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Research paper

# Isolation of drugs from biological fluids by using pH sensitive poly(acrylic acid) grafted poly(vinylidene fluoride) polymer membrane *in vitro*

Jouni Karppi [a,b,*], Satu Åkerman [c], Kari Åkerman [d], Annika Sundell [e], Kristiina Nyyssönen [b], Ilkka Penttilä [a,f]

[a] *Laboratory Centre, Kuopio University Hospital, Kuopio, Finland*
[b] *Research Institute of Public Health, University of Kuopio, Kuopio, Finland*
[c] *Kajaanin I Apteekki, Kajaani, Finland*
[d] *Laboratory Department, Kainuu Central Hospital, Kajaani, Finland*
[e] *Department of Polymer Technology, Åbo Akademi University, Åbo, Finland*
[f] *Kuopio Research Institute of Exercise Medicine, Kuopio, Finland*

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**Abstract**

Isolation of acidic and basic model drugs by using pH sensitive poly(acrylic acid) grafted poly(vinylidene fluoride) (PAA–PVDF) cation-exchange membrane from biological fluids was reported. Effects of drug charge and lipophilicity on adsorption were also investigated. In the present study, basic model drugs adsorbed to a considerably greater extent onto the membrane than acidic drugs. Albumin was not adsorbed onto the membrane. Results of our study exposed, that electrostatic interactions between positively charged basic drug and negatively charged PVDF–PAA membrane were the most important factor affecting drug adsorption onto the membrane. Adsorption of acidic and basic drugs onto the PVDF–PAA membrane was not related to drug lipophilicity. The results of present study demonstrated that basic drugs adsorbed extensively onto the membrane, but albumin did not, proposing that PAA–PVDF membrane may be suitable for isolating basic drugs from proteinaceous biological fluids (i.e. serum) for subsequent monitoring and evaluation.


*Keywords:* Charge; Electrostatic interactions; Isolation; Lipophilicity; PDVF–PAA; Serum

## 1. Introduction

Nowadays solid-phase extraction is a major sample preparation tool [1]. Membranes (e.g. ion-exchange), like chromatography columns, can be used for repeated separations [2]. Surface modified micro-filtration membranes, particle-loaded membranes and particle-embedded glass fiber disks have widely been used to isolate and concentrate selected compounds from liquid solutions prior to chromatographic analyses. Particle-loaded membranes were reported to be more efficient than packed solid-phase extraction cartridges in sample preparation [1,3].

Anion- and cation-exchange membranes have been used for separation of compounds of interest from different kind of liquid solutions. In previous work drug adsorption onto pH responsive poly(*N*,*N*-dimethyl aminoethyl methacrylate) grafted poly(vinylidene fluoride) anion-exchange membrane (PVDF–DMAEMA) was studied. The results clarified that acidic drugs and albumin adsorbed onto the membrane, which suggests that PVDF–DMAEMA membrane may be suitable for separating acidic drugs from

* Corresponding author. Research Institute of Public Health, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. Tel.: +358 17 162953; fax: +358 17 162936.
*E-mail address:* jouni.karppi@uku.fi (J. Karppi).

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protein-free medium for subsequent monitoring and evaluation [4]. Adsorption of drugs onto PVDF–PAA membrane was studied by Åkerman et al. [5]. It was found that studied membrane may be suitable for separating basic drugs from proteinaceous substances, because albumin was not adsorbed onto the membrane. Basic drugs adsorbed to a considerable greater extent onto the membrane than acidic drugs, respectively. Ware et al. [6] have described a clean up method for ergot alkaloids by using a solid-phase extraction (SPE) disk. Ergot alkaloid salts are positively charged, they can easily and selectively adsorb onto negatively charged strong cation-exchange SPE disk. Mean recovery was 88%. Avramescu and co-workers [7,8] have used an ion-exchange mixed-matrix membrane for isolation of bovine serum albumin and bovine haemoglobin or ethylene vinyl alcohol membrane for bilirubin removal from liquid solutions. Anion-exchange membranes were used for separation of glucosinolates from seed suspensions. In extraction procedure glucosinolates adsorbed onto the membrane and thereafter they were released from the membrane by using releasing medium. Using this procedure, a recovery of 80% was obtained [9,10].

The aim of present investigation was to study the adsorption of acidic and basic drugs from serum onto the PVDF–PAA cation-exchange membrane *in vitro*. Drug adsorption was not investigated from buffer solution, since that was evaluated briefly in previous study [5]. Poly(acrylic acid) (PAA) is an environmentally sensitive polymer which undergoes conformational changes as a function of pH and ionic strength [11]. At low pH or high ionic strength PAA-chains are non-dissociated and thus in compact conformation. At high pH or low ionic strength PAA-chains are able to dissociate and get into expanded ''swollen'' form. Due to the dissociation of the carboxylic acid groups in grafted PAA-chains, the membrane carries a negative surface charge, which gives the cation-exchange properties [12]. Suitability of studied membrane for isolating drugs from biological fluids (e.g. blood serum) was also evaluated.

## 2. Materials and methods

### 2.1. Reagents

All the drugs studied were in HCl-form. Alprazolam, chlorpromazine, chloroprotixen, haloperidol, levomepromazine, mianserin, oxazepam, pentobarbital, phenobarbital, phenytoin, temazepam, thioridazine and trazodone were purchased from Orion Co. (Helsinki, Finland). Amitriptyline, citalopram, desmethylcitalopram and nortriptyline were obtained from H. Lundbeck A/S (Copenhagen-Valby, Denmark). Carbamazepine was from Lääkefarmos Co. (Turku, Finland). Clobazam and norclobazam were obtained from Hoechst Ag. (Frankfurt-am-Main, Germany). Clomipramine, desipramine, desmethylmaprotilin, hydroxycarbazepine, imipramine, maprotilin, norclomipramine, oxcarbazepine and protriptyline were obtained from Ciba-Geigy Ag. (Basel, Switzerland). Clonazepam and flunitrazepam were from Roche Co. (Basel, Switzerland). Clozapine and norclozapine were obtained from Sandoz Co. (Berne, Switzerland). Diazepam and nordiazepam were obtained from Dumex Co. (Copenhagen, Denmark). Doxepin, nordoxepin, medazepam, midazolam and thiotixene were obtained from Pfizer Co. (Brussels, Belgium). Fluoxetine and norfluoxetine were from E. Lilly Co. (Indianapolis, USA). Lamotrigine was obtained from The Wellcome Foundation Ltd. (London, England). Nitrazepam was obtained from Leiras Co. (Turku, Finland). Primidone was obtained from Cambridge Research Biochemicals Co. (Chesire, UK). Nortrimipramine, trimipramine and zopiclone were from Rhone-Poulenc Rorer Co. (Birkenrod, Denmark). Hepes (>99.5%) (4-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) and human serum immunoglobulin G (IgG) were purchased from Sigma Co. (St. Louis, MO, USA). Human serum albumin (HSA) was obtained from Red Cross Finland (Helsinki, Finland). Deionized (Millipore™) Milli-Q water (resistivity $\geqslant 18$ MΩ/cm) was used to prepare albumin and IgG solutions. HPLC grade acetonitrile and methanol were purchased from VWR International AB (Darmstadt, Germany). Analytical grade reagents were obtained from Riedel-DeHaén Co. (Seelze, Germany) and FF-Chemicals Co. (Yli-Ii, Finland).

### 2.2. Preparation of membranes

Hydrophobic PVDF membranes, (Millipore) with pore sizes of 0.22 μm, and poly(acrylic acid) (PAA) (Aldrich, Steinheim, Germany) stabilized with 200 ppm hydroquinone, were used as received. Ion-exchange water was used throughout.

Pre-irradiation grafting was accomplished by irradiating the PVDF membranes under nitrogen atmosphere (<200 ppm $O_2$) using Electrocurtain electron accelerator (Energy Sciences Inc., Wilmington, MA, USA) operating at an acceleration voltage of 175 kV. The membranes were irradiated with 25 kGy. After irradiation the membranes were immersed at ambient temperature in a graft solution containing poly(acrylic acid). This solution was purged continuously with nitrogen in order to remove oxygen.

After grafting, the membranes were Soxhlet extracted with water to remove the remaining monomer and dried in vacuo at 40 °C overnight. The degree of grafting (wt%) was determined gravimetrically according to:

$$G = \frac{m_1 - m_0}{m_0} \times 100\% \ (\text{wt}\%)$$

where $m_0$ represents the mass of original PVDF membrane and $m_1$ represents the mass of the grafted, washed and dried membrane. Studies were performed using 43 wt% and 50 wt% grafted membranes. Membranes were pre-treated in a drug-free 25 mM Hepes buffer solution (pH 7.0) for 1 h before immersing membranes in the protein solutions and serum (pH 7.0 and 7.4).

### 2.3. Surface morphology

Surface morphology of the PVDF–PAA membrane was investigated in the previous study [13]. In order to find out the effects of pH on the conformational state of the PAA chains, the morphology of the membranes was examined by scanning electron microscopy (SEM) (JSM-35 Scanning microscope, Jeol Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV. Membranes were presoaked in 6 mM phosphate buffer at pH 2.0 and pH 8.6 at room temperature for 48 h and dried in the air. The dried membranes were coated with gold before SEM. The SEM pictures of the 58 wt% grafted membrane (Fig. 1) seem to confirm the changes in the membrane porosity. The membrane equilibrated at pH 2.0 appears to be more porous than the one equilibrated at pH 8.6. At pH 2.0, the carboxyl groups are practically undissociated resulting in a compact conformation of the PAA chains. Thus the pores in the membrane are left open. However, when the pH is raised from 2.0 to 5.8 the conformation of the grafted PAA chains is greatly expanded due to the repulsion between dissociated carboxyl groups. Expanded chains partially block the pores [13]. It is obvious that the surface morphology and the physicochemical properties of the polymer membrane affect isolation efficacy. Dissociated carboxylic acid groups of PAA are assumed to adsorb basic drugs more effectively than undissociated groups [5], which improve the isolation process.

### 2.4. Determination of drug adsorption onto 50 wt% grafted PVDF–PAA membrane

#### 2.4.1. Drug adsorption from spiked serum

Stock solutions of acidic and basic drugs were made by dissolving 10 mg of each drug in 10 ml methanol in separate bottles and stored at −20 °C. Spiked serum was prepared by adding the drugs to serum immediately prior to use. Concentrations of the drugs in the final solutions were ~0.80–500 μmol/l. Serum was collected from drug-free patient blood samples routinely submitted to our laboratory as follows: the serum was allowed to clot at room temperature for 30 min. The blood samples were centrifuged at 1500*g* for 10 min and the serum was separated, pooled and stored at 4 °C (maximum a week) until the samples were prepared and analyzed.

Drug adsorption studies were performed by shaking test tubes containing 50 wt% grafted PVDF–PAA membranes (weight varied between 8.5 and 13.6 mg, area ~0.75 $cm^2$) and spiked serum (pH 7.4, 1 ml) at room temperature for 24 h. The membrane-free spiked serum samples were treated under identical experimental conditions. Prior to the drug analysis, the serum samples were extracted with an automatic (Gilson Medical Electronics, Villiers-Le-Bel, France) or manual sample preparator (Vac-Elut SPS 24, Analytichem International, Harbor City, CA, USA) using 100 mg Bond-Elut® C18 solid-phase extraction columns (Varian Sunnyvale, CA, USA), and methanol for acidic drugs and 10 mM acetic acid/5 mM diethyl amine in methanol for basic drugs as extraction solvents [14–17]. Eluates were evaporated to dryness using a Techne Sample Concentrator (Techne, Cambridge, UK) with a gentle stream of air at +37 °C and reconstituted in 1 ml of the mobile phase. The rest of the drugs were analyzed by HPLC with methods described below. Adsorbed amounts of both acidic and basic drugs were calculated from peak-height ratios.

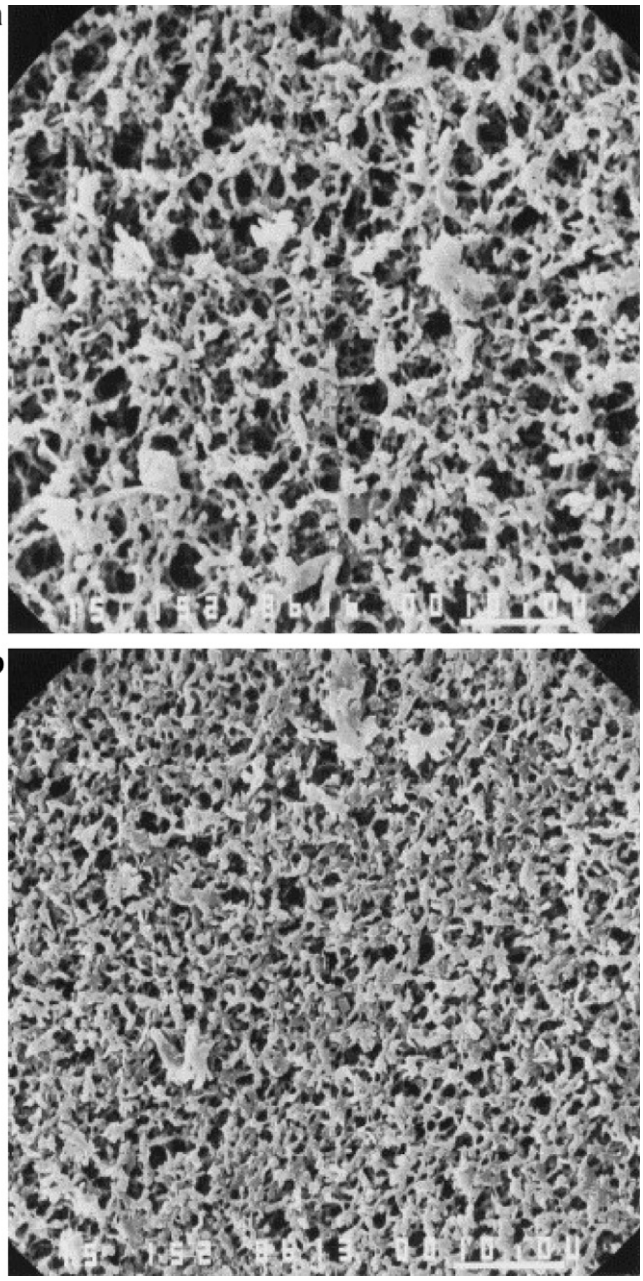


Fig. 1. Scanning electron microscope micrographs demonstrating the effect of pH on the pore size of the 58 wt% grafted membrane. Membranes were presoaked in buffer solution at (a) pH 2 and (b) pH 8.6 for 48 h and dried in the air. (Bar = 10 μm). Reprinted from Journal of Controlled Release, 50, Åkerman S, Viinikka P, Svarfvar B, Jarvinen K, Kontturi K, Nasman J, Urtti A, Paronen P, Transport of drugs across porous ion exchange membranes, 153–166, Copyright (1998), with permission from Elsevier.

### 2.5. Analysis of the drug concentrations

The concentrations of the drugs were analyzed by HPLC [14–17] or with other slightly modified HPLC methods. The drugs were separated chromatographically by using a Select-B (C8 5 μm) 125 mm × 4.0 mm (VWR International AB), Symmetry (C8 5 μm) 150 mm × 4.6 mm. (Waters, Milford, Massachusetts, USA), LiChroCart (C18 5 μm) 250 mm × 4.0 mm (VWR International AB) or NovaPak (C18 5 μm) 150 mm × 4.6 mm analytical column (Waters). The elution was isocratic with a mobile phase of acetonitrile/50 mM dipotassium hydrogen phosphate (pH 4.7): 40%/60% at a flow-rate of 1.2 ml/min or with a mobile phase of methanol/acetonitrile/10 mM dipotassium hydrogen phosphate (pH 3.7): 2%/30%/68% at a flow-rate of 1.5 ml/min. The drugs were detected at 220, 240 or 257 nm and peak purity analyses were performed at 210–365 nm. A Hewlett Packard Series 1050 liquid chromatography system (HP Series 1050 sampler, HP Series 1050 Quaternary pump, HP Series 1050 Diode Array Detector) controlled by ChemStation chromatography workstation (Palo Alto, CA, USA), a Hewlett Packard Series 1100 liquid chromatography system (HP Series 1100 sampler, HP Series 1100 Quaternary pump, HP Series 1100 Diode Array Detector) controlled by ChemStation chromatography workstation and a Perkin-Elmer liquid chromatography system (ISS 200 autosampler, Binary LC 250 pump, 235C diode-array detector), controlled by a Turbochrom chromatography workstation (Perkin-Elmer, Norvalk, CT, USA), were used.

### 2.6. Determination of albumin and IgG adsorption

Albumin and IgG adsorption were studied by shaking test tubes containing 50 wt% grafted PVDF–PAA membranes (weight varied between 4.7 and 6.0 mg, area ~0.40 $cm^2$) and 1 ml of 0.005–50 g/l albumin solution or 0.005–20 g/l IgG solution in 25 mM Hepes buffer (pH 7.0) at room temperature for 24 h. The membrane-free albumin and IgG solutions were treated under identical experimental conditions. Their levels were measured by kinetic nephelometry with a Beckman array protein analyzer (Beckman Co, Brea, CA, USA). Adsorbed amounts were calculated from concentration ratios.

### 2.7. Determination of cortisol, free thyroxine ($FT_4$) and thyrotropin (TSH) adsorption

The blood samples were collected as described in Section 2.4.1. Specimens were authentic patient serum samples. Adsorption of cortisol, $FT_4$ and TSH onto the membrane was studied by shaking test tubes containing 43 wt% grafted PVDF–PAA membranes (weight varied between 7.3 and 8.0 mg, area ~0.55 $cm^2$) and 1 ml of serum for 24 h. Serum samples in the absences of membranes were treated under identical experimental conditions. The levels of TSH and $FT_4$ were measured by AutoDelfia hTSH Ultra kit and AutoDelfia free thyroxine ($FT_4$) kit with Wallac Autodelfia analyzer (Turku, Finland). The levels of cortisol were measured by the Immulite Corsol kit with the Immulite analyzer (Immulite®, diagnostic Products Co., Los Angeles, CA, USA). Adsorbed amounts were calculated from concentration ratios.

### 2.8. Statistical analyses

Results are expressed as means ± SD. Statistical analyses were performed with the regression analysis. A probability level of $<0.05$ was considered statistically significant. SPSS software (version 14.0; SPSS, Inc., Chicago, IL, USA) was used for statistical analyses.

## 3. Results and discussion

In this case, drugs will be absorbed on the PAA-chains through the mechanisms of ion-exchange, i.e. the negatively charged polymer chains will exchange their positively charged counter ion ($H^+$) for preferably a positively charged drug as described in Section 3.1

However, the amount of absorbed drug (Table 1) is affected by several things: (1) The concentration of the drug in the drug solution; higher drug concentrations give higher amount of adsorbed drug since the ion exchange process is faster. (2) The maximum binding capacity of the membrane; membrane with a higher binding capacity binds drug with a higher rate, for comparable results only membranes with identical binding capacities should be tested. If there are differences in the binding capacities and testing time is predetermined → difference in the amount of drug absorption may exist. (3) The counter ion originally "attached" on each exchange site; the membrane prefers counter ions in a certain order, depending on which counter ion originally attached to the membrane ion-exchange will occur or not. (4) The molecular size and complexity of the drug; complex molecules with high molecular mass (proteins) will occupy more than one binding site → reduced binding capacity. They might also act as "crosslinkers" → reducing drug flux through the membrane.

### 3.1. Effect of charge of the drug on adsorption onto the 50 wt% grafted PVDF–PAA membrane

Amounts of model drugs adsorbed onto the membrane (disappearance of the drugs from the sample) are presented in Table 1. The pH of the external adsorption medium affects both the drug and poly(acrylic acid) ionization. At physiological pH PAA is able to dissociate completely ($pK_a \sim 4.0$) due to the surface of the membrane being negatively charged that assists drug adsorption [18]. Basic antidepressant drugs adsorbed onto the membrane to a considerably greater extent than acidic drugs. Basic drugs adsorbed onto the membrane approximately doubly more than acidic drugs. However, basic thioridazine adsorbed only weakly (adsorbed amount 26.8 ± 15.1% of the initial

Table 1
Amounts of the drugs adsorbed from spiked serum pool onto the 50 wt% grafted PVDF–PAA membrane

| Drug | $pK_a$ [20,21] | $\log P$ [20,21] | Adsorbed amount (%) |
|---|---|---|---|
| Alprazolam (A) | 2.4 | 3.2 | 65.9 ± 6.4 |
| Carbamazepine (A) | | 2.5 | 7.8 ± 6.7 |
| Clobazam (A) | <6.0 | 1.0 | 32.0 ± 17.7 |
| Clonazepam (A) | 1.5,10.5 | 2.4 | 23.9 ± 3.6 |
| Diazepam (A) | 3.4 | 2.8 | 15.1 ± 2.9 |
| Flunitrazepam (A) | 1.8 | 2.1 | 54.7 ± 17.4 |
| Hydroxycarbazepine (A) | | | 6.1 ± 3.1 |
| Lamotrigine (A) | 5.5 | 0.1 | 0.4 ± 5.0 |
| Medazepam (A) | 6.2 | 4.4 | 27.2 ± 6.9 |
| Midazolam (A) | 6.2 | 3.7 | 56.4 ± 14.1 |
| Nitrazepam (A) | 3.2,10.8 | 2.3 | 13.4 ± 2.4 |
| Norclobazam (A) | | | 38.5 ± 11.3 |
| Nordiazepam (A) | 3.5,12.0 | | 11.5 ± 2.0 |
| Oxazepam (A) | 1.7,11.3 | 2.2 | 7.4 ± 1.7 |
| Oxcarbazepine (A) | | | 45.0 ± 18.6 |
| Pentobarbital (A) | 7.4 | 1.5 | 4.9 ± 5.6 |
| Phenobarbital (A) | 8.1 | 1.9 | ND |
| Phenytoin (A) | 8.3 | 2.5 | ND |
| Primidone (A) | 13 | 0.9 | 9.5 ± 7.4 |
| Temazepam (A) | 1.3 | 2.2 | 42.4 ± 18.5 |
| Zopiclone (A) | 6.7 | 1.0 | 35.0 ± 17.9 |
| Amitriptyline (B) | 9.4 | 5.0 | 94.1 ± 7.5 |
| Chloropramazine (B) | 9.3 | 3.4 | 83.2 ± 18.6 |
| Chloroprotixen (B) | 8.8 | 2.7 | 82.3 ± 3.5 |
| Citalopram (B) | 9.5 | 0.6 | 98.7 ± 0.2 |
| Clomipramine (B) | 9.5 | 5.2 | 92.6 ± 0.6 |
| Clozapine (B) | 8.0 | 4.3 | 93.4 ± 0.6 |
| Desipramine (B) | 9.4 | 4.9 | 95.8 ± 0.8 |
| Dm-citalopram (B) | | | 97.5 ± 0.2 |
| Dm-maprotiline (B) | | | 85.7 ± 5.1 |
| Doxepin (B) | 9.0 | 2.4 | 96.8 ± 0.3 |
| Fluoxetine (B) | | 0.6 | 92.2 ± 1.3 |
| Haloperidol (B) | 8.3 | 3.4 | 96.1 ± 0.6 |
| Imipramine (B) | 9.5 | 2.5 | 95.3 ± 0.9 |
| Levomepromazine (B) | 9.2 | 4.7 | 89.6 ± 3.8 |
| Maprotiline (B) | 10.2 | 4.2 | 90.6 ± 3.3 |
| Mianserin (B) | 7.1 | 4.3 | 95.2 ± 1.4 |
| Norclomipramine (B) | | | 89.8 ± 0.7 |
| Norclozapine (B) | | | 92.4 ± 0.5 |
| Nordoxepin (B) | | | 94.9 ± 0.4 |
| Norfluoxetine (B) | | | 93.4 ± 1.6 |
| Nortrimipramine (B) | | | 92.1 ± 1.3 |
| Nortriptyline (B) | 9.7 | 1.7 | 93.0 ± 0.6 |
| Protriptyline (B) | | 1.2 | 95.5 ± 0.5 |
| Thioridazine (B) | 9.5 | 5.9 | 26.8 ± 15.1 |
| Thiothixen (B) | | | 65.0 ± 10.3 |
| Trazodone (B) | 6.7 | | 75.6 ± 12.8 |
| Trimipramine (B) | 7.7 | 4.7 | 93.8 ± 1.5 |

Mean ± SD, $n = 5$. ND: not detectable, A: behaves like acid, B: behaves like base, [20,21]$pK_a$ and [20,21]$\log P$ values were obtained from Jack (1992) and Dollery (1999).

drug dose) onto the membrane. Adsorption of basic drugs varied from 26.8% to 98.7% of the initial drug doses (mean adsorbed amount 88.0 ± 14.3%). At pH values greater than two units above $pK_a$ values of basic drugs, they are non-dissociated; and at physiological pH at the $pK_a$ of the drug >7.0, they are fully dissociated and positively charged [19]. Acidic antiepileptic drugs and benzodiazepines adsorbed onto the PVDF–PAA membrane only slightly. Adsorption varied between not detected and 56.4% of the initial drug doses (mean adsorbed amount 27.1 ± 19.8%). At studied pH 7.4, when the $pK_a$ of the drug is <7.0, acidic drugs are dissociated and negatively charged. When pH is equal to the $pK_a$, all the drug molecules are dissociated exactly 50% [19]. The $pK_a$ values of each studied drugs are given in Table 1 [20,21].

In the present study basic model drugs adsorbed onto the PVDF–PAA membrane considerably to a greater extent than acidic model drugs. Results of the study clearly indicate that the ionic interaction between a basic positively charged drug molecule and the negatively charged carboxylic acid group of the PAA was the most important factor affecting drug adsorption onto the membrane. In previous studies drug adsorption onto the PVDF–PAA membrane was investigated. Åkerman et al. [5] observed that electrostatic interactions between the basic drugs and the membrane were much stronger than the interactions between acidic and neutral drugs, and the membrane. The formation of complex between the anionic polymer (PAA) and procaine HCl (basic drug) was studied by Govender et al. [22]. They found that non-electrostatic attractions to the interaction of PAA with procaine HCl were greater than those of the electrostatic attractions. Similar electrostatic interactions between drugs and other kind of ion-exchange polymers have been reported [4,23–25]. Our previously published results proposed that acidic drugs adsorbed onto the PVDF–DMAEMA membrane due to positive surface charge via electrostatic interactions [4]. Jenquin et al. [23] have characterized acrylic resin matrix films (Eudragits RL and RS) and mechanisms of drug–polymer interactions. Salicylic acid and chlorpheniramine maleate were used as model drugs. Acidic salicylic acid interacted with these Eudragit polymers (contain quaternary ammonium groups) primarily via ionic electrostatic interactions. Pignatello and co-workers [24] studied the mechanisms of interaction between Eudragit RS100 and RL100 polymers with three nonsteroidal anti-inflammatory drugs: diflunisal, flurbiprofen, and piroxicam. Drugs strongly interacted with the ammonium groups present in polymers with electrostatic interactions. Rodriguez et al. [25] have evaluated the interaction of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels. The drug molecules interacted weakly with the polymers through ionic interactions. However, instead of ionic forces, there could be non-ionic interactions like van der Waals forces or hydrophobic interactions that affect drug adsorption [25,26]. In the present study, adsorption of acidic model drugs onto the membrane may have occurred via non-electrostic forces.

### 3.2. Effects of proteins and hormones on drug adsorption

Albumin was not adsorbed onto the PVDF–PAA membrane (Table 2). Drug adsorption onto the PVDF–PAA membrane with and without albumin was studied by Åkerman et al. [5]. It was observed that since albumin binds desipramine and thioridazine at physiological pH, the

Table 2
Amounts of albumin adsorbed from 25 mM Hepes buffer (pH 7.0) onto the 43 wt% grafted PVDF–PAA membrane

| Concentration added (g/l) | Adsorbed amount (%) |
|---|---|
| 0.005 | ND |
| 0.02 | ND |
| 0.04 | 3.9 ± 3.9 |
| 0.08 | ND |
| 0.1 | ND |
| 0.5 | ND |
| 0.7 | ND |
| 0.8 | ND |
| 23.9 | 2.0 ± 1.9 |
| 47.3 | 2.3 ± 4.9 |

Mean ± SD, $n = 3$. ND: not detectable.

reduced drug adsorption onto the PVDF–PAA membrane in the presence of albumin at pH 7.0 was most probably due to the distribution of the drug between albumin and the PVDF–PAA membrane. It could be suggested that albumin did not affect the binding capacity of the membrane or reduce the drug adsorption via steric effects. Albumin binds thioridazine very tightly in serum (>99.5%) [20,21]. It would be suggested that this phenomenon may also explain weak adsorption of thioridazine onto the PVDF–PAA membrane in the present study. Adsorbed amount of IgG varied between not detected and 53.8% (mean adsorbed amount 27.1 ± 22.9%; Table 3). In physiological concentration (reference range: 7.0–16.1 g/l; Laboratory Centre, Kuopio University Hospital, Kuopio, Finland) the adsorbed amount of IgG was 29.2 ± 2.8% of the initial dose. The highest adsorption was in IgG concentration of 1.0 g/l. Cortisol adsorbed 26.0 ± 6.0% onto the membrane (Table 4). However, TSH and $T_4F$ were not adsorbed onto the membrane. Reference ranges of studied hormones are given in Table 4. We would propose that cortisol and IgG may decrease drug adsorption onto the PVDF–PAA membrane from serum, but that should be examined further in future studies.

### 3.3. Effect of lipophilicity of the drug on adsorption

Effect of lipophilicity on adsorption of basic model drugs from serum onto the 50 wt% grafted PVDF–PAA membrane was evaluated. Results of previous study proposed that adsorption of basic drug onto the PVDF–PAA membrane was related to the lipophilicity of the drug [5]. There was much bigger number of basic model drugs in the present study. Basic model drugs that we have evaluated are hydrophilic and lipophilic ($\log P = 0.6$–$5.9$), and they all adsorbed onto the membrane extensively except for thioridazine that is the most lipophilic basic drug ($\log P = 5.9$). Based on results of the present study, it would be proposed that lipophilicity did not enhance the adsorption of basic model drugs onto the PVDF–PAA membrane from serum ($R = 0.1583$, $p = 0.102$). Adsorption of acidic drugs was also not related to drug lipophilicity ($R = 0.1894$, $p = 0.105$). Other authors have observed a favourable effect of drug lipophilicity on adsorption to the ion-exchangers [27–29]. Adsorption of clomipramine and viloxazine hydrochlorides into a new multilayer polyethylene-lined film (Stedim 6) and polyvinyl chloride (PVC) bags was studied by Airaudo et al. [27]. Behavioural differences observed between the two drugs with regard to PVC are explained in terms of differences of lipophilicity of the drugs. Jaskari et al. [28] have observed that the lipophilic drugs, tacrine and propranolol, were adsorbed to the ion-exchange fibers more strongly and longer than the more hydrophilic nadolol. The highest amount of binding was observed for the most lipophilic salicylate (5-Cl) and the most lipophilic fiber (Smopex®-105pe). In the other report Vuorio et al. [29] found that lipophilic tacrine and propranolol bound into ion-exchange materials consisting of a poly(ethylene) framework more effectively than hydrophilic drugs.

Table 3
Amounts of adsorbed IgG from 25 mM Hepes buffer (pH 7.0) onto the 43 wt% grafted PVDF–PAA membrane

| Concentration added (g/l) | Adsorbed amount (%) |
|---|---|
| 0.01 | ND |
| 0.03 | ND |
| 0.07 | ND |
| 0.1 | 4.5 ± 1.3 |
| 0.4 | 3.4 ± 10.1 |
| 0.7 | 44.8 ± 2.6 |
| 1.0 | 53.8 ± 2.6 |
| 18.5 | 29.2 ± 2.8 |

Mean ± SD, $n = 3$. ND: not detectable.

Table 4
Amounts of hormones adsorbed from authentic patient serum samples onto the 43 wt% grafted PVDF–PAA membrane

| Hormone | Concentration range | Reference range[a] | Adsorbed amount (%) |
|---|---|---|---|
| Cortisol | 170–1208 nmol/l | 170–540 nmol/l[b] | 26.0 ± 6.0 |
| $FT_4$ | 9–25 pmol/l | 12–22 pmol/l | ND |
| TSH | 0.5–2.7 mU/l | 0.3–4.2 mU/l | ND |

ND: not detectable.
[a] Laboratory Centre, Kuopio University Hospital, Kuopio, Finland.
[b] Reference range at 8.00–10.00 a.m. Mean ± SD, $n = 10$.

## 4. Conclusion

The effects of charge and lipophilicity of model drugs on interactions between the drug and the PVDF–PAA cation-exchange membrane were studied. Basic model drugs adsorbed to a greater extent than acidic drugs onto the PAA grafted PVDF membrane from serum. It could be concluded that electrostatic interactions between positively charged basic drug and negatively charged PVDF–PAA membrane was one of the most important factors affecting drug adsorption onto the membrane. Lipophilicity was not related to the adsorption of acidic and basic drugs onto the PVDF–PAA membrane. Albumin was not adsorbed onto

the membrane, which suggests that studied membrane might be suitable for isolation of basic antidepressant drugs from proteinaceous biological fluids (i.e. serum) for subsequent evaluation and monitoring.

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