Chemical Engineering Journal, Volume 300, 15 September 2016, pp. 358-366
DOI:10.1016/j.cej.2016.04.121

Development of functionalized nanostructured polymeric membranes for water purification

Zeynep Altintas[a,b]*, Iva Chianella[a], Gabriella Da Ponte[c], Sabine Paulussen[c], Soccorso Gaeta[d], Ibtisam E. Tothill[a]*

[a] Cranfield University, Bedfordshire, MK43 0AL, England, UK

[b] Technical University of Berlin, Straße des 17. Juni 124, Berlin 10623, Germany

[c] VITO Materials Technology, Flemish Institute for Technological Research, Boeretang 200, B-2400 Mol, Belgium

[d] GVS Filter Technology, 40069, Zola Predosa, Bologna, Italy

*Corresponding authors:

Z. Altintas

I.E. Tothill

Tel: +44 (0) 7500766487, Fax: +44 (0) 1234 758380

E-mail: zeynep.altintas@cranfield.ac.uk

i.tothill@cranfield.ac.uk

ABSTRACT

Pharmaceuticals specific molecularly imprinted polymers nanoparticles (MIPNPs) were synthesized and applied onto the polyvinylidene fluoride (PVDF) membranes previously subjected to the plasma treatment. Diclofenac-, metoprolol- and vancomycin-MIPs were applied onto the membranes and scanning electron microscopy was employed to visualize MIPNPs on the membrane. After functionalization of the membranes with target-specific MIPs the molecularly imprinted membranes (MIMs) affinity against their targets was evaluated using solid phase extraction (SPE) technique coupled with high performance liquid chromatography (HPLC). MIMs were used as filters to load the target solutions through employing a vacuum pump to evaluate the amount of pharmaceuticals in filtrate. Moreover, a comparative study was performed by comparing the efficiency of MIMs functionalized either by adsorption or covalent immobilization. The capacity analysis of MIPNPs by SPE-HPLC revealed 100%, 96.3%, and 50.1% uptake of loaded solution of metoprolol, diclofenac and vancomycin, respectively. MIMs showed 99.6% uptake with a capacity of 60.39 ng cm^2 for metoprolol; 94.7% uptake with a capacity of 45.09 ng cm^2 for diclofenac; and 42.6% uptake with a capacity of 16.9 ng cm^2 for vancomycin. HPLC detection limits of targets were found as 3.7, 7.5 and 15 ng mL^{-1} for diclofenac, metoprolol and vancomycin respectively. A small scale pilot test was also conducted which indicates the promising future applications of the developed MIMs for high volume of filtrates especially in the case of the plasma-treated PVDF membranes prepared by covalent immobilization of the MIPs.

Keywords: Polyvinylidene fluoride (PVDF) membranes, Molecularly imprinted polymers nanoparticles (MIPNPs), Plasma treatment, Water purification, Pharmaceuticals.

1. Introduction

Pharmaceuticals are active compounds with biological effects and they are used in many applications for human and veterinary medicine. However, part of the administered dose is excreted as the active substance and/or as metabolite, essentially through the organisms' urine system and the biliary system leading to a release of drugs in the environment [1-3]. This problem has been recognized in the US in the 1970s, and around ten years later in England. To date, the continuous advances in analytical techniques have raised concern about the levels of these compounds in wastewater. Sewage treatment is not efficient enough to eliminate most of these compounds which remain in the effluents and then get into the surface and groundwater. So far antibiotics, beta-blockers, antiphlogistics, vasodilatators, antiepileptics, sympathomometics, lipid regulators and anti-epileptics have been found in manure, sewage, wastewater, groundwater and drinking water [4,5].

Concentrations up to mg L^{-1} have been detected in effluents for single substances in Asian countries [6]. Pharmacodynamic and pharmacokinetic studies are largely carried out during the drug development process and environmental risk is also assessed. However, a risk assessment needs to be developed as well as assessment procedures within a case-by-case approach [6-7]. With the presence of trace level of pharmaceutical in drinking water supplies, the issue has become a public health concern. Further studies pointed out the adverse effects including endocrine disruption, genotoxicity, resistance in pathogenic bacteria and aquatic toxicity, nevertheless chronic health effects are not well known yet [8]. Constant development of analytical techniques is continuously improving pharmaceuticals detection in the aquatic environment and nowadays detections of residues at the amount of nanogram per litre are possible [9]. One of the main causes for the dispersion of pharmaceuticals after human treatment is the lack of efficiency of sewage

treatment plants (STPs) in their mineralization, with evidences of the occurrence of more than 160 different pharmaceuticals in STP effluent, groundwater and surface water [6, 10]. In wastewater treatment, two elimination procedures are important, biodegradation which occurs in the aerobic treatment and adsorption to suspended solids. If not removed in the waste water treatment plants (WWTPs), the drugs will spread into the ecosystem. Most WWTPs employ activated sludge operation in which microorganisms are used to mineralize the compounds to carbon dioxide and water, or reduce the pollutant to an acceptable structure. Another way to remove the substances is by stripping into air or by sorption onto sludge. Moreover, some residues may be subject to phototransformation. To summarize, the five mechanisms to remove pharmaceutical substances include phototransformation, sorption, air stripping, uptake by plants and biotransformation [9, 11].

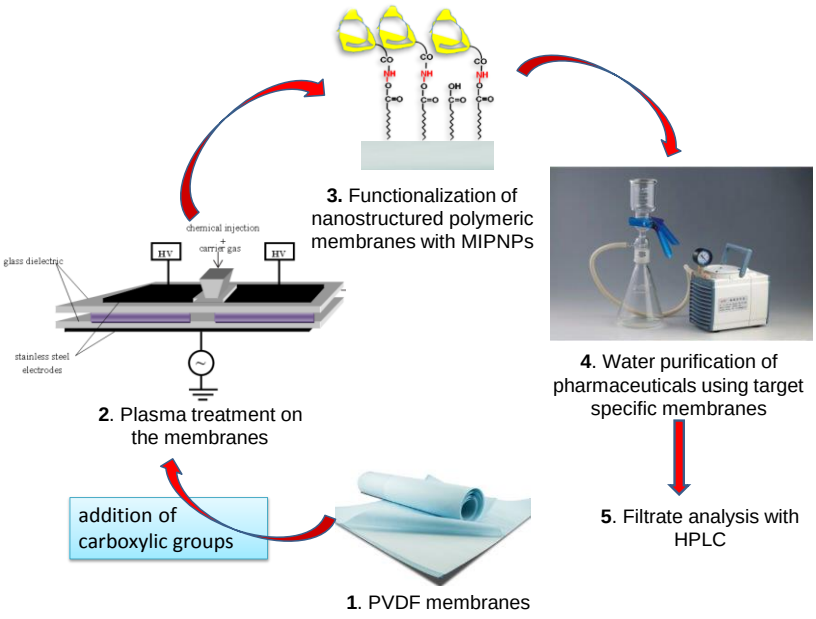
Since many years, researchers have been trying to develop membranes to detect or extract pharmaceuticals from water. Membrane filtration has been exploited to optimize the removal of pollutants such as pesticides and pharmaceuticals. In wastewaters treatment plants, membrane bioreactor (MBR) appears to be an interesting advanced technology. In fact, MBR encompasses organic matter degradation with membrane filtration more efficient than the conventional activated sludge (CAS) process with 56% elimination of diclofenac residues for MBR versus 26% for the CAS. MBR can be equipped with hollow-fibre ultrafiltration membranes, microfiltration-membrane or flat-sheet membrane [12]. Others studies characterized the removal of uncharged trace organics by nanofiltration (NF) membranes due to steric hindrance, whereas polar trace organics removal was influenced by electrostatic interaction with the charged membrane. Several studies compared the removal of pharmaceuticals with different kind of membrane systems. Reverse osmosis (RO) membranes with a molecular weight cut-off inferior to 200 Daltons

provided a good removal with more than 90% removal of the tested compounds; largely more efficient than NF membranes. These significant results suggest that MBR-RO would provide efficient removal of the tested micropollutants [13].

Martínez and colleagues coupled membrane separation and photocatalytic oxidation processes for the degradation of pharmaceuticals [14]. They explored nanofiltration and reverse osmosis method and concluded that nanofiltration exhibits better conditions, in terms of power operation and time saving. They also suggested that the combination of photocatalytic oxidation with membrane separation would be a feasible alternative for pharmaceutical removal for wastewaters [14]. However, the occurrence of pharmaceuticals in environment and drinking water are still high in both developed and undeveloped countries. This emphasizes the lack of efficiency of WWTP and explains the large number of studies focusing on membrane development. Nanomaterials such as graphene and carbon nanotubes have also been used to develop efficient filtration systems for water purification due to their superior characteristics [15-19].

With the lack of efficiency of pharmaceuticals removal in water, researchers focused on improving the selectivity of membranes for toxins and drugs [20-22]. Therefore, the incorporation of selective ligands to the PVDF membrane for the selective removal of pharmaceuticals has been investigated in this work for the first time. Three commonly used drugs including diclofenac as a pain killer, metoprolol as a β-blocker and vancomycin as an antibiotic were selected. Molecular imprints of these molecules were created in the form of nanoparticles using a novel solid phase synthesis method [23-25]. After obtaining MIPs nanoparticles (MIPNPs) with high quality and uniform size, the capacity analysis was conducted by employing solid phase extraction (SPE) technique coupled with HPLC. The PVDF membranes were processed with plasma treatment for surface modification to add functional groups to the membranes prior to incorporating high

capacity and affinity MIPs both by adsorption and covalent immobilization. The results provide a new and promising technology for the purification of water sources from pharmaceutical products by using nanostructured molecularly imprinted membranes (MIM). **Scheme 1** illustrates the entire work with major steps.



Scheme 1. Development of functionalized nanostructured polymeric membranes for water purification of pharmaceuticals using target specific membranes.

2. Experimental section

2.1. Reagents and chemicals

Metoprolol, diclofenac, vancomycin hydrochloride, ethanol, 60 mL SPE tubes and 20 µm pore frits, acetonitrile (ACN), acrylic acid and sodium dihydrogen phosphate monohydrate were all obtained from Sigma-Aldrich (Poole, UK). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Fisher Scientific (Loughborough, UK). Glass beads (Spheriglass® 2429, 53 µm < diameter < 106 µm) were from

Blagden Chemicals (UK). Nitrogen gas was obtained from BOC gases (Manchester, UK). All chemicals and solvents were analytical or HPLC grade with more than 95% purity and were used without further purification.

2.2. Apparatus and equipment

A Sartorius (Göttingen, Germany) analytical balance was used to weigh compounds and membranes. For MIPs and buffers productions a KNF LABOPORT® (KNF Neuberger, Inc., USA) pump was used to apply a vacuum atmosphere. The polymerization of the MIPs has been carried out thanks to a Philips Facial tinner HB175 (Philips, UK) UV source. After the production, MIPs were analyzed by DLS Zetasizer Nano (Nano-S) from Malvern Instruments Ltd (Malvern, UK) and the absorbance was determined using Shimadzu UV-2100 (Shimadzu, Japan). After SPE analysis, samples were analyzed using an Agilent 1200 Series Rapid Resolution System with a reverse phase C18 phenomenex column (Torrance, California, U.S.). For the dissolution of compounds and break down of MIP agglomerates Hilsonic sonicator (Hilsonic, UK) and vortex genie 2 (Scientific instruments, Inc., USA) were employed.

2.3. MIP synthesis

A novel solid phase production technique was used to synthesize the MIPs by immobilizing the templates on micro glass beads. The detail procedures for the preparation of derivatized glass beads and immobilization of the template on the solid support were previously reported [2326]. After the preparation of the template immobilized beads, MIPs were produced as nanoparticles by employing a three-step production method [23, 26]. Briefly, the polymerization mixture immersed the bulk of micro glass beads, and the polymerization was then performed at room temperature under UV source during 2 minutes (step 1). The temperature was then adjusted

to 0 °C to discard low affinity particles using 50 mL ACN (step 2). Finally, the high affinity MIPs were eluted from the affinity media by passing three fractions of 50 mL of ACN at 60 °C (step 3). The characterization of the MIPs size and quality were determined by employing DLS and transmission electron microscopy technique [30]. The size of diclofenac-, metoprolol- and vancomycin-MIPNPs were found to be 132.3 ± 3.2 nm, 169.4 ± 3.5 nm and 263 ± 10 nm, respectively.

2.4. Capacity analysis of MIPs by solid phase extraction-HPLC

SPE-HPLC was carried out to evaluate the MIPs capacity by loading pharmaceuticals onto the MIPNPs and measuring the remaining pharmaceuticals concentration by reverse phase HPLC. SPE columns were set up in duplicate for each pharmaceutical. First EDC-NHS chemistry was used to bind the MIPNPs (containing primary amino groups) to glass beads (containing carboxylic groups). MIPNPs solutions (9 mL) were mixed with 1mL of EDC-NHS (0.4 M-0.1 M) prior to be poured onto 1 g of derivatized glass beads and incubated for 2 hours at room temperature. The mixture was then transferred into the empty SPE columns. Glass beads were retained into the column by two pore frits before processing 8 washing steps with double distilled water using a vacuum system. Samples of 200 ng mL^{-1} of each pharmaceutical were prepared and loaded (1 mL) throughout the columns. The eluted samples were collected in glass vials prior to HPLC analysis. Buffer (water) was loaded (1 mL) after the pharmaceutical in order to determine a possible release of the analytes. Finally washing steps with 1 mL of cold (25°C) and hot acetonitrile (60°C) were carried out to evaluate drugs removal. For the HPLC analysis an Agilent 1200 Series Rapid Resolution System was employed. The column used was a reverse-phase C18 Gemini phenomenex column (150 x 4.6 mm with 5 µm particles). The mobile phase was applied in a gradient mode for vancomycin and metoprolol starting with acetonitrile (ACN) and sodium

dihydrogen phosphate monohydrate (25 mM, pH 3.4) within the HPLC instrument and eluted with a 1 mL min^{-1} flow rate before being submitted to analyse at 210 nm. ACN and the buffer were mixed over 10 minutes with a gradual increase of ACN from 10% to 35%. For diclofenac, an isocratic mode was employed using 70% ACN and 30% buffer after optimization studies. Each sample was analyzed in triplicate at 25 ^{o}C.

2.5. Plasma treatment on membranes

An atmospheric pressure plasma method based on dielectric barrier discharge (DBD) technology was employed for functionalization of PVDF microfiltration membranes with carboxylic groups. The DBD plasma reactor consists of 2 parallel electrodes one of which is covered with a 3 mm thick insulating glass plate and connected to a high voltage power supply (**Fig.1**). In order to guarantee homogeneous plasma treatment, the top electrode moves back and forth in 10 passes over the grounded bottom electrode at a speed of 2 m/min. The gap between the electrodes was limited to 2 mm to ensure stable plasma operation. Plasma discharges were generated at a fixed frequency of 1.5 kHz and a dissipated power of 0.1 W/cm^2 of electrode surface. Argon was used as carrier gas. Acrylic acid was nebulized during the plasma treatment with an atomizer (TSI model 3076) to produce a fine aerosol. Droplet sizes were measured with a particle size analyzer (TSI model 3080) and were found in the range of 10-300 nm with a maximum concentration around 50 nm. The small particle size generated by this atomizer ensures optimum reaction conditions in the plasma. Immediately after plasma treatment, the water contact angle of the functionalized membranes and the time needed for a water drop to penetrate into the membrane were determined with a drop shape analyser (Krüss DSA100). Prior to MIPNPs application, plasma-treated membranes were cut in small pieces (2.4 x 7 cm, area= 16.8 cm^2) and their weight

assessed using analytical balance. MIPNP immobilized and standard membranes were also characterized by employing scanning electron microscopy (Philips XL30 ESEM).

2.6. Molecular imprinted membrane (MIM) characterization

The membranes properties were evaluated by measuring their capacity to absorb the drugs from aqueous solution during a fast filtration. Before use, the membranes were subjected to a washing application and a drying process at 80°C. Two membranes coated with MIPNPs were used per assay to filter 4 mL of solution. A vacuum pump with a pressure level of 0.5 bar and the frequency of 50Hz (230V, 0.6A, 100W) was employed for the filtration. Membranes were initially washed with double distilled water prior to be fitted on the filtration system as recommended by GVS Filter Technology. A small aliquot (4 mL of 200 ng mL^{-1} of each drug) was loaded onto the membrane and filtration was executed with a vacuum system. Filtrates were collected in glass vials prior to HPLC analysis.

2.7. Comparative study for MIP immobilization on membrane

MIPNPs were immobilized on one set of membranes by adsorption without EDC-NHS chemistry. Another set of membranes was subjected to an EDC (0.2 M)-NHS (0.05 M) solution for 30 minutes before MIPNP application. The principle of this application relies on a common method which activates carboxyl groups on the surface of the membranes and allows the immobilization of MIPNPs via their amino groups [23]. All membranes were then dried for one hour in an oven at 80ºC. The membranes were used in a filtration system to filter the solutions of targets (200 ng mL^{-1} diclofenac on diclofenac-MIP immobilized membrane, 200 ng mL^{-1} metoprolol on metoprolol-MIP immobilized membrane, 200 ng mL^{-1} vancomycin on vancomycin-

MIP immobilized membrane). For control experiment, 200 ng mL^{-1} diclofenac was filtered through MIP-free membranes.

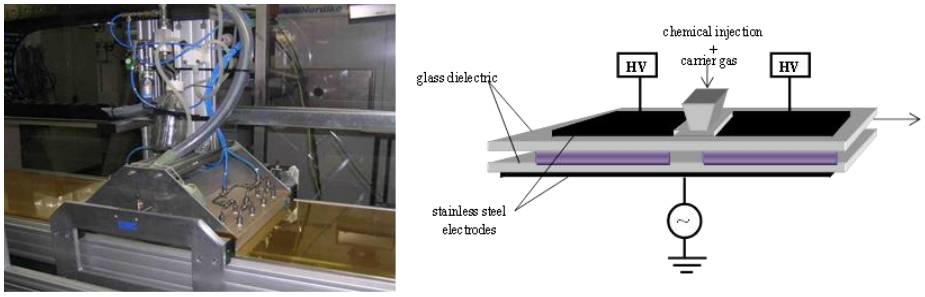


Fig 1. Picture and schematic drawing of the atmospheric plasma set-up.

3. Results and discussions

3.1. Diclofenac MIPs capacity analysis by SPE-HPLC

MIPNPs specific for diclofenac, metoprolol and vancomycin were prepared as already explained elsewhere [30]. After characterization, the MIPNPs were attached to a solid support (glass beads) [26-28], packed in empty SPE columns and their capacity was assessed by SPE-HPLC. To detect diclofenac in samples, an HPLC detection method was developed and a detection limit of 3.7 ng mL^{-1} was obtained. The investigation range of 1.5-1000 ng mL^{-1} allowed linear regression analysis to be carried out with average data of three analysis and R^2 value was found as 0.9995 with very low standard deviation (<0.07%). Diclofenac (200 ng mL^{-1}) was then loaded (1 mL) onto the MIPNPs columns; the filtrates were collected and the residual amount of diclofenac was determined by HPLC (**Table 1, Fig.2**a). Four diclofenac standards were also analyzed with the real samples to calculate the response and to do comparative data analysis. The graph shows that the HPLC responses after drug loading on SPE were relatively low with an average concentration of 27.1 ng mL^{-1} which indicated high level of uptake of diclofenac by diclofenac MIPNPs with an average uptake of 96.3%. Buffer, cold acetonitrile (ACN) and hot ACN were also

loaded on SPE columns after diclofenac and these solutions were also analyzed by HPLC to determine the affinity between diclofenac MIPNP and its target. Diclofenac was not removed significantly from the SPE column either by buffer or ACN. The HPLC response was found very little for buffer loading, whereas it was zero level for both cold and hot ACN (**Fig.2**a).

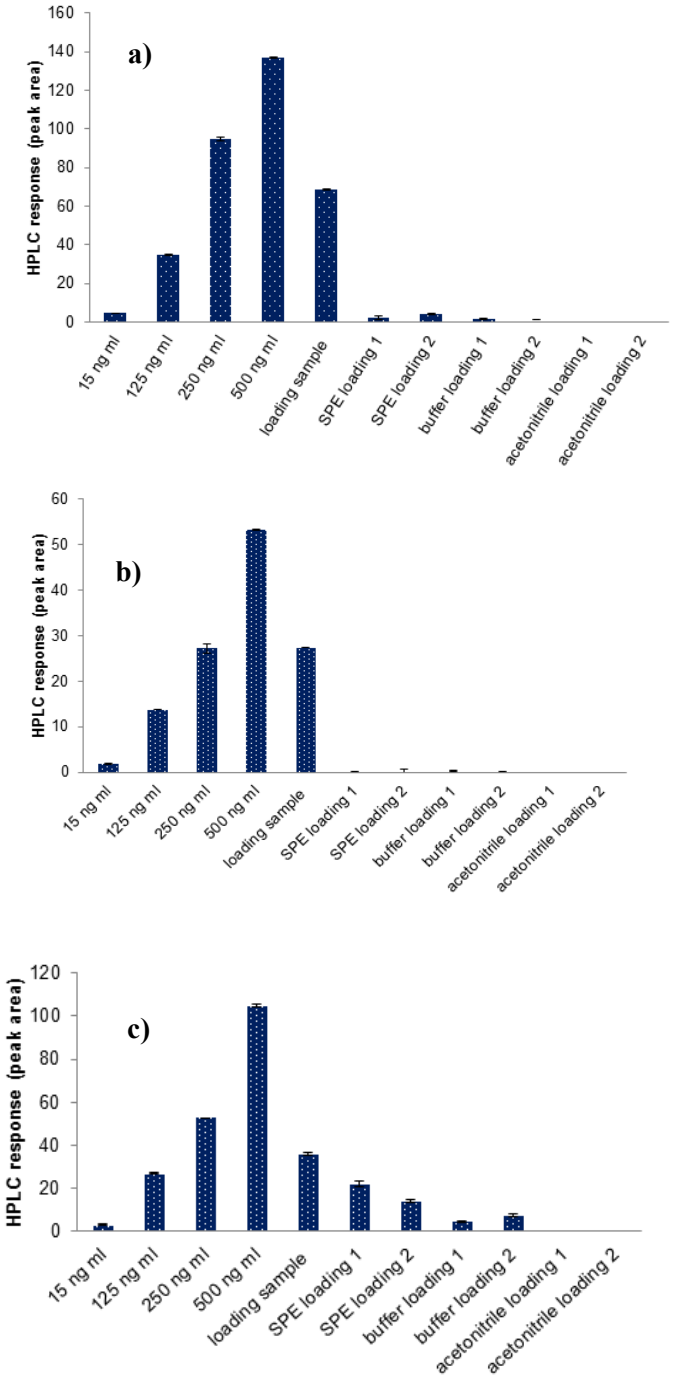


Fig 2. Diclofenac (a), metoprolol (b) and vancomycin (c) concentrations determined by HPLC in standards and before and after SPE application. Loading sample represents the starting solution of each pharmaceutical.

Table 1: Pharmaceutical detection by HPLC after SPE analysis.

	SPE Application	SPE loading 1	SPE* loading 2	Buffer loading 1	Buffer* Loading 2	Cold ACN loading 25°C	Hot ACN loading 60 °C
Diclofenac	Residual concentration in filtrates ng mL^{-1}	17.33	36.89	10.46	7.29	0	0
	Capacity percentage	97.6% uptake	94.9% uptake	5.3% release	1.45% release	0% release	0% release
Metoprolol	Residual concentration in filtrates ng mL^{-1}	0	0	0	0	0	0
	Capacity Percentage	100% uptake	100% uptake	0% release	0% release	0% release	0% release
Vancomycin	Residual concentration in filtrates ng mL^{-1}	100.68	63.83	20.07	33.66	0	0
	Capacity percentage	38.9% uptake	61.3% uptake	12.2% release	20.4% release	0% release	0% release

*The experiments were performed on two columns used in parallel.

3.2. Metoprolol MIPs capacity analysis by SPE-HPLC

An HPLC detection method for metoprolol was developed and a detection limit of 7.5 ng mL^{-1} was achieved in the investigation range of 1.5-1000 ng mL^{-1}. Linear regression analysis was conducted with average data of three analysis and R^2 value was found as 1 with minimal standard deviation (<0.05%). After the development of a successful metoprolol detection method with HPLC, SPE application was conducted as described above for diclofenac. Residual

concentrations of metoprolol in samples collected after SPE analysis could then be determined by HPLC and the results are summarized in **Table 1** and in **Fig.2**b. Four metoprolol standards were also analyzed to calculate the response and to do comparative data analysis. The concentration of the metoprolol starting solution used for the loading was calculated as 257.13 ng mL^{-1} using the HPLC calibration curve. The responses after the pharmaceutical loading on SPE reveals a high level uptake of metoprolol by metoprolol MIPNPs immobilized on SPE with 0 ng mL^{-1} eluted and 100% uptake. Buffer, cold ACN and hot ACN were loaded on SPE column after metoprolol loading to establish the affinity between metoprolol MIP and its target. Metoprolol was not removed from the SPE column with either buffer or ACN. The HPLC response indicated zero level for buffer loading, cold and hot ACN.

3.3. Vancomycin MIPs capacity analysis by SPE-HPLC

An HPLC vancomycin detection method was developed with an investigation range of 1.5-1000 ng mL^{-1} and a detection limit of 15 ng mL^{-1} was obtained. The linear regression analysis was carried out with average data of three analysis and R^2 value was found as 0.9998 with very low standard deviation (<0.08%). Once the standards were analyzed, the SPE loading samples were then measured by HPLC and peak area allowed to determine vancomycin concentration. Results are reported in **Table 1** and **Fig.2**c. The vancomycin concentration in the starting solution was evaluated by HPLC and a value of 164.7 ng mL^{-1} was obtained. The residual vancomycin in the filtrates after loading it on SPE was evaluated by HPLC as 82.25 ng mL^{-1}. This reveals an average uptake of vancomycin of 50.1% by vancomycin MIPNPs immobilized on SPE. Buffer, cold ACN and hot ACN were loaded on SPE column after vancomycin. A small amount of the drug (26.9 ng mL^{-1}) was removed by the buffer, which indicates a lower affinity between vancomycin MIPNPs and its target than for the two other drugs. Nevertheless vancomycin was not

removed from the SPE column by ACN, as the HPLC response indicated zero level for both cold and hot ACN loading. Due to the complex structure and bigger size of vancomycin, its imprinting is more difficult than the other two pharmaceuticals and this might have caused a less stabile yield with lower monodispersity. Therefore, the uptake of vancomycin by its MIPNP on the SPE column was found lower than the other drugs.

3.4. Plasma treatment on PVDF membranes

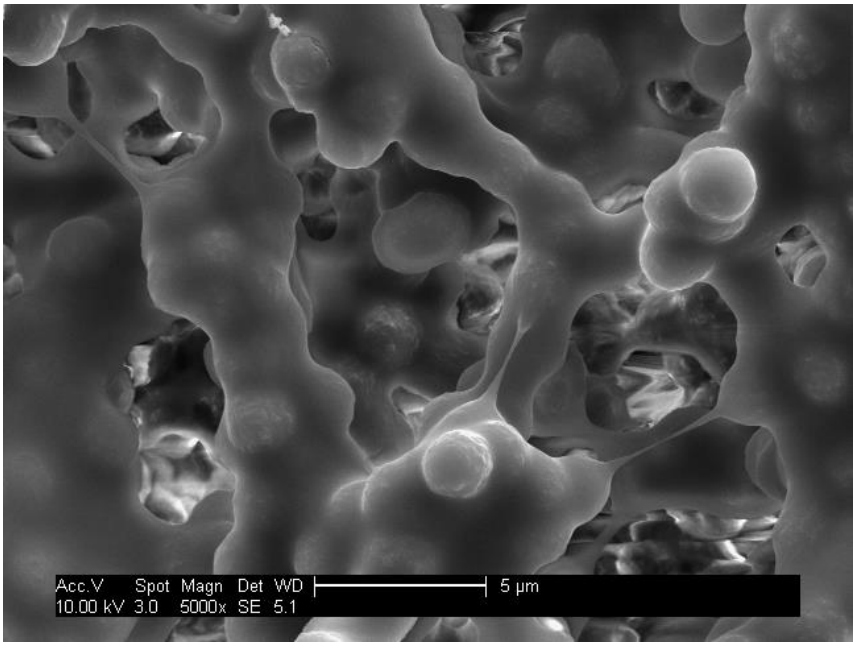
Membranes supplied by GVS Filter Technology (Zola Predosa, Italy) were PVDF membranes produced by means of vapour induced phase separation (VIPS) and have pores with an average size of 3 μm. The membranes were modified by plasma treatment which is a common method used for surface modification. The process consists of plasma exposure to generate free radicals and graft hydrophilic monomers. In this case, acrylic acid was used as the monomer during the plasma deposition process and a high density of carboxylic groups could be obtained at the surface of the membranes. By means of a dye method the amount of grafted carboxylic groups on the membrane surface could be quantified [27, 28]. The untreated PVDF membrane contains $1.44x10^9$ carboxylic groups per square mm of membrane surface, while after plasma treatment; the density of carboxylic groups is increased up to $8.93x10^9$ sites per square mm. The presence of the carboxylic groups at the surface of the plasma-treated membranes also gives rise to a drastic decrease of the water contact angle of the membrane surface: while the water contact angle of the untreated membrane is 128°, this value drops to less than 10° after plasma treatment. After 5 seconds of contact with the plasma treated membrane, the water droplet is completely absorbed. These values remain unchanged after immersion of the plasma treated membrane in MilliQ water for 200 hours, indicating that the effect of the plasma treatment in stable in water. Thus, it can

concluded that the carboxylic groups grafted on the PVDF-membrane are stable in water and are ideal anchorage sites for the MIPNPs which possess amine groups.

3.5. Functionalization of nanostructured polymeric membranes

One batch of MIP production yielded ~15 mg of MIPNPs in 150 mL of aqueous solution [26, 29-30]. For each membrane piece (2.4×7 cm), 3 mL of MIP solution was applied and the MIPNP immobilized both by adsorption and covalent immobilization. After the incubation time, the MIP solutions remained in Petri dishes were collected and measured by UV spectrometer. The level of MIP immobilization on the membranes were analyzed and found to be 69%±1.5%, 67%±2% and 62%±1.8% for diclofenac-, metoprolol- and vancomycin-MIPs, respectively. The standard and functionalized membranes were comparatively visualized by employing SEM and a clear difference was observed before and after MIP immobilization (**Fig.3**). After MIP immobilization the membranes surface appears to be more roughened, possibly due to etching and acrylic acid deposition that take place during the plasma process as well as the presence of MIP nanoparticles on the membrane which resulted in a less smooth membrane surface since the MIP surrounded the membrane pores.

a)



b)

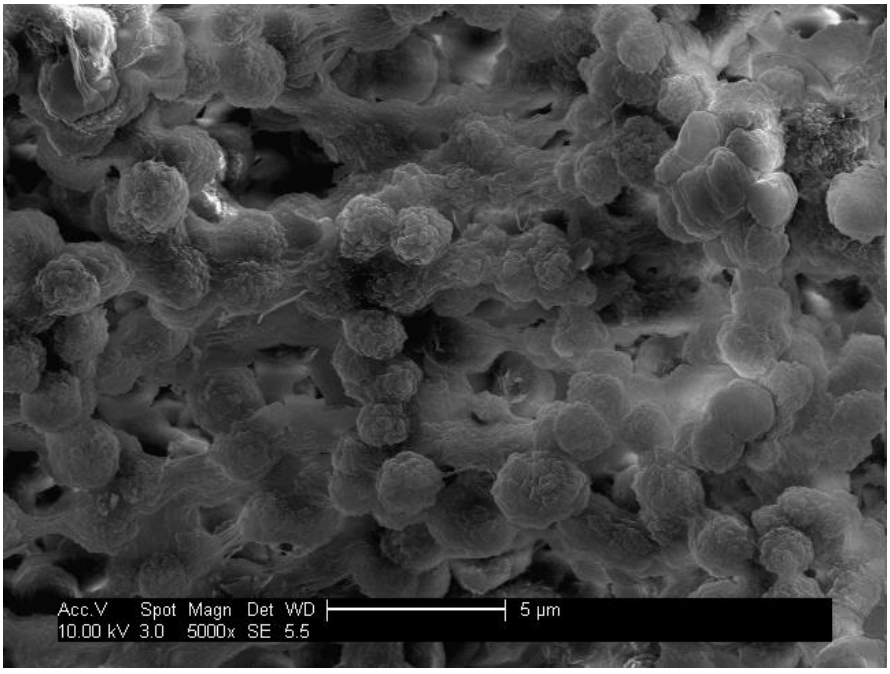


Fig 3. Scanning electron microscopy images of standard (a) and MIP immobilized (b) nanostructured polymeric membranes.

3.6. Diclofenac MIM performance analysis

The capacity of MIMs was evaluated with 4 mL filtration of diclofenac solution (200 ng mL^{-1}) using a vacuum system. The HPLC analysis of the filtrates was performed in parallel of the SPE analysis, with the same standards. Two membranes were also used as the control, which refers to the drug loading on the membrane without MIPNPs. The amount of diclofenac found in the filtrates after drug loading was 10.58 ± 0.11 ng mL^{-1}, meaning that the imprinted membrane were able to bind 94.7 ± 0.05 % of the pharmaceutical from aqueous solution (**Table 2**). This corresponds up to 45.09 ng cm^2. At the same time, the control membranes did not retain the pharmaceutical as most of the drug was found in the filtrates. This could be due to the large pore size of the membranes as compared with the pharmaceuticals. HPLC responses are summarized in **Fig.4**a.

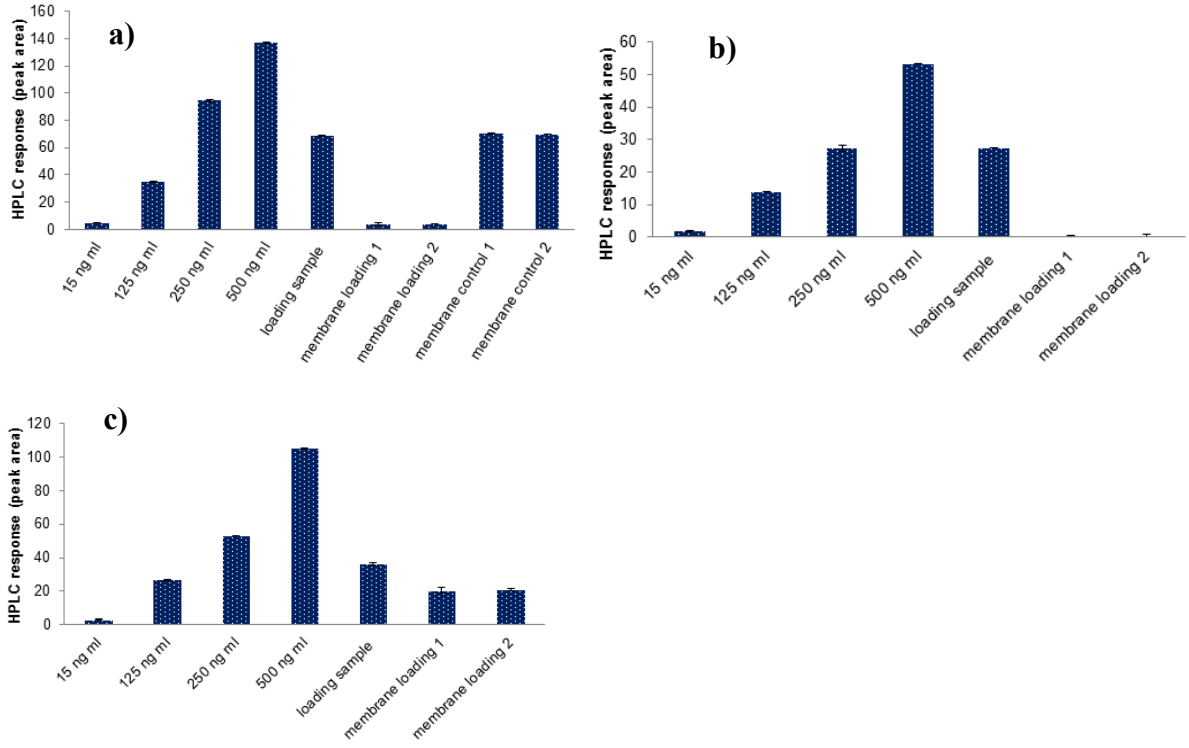


Fig 4. Diclofenac (a), metoprolol (b) and vancomycin (c) concentrations for standards and filtrates determined by HPLC after loading on the membranes. Loading sample represents the starting solution of each pharmaceutical.

Table 2. Pharmaceutical detection by HPLC after membrane filtration.

Membrane Application		Membrane loading 1	Membrane loading 2
Diclofenac	Residual concentration in filtrates ng mL^{-1}	38.47	37.94
	Capacity percentage	94.67% uptake	94.75% uptake
Metoprolol	Residual concentration in filtrates ng mL^{-1}	0.97	1.11
	Capacity percentage	99.62% uptake	99.57% uptake
Vancomycin	Residual concentration in filtrates ng mL^{-1}	93.08	96.07
	Capacity percentage	43.49% uptake	41.67% uptake
Control	Residual concentration in filtrates ng mL^{-1}	200	200
	Capacity percentage	0% uptake	0% uptake

3.7. Metoprolol MIM performance analysis

Metoprolol MIM performance analysis was performed as described before. The concentration for metoprolol in the starting solution was 257.13 ng mL^{-1} and this amount was calculated based on the HPLC standards (**Fig.4**b). The residual amount of metoprolol found in the filtrates was 1.04 ± 0.10 ng mL^{-1}, meaning that the imprinted membranes were able to bind 99.59 ± 0.04% of the pharmaceutical from aqueous solution (**Table 2**). This corresponds to up to 60.39 ng cm^2.

3.8. Vancomycin MIM performance analysis

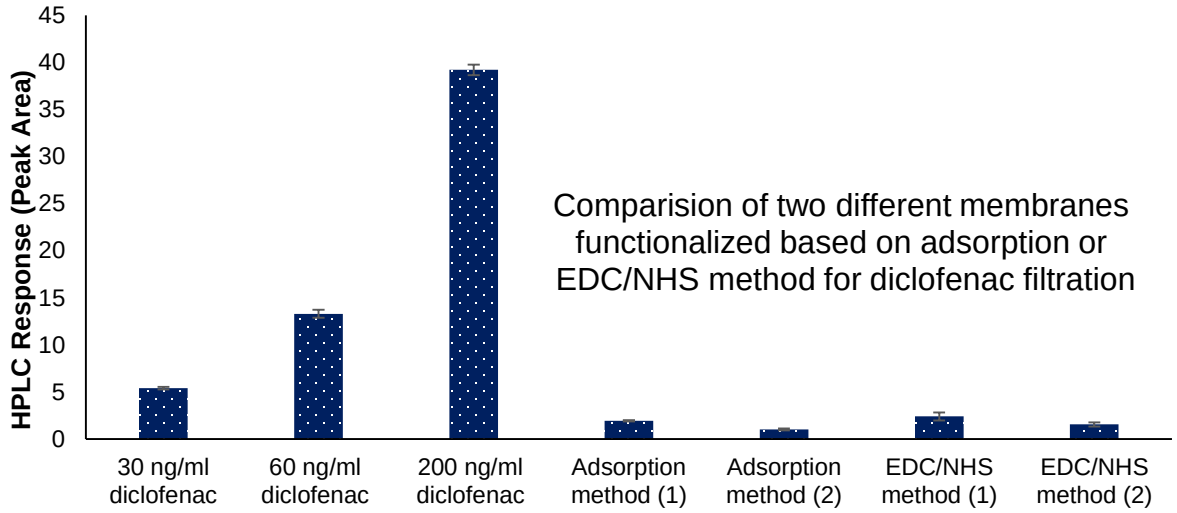
The concentration of vancomycin in the starting solution was 164.7 ng mL^{-1}, which was determined using the HPLC standards (**Fig.4**c). The concentration of vancomycin in the filtrates after loading was of 94.58 ± 2.12 ng mL^{-1}, meaning that the imprinted membranes were able to

bind 42.6 ± 1.3 % of the pharmaceutical from aqueous solution (**Table 2**). This corresponds to up to 16.8 ng cm^2.

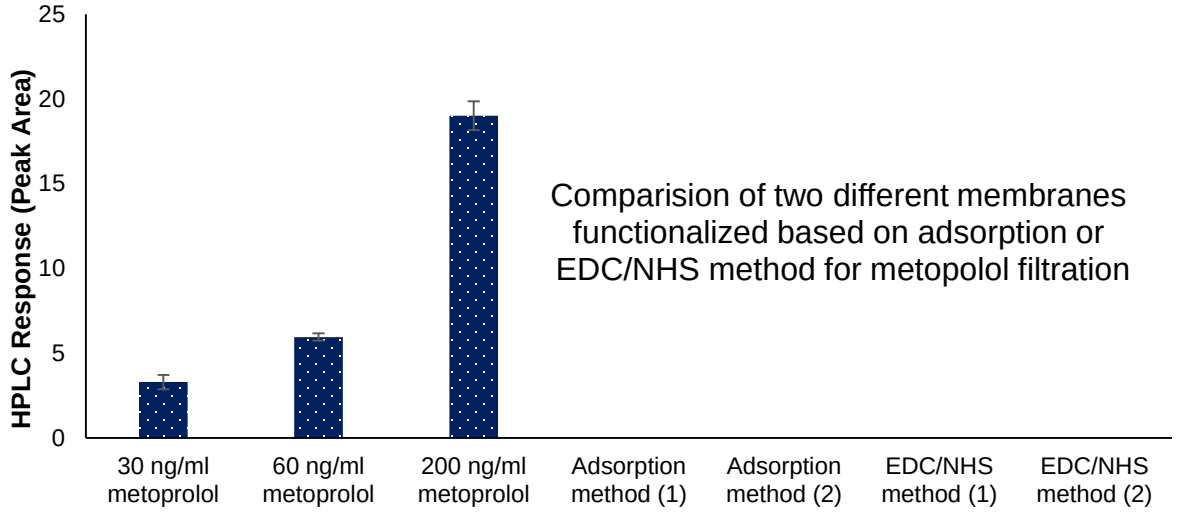
3.9. Comparative study for MIP immobilization on membrane

Here, we aimed to observe the effect of covalent immobilization of MIPNPs onto membranes by applying EDC-NHS coupling chemistry as compared with simple adsorption. For the experiment one set of membranes was processed without EDC-NHS treatment prior to MIPNP immobilization. Another set of membranes were processed with EDC-NHS chemistry. The membranes were used in a filtration system to filter the solutions of the target analytes (200 ng mL^{-1} diclofenac on diclofenac-MIP immobilized membrane, 200 ng mL^{-1} metoprolol on metoprolol-MIP immobilized membrane and 200 ng mL^{-1} vancomycin on vancomycin-MIP immobilized membrane). The same concentration (200 ng mL^{-1}) of each target was used as starting solutions, whereas 30 ng mL^{-1}, 60 ng mL^{-1} and 200 ng mL^{-1} were used as standards. A small percentage (4.89%) of 200 ng mL^{-1} diclofenac was found in filtrate samples in the case of adsorption based MIPNP immobilization, whereas the amount was slightly higher (6.1%) for MIPNP immobilized by EDC-NHS chemistry. After loading the drug samples, buffer loading was also performed on two sets of membranes to calculate the residual amount of the drug after buffer loading which also shows the removal of the drug from the membrane by buffer.. Only 3.9% and 2.6% release of diclofenac was observed on EDC-NHS treated/untreated diclofenac-MIP immobilized membranes, respectively (**Fig.5**a). However, the HPLC response obtained for these filtrates were under the HPLC detection limit of 3.75 ng mL^{-1}.

a)


HPLC Response (Peak Area)
45
40
35
30
25
20
15
10
5
0
Comparision of two different membranes functionalized based on adsorption or EDC/NHS method for diclofenac filtration
30 ng/ml diclofenac
60 ng/ml diclofenac
200 ng/ml diclofenac
Adsorption method (1)
Adsorption method (2)
EDC/NHS method (1)
EDC/NHS method (2)

b)


HPLC Response (Peak Area)
25
20
15
10
5
0
Comparision of two different membranes functionalized based on adsorption or EDC/NHS method for metopolol filtration
30 ng/ml metoprolol
60 ng/ml metoprolol
200 ng/ml metoprolol
Adsorption method (1)
Adsorption method (2)
EDC/NHS method (1)
EDC/NHS method (2)

c)

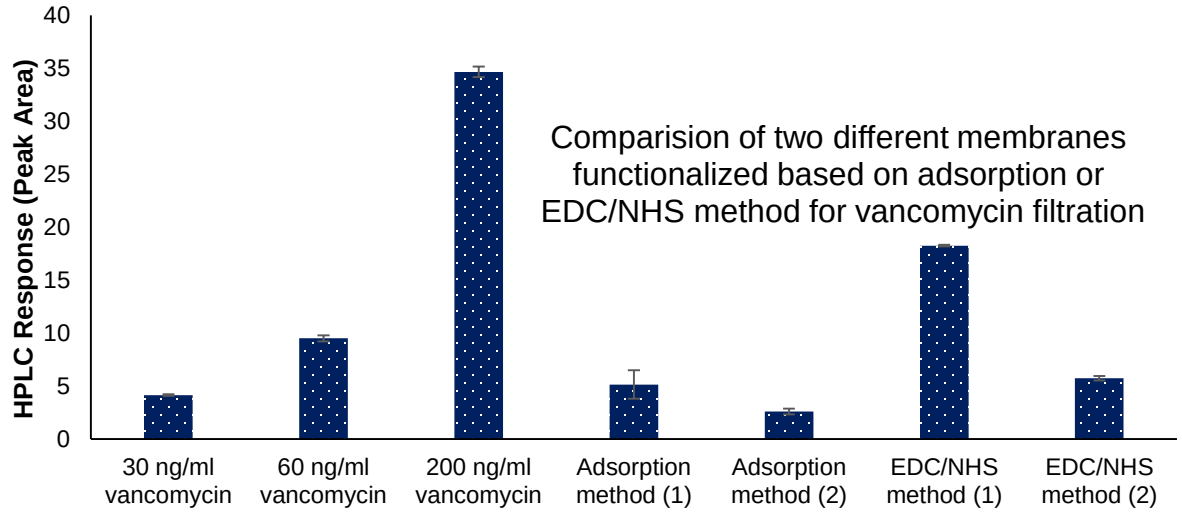


Fig 5. Comparative membrane studies for diclofenac (a), metoprolol (b) and vancomycin (c) with/out EDC-NHS chemistry prior to MIP immobilization. (1: HPLC response of pharmaceutical in filtrate after filtration on the membrane, 2: Removal of pharmaceutical from the membrane after buffer filtration. First three columns of each graph display the HPLC results of three standards for each pharmaceuticals. The starting solution of each pharmaceutical sample was also 200 ng mL^{-1})

The results for metoprolol-MIP immobilized membranes are also summarized in **Fig.5**b. No metoprolol (0% of 200 ng mL^{-1}) was found in any filtrated solutions in all cases. A difference between EDC-NHS treated and untreated membranes was not observed as well as there was no release of metoprolol from metoprolol-MIPNP immobilized membranes in the case of buffer loading.

The success of membrane application for vancomycin filtration was clearly less than the other two targets (**Fig.5**c). This is due to the low quality and uniformity of vancomycin-MIPNP

because of difficulties during the imprinting process. However, the positive effect of EDC-NHS application for vancomycin-MIP was obvious in this case. A relative high percentage (14.86% of 200 ng mL^{-1}) of vancomycin was found in filtrates of vancomycin-MIPNP immobilized membrane without EDC-NHS application, whereas the percentage was halved (7.5%) in the case of EDC-NHS treated membrane. Moreover, due to the higher stability of MIPNP immobilization on the membrane with the aid of EDC-NHS, buffer loading on this membrane did not cause high level of release (16.59%) from the membrane, as compared to buffer loading on the adsorption membrane (47.34%) which was not treated with EDC-NHS.

Based on the results reported above, we can conclude that there is no effective difference between adsorption and covalent immobilization of diclofenac-MIPNP and metoprolol-MIPNP on the membrane, whereas there is a clear difference for vancomycin-MIPNP. In our application, the filtration time was quite short (~2 seconds) and the volume of filtrated solution was small (~4 mL). Another important point is that each membrane was used only for one set of experiment. In the case of long time processes with high volume of solution to be filtered, MIPNP immobilization by EDC-NHS can be more important. The benefit of EDC-NHS chemistry could be even more significant when membranes are reused for many times, since this chemistry can strongly stabilize the MIPNPs on the membrane for a long time. The results of vancomycin-MIP immobilized membranes have been supporting this idea. To see the efficiency of covalent immobilization with much higher volumes and membrane reuse, we also conducted a pilot test in which different volumes of water (1 litre, 2 litres and 5 litres) were filtered on the nanostructured polymeric membranes. The same membranes were used during all filtration period and the total removal of MIPNPs from the membranes was found as 10.01%±2% (n=3) and 19.01%±3.5% (n=3) for covalent and adsorption-based immobilization, respectively. The results indicate both a clear

difference between the two immobilization methods and a gradual removal when higher volumes of water filtration are used. To decrease the level of MIP removal from the membrane during an extensive time period and in the case of high filtration volumes, membrane characteristics and/or plasma treatment process can be further improved.

4. Conclusions

Pharmaceuticals and their metabolites can reach the aquatic environment and drinking waters if they are not eliminated during sewage treatments. Moreover, some compounds used for landfills or animals treatment enter directly environmental waters without going through sewage treatments. The development of an efficient filtration system has crucial importance to prevent toxic effect of the drugs, which may cause health problems. In this work, we successfully developed a functionalized nanostructured polymeric membrane with the aid of molecular imprinting technology and plasma treatment for the first time. The membranes are capable of selective removal of three commonly used pharmaceuticals. MIPNPs capacity analysis by SPE-HPLC revealed 100%, 96.3%, and 50.1% uptake of loaded solutions of metoprolol, diclofenac and vancomycin, respectively. MIMs performance analysis using HPLC achieved 99.6% uptake with a capacity of 60.39 ng cm^2 for metoprolol; 94.7% uptake with a capacity of 45.09 ng cm^2 for diclofenac and 42.6% uptake with a capacity of 16.9 ng cm^2 for vancomycin. A small scale pilot test also indicated the promising future application of the membrane with high volume of filtrates in the case of covalent immobilization of the MIPNPs on the plasma-treated PVDF membranes.

Acknowledgment

This research was financially supported by European Commission under the project code FP7-280595.

References

[1] A. Haiß, K. Kümmerer, Biodegradability of the X-ray contrast compound diatrizoic acid, identification of aerobic degradation products and effects against sewage sludge microorganisms, Chemosphere 62 (2006) 294-302.

[2] A.T. Ternes, J. Stüber, N. Herrmann, D. McDowell D, A. Ried, M. Kampmann, B. Teiser B, Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. 37 (2003) 1976-1982.

[3] J. Gröning, C. Held, C. Garten, U. Claußnitzer, S.R. Kaschabek, M. Schlömann, Transformation of diclofenac by the indigenous microflora of river sediments and identification of a major intermediate, Chemosphere 69 (2007) 509-516.

[4] P.K. Jjemba, Excretion and ecotoxicity of pharmaceutical and personal care products in the environment, Ecotox Environ Safe 63 (2006) 113-130.

[5] L.H. Santos, A.N. Araújo, A. Fachini, A. Pena A, C. Delerue-Matos, M. Montenegro, Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment, J. Hazard Mater. 175 (2010) 45-95.

[6] K. Kümmerer, Pharmaceuticals in the environment: sources, fate, effects and risks: Springer Science & Business Media, Heidelberg, 2008.

[7] R. Laenge, T. Steger-Hartmann, H. Schweinfurth, The environmental risk assessment of human pharmaceuticals in the overall EU regulatory affairs process, Regul Toxicol Pharm. 45 (2006) 223-228.

[8] J.L. Tambosi, L.Y. Yamanaka, H.J. José, R.F.P.M. Moreira, H.F. Schröder, Recent research data on the removal of pharmaceuticals from sewage treatment plants (STP), Quim Nova 33 (2010) 411-420.

[9] Y. Zhang, S.U. Geißen, C. Gal, Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies, Chemosphere 73 (2008) 1151-1161.

[10] M. Goldstein, M. Shenkert, B. Chefetz, Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables

[11] M. Sammartino, F. Bellanti, M. Castrucci, D. Ruiu, G. Visco, T. Zoccarato, Ecopharmacology: Deliberated or casual dispersion of pharmaceutical principles, phytosanitary, personal health care and veterinary products in environment needs a

multivariate analysis or expert systems for the control, the measure and the remediation, Microchem J. 88 (2008) 201-209.

[12] J. Radjenović, M. Petrović, D. Barceló, Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment, Water Res. 43 (2009) 831-841.

[13] A.M. Comerton, R.C. Andrews, D.M. Bagley, C. Hao, The rejection of endocrine disrupting and pharmaceutically active compounds by NF and RO membranes as a function of compound and water matrix properties, J Membrane Sci. 313 (2008) 323-335.

[14] F. Martínez, M. López-Muñoz, J. Aguado, J. Melero, J. Arsuag, A. Sotto, R. Molina, Y. Segura, M. Pariente, A. Revilla, Coupling membrane separation and photocatalytic oxidation processes for the degradation of pharmaceutical pollutants, Water Res. 47 (2013) 5647-5658.

[15] Y. Han, Z. Xu, C. Gao, Ultrathin graphene nanofiltration membrane for water purification, Adv Funct Mater. 23 (2013) 3693-3700.

[16] M. Majumder, N. Chopra, R. Andrews, B.J. Hinds, Nanoscale hydrodynamics: enhanced flow in carbon nanotubes, Nature 438 (2002) 44-44.

[17] G. Cicero, J.C. Grossman, E. Schwegler, F. Gygi, G. Galli, Water confined in nanotubes and between graphene sheets: A first principle study, J. Am. Chem. Soc. 130 (2008) 1871-1878.

[18] X. Yang, L. Qiu, C. Cheng, Y.Wu, Z.F. Ma, D. Li, Ordered gelation of chemically converted graphene for next-generation electroconductive hydrogel films, Angew Chem Int Edit. 50 (2011) 7325-7328.

[19] R. Nair, H. Wu, P. Jayaram, I. Grigorieva, A. Geim, Unimpeded permeation of water through helium-leak–tight graphene-based membranes, Science 335 (2012) 442-444.

[20] F. Liu, Q. Liu, Y. Zhang, Y. Liu, Y. Wan, K. Gao, Y. Huang, W. Xia, H. Wang, Y. Shi, Molecularly imprinted nanofiber membranes enhanced biodegradation of trace bisphenol A by Pseudomonas aeruginosa, Chem Eng J. 262 (2015) 989-998.

[21] C. He, W. Zhao, Y. Liu, S. Sun, C. Zhao, Molecularly imprinted polyethersulfone membranes for the sieving, binding and recognition of bisphenol A, Desalin Water Treat.52 (2014) 5781-5489.

[22] Y.L.F. Musico, C.M. Santos, M.L.P. Dalida, D.F. Rodrigues, Surface modification of membrane filters using graphene and graphene oxide-based nanomaterials for bacterial inactivation and removal, ACS Sustain Chem Eng. 2 (2014) 1559-1565.

[23] Z. Altintas, M. Gittens, A. Guerreiro, K.A. Thompson, J. Walker, S. Piletsky, I.E. Tothill, Detection of waterborne viruses using high affinity molecularly imprinted polymers, Anal. Chem. 87 (2015) 6801−6807.

[24] Z. Altintas, I.E. Tothill, Molecularly imprinting polymer-based affinity nanomaterials for pharmaceuticals capture, filtration and detection, GB Patent. GB1413210.4, 2014.

[25] Z. Altintas, A. Guerreiro, S. Piletsky, I.E. Tothill, Affinity molecular receptor for viruses capture and sensing, GB Patent, GB1413209.6, 2014.

[26] Z. Altintas, A. Guerreiro, S.A. Piletsky, I.E. Tothill, NanoMIP based optical sensor for pharmaceuticals monitoring, Sens. Actuators, B. 213 (2015) 305-313.

[27] C. Geismann, M. Ulbricht, Photoreactive functionalization of poly (ethylene terephthalate) track-etched pore surfaces with smart polymer systems, Macromol Chem Physic. 2005, 206, 268-281.

[28] A. Papra, H.G. Hicke, D. Paul, Synthesis of peptides onto the surface of poly (ethylene terephthalate) particle track membranes, J Appl Polym Sci. 74 (1999) 1669-1674.

[29] Z. Altintas, M.J. Abdin, I.E. Tothill, MIP-NPs for endotoxin filtration and monitoring, GB Patent, GB1413206.2, 2014.

[30] Z. Altintas, B. France, J.O. Ortiz, I.E. Tothill, Computationally modelled receptors for drug monitoring using an optical based biomimetic SPR sensor, Sens. Actuators, B. 224 (2016) 726-737.

2016-04-24

Development of functionalized nanostructured polymeric membranes for water purification

Altintas, Zeynep

Elsevier

Zeynep Altintas, Iva Chianella, Gabriella Da Ponte, Sabine Paulussen, Soccorso Gaeta, Ibtisam E. Tothill, Development of functionalized nanostructured polymeric membranes for water purification, Chemical Engineering Journal, Volume 300, 15 September 2016, pp358-366
http://dx.doi.org/10.1016/j.cej.2016.04.121