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Equilibrium sampling through membrane based on a single hollow fibre for determination of drug–protein binding and free drug concentration in plasma

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Abstract

The determination of drug–protein binding and free drug concentration in plasma applying the equilibrium sampling through membrane (ESTM) technique has been studied using supported liquid membrane extraction in a single hollow fibre without any membrane carrier. In the extraction setup, the donor phase (plasma or buffer) was placed in the vial, into which was immersed the hollow fibre with the acceptor phase situated in the lumen. This proposed technique was applied to study the drug–protein binding of five local anaesthetics and two antidepressants as model substances, and the influence of the total drug concentration on the drug–protein binding was investigated. The brief theoretical background for determination of the drug–protein binding under equilibrium conditions is described. The developed method shows a new, improved and simple procedure for determination of free drug concentration in plasma and extent of drug–protein binding.

Keywords: Equilibrium sampling through membrane (ESTM); Hollow fibre; Drug–protein binding; Local anaesthetic; Antidepressant

1. Introduction

Drug–protein binding is the reversible interaction of drugs with proteins in plasma. The binding of a drug to a protein binding site is a saturable process and it can be expressed by the ratio of free to total drug concentration (the free fraction) or by the ratio of bound drug amount to the total amount (drug–protein binding ratio) [1]. The extent to which binding occurs varies and depends on the physico-chemical nature of the drug, the affinity between the drug and protein, the drug and protein concentrations and the presence of other substances which either compete with the drug for binding sites or displace it through the allosteric effects [1,2]. There are several proteins in plasma, including serum albumin, α_1-acid glycoprotein (AAG) and lipoproteins that contribute to plasma protein binding of a drug. Increased concentration of the binding protein results in a decreased free drug concentration, and increased drug concentration results in reduced drug–protein binding ratio due to saturation of the protein. In view of the high variation in the free fraction of different proteins and drug concentration, it is of interest from a pharmacological point of view to measure the free drug concentration, since the pharmacological effect is considered to be proportional to the free drug concentration [3]. There are clinical situations where monitoring free drug concentrations provide the clinician with information and insights into a patient's pharmacologic status. For those drugs that are highly bound (>80%) to plasma proteins, a relatively small change in the degree of binding may have a significant effect on the free fraction. Some drugs also exhibit a concentration-dependent (saturable) protein binding within their therapeutic range so that higher concentrations are associated with lower drug–protein binding ratios [1]. AAG binds most of the basic drugs and some hormones. It is an acute-phase reactant which has one binding site selective for basic drugs such as disopyramide and lidocaine. The plasma concentration of AAG increases in the presence of stress, inflammation, malignancy, myocardial infarction and various haematological disorders. This AAG increase causes more binding sites to become available thus causing a decrease in free drug level [4].

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Several methods have been used for determination of free drug concentration in plasma as well as drug–protein binding ratio. These include affinity chromatography [5], ultrafiltration [6], ultracentrifugation [3], equilibrium dialysis [7], microdialysis [8], capillary electrophoresis [9] solvent microextraction [10] and supported liquid membrane extraction [11,12]. These methods differ in their speed, data quality and complexity [13]. Experimental artefacts associated with these techniques affect the drug equilibrium and have led to erroneous values. Such artefacts include: non-specific binding of the drug to the plastic housing and ultrafiltration membrane, non-physiological conditions (pH, presence of organic solvents), low concentration of free drug (even under the detection limit), long periods of analysis, etc. [14].

Supported liquid membrane (SLM) equilibrium extraction (equilibrium sampling through microporous membrane) with a flat membrane has recently been demonstrated as a new method for in vitro determination of drug–protein binding under physiological conditions without disturbing the equilibrium between a drug and protein in plasma [15]. When the extraction is performed with a very low enrichment factor (equilibrium extraction with incomplete trapping), only the free (unbound) fraction of the drug is removed from the aqueous donor phase, so that the binding equilibrium is not significantly perturbed [10,15]. With these conditions met, the free concentration of the drug can be determined directly as well as the drug–protein binding ratio.

The aim of the present study was to develop an improved and simple method based on the equilibrium sampling through a microporous membrane with a single hollow fibre for determination of drug–protein binding and free drug concentration in plasma. The single hollow fibre extraction device was developed in our laboratory and previously applied to environmental sampling for the determination of freely dissolved concentration of chlorophenol pollutants [16]. Simple sample preparation, short separation time and physiological conditions were anticipated when the study started.

2. Theoretical background

Since in our previous paper, the theory for determination of drug–protein binding using SLM extraction under equilibrium conditions was described in detail [15], here is only given a brief description of the theory.

In the SLM extraction in a single hollow fibre, an aqueous donor (sample) solution containing the analyte (local anaesthetic or antidepressant in this study) is placed outside a microporous hollow fibre. The drug is extracted in a three-phase system through an organic solvent immobilized in the pores into an aqueous acceptor solution inside the lumen of the hollow fibre [16,17]. The rate of the mass transfer from the donor to the acceptor is proportional to the concentration difference, ΔC, over the membrane, which can be written as [18]:

$$\Delta C = \alpha_D C_D K_D - \alpha_A C_A K_A \quad (1)$$

where the symbols are defined in the List of Symbols. If the extraction conditions are set so that $\alpha_A > 0$, i.e. with an acceptor pH value not much lower than the pK_a (for basic compounds), the system can reach equilibrium ($\Delta C = 0$) in a reasonable time period. This is in contrast to other applications of membrane extraction [17,18] intending to perform a more or less exhaustive extraction, where acceptor pH at least 3.3 units lower than pK_a is used leading to $\alpha_A \approx 0$. This ascertains high enrichment factors, so that a true equilibrium is not reached within the experiment time. This mode of extraction is not further considered here.

The maximum concentration enrichment factor is reached at equilibrium and can be expressed by:

$$E_{e(max)} = \left(\frac{C_A}{C_I}\right)_{max} = \frac{\alpha_D K_D}{\alpha_A K_A} \quad (2)$$

When plasma is spiked with a weakly basic drug (all local anaesthetics and antidepressants investigated in the work reported herein are weakly basic amines), two equilibria are established: the equilibrium between charged (unextractable) and uncharged (extractable) drug molecules (i.e. the dissociation equilibrium, described by pK_a), and the equilibrium between the protein drug complex and the free drug in plasma. Both equilibria depend on pH of the donor solution. If the extraction is performed under equilibrium conditions and the total concentration of the drug in the plasma sample in equilibrium with the organic and acceptor phase does not change significantly during the extraction, then none of these equilibria will be influenced during the extraction. Therefore, it is possible to determine the true free concentration of the drug in plasma as well as the extent of the drug–protein binding.

It is necessary that the volume of a sample is large enough or that the enrichment factor is very low, so that the total analyte concentration in the sample is not influenced by the extraction (depleting of the analyte in the donor phase should not be more than 5%) [19]. When extraction is performed in a flow system as in [15], the extraction equilibrium condition in the donor represents the situation in the original sample; any equilibrium within the sample in which the analyte participates is undisturbed, in static systems it is important to ascertain that the depletion is negligible.

Considering equilibrium between all phases in the plasma sample, the fraction of analyte in the extractable form in the donor, α_D, has to be modified in order to include the effects of the protein binding:

$$\alpha_D = \alpha_P \alpha_{Dd} \quad (3)$$

where α_P is the fraction of free drug, and α_{Dd} is the uncharged fraction of the non-bound drug due to the dissociation equilibrium. Then the maximum enrichment factor for equilibrium extraction from spiked plasma sample is:

$$E^P_{e(max)} = \frac{\alpha_P \alpha_{Dd} K^P_D}{\alpha_A K_A} \quad (4)$$

Taking into account the experimentally determined equilibrium values of C_A and calculated values of α_A and α_{Dd} [20] and using Eq. (4), it is possible to calculate the fraction of free drug

Table 1
The main characteristics of studied local anaesthetics and antidepressants

Name/abbreviation	pK_a		Log P	PB (%)
Prilocaine/Pri.	8.0[a]	7.9[b]	2.73[d]	55[e]
Ropivacaine/Rop.	8.9[a]	8.1[c]	–	90[f]
Lidocaine/Lid.	8.5[a]	7.8[c]	3.40[d]	40–60[e]
Bupivacaine/Bup.	8.9[a]	8.1[c]	4.05[d]	95[f]
Mesocaine/Mes.	8.6[a]	–	–	50–70[g]
Reboxentine/Reb.	8.3[a]	–	3.17[a]	97[h]
Fluvoxamine/Flu.	9.4[a]	–	2.82[a]	77[i]

[a] Calculated by the program ACD/pK_a DB (Advanced Chemistry Development Inc., Toronto, Canada; Ref. [15,21].
[b] Ref. [22].
[c] Ref. [23].
[d] Ref. [24].
[e] Ref. [25].
[f] Ref. [26].
[g] Estimated, T. Arvidsson, personal communication.
[h] Ref. [27].
[i] Ref. [28].

α_P, as well as to estimate the drug–protein binding ratio. This additionally requires either the assumption that $K_D \cong K_A$ or an experimental determination of K_A and K_D.

In our previous paper [15], a simplified method for determination of protein binding was described that used the experimentally obtained values of C_A after the equilibrium extraction with the same total drug concentrations from both plasma (C_A^P) and buffer (C_A). From Eqs. (2) and (4), α_P can then be calculated:

$$\alpha_P = \frac{C_A^P \alpha_D K_D}{C_A \alpha_{Dd} K_D^P} \quad (5)$$

where α_{Dd} refers to the plasma sample, and α_D refers to the buffer solution. Then, assuming that $\alpha_D = \alpha_{Dd}$ and $K_D = K_D^P$ under the experimental conditions, a simple relation for protein binding is obtained:

$$\alpha_P = \frac{C_A^P}{C_A} \quad (6)$$

The assumptions leading to Eq. (6) require that the buffer has the same pH and ionic strength as the plasma [15].

3. Experimental

3.1. Chemicals and materials

The local anesthetics studied (prilocaine, lidocaine, ropivacaine and bupivacaine) were obtained as hydrochloride salts from Astra Pharmaceutical Production (Södertälje, Sweden). Mesocaine was synthesized at Astra Pain Control (Södertälje, Sweden). The antidepressants: reboxetine methane-sulfonate and fluvoxamine maleate were obtained from Solvay pharmaceuticals (Weesp, the Netherlands) and Pharmacia & Upjohn S.p.A (Milano, Italy), respectively. The main characteristics of the studied compounds are given in Table 1.

Stock solutions (200 mg dm^{-3}) were prepared in water and were stable for months when stored at 4 °C and protected from light. Aqueous working solutions were prepared daily from the stock solution.

The donor solution contained 0.25–10.0 mg dm^{-3} of a single drug in the buffer solution (0.067 mol dm^{-3} phosphate buffer at pH 7.53). The acceptor solution was 0.067 mol dm^{-3} phosphate buffer at pH ranging from 7.0 to 7.4 depending on the studied drug. Di-hexyl ether with 5% tri-octylphosphine oxide (TOPO) was used as an organic phase.

Blank plasma was obtained from the blood center, Lund University Hospital (Lund, Sweden) and kept frozen at −20 °C. According to information from the blood center, the plasma contained 0.01 mol dm^{-3} citrate acid, 0.07 mol dm^{-3} sodium citrate, 0.01 mol dm^{-3} sodium dihydrogenphosphate and 0.08 mol dm^{-3} glucose. The plasma was thawed and kept at room temperature during the day of analysis. The spiked plasma solutions were obtained by adding the appropriate amounts of the stock solution to the blank plasma.

3.2. HPLC analysis

The HPLC system consisted of a pump (Varian 9012), a column (250 mm × 4.6 mm, 3 μm, Ace 3 C_{18}, Advanced Chromatography Technologies, Scotland), a LC-detector (Spectroflow 755 ABI Analytical Kratos Division) at 210 nm for the local anaesthetics and at 230 nm for the antidepressants. A mobile phase consisting of 30% methanol and 70% 0.025 mol dm^{-3} phosphate buffer at pH 2.5 with a flow rate of 1.0 cm^3 min^{-1} was applied for the analysis of the local anaesthetics. Thirty-five percent acetonitrile and 65% 0.01 mol dm^{-3} triethylamine aqueous solution, adjusted to pH 2.90 by phosphoric acid, with flow rate of 1.0 cm^3 min^{-1} was used for analysis of the antidepressants. The chromatographic data were evaluated using a PeakSimple Model 203 Single Channel Data System (SRI Instruments, Torrance, CA, USA).

3.3. Procedure of SLM extraction in a single hollow fibre

The microporous polypropylene hollow fibre membrane (ACCUREL, Type PP 50/280, Membrane GmbH Wuppertal, Germany) was used in the study. The inner diameter of the hollow fibre was 280 μm, the thickness of the wall was 50 μm and the length of the hollow fibre was 150 mm. The effective volume was calculated after extraction for each hollow fibre separately.

The lumen of a single hollow fibre membrane was filled with acceptor solution using a 0.5 cm^3 syringe with 0.3 mm needle (BD Micro-Fine Syringe, BD Consumer Healthcare, NJ, USA). The membrane was impregnated by soaking in the organic phase for 5 s, which was followed by washing in water in order to remove excess of the organic phase. Then, the lumen of the hollow fibre was washed with the acceptor buffer and the ends of the hollow fibre were sealed to prevent leakage. This was made by bending the fibre ends over and fixing with a piece of Al-foil and a 50 μL limited volume vial (Alltech). The membrane was placed in the donor solution (spiked buffer or plasma) present in a 10 cm^3 vial (see Fig. 1). During the extraction, the sample vials were shaken at 100 rpm using a shaker (INFORS AG, Bottmingen, Switzerland). The final collection of the acceptor

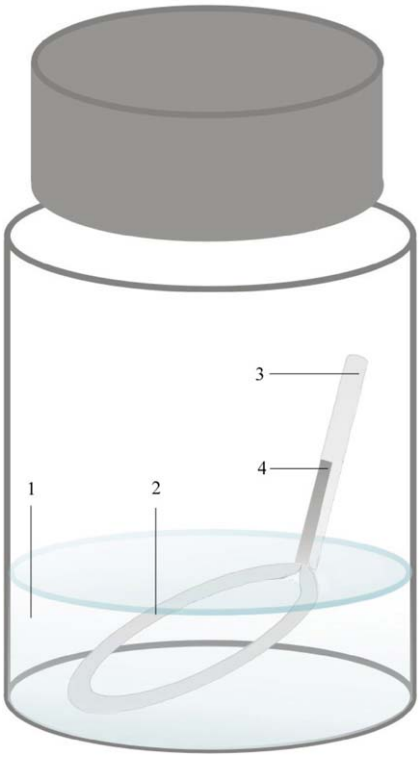

Fig. 1. Schematic of SLM extraction in a single microporous hollow fibre. 1: donor aqueous phase; 2: microporous hollow fibre with organic phase placed into membrane pores and the acceptor phase in the lumen of hollow fibre; 3: 50 μL limited volume vial (Alltech); 4: Al-foil.

solution was accomplished by pressing the contents of the fibre into a 50 μL vial with a 0.5 cm^3 syringe, followed by injection into the HPLC. Similar experimental devices were described in more details in [16].

4. Results and discussion

The main characteristics of the investigated local anaesthetics and antidepressants are given in Table 1. All these drugs are weakly basic amines. For some drugs, two different values of pK_a are given: both calculated using the computer program ACD/pK_a DB (Advanced Chemistry Development Inc., Toronto, Canada) and obtained from the literature. The values of pK_a from the literature are specified at 37 °C [23].

4.1. Optimization of the extraction procedure

4.1.1. Donor pH

The pH of the sample has a large influence on the equilibrium between protein-bound and free drug in plasma [29]. The free concentration of dissociable drugs is strongly affected by the pH of the plasma. As an example, when the pH was raised from 7.2 to 7.6, the free fraction of ropivacaine and bupivacaine (which are basic drugs) decreased by a factor of two [3]. In the view of this and in order to be able to study the drug–protein binding at native conditions, the pH of the donor solution, i.e. the buffer solution of the drug or plasma spiked with drug, was kept constant and equal to 7.5 in all of the experiments. This condition was the same as in our previous work [15].

Table 2
The influence of the acceptor pH on the total concentration of the analyte in the donor phase under the equilibrium conditions expressed as a percentage of drug depletion in the donor phase

pH	Drug depletion in donor phase (%)						
	Rop.	Pri.	Lid.	Bup.	Mes.	Reb.	Flu.
7.0	10.0	2.5	6.5	20	8.7	3.6	4.2
7.1	6.3	1.0	5.1	17	3.5	–	–
7.2	2.0	–	3.0	14	–	–	–
7.3	–	–	–	11	–	–	–
7.4	–	–	–	8	–	–	–

4.1.2. Acceptor pH

The selection of the acceptor pH is less straightforward. On one side, a low pH_A leads to a higher enrichment factors, and thus a more sensitive determination of low drug concentrations, but this is not crucial in this application. High enrichment factors could lead to depletion of the donor causing changes in the drug protein binding equilibrium. Because of this, the influence of the acceptor pH on depleting the analyte concentration in the donor phase was investigated for each drug separately. These results are given in Table 2. Depleting partly depends on hydrophobicity of the drug, as a hydrophobic drug tends to accumulate in the organic phase in the membrane. More hydrophobic drugs (higher log P) are more depleted from the donor phase. For example, lidocaine has higher log P (3.4) [24] than prilocaine (2.73) (both of these drugs have low protein binding), thus the pH of the acceptor phase should be higher for lidocaine (pH 7.2) than for prilocaine (pH 7.0) in order to reach equilibrium without changing in the total drug concentration in the donor phase. Also, in the case of extraction of bupivacaine which is the most hydrophobic of the investigated local anaesthetics (log P = 4.05), depleting of the drug in the donor phase is more than 5%, when the acceptor pH 7.4.

4.1.3. Time to attain equilibrium

The time required to obtain results in biomedical studies is often considered to limit the usefulness of the method. Fig. 2 shows the time variation of the enrichment factor for ropivacaine and lidocaine. These drugs are extracted separately from the buffer solution and plasma. The total concentration in donor phase was 5 mg dm^{-3}, the donor volume was 2 cm^3 and the membrane length was 150 mm. It is evident from this figure that the time required for the establishment of the equilibrium conditions is no longer than 25 min. It means that the total time for determination of drug–protein binding is 30–40 min depending on the retention time of the drugs in HPLC analysis. This compares favorably with the application of dialysis and microdialysis for determination of drug–protein binding where very long experiments time is needed (at least 12 h) [30].

4.1.4. Phase volumes

The volumes of biological material are highly limited. In the majority of membrane extraction studies reported on human

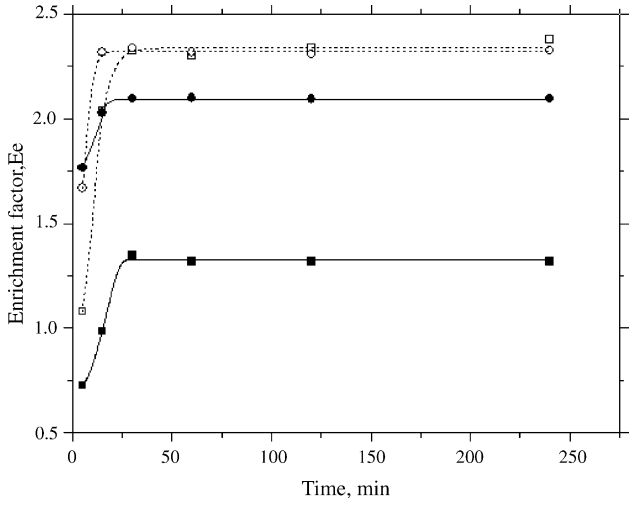

Fig. 2. The time variation of the enrichment factor for ropivacaine and lidocaine. The total concentrations of drug in donor phase (buffer or spiked plasma) was 5 mg dm^{-3}. (◯) lidocaine in phosphate buffer; (●) lidocaine in blood plasma; (□) ropivacaine in phosphate buffer; (■) ropivacaine in blood plasma. Acceptor was phosphate buffer pH$_A$ 7.00.

plasma [11,31–33] extractions were accomplished from a 4 cm^3 sample, and in many cases, smaller plasma volumes (0.5–2 cm^3) were diluted with the buffer to give a total donor volume of 4 cm^3. Dilution is not advisable for drug–protein binding studies as the equilibrium could be shifted. Therefore, the influence of the donor volume on the extraction was investigated. Three different sample volumes: 1, 2 and 4 cm^3, were used for SLM extraction of ropivacaine (C_I = 2 mg dm^{-3}) from the buffer solution, while the acceptor pH was 7.0. Also, two different hollow fibre lengths were investigated in these experiments. The obtained results are given in Table 3. The enrichment factor is slightly higher in the extraction with the longer hollow fibre (150 mm) than in the extraction with the smaller length (100 mm) of the hollow fibre but this is within the measurement errors. Also, the enrichment factor was higher when the drug was extracted from 2 and 4 cm^3 than from 1 cm^3 of the donor solution, i.e. depletion might occur at 1 cm^3 sample volume. There is no significant difference in enrichment between SLM extraction from 2 and 4 cm^3 donor volumes (the difference is within the measurement error). Therefore, in the following experiments, 2 cm^3 donor volume and hollow fibres of 150 mm were used.

Table 3
The dependence of the enrichment factor on donor volume and length of hollow fibre (L_{HF})

Donor volume (cm^3)	Enrichment factor (E_e)	
	L_{HF} = 100 mm	L_{HF} = 150 mm
1	1.9 (2.8)	2.1 (4.8)
2	2.2 (5.1)	2.4 (4.8)
4	2.1 (3.4)	2.3 (3.2)

Ropivacaine (C_I = 2 mg dm^{-3}) was extracted from buffer solution pH$_D$ 7.5 and pH$_A$ 7.0. The time of extraction was 60 min. The values of relative standard deviation for five replicates are given in brackets.

5. Drug–protein binding determination

The drug–protein binding was determined for five local anaesthetics and two antidepressants, all weakly basic amines and dominantly bounding to α_1-acid glycoprotein (AAG). Although the affinity of local anaesthetics to HSA is less than to AAG, the enormous binding capacity of HSA renders this protein important in the binding equilibrium process: when binding to AAG is saturated, HSA continues to bind these drugs [23].

The same amount of a single drug was extracted both from the buffer solution and from spiked plasma in triplicate. After 60 min of SLM extraction, the hollow fibre was removed from the donor phase and the acceptor phase was collected from the lumen of the hollow fibre. The acceptor concentration of the drug was determined using HPLC, both after extraction from buffer and from plasma solutions. Fig. 3 shows HPLC chromatograms obtained after the extraction of reboxetine and fluvoxamine from buffer and spiked plasma. It is clear that the peak area after extraction of the drug from plasma is significantly smaller than the peak area after extraction of the drug from the buffer. The fraction of the free drug in plasma is calculated from Eq. (6) using experimentally obtained values of C_A and C_A^P. The obtained values of protein binding, PB, (in percent) for the local anaesthetics are given in Fig. 4 and Table 4. (PB = 100 $(1 - \alpha_P)$).

The influence of total drug concentration on protein binding was investigated in this study. In most cases, drug concentrations at therapeutic doses are well below those of the binding protein

Table 4
The obtained values of protein binding (PB) of local anaesthetics calculated on the bases of experimental results of SLM extraction under equilibrium conditions in a single hollow fibre at different total drug concentrations

Drug	C (mg dm^{-3})	PB (%)		
		Hollow fibre	Flat membrane [15]	Literature (cf. Table 1)
Prilocaine	5.1	12 (4)		55
	2.4	21 (4)	19	
	1.0	45 (5)	36	
	0.5	49 (7)	35	
Ropivacaine	10.7	60 (4)	–	90
	5.4	68 (4)	–	
	2.6	70 (5)	70	
	1.1	73 (4)	73	
	0.6	74 (6)	68	
Lidocaine	6.6	11 (5)	–	40–60
	2.9	31 (5)	–	
	1.5	42 (5)	–	
	0.5	56 (7)	44	
Bupivacaine	10.7	64 (3)	–	95
	5.6	69 (5)	–	
	3.0	73 (5)	–	
	1.2	74 (6)	58	
Mesocaine	10.0	54 (5)	–	50–70
	4.9	74 (5)	–	
	2.4	76 (7)	50	

The values of relative standard deviation for three replicates are given in brackets.

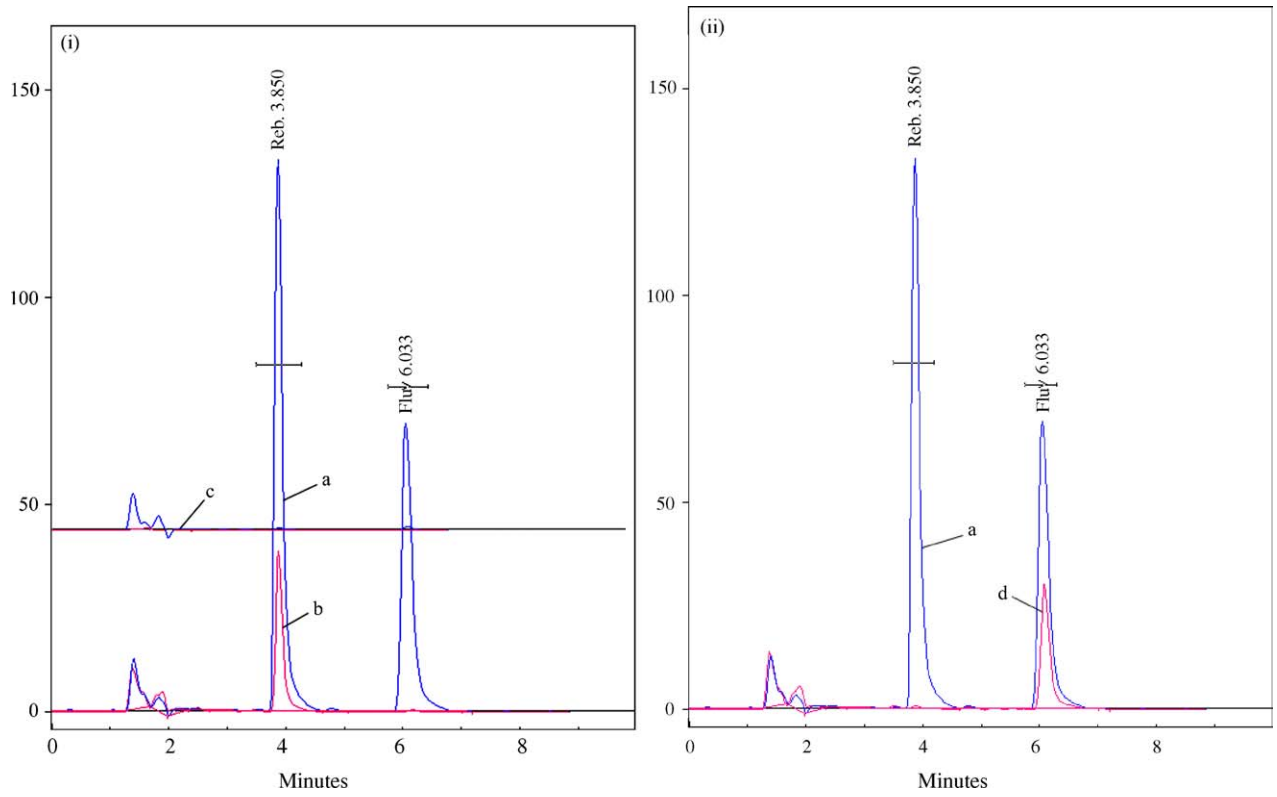

Fig. 3. HPLC chromatogram of the acceptor phase after SLM extraction in a single hollow fibre. (i) a: Extraction of reboxetine and fluvoxamine (0.5 mg dm^{-3}) from buffer solution; b: extraction of reboxetine from plasma spiked with 0.5 mg dm^{-3} of reboxetine; c: blank after enrichment from blood plasma. (ii) a: Extraction of reboxetine and fluvoxamine (0.5 mg dm^{-3}) from buffer solution; d: extraction of fluvoxamine from plasma spiked with 0.5 mg dm^{-3} of fluvoxamine.

and the fraction unbound is constant across the therapeutic range of the drug concentration. However, the concentration of α-acid glycoprotein is relatively low, and saturation of the binding sites can occur in the therapeutic range. Therapeutic plasma concentrations of local anaesthetics are usually in the range of 2–5 mg dm^{-3} [34]. It is clear from Fig. 4 that there is highly significant influence of total drug concentration on drug–protein binding for local anaesthetics with low protein-binding (about 50%). For example, in the cases of prilocaine, with the increasing of total drug concentration in plasma from 0.25 to 5 mg dm^{-3}, drug–protein binding decreases from 49 to 12%. However, local anaesthetics with high drug–protein binding such as bupivacaine and ropivacaine do not show any significant dependence of drug–protein binding on the total drug concentration in the concentration range from 0.5 to 10 mg dm^{-3}. The values of drug–protein binding of the local anaesthetics obtained in the work reported herein are in good agreement with previously published values obtained using equilibrium SLM extraction with the flat membrane in a flow mode operation [15].

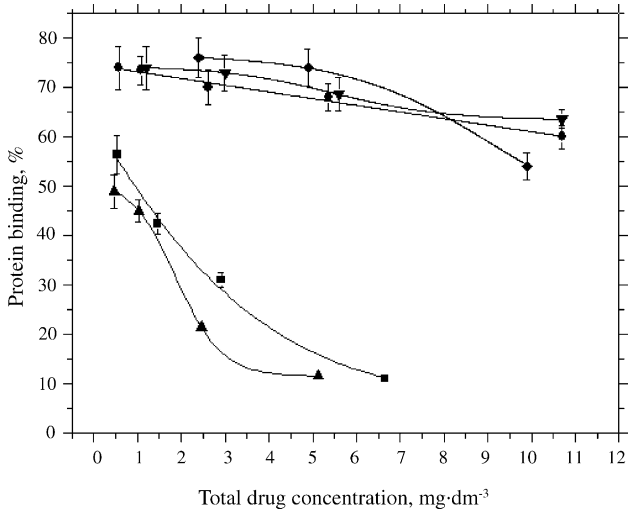

Fig. 4. The influence of the total drug concentration on protein binding of local anaesthetics: (■) lidocaine; (●) ropivacaine; (▲) prilocaine; (▼) bupivacaine; and (♦) mesocaine.

The protein binding for two antidepressants (reboxetine and fluvoxamine) is determined and given in Fig. 5 and Table 5. Also here, a clear dependence of protein binding on the total drug concentration was observed.

The obtained maximum values of drug protein binding are lower than the literature values, but the comparison is uncertain, as there is usually no information in literature about which plasma drug concentration the given protein binding refers to. Also, the literature values are mainly obtained using the ultrafiltration technique, which can be suspected to have a tendency to provide systematically high protein binding values, either by shifting the protein–drug equilibrium during the ultrafiltration procedure or by absorption of the drug to the filter. Therefore, the values obtained here could be more accurate as they are based on a true equilibrium and would be insensitive to absorption in the membrane.

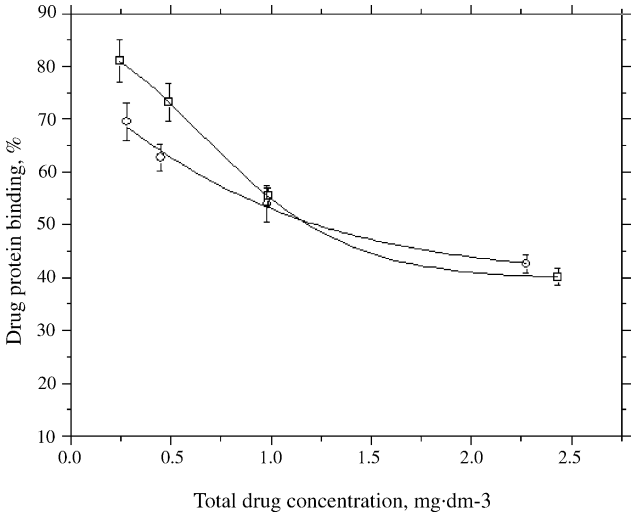

Fig. 5. The influence of the total drug concentration on protein binding of antidepressants: (□) reboxetine; (◯) fluvoxamine.

Table 5
The obtained values of protein binding (PB) of antidepressants calculated on the bases of experimental results of SLM extraction under equilibrium conditions in a single hollow fibre at different total drug concentrations

Drug	C (mg dm^{-3})	PB (%)	
		Hollow fibre	Literature
Reboxetine	2.4	40 (4)	97
	1.0	55 (3)	
	0.5	73 (5)	
	0.2	81 (5)	
Fluvoxamine	2.3	43 (4)	77
	1.0	59 (6)	
	0.4	63 (4)	
	0.3	70 (5)	

The values of relative standard deviation for three replicates are given in brackets.

6. Conclusion

The main goal in the approach discussed in this study has been to develop a technically simple realisation of the ESTM principle for measurement of drug–protein interaction and the free drug concentration under equilibrium and physiological conditions. The equilibrium sampling through membrane in a single hollow fibre represents a simple and fast method for drug–protein binding determination. Strong influence of the total drug concentration on percentage of drug–protein binding was observed only for low protein binding drugs.

7. List of Symbols

C_A total concentration of analyte in acceptor phase
C_D mean concentration of analyte in donor phase
C_I initial concentration of analyte in donor phase
E_e concentration enrichment factor
K_A partition coefficient between organic phase and acceptor phase
K_D partition coefficient between donor phase and organic phase
K_a dissociation constant of the analyte
PB drug–protein binding, expressed in percent

Greek letters
α_A fraction of analyte in extractable form in acceptor phase
α_D fraction of analyte in extractable form in donor phase
α_P fraction of analyte not bound to plasma proteins

Subscript and superscript
P denotes the conditions in the plasma solution

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