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Adsorption of drugs onto a pH responsive poly(*N*,*N*-dimethyl aminoethyl methacrylate) grafted anion-exchange membrane in vitro

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Abstract

The influence of charge and lipophilicity of acidic and basic model drugs on their adsorption onto poly(*N*,*N*-dimethyl aminoethyl methacrylic acid) grafted poly(vinylidene fluoride) (DMAEMA–PVDF) membranes was evaluated. The effect of serum proteins (albumin, IgG) and hormones (cortisol, free thyroxine (T_4F) and thyrotropin (TSH)) on drug adsorption was also studied. Acidic model drugs (antiepileptics and benzodiazepies) adsorbed to a greater extent onto the membrane from Hepes buffer at ionic strength of 25 mM and pH 7.0 than basic drugs (antidepressants) did. Adsorption of acidic model drugs was based on electrostatic interactions between positively charged tertiary amino groups of DMAEMA side-chain and acidic negatively charged drug. Albumin diminished the adsorption of drugs from serum onto the membrane. Lipophilicity was related to the adsorption of acidic model drugs from serum onto the membrane. The degree of grafting had the greatest effect on adsorption of lipophilic drugs, but no influence was observed on adsorption of hydrophilic drugs. The present results showed that acidic drugs and albumin adsorbed onto the membrane, which suggests that the PVDF–DMAEMA membrane may be suitable for separating acidic drugs from protein-free substances for subsequent monitoring and evaluation.

Keywords: Albumin; Degree of grafting; Drug adsorption; Drug lipophilicity; PVDF–DMAEMA; Serum

1. Introduction

Solid-phase extraction is the most popular method for sample purification (Lingeman and Hoekstra-Oussoren, 1997). Membranes (e.g. ion-exchange), like chromatography columns, can be used for repeated separations of analytes (Klein, 2000). It was reported that 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 (Lingeman and Hoekstra-Oussoren, 1997; Lensmeyer et al., 1995).

Ion-exchange membranes have been used to separate molecules of interest from different kinds of liquid mixtures. In previous work we studied drug adsorption onto poly(acrylic acid) grafted poly(vinylidene fluoride) membrane (PAA–PVDF). We found that studied membrane may be suitable for separating basic drugs from proteinaceous substances, because albumin was not adsorbed onto the membrane (Åkerman et al., 1999a). Beta-lactam antibiotic drugs (penicillins and cephalosporins) were isolated from fermentation broth for further analysis by using liquid membrane (Ghosh et al., 1996). Mixed-matrix ion-exchange membrane was used in purification process of bovine serum albumin and bovine haemoglobin. Analytes were separated by adsorption onto the

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membrane (Avramescu et al., 2003). Anion-exchange membranes were used for separation of glucosinolates from seed suspensions. In an extraction procedure glucosinolates adsorbed onto the membrane and thereafter they were released from the membrane by using releasing medium. A recovery of 80% was obtained by using this procedure (Szmigielska et al., 2000; Szmigielska and Schoenau, 2000). A clean up method for ergot alkaloids by using a solid-phase extraction (SPE) disk was described. Since ergot alkaloid salts are positively charged, they can easily and selectively adsorb onto negatively charged strong cation-exchange SPE disk in separation process. Mean recovery was 88% (Ware et al., 2000).

The aim of the present investigation was to study adsorption of acidic and basic model drugs from 25 mM Hepes buffer (pH 7.0) solution and serum onto the pH responsive PVDF–DMAEMA membrane. DMAEMA is a water-soluble cationic polymer with tertiary amine side groups that have a pK_a of 8.0. Almost all amine side groups are dissociated and positively charged under physiological conditions (Van de Wetering et al., 1998, 2000). The suitability of the membrane for separation of drugs from biological fluids such as blood serum was also investigated.

2. Materials and methods

2.1. Chemicals

All the drugs studied were in HCl-form. Alprazolam, chlorpromazine, chlorprotixen, 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). Deionized (Millipore^{TM}) Milli-Q water (resistivity $\geq$ 18 MΩ/cm) was used to prepare buffered drug, albumin and IgG solutions. Hepes and human serum immunoglobulin G (IgG) were purchased from Sigma Co. (St. Louis, USA). Human serum albumin (HSA) was obtained from Red Cross Finland (Helsinki, Finland). Both 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

Preweighed hydrophobic PVDF membranes (Millipore, Co., Bedford, Massachusetts, USA) with a pore size of 0.22 μm were graft modified with 2-*N*,*N*-dimethyl aminoethyl methylacrylate (Polysciences, Inc., Warrington, PA, USA). The PVDF membranes were irradiated under nitrogen atmosphere (<200 ppm O_2) using an Electrocurtain electron accelerator (Energy Sciences Inc., Wilmington, MA, USA) operating at an acceleration voltage of 175 kV. The membranes were irradiated with 25 kGy on each side. Immediately after irradiation, the membranes were immersed at ambient temperature in a graft solution containing 30 wt.% DMAEMA for a predetermined time. In order to remove oxygen, the graft solution was purged with nitrogen for at least 30 min before grafting and then continuously throughout the experiment.

After grafting, the membranes were washed overnight with ion-exchanged water, dried in vacuo at 40 °C to constant weight and reweighed to determine the extent of grafting gravimetrically (wt.%) according to

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

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 10–150 wt.% grafted membranes. Membranes were pre-treated in a drug-free 25 mM Hepes buffer solution (20 ml, pH 7.0) for 1 h before immersing membranes in the drug and protein solutions and serum (pH 7.0 and 7.4).

2.3. Determination of drug adsorption onto the PVDF–DMAEMA membrane

2.3.1. Samples

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. Drug solutions and spiked serum were prepared by adding the drugs to 25 mM Hepes buffer (pH 7.0) solution and 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 were separated, pooled and stored at 4 °C (maximum a week) until the samples were prepared and analyzed.

2.3.2. Drug adsorption from 25 mM Hepes buffer (pH 7.0) solution

Adsorption of acidic and basic model drugs onto the membrane was studied by shaking test tubes containing 75 wt.%

grafted PVDF–DMAEMA membranes (weight varied between 6.7 and 7.5 mg, area ~0.54 cm^2) and drug solutions (1 ml; pH 7.0) at room temperature for 24 h. The membrane-free drug solutions were treated under identical experimental conditions. Concentrations of the drugs were measured by HPLC with the methods described in chapter 2.4. Adsorbed amounts of the drugs (%) were calculated from peak-height ratios.

2.3.3. Effect of degree of grafting on drug adsorption

The effect of degree of grafting on drug adsorption was studied by using 10 wt.% (weight varied between 4.2 and 6.7 mg, area 0.50 cm^2), 50 wt.% (weight varied between 4.2 and 6.7 mg) and 75 wt.% (weight varied between 5.2 and 7.5 mg, area 0.54 cm^2) grafted PVDF–DMAEMA membranes. Citalopram (basic), clonazepam (acidic), mianserin (basic), phenytoin (acidic), primidone (acidic) and thioridazine (basic) were used as model drugs. Preparation of the drug solutions, drug adsorption studies and calculations of results were carried out as described above. Drug adsorption was performed from Hepes buffer solution.

2.3.4. Drug adsorption from spiked serum

We have chosen drugs that adsorbed >10% from Hepes buffer (pH 7.0) solution onto the membrane for this experiment. Adsorption of drugs onto the membrane was studied by shaking test tubes containing 150 wt.% grafted PVDF–DMAEMA membranes (weight varied between 8.5 and 13.6 mg, area ~0.75 cm^2) and spiked serum (1 ml; pH 7.4) at room temperature for 24 h. Samples in the absence of membranes 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) using 100 mg Bond-Elut® C_{18} 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 (Åkerman et al., 1996, 1998; Åkerman, 1996, 1997). 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 in Section 2.4. Adsorbed amounts of the drugs were calculated from peak-height ratios.

2.4. Analysis of the drugs

The concentrations of the drugs were analyzed by HPLC (Åkerman et al., 1996, 1998; Åkerman, 1996, 1997) or with other slightly modified HPLC methods. The drugs were separated chromatographically by using a Select-B C_8 4.0 mm × 125 mm (VWR International AB), Symmetry C_8 4.6 mm × 150 mm (Waters, Milford, MA, USA), LiChroCart C_{18} 4.0 mm × 250 mm (VWR International AB) or NovaPak C_{18} 4.6 mm × 150 mm analytical column (Waters). The elution was isocratic with a mobile phase of acetonitrile–50 mM dipotassium hydrogen phosphate, pH 4.7 (40:60, v/v), 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, v/v/v), 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.

2.5. Determination of albumin and IgG adsorption

Adsorption of albumin and IgG onto the membrane was studied by shaking test tubes containing 75 wt.% grafted PVDF–DMAEMA membranes (weight varied between 5.5 and 6.2 mg, area ~0.45 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 concentrations were measured by kinetic nephelometry with a Beckman Array protein analyzer (Beckman Co., Brea, CA, USA). Adsorbed amounts were calculated from concentration ratios.

2.6. Determination of cortisol, T_4F and TSH adsorption

The blood samples were collected as described in Section 2.3.1. Specimens were authentic patient serum samples. Adsorption of cortisol, T_4F and TSH onto the membrane was studied by shaking test tubes containing 75 wt.% grafted PVDF–DMAEMA membranes (weight varied between 7.3 and 7.9 mg, area ~0.55 cm^2) and 1 ml of serum for 24 h at room temperature. The membrane-free serum samples were treated under identical experimental conditions. The concentrations of TSH and T_4F 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.

3. Results and discussion

In this case, drugs will be absorbed on the DMAEMA-chains through the mechanisms of ion exchange, i.e. the positively charged polymer chains will exchange their negatively charged counter ion (OH^-, Cl^-) for preferably a negatively charged drug as described in Section 3.1.1.

However, the amount of absorbed drug (Table 1) is affected by several things:

Table 1
Amounts of drugs adsorbed from 25 mM Hepes buffer (pH 7.0) and spiked serum pool onto the PVDF–DMAEMA membrane

Drug	pK_a [a]	log P[b]	Adsorbed amount (%)	
			25 mM Hepes buffer (pH 7.0) (75 wt.% DMAEMA)	Serum (150 wt.% DMAEMA)
Alprazolam (A)	2.4	3.2	ND	NT
Carbamazepine (A)		2.5	9.9 ± 8.2	NT
Clobazam (A)	<6.0	1.0	21.2 ± 3.4	12.9 ± 2.6
Clonazepam (A)	1.5, 10.5	2.4	50.8 ± 3.0	31.2 ± 7.7
Diazepam (A)	3.4	2.8	15.6 ± 1.2	18.0 ± 4.9
Flunitrazepam (A)	1.8	2.1	31.1 ± 3.3	27.0 ± 8.4
Hydroxycarbazepine (A)			35.4 ± 9.7	12.0 ± 1.9
Lamotrigine (A)	5.5	0.1	17.7 ± 11.6	11.8 ± 6.5
Medazepam (A)	6.2	4.4	54.1 ± 11.4	52.7 ± 13.0
Midazolam (A)	6.2	3.7	44.1 ± 2.8	7.1 ± 6.1[c]
Nitrazepam (A)	3.2, 10.8	2.3	39.7 ± 5.7	24.0 ± 8.6
Norclobazam (A)			23.6 ± 3.3	15.9 ± 1.5
Nordiazepam (A)	3.5, 12.0		17.6 ± 1.2	16.1 ± 4.2
Oxazepam (A)	1.7, 11.3	2.2	15.6 ± 1.5	19.1 ± 3.5
Oxcarbazepine (A)			53.6 ± 9.8	69.8 ± 4.1
Pentobarbital (A)	7.4	1.5	49.3 ± 3.6	19.2 ± 4.3
Phenobarbital (A)	8.1	1.9	48.7 ± 6.7	20.8 ± 3.7
Phenytoin (A)	8.3	2.5	72.6 ± 4.2	27.4 ± 7.4
Primidone (A)	13	0.9	16.7 ± 13.1	17.4 ± 7.8
Temazepam (A)	1.3	2.2	26.3 ± 2.4	11.5 ± 4.7
Zopiclone (A)	6.7	1.0	9.2 ± 2.9	NT
Amitriptyline (B)	9.4	5.0	7.6 ± 2.1	NT
Chloropramazine (B)	9.3	3.4	24.6 ± 0.9	8.1 ± 5.3
Chloroprotixen (B)	8.8	2.7	41.9 ± 5.5	5.1 ± 3.7
Citalopram (B)	9.5	0.6	1.8 ± 2.1	NT
Clomipramine (B)	9.5	5.2	11.7 ± 3.0	7.8 ± 3.3
Clozapine (B)	8.0	4.3	35.4 ± 4.0	11.3 ± 6.9
Desipramine (B)	9.4	4.9	1.3 ± 1.2	NT
Dm-citalopram (B)			1.9 ± 0.3	NT
Dm-maprotiline (B)			1.5 ± 3.8	NT
Doxepin (B)	9.0	2.4	5.6 ± 1.1	NT
Fluoxetine (B)		0.6	6.2 ± 1.6	NT
Haloperidol (B)	8.3	3.4	14.7 ± 0.6	9.4 ± 2.2
Imipramine (B)	9.5	2.5	4.4 ± 0.8	NT
Levomepromazine (B)	9.2	4.7	15.6 ± 4.8	8.0 ± 3.0
Maprotiline (B)	10.2	4.2	1.4 ± 3.1	NT
Mianserin (B)	7.1	4.3	44.3 ± 3.1	11.5 ± 3.5
Norclomipramine (B)			3.5 ± 4.3	NT
Norclozapine (B)			10.1 ± 2.6	16.6 ± 5.4
Nordoxepin (B)			3.5 ± 1.1	NT
Norfluoxetine (B)			10.7 ± 1.5	6.6 ± 3.6
Nortrimipramine (B)			5.9 ± 1.5	NT
Nortriptyline (B)	9.7	1.7	3.5 ± 1.0	NT
Protriptyline (B)		1.2	2.7 ± 2.7	NT
Thioridazine (B)	9.5	5.9	23.4 ± 2.7	0.2 ± 5.9
Thiothixen (B)			9.0 ± 2.3	13.4 ± 5.7
Trazodone (B)	6.7		10.2 ± 3.3	5.6 ± 17.1
Trimipramine (B)	7.7	4.7	10.5 ± 2.5	NT

Mean ± S.D. ($n = 5$); ND: not detectable; NT: not tested; A: behaves like acid; B: behaves like base.
[a] pK_a values were obtained from Jack (1992).
[b] log P values were obtained from Dollery (1999).
[c] Midazolam was omitted from Fig. 1.

1. The concentration of the drug in the drug solution: higher drug concentrations gives 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 amount of drug adsorption.
3. The counter ion originally "attached" on each exchange site: the membrane prefer counter ions in a certain order,

depending on which counter ion originally is attached to the membrane ion-exchange will occur or not.

4. The molecular size and complexicity 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. Drug adsorption onto the PVDF–DMAEMA membrane from 25 mM Hepes buffer (pH 7.0) and serum

3.1.1. Acidic model drugs

Amounts of drugs adsorbed onto the DMAEMA grafted PVDF membrane (disappearance of the drugs from the sample) from Hepes buffer solution (pH 7.0, $\mu = 25$ mM) and serum are presented in Table 1. All drugs tested in Hepes were not tested in serum. Environmental pH affects ionization of both the acidic drug and the DMAEMA groups. The pK_a value of DMAEMA is ~8.0 and at pH 7.0 it is able to dissociate carrying a positive surface charge (Van de Wetering et al., 1998, 2000). Interactions between acidic drugs and the membrane were much stronger than interactions between basic drugs and the membrane. Adsorption varied between not detected and 72.6% from buffer solution ($n = 20$) and from 7.1% to 69.8% from serum ($n = 18$), respectively. Clonazepam, medazepam, oxcarbazepine, pentobarbital, phenobarbital and phenytoin adsorbed most extensively onto the membrane (adsorption varied between 49% and 73% from buffer solution). These acidic antiepileptic drugs studied are highly lipophilic ($\log P > 1.5$). Medazepam and oxcarbazepine adsorbed onto the membrane most effectively from serum (adsorbed amount was $52.7 \pm 13.0\%$ and $69.8 \pm 4.1\%$, respectively). Medazepam is known to be the most lipophilic acidic model drug in this study ($\log P = 4.4$). The mean adsorbed amount of acidic model drugs tested both in Hepes buffer and serum ($n = 18$) was $35.2 \pm 17.1\%$ of the initial dose from buffer solution and that of $23.0 \pm 15.5\%$ from serum, respectively. At pH values greater than 2 units below their pK_a value, acidic drugs are undissociated. At the pH range studied, 7.0–7.4, at pK_a of drug <7.0, they are dissociated and negatively charged. When pH is equal to the pK_a, the drug molecules are dissociated exactly 50% (Florence and Attwood, 1998). In the present study the pK_a values of acidic drugs varied from 1.3 to 13 due to the variation in their adsorption onto the membrane. The pK_a values are given in Table 1. We observed a trend of decrease of adsorption of clobazam, clonazepam, hydroxycarbazepine, midazolam, nitrazepam, norclobazam, pentobarbital, phenobarbital, phenytoin and temazepam from serum onto the 150 wt.% grafted PVDF–DMAEMA membrane compared with mean adsorbed amounts obtained from Hepes buffer (pH 7.0) (see Table 1).

Results of our study clearly indicate that the ionic interaction between a negatively charged acidic drug molecule and the positively charged tertiary amine group of DMAEMA was the most important factor affecting drug adsorption onto the membrane. Previous studies have also shown similar electrostatic interactions between drugs and polymer materials. We have studied drug adsorption onto anionic PVDF–PAA membrane and found that basic drugs adsorbed most onto the membrane due to electrostatic interactions (Åkerman et al., 1999a). Bioadhesive properties of the hydrophobic, basic polyelectrolyte hydrogel discs containing crosslinked *N*,*N*-dimethylaminoethyl methacrylate-*co*-methyl methacrylate were evaluated. The results indicated that initial bioadhesive contact may be the result of surface energy effects and electrostatic interactions of oppositely charged groups between mucin and the gel (Quintanar-Guerrero et al., 2001). The mechanisms of interaction between Eudragit RS100 and RL100 polymers with diflunisal, flurbiprofen and piroxicam that belong to non-steroidal anti-inflammatory drug group were evaluated (Pignatello et al., 2002). They observed that drugs interacted strongly with the ammonium groups present in polymers by electrostatic interactions. Acrylic resin matrix films (Eudragits RL and RS) and mechanisms of drug–polymer interactions have been characterized. Salicylic acid and chlorpheniramine maleate were used as model drugs. Acidic salicylic acid interacted with these Eudragits polymers (contain quaternary ammonium groups) primarily via ionic electrostatic interactions (Jenquin and McGinity, 1994). The interaction of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels was studied. The drug molecules interacted weakly with the polymers through ionic interactions (Rodriguez et al., 2003). However, in addition to ionic interactions, there could also be loose non-ionic interactions like Van der Waals forces or hydrophobic interactions that affect on drug adsorption (Van de Wetering et al., 2000; Rodriguez et al., 2003; Dahlstrom et al., 2004).

3.1.2. Basic model drugs

Amounts of drugs adsorbed onto the membrane are shown in Table 1. All drugs tested in Hepes buffer were not tested in serum. Basic model drugs adsorbed onto the PVDF–DMAEMA membrane only slightly. Adsorption onto the membrane varied between 1.3% and 44.3% from buffer solution ($n = 27$) and between 0.2% and 16.6% from serum ($n = 12$), respectively. The greatest adsorbed amount was observed for mianserin and chloroprotixen from Hepes buffer (mean adsorbed amounts 44% and 42%, respectively). The mean adsorbed amount of drugs tested both in Hepes buffer and the serum ($n = 12$) was $21.0 \pm 13.0\%$ from buffer solution and that of $8.6 \pm 4.3\%$ of the initial drug doses from serum. At pH values greater than two units above their pK_a value, basic drugs are non-dissociated; and so in physiological conditions at the pK_a of the drug >7.0, they are dissociated and positively charged (Florence and Attwood, 1998). The mean adsorbed amounts of chlorpromazine, chlorprotixen, clozapine, haloperidol, levomepromazine, mianserin and thioridazine from serum onto the 150 wt.% grafted PVDF–DMAEMA membrane decreased compared with results obtained from Hepes buffer (pH 7.0) (see Table 1). The results indicate that, at the pH studied, there is electrostatic repulsion between a dissociated positively charged drug and a positively charged membrane. As discussed in Section 3.1.1, non-ionic interactions, such as hydrogen bonding, and van der Waals forces may affect loose interactions between drugs and the studied polymers (Van de Wetering et al., 2000; Rodriguez et al., 2003; Dahlstrom et al., 2004). Basic

drugs may have adsorbed onto the membrane via non-ionic interactions.

3.2. Effects of proteins and hormones on drug adsorption

Adsorption of acidic drugs onto the PVDF–DMAEMA membrane decreased 12.2% and basic drugs 12.4% from serum, respectively. Albumin concentration in test buffer varied from 0.005 to 50 g/l and adsorbed between not detected to 94.8%. The highest adsorption was in albumin concentration of 0.5 g/l. In physiological albumin concentration (reference range: 36–48 g/l; Laboratory Centre, Kuopio University Hospital, Kuopio, Finland) the adsorption onto the membrane varied from 8.1% to 16% (see Table 2). Since albumin binds acidic and basic drugs in serum, the reduced drug adsorption onto the PVDF–DMAEMA membrane from serum was most probably due to the distribution of the drugs between albumin and the PVDF–DMAEMA membrane. We observed that albumin adsorbed onto the membrane and that may also reduce drug adsorption. Drug adsorption onto the PVDF–PAA membrane in the presence and absence of albumin was studied (Åkerman et al., 1999a). We found that since albumin binds desipramine and thioridazine at physiological pH, the 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. The structural characteristics of activated carbons and ibuprofen adsorption affected by bovine serum albumin (BSA) were evaluated. It was found that the non-uniformity of ibuprofen adsorption to complexes diminished with the presence of BSA. This effect may be explained by a partial adsorption of ibuprofen onto protein molecules immobilized on carbon particles and blocking of a portion of narrow pores (Melillo et al., 2004). In addition, we found that IgG adsorbed <10% onto the 50 wt.% grafted PVDF–DMAEMA membrane (Table 3). The amount of adsorbed cortisol was 6.6 ± 10.9% and that of T_4F 10.7 ± 2.4%. TSH, on the other hand, did not adsorb onto the membrane (Table 4). Reference ranges of studied hormones are shown in Table 4. We propose that they may also prevent drug adsorption onto the membrane from serum, but that was not properly investigated.

Table 2
Amounts of albumin adsorbed from 25 mM Hepes buffer (pH 7.0) onto the 75 wt.% grafted PVDF–DMAEMA membrane

Concentration added (g/l)	Adsorbed amount (%)
0.005	ND
0.02	15.5 ± 16.1
0.04	44.6 ± 6.0
0.08	72.4 ± 2.5
0.1	79.2 ± 1.8
0.5	94.8 ± 0.4
0.7	71.8 ± 1.6
0.8	75.7 ± 0.9
23.9	16.0 ± 2.4
47.3	8.1 ± 4.7

Mean ± S.D. (n = 3); ND: not detectable.

Table 3
Amounts of IgG adsorbed from 25 mM Hepes buffer (pH 7.0) onto the 50 wt.% grafted PVDF–DMAEMA membrane

Concentration added (g/l)	Adsorbed amount (%)
0.01	ND
0.03	ND
0.07	ND
0.1	ND
0.4	7.5 ± 1.3
0.7	6.4 ± 0.9
0.9	9.6 ± 1.5
18.5	4.1 ± 1.6

Mean ± S.D. (n = 3); ND: not detectable.

3.3. Influence of degree of grafting on drug adsorption

Adsorption capacity of the membrane was studied as a function of the grafting. We found that the degree of grafting did not affect on the adsorption of citalopram (basic), primidone (acidic) and thioridazine (basic) onto the membrane. Adsorbed amounts of clonazepam (acidic), mianserin (basic) and phenytoin (acidic) increased with degree of grafting (Table 5). If the degree of grafting increased from 50 to 75 wt.%; however, adsorption of drugs did not increase. These drugs are reported to be very lipophilic ($\log P$ = 2.4–5.9). These results emphasized the fact that the degree of grafting had the greatest impact on adsorption of lipophilic drugs. In some other publication it was observed that when the degree of grafting was increased from 7 to 58 wt.% the adsorption of propranolol (basic) onto the PVDF–PAA membrane increased slightly, but a plateau in adsorption was observed with higher grafting rates of the membrane. However, binding of propranolol–HCl per mole of PAA decreased at higher degrees of grafting. This result indicated that increasing the grafting does not linearly increase the accessible adsorption sites in the membrane. These results suggested that a highly grafted polymer forms a compact structure and the reduced electrostatic repulsion of the charged carboxyl groups also prevents drug adsorption to the inner part of membrane (Åkerman et al., 1999b). The same phenomena may also explain our parallel results. The effect of degree of grafting on fibronectin adsorption was studied. When degree of grafting increased, the adsorption of fibronectin onto hydrophilic poly(ethylen glycol) polymer decreased (Altankov et al., 2000). Study was not comparable with systems based on ion exchange (e.g. DMAEMA-membranes).

Table 4
Amounts of hormones adsorbed from authentic patient serum samples onto the 75 wt.% grafted PVDF–DMAEMA membrane

Hormone	Concentration range	Reference range[a]	Adsorbed amount (%)
Cortisol	116–822 nmol/l	170–540 nmol/l[b]	6.6 ± 10.9
T_4F	11–24 pmol/l	12–22 pmol/l	10.7 ± 2.4
TSH	0.01–4.3 mU/l	0.3–4.2 mU/l	ND

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

Table 5
The effect of degree of grafting on the adsorption of drugs from 25 mM Hepes buffer (pH 7.0) onto the PVDF–DMAEMA membrane

Drug	pK_a [a]	log P [b]	Adsorbed amount (%)		
			10 wt.% DMAEMA	50 wt.% DMAEMA	75 wt.% DMAEMA
Clonazepam (A)	1.5, 10.5	2.4	10.5 ± 2.5	46.4 ± 3.3	49.4 ± 1.6
Phenytoin (A)	8.3	2.5	22.8 ± 7.5	61.0 ± 0.8	64.3 ± 2.8
Primidone (A)	13.0	0.9	7.1 ± 7.4	0.7 ± 1.0	6.5 ± 3.9
Citalopram (B)	9.5	0.6	0.5 ± 1.6	1.0 ± 0.7	ND
Mianserin (B)	7.05	4.3	14.8 ± 3.7	33.0 ± 4.0	39.7 ± 5.2
Thioridazine (B)	9.5	5.9	6.5 ± 2.8	13.6 ± 3.9	12.2 ± 6.6

Mean ± S.D. ($n = 3$); ND: not detectable; A: behaves like acid; B: behaves like base.
[a] pK_a values were obtained from Jack (1992).
[b] log P values were obtained from Dollery (1999).

3.4. Effect of lipophilicity of the acidic drug on the adsorption

The effect of lipophilicity was evaluated on adsorption of acidic model drugs from Hepes buffer solution (pH 7.0) and the serum onto the 75 wt.% grafted PVDF–DMAEMA membrane. Lipophilicity is an important factor that affects on the drug partitioning within the polymers (Sung et al., 1990). Lipophilic drugs with high partition coefficients are able to interact more extensively with the hydrophobic phases than the hydrophilic drugs (Schoenwald and Huang, 1983). In our previous study we have proposed that adsorption of basic drugs onto the PVDF–PAA membrane was related to the lipophilicity of the drug (Åkerman et al., 1999a). The log P values of each of the studied drugs are given in Table 1. Lamotrigine is the hydrophilic acidic drug (log $P = 0.1$) that adsorbed from Hepes buffer solution and serum onto the membrane as effectively as other acidic lipophilic model drugs. Although, like lamotrigine and primidone are hydrophilic acidic drug (log $P = 0.9$), they adsorbed onto the membrane only slightly from both adsorption media. Adsorption was greatest for highly lipophilic acidic drugs (log $P > 1.5$) from Hepes buffer solution (pH 7.0) (see Table 1). However, there was no relationship between drug lipophilicity and adsorption onto the membrane in the Hepes buffer solution (pH 7.0) observed ($R^2 = 0.2211$). When drugs were adsorbed from serum onto the membrane, the adsorption of acidic drugs was related to drug lipophilicity ($R^2 = 0.6483$, $p < 0.001$) (Fig. 1). The result of midazolam was omitted from the Fig. 1, because its adsorption differed significantly from the adsorption of other acidic drugs tested in this experiment. Thus, we propose that lipophilicity enhances the adsorption of acidic drugs from the serum onto the PVDF–DMAEMA membrane.

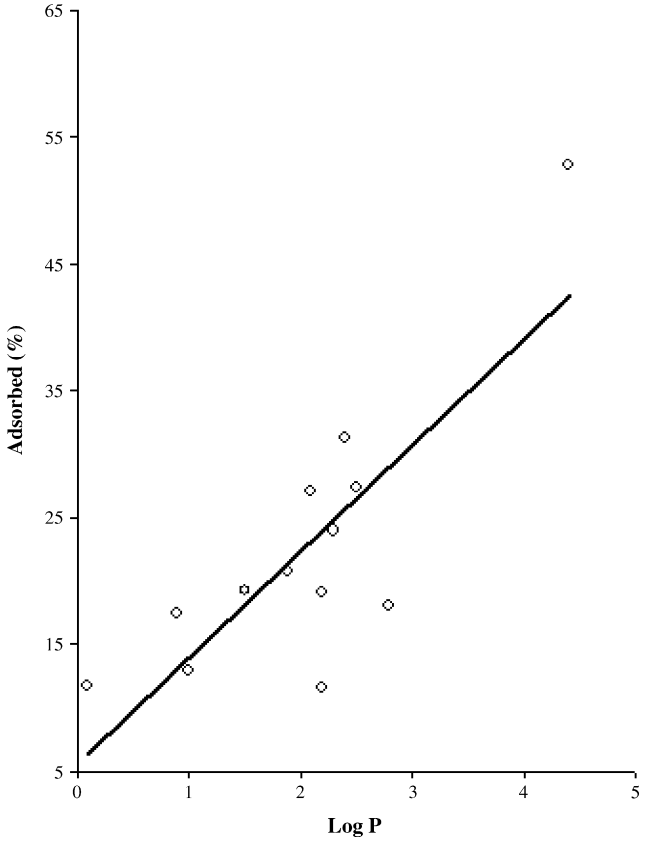

Fig. 1. Effect of lipophilicity (log P) on adsorption of acidic model drugs from serum onto the 150 wt.% grafted PVDF–DMAEMA. $R^2 = 0.6483$, $p < 0.001$ ($n = 13$). *Result of midazolam was omitted from the figure (see Table 1).

4. Conclusions

The effect of the charge of model drugs was studied to determine the interaction between these drugs and PVDF–DMAEMA anion-exchange membranes. In the case of acidic model drugs that emphasize the importance of ionic interactions between the drug and the membrane in the adsorption phenomena, the extent of dissociation of DMAEMA chains at physiological pH strongly affects the amount of adsorption. Lipophilicity of the acidic drug enhanced its adsorption onto the hydrophobic membrane from the serum. When the effect of degree of grafting on adsorption of the drug onto the membrane was evaluated, the results emphasized that degree of grafting influenced most on adsorption of lipophilic drugs. Albumin in serum diminished the adsorption of drugs onto the membrane. In conclusion, the PVDF–DMAEMA membrane may be suitable for separating acidic drugs such as clonazepam, flunitrazepam, hydroxycarbazepine, medazepam, midazolam, nitrazepam, oxcarbazepine, pentobarbital, phenobarbital and phenytoin from protein-free medium for subsequent monitoring and evaluation.

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