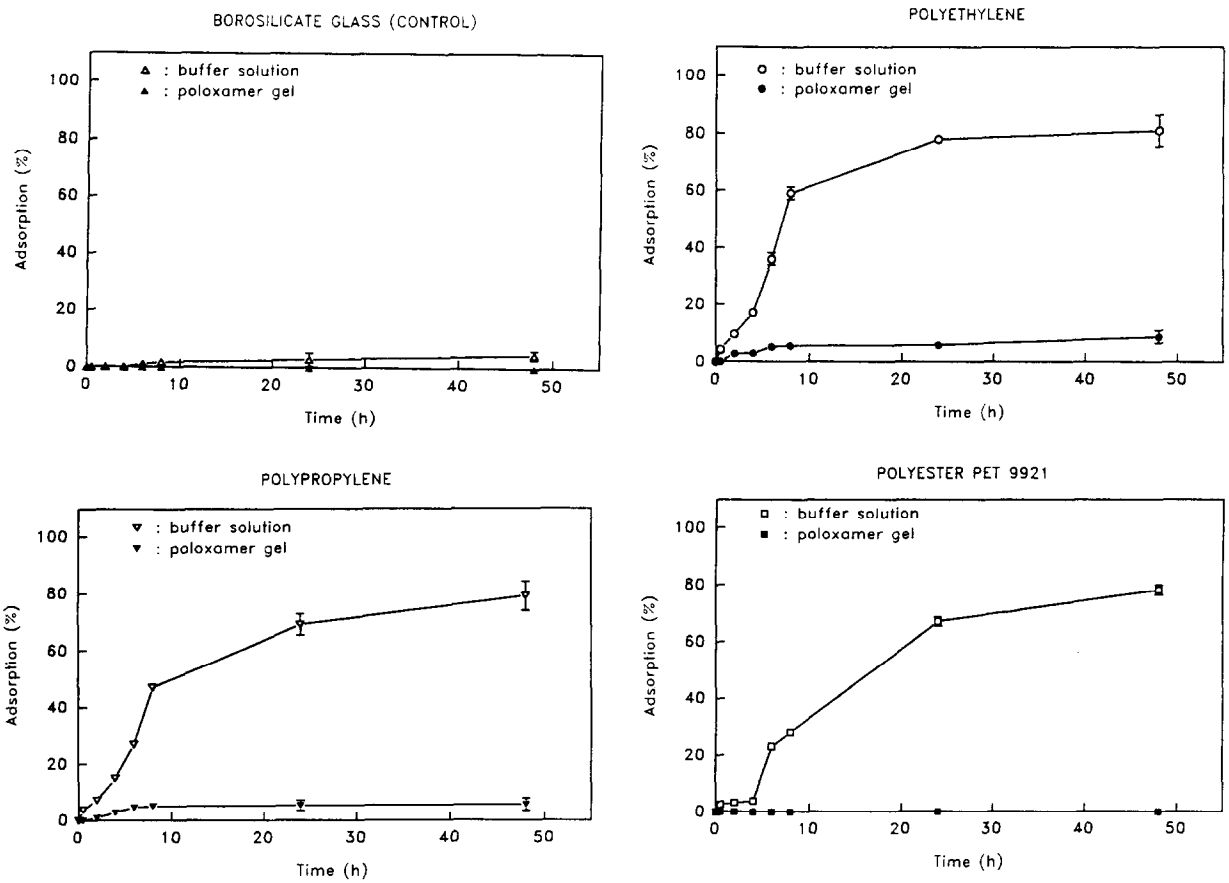

Fig. 1. Percentage of initial (t_0) ANF concentration adsorbed to polyethylene (PE), polypropylene (PP), polyester (PET 9921) surfaces and borosilicate glass vials (control) at 37 °C (pH 7.3) as a function of time. Each point represents the mean ± SE of three separate experiments.

correct. Adsorption of ANF on the polymeric surfaces listed in Table 1 was studied at 37 °C by placing three disks of polymer, each of 0.283 cm^2 surface area into the solutions in borosilicate glass containers. ANF was assayed in samples collected after 30 min, 1, 2, 4, 6, 24 and 48 h. Data of triplicate experiments were treated according to Bonferroni's method of ANOVA analysis.

Studies performed at 4 °C show no reduction of ANF concentration in aqueous solution over a 3 month period. No loss by adsorption to the borosilicate glass walls was observed, irrespective of the pH (4.0 or 7.3) and of the presence or absence of poloxamer 407. Since no loss was observed, hydrolysis can be disregarded. Furthermore, aggregation was definitely excluded by the use of a very specific RRA to the native form of ANF (Ong et al., 1988). At 37 °C, a marked loss of ANF was observed which was more pronounced in the presence of polymeric sheets (Fig. 1). Owing to the absence of aggregates or hydrolysis products, this loss may be attributed to the adsorption of ANF to the container walls and polymeric sheets. Adsorption of ANF was faster on PE than on PP which in turn was slightly more rapid than PP. Using Bonferroni's ANOVA method, significant differences ($P < 0.05$) were observed between PE and PP at 2, 4, 6 and 8 h, between PP and PET at all times except 24 and 48 h, and between PE and PET at all times except 48 h. These polymers also have surface free energies in the order: PE < PP < PET, with major differences in the dispersive component of surface free energy. Thus, our findings are consistent with earlier reports indicating that protein adsorption becomes increasingly rapid on surfaces of decreasing free energy (Van Oss et al., 1981). The addition of poloxamer 407 prevented of adsorption of ANF on all surfaces tested, including the container walls. It is well established that poloxamer can adhere to various surfaces, such as polymethylmethacrylate (Wallis et al., 1990), polystyrene (Lee et al., 1989) or phosphatidylcholine (Jamshaid et al., 1988). Anchoring is achieved by the central POP group (Kayes et al., 1979), whereas the more hydrophilic POE groups extend into the water solution at 37 °C. It is assumed that the surface adsorption of the peptide is hindered as a result of the overcrowding effect of the poloxamer coating.

Finally, the stability of ANF solutions during 10 consecutive freeze-thaw cycles (−40 °C to +4 °C) was studied. Over 96% of the ANF initially present was found to remain intact as determined by HPLC and RRA after 10 consecutive freeze-thaw cycles with and without addition of poloxamer 407 and using either borosilicate glass or polypropylene containers.

In conclusion, poloxamer 407 appears to be an acceptable vehicle for the delivery of ANF and can even reduce ANF loss by preventing adsorption to container walls. In vivo testing of transnasally administered ANF in poloxamer 407 solutions therefore appears feasible and is presently in progress.

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