Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1381–1384

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Adsorbed Surfactants for Affinity Chromatography: End-Group Modification of Ethylene Glycol Polymers

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Received 12 April 2002; accepted 13 December 2002

**Abstract**—The hydroxyl end-groups of Pluronic®F108 {a tri-block copolymer surfactant of poly(ethylene glycol) and poly (propylene glycol) [PEG-PPG-PEG]} were converted into primary amine and quaternary ammonium equivalents for use in a new approach to affinity chromatography. The preparation of sulphonic acid end-groups was also attempted.


The non-covalent adsorption of surfactants onto the surface of lipophilic membrane filters has been reported as a means to reduce fouling by organic compounds.[1–6] Modification of the end-groups of the surfactants, such as by sulphonation, may render them even more suited as anti-foulants. Ligands can also be attached to the end-groups of surfactants. If the modified surfactant is adsorbed onto particle surfaces, the coated particles could be used for affinity separation.[7]

In this paper, the conversion of the end-groups of Pluronic®F108 (BASF; 14 600 Da) into amines and cations, and the attempted conversion into anions is reported. Furthermore, by attaching a ligand to the amine-terminated Pluronic®F108 and immobilising the modified surfactant by adsorption onto polysulphone capillary membranes, serum albumin was extracted from serum by affinity chromatography.

The synthetic work was initiated with model compound studies. Di(ethylene glycol) methyl ether (DEG) simulates the first two ethylene oxide nodes of PluronicF108 with the hydroxyl terminus. Hydroxyl-terminated poly(ethylene glycol) [PEG] with a molecular mass of 600 Da was used as a transition model compound between DEG and Pluronic®F108. The use of PEG allowed for the development of the reactions on a polymer that is relatively easily characterisable.

Yields reported for Pluronic derivatives are yields by mass. No effort was made to remove the polymers with unreacted or by-product end-groups. NMR investigation at each intermediate reaction step indicated primarily the expected compounds, with no significant amount of unreacted or wrongly reacted termini.[8] Due to the difficulties involved in separating the correctly-reacted polymers from those that did not react correctly, the presence of the undesired polymers will be characterised and quantified in a later paper. The potential use of the modified Pluronic®F108 for affinity chromatography was ascertained first and is reported herein.

Amine-terminated Pluronic®F108 was synthesised as follows (Scheme 1): the hydroxyl group was activated by conversion to a tosylate,[9] that was in turn displaced with an azide,[10] followed by reduction to the desired primary amine.[11–13]

The hydroxyl group(s) of DEG and PEG were converted to the tosyl equivalents by sulphonylation with 50% excess *p*-toluenesulphonyl chloride in pyridine at 0 °C for 12 h (84% yield for DEG). These reaction mixtures and the products that were obtained were never heated beyond 20 °C since these tosylates disintegrate above that temperature. Satisfactory conversion was obtained on treating a solution of 14.6

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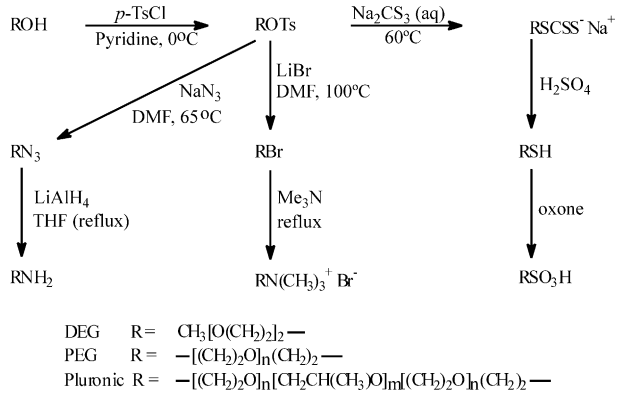
doi:10.1016/S0960-894X(03)00098-2




**Scheme 1.**

g Pluronic®F108 in pyridine (50 mL) with 19.06 g of tosyl chloride for 7 days. The reaction mixture was poured onto ice and extracted with chloroform. The chloroform extracts were washed with 6 M HCl, then with water, and dried over $K_2CO_3/Na_2SO_4$, yielding 13.27 g pluronic tosylate (89% yield, 89% conversion). Conversion was determined by the addition of trichloroacetyl isocyanate to an NMR-sample and integrating the terminal methylene $^1$H-signal of the resultant carbamate thus derived from unconverted Pluronic®F108, relative to the equivalent tosylate signal.[8]

DEG tosylate and PEG tosylate were converted to the azides with a 20-fold excess of $NaN_3$ (80% yield for DEG tosylate). Sodium azide (7.95 g) was added to a solution of pluronic tosylate (1.52 g) in dry DMF (25 mL) under Ar atmosphere at 65 °C and allowed to react for 18 h. The reaction mixture was then poured onto ice water, extracted with chloroform, the extract washed with 2.5 M HCl and distilled water, sequentially, and dried over $MgSO_4$ to yield 1.31 g pluronic azide (87%).

DEG azide and PEG azide were reduced to the amine using 8 mol equivalents of $LiAlH_4$ per azide in ether, at reflux temperature for 6 h. For pluronic azide, 0.50 g of $LiAlH_4$ was added to a solution of pluronic azide (0.40 g) in THF (15 mL). The reaction mixture was refluxed for 6 h, then slowly quenched with water. The mixture was made basic (pH 10) with 25 mL of 5 M sodium hydroxide and then continuously extracted with chloroform for 12 h, furnishing 0.30 g (76%) amino-pluronic.

Two synthetic routes were considered to convert the hydroxyl end-groups of Pluronic®F108 into trimethyl-ammonium end-groups: alkylation of amino-pluronic by exhaustive methylation; or use of trimethylamine as a nucleophile to substitute the tosylate of pluronic tosylate. The one-step conversion of the tosylate to quaternary ammonium equivalents using trimethyl-amine[14,15] seemed more expedient.

The synthesis of DEG-trimethylammonium salt was first attempted using DEG tosylate as precursor. A mixture of DEG tosylate, trimethylammonium chloride and sodium hydrogencarbonate in dry methanol was refluxed. The reflux condenser was maintained at −5 °C to minimise the loss of trimethylamine (bp: 3–4 °C). No reaction took place.

As an alternative to the direct displacement of tosylate with trimethylamine, the following was considered more appropriate: conversion of the tosylate to the alkyl bromide[16,17] and then displacement of the halide, nucleophilically, with trimethylamine (Scheme 1).[14,15]

DEG tosylate was converted to DEG bromide with 2 mol equivalents of lithium bromide in dimethyl formamide for 3 h under an Ar atmosphere. The preparation of PEG bromide from the tosylated equivalent was similar to that of DEG bromide. Lithium bromide (2.64 g) was dissolved in a solution of pluronic tosylate (2.81 g) in dry DMF (20 mL) and maintained at 100 °C under Ar for 3 h. 3 M HCl (50 mL) was added to the mixture, which was then extracted with chloroform, to yield (2.41 g) pluronic bromide (87%).

DEG bromide was converted to DEG-trimethyl-ammonium bromide with 3.0 mol equivalents of trimethylamine. PEG- and pluronic-trimethyl-ammonium salts were prepared likewise, using the same mass to mass amount of trimethylamine. Pluronic bromide (1.5 g) was dissolved in 10 mL of 40% (m/m) aq trimethylamine and refluxed for 4 h. The reaction mixture was evaporated in vacuo, yielding 1.04 g pluronic-trimethylammonium bromide (69%).

The strategy for the transformation of Pluronic®F108 to pluronic sulphonic acid was the conversion of pluronic tosylate to pluronic-thiol,[18,19] followed by oxidation with oxone® to the corresponding sulphonic acid.[20,21]

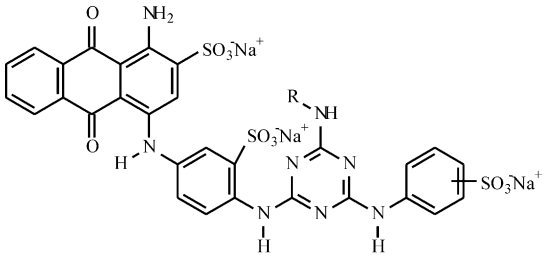
Focusing on the model compounds first, DEG tosylate and PEG tosylate were converted to the corresponding thiols with 2 mol equivalents of aqueous $Na_2CS_3$ (33%).[18] per tosylate at 65 °C for 5 h. The thiolation reaction is postulated to proceed via the sodium mono-alkyl trithiocarbonate which, upon acidification, decomposes spontaneously to liberate a thiol and carbon disulphide.[22] Besides the formation of the desired DEG-thiol, significant amounts of diDEG disulphide and diDEG sulphide also formed. Oxidation of diDEG disulphide should readily oxidise to the desired sulphonic acid,[23,24] however, the diDEG sulphide will most likely yield the sulphone. A rationale for the formation of the sulphide is nucleophilic attack of the tosylate by the DEG sulphide ion instead of trithiocarbonate. The sulphide is likely to originate from the dissociation of the sodium alkyltrithiocarbonate into the alkyl sulphide and $CS_2$ in a reversible process. With this rationale in mind the reaction of DEG tosylate was repeated in the presence of $CS_2$ (75% yield for DEG sulphide). This modified procedure circumvented the formation of the sulphide. The preparation of pluronic thiol follows: pluronic tosylate (2.00 g) was added to a 33% solution of sodium trithiocarbonate and 41% $CS_2$ in water (9 mL) under Ar. The reaction mixture was stirred and gradually heated to 60 °C. After 5 h the mixture was

acidified (concd $H_2SO_4$) to between pH 1 and 2, extracted with chloroform and dried ($MgSO_4$), yielding 1.75 g pluronic-thiol (89%).

DEG- and PEG-thiols were successfully oxidised to the corresponding sulphonic acids with a stoichiometric amount (oxone–mercaptan, 3:1) of oxone® ($2KHSO_5KHSO_4K_2SO_4$) for 1 h at ambient temperature. The same amount of oxone® to substrate mass was used for pluronic-thiol as was used for PEG-thiol (i.e., 22-fold excess). Characterisation of the product by $^1H$ and $^{13}C$ NMR spectroscopy indicate very little, if any, of the sulphonic acid derivative. The major derivative was Pluronic®F108, which had not been present before oxidation. Oxone® had thus cleaved off the sulphur atom.

The preparation of DEG-sulphonic acid was carried out as follows: DEG-thiol (0.51 g) was dissolved in a 25% (m/v) aqueous solution of oxone® (14 mL) and stirred for 1 h. Concd HCl (14 mL) was added and the mixture stirred for a further 15 min. The reaction mixture was then extracted with ether and the ethereal extract dried ($MgSO_4$), and the organic solvents removed by evaporation, yielding 0.23 g DEG-sulphonic acid (33%).

Isolation of albumin from sheep serum was considered to investigate the suitability of aminated Pluronic®F108 as a means to immobilise ligands for affinity chromatography. Cibacron Blue 3GA is used as a ligand for the isolation of albumin.[25–27] 2-Methoxyethylamine was successfully used as a model amine for developing the coupling reaction of Cibacron Blue 3GA to an amino end-group (Scheme 2). Thereafter an aqueous solution of 120 mg Cibacron Blue 3GA and 318 mg $Na_2CO_3$ was added to an aqueous solution of 570 mg amino-pluronic. The reaction mixture was maintained at 45 °C for 3 days.[28] Cibacron Blue-amino-pluronic (CBAP) and unreacted dye were separated by eluting through a Sephadex G-50-40 column. The fractions were analysed by UV spectroscopy at 610 nm (absorption maximum of Cibacron Blue). Product fractions were freeze dried, and investigated by $^{13}C$ and $^1H$ NMR spectroscopy. Even though the efficiency of conversion of amino-pluronic to CBAP could not be ascertained by NMR spectroscopy, because of the coincidence of the terminal $CH_2$-signal of amino-pluronic with the signal of the deuterated solvent, the chemical shift of the terminal $CH_2$-group of the PEG-block of CBAP was observed at




**Scheme 2.**

δ 60.7, indicating coupling of the PEG-block to Cibacron Blue 3GA. The proton NMR spectrum did not prove of any value in indicating the process of coupling because of the signals of the bulk polymer at the critical chemical shifts.

The utility of Cibacron blue 3GA coupled amino-pluronic (CBAP) adsorbed onto polysulphone fibers as a non-covalent ligand for the affinity separation of albumin was then investigated. A single polysulphone capillary membrane (PSf matrix) was first used to test the isolation of commercial bovine serum albumin. Thereafter, a multi-fibre system was used to enable the characterisation and quantification of the isolated albumin.[29] The PSf membranes in the multi-fibre module were coated in the following manner. A 0.1% aqueous solution of CBAP was circulated through the multi-fibre PSf matrix for 25°h at room temperature, using a peristaltic pump. The fibres were then rinsed with distilled-deionised water until the fractions emerging from the fibre showed no absorbency at 610 nm.

Two buffer solutions were used to process the protein-loaded, uncoated (control) and coated PSf matrices (CBAP): Buffer A (washing buffer) 0.05 M Tris/HCl, pH 8.0, containing 0.05 M NaCl, and Buffer B (elution buffer) 0.05 M Tris/HCl, pH 8.0, containing 0.2 M NaSCN.

Sheep serum (5 mL) containing 5 mg/mL of protein was introduced into the fibres. buffer A was passed through the system for 6 h under constant UV detection at 280 nm. Sheep serum proteins that were retained by the system were eluted thereafter by passing buffer B through the fibres for 4 h. Albumin retention was monitored qualitatively by the Kaiser ninhydrin test[30,31] and quantitatively by the Pierce$^M$ Protein Assay.[32,33] Fractions which contained sheep serum albumin were further characterised by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis[34–36] and Electrospray Mass Spectrometry. A 32 mL fraction containing 35 μg/mL of highly purified sheep serum albumin, as determined by ESMS, was thus obtained.

The potential use of non-covalent anchoring of ligands for affinity separation has been proved. Improved procedures to prepare the ligand carrier, such as a triethylamine-[37–39] or alkoxide-based[40] preparation of pluronic-tosylate instead of pyridine, are currently being investigated. The use of propane sultone[41,42] as a means of sulphonylating the termini of Pluronic®F108 is also under investigation. The economic viability of this procedure for the isolation of albumin from abattoir effluent is now also being studied. Other applications of affinity separation by non-covalent adsorption onto membranes are also being explored.

## Acknowledgements

The Water Research Commission and the National Research Foundation of South Africa and the University of Stellenbosch are thanked for funding this research.

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