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Purification of Pluronic F-68 for perfluorochemical emulsification*

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Abstract—A novel technique for purification of Pluronic F-68 has been developed involving passage through a silica-Amberlite resin column. Impurities present in commercial grade Pluronic could be readily removed, as confirmed by analysis of absorption and fluorescence emission bands. Intravenous injection of a 4% (w/v) unpurified Pluronic solution in male rats increased mean liver weight by 14% ($P < 0.05$) after 24 h and increased spleen weight in female rats by 29% ($P < 0.01$) after 7 days. No corresponding tissue weight changes occurred following injection of purified Pluronic solution. Haematocrit and red cell counts were also unchanged throughout. We propose that the use of purified Pluronic for perfluorochemical emulsification may reduce previously reported side-effects.

Pluronic F-68 (Poloxamer 188) is a non-ionic polyoxyethylene-polyoxypropylene block co-polymer surfactant which has a number of industrial and pharmaceutical applications owing to its surface-active properties. It has, for example, been used to emulsify perfluorochemicals (PFCs) for biological uses related to oxygen transport (Davis et al 1985; Lowe 1987). Commercial grade Pluronic is known to contain variable amounts of low molecular weight impurities, including aldehydes and both formic and acetic acids (Riess & Le Blanc 1988; Bentley et al 1988). In addition, peroxide derivatives of Pluronic, formed during steam sterilization of the commercial PFC emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan), have been implicated in adverse physiological effects of this preparation in-vivo (McCoy et al 1984). Moreover, it has been claimed that Pluronic can induce hepatomegaly in rats (Goodman et al 1984) and may thus contribute to the increase in lymphoid tissue weights which can follow injection of emulsified PFCs (Sharma et al 1987; Lowe 1987, 1988).

We have recently reported the development and physicochemical properties of novel PFC emulsions based on perfluorodecalin (FDC), emulsified with Pluronic F-68 and further stabilized against Ostwald ripening by the addition of small quantities of polycyclic, perfluorinated, higher boiling point oil additives (Davis et al 1986; Sharma et al 1987). In order to minimize possible adverse physiological reactions to such emulsions that may be caused by Pluronic impurities, we now report a novel technique for purification of this surfactant together with its effects on tissue weights and haematological variables in rats. A preliminary report of some of these results has already been published (Bentley et al 1988).

Materials and methods

Purification procedure. A 4% (w/v) solution of commercial grade Pluronic F-68 (Atochem/ICI Petrochemicals, Runcorn) in distilled water was purified by passage through a 30 cm × 2 cm column, the bottom half of which was packed with Amberlite MB-1 16–50 mesh (Sigma, Poole) and the upper half packed with silica 60–120 mesh (BDH, Atherstone). In addition, some samples were purified by passage through silica alone. The column packing (ca 200 mL) was routinely discarded after purification of 1L Pluronic solution. Fehling's test for reducing agents was used to monitor the removal of reducing impurities.

Measurement of absorption/fluorescence emission. UV absorption (200–400 nm) of Pluronic solutions was measured using a Kontron Uvikon 860 spectrometer. Fluorescence emission (280 nm excitation; 5 nm slit width; emission wavelength 280–400 nm) was measured using a Perkin-Elmer 3000 spectrofluorimeter.

Care of animals and experimental procedures. Male and female Wistar rats (150–330 g, n = 96) were maintained in the laboratory animal house under controlled conditions (13 h light, 11 h dark; temperature 24 ± 1°C) and had free access to standard food concentrate diet (Rat and Mouse Breeding Diet; Haygates, Birmingham). Before experimentation, they were allocated randomly into one of four experimental groups of 24 animals as follows: Group I: saline controls; Group II: unpurified Pluronic F-68; Group III: Pluronic F-68 purified by silica alone; Group IV: Pluronic F-68 purified by both silica and amberlite.

All animals were initially anaesthetized with ether and then injected intravenously (i.v.) via a tail vein with 10 mL kg^{-1} body weight of a 4% (w/v) solution of either unpurified or purified Pluronic or saline. Control animals (Group I) were injected i.v. with an identical dose of sterile saline. Pluronic solutions were made isotonic with NaCl (0·9% w/v) and sterilized by filtration through a 0·22 μm Millipore filter before injection.

Animals were killed either at 24 h, 72 h or 7 days after injection by stunning followed by cervical dislocation; they were then exsanguinated by cardiac puncture. The weights of liver, spleen, heart and kidneys were measured following dissection of individual tissues.

Treatment of blood samples and haematological procedures. Blood samples were placed into Eppendorf tubes (Sarstedt, Leicester) and stored on ice. The haematocrit was measured using an automatic Hawksley 1500 microhaematocrit centrifuge while red blood cell counts were performed manually using a haemacytometer.

Statistical analyses. Statistical analyses were performed according to the methods of Snedecor & Cochran (1980). Means and standard errors (s.e.m.) have been used throughout unless indicated otherwise and statistical significance between mean values was assessed using a conventional two-tailed Student's *t*-test. A probability of $P < 0.05$ was considered significant.

Results

Assessment of Pluronic purity. The unpurified Pluronic solution showed an absorption band with a peak at 278–280 nm (typical absorbance 0·3 A.U.; Fig. 1) and strong fluorescence emission bands at ca 315 and 330 nm (Fig. 2). It also gave a positive reaction to Fehling's test for reducing agents. After a single passage through the silica-amberlite column, the ultraviolet absorption band was no longer detectable (< 0·005 A.U.; Fig. 1) while the fluorescence emission was reduced by a factor of 5 (Fig. 2). The purified Pluronic solution no longer gave a positive Fehling's test.

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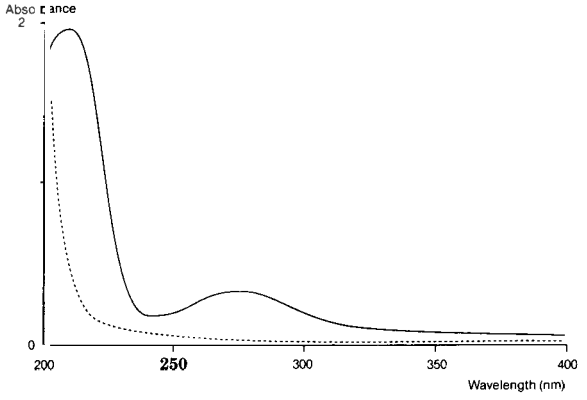

FIG. 1. Ultraviolet spectra of unpurified (——) and purified (– – –) Pluronic F-68 solutions (4% w/v in distilled water).

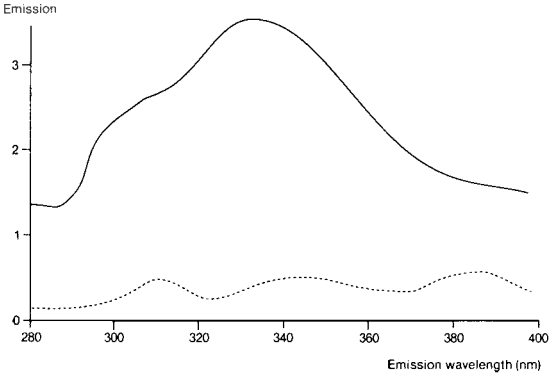

FIG. 2. Fluorescent emission of unpurified (——) and purified (– – –) Pluronic F-68 solutions (4% w/v in distilled water).

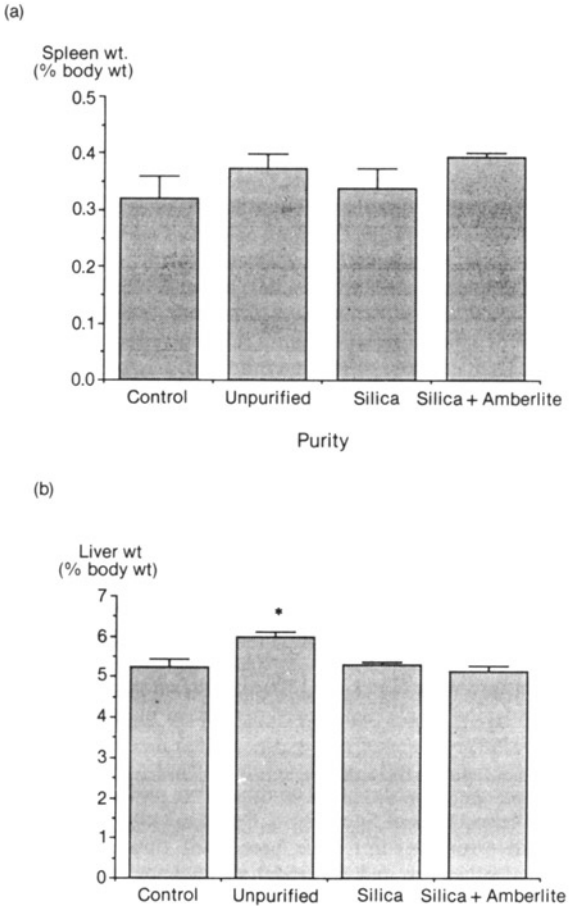

FIG. 3. Mean weights of (a) spleen and (b) liver in male rats 24 h after injection of Pluronic solution. Vertical bars represent s.e.m. (n = 24). $*P < 0.05$.

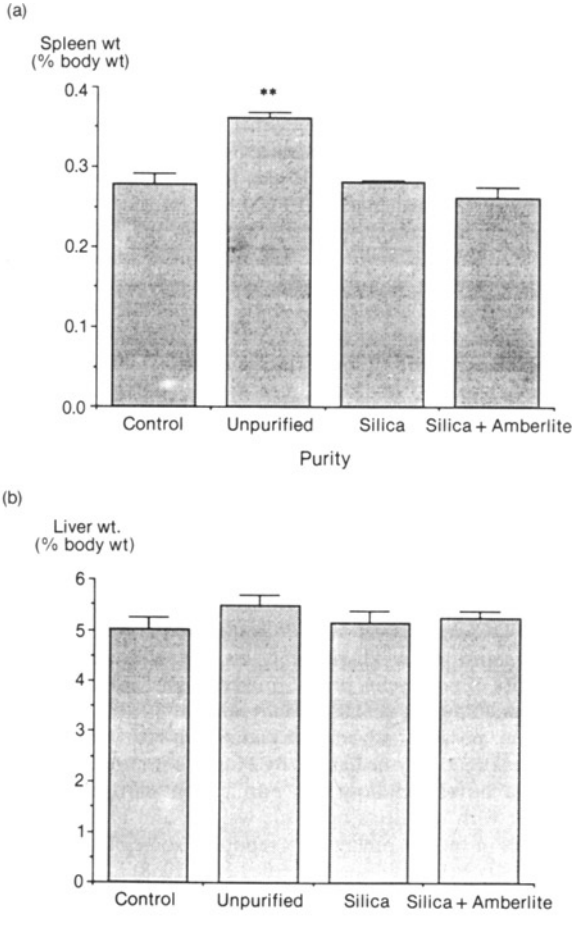

FIG. 4. Mean weights of (a) spleen and (b) liver in female rats 7 days after injection of Pluronic solution. Vertical bars represent s.e.m. (n = 24). $**P < 0.01$.

Changes in tissue weights and haematological parameters. Mean liver weight was increased by a maximum of 14% at 24 h after injection of unpurified Pluronic in male rats ($P < 0.05$), but was similar to control after injection of Pluronic purified either by silica or silica-amberlite (Fig. 3). Mean spleen weights after 24 h were not significantly changed in response to any of the treatments.

At 7 days, spleen weight in female rats was significantly ($P < 0.01$) increased following injection of unpurified Pluronic (Fig. 4) but was similar to control in all other cases (Fig. 4). Liver weights at 7 days were unchanged in response to any of the treatments.

Tissue weights in both male and female rats were unchanged at 48 h after injection of Pluronic, irrespective of purity.

Moreover, no significant changes were noted in the weights of heart, lungs or kidneys throughout the experiments. Both haematocrit and red cell counts were also unchanged throughout.

Discussion

These results show that commercial grade Pluronic contains impurities which can be readily removed by the chromatographic purification method described. While the nature of the impurities is uncertain, the absorption and fluorescence emission spectra suggest the presence of an aldehyde (supported by positive Fehling's test) and a fluorescent chromophore. This was in accord with previous reports that commercial grade Pluronic contains various impurities including traces of acetaldehyde, propionaldehyde, formic and acetic acids together with an antioxidant, *O,O'*-ditert-butylcresol (Riess & Le Blanc 1988). We have, however, observed that all samples of commercial Pluronics examined in our laboratory show absorption and emission data comparable to those presented here. Others have also noted considerable variation in the composition of Pluronic samples obtained from different manufacturers (Follana et al 1988).

unpurified Pluronic was in accordance with previous suggestions (Goodman et al 1984). However, the absence of a corresponding hepatomegaly following injection of purified Pluronic suggests that impurities were responsible. The observed differences in tissue responses between male and female animals were in broad agreement with previous work showing that induction of hepatic cytochromes P-450 produced by injection of emulsified PFCs (containing Pluronic F-68) also differed between the sexes (Armstrong & Lowe 1988). The increase in tissue weights may be due to alterations in cell membrane function and nutrient uptake as has been seen in related cell culture studies (Mizrahi 1975; King et al 1988). If this is the case, the present results suggest that impurities in commercial grade Pluronic are the active principle(s) involved but this needs confirmation.

We propose that the use of purified Pluronic as surfactant for PFC emulsification and other intravenous uses may reduce side-effects. Further improvements in purity of commercial grade samples are an essential pre-requisite to such pharmaceutical uses of this compound.

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References

Armstrong, F. H., Lowe, K. C. (1988) Changes in rat liver cytochromes P-450 in response to injection of emulsified perfluorochemicals and their components. J. Pharm. Pharmacol. 40 (Suppl.): 141P

Bentley, P. K., Lowe, K. C., Johnson, O. L., Sharma, S. K., Washington, C., Davis, S. S. (1988) Purification of Pluronic F-68 and its effects on lymphoid tissues in rats. Ibid. 40 (Suppl.): 143P

Davis, S. S., Hadgraft, J., Palin, K. J. (1985) Medical and pharmaceutical applications of emulsions, in: Becher, P. (ed) Encyclopedia of Emulsion Technology, Vol. 2. Marcel Dekker, New York, pp 159–238

Davis, S. S., Lowe, K. C., Sharma, S. K. (1986) Novel compositions of emulsified perfluorocarbons for biological applications. Br. J. Pharmacol. 89: 665P

Follana, R., Le Blanc, M., Mahe, A. M., Pace, S., Riess, J. G., Valla, A. (1988) The controversial question of the biocompatibility of Pluronic F-68. An experimental study. Proc. Soc. Exptl. Biol., Lancaster, p. 36 (Abstract 167)

Goodman, R. L., Moore, R. E., Davis, M. E., Stokes, D., Yuhas, J. M. (1984) Perfluorocarbon emulsions in cancer therapy: preliminary observations on presently available formulations. Int. J. Radiat. Oncol. Biol. Phys. 10: 1421–1424

King, A. T., Lowe, K. C., Mulligan, B. J. (1988) Microbial cell responses to a non-ionic surfactant. Biotechnol. Lett. 10: 177–180

Lowe, K. C. (1987) Perfluorocarbons as oxygen-transport fluids. Comp. Biochem. Physiol. 87: 825–838

Lowe, K. C. (1988) Emulsified perfluorochemicals for oxygen transport to tissues: effects on lymphoid system and immunological competence in: Mochizuki, M., Honig, C. R., Koyama, T., Goldstick, T. K., Bruley, D. F. (eds), Vol X. Plenum, New York, pp 655–663

McCoy, L. E., Becker, C. A., Goodin, T. H., Barnhart, M. I. (1984) Endothelial response to perfluorochemical perfusion. Scan. Electron Micros. 16: 311–319

Mizrahi, A. (1975) Pluronic polyols in human lymphocyte cell line cultures. J. Clin. Microbiol. 2: 11–13

Riess, J. G., Le Blanc, M. (1988) Preparation of perfluorochemicals emulsions for biomedical use: principles, materials and methods, in: Lowe, K. C. (ed) Blood Substitutes: Preparation, Physiology and Medical Applications. Ellis Horwood, Chichester, pp 94–129

Sharma, S. K., Bollands, A. D., Davis, S. S., Lowe, K. C. (1987) Emulsified perfluorochemicals as physiological oxygen-transport fluids: assessment of a novel formulation in: Silver, I. A., Silver, A. (eds) Oxygen Transport to Tissue Vol. IX. Plenum, London, pp 97–108

Snedecor, G. W., Cochran, W. G. (1980) Statistical Methods, 7th Ed. Iowa State College Press, Ames