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Suitability of Smopex®-102 cation-exchange fiber for analytical purposes and drug monitoring

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The present study aimed to evaluate the suitability of Smopex®-102 cation-exchange fiber for the separation of acidic and basic model drugs from biological fluids (e.g. serum) prior to chromatographic analysis. In addition, the interactions of the drugs with the fiber were studied. The study found that basic antidepressant model drugs bound to a considerably greater extent than acidic drugs to poly(acrylic acid) (PAA) grafted Smopex®-102 cation-exchange fiber from 25 mM HEPES buffer (pH 7.0) and spiked serum. Drug binding from serum decreased except for acidic drugs due to drug distribution between serum proteins and cation-exchange fiber. Electrostatic interactions were possibly the most important factors affecting drug binding to the fiber. Basic drugs were released most effectively from the fiber by using acetic acid (mean released amount 123.7 ± 36.3% and mean absolute recovery 95.4 ± 23.8%). Results demonstrated that the cation-exchange fiber evaluated might be a potential material for separating basic drugs from protein–free and proteinaceous (e.g. serum) liquid solutions for subsequent monitoring and evaluation. However, the drug release solution and release time must be optimized more precisely in order to validate described sample preparation method for each basic drug.

1. Introduction

Nowadays traditional solid-phase-extraction is the most commonly used method for sample preparation (Lingeman et al. 1997). Ion-exchange fibers have been widely used in many separation processes due to their high drug loading capacity, easy drug loading procedure and fast ion-exchange rate (Chen et al. 1996). Over the years published applications have described separation of rare earth elements (Asami et al. 1985), enrichment of uranium from seawater (Chen et al. 1996), air purification (Soldatov et al. 1988) and chromatographic methods (Stevens et al. 1982). Furthermore, surface modified micro-filtration membranes, particle-loaded membranes and particle-embedded glass fiber disks have been widely used to isolate and concentrate selected compounds from liquid solutions prior to chromatographic analyses (Lingeman et al. 1997; Lensmeyer et al. 1995).
Numerous recently published papers deal with drug binding affinity to ion-exchange fibers (Yu et al. 2006; Jaskari et al. 2000, 2001; Kankkunen et al. 2002; Hänninen et al. 2003, 2005). Ion-exchange fibers are usually similar like cotton clothing consisting of a backbone that is typically a non-crosslinked water insoluble hydrophobic hydrocarbon chain, such as polyethylene, polypropylene or viscose backbone with positively (anion-exchange) or negatively charged (cation-exchange) functional grafted groups. The charged groups are grafted to the backbone by radiation under an electron beam. The ion-exchangers may be strong or weak depending on the acidic or basic character of the ionic group. Weakly acidic groups such as poly(acrylic acid) (PAA) are dissociated only under high pH conditions (Jaskari et al. 2001). The poly(ethylene-g-acrylic acid) of the Smopex®-102 cation-exchange fiber is an environmentally sensitive polymer, which undergoes conformational changes as a function of pH and ionic strength. The fiber has a negative charge, which gives it cation-exchange properties due to the dissociation of the carboxylic acid groups in the grafted PAA-chains. Negatively charged PAA is thought to bind positively charged molecules, such as drugs (Tarvainen et al. 1999; Åkerman et al. 1999).
Anion- and cation-exchangers have been used for the separation of compounds of interest from different kinds of liquid solutions. In previous work drug adsorption on to a pH-responsive PVDF-DMAEMA anion-exchange membrane was studied. The results demonstrated that acidic drugs and albumin were adsorbed on to the membrane, which suggests that the PVDF-DMAEMA membrane may be suitable for separating acidic drugs from a protein-free solution for subsequent monitoring and evaluation (Karppi et al. 2007a). Adsorption of drugs on to a PVDF-PAA ca-

tion-exchange membrane has been evaluated in many studies (Tarvainen et al. 1999; Åkerman et al. 1999a; Karppi et al. 2007b). The membrane studied may be suitable for isolating basic drugs from protein containing liquids, because albumin was not adsorbed onto the membrane. Basic drugs are adsorbed on to the membrane to a considerably greater extent than acidic drugs. Ware et al. (2000) have described a clean up method for ergot alkaloids using a solid-phase extraction (SPE) disk. Ergot alkaloid salts are positively charged, so they can be adsorbed easily and selectively on to the negatively charged strong cation-exchange SPE disk. Mean recovery was 88%. Ion-exchange mixed-matrix membranes have been used for isolation of bovine serum albumin and bovine haemoglobin and ethylene vinyl alcohol membranes for bilirubin removal from liquid solutions (Avramescu et al. 2003, 2004). Anion-exchange membranes have been used for separation of glucosinolates from seed suspensions. In the extraction procedure, the glucosinolates were adsorbed on to the membrane and were released from the membrane afterwards using releasing medium. A recovery of 80% was obtained using this procedure (Szmigielska et al. 2000a, b).

The aim of the present study was to evaluate the suitability of Smopex®-102 cation-exchange fiber for the separation of acidic and basic drugs from biological fluids (e.g. serum) prior to chromatographic analysis. Drug binding and release were examined.

2. Investigations and results

2.1. Drug binding to Smopex®-102 cation-exchange fiber

The amounts of model drugs bound to the cation-exchange fiber from HEPES buffer solution (pH 7.0) and spiked serum (shown by disappearance of the drugs from the sample) are presented in the Table. Results are expressed as mean ± SD. The binding capacity of staple fiber has been reported to be 8 mmol/g (Jaskari et al. 2001). Basic model drugs (antidepressants) bound to the fiber to a considerably greater extent than did acidic drugs. Binding of basic drugs ranged from 86.2 to 100.0% of the initial drug doses in HEPES buffer solution (mean 96.8 ± 2.8%) and were from 22.2 to 92.3% of the initial drug doses in spiked serum (mean 74.3 ± 15.6%). However, though basic the most lipophilic drug thioridazine bound only slightly to the fiber (22.2 ± 8.6%). Binding of basic drugs from spiked serum was 22% lower. Acidic model drugs (antiepileptic drugs and benzodiazepines) bound to the cation-exchange fiber as effectively from HEPES buffer solution as from serum. Adsorption ranged between 34.9 and 92.1% of the initial drug doses from HEPES buffer (mean 56.3 ± 14.6%). Amounts bound from spiked serum varied from 33.1 to 91.6% of the initial drug doses (mean 57.1 ± 15.9%).

2.2. Drug release and absolute recoveries

2.2.1. Drugs bound from buffer solution

Results are expressed as mean ± SD. Acidic model drugs (n = 21) were released most effectively from the fiber by using solutions: 10% formic acid in methanol (v/v) (pH 1.6) and methanol. Mean amounts released were 72.4 ± 21.5% and 73.7 ± 29.7% respectively. Corresponding mean absolute recoveries were 43.7 ± 22.1% and 38.2 ± 22%. For basic drugs (n = 27) 10% formic acid in methanol was the most suitable (mean amount released 71.2 ± 26.5% and mean absolute recovery 69.3 ± 26%, respectively).

2.2.2. Drugs bound from spiked serum

When using acetic acid (>99.7%) the mean amount of acidic drugs (n = 20) released was 62.9 ± 36.1% and the mean absolute recovery was 39.7 ± 22.6%. The mean amount released for basic drugs (n = 25) was 123.7 ± 36.3% and the mean absolute recovery was 95.4 ± 23.8%, respectively. The mean absolute recovery of acidic drugs was poor.

3. Discussion

3.1. Effect of charge on drug binding

In the present study basic model drugs were bound to the Smopex®-102 cation-exchange fiber to a considerably greater extent than were acidic model drugs from both adsorption media. The binding of basic model drugs from serum was reduced due to drug distribution between serum proteins and the cation-exchange fiber. Åkerman et al. (1999a) observed that PAA does not bind albumin and it might be suggested that PAA grafted Smopex®-102 cation-exchange fiber does not bind albumin, but that was not tested in the present study.

The pH of the external adsorption medium affects both the drug and the dissociation of grafted poly (acrylic) acid. The pK_a value of the PAA is ~4.0 due to PAA being able to dissociate at high pH (7.0) as a weak cation-exchanger (Park et al. 1987). Smopex®-102 cation-exchange fiber is therefore fully dissociated and negatively charged at physiological pH. Drugs will be bound to the PAA-chains of the Smopex®-102 cation-exchange fiber by an ion exchange mechanism, i.e. the negatively charged polymer chains will exchange their positively charged counter ion (H^+) preferentially for a positively charged drug. The amount of bound drug (Table) is affected by several things: The concentration of the drug in the drug solution; higher drug concentrations give a higher amount of bound drug since the ion exchange process is faster. The counter ion originally "attached" on each exchange site; the fibers prefer counter ions in a certain order, so depending on which counter ion is attached to the fiber originally, ion-exchange will occur or not. The molecular size and complexity of the drug; complex molecules with high molecular mass will occupy more than one binding site → reduced binding capacity for the drugs (for example in protein containing solutions). Other effects that have an influence on drug binding are for example ionic strength, the dielectric value of the solution, the degree of drug molecule ionization, and non-ionic interactions.

Ion-exhange fibers are able to bind ionic compounds by two mechanisms. The first layer of molecules adsorb strongly via electrostatic interactions. These strong bonds have a chemical nature, and only dissociated molecules are able to adsorb into that layer, where the concentration of adsorbed molecules is very high. Molecules may also adsorb into a second layer via weak non-electrostatic forces (hydrophobic interactions, hydrogen bonding). Hydrophobic interactions may occur between the side chains of the adsorbed molecules. It has been observed that both dissociated and non-dissociated molecules will be present in the second layer. These non-ionic interactions may explain the incorporation of drugs into ion-exchange fibers of similar charge (Kankkunen et al. 2002; Hänninen et al. 2003). The same phenomenon was observed in the present study, because acidic negatively charged drugs were adsorbed to Smopex®-102 cation-exchange fiber of similar charge.

Table: Amounts of the drugs bound to poly(acrylic acid) (PAA) grafted Smopex®-102 cation-exchange fiber from 25 mM HEPES buffer (pH 7.0) and spiked serum

Drug	pK_a[a]	log P[a]	Binding (%)	
			25 mM Hepes buffer, pH 7.0	Serum
Alprazolam (A)	2.4	2.12; 2.30[b]	67.3 ± 8.6	72.0 ± 4.1
Carbamazepine (A)	7.0	2.45; 1.98	69.1 ± 7.6	68.5 ± 5.2
Clobazam (A)	<6.0	0.95; 2.65	55.4 ± 7.9	49.7 ± 4.1
Clonazepam (A)	1.5, 10.5	2.41; 2.84	59.0 ± 7.8	49.7 ± 4.4
Diazepam (A)	3.3	2.80; 3.18	73.1 ± 3.2	56.5 ± 7.5
Flunitrazepam (A)	1.8	2.06; 2.36	59.2 ± 7.7	60.2 ± 4.3
Hydroxycarbazepine (A)			33.1 ± 3.8	41.9 ± 2.5
Lamotrigine (A)	5.5	2.08	91.6 ± 5.2	92.1 ± 3.8
Medazepam (A)	6.2	4.41; 4.47	67.9 ± 11.6	71.0 ± 4.2
Midazolam (A)	6.15	3.37	81.0 ± 8.6	79.4 ± 8.3
Nitrazepam (A)	3.2, 10.8	2.25; 2.53	59.9 ± 7.9	55.6 ± 5.5
Norclobazam (A)			50.7 ± 7.1	47.9 ± 4.4
Nordiazepam (A)	3.5, 12.0	2.93; 3.01[b]	72.8 ± 3.5	63.9 ± 6.1
Oxazepam (A)	1.7, 11.3	2.24; 2.1	56.4 ± 3.7	60.0 ± 7.8
Oxcarbazepine (A)			42.2 ± 4.1	55.6 ± 5.3
Pentobarbital (A)	8.0	2.07; 2.11	40.3 ± 3.9	47.2 ± 7.4
Phenobarbital (A)	7.4	1.47; 1.36	32.8 ± 4.2	42.9 ± 5.8
Phenytoin (A)	8.3	2.47; 2.09	49.5 ± 4.9	34.9 ± 5.0
Primidone (A)	13.0	0.91; 1.74	34.2 ± 4.1	40.9 ± 12.2
Temazepam (A)	1.6	2.19; 2.4	58.8 ± 8.8	35.0 ± 3.0
Zopiclone (A)	6.7	0.98	45.7 ± 4.9	56.4 ± 6.0
Amitriptyline (B)	9.42	5.04; 4.64	98.6 ± 1.3	76.3 ± 6.8
Chloropromazine (B)	9.3	5.35; 5.20	86.2 ± 2.4	50.8 ± 7.9
Chloroprothixene (B)	7.6	5.18; 5.30	98.8 ± 0.7	65.0 ± 6.9
Citalopram (B)	9.5	2.98	98.4 ± 0.8	87.8 ± 1.6
Clomipramine (B)	9.38	5.19; 5.30	95.6 ± 1.3	73.4 ± 5.1
Clozapine (B)	8.0	4.30	95.1 ± 1.7	85.6 ± 5.9
Desipramine (B)	10.44	4.9; 4.09	93.5 ± 0.8	85.4 ± 6.5
Dm-citalopram (B)			99.0 ± 0.5	90.0 ± 1.8
Dm-maprotiline (B)			96.2 ± 1.6	66.0 ± 3.9
Doxepin (B)	9.0	3.88	98.9 ± 0.4	81.6 ± 6.2
Fluoxetine (B)	8.7	4.05	100.0 ± 0.0	71.0 ± 10.7
Haloperidol (B)	8.3	3.36; 3.52	95.1 ± 0.3	92.3 ± 1.8
Imipramine (B)	9.5	4.8; 4.41	98.2 ± 0.9	66.7 ± 12.5
Levomepromazine (B)	9.2	4.70	95.8 ± 1.8	62.1 ± 4.2
Maprotiline (B)	10.5	4.22	96.0 ± 1.9	64.6 ± 5.3
Mianserin (B)	7.05	4.26	97.3 ± 0.3	82.2 ± 4.4
Norclomipramine (B)			97.7 ± 1.1	74.3 ± 4.6
Norclozapine (B)			95.6 ± 1.6	87.5 ± 5.8
Nordoxepin (B)			99.2 ± 0.4	82.8 ± 5.9
Norfluoxetine (B)			100.0 ± 0.0	85.1 ± 7.8
Nortrimpramine (B)			98.8 ± 0.7	75.6 ± 6.3
Nortriptyline (B)	9.7	4.28; 4.32	96.2 ± 0.5	79.2 ± 5.9
Protriptyline (B)	10.0	4.32	94.9 ± 1.3	84.5 ± 5.3
Thioridazine (B)	9.5	5.9; 6.42	99.3 ± 0.6	22.2 ± 8.6
Thiotixene (B)	7.67; 4.8	3.78; 4.80	96.2 ± 0.1	50.4 ± 5.5
Trazodone (B)	6.14	4.0	94.3 ± 2.1	81.2 ± 3.1
Trimipramine (B)	8.0	4.73	98.8 ± 0.7	74.9 ± 6.5

A: behaves like acid; B: behaves like base.
[a]Hansch 1990.
[b]Zhao et al. (2001)
Mean ± S.D. % (n = 5)

In many previous studies drug binding to ion-exchange fibers has been widely evaluated. Kankkunen et al. (2002) observed that zwitterionic levodopa was adsorbed on to both cation-exchange fibers (S-101 and S-102) and anion-exchange fibers (S-103 and S-105) via electrostatic and non-electrostatic interactions. In previous studies the binding of salicylates to fibrous ion-exchangers was studied (Hänninen et al. 2005, 2003). It was found that binding was affected by the physicochemical properties of both the salicylates and the ion-exchange fibers. The highest molar amount of binding was obtained with 5-chlorosalicylic acid (5-Cl) and weak base fibers (Smopex®-105v and Smopex®-105pe). Binding of salicylates was based on electrostatic and non-electrostatic interactions. 5-Hydroxyisophthalic acid also interacted with anion-exchange fibers (Smopex®-103pe and Smopex DS-218v) with ionic forces (Kankkunen et al. 2002; Hänninen et al. 2005). Jaskari et al. (2001) investigated the mechanisms of drug binding to cation-exchange fibers. The results suggested both electrostatic and hydrophobic interactions between basic drugs (nadolol, propranolol and tacrine) and cation-exchange fibres (Smopex®-101, 102 and 107). Yu et al. (2006) studied the delivery of ketoprofen from a system containing ion-exchange fibers. Ketoprofen was adsorbed on to anion-exchange fiber (poly(propylene-g-vinylbenzyl-trimethyl-ammonium-chloride) by ionic interactions (Yu

et al. 2006). Besides ion-exchange fibers, drug adsorption on to a PAA grafted PVDF membrane was studied, and it was observed that interactions between the basic drugs and the membrane were much stronger than the interactions between acidic and neutral drugs and the membrane (Tarvainen et al. 1999; Åkerman 1999a; Karppi et al. 2007b). Other studies have also shown similar electrostatic interactions between drugs and different kinds of ion-exchange polymers. Pignatello et al. (2002) studied the mechanisms of interaction between Eudragit RS100 and RL100 polymers and three nonsteroidal anti-inflammatory drugs: diflunisal, flurbiprofen, and piroxicam. The drugs interacted strongly with the ammonium groups present in the polymers by electrostatic interactions. The research group of Rodriguez (2003) evaluated the interaction of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels. The drug molecules interacted weakly with the polymers through ionic interactions.

3.2. Drug release from the fiber

In the present study, basic drugs were released most effectively by using acidic solutions (10% formic acid in methanol and acetic acid). Acetic acid turned out to be the most suitable solution, because the mean absolute recovery of basic drugs increased by 26% (95.4 ± 23.8%). The absolute recovery of acidic drugs was poor when using all the solutions evaluated.

Drug release from PAA depends on the physicochemical properties of the drugs and on environmental conditions (pH and ionic strength of release solution). At low pH or high ionic strength the grafted PAA-chains are practically non-dissociated and are in a compact conformation. In contrast, at high pH or low ionic strength, the chains remain dissociated and in expanded form (Park et al. 1987). In a previous study it was reported that drug release from a PVDF-PAA membrane was considerably faster at pH 2.0 than at pH 7.0. At low pH basic drugs are also non-dissociated accelerating their release (Järvinen et al. 1998). There are many previous studies where drug release from membranes or ion-exchange fibers have been studied by changing the salt concentrations of the dissolution solutions (e.g. Tarvainen et al. 1999; Åkerman et al. 1999b; Jaskari et al. 2001; Kankkunen et al. 2002; Hänninen et al. 2003; Vuorio et al. 2004; Hänninen et al. 2005). In these studies, the drug release process was too slow to be considered for analytical purposes. In drug monitoring (e.g. in the clinical laboratory) sample preparation must be easy and fast to perform while giving acceptable recoveries. Therefore we used acidic solutions for drug release from the fiber. The first limitation of the present study was optimization of the drug release time, because different incubation times were not evaluated. Consequently, 10% formic acid in methanol might be a suitable solution for drug release if incubation time were prolonged, e.g. to 1 h. Organic acids also present a risk for laboratory staff in routine work. Thus acids diluted with methanol are quite safe and such solutions evaporate to dryness even faster. Secondly, removal of fiber bundles from cartridges for the drug release step delayed the work. Future aspects: incubation time must be optimized and drug release from the fiber in cartridges needs to be evaluated. Validation of the exact method needs to be done for each basic drug (absolute and relative recoveries, linearity and repeatability experiments, limits of detection (LOD) and quantification (LOQ), method comparisons etc.). The present study was an approximate screening investigation of the suitability of Smopex®-102 cation-exchange fiber for the separation of drugs from serum prior to quantitative or qualitative analysis.

In conclusion, basic model drugs were found to have greater binding to cation-exchange fibers than acidic drugs. Based on the results achieved, we would propose that the cation-exchange fiber evaluated might be a potential material for separation of basic drugs from protein–free and proteinaceous (e.g. serum) liquid solutions for subsequent monitoring and evaluation.

4. Experimental

4.1. Reagents

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. (Cheshre, UK). Nortrimipramine, trimipramine and zopiclone were from Rhone-Poulenc Rorer Co. (Birkenrod, Denmark). HEPES (>99.5%) (*N*-[2-hydroxyethyl] piperazine-*N′*-[2-ethanesulfonic acid]) was purchased from Sigma Co. (St. Louis, USA). Deionized (Millipore™) Milli-Q water (resistivity $\geq 18\ M\Omega/cm$) was used to prepare buffered drug 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). The Smopex®-102 [–COOH ion-exchange groups, poly (ethylene-g-acrylic acid)] cation-exchange fiber was obtained from SmopTech Co. (Turku, Finland).

4.2. Drug binding from 25 mM HEPES buffer (pH 7.0) and spiked serum

Stock solutions of acidic and basic drugs were prepared by dissolving 10 mg of each drug in 10 mL methanol in separate bottles and were stored at −20 °C. Drug solutions and spiked serum were prepared by adding appropriate amounts of the drugs to 25 mM HEPES buffer (pH 7.0) or serum immediately prior to use. Concentrations of the drugs in the final solution were ~0.80–500 μM. 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 for a maximum of 1 week at 4 °C until the samples were prepared and analyzed.

The polyethylene backbone of the Smopex®-102 cation-exchange fiber was grafted by radiation with poly(acrylic) acid, the ionic resin being a weak cation-exchanger ($-COO^-H^+$). The fiber resembled cotton textile fibre. Bundles were cut from the cation-exchange fiber, with weight ranging from 100 to 110 mg. In order to activate the ion-exhange groups, the fiber bundles were treated with HPLC-grade methanol for 1 h and then in 25 mM HEPES buffer solution (pH 7.0) with no drug at room temperature for another 1 h. The fiber bundles were then squeezed into a 2 mL plastic syringe (Terumo Europe N.V., Leuven, Belgium) and additional buffer was removed by using the syringe piston. Then, the 1 mL sample containing the drug (HEPES buffer solution and spiked serum) was loaded into the syringe and eluted. Concentrations of each acidic and basic drug were measured from this eluate and the initial non-loaded drug solution by reversed-phase HPLC by methods described below. The amount of drug bound to the fiber was determined as the difference between the amount of the drug in the initial non-loaded drug solution and the collected eluate (peak-height ratios).

Prior to drug analysis, the serum samples (non-loaded serum and eluted serum) were extracted with an automatic (Gilson Medical Electronics, Villiers-Le-Bel, France) or manual (Vac-Elut SPS 24, Analytichem International, Harbor City, CA) sample preparator 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–methanol for basic drugs as extraction solvents (Åkerman et al., 1996a, b, 1997,

1998). The 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 drugs remaining were analysed by HPLC by methods described below. The amount of the drugs bound to the fiber was determined as the difference between the amount of the drug in the non-loaded serum and eluted serum (peak-height ratios).

4.3. Drug release from cation-exchange fiber

After drug binding from the buffer solution, the cation-exchange fiber bundles were washed with 2 mL of distilled water and removed from the cartridges. Washed fiber bundles were placed in polypropylene test tubes containing 1 mL of five different drug release solutions in order to find the most suitable solution for drug release from the fiber. Drug release solutions were as follows: I: 10% formic acid in methanol (v/v) (pH 1.6), II: methanol, III: 100 mM acetate buffer (pH 5.3), IV: 50 mM ammonium hydroxide (pH 11.0) and V: acetonitrile. The drug release step was performed by incubating the fiber bundles at room temperature for 30 min. After drug release, the fiber bundles were removed from the drug release solutions and solutions I, II and V were evaporated to dryness with a gentle stream of air at 37 °C and reconstituted in 1 mL of the mobile phase. Solutions III and IV were ready for analysis after drug release. When drugs were bound from serum, drug release was performed in the same manner using acetic acid. Before drug analysis, all solutions were filtered using 0.22 μm PVDF membrane filters (Millipore). Absolute recoveries were calculated by comparing peak height from the drug release solution with peak height from the initial non-loaded sample.

4.4. Analysis of drug concentrations

The concentrations of antidepressant drugs were analysed by 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 a ChemStation chromatography workstation (Palo Alto, CA, USA). The column was a Select-B C-8 (125 × 4.0 mm) (VWR International AB) or Symmetry C-8 (150 × 4.6 mm) (Waters, Milford, Massachusetts, USA) reversed phase column. The elution was isocratic with a mobile phase consisting of acetonitrile-50 mM dipotassium hydrogen phosphate (40 : 60, v/v), pH 4.7 at a flow-rate of 1.2 mL/min. Benzodiazepines, hydroxycarbazepine and oxcarbazepine were analysed by 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 a ChemStation chromatography workstation. Columns were NovaPak C-18 (150 × 4.6 mm) or LiChroCart C-18 (250 × 4 mm) (VWR International AB) analytical column (Waters). Analysis employed a mobile phase of methanol-acetonitrile-10 mM dipotassium hydrogen phosphate (2 : 30 : 68, v/v/v), pH 3.7, at a flow-rate of 1.5 mL/min. The levels of primidone, lamotrigine, phenobarbital, pentobarbital, carbamazepine and phenytoin were analysed by 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). The column was a Select-B C-8 (125 × 4.0 mm) (VWR International AB) column. The elution was isocratic with a mobile phase of acetonitrile-50 mM dipotassium hydrogen phosphate (40 : 60, v/v), pH 4.7, at a flow-rate of 1.5 mL/min. All the drugs were detected at 220, 240 or 257 nm and peak purity analyses were performed at 210–365 nm HPLC (Åkerman et al., 1996a, b, 1997, 1998).

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References

Åkerman KK, Jolkkonen J, Huttunen H, Penttilä I (1998) High-performance liquid chromatography method for analysing citalopram and desmethylcitalopram from human serum. Ther Drug Monit 20: 25–29.

Åkerman KK, Jolkkonen J, Parviainen M, Penttilä I (1996a) Analysis of low-dose benzodiazepines by HPLC with automated solid-phase extraction. Clin Chem 42: 1412–1416.

Åkerman KK (1996b) Analysis of clobazam and its active metabolite norclobazam in plasma and serum using HPLC/DAD. Scand J Clin Lab Invest 56: 609–614.

Åkerman KK (1997) Analysis of clozapine and norclozapine by high-performance liquid chromatography. J Chromatogr B 696: 253–259.

Åkerman S, Åkerman K, Karppi J, Koivu P, Sundell A, Paronen P, Jarvinen K (1999a) Adsorption of drugs onto a poly(acrylic) grafted cation exchange membrane. Eur J Pharm Sci 9: 137–143.

Åkerman S, Svarfvar B, Kontturi K, Näsman J, Urtti A, Paronen P, Järvinen K (1999b) Influence of ionic strength on drug adsorption onto and release from a poly(acrylic acid) grafted poly(vinylidene fluoride) membrane. Int J Pharm 178: 67–75.

Asami T, Suehiro T, Ichijo H, Yamauchi A, Ogawa S, Suzuki M, Uzumaki M (1985) US Patent 4514367.

Avramescu ME, Borneman Z, Wessling M (2003) Mixed-matrix membrane adsorbers for protein separation. J Chromatogr A 18: 171–183.

Avramescu ME, Sager WFC, Borneman Z, Wessling M (2004) Adsorptive membranes for bilirubin removal. J Chromatogr B 803: 215–223.

Chen L, Yang G, Zhang J (1996) A study of the exchange kinetics of ion-exchange fiber. React Funct Polym 29: 139–144.

Hansch C (1990) Comprehensive Medical Chemistry, Pergamon, 6th ed. Oxford.

Hänninen K, Kaukonen AM, Kankkunen T, Hirvonen J (2003) Rate and extent of ion-exchange process – the effect of physico-chemical properties of salicylates. J Control Release 91: 449–463.

Hänninen KR, Kaukonen AM, Murtomaki LS, Hirvonen JT (2005) Effect of ion-exchange fiber structure on the binding and release of model salicylates. J Pharm Sci 94: 1772–1781.

Jaskari T, Vuorio M, Kontturi K, Manzanares JA, Hirvonen J (2001) Ion-exchange fibers and drugs; an equilibrium study. J Control Release 70: 219–229.

Jaskari T, Vuorio M, Kontturi K, Urtti A, Manzanares JA, Hirvonen J (2000) Controlled transdermal iontophoresis by ion-exchange fiber. J Control Release 67: 179–190.

Järvinen K, Akerman S, Svarfvar B, Tarvainen T, Viinikka P, Paronen P (1998) Drug release from pH and ionic strength responsive poly(acrylic acid) grafted poly(vinylidenefluoride) membrane bags in vitro. Pharm Res 15: 802–805.

Kankkunen T, Huupponen I, Lahtinen K, Sundell M, Ekman K, Kontturi K, Hirvonen K (2002) Improved stability and release control of levodopa and metaraminol using ion-exchange fibers and transdermal iontophoresis. Eur J Pharm Sci 16: 273–280.

Karppi J, Åkerman S, Åkerman K, Sundell A, Nyyssönen K, Penttilä I (2007a) Adsorption of drugs onto a pH responsive poly(N,N-dimethyl aminoethyl methacrylate) grafted anion-exchange membrane in vitro. Int J Pharm 338: 7–14.

Karppi J, Åkerman S, Åkerman K, Sundell A, Nyyssönen K, Penttilä I (2007b) Isolation of drugs from biological fluids by using pH sensitive poly(acrylic acid) grafted poly(vinylidene fluoride) polymer membrane in vitro. Eur J Pharm Biopharm 67: 562–568.

Lensmeyer GL, Onsager C, Carlson IH, Wiebe DA (1995) Use of particle-loaded membranes to extract steroids for high-performance liquid chromatographic analyses improved analyte stability and detection. J Chromatogr A 691: 239–246.

Lingeman H, Hoekstra-Oussoren SJ (1997) Particle-loaded membranes for sample concentration and/or clean-up in bioanalysis. J Chromatogr B Biomed Sci Appl 689: 221–237.

Park H, Robinson JR (1987) Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels. Pharm Res 4: 457–464.

Pignatello R, Ferro M, Puglisi G (2002) Preparation of solid dispersions of nonsteroidal anti-inflammatory drugs with acrylic polymers and studies on mechanisms of drug-polymer interactions. AAPS Pharm Sci Tech 3: E10.

Rodriguez R, Alvarez-Lorenzo C, Concheiro A (2003) Interactions of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels. Rheological and diffusional implications. Eur J Pharm Sci 20: 429–438.

Soldatov VS, Shunkevich AA and Sergeev GI (1988) Synthesis, structure and properties of new fibrous ion exchangers. React Polym 7: 159–172.

Stevens TS, Jewett GL and Bredeweg RA (1982) Packed hollow fiber suppressors for ion chromatography. Anal Chem 54: 1206–1208.

Szmigielska AM, Schoenau JJ, Levers V (2000a) Determination of glucosinolates in canola seeds using anion exchange membrane extraction combined with the high-pressure liquid chromatography detection. J Agric Food Chem 48: 4487–4491.

Szmigielska AM, Schoenau JJ (2000b) Use of anion-exchange membrane extraction for the high-performance liquid chromatographic analysis of mustard seed glucosinolates. J Agric Food Chem 48: 5190–5194.

Tarvainen T, Svarfvar B, Akerman S, Savolainen J, Karhu M, Paronen P, Jarvinen K (1999) Drug release from a porous ion-exchange membrane in vitro. Biomaterials 20: 2177–2183.

Ware GM, Price G, Carter L, Eitenmiller RR Jr (2000) Liquid chromatographic preparative method for isolating ergot alkaloids, using a particle-loaded membrane extracting disk. J AOAC 83: 1395–1399.

Vuorio M, Murtomäki L, Hirvonen J, Kontturi K (2004) Ion-exchange fibers and drugs: A novel device for the screening of iontophoretic systems. J Control Release 97: 485–492.

Yu L, Li S, Yuan Y, Dai Y, Liu H (2006) The delivery of ketoprofen from a system containing ion-exchange fibers. Int J Pharm 319: 107–113.

Zhao YH, Le J, Abraham MH, Hersey A, Eddershaw PJ, Luscombe CN, Boutina D, Beck G, Sherborne B, Cooper I, Platts JA (2001) Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors. Eur J Med Chem 90: 749–784.