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Removal of heavy metals from solution using biocompatible polymers

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ABSTRACT

Well characterised, hydrophobic, poly(vinylidene fluoride) and poly(sulfone) membranes, a physisorbed amphiphillic surfactant as an affinity linker with covalently attached bio-specific ligands to demonstrate heavy metal ion binding and re-use is reported. Central to this technology are the easy to manufacture membranes and a biocompatible Pluronic^{TM} surfactant that serves multiple functions, where it acts not only as an affinity linker, preventing non-specific protein adsorption to the membrane surface but also non-covalently hydrophilises the membrane matrix. This work describes the fabrication and characterisation of non-porous, hydrophobic membranes for the attachment of a robust, bio-compatible ligand, in order to specifically chelate divalent cations (Cd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}) and subsequently, histidine tagged proteins. These affinity tags are frequently used by bioengineers and biochemists to produce recombinant enzymes, peptides, hormones and antibodies. Successful coupling of ligands to Pluronic was achieved followed by quantification of the ligand binding sites on the surface. This technology is scalable (due to the multi-variant membrane module design), able to resist non-specific protein adsorption and can be regenerated and re-used (up to five times) with a simple anionic surfactant solution.

Keywords: Heavy metals; Affinity membranes; Chelating ligand; Pluronic; Regeneration

1. Introduction

Heavy metal pollution is becoming an increasing environmental concern exacerbated by the unprecedented increase in urbanisation and industrialisation. Toxic pollutants such as cadmium, lead, mercury, nickel, zinc and copper enter aquatic systems naturally via weathering and also through mining, air-pollution and processing. Cadmium in particular derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans [1]. Chronic dust or fume exposure of cadmium is bio-persistent and, once absorbed by an organism, remains resident for many years (over decades for humans) resulting in irreversible lung damage, emphysema, nephrotoxicity and carcinogenisis [2]. Copper and nickel are commonly leached from pipes and industrial materials and are implicated in liver and kidney damage. Removal strategies from both treated water and waste water streams are therefore of both environmental and industrial significance with human and animal health as a driving force [3].

Membrane technology is a widely accepted and a relatively low-cost (eg. ultrafiltration and microfiltration)

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large-scale technology for waste treatment and potable water production [4]. Metal ion removal however, is not so trivial, necessitating higher process costs associated with nanofiltration and reverse osmosis [5]. Membrane based applications in water engineering [6] are often complicated by the inherent tendency of hydrophobic surfaces to foul via non-specific adsorption of molecules from solution [5]. Many reports focus on surface modification techniques such as the introduction of hydrophilic groups via chemical reactions, plasma treatment, adsorption and *in situ* polymerisation. Adsorption arguably represents the most economical and shortest route to membrane surface modification. Additionally, there are increasing reports of the use of surfactant formulations for the multi-functional modification of surfaces [7, 8].

A promising surfactant based system uses poly(ethylene glycol) (PEG) or Pluronic block copolymers, which are amphiphilic surfactants for reducing membrane fouling and to introduce functional groups on the membrane surface for ligand binding. The practical and user-friendly advantage of the Pluronic family of surfactants is that they are both FDA and EPA approved, making them compatible with environmental and medical applications. Recent studies [9] have also shown that such surfactant modified membranes can also be effectively regenerated and re-used with SDS based detergents which further increases the process lifespan of the attendant membranes.

This work reviews the fabrication and solid state characterisation of non-porous, hydrophobic poly(sulfone) (PSU) and poly(vinyldiene) (PVDF) membranes for the attachment of a novel EDTA type metal chelating ligand Pluronic-*N*,*N*-dicarboxymethyl-3,6-diazaoctanedioate (Pluronic-DMDDO). The DMDDO ligand was coupled to Pluronic F108 in order to specifically remove divalent cations (Cd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}) from solution. This report focuses on the ability of the functionalised polymer to bind and remove said heavy metal ions from solution and to quantify the ligand binding capacity of the chelated membranes for the attendant metal ions. Concomitant protein affinity binding and membrane re-use strategies are also described.

2. Experimental details

2.1. Reagents and chemicals

Proteins such as bovine serum albumin (BSA), lysozyme and enzyme solutions were obtained from (Roche, Penzberg Germany) and were reconstituted as 0.25 mg mL^{-1} solutions in 0.1 M phosphate buffer, pH 7.4. SDS (Merck, Darmstadt, Germany) was used as a desorption agent. Pluronic® F108 (14 600 g mol^{-1}) was obtained from BASF corporation (NJ, USA). Unless otherwise stated, all other reagents were purchased from Merck (Darmstadt, Germany).

2.2. Polymer fabrication

Planar nonporous membranes were cast from solutions containing 27% [m/m] (PSU and PVDF) respectively and 73% (m/m) *N*,*N*-Dimethylacetamide (DMAc). PSU was dissolved in DMAc by rotating the solution container for more than 48 h at room temperature to obtain a homogeneous solution. PVDF required sonication in an ultrasonic water bath for 30 min and further heat treatment at 55°C for 48 h to dissolve. The solutions were then degassed before being used to cast the 200 µm planar membranes. Nonporous hollow fibre (HF) and hollow fine fibre (HFF) membranes and externally unskinned ultrafiltration (UF) membranes were produced by the phase inversion technique [7] using a dry-wet spinning process [6]. The dimensions of the capillary membranes were measured using an optical light microscope with a vernier scale and these measurements were verified with scanning electron microscopy (SEM). In all investigations 1 cm^2 membranes were used in static adsorption experiments.

2.3. Spectrophotometric analysis

A biphasic colorimetric assay for Pluronic quantification was performed as described by Govender et al. [7]. Protein concentration was measured using a bicinchoninic acid protein assay kit from Pierce™, (Rockford, USA), with bovine serum albumin as a protein standard. Pantothenate kinase (PK) activity was based on the measurement of the decrease in the absorbance at 340 nm, where an extinction coefficient of 6220 M^{-1} cm^{-1} was used for the calculation of NADH concentrations. Reactions were monitored at 25°C in a CARY 110 UV-Vis spectrophotometer [10].

2.4. Membrane fouling and surface modification

Pluronic modified membranes were stripped of adsorbed Pluronic using an aqueous SDS solution. These membranes were initially statically equilibrated in 10 mL of the SDS solution for 1 h and then transferred to a Stoval Belly Dancer™ shaker for 20 h of vigorous shaking. After incubation in SDS, the membranes were washed in a solution of 100 mL dH_20 for 12 h and finally rinsed three times in dH_2O. Pluronic was separated from SDS after solvent evaporation, followed by the addition of 10 mL $CHCl_3$. SDS is insoluble in $CHCl_3$ and can be separated from Pluronic by filtration through Whatman filter paper [9].

Ligand modification was initiated using model halogenated (Br and I) derivatives with PEG and Pluronic surfactants. Thereafter polymeric membranes were functionalised via physisorption with 5 mg mL^{-1} derivatised Pluronic for 8 h at 20°C. The modified polymers were then washed in dH_2O, rinsed and transferred to scintillation vials containing ligate solutions. PVDF~F108-DMDDO membranes were chelated with 0.05 M solutions of the following divalent cations (Ni, Zn, Cu and Cd). The synthesis of the DMDDO ligand is a simple 3 step synthesis scheme with a 68% yield [12].

2.5. Solid state analysis

Ligand binding was studied using proton induced x-ray emission (PIXE) which was also effective in quantifying the metal chelate capacity of the surfactant modified membranes. Measurements were performed at the nuclear microprobe facility at the Materials Research Group, iThemba LABS, South Africa [10]. For atomic force spectroscopy analysis, a topographical map was obtained by scanning a silicon nitride tip attached to a cantilever over the air-dried membrane surface, while maintaining a constant force between the tip and the sample. The deflection of the tip and cantilever was measured optically by reflecting a laser light beam off the back-face of the cantilever (AFM, TMX 2000 Explorer, Topometrix, Santa Barbara, CA, USA).

2.6. Module design

The schematic cross sectional area of an experimental plate and frame module is illustrated in Fig. 1. The module designed for housing the affinity membranes was fabricated from clear Perspex with a plate and frame geometry consisting of multiple cassettes. This is a simple geometry that was designed as a relatively inexpensive, reusable unit for contacting with a comparatively pure or clean feed solution. The unit can be easily sealed and opened with the easy to access stainless steel bolts. The pseudo-circular flat sheet membranes, with separator plates were also designed as cassettes. The cassettes allow membranes to be inserted and removed individually without the necessity of dismantling the module. However this would limit the module to operating pressures used during operation, but are nevertheless more than adequate for affinity separation and microfiltration or ultrafiltration processes. The flow rate of the recirculating liquid can also be regulated using flow control valves.

3. Results and discussion

3.1. Membrane characterisation

The SEM images in Figs. 2–3, depict fairly heterogeneous surfaces, with thin (nano scale) abrasions on the surface. This was a consequence of the fabrication process of the candidate planar membranes, where a stainless steel bar and a 2 mm spacer blade were used to spread the polymer films to form membranes. After coating with 5 mg mL^{-1} Pluronic solutions, more homogenous and smoother surfaces were noticed (Fig. 2B). The adsorbed Pluronic 'masking' of the inherent markings on the native membrane suggest that Pluronic adsorption was uniform over the 1 cm^2 membrane surface. AFM analysis was performed under hydrated conditions, with the less destructive intermittent contact mode or 'tapping mode'. This involved performing vertical oscillations with the cantilever across the membrane surface using a constant force. The atomic force micrographs in Fig. 2

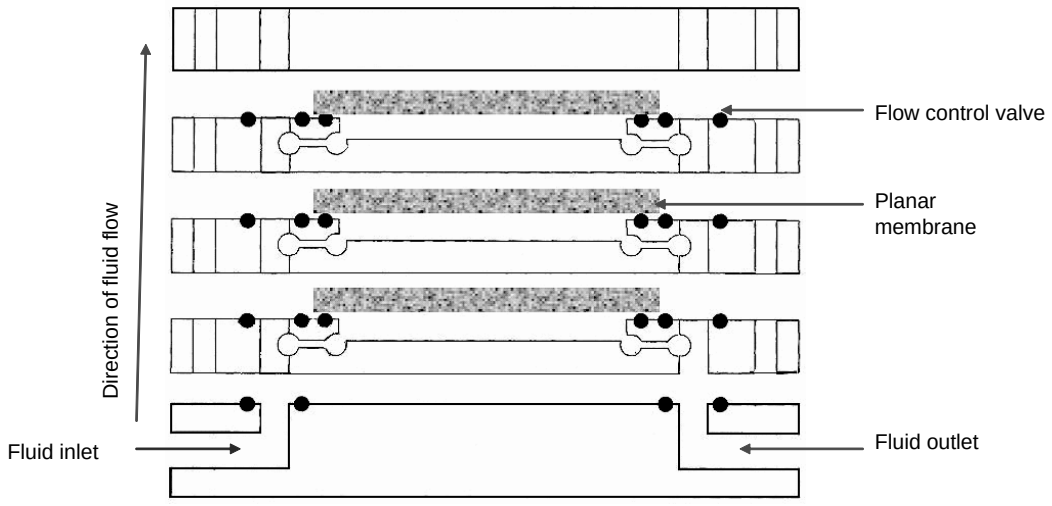

Fig. 1. Schematic illustration of the cross section of the modular membrane unit.

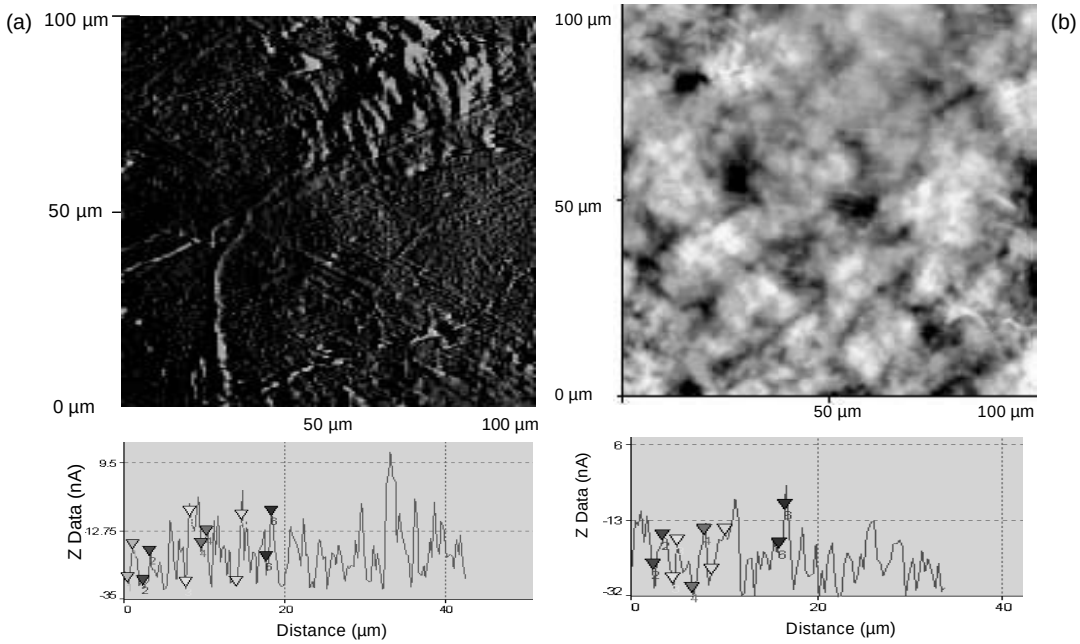

Fig. 2. Atomic force topographical micrographs showing (A) native planar PVDF membrane and (B) pluronic modified surface. The inset beneath each picture represent force distance curves, which indicate the surface roughness.

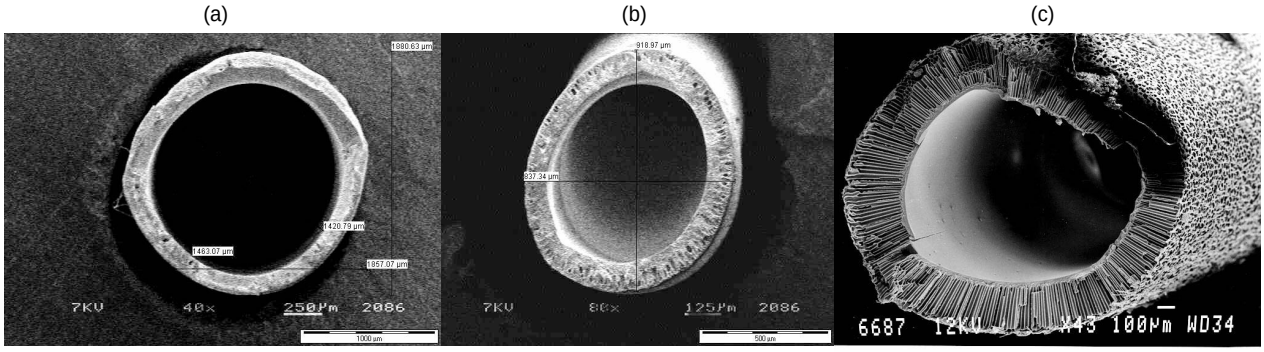

Fig. 3. Electron micrographs of (A) nonporous hollow fiber (HF), (B) nonporous hollow fine fiber (HFF) and (C) externally unskinned ultrafiltration PSU membranes (HF).

and the attendant force curves suggest that the average surface roughness decreases with Pluronic modification of the surface. A similar trend was observed with PSU and poly(ether imide) membranes.

Pluronic adsorption on each of the membrane matrices are summarised in Table 1. The results show an increase in Pluronic adsorption (0.055–0.14 mg cm^{-2}) with an increase in membrane radius (0.9–1.88 mm). The capillaries with a larger lumen diameter, hence less convex than the HFF showed greater pluronic adsorption. This correlates with the trend seen with Pluronics adsorbed onto polystyrene lattices of varying thickness [11]. However, reported results in literature appear to be plagued with inconsistencies, and this is due to a combination of the instability of the adsorption matrix used and the constraints imposed by the analytical instrumentation or technique [7]. The reliable hexane:isopropanol protocol and the sensitive biphasic $NH_4FeSCN/CHCl_3$ assay system is well-suited to Pluronic analysis on synthetic polymer membranes which limits the possibility of

Table 1
The dependence of copolymer adsorption on membranes of varying surface hydrophobicity and interfacial curvature.

Membrane type	Desorbed pluronic (mg. cm^{-2})	±SD	N
Planar PSU	0.063	0.0084	3
Planar PVDF	0.21	0.024	3
PSU HF	0.14	0.0025	3
UF PSU HF	0.17	0.0098	3
PSU HFF	0.055	0.0010	3

drawing convoluted conclusions from the interfacial curvature data. The larger interfacial curvature of HFF membranes causes additional lateral crowding in the adsorbed layer, which might sterically hinder the adsorption of further PPO chains. The capillary membrane has a larger radius of curvature thus limiting lateral crowding and this is reflected in the much greater amounts of Pluronic adsorbed per cm^2 of membrane.

3.2. Metal chelation and stability

A method for the direct solid-state quantification of ligand binding sites on derivatised Pluronic, using a halogenated derivative of Pluronic could then also be used to quantify the metal affinity of a chelating Pluronic ligand. Halogenation of Pluronic was accomplished by covalently coupling the halogens Br and I to the terminal hydroxyl group of Pluronic, which was conventionally used for ligand attachment [8]. The protocols developed in this study were based on initial experiments performed under empirical conditions. An important characteristic of chelating ligands is their ability to be regenerated and reused [9] with minimal leaching of metal ions. The effect on repeated use of the chelated membrane for Ni removal is depicted in Table 2. The effects of five cycles of repeated loading, washing and elution were investigated with a synthetic Ni^{2+} containing stream. After five cycles of use, the membrane lost its chelate capacity for heavy metals. In the absence of biological contaminants, it is highly likely that metal leakage occurred as a result of the high salt concentration (0.3 M NaCl) used in the loading, washing and elution steps. If the application is purely for metal ion removal (as opposed to industrial biotechnology applications focusing on histidine tagged proteins), then a reduced salt concentration of 0.1–0.2 M would be more suitable.

The stability of the repeated application of the chelating copolymer can be estimated from results obtained in several sorption/desorption cycles. Previous studies related to the stability and regeneration of membrane associated metal chelating ligands make use of either the relatively non-specific monochlorotriazinyl dye cibacron blue F3GA in conjunction with strong acids (2 M HCl) or conventional ligands like IDA [8]. The pH plays a complex role in the chelation, retention and elution processes because of its affects on the nucleophilic behaviour of the buffer components. Metal coordination can therefore be controlled by varying the pH [13, 14]. The carboxymethyl groups on the Pluronic-DMDDO chelator need to be deprotonated for metal ion chelation. This is usually achieved at pH 4 or higher. Metal ion displacement, however, is relatively simple to implement via displacement with 0.1 M EDTA. Treatment of chelated membranes in the elution buffer set at a pH range from 2.5 to 8.5 suggested that the stability of the Ni^{2+} chelate was very high above pH 5.5, with Ni^{2+} most stable at physiological pH (7.5). However, metal leaching (5–10%) was observed below pH 5.

3.3. Metal binding and affinity separation

Heavy metal binding was of the order Cd>Zn>Ni>Cu (Table 3). The 0.05M bulk metal stream contained about 2 g L^{-1} metal ions, which was approximately 2–3 orders of magnitude more than what one would normally find in a typical waste water stream. The data pertaining to metal removal percentages would therefore be higher in a 'real' waste water stream, while further studies on binding kinetics, removal rates and membrane geometry would contribute to creating optimum treatment conditions.

PIXE was used to quantify the heavy metal binding capacity of PVDF~F108-DMDDO membranes (Fig. 4). The homogeneity maps in Fig. 5 relate the surface distribution of the attendant metal ions on the surface of each membrane. In another study [10] a 2 mL solution of 0.2 mg mL^{-1} His_6(PK) was incubated with ligand modified Pluronic, unmodified Pluronic and native PVDF membranes. Affinity adsorption of his_6(PK) was confirmed by analysis of NADH conversion after incubation of PVDF~F108-DMDDO-Ni membranes with recombinant proteins. A single 1 cm^2 Ni-chelated membrane was capable of removing 0.17 mg his_6(PK) from solution and this system was regenerated and re-used up to five times.

Table 2
The effect of cyclical use of the chelated membranes on the metal binding capacity.

	$[Ni^{2+}]$	±SD	Ni^{2+} loss	N
cycle 1	27.00	1.407		3
cycle 2	19.59	2.086	27.44%	3
cycle 3	18.66	2.220	30.89%	3
cycle 4	4.976	0.0827	81.57%	3
cycle 5	2.823	0.1096	89.54%	3

Table 3
Heavy metal binding capacity of the chelated membranes after 1 cycle in 10 mL of bulk metal solution (0.05 M).

	Chelated metal after 1 cycle	% stat error	Metal Removal	N
Ni^{2+}	27.00 $mg.cm^{-2}$	0.16	0.0954%	3
Cu^{2+}	20.77 $mg.cm^{-2}$	0.21	0.0653%	3
Zn^{2+}	34.22 $mg.cm^{-2}$	0.18	0.1046%	3
Cd^{2+}	79.14 $mg.cm^{-2}$	0.15	0.1408%	3

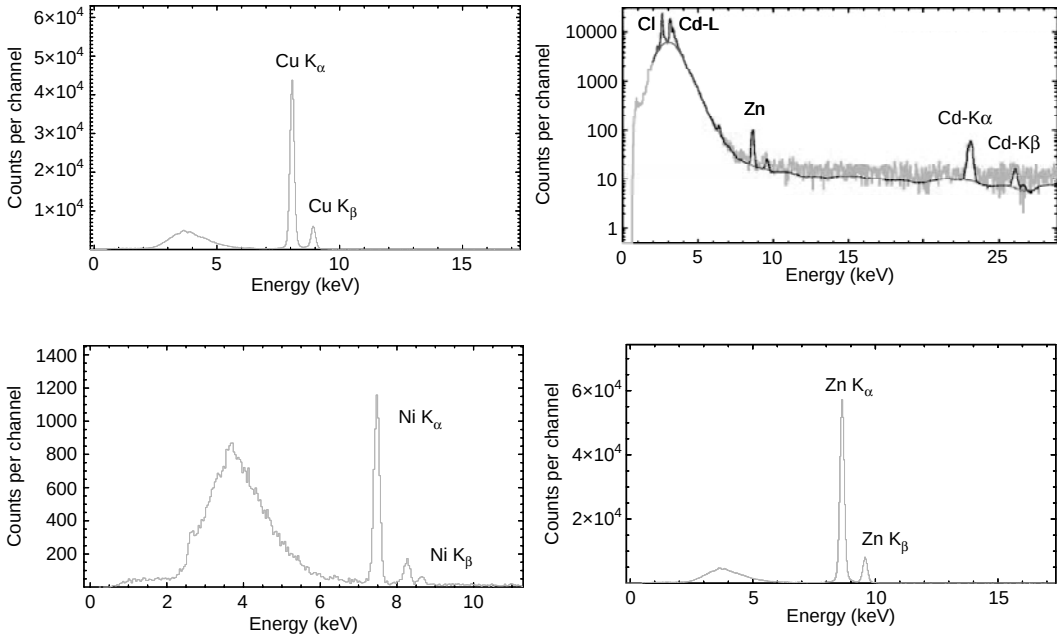

Fig. 4. PIXE spectra showing specific divalent cation binding on PVDF~F108-DMDDO membranes. The sorption capacities of Ni, Zn, Cu and Cd were 27.99 μg cm^{-2}, 34.22 μg cm^{-2}, 20.74 μg cm^{-2} and 79.14 μg cm^{-2} respectively.

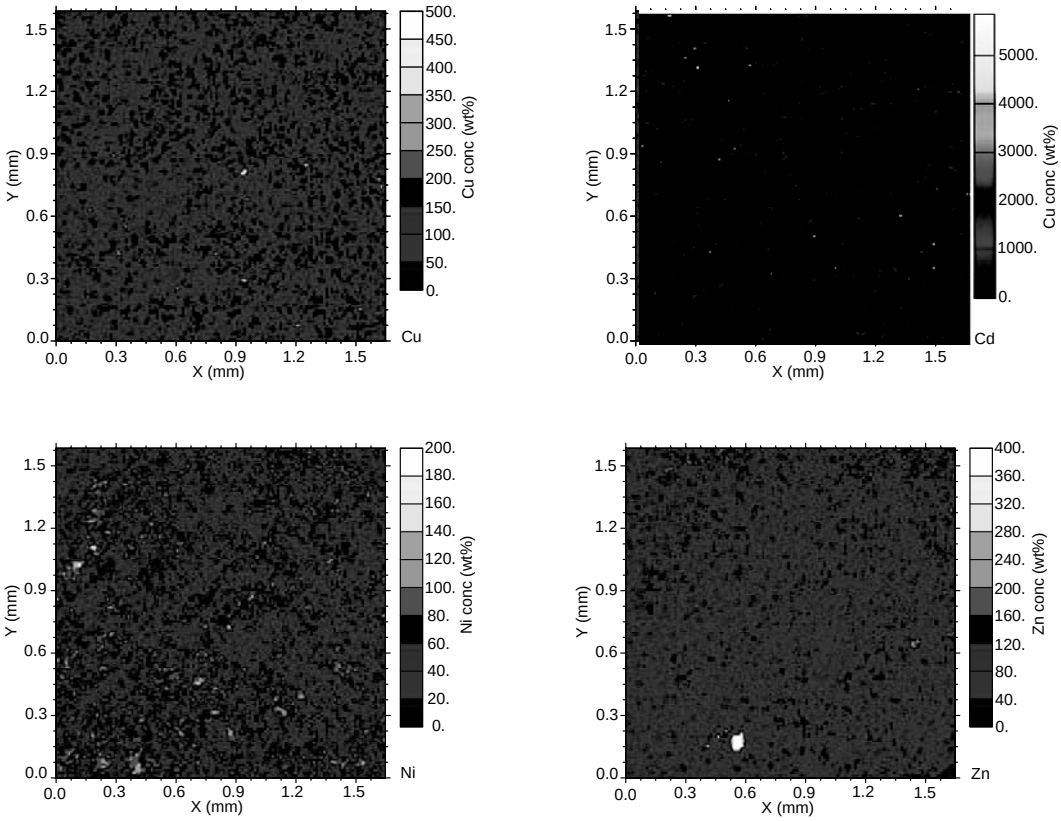

Fig. 5. PIXE elemental maps indicating the surface distribution of Cu, Cd, Ni and Zn on planar PVDF membranes, surface modified with Pluronic-DMDDO. Note that the inset colour scale is a reflection of metal ion concentration and not intensity.

4. Conclusions

The non-porous membranes developed in this study were well characterised and suited to affinity-based separations where surface contacting was not dependent on possible filtration benefits. This work also showed the effectiveness of Pluronic tri-block copolymers as surface modifying agents with minimal effort required to generate homogenous hydrophilised surfaces that could be used for heavy metal binding. The Pluronic-DMDDO chelator retained its chelation properties following surface modification, showing specific affinity for all the heavy metals under investigation. Binding capacities on PVDF~F108-DMDDO membranes for Ni, Zn, Cu and Cd were 27.99 μg cm^{-2}, 34.22 μg cm^{-2}, 20.74 μg cm^{-2} and 79.14 μg cm^{-2} respectively. The biocompatible, chelated membrane system shows potential for the removal of trace quantities of metals in either purified water or waste streams.

This technology differs from other reports in that it is scalable (due to the multi-variant membrane module design), able to resist non-specific protein adsorption and the Pluronic copolymer can be regenerated and reused (up to five times). To minimise metal ion leakage, reduced salt concentration buffers (~ 0.1 M) should be used. Successful coupling of both ligands to Pluronic was achieved followed by quantification of the ligand binding sites on the surface. For industrial biotechnology applications, the Ni^{2+} chelated membranes bound up to 0.8 mg cm^{-2} His_6(PK) and was regenerated/reused up to five times before non-specific protein adsorption dominated the surface architecture. Further studies include incorporating the ligand-modified membrane into the scalable membrane module described in Fig.1 such that divalent heavy metals can be removed from solution.

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