Determination of Drugs and Metabolites in Water by use of Liquid Membrane Systems and HPLC

-Method development and application-

Der Fakultät für Naturwissenschaften
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Von
Nabil N. Ahmad AL-Hadithi, M.Sc. in Chemistry
aus AL-Anbar/Irak

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The present work has been carried out during April 2004 and April 2007 at the University of Paderborn, Faculty of Science, Department of Chemistry under supervision of Prof. Dr. M. Grote.

1. Referent	**Prof. Dr. M. Grote**
2. Referent	**Prof. Dr. M. Weiß**

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Paderborn, June 2007 **Nabil AL-Hadithi**

Dedicated to:

Mum's spirit and dad

Brothers and sisters,

My wife,

My relatives and friends,

Table of contents

Abbreviations

A^-	Dissociated acidic compound
AH	Undissociated acidic compound
aq.	Aqueous phase
B	Undissociated basic compound
BH^+	Dissociated basic compound
BFR	Biofilm reactors
BLM	Bulk liquid membrane
C	Carrier
CE	Capillary electrophoresis
C_F	Initial concentration of analyte in feed phase
$C_{F,i}$	Analyte concentration in feed phase at x = 0
C_{FM}	Analyte concentration in membrane phase at x = 0
C_M	Analyte concentration in membrane phase
C_{MS}	Analyte concentration in membrane phase at x = h_M
CoA	Acetyl coenzyme A
C_S	Concentration of analyte in strip phase
$C_{S,i}$	Initial concentration of analyte in strip phase
D	Diffusion coefficient
DAD	Diode array detector
dC_F/dt	Mass balance equation in feed phase
dC_S/dt	Mass balance equation in feed phase
DEHPA	Di-(2-ethylhexyl)phosphoric acid
D_F	Diffusion coefficient for active form of analyte in feed phase
DHE	Dihexyl ether
D_M	Diffusion coefficient for active form of analyte in membrane phase
D_S	Diffusion coefficient for active form of analyte in strip phase
E	Extraction efficiency
ECD	Electron capture detector
EC_{50}	Molar concentration of an agonist, which produces 50 % of the maximum possible response for that agonist

ED	Electrodialysis
$E_{e(max)}$	Maximum enrichment factor
EROD	Ethoxyresorufin-O-deethylase
F	Feed phase
f_F	Linear flow velocity in feed phase
GC	Gas chromatography
HPLC	High performance liquid chromatography
h_F	Height (thickness) of feed phase
h_M	Height (thickness) of membrane
h_S	Height (thickness) of strip phase
IC	Ion chromatography
J	Overall flux
J_F	Flux of active form of the analyte in feed phase
J_F,	Flux of inactive form of the analyte in feed phase
J_M	Flux of analyte in membrane phase
J_S	Flux of active form of the analyte in strip phase
J_S,	Flux of inactive form of the analyte in strip phase
k	Overall mass transfer coefficient
K_a	Dissociation constant
k_F	Mass transfer coefficient in feed phase
K_F	Partition coefficient of the analyte between feed and membrane phase
k_S	Mass transfer coefficient in strip phase
K_S	Partition coefficient of the analyte between strip and membrane phase
kV	Kilo volt
LC	Liquid chromatography
LLE	Liquid liquid extraction
Log *P*	Partition coefficient
MDL	Method detection limit
mg	Microgram
min	Minute
ml	Milliliter
mol	Mole
n	Number of samples

nm	Nanometer
OcSA	1-octanesulfonic acid (sodium salt)
org.	Organic phase
Pc	Critical displacement pressure
pH	The negative logarithm of the hydrogen ion (H^+) concentration
PME	Polymeric membrane extraction
PP	Polypropylene
PTFE	Poly(tetra fluoro ethylene)
r	Pore radius
$\mathbf{S_{rel}}$	Relative standard deviation
S	Strip phase
SLM	Supported liquid membrane
SL-FM	Supported liquid flat-membrane
SL-BM	Supported liquid bag membrane
SL-4-BM	Supported liquid membrane-4-bag membrane
SPE	Solid phase extraction
STP	Sewage treatment plants
TOF	Time-of-flight
TOPO	Tri-n-octylphosphine oxide
TWA	Time-weighted average
UV	Ultraviolet
$\boldsymbol{V_F}$	Volume of feed phase
$\boldsymbol{V_S}$	Volume of strip phase
w/w	Weight/ weight
α	Fraction of analyte in active form
$\boldsymbol{\alpha_F}$	Fraction of analyte in active form in feed phase
$\boldsymbol{\alpha_S}$	Fraction of analyte in active form in strip phase
Γ	Interfacial tension
Θ	Contact angle between the water and the membrane
ξ	Membrane tortuosity
E	Membrane porosity
ΔC	Concentration difference of neutral extractable analyte between feed and strip

Abbreviation of active drugs and metabolites

Active drug		Metabolite	
CBZ	**carbamazepine**	**CBZ-DiOH**	**10,11-dihydroxycarbamazepine**
		CBZ-EP	10,11-epoxycarbamazepine
		CBZ-OH	1-hydroxycarbamazepine
		CBZ-2OH	2-hydroxycarbamazepine
		CBZ-3OH	3-hydroxycarbamazepine
		CBZ-10OH	10,11-dihydro-10-hydroxycarbamazepine
DCF	**Diclofenac**	**DCF-4OH**	**4'-hydroxydiclofenac**
		DCF-Glu	Diclofenac-N-glucuronid
		DCF-M	3'- methoxydiclofenac
		DCF-MOH	3'-hydroxy-4'-methoxydiclofenac
		DCF-3OH	3'-hydroxydiclofenac
		DCF-DiOH	4', 5-dihydroxydiclofenac
		DCF-5OH	5-hydroxydiclofenac
IBU	**Ibuprofen**	**IBU-2OH**	**2-hydroxyibuprofen**
		IBU-OH	1-hydroxyibuprofen
		IBU-CA	Carboxyibuprofen
		IBU-3OH	3-hydroxyibuprofen
SFM	**sulfamethoxazole**	**SFM-Ac**	**N4-acetylsulfamethoxazole**
		SFM-Glu	**Sulfamethoxazole-N1-glucuronide**
		SFM-Me	5-methylhydroxysulfamethoxazole
		SFM-MOH	N4-acetyl-5-methylhydroxylsulfamethoxazole
		SFM-NOH	N-hydroxysulfamethoxazole

***Bold letters:** compound was used in this investigation

1 Introduction

1.1 Preface

The issue of pharmaceuticals in the aquatic environment has raised increasing concern in recent years. Human and veterinary pharmaceuticals are a group of “emerging” contaminants [1], some of which are produced and used in increasingly large volume every year. The amounts produced are reaching quantities similar to those of pesticides and other organic pollutants. Residues of these biologically active compounds can enter the environment via transport pathway-emissions during manufacture, disposal of unused or expired medicines, human and animal excretion in urine and faeces, direct discharge of aquaculture products, and manure and slurry spreading [2].

The majority of pharmaceutical compounds enter aquatic systems after ingestion and subsequent excretion in the form of the non-metabolized parent compounds or as metabolites via the sewage treatment network [3]. Several investigation have shown evidence that sewage treatment plants (STPs) are not able to remove these drugs and their excretion metabolites completely and they are discharged to different environmental compartments at concentrations ranging from ng/L to μg/L [4]. In addition to river and sea water, recent studies have shown they may even enter drinking water produced from ground water [4, 5]. Many believe that of all the emerging contaminants, antibiotics are the biggest concern because of the potential for antibiotics resistance. The increasing use of these drugs in the livestock, poultry production, and fish farming during the last fives decades has caused a genetic selection of more harmful bacteria, which is a matter of great concern [6]. However, other pharmaceuticals compounds, especially polar one, such as anti-epileptics [7], analgesic and anti-inflammatory drugs [8] also deserve particular attention.

Elimination of these pharmaceuticals in STPs was found to be rather low and consequently sewage effluents are one of the main sources for these compounds and their recalcitrant metabolites. Due to their physico-chemicals properties (high water solubility and often poor degradability) they are able to penetrate through all natural filtration steps and enter groundwater as well as drinking water [9]. In comparison with conventional pollutants, these substances are designed to have specific pharmacological and physiological functions and thus are inherently potent, often with unintended health outcomes in wildlife [10]. Particularly, there is an urgent need for the additional detection of metabolites as has been demonstrated in the case of clofibrate or erythromycin: in both cases the active compounds

could not be found in water samples but their main metabolites [10], which may also cause severe (eco-) toxicological effects. The lack of information on the environmental fate of both active drugs and their metabolites should be completed for many of the pharmaceuticals applied. Consequently there is a necessity to monitor the input of pharmaceutical residues in the different waterways, e.g. surface and groundwater, by means of sensitive chromatographic methods. Hyphenated techniques such as LC-MS or GC-MS are preferred for the predominantly polar compounds [11-13]. However, even those sophisticated methods may need efficient sample pre-treatment to minimise the effects of the sample matrix and to enrich the analytes.

To achieve the separation of pharmaceuticals from water samples, adsorption techniques such as solid phase extraction (SPE) are mostly applied, however, the enrichment factors obtained are not high (<100). Likewise synthetic membranes are often used but not with satisfactory results particularly to small molecules [14]. In principal the liquid membrane extraction, provide several advantages compared to other extraction methods. It offers a high selectivity and, thus, an efficient cleanup from complex matrices by achieving high enrichment factors and reduced use of organic solvent [15, 16]. Although liquid membrane extraction systems were found to be effective for the acidic and basic drugs [17, 18], no information is currently available on liquid membrane extraction of highly polar drugs from water samples. In the present work attention was focused to develop certain types of liquid membranes: bulk liquid membranes (BLM) and supported liquid membranes (SLM). Some of these systems have been already successfully used for the separation of diclofenac and ibuprofen [20].

1.2 Aim of the study

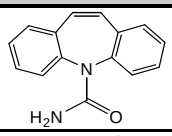
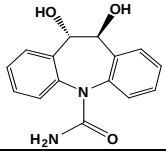
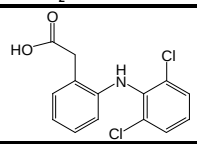
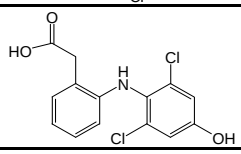
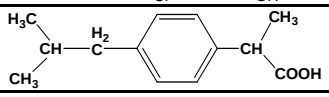
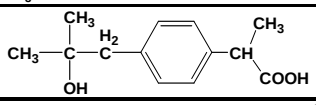
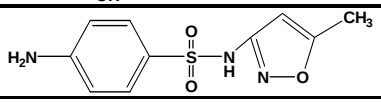
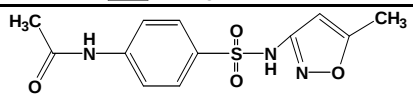
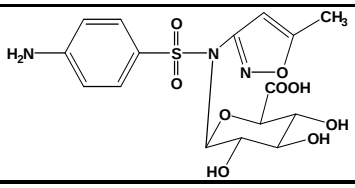
The aim of the present study was to develop analytical methods for the determination of selected metabolites and the corresponding active drugs of environmental concern in water samples. The methods will be based on enrichment steps by means of certain liquid membrane-systems (bulk type and SLM) and liquid chromatography (HPLC-UV or LC-MS).

The target metabolites were: 10,11-dihydroxycarbamazepine (CBZ-DiOH), 4′-hydroxydiclofenac (DCF-4OH), 4-hydroxyibuprofen (IBU-2OH), N-4-acetylsulfamethoxazol (SFM-Ac) and sulfamethoxazol-N1-glucuronid (SFM-Glu) and their parent drugs carbamazepine (CBZ), diclofenac (DCF), Ibuprofen (IBU) and sulfamethoxazole (SFM) (Table-1.1). The selection of these pharmaceuticals is based on their amounts applied for medical purposes [21], their relative high concentrations found in the aquatic environment

[22], their occurrence in the environment [23- 28] and their structural diversity (Table-1.1). The selected metabolites CBZ-DiOH, DCF-4OH, IBU-2OH, SFM-Ac and SFM-Glu are not commercially available; however, they are required to perform the membrane tests and to use them as reference substances for the calibration of the chromatographic systems. Therefore, the metabolites had to be synthesized. As a consequence the present study is divided into three scopes:

- Synthesis of the selected metabolites.
- Investigation of the mass transfer of these compounds in liquid membrane systems.
- Development of analytical methods by combining membrane extraction and determination by HPLC.

Table-1.1: Structure of selected pharmaceuticals

Pharmaceutical class	Compound	Chemical structure
Antiepileptics	Carbamazepine (CBZ)	
	Carbamazepine metabolite: 10,11-Dihydroxycarbamazepine (CBZ-DiOH)	
Analgetics and anti-inflammatories	Diclofenac (DCF)	
	Diclofenac metabolite: 4′-hydroxydiclofenac (DCF-4OH)	
	Ibuprofen (IBU)	
	Ibuprofen metabolite: 4-Hydroxyibuprofen (IBU-2OH)	
Antibiotics	Sulfamethoxazole (SFM)	
	Sulfamethoxazole metabolite: N-4-acetylsulfamethoxazol (SFM-Ac)	
	Sulfamethoxazole metabolite: Sulfamethoxazol-N1-glucuronide (SFM-Glu)	

1.3 Pharmaceuticals in the aquatic environment: Theoretical background

1.3.1 Pharmacokinetics and drug metabolism

A major factor determining the occurrence of pharmaceuticals in the aquatic environment is their pharmacokinetic behavior which describes the times course of a drug and metabolites in the human body after any kind of administration [33]. Drug metabolism, or biotransformation, is a major route by which drugs are eliminated from the body [29]. The metabolism of pharmaceuticals occurs by (phase I) and/ or conjugation (phase II) functionalization reactions, which usually resulted into polar, water-soluble, and extractable metabolites via urine and faeces [30].

The clinical response to a therapeutic agent is related to the plasma concentration of the drug and/ or its metabolism. It has been well established that the metabolism of a drug is often a major determinant of the duration and intensity of its clinical effects. Metabolic processes are necessary to convert a lipophilic drug into one or more metabolites, which are more water soluble than the parent drug, facilitating urinary excretion [30]. Drug metabolism may yield inactive metabolites, but this is not always true. In some cases, metabolites have pharmacological activity similar to that of parent drug: well known examples are many benzodiazepines, whose long-lived active metabolites cause their effects to persist after the parent drug has been eliminated, or some antidepressants, such as imipramine and amitriptyline, whose antidepressant action is partially due to their metabolites desmethylimipramine and nortriptyline [30]. In some cases, a drug referred to as pro-drug, becomes pharmacologically active only after metabolism: an example is the angiotesin-converting enzyme inhibitor enalapril that exerts its pharmacological activity through is metabolite enalaprilat. Metabolism can alter the pharmacological properties of a compound qualitatively: for example, salicylic acid shares with its parent drug acetylsalicylic acid the anti-inflammatory, but not the antiplatelet activity. There are also cases in which the metabolism yields toxic compounds: an example is the hepatotoxicity of paracetamol, caused by drug metabolising N-acetyl-p-benzoquinone imine [31]. Hence, variability in activity of drug metabolising enzymes can lead to interindividual differences in drug effects, one of the major problems in drug therapy [31].

Drug metabolism involves a wide rang of chemical reactions, including oxidation, reduction, hydrolysis, hydration, conjugation, condensation, and isomerization [30]. The enzymes

involved are present in many tissues (like brain, lung, intestine and testicle) but generally are more concentrated in the liver. For many drugs, metabolism occurs in two apparent phases:

Phase I reactions include oxidative, reductive and hydrolytic biotransformation. The purpose of these reactions is to introduce a polar functional group (e.g. -OH, -COOH, $-NH_2$, -SH) into the drug molecule. This can occur through direct introduction of the functional group (e.g. aromatic and aliphatic hydroxylation) or by modifying existing functionalities (e.g. reduction of ketone and aldehydes to alcohols; oxidation of alcohols to acids; hydrolysis of ester and amides to yield COOH, NH_4 and OH groups; reduction of azo and nitro compounds to give NH_2 moieties and oxidative -N, -O, -S dealkylation to give $-NH_2$, -OH and -SH groups. Phase I products are often more reactive and sometimes more toxic than the parent drugs [31].

***Cytochrome P-450*:** The most important enzyme system of phase I metabolism is cytochrome P-450, a microsomal superfamily of isoenzymes that transfer electrons and thereby catalyze the oxidation of many drugs, which located in the membrane of the smooth endoplasmic reticulum, mainly in the liver, but also in extrahepatic tissues (e.g. intestinal mucosa, lung, kidney, brain, lymphocytes, placenta, etc.) [31]. The electrons are supplied by NADPH-cytochrome P-450 reductase, a flavoprotein that transfers electrons from NADPH (the reduced form of nicotinamideadenine dinucleotide phosphate) to cytochrome P-450 (see Figure-1.1) [32]. Cytochrome P-450 enzymes are grouped into 14 mammalian gene families that share sequence identity and 17 subfamilies. Enzymes in the 1A, 2B, 2C, 2D and 3A are most important in mammalian metabolism; CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are important in human metabolism.

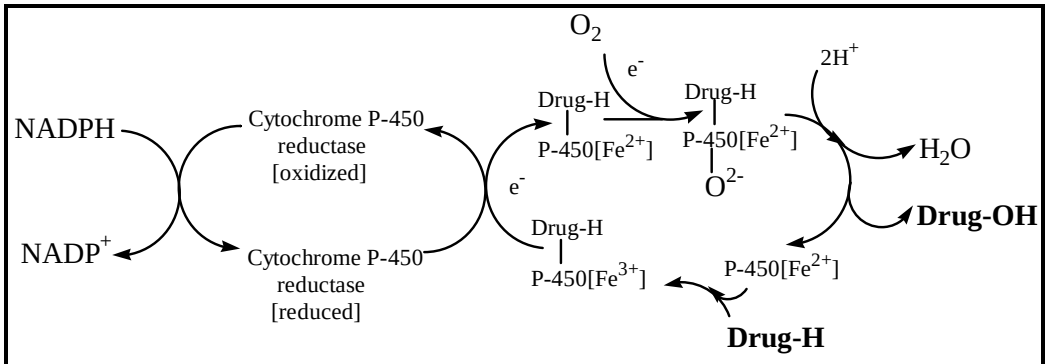


Figure-1.1: Phase I metabolism pathways (oxidation reactions) [31, 32]

Phase II reactions from water-soluble conjugated products by reaction with polar and ionisable endogenous compounds such as glucuronic acid, sulphate, glycine and other amino acids to the functional groups of phase I metabolites. Conjugated metabolites are readily

excreted in the urine and are generally pharmacologically inactive and non-toxic. Other phase II pathways, such as methylation and acetylation, serve to terminate biological activity, while glutathione conjugation serves to protect the body against chemically reactive compounds. Drugs that already have existing functional groups, such as OH, COOH, and NH_2, are often directly conjugated by reactions with phase II enzymes [30].

1- Glucuronidation: Glucuronidation is the most widespread of the conjugation reactions probably due to the relative abundance of the cofactor for the reaction, UDP-glucuronic acid. UDP-glucuronic acid (uridine diphosphoglucuronic acid), being part of intermediary metabolism and closely related to glycogen synthesis, is found in all tissues of the body. The enzymes involved are located in the cytosol. UDP-glucuronic acid can be considered as an energy-rich intermediate for the transfer of the glucuronic acid moiety [30].

N-glucuronide conjugation is one of the conjugation reactions which come from the reaction between amines (mainly aromatic), amides and sulfonamides with UDP-glucuronic acid as shown in Figure-1.2.

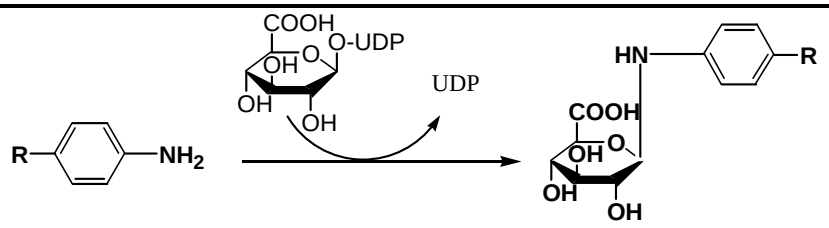


Figure-1.2: Phase II metabolism (N-glucuronide conjugation reaction) [30]

***2- Acetylation*:** acetylation reactions are typical for aromatic amines and sulfonamides and require the co-factor, acetyl-CoA (Figure-1.3). Acetylation takes place mainly in the liver and can be also in the reticuloendothelial cells of the spleen, lung and gut [30].

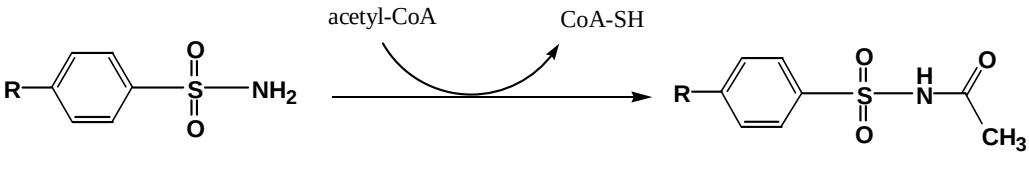


Figure-1.3: Phase II metabolism (acetylation reaction)[30]

1.3.2 Exposure pathway

Production and application of human and veterinary pharmaceuticals lead to a potential environment exposure and potentially to an accumulation in certain environmental

compartment. After their use, pharmaceuticals are excreted uncharged and/or as metabolites in feces and urine and hence are present in wastewater [10].

Similar to other compounds of anthropogenic origin, the fate of the pharmaceuticals residues during sewage treatment can follow one or a combination of three types of behavior: a) biodegradation, b) sorption of the residues onto sewage sludge or c) elimination [21]. The proportion of the pharmaceutical that is retained in sewage treatment either due to biodegradation or by sorption to sludge strongly depends on its chemical structure and physico-chemical properties, but also on the specific conditions within the respective plant. Water temperature, residence times corresponding to flow rates, dilutions with rainwater and sludge age were found to have influence on elimination efficiencies [10].

Hence, compounds that are not readily degradable enter the environment either with the digested sludge or as dissolved pollutants in the sewage treatment plant (STP) discharges. The latter scenario results in the contamination of the receiving waters and finally, the aquatic environment [21].

1.3.3 Occurrence and fate

Numerous studies have been conducted to investigate various aqueous matrices for the presence of pharmaceuticals residue, comprising the target compounds and metabolites. In fact, these residues have been found to be ubiquitous in environmental waters. The main contributing factor for the occurrence of pharmaceuticals in the aquatic environment is the elimination efficiency of the STP [34].

As described before, many pharmaceuticals are excreted to a large extent as transformed phase I metabolites and/or after conjugation to hydrophilic groups as phase II metabolites. Conjugates are easily cleaved in the STP, causing a re-formation of original pharmaceuticals [34]. This might lead to higher concentration in the STP effluent than in the raw wastewater.

Residues of various pharmaceuticals are present in the low µg/L rang in STP effluents. Discharge of the STP effluent into the receiving waters leads to a dilution of the pharmaceuticals residues which occur up to the ng/L range in contaminated surface water. Once introduced into the surface waters, pharmaceuticals may undergo biodegradation, most likely due to co-metabolic processes. For some pharmaceuticals, photo induced degradation may occur from natural solar radiation [35].

The determination of the environmental fate of a compound is a complex issue. Transformation and distribution processes are strongly dependant on the specific

environmental conditions, which lead to a sophisticated linkage of individual systems parameters.

By (continuous) exposure to low concentrations of pharmaceuticals in the theory, the following negative effects on aquatic organisms are possible:

- Ecotoxicological effects.
- Pharmaceuticals effects.
- Resistance development of micro-organism.

It is clear that during the past few years a wealth of data has become available on the levels of pharmaceuticals in the environment and their effects on the aquatic and terrestrial organisms. There are however, still many questions that need to address before we can eventually determine whether residues in the environment are a threat to human and environment health.

1.3.4 Pharmaceuticals under study

1.3.4.1 Carbamazepine (CBZ)

Carbamazepine (CBZ) is an established drug for the control of grand and psychomotoric epilepsy and it is also effective in the treatment of trigeminal neuralgia. Furthermore, it is presently used in bipolar depression. It is predominantly eliminated in the liver, where it is metabolised to 10,11-epoxycarbamazepine (CBZ-EP) and other derivatives (Figure-1.4). CBZ-EP seems to have antiepileptic properties as well as CBZ itself [36, 37].

CBZ undergoes extensive hepatic metabolism by the cytochrome P-450 (CPY) system. Thirty-three metabolites of CBZ have been identified from human and rate urine. The main metabolic pathway of CBZ is oxidation to 10,11-epoxycarbamazepine (CBZ-EP), hydration to 10,11-dihydroxycarbamazepine (CBZ-DiOH) and 10-hyroxycarbamazepine (CBZ-10 OH), then conjugation of these compounds with glucuronide. The second minor distinct pathway for the biotransformation of CBZ, catalyzed by cytochrome P-450 (CPY), involving oxidation to 1-hydroxcarbamazepine, 2-hydroxycarbamazepine and 3-hydroxycarbamazepine, and subsequent conjugation glucuronide see Figure-1.4 [38-41].

In human lymphocytes, CBZ is metabolized by CYP dependant monooxygenase into CBZ-EP, an active and toxic metabolite which is then transformed into the corresponding CBZ-DiOH by an epoxide hydrolase (Figure-1.4). A strong and specific CYP1A inhibition was observed from the CBZ biotransformation into reactive metabolites [42] (Table-1.2).

Environmental field studies have shown that the CBZ is one of the most frequently detected pharmaceuticals in sewage treatment plant effluent, in river water, and in seawater [43]. Investigations of influent and effluent samples from different municipal STPs have shown that CBZ is not significantly removed (less than 10 %) during sewage treatment (Table-1.3). Thus, CBZ has been detected at concentrations more than 1 μg/L in surface water [44- 46]. Also different studies have shown that CBZ is not biological degradable (Table-1.3), this explains why CBZ has been detected in a number of groundwater samples [47- 50] (Table-1.4). In addition all five metabolites of CBZ (Figure-1.4) were detected in the STP influent and effluent samples. Only CBZ and CBZ-DiOH were detected in the surface water (Table-1.4).

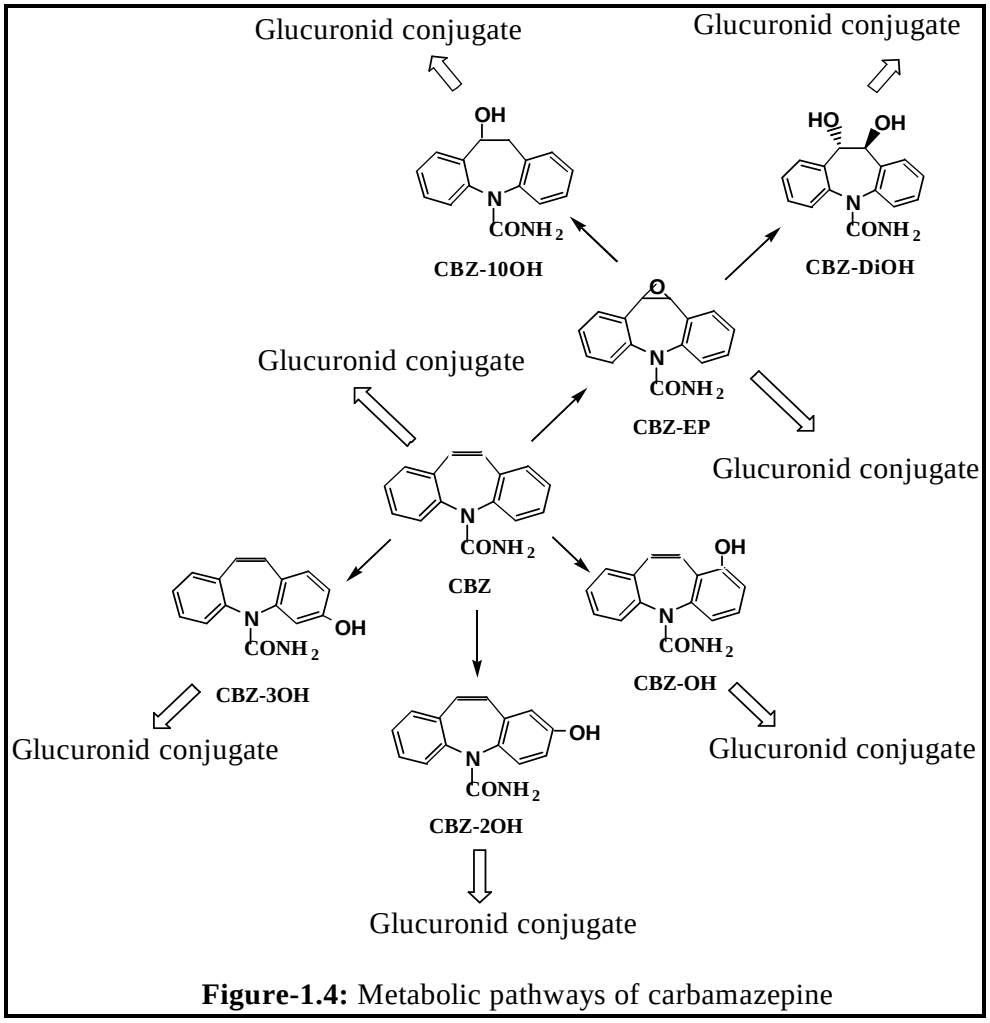


Figure-1.4: Metabolic pathways of carbamazepine

1.3.4.2 Diclofenac (DCF)

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID). In pharmacologic studies, DCF has shown anti-inflammatory, analgestic, and antipyretic activity. DCF is indicated for the acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis.

In addition, DCF is indicated for the treatment of ankylosing spondylitis and for the management of pain and primary dysmenorrheal [51].

In human, metabolism of DCF is mediated by both glucuronidation and oxidative biotransformation [51]. Oxidation of the aromatic rings is mediated by cytochromes P-450 (CYP). Hydroxylation of the dichlorophenyl ring is catalyzed specifically by CYP2C9 to produce 4′-hydroxydiclofenac (DCF-4OH) as the major metabolite [52]. Another five metabolites of DCF have been identified in human plasma and urine [53]. The metabolites include 3′-hydroxydiclofenac (DCF-3OH), 5-hydroxydiclofenac (DCF-5OH), 4',5-dihydroxydiclofenac(DCF-DiOH), 3′-hydroxy-4′-methoxydiclofenac (DCF-MOH), 3′-methoxydiclofenac (DCF-M), and diclofenac-N-glucuronid (DCF-Glu), [53] (Figure-1.5). Approximately 65 % of the dose is excreted in the urine, and approximately 35 % in the bile. Little Conjugates of uncharged DCF account for < 1 % of the dose excreted in the urine and for less than 5 % excreted in the bile. 15 % of unchanged unconjugated drug is excreted by via urine see Table-1.3.

The DCF exerted a cytotoxic effect at 500 µM, which is in agreement with previous results on rat or human hepatocytes [54]. The mechanism of DCF cytotoxicity is not fully understood but there is some evidence that both uncoupling of mitochondrial oxidative phosphorylation and CYP-mediated metabolism are involved in human and rat hepatocytes acute toxicity [55]. It was observed a clear inhibition of EROD (Ethoxyresorufin-O-deethylase) at 36 µM, suggesting a specific interaction between DCF or its metabolites with this enzyme in rainbow trout [42] (Table-1.2).

Approximately, 86 tons of the prescriptions DCF are annually sold in Germany (Table-1.3). In long-term monitory investigations of sewage and surface water samples (Table-1.4), DCF identified as one of the most important pharmaceuticals present in the water-cycle. The removal rates which have been reported of DCF in STPs were between 9-75 % [56, 57] (Table-1.3).

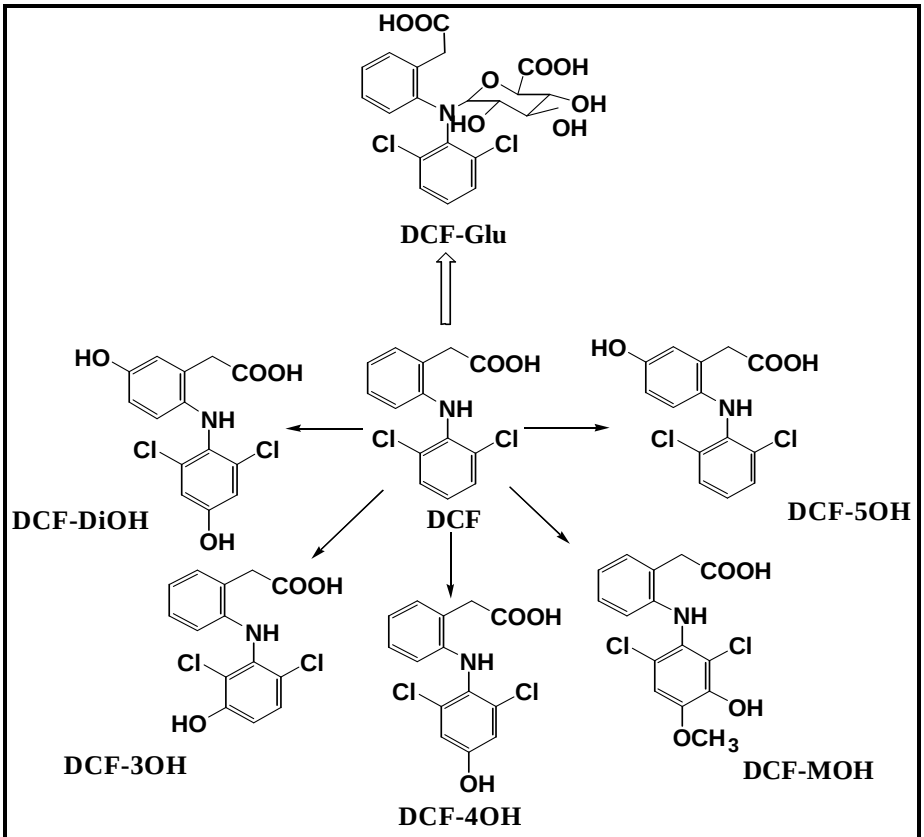


Figure-1.5: Metabolic pathway of diclofenac[53]

1.3.4.3 Ibuprofen (IBU)

Ibuprofen (IBU) is a nonsteroidal anti-inflammatory drug (NSAID). IBU possesses analgetic and antipyretic activities. Its mode of action, like that of other NSAIDs, is not completely understood, but may be related to prostaglandin synthetase inhibition. IBU is indicated for relief of the signs and symptoms of rheumatoid arthritis and osteoarthritis. IBU is also indicated for relief of mild to moderate pain and for the treatment of primary dysmenorrheal [51]. It is on the most important pharmaceuticals in term of quantities consumed seeing Table-1.3.

Oxidative metabolism by using cytochrom P-450 is the major route for biotransformation of IBU. Four oxidative metabolites have been identified in urine and plasma samples obtained from human after oral intake of IBU [58] (Figure-1.6). In humans the parent drug, as well as the metabolites, are found to be conjugated with glucuronic acid [59, 60], and glucuronidation has in all cases taken place at the carboxyl group in the propionic acid side chain. Studies have shown that following ingestion of the drug, 45 % to 79 % of the dose was recovered in urine within 24 hours as metabolites: 2-hydroxyibuprofen (IBU-2OH) 25 %, and 37 % as

carboxyibuprofen (IBU-CA); the percentages of free and conjugated ibuprofen were 1-8 % and 14 %, respectively see Table-1.3 [51].

IBU has been shown to significantly affect the growth of several bacterial and fungal [61-63]. Some studies suggests depending with evidences that IBU metabolites are non toxic for aquatic organisms tested, and may however have growth stimulating properties [64, 65] (Table-1.2).

Because of large amounts produced and used (Table-1.3), IBU is an environmentally relevant compound. In sewage water, sewage effluents, and surface water ibuprofen was detected among other pharmaceuticals residues in the range of ng- μg/L [59] (Table-1.4). IBU is degradable in the human body to its principal metabolites 1-hydroxyibuprofen (IBU-OH) and IBU-CA, which are found together with IBU in raw sewage. Some studies observed a significant removal of IBU and especially of IBU-CA during sewage treatment, whereas the concentration of IBU-OH in the sewage effluents was almost similar to those in the influents [44]. Degradation experiment in both biofilm reactors (BFR) and batch experiments with activated sludge (BAS) reveal IBU-OH as the major metabolite of IBU under oxic conditions, and IBU-CA under anoxic conditions. Efficient elimination (95-99 %) of all these compounds (IBU, IBU-CA, and IBU-OH) was found in the municipal STPs [60]. Other studies indicate that microbial biofilm and other microbial activity play an important role in the degradation of IBU in the surface water systems and the degradation pathway and resultant metabolism differ from those observed in human metabolism. Thus, IBU-OH was found in surface water at much higher concentration than IBU or IBU-CA [66].

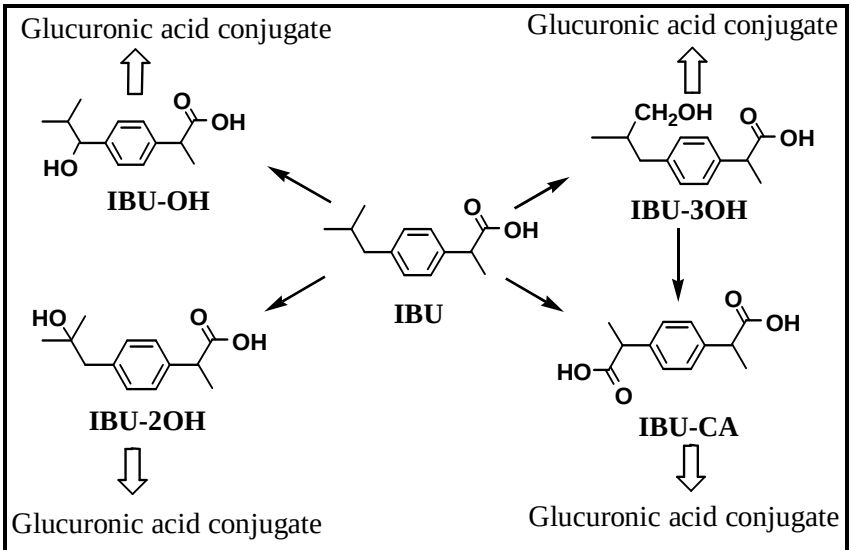


Figure-1.6: Metabolic pathway of ibuprofen [60]

1.3.4.4. Sulfamethoxazole (SFM)

Sulfamethoxazole (SFM) is a member of the sulfonamide family of antibiotics for human and animal. It is one of the most widely used anti-bacterial agents [67].

The known metabolism of SFM involves acetylation and oxidation leading to N4-acetylsulfamethoxazol (SFM-Ac), and N-hydroxysulfamethoxazole (SFM-NOH) (Figure-9). Hydroxylation take also place to SFM metabolism leading to 5-methylhydroxy-sulfamethoxazole (SFM-Me), and N4-acetyl-5-methylhydroxylsulfamethoxazole (SFM-MOH). Moreover SFM is glucuronidated leading to sulfamethoxazole-N1-glucuronide (SFM-Glu) [68] (Figure-4.4). About 50-60 % of applied dose in human body was excreted as the inactive metabolite (SFM-Ac), 15 % as the conjugate metabolite (SFM-Glu), and only 15-20 % as the uncharged active compound [6] (Table-1.3).

The SFM was not cytotoxic enough to calculate EC_{50} values; it inhibited EROD activity right from 125 µM (Table-1.2). In human liver microsomes, SFM is described as a selective inhibitor of CYP2C8 and CYP2C9 that would loose selectivity for the CYP isoforms at concentrations higher than 500 µM [69]. As a result, SFM must be a selective inhibitor of CYP1A enzymes in fish hepatocytes [42] (Table-1.2).

Most of antibiotics are metabolized only incompletely by patients after administration and enter municipal sewage and sewage treatment plants. If they are not eliminated during sewage treatment plants they are emitted into surface water and may reach drinking water [70].

SFM has been detected in sewage discharge at concentration of 0.62 µg/L [71], and detected SFM in 12.5 % of surface water samples at a median concentration of 0,15 µg/L [72, 73]. Also it was reported that SFM could be removed about 67 % during the biological step in municipal sewage treatment plant [44] as shown in Table-1.3.

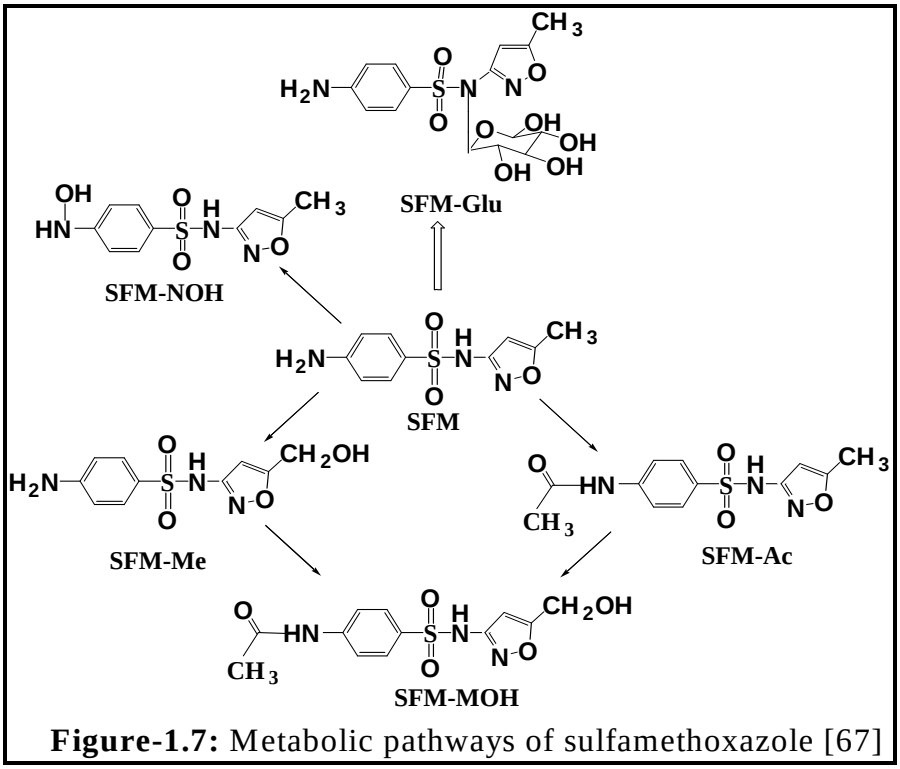


Figure-1.7: Metabolic pathways of sulfamethoxazole [67]

Table-1.2: Reported effects of active parent drugs on aquatic and terrestrial organisms

Substance	Reported effect	Reference
Ibuprofen	Stimulation of growth of cyanobacteria and inhibition of growth of aquatic plants	[62]
Diclofenac	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes	[42]
Carbamazepine	Inhibition of basal EROD activity in cultures trout hepatocytes. Inhibition of emergence of *Chironomus riparius*	[42, 81]
Sulfamethoxazole	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes	[42]

Table-1.3: Active drugs under study: basic properties and ecotoxicological data

Parameter	SFM	CBZ	DCF		IBU	
Formula [74]	$C_{10}H_{11}N_3O_3S$	$C_{15}H_{12}N_2O$	$C_{14}H_{11}Cl_2NO_2$		$C_{13}H_{18}O_2$	
Molecular weight (g/mol) [74]	253.28	236.27	296.15		206.28	
Solubility in water (mg/L) [74]	610	112	242		291	
Melting point [74]	167-169 ^oC	190-192 ^oC	156-158 ^oC		78.87 ^oC	
Dissociation constant (p*Ka*) [74]	5.81±0.5 1.39±0.1	13.94±0.2	4.18±0.2		4.41±0.2	
Octanol-water partition coefficient (Log *P*) [74]	0.887±0.419	2.673±0.376	3.284±0.361		3.722±0.227	
Estimated amount used (tons/year) [22]	54[a]	88[a], 40[b], 10[c], 38[d]	86[a], 26[b]		344[a], 162[b], 14[c], 34[e]	
Total removal via wastewater treatment [75]	Activated sludge	Activated sludge	Activated sludge	Biologic filter	Activated sludge	Biologic filter
	67 %	7-10 %	69-75 %	9 %	90-99 %	65 %
Predicted environmental concentrations for surface water (PEC_{SW}) (ng/L) [74]	895[a]	1460[a]	….		…..	
Environmental risk indicators [75]	High volumes; detected in the environment; concerns over toxicity and antibacterial resistance	High volumes; long-term prescription; persistent	Very high prescription and over-the-counter; detected in the environment		Very high prescription and over-the-counter; detected in the environment	

[a]Germany in 2001
[b]UK in 2000
[c]Australia in 1998
[d]France in 1998
[e]Denmark in 1997/1998

Table-1.4: Detected concentrations (μg/L) for active drugs and their selected metabolites in different water sources

Substance	Inflow wastewater	Effluent wastewater	Surface water	Ground water	Drinking water	References
IBU	3.3- 0.99	0.1, 0.37, 2				[21]
		0.37	0.07			[54]
	1.642	0.12		n.d.		[77]
		1.5, 0.87, 85	2.7			[2]
IBU-2OH	0.92		0.34			[2]
IBU-CA			0.02			[2]
DCF	3.55-0.5	3 - 1.18				[77]
	3.02	2.51	>1			[2]
		0.81		0.15		[54]
		0.359	0.194			[43]
CBZ	2.410-0.55	1.67-0.73				[77]
		2.1	1.075	1.1	0.03	[2]
		0.8-0.1	0.25-0.03			[46]
	6.3	2.1				[78]
	1.78	1.63, 2.1				[21]
	0.368	0.426	0.0007			[43]
CBZ-EP	0.047	0.0523	n.d.			[43]
CBZ-DiOH	1.571	1.325	0.0022			[43]
CBZ-2OH	0.121	0.132	n.d.			[43]
CBZ-3OH	0.094	0.101	n.d.			[43]
SFM		> 1.0	0.1-0.2	0.4		[6]
	0.243-0.871	0.008	0.1			[54]
	0.1-1.7	0.05-0.09	6			[46]
				0.41		[2]
	n.d.	0.4, 0.9				[21]
	0.343	0.352				[25]
SFM-Ac		2.2				[79]
			0.316			[80]
		2.2-0.690	0.24			[70]
	0.518	0.082				[25]

n.d.: not detected

1.4 Analytical extraction techniques

In recent years, several analytical techniques and methods have been developed for analysis of various pharmaceuticals and corresponding metabolites in environmental and biological samples. Despite the achievements in analytical science, there are still challenges. One challenge lies in determining pharmaceuticals in various complex matrices such as wastewaters, surface waters, sediments, and biological fluids. Most developed analytical methods require several steps consuming time and solvents. In ecological risk assessment for chemical pollutants, it is important to quantify the concentrations of freely dissolved in aqueous samples for approximate characterization of the bioavailable fraction. The challenge of chemical analysis, especially speciation studies, and determining the freely dissolved pharmaceuticals in a complex sample is staggering. Moreover, components of interest exist at trace levels. These challenges have made sample preparation become a key step in modern chemical analysis. It is essential part of any analytical procedure because of the reasons: sample preconcentration or enrichment and removal of contaminants [82].

The most widely used sample preparation techniques are liquid-liquid extraction (LLE) [83] and solid-phase extraction (SPE) [14]. LLE is the traditional technique for extraction of organic analytes from aqueous solutions. The basis is the partition of the dissolved analytes between the organic phase (extraction liquid) and the aqueous solution (sample solution) according to their partition coefficients. Further shifting of the equilibrium toward the organic phase brings about increased enrichment in the organic phase. The technique is well known and still widely used, although now it is less attractive and is being replaced by other techniques. This is because LLE:

- is tedious and time-consuming especially when extracting aqueous complex samples, which demands many steps before a clean extraction can be obtained;
- forms emulsions which at times makes it difficult to separate the two phases;
- is environmentally unfriendly due to large volumes of organic solvents used. However, with LLE, large enrichment factors can be obtained despite the cited drawbacks.

SPE techniques are perhaps the most popular in sample preparation especially for organic analysis. The principle of SPE is based on sorption of analytes on a sorbent. The aqueous sample solution passes the SPE column, and the analytes are first trapped on the sorbent and then eluted with a suitable small volume of organic solvent. Extraction and enrichment of the analytes is thereby simultaneously achieved. Most sorbents are now available as disks,

cartridges, or precolumns. Despite its simplicity, it lacks selectivity when extracting analytes in complex matrices such as plant extracts, foodstuffs, and wastewater [84].

There are a number of different membrane techniques, which have been suggested as alternative to the SPE and LLE techniques. An area enjoying much attention by various research groups is developing membrane-based extraction techniques that are simple, cheap, and miniaturized [85, 86].

1.4.1 Liquid membrane extraction techniques

Generally, a membrane can be classified as a selective barrier between two phases [15, 16]. With a driving force applied across a membrane, analytes can be transferred from one phase (feed phase) to another (strip phase). The main membrane techniques that have been used for analytical applications can be classified based on whether membrane is porous or nonporous during the extraction of the sample solution [84].

In porous membrane systems, the liquids on either side of the membrane are physically connected through the pores. These membranes are used in dialysis, electrodialysis, and filtration (Table-1.5) to separate low-molecular-mass analytes from high-molecular-mass matrix components, leading to an efficient clean-up but no discrimination between different small molecules. No enrichment of the small molecules is possible; instead the analytes are diluted since the driving force of the mass transfer process is a simple concentration difference across the membrane.

Nonporous membrane is utilized in membrane extraction techniques. Such a membrane is liquid or solid (such as a polymeric) phase that is placed between two other phases-usually liquid but can also be gaseous [16]. A nonporous membrane may in fact have pores, but these are usually micropores. The nonporous membrane extraction techniques include bulk liquid membrane (BLM), supported liquid membrane (SLM), microporous membrane liquid-liquid extraction, semipermeable membrane devices, polymeric membrane extraction, and membrane extraction with a sorbent interface [87] see Table-1.5.

A wide variety of membrane materials are available. Porous synthetic membranes, such as polypropylene, polysulphone and cellulose derivative, are most widely employed. Ion-exchange membranes and nonporous membrane are commonly used as well. The pore sizes of the membranes vary with the technique applied. In dialysis, for example, the pore sizes range from a few nanometers to 100 nm, while in SLM from 0.1-10 μm. Most of the applications involve the use of a planar flat-sheet membrane, but hollow fibre membranes are also

available. The liquid membrane techniques have been coupled with liquid chromatography and gas chromatography and they have been applied to gaseous and liquid samples [84].

Table-1.5: Different major membrane techniques used in analytical applications [85]

Name	Abbr.*	Type	Membrane contents	Phase combinations used feed/membrane/strip
Dialysis	…..	porous membrane	porous membrane	aq/membrane/aq
Electrodialysis	ED	porous membrane	porous membrane	aq/membrane/aq
Filtration	….	porous membrane	porous membrane	aq/membrane
Bulk liquid membrane	BLM	nonporous membrane	organic liquid	aq/org/aq
Supported liquid membrane	SLM	nonporous membrane	porous polymer soaked with organic liquid	aq/membrane/aq
Microporous membrane liquid-liquid extraction	MMLLE	nonporous membrane	porous polymer soaked with organic solvent	aq/membrane/org
Semipermeable membrane devices	SPMDs	nonporous membrane	polymer	aq/polymer/org
Polymeric membrane extraction	PME	nonporous membrane	polymer	aq/polymer/aq, org/polymer/aq, aq/polymer/org
Membrane extraction with sorbent interface	MESI	nonporous membrane	polymer	gas/polymer/gas, liquid/polymer/gas

Abbr.*: Abbreviation

1.4.1.1 Bulk liquid membrane (BLM)

It is a liquid phase, usually organic, interposed between two miscible aqueous solutions. At one side of the membrane (feed phase) the material to be transported is extracted, while at the other side (strip phase) re-extraction occurs. Since in each of the aqueous phase some specific, and different for each of them, thermodynamic conditions exist, the extraction and re-extraction occur simultaneously.

The BLM technique requires a simple design of the cell for the transport process to be realized (Figure-1.8). The membrane liquid is placed above the feed and strip solutions, separated with a solid barrier, thus being in contact with both of these solutions [88-91].

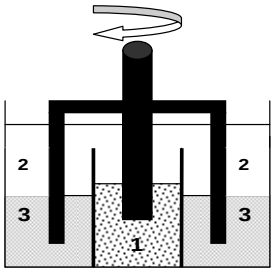


Figure-1.8: Bulk liquid membrane, (1) feed phase (2) membrane (organic solvent) and (3) strip phase

There are two basic principle how the BLM operate (Figure-1.9). In the first, simple case, the analyte, A, is transferred from the feed phase into the membrane, because of its larger solubility in the organic phase, and then from the membrane into the strip phase, where conditions prevent back-extraction of the analyte (Figure-1.9a), this is the so-called "unfacilitated transport". For the second case, "facilitated transport" (Figure-1.9b), the appropriate solubility of the analyte A in the membrane phase is not required since the analyte interacts with the carrier molecule, C, dissolved in the membrane phase. This carrier should be totally insoluble in both feed and the strip phases and should react and reversibly with the analyte [88, 92, 93].

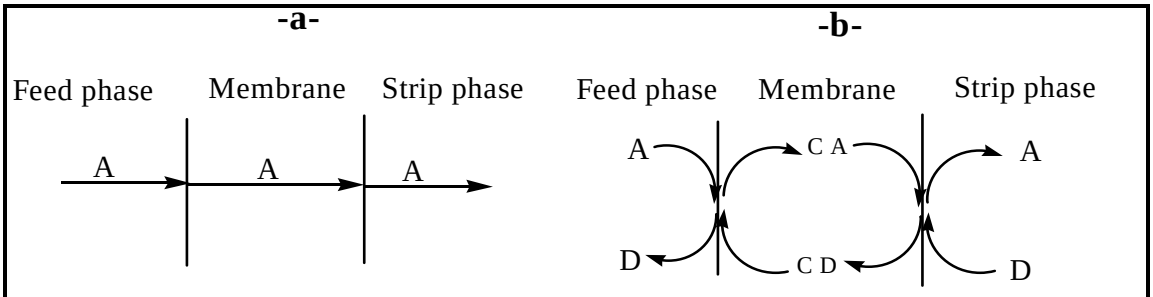


Figure-1.9: Schematic representation of transport mechanisms of analyte through bulk liquid membrane (a) unfacilitated transport, (b) facilitated transport [88]

1.4.1.2 Supported liquid membrane (SLM)

In an SLM extraction, an organic solvent is immobilized in the pores of an inert support material, separating the aqueous feed and the strip phases (Figure-1.10). The analytes are partitioned from the aqueous sample stream into the organic membrane and are then re-extracted into the aqueous strip phase. The driving force is the difference of the analyte concentration between the feed and strip phases. In order to maintain the concentration gradient across the two phases, the solutes must be able to exist in two forms: in a nonionic form on the feed side to extract into the membrane and in an ionic form on the strip side in

order to irreversibly trapped [15, 16, 82, 84, 85]. This is most simply achieved by pH adjustments in the two phases, and the method is, therefore, particularly well suited for ionizable compounds such as medium-to-weak acids and bases [94- 97]. SLM extraction can provide very selective enrichment. Selectivity can be fine-tuned by proper choice of the conditions in the three phases as seen in Figure-1.10 [98]. This creates a selectivity window such that by the time the analytes are enriched in the strip phase, an indirect structural recognition is achieved and only analytes belonging to the same family are generally trapped at a time. Macromolecules are discriminated on the basis of their size while charged compounds are too polar dissolve into the organic liquid. Natural molecules merely distribute between the three phases without any enrichment.

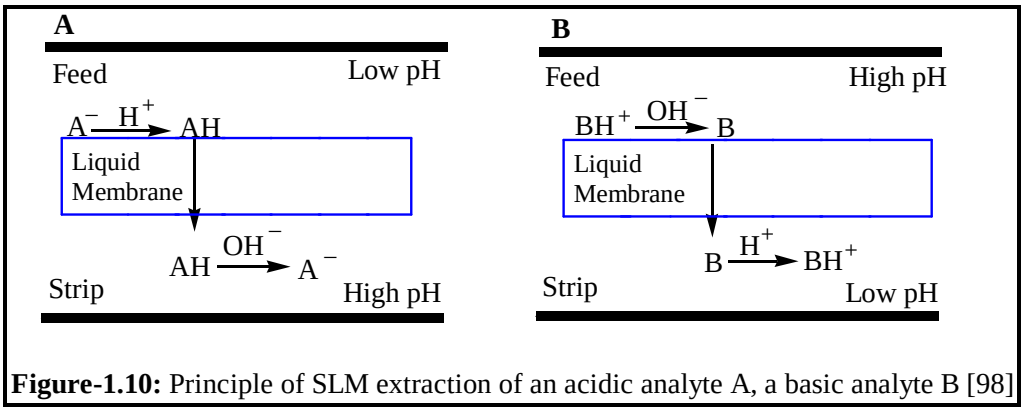


Figure-1.10: Principle of SLM extraction of an acidic analyte A, a basic analyte B [98]

Often, selective transport based on relative differences in solubility in the membrane and trapping in the strip phase may be difficult to achieve. In another case, the solubility of the analyte may be too low to give efficient extraction even when the trapping in the strip can easily be realized. A good approach in such a case is to incorporate a mobile carrier into the membrane that selectively binds the analytes. The idea of incorporating a carrier also allows SLM extraction to be a variety of compounds such as permanently charged chemical species like metal ions. It also gives different versions of carrier-mediated transport mechanism such as simple carrier transport (with chemical reaction in the strip), coupled co-transport [15, 99, 100], and coupled counter-transport [83]. In simple carrier transport, the carrier in the membrane forms a complex with the analyte in the feed that diffuses to the strip, where the analyte is converted to a non-extractable form. This type of transport was used in the extraction of short chain aliphatic carboxylic acidic feed solution to an alkaline strip solution with liquid membrane containing tri-*n*-octylphosphine oxide (TOPO) as a neutral carrier [100]. Changed carriers can be used, such as the anionic di-(2-ethylhexyl)phosphoric acid

(D2EHPA) and octansulfonic acid (OcSA) [19, 101]. In such case, dissolution of the analyte into the membrane occurs through ionic interactions with the charge carrier. Once the analyte reaches the strip phase, it is exchange for a proton and converted to a non-extractable form. The proton gradient across the membrane in this case is the driving force [102].

Stability of the membrane

The reason for SLM to become instable is the loss of liquid phase (solvent and/or carrier) out of the pores of the membrane. This loss can be due to several parameters, such as a pressure difference over the membrane, solubility of membrane solvent and carrier in adjacent feed and strip solutions, wetting of membrane pores by the aqueous phases, blockage of membrane pores by precipitation of the carrier or by water, the presence of an osmotic pressure gradient over the membrane or the emulsion formation of the liquid membrane phase in water induced by lateral shear forces [103].

An organic solvent filling the pores of a hydrophobic membrane cannot be displaced by water due to the surface tension of water against the membrane material and the organic solvent. It prevents the surface deformation of the water caused by penetration into the pores. The critical displacement pressure P_c for an SLM can be defined as the minimum transmembrane pressure required to displace the impregnating out of the largest pore.

For a cylindrical capillary, this can be quantified by the equation of Young and Laplace:

$$P_c = \frac{2\gamma.\cos\theta}{r} \quad (1)$$

with P_c the critical displacement pressure (lowest pressure needed to force water through the pores) (Pa), γ the interfacial tension (Nm^{-1}), θ the contact angle between water and the membrane (dimensionless) and r the pore radius (m) [104].

Recent directions in SLM

Recently, there has been a shift towards designing simple and easily miniaturized SLM extraction modules. For the most part the miniaturized SLM extraction designs have been a strip-phase volume of less than (1 mL) to a few microliters. These new designs are very much suited for speciation studies. Many of the reported miniaturized SLM extractions to this point have been used to extract the bioavailable fraction of organic compounds in water or biological fluids [105]. Most of miniaturized SLM have been based on polypropylene fibers, but some efforts with porous polyvinyldene difluoride tubing have also been reported.

Polypropylene was selected because it is highly compatible with a broad range of organic solvents. In addition, with a pore size of approximately 0.2 μm, polypropylene strongly immobilizes the organic solvents used in SLM. This strong immobilization is important for ensuring that the organic phase does not leak during extraction, as that may alter extraction performance and the characteristics of the system. Jönsson and Mathiasson introduce SLM flat membrane extraction module as an enrichment technique for polar and ionisable analytes. SLM flat membrane is based on a three phase system, with the organic phase being immobilized in the pores of polypropylene membrane sandwiched between two aqueous phases with 10 μL volume of strip channel (Figure-1.11). This module of SLM extraction is usually constructed as flow system (on-line), where the strip flow is directly interfaced to HPLC [15, 106, 107].

The on-line coupling of SLM extraction units to analytical instruments poses the problem of controlling memory effects. In order to avoid this problem, Pedersen-Bjergaard and Rasmussen established a liquid-phase microextraction technique (LPME) based on a disposable porous polypropylene hollow fibre [15, 108]. The fibre is impregnated with an organic phase, filled with a (1- 25 μL) volume of strip phase solution and place in a small volume of biological or environmental samples within a (4 mL) vial (Figure-1.12). The device is agitated and the extract is finally transferred to an autosmapler vial. No memory effects occur, as each membrane is used only for once. Extraction is carried out off-line, but, because of the simplicity of the extraction devices, high sample throughput can be achieved by performing many extractions in parallel. Similar device have been described by Lee et al. [109], Müller et al. [110], and Hauser et al [111]. This method has been used successfully to trap and enrich of various types of organic pollutant such as acidic drugs like DCF and IBU, basic drugs and metabolites from water samples as listed in Table-1.6.

The technique of SL-bag membrane (SL-BM) was introduced by Kurtulus et al. [112]. It is base of a three phase liquid membrane with a bag-type membrane geometry separating the aqueous sample (feed phase) with a volume 500 mL (or higher) from the strip phase with a volume of 300- 400 μL. This technique was successfully applied to extract and enrich DCF and IBU from real water samples [112]. Efficient preconcentration was achieved by immobilizing dihexyl ether (DHE) loaded with octansulfonic acid (OcSA) inside the pores of polypropylene membrane, resulting in enrichment factors of up to 1100 [112]. Like LPME, the SL-BM technique is carried out within a vial and off-line system. Despit the many advantages of SL-BM, the technique failed to extract CBZ and SFM from real water samples.

Different ways can be used to overcome this problem: In case of acidic and basic compounds the enrichment can be achieved by adjusting the pH of the feed and strip phase to appropriate values [94, 95]. In order to extract highly polar compounds (like target metabolites), it is necessary to use a carrier incorporated into the membrane organic phase. Of great importance is the specific of carrier-analyte interactions allowing highly selective separations. The choice of the carrier will be made, therefore, considering the analyte to be transported, as well as the experimental conditions [19, 100]. This technique may therefore be of vital important in the current situation where much attention has been focused on the presence of selected pharmaceuticals that also tend to contaminate the aquatic environment.

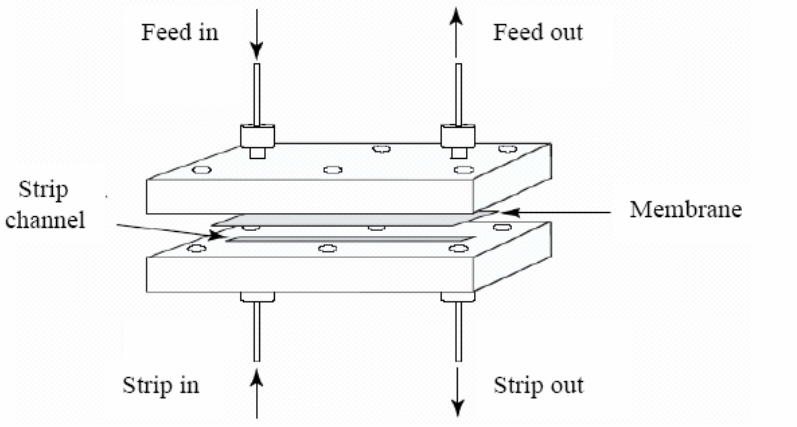


Figure-1.11: On-line SLM flat membrane module with 10 µL volume of strip channel [106]

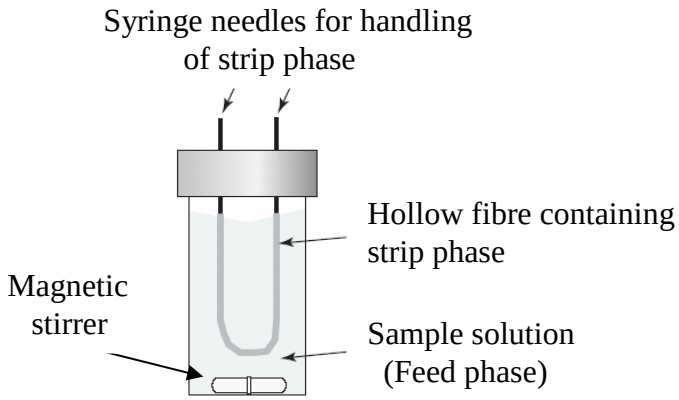


Figure-1.12: Off-line liquid-phase microextraction (LPME) with 1- 100 µL of strip phase[108]

Table-1.6: Applications of SLM to the determination of trace pollutants in natural water

Type of analyte	Enrichment factor	Liquid membrane		Detection method	Ref.
		Organic solvent	Carrier		
Acidic Pharmaceuticals: Piroxicam Ketorolac Clofibric acid Naproxen Bezafibrate Fenoprofen Ibuprofen Diclofenac Indomethacin	38- 234	1-octanol	…..	LC-MS-MS	113
Ibuprofen 2-(4-chlorophenoxy)-2-methylpropionic acid	15000	1-octanol	…..	LC-UV	109
Ibuprofen Naproxen Ketoprofen	100	DHE	…..	CE	115
Basic pharmaceuticals: 2-amino-1-phenylethanol Norephedrine	34-79	1-octanol		CE	115
Pindolol Atenolol	19- 55	DHE		CE	
Haloacetic acids	60	DHE	5 % (w/w) TOPO	LC-UV	113
	3000	DHE	DEHP	LC-UV	
Fungicides	67	DHE	15 % (w/w) TOPO	LC-UV	113
Phenols	400	1-octanol	…...	LC-UV	113
	100	hexane	…..	LC-UV	
	300	heptane-toluene (1:1)	…..	LC-UV	
Phenoxy herbicides	490	1-octanol	…..	LC-UV	113
Aromatic amines	6000	benzyl alchohol-ethyl acetate (8:2)	…..	LC-UV	113
	500	DHE	…..	LC-UV	
	250	DHE	…..	LC-UV	
Triazine herbicides	390	DHE	…..	LC-UV	113
Trihalomethanes	62	1-octanol	…..	GC-ECD	116
Dinitrophenols	300	undecane-toluene(1:1)	…..	HPLC-DAD	113

Ref.: References

2. Results and discussion

2.1. Methodical approach

This study is divided into three steps:

At first metabolites were synthesized and characterized according to the methods described in literature. In the second part the mass transfer of metabolites and active drugs in the liquid membrane systems was investigated. In the third part analytical methods were developed by combining membrane extraction and HPLC-UV to determine metabolites and active drugs in surface water.

Synthesis of metabolites

The metabolites 10,11-dihydroxycarbamazepine (CBZ-DiOH), 4`-hydroxydiclofenac (DCF-4OH), 4-hydroxyibuprofen (IBU-2OH), N-4-acetylsulfamethoxazole (SFM-Ac), and Sulfamethoxazol-N1-glucuronide (SFM-Glu) are not commercially available. They were synthesized and identified according to common spectroscopic methods (see section 3.1).

Mass transfer of metabolites and active dugs in liquid membrane systems

The mass transfer of metabolites through the liquid membrane systems was carried out in a three-compartment transport cell and supported liquid membrane-chambers which have been developed at the University of Paderborn [17-21].

The different liquid membrane systems consist of an aqueous (acidified or alkaline) feed solution containing the analytes, a bulk (organic solvent with and/or without a dissolved mobile carrier) and an aqueous stripping solution, e.g. mineral acids or alkalis. For the preliminary experiment further solvent systems were tested as organic bulk phase. Organic solvent and mixtures were varied systematically to increase transport efficiency. Acidic or neutral carriers (such as OcSA and TOPO) were admixed to the organic phase to support the forward and back-extraction. pH-gradients between the aqueous feed and strip phase were optimized to increase the effectiveness of transport processes.

Optimal combinations of liquid phases were transferred to the SLM technique. The supported flat-membrane impregnated with certain selected water-immiscible organic phase is mounted between two PTFE-chambers of equal size. Moreover, direct influences on the extraction efficiencies of the metabolites like concentration of selected analytes, extraction time and concentration of carrier in liquid membrane are very important to investigate.

Finally miniaturized SL-BMs were prepared and employed as enrichment devices for metabolites and their parent drugs. The following steps were carried out:

- Optimization of SL-BM extraction conditions
- Development of enrichment procedures for metabolites and active drugs
- Comparison of enrichment and clean up properties: SL-BM and SPE (Solid Phase Extraction)

Development of analytical methods by combining membrane extraction and determination by HPLC-UV

The final stage of this study the enrichment SLM-devices was tested for the analysis of real water samples: tap water and surface water. For this purpose the extraction procedure adjusted and modified to chromatographic conditions of the HPLC-UV or LC-MS system.

The surface water was sampled from the river Ruhr by the Institut für Wasserforschung (IFW) Dortmund. The water quality is affected by effluents of a sewage treatment plant (STP).

2.2 Three liquid membrane extraction systems

a) Extraction principles

The enrichment principle of three phase liquid membrane systems is depending on the liquid extraction and back extraction of the analytes from agitating aqueous feed phase, through a hydrophilic or hydrophobic liquid membrane, into a stagnant aqueous phase. By careful choice of solvent for the liquid membrane, combined with a proper composition of feed and strip phases, a selective preconcentration and efficient clean-up of the sample can be achieved simultaneously. The organic solvent has to satisfy the following variety of requirements:

1) its water solubility and also volatility should be low in order to separate it from the aqueous phases (feed and strip) and also to prevent solvent loss during extraction;
2) it should have a low tendency to dissolve or take up interferences existing in the feed phase;
3) it should low viscosity because this promotes high analyte fluxes through the membrane;
4) and finally it is better that the solvent have low toxicity due to occupational health and safety[130].

Considering the extraction of acidic and basic compounds, the selectivity in the process is achieved by adjusting the pH of the two phases so that the uncharged species pass the liquid membrane by diffusion and are trapped by ionization in a stagnant acceptor phase [131]. In case of highly polar compounds like selected metabolites and some of active drugs, molecules are charged over the pH range and thus, are not directly extractable. As a consequence, it is necessary to use carrier dissolved in the membrane phase to facilitate the analyte transport. Typically, for polar analytes, diffusion through the organic membrane can be enhanced by doping the organic solvent with several carriers. Thus, tri-n-octylphosphine oxide (TOPO), a strong H-bonding carrier [130, 131], has been used to promote the extractability of polar acids [131], bases [94] and some of fungicide metabolites (see Table-1.6). 1-octanesulfonic acid sodium salt monohydrate (OcSA) as an ion-pair carrier can also be employed for extraction of polar compounds [19, 132, 133]. The ion pairs formed are extracted into the organic interface and broken by selecting the appropriate pH in the strip solution, which releases the free analytes. In Table-2.1, some physical and toxicological data of TOPO and OcSA are listed.

Table-2.1: Physical properties and toxicological data of OcSA and TOPO [74]

Carrier	Molecular weight	Melting point (^{o}C)	p*Ka*	Log *P* (Octanol/water)	Toxicological data
OcSA	194.29	……..	1.86	1.829±0.394	Not available
TOPO	386.63	51.0- 51.5	……	9.398±0.552	ORAL (LD50): >10000 mg/kg [Rat] DERMAL (LD50): 2830 mg/kg [Rabbit]

Carrier transport model: The transport model of carrier in three liquid membranes is based on five different steps which can be identified during the extraction of analyte [134]:

1) Diffusion of analyte through the feed side boundary layer to reach the feed- liquid membrane interface;
2) complexation reaction between the analyte and the carrier as a carrier present in the liquid membrane at feed-membrane interface to form a carrier-analyte complex;
3) diffusion of the carrier-analyte complex through liquid membrane;
4) de-complexation reaction between the carrier-analyte complex and the strip agent at the strip-membrane interface and release of analyte;
5) diffusion of analyte through the strip side boundary layer to reach the bulk of the strip phase.

Anionic carrier OcSA

With OcSA as an anion carrier solved in liquid membrane a proton gradient between the strip and feed phase is the driving force for the mass transfer of polar analytes in three liquid membranes as shown in Figure-2.1. This type of carrier is associated with counter-ions to maintain electroneutrality in the polar membrane phase [19, 132, 133].

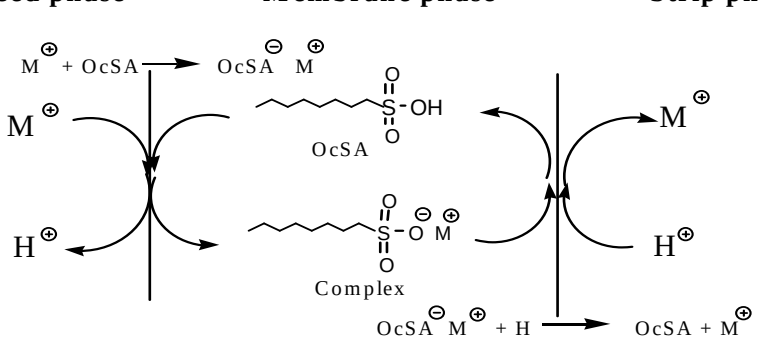


Figure-2.1: Mechanisum transport of polar compounds (M) across a liquid membrane containing OcSA as carrier

Neutral carrier TOPO

TOPO is known as an efficient carrier for polar compounds [16], owing to the two lone electron pairs on the oxygen atom. TOPO has the ability to form hydrogen-bond complexes of various compositions [131, 136]. Therefore, it is particularly useful in the extraction of acidic and basics, especially highly polar. Moreover, TOPO is very stable when used in a liquid membrane, as it is soluble in organic solvents but insoluble in water (see Table-2.1). The whole reaction scheme for polar acidic and basic drugs by TOPO can be presented as follows:

Extraction of acidic drug [130]:

$$A^- + H^+_{(aq)} \rightleftharpoons HA_{(aq)} \quad (2)$$

$$HA_{(aq)} + TOPO_{(org)} \rightleftharpoons [TOPO..HA]_{(org)} \quad (3)$$

$$[TOPO..HA]_{(org)} + OH^-_{(aq)} \rightleftharpoons A^-_{(aq)} + TOPO_{(org)} + H_2O \quad (4)$$

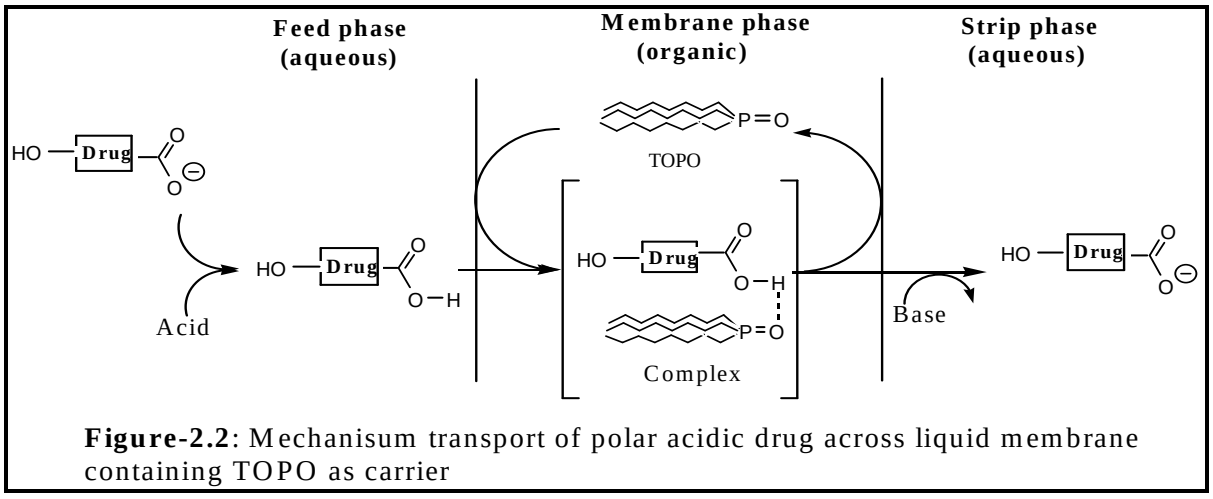


Figure-2.2: Mechanisum transport of polar acidic drug across liquid membrane containing TOPO as carrier

Extraction of basic drug [131]:

$$BH^+ + OH^-_{(aq)} \rightleftharpoons B_{(aq)} \quad (5)$$

$$B_{(aq)} + TOPO_{(org)} \rightleftharpoons [TOPO..B]_{(org)} \quad (6)$$

$$[TOPO..B]_{(org)} + H^+_{(aq)} \rightleftharpoons BH^+_{(aq)} + TOPO_{(org)} + H_2O \quad (7)$$

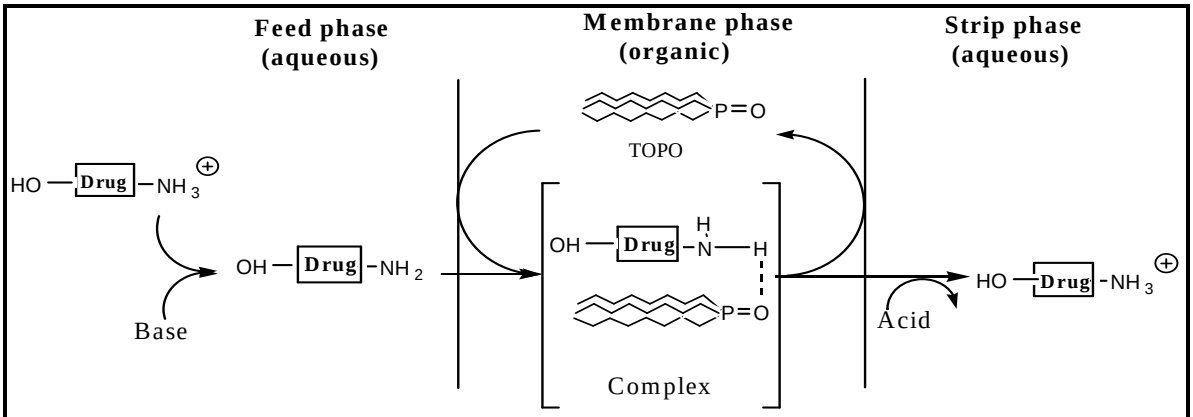


Figure-2.3: Mechamisum transport of polar basic drug across liquid membrane containing TOPO as carrier

b) Development of HPLC-UV methods

In order to monitor the transport of the metabolites and active drugs through the membrane systems, aliquots were taken from the liquid phases (feed and strip) in intervals by means of a micro-liter syringe which is introduced through self-closing seals (bulk liquid membrane) or directly from the open SLM-chambers and then analyzed by HPLC.

The samples are introduced into the chromatographic systems by an autosampler and an isocratic or gradient pump. UV-Vis-PAD-Detector was connected with a chromato-integrator. RP 18 analytical column was used (details see section 3.2.1).

2.2.1 Bulk liquid membrane (BLM)

The equipments used are based on earlier developments [17-20]. As shown schematically in Figure-2.4, the three-phase system was established in a home-made glass cell equipped with an agitator (PTFE) which allows extraction and back-extraction in one unit. The cell consists of two concentric chambers dividing it into separate compartments. Thus, the feed is allowed to contact the bulk membrane and the strip solution to contact the membrane. The whole cell covered by a fitting glass lid in order to minimize loss of solvent by evaporation.

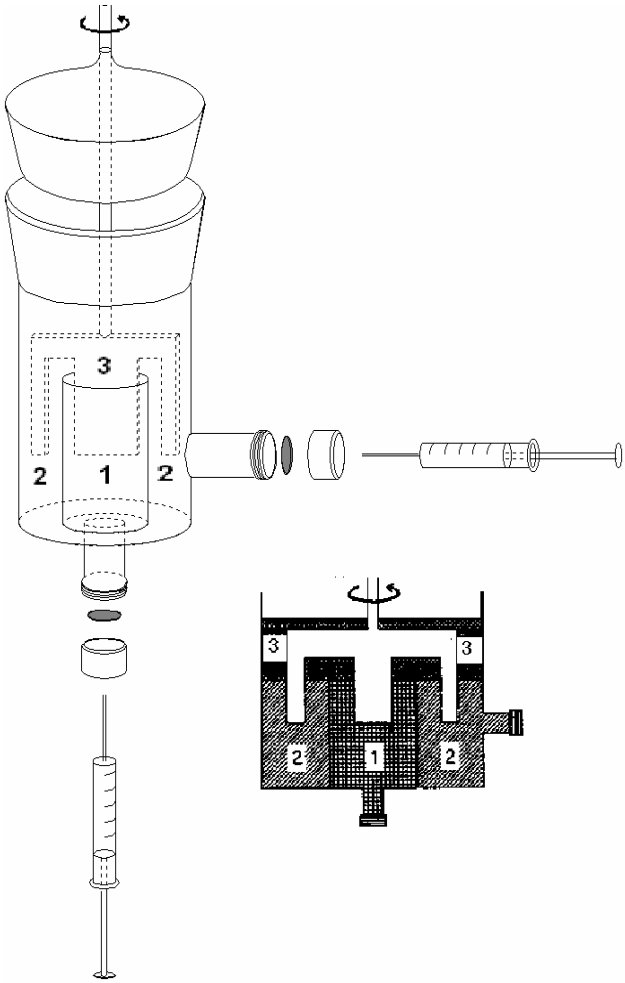


Figure-2.4: Three-phase liquid bulk membrane system (1: strip phase, total volume:25 mL, 2: feed phase 32 mL, 3: membrane phase 20 mL) [19]

The efficiency and selectivity of membranes will be influenced by the composition of organic phase. Therefore, we used common water-immiscible organic solvents of different polarities as shown in Table-2.2. The different combinations of feed and strip phases, which were used in the BLM systems, are summarized in Table-2.3.

Table-2.2: Physical properties of organic solvents used as liquid membrane [74]

Solvent	**Log *P* (octanol/water)**	**Density (g/mL)**
1-pentanol	1.407±0.176	0.811±0.06
DHE	5.232±0.206	0.799±0.06
Undecane	6.600±0.166	0.743±0.06
Decane	6.069±0.166	0.734±0.06

Table-2.3: Composition of three-phase membrane system used

Feed phase Volume: 32 mL	Membrane phase volume: 20 mL	Carrier	Strip phase volume: 25 mL
0.1 mol/L NaOH	1-pentanol	-	0.1 mol/L HCl
0.1 mol/L HCl	1-pentanol	-	0.1 mol/L NaOH
0.1 mol/L HCl	DHE	-	0.1 mol/L NaOH
0.1 mol/L HCl	undecane	-	0.1 mol/L NaOH
0.1 mol/L HCl	decane	-	0.1 mol/L NaOH
0.1 mol/L HCl	DHE	0.025 g/L OcSA	0.1 mol/L NaOH
0.1 mol/L HCl	DHE	1 % (w/w) TOPO	0.1 mol/L NaOH
0.1 mol/L HCl	undecane	1 % (w/w) TOPO	0.1 mol/L NaOH
0.1 mol/L HCl	decane	1 % (w/w) TOPO	0.1 mol/L NaOH

2.2.1.1 Influence of organic solvent

By using organic solvents (Table-2.2), the distribution of the metabolites governs the extent of extraction from the aqueous feed matrix into the organic bulk phase. As can be expected from other classes of compounds, the magnitude of the calculated partition coefficients in the octanol/water systems (Log *P*) of the individual drugs corresponds to the extraction yields determined (Table-2.4) [19]. Obviously the metabolites with high Log *P* such as IBU-2OH and SFM-Ac lead to different extractabilities in polar and nonpolar liquid phases. These compounds were highly dissolved in 1-pentanol and too much lower yields in DHE and undecane were obtained (Table-2.4).

By contrast, SFM-Glu and CBZ-DiOH with very low partition coefficient gave drastically much lower extraction efficiencies in polar and nonpolar membrane as seen in Table-2.4.

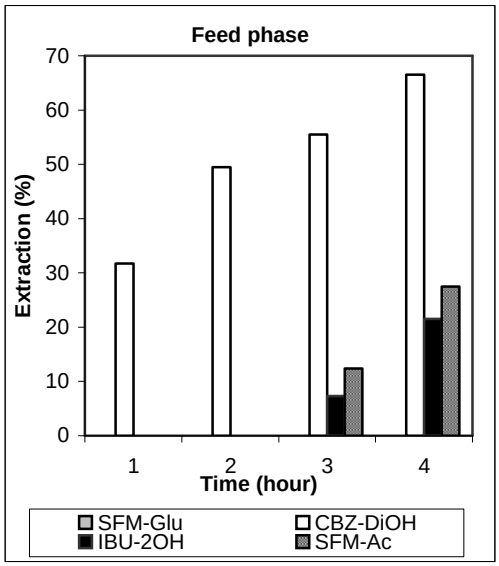
Table-2.4: Extraction efficiency *E* (%) of metabolites in liquid membrane phase after 4 hours of extraction by using BLM (feed: 0.1 mol/L HCl, strip: 0.1 mol/L NaOH)

Metabolite	pK_a*	Log *P**	*E* (%) after 4 hours		
			1-pentanol	DHE	Undecane
IBU-2OH	4.44	1.690±0.242	~100	19.7	19.3
SFM-Ac	5.60	1.478±0.436	~100	40.7	45.2
SFM-Glu	2.70 (pK_{a1}) 0.36 (pK_{a2})	0.561±0.624	22.6	9.9	11.7
CBZ-DiOH	12.62	0.132±0.405	54.8	11.8	17.6

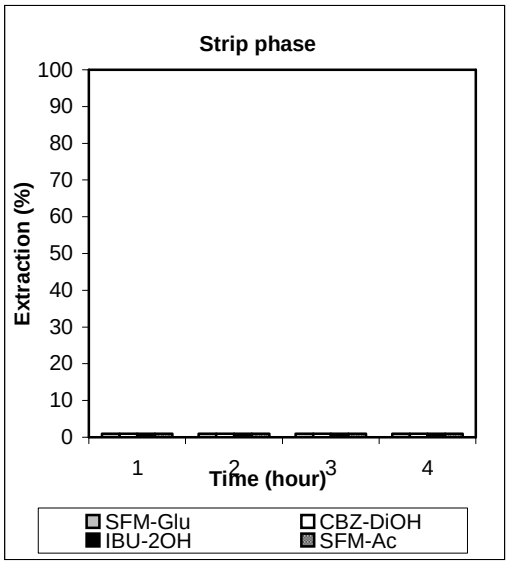
*Dissociation constants (pK_a) and octanol-water partition coefficients (Log *P*) are taken from reference [74]

2.2.1.2 Influence of pH-gradient between feed and strip phase

Besides the polarity of the organic bulk phase the acid-base properties of the metabolites were utilized to facilitate their transfer from the feed into the strip phase. pK_a values of the metabolites correspond from acidic (like: SFM-Glu, IBU-2OH, and SFM-Ac) to basic compounds (like; CBZ-DiOH) as shown in Table-2.4. Since the analytes should be in their neutral state in order to be extracted into the organic phase, and to trap charged analytes into the strip phase. Different pH values of feed and strip were used to adjust the optimum extraction efficiency (Table-2.3) of metabolites in strip phase. The best results were found when the pH of the feed phase was kept at pH ~1 by using 0.1 mol/L HCl and the strip at pH ~13 by using 0.1 mol/L NaOH. About 50 % of IBU-2OH and ~35 % of SFM-Ac are released into the strip solution after 4 hours of extraction (Figure-2.6).

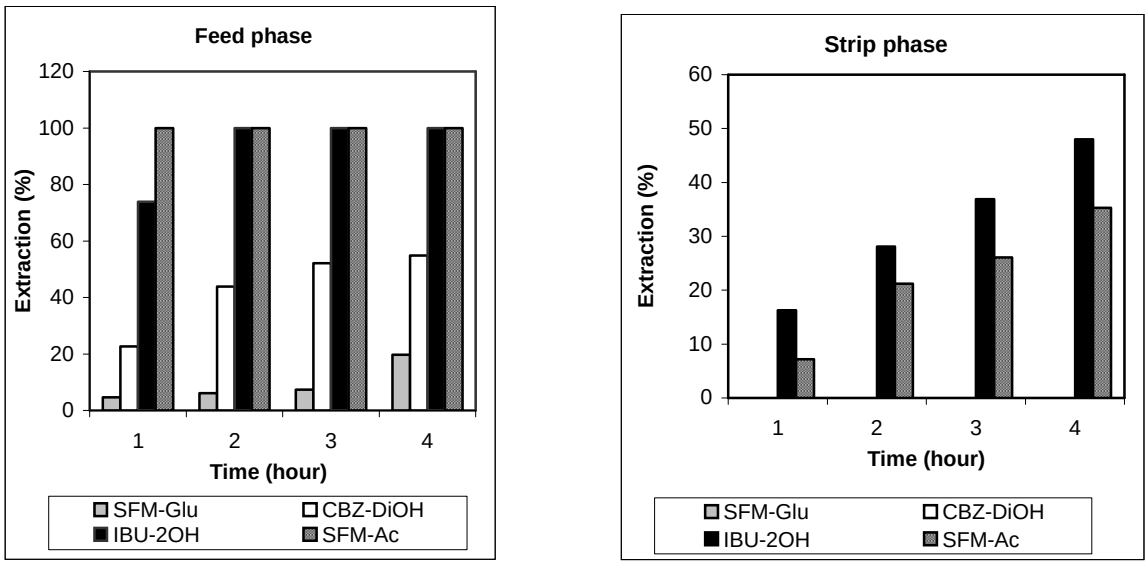


a)



b)

Figure-2.5: Transport of metabolite mixtures in BLM, by using basic feed phase and acidic strip phase, a) Forward-extraction, b) back-extraction (Extraction conditions: feed 1 mg/L of metabolites in 32 mL of 0.1 mol/L NaOH, strip: 25 mL of 0.1 mol/L HCl, and liquid membrane: 1-pentanol)

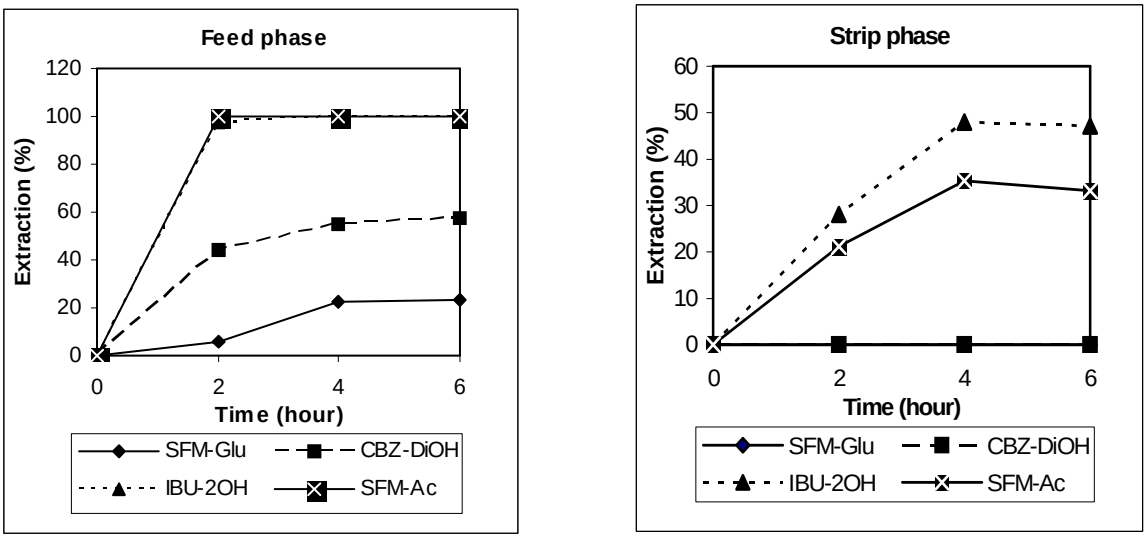


a) b)

Figure-2.6: Transport of metabolite mixtures in BLM by using acidic feed phase and basic strip phase, a) Forward-extraction, b) back-extraction (Extraction conditions: feed 1 mg/L of metabolites in 32 mL of 0.1 mol/L HCl, strip: 25 mL of 0.1 mol/L NaOH and liquid membrane: 1-pentanol)

2.2.1.3 Influence of extraction time

The influence of extraction time on the transport of metabolites was investigated, 2, 4, and 6 hours were applied. Complete extraction of IBU-2OH and SFM-Ac from feed solution achieved within 2 hours. The extracted amount of IBU-2OH and SFM-Ac in the strip phase increased with increasing extraction time and maximum extraction was found after 4 hours (Figure 2.7). After then the extraction efficiencies were slightly decreased as some of the analytes re-entered to the membrane phase, therefore a time interval of 4 h was chosen for further experiments.



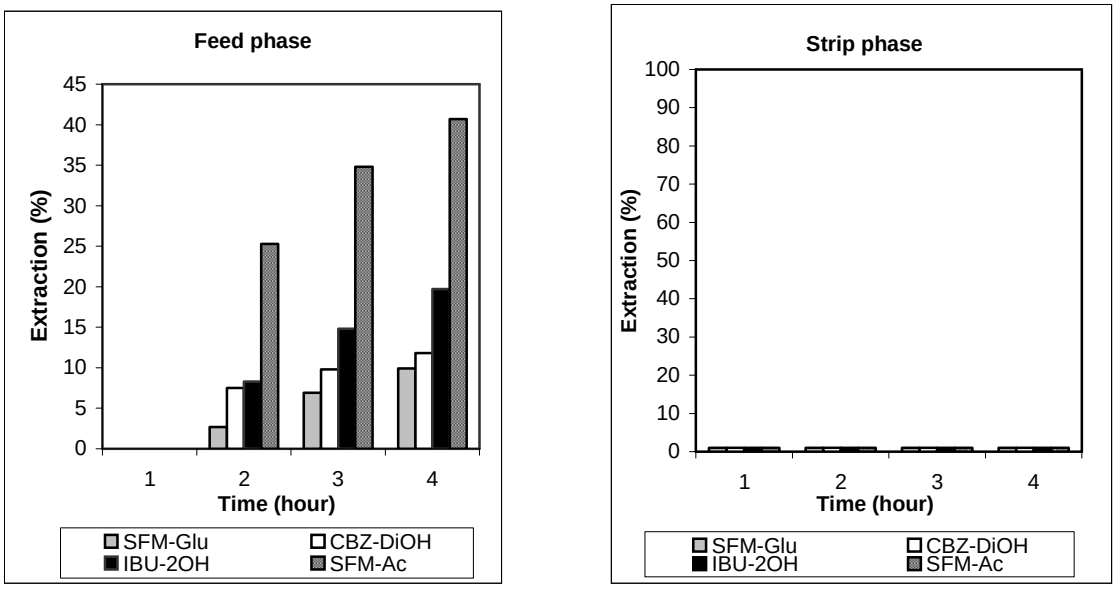
a) b)

Figure-2.7: Transport of metabolite mixtures in BLM with different extraction times, a) Forward-extraction, b) back-extraction (Extraction conditions: feed 1 mg/L of metabolites in 32 mL of 0.1 mol/L HCl, strip: 25 mL of 0.1 mol/L NaOH, and liquid membrane: 1-pentanol)

2.2.1.4 Influence of carrier in liquid membrane

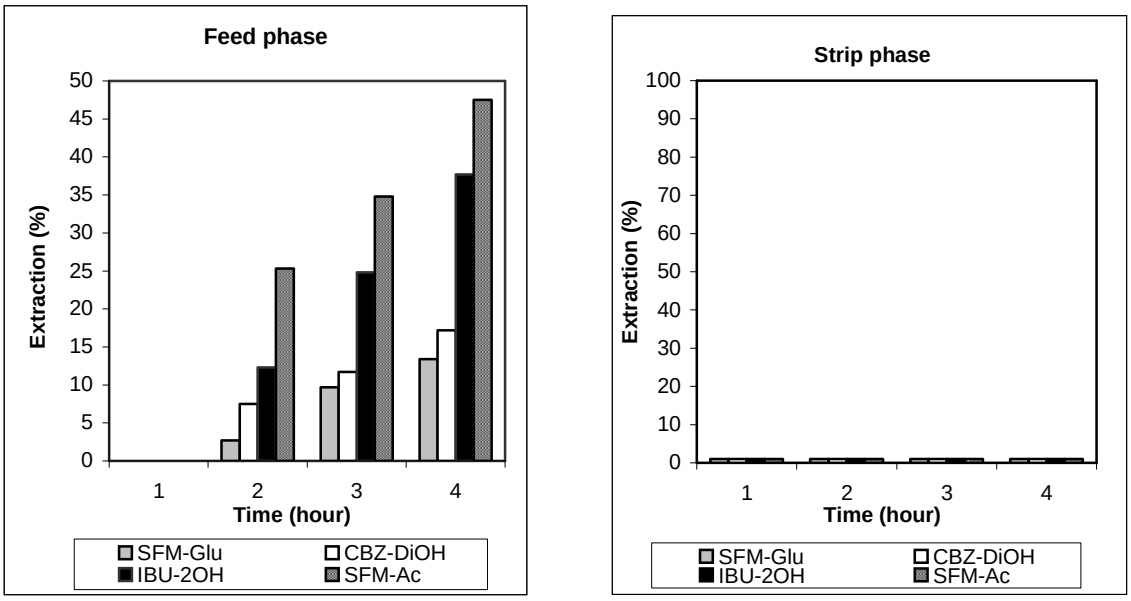
Since all the metabolites are polar (Table-2.4) and are less extractable by DHE, undecane and decane as expected [130]. OcSA dissolved in DHE was already successfully used as liquid membrane to extract active drugs (IBU and DCF) from water samples [19]. In another application, TOPO was used as a carrier to increase the dissolution into the liquid membrane of DHE and undecane in the extraction of polar acidic and basic compounds [94, 131]. In order to enhance the dissolution into the liquid membrane and thus the extraction efficiency, the added amounts of 0.025 g/L of OcSA or 1 % (w/w) of TOPO as carrier were applied (Table-2.3). Generally, the results indicate a significant increase of extraction efficiency for IBU-2OH and SFM-Ac. OcSA with DHE as a liquid membrane was not able to extract the metabolites into the strip phase (see Figure-2.9).

TOPO has a good but not unlimited solubility in organic solvents as in some cases precipitations were observed in liquid membranes at a concentration higher than 1 % (w/w) after 2 h of extraction.



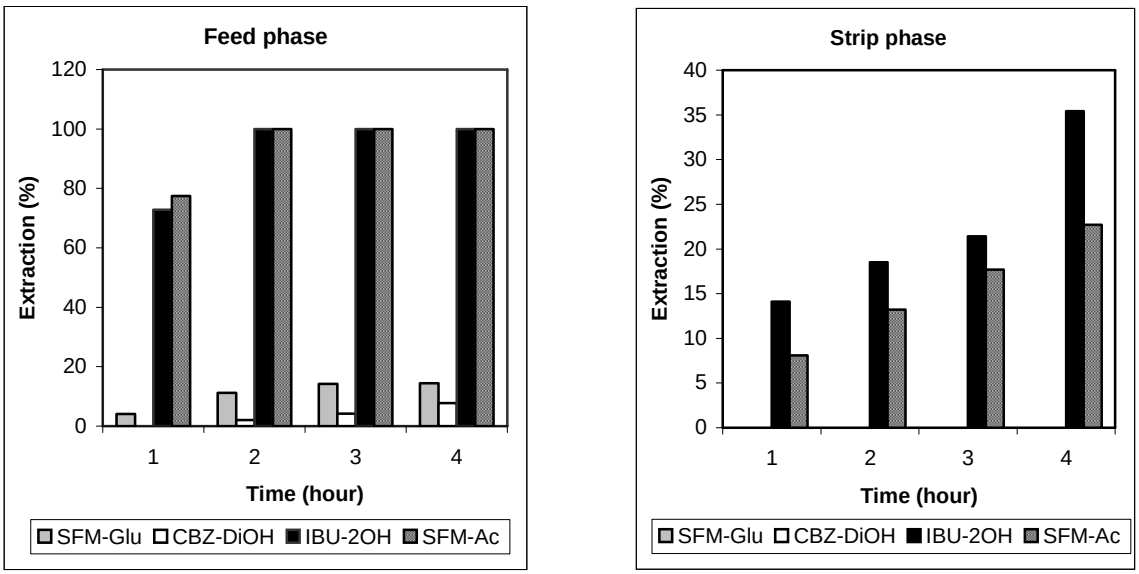
a) b)

Figure-2.8: Transport of metabolite mixtures in BLM using DHE as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: feed 1 mg/L of metabolites in 32 mL of 0.1 mol/L HCl, strip: 25 mL of 0.1 mol/L NaOH)



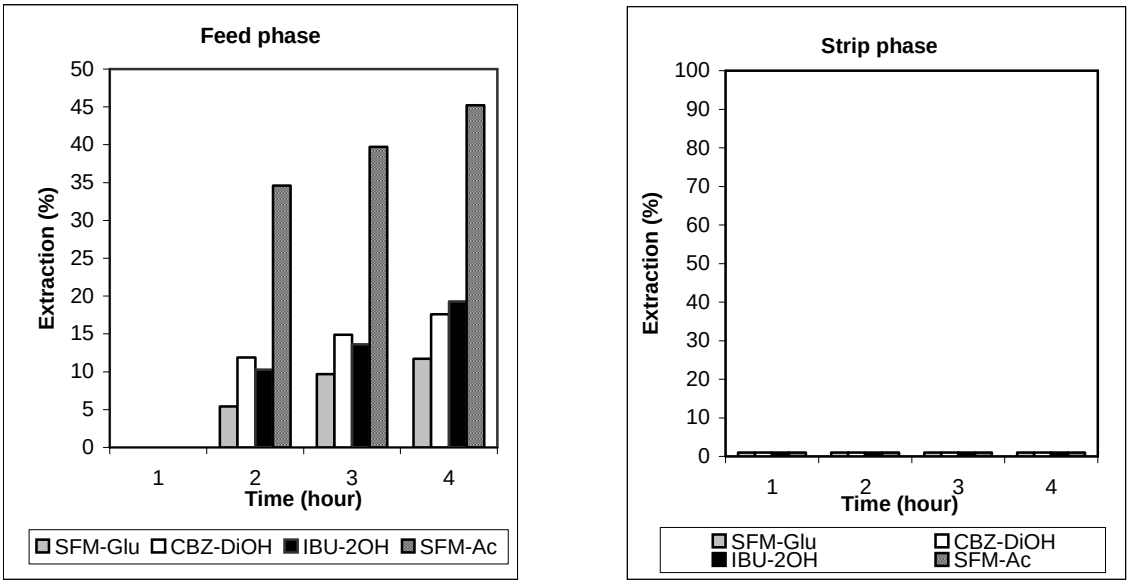
a) b)

Figure-2.9: Transport of metabolite mixtures in BLM using DHE with 0.025 g/L OcSA as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: see Figure-2.8)



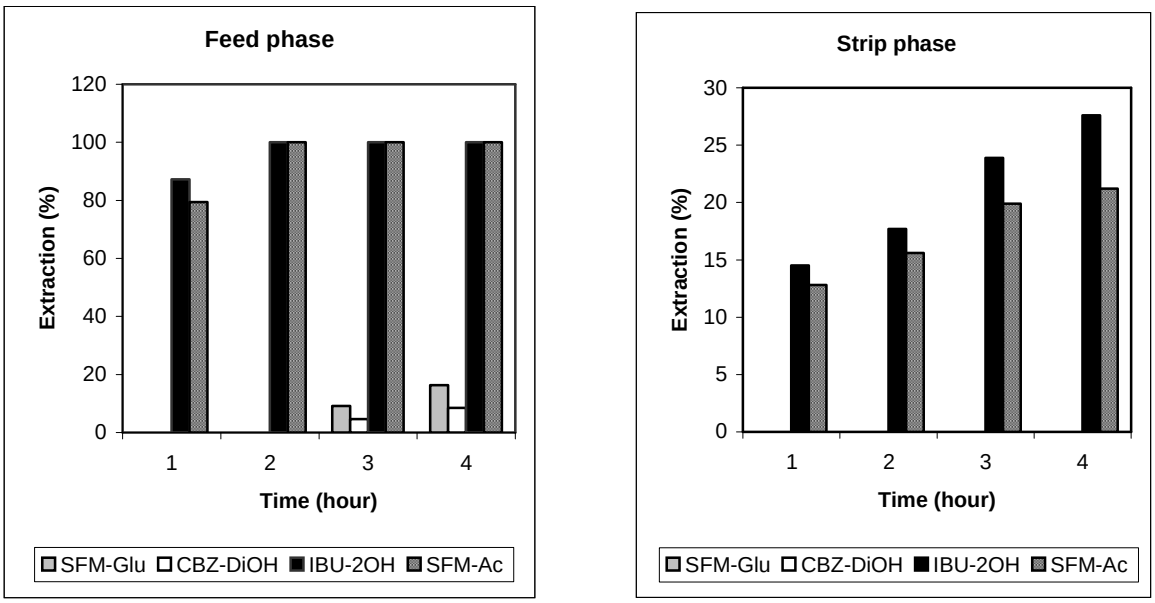
a) b)

Figure-2.10: Transport of metabolite mixtures in BLM using DHE with 1 % (w/w) TOPO as Liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: see Figure-2.8)



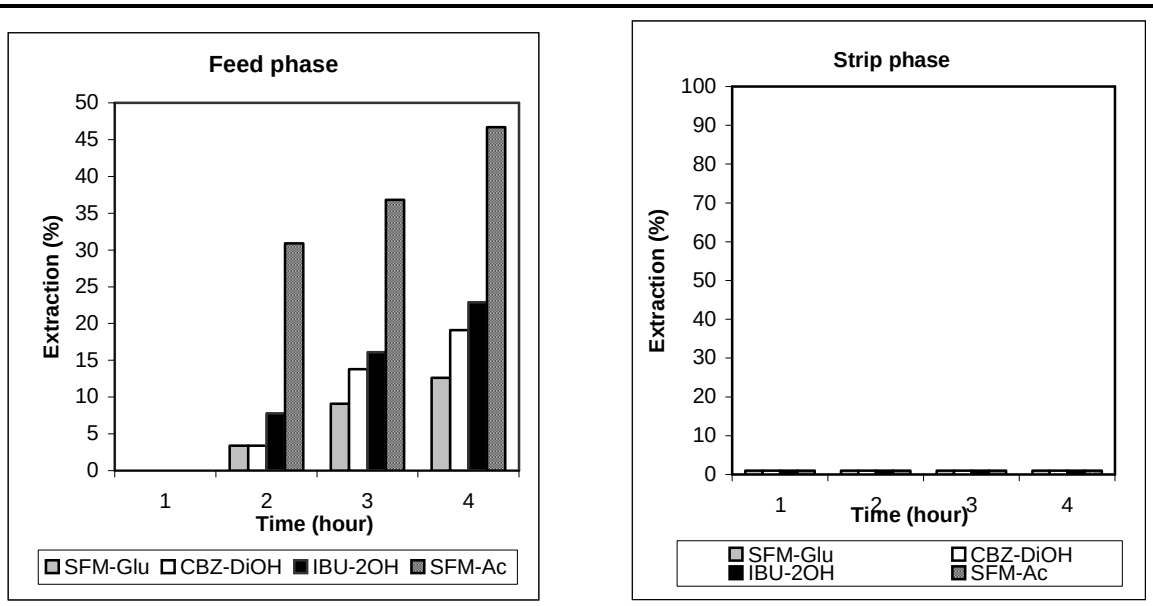
a) b)

Figure-2.11: Transport of metabolite mixtures in BLM using undecane as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: see Figure-2.8)



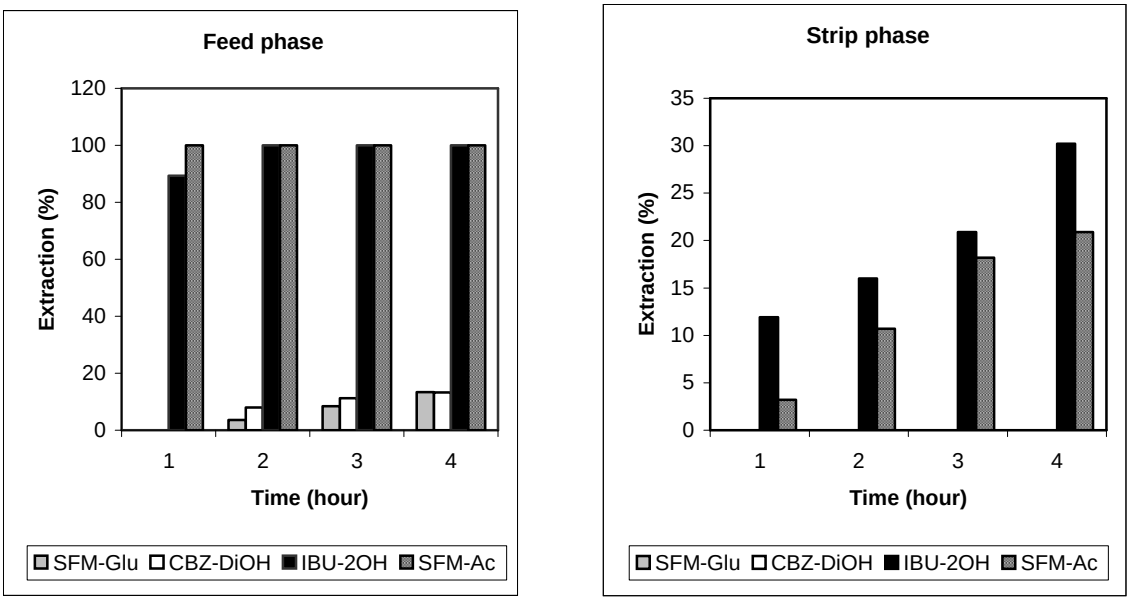
a) b)

Figure-2.12: Transport of metabolite mixtures in BLM using undecane with 1 % (w/w) TOPO as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: see Figure-2.8)



a) b)

Figure-2.13: Transport of metabolite mixtures in BLM using decane as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: see Figure-2.8)



a) b)

Figure-2.14: Transport of metabolite mixtures in BLM using decane with 1 % (w/w) of TOPO as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions: see Figure-2.8)

2.2.2 Supported liquid flat-membrane (SL-FM) system

This SLM technique is base on a polypropylene (PP) membrane (pore size 0.1 μm, total thickness 90 μm). It is impregnated with a water-immiscible organic solvent (e.g. DHE, undecane or decane) containing the carrier (e.g. TOPO) which is held by capillary forces placed between two aqueous phases, feed and strip.

The supported liquid flat-membrane (SL-FM) system 150:150 (v/v) (Figure-2.15) has been used to extract the metabolites. Certain membranes used for the bulk liquid membrane were selected to test their applicability in the SL-FM system, applying different concentrations of TOPO as a carrier.

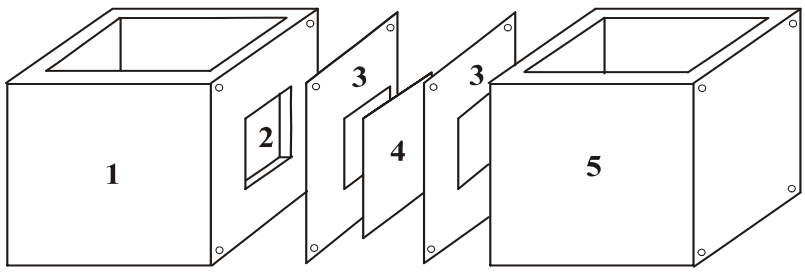


Figure-2.15: SL-FM device, 1 strip-chamber, 5 feed-chamber (volume of both chambers 150 mL), 2 contact window, 3 fixing plants, 4 membrane [19]

2.2.2.1 Theoretical approach

SLM-transport of compounds is based on two processes: extraction of an analyte from the feed phase into an organic solvent situated in membrane pores, and a simultaneous back-extraction from the organic phase into an aqueous stripping phase, where the analyte is converted to a non-extractable form and thus trapped.

The schematic transport mechanism of an analyte from an bulk agitating aqueous feed phase to a bulk stagnant strip phase (Figure-2.16) (where the volume of both phases is equal) through an organic phase in the microporous polymeric membrane is shown in Figure-2.17 [137].

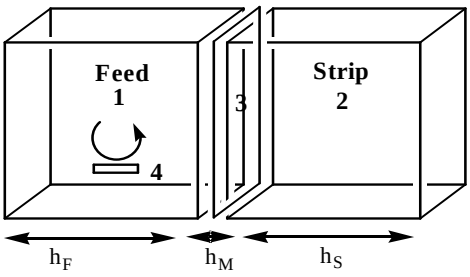


Figure-2.16: SLM device , (1) feed phase, (2) strip phase, (3) membrane, (4) agitator

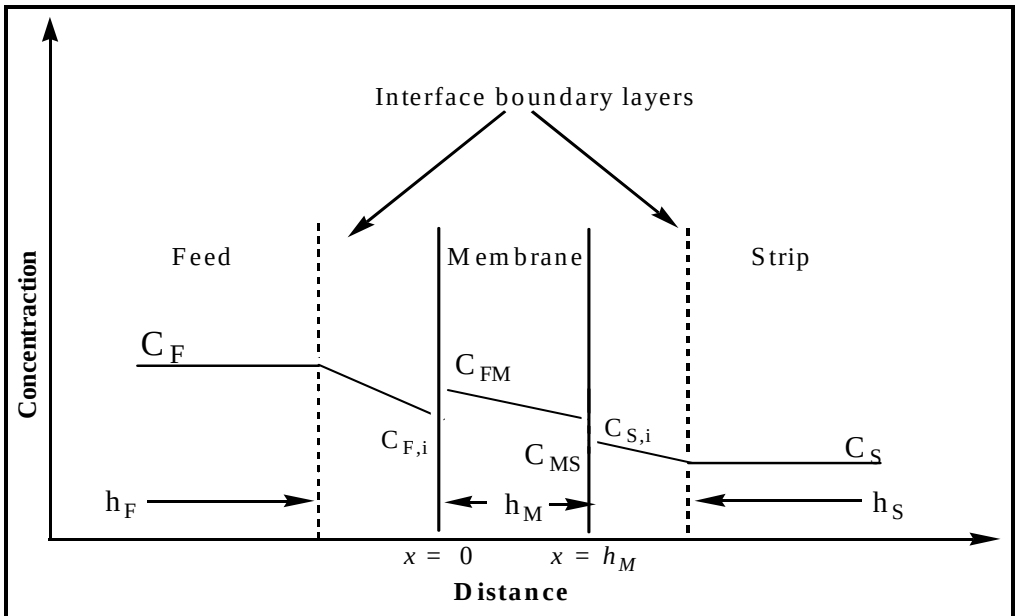


Figure-2.17: Schematic transport of analyte inSLM[137]

Overall mass transfer

The overall mass transfer under steady-state condition, consist of three mass transfer processes: the mass transfer in feed phase, in the membrane phase and in the strip phase.

To obtain an expression for mass transfer coefficient, *k*, Fick's first law and definition of mass transfer coefficients are applied [138]:

$$J = D\frac{dC}{dx}\Big|_{x=h} = k[C_0(t) - C] \tag{8}$$

Where J = mass flux of analyte per unit area per unit time

D = diffusion coefficient

$C_0(t)$ = analyte concentration at $t = 0$

$$C = \frac{1}{h}\int_0^h C dx$$

dC/dx = spatial concentration gradient along the axis of flow path

Assuming equilibrium at feed-membrane interface, and between the active and inactive forms of analytes, the mass transfer in feed phase is described as following equation (Eq.) [139]:

$$J_F = k_F \alpha_F (C_F - C_{F,i}) \tag{9}$$

$$J'_F = k'_F (1 - \alpha_F)(C_F - C_{F,i}) \tag{10}$$

Where, J_F and J'_F are the mass transfer of the analyte in active and inactive form in feed phase respectively. α_F is the fraction of total analyte that is in active form in the feed phase, i.e., in such a form that it may transverse the membrane $(0 \leq \alpha_F \leq 1)$. It is assumed that the conditions are such that α_F is constant throughout the feed phase, also in the vicinity of membrane surface.

The mass transfer coefficient for active form in the feed phase (k_F) is derived from the penetration theory as [138]:

$$k_F = \sqrt{\frac{3D_F f_F}{2\pi x}} \tag{11}$$

Where, D_F is the diffusion coefficient of analyte in active form in feed phase, and f_F, is the linear flow velocity in feed phase.

The fluxes of membrane (J_M) and strip phase (J_S) can be written analogously to Eq. (9), noting that no inactive form of the analyte exists in the membrane phase:

$$J_M = k_M (K_F \alpha_F C_{FM} - K_S \alpha_S C_{MS}) \tag{12}$$

$$J_S = k_S \alpha_S (C_{S,i} - C_S) \tag{13}$$

$$J'_S = k'_S(1-\alpha_S)(C_{S,i} - C_S) \tag{14}$$

The distributions constant, K_F and K_S are the partition coefficient of feed and strip phases respectively. α_S is assumed to be constant throughout the strip phase, even at high degree of enrichment.

For the mass transfer in the membrane phase (k_M), the film theory for mass transfer gives [139]:

$$k_M = \frac{D_M \varepsilon}{\xi h_M} \tag{15}$$

Where, D_M is the diffusion coefficient for active form of analyte in membrane phase, ε, the membrane porosity and ξ is the membrane tortuosity.

An expression of the mass transfer coefficient in the stagnant strip phase (*ks*) can be derived from the film theory as [139]:

$$k_S = \frac{D_S}{h_S} \tag{16}$$

Where, *Ds* is the diffusion coefficient for active form of analyte in strip phase, and *hs* is the thickness of strip phase.

The total flux of analyte from bulk of feed phase to bulk of the stripping phase (*J*) is described by [139]:

$$J = k(\alpha_F C_F - \alpha_S \frac{K_S}{K_F} C_S) \tag{17}$$

Where, k is the overall mass transfer coefficient.

Implicit in this equation is zero flux $(J = 0)$ between all bulks at equilibrium. Thus, at steady state transfer it holed that:

$$J = J_F + J'_F = J_M = J_S + J'_S \tag{18}$$

From Eqs. (8), (9), (12), (13), (17), and (18) an expression for the overall mass transfer coefficient can be derived [140]:

$$\frac{1}{k} = \frac{\alpha_F}{k_F} + \frac{1}{k_M K_F} + \frac{\alpha_S K_S}{k_S K_F} \tag{19}$$

, *where* K_F is the partition coefficient between the membrane and the feed, and K_S is the partition coefficient between the membrane and the strip [140].

Mass balance equations

With the agitating feed phase, the mass balance equation in the feed phase is:

$$\frac{dC_F}{dt} = -f_F \frac{dC_F}{dx} - \frac{k}{V_F}(\alpha_F C_F - \alpha_S C_S) \tag{20}$$

And for the stagnant stripping phase, the mass balance equation in the strip phase as following [139]:

$$\frac{dC_S}{dt} = \frac{k}{V_S}(\alpha_F C_F - \alpha_S C_S) \tag{21}$$

The rate of mass transfer

The rate of mass transfer across the liquid membrane is proportional to the concentration difference ΔC of neutral extractable analyte between feed and strip phase, which can be written as [141]:

$$\Delta C = \alpha_F C_F K_F - \alpha_S C_S K_S \tag{22}$$

In most applications of supported liquid membrane, the conditions as shown in Figure-2.18 are set so that the second term in Eq. (22) is negligible (α_S ~ 0) and α_F is closed to 1.

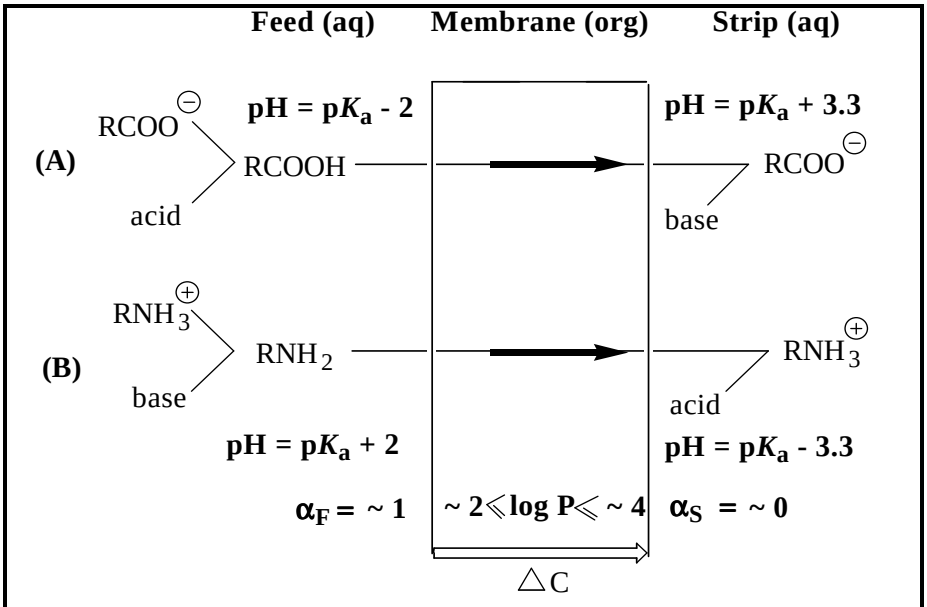


Figure-2.18: The optimum extraction conditions of an acidic analyte (A), and basic analyte (B) by SLM [141]

2.2.2.2 Influence of the composition of liquid membrane

The certain liquid membranes which have been used in BLM systems were also tested in an SL-FM system. As shown in Table-2.5, the extraction efficiencies of metabolites in SL-FM membrane phase were approximately the same as in BLM systems. By increasing the

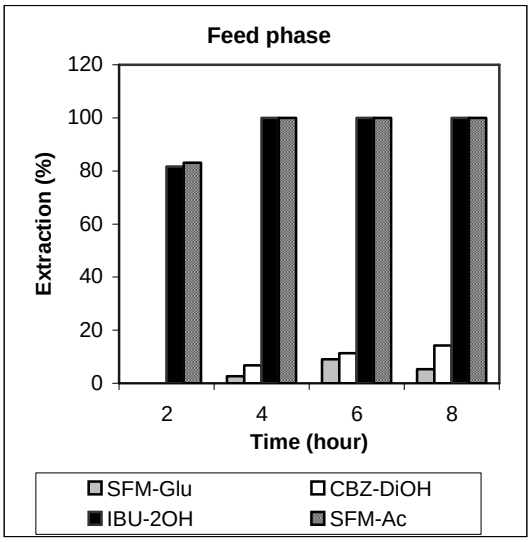
concentration of TOPO from 1 to 10 % (w/w) as carrier in DHE, undecane and decane (Table-2.6) gave a quantitative separation of IBU-2OH and SFM-Ac as can see in Figures-2.19, 2.20, and 2.21.

Table-2.5: Extraction efficiency, *E,* (%) of the metabolites after 8 hours by using SL-FM system (with feed: 0.1 mol/L HCl, and strip: 0.1 mol/L NaOH)

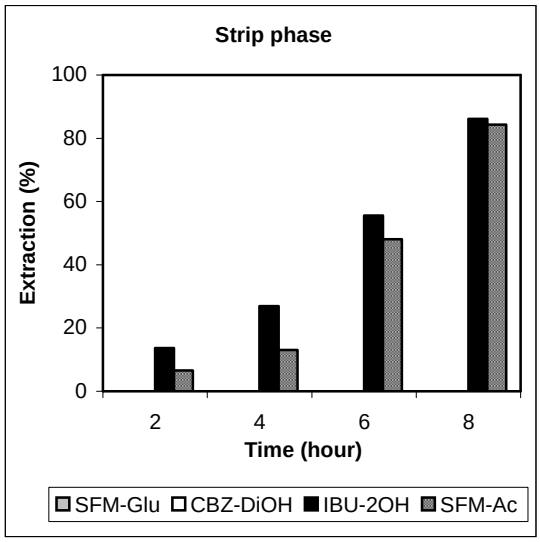
Metabolite	*E* (%) after 8 hours			
	1-pentanol	DHE with 1 % (w/w) of TOPO	Undecane with 1 % (w/w) of TOPO	Decane with 1 % (w/w) of TOPO
IBU-2OH	~100	~100	~100	~100
SFM-Ac	~100	~100	~100	~100
SFM-Glu	~0	~0	~0	~0
CBZ-DiOH	33.4	~0	~0	~0

Table-2.6: Compositions of liquid membranes in SL-FM systems

Membrane	Carrier (w/w)
1-pentanol	…..
DHE	1 , 3 , 5 , and 10 % of TOPO
Undecane	1 , 3 , 5 , and 10 % of TOPO
Decane	1 , 3 , 5 , and 10 % of TOPO

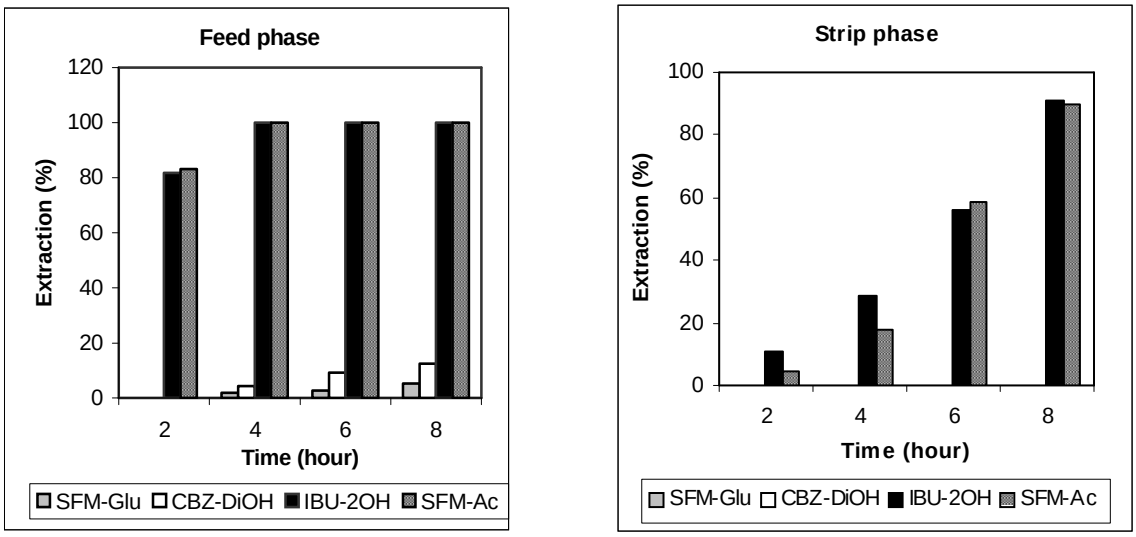


a)



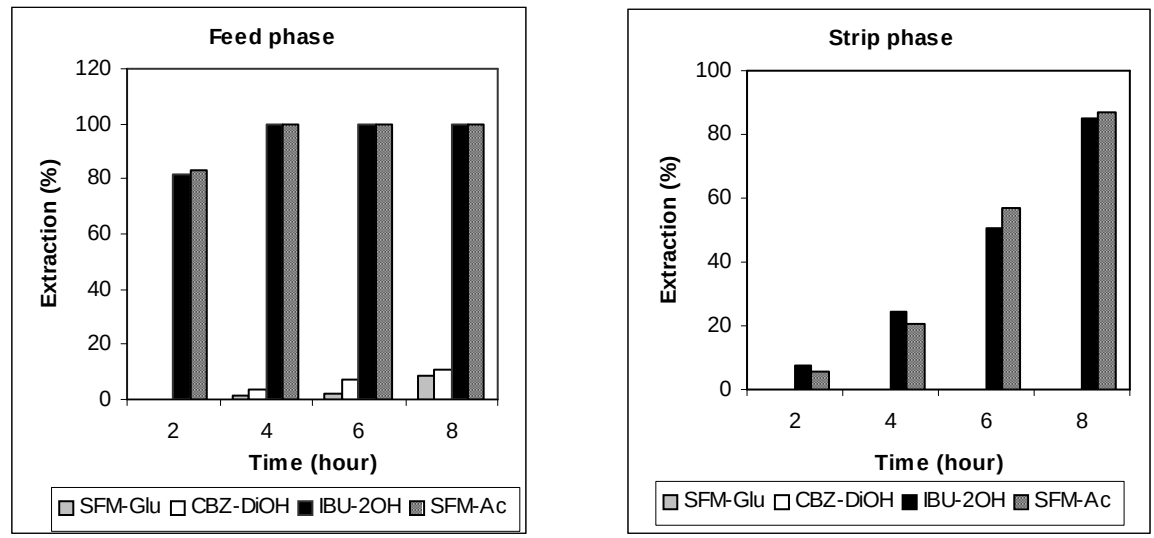
b)

Figure-2.19: Transport of metabolite mixtures in SL-FM, DHE with 10 % (w/w) of TOPO as liquid membrane, a) Forward-extraction, b) back-extraction (Extraction conditions, feed: 1 mg/L of metabolites in 150 mL of 0.1 mol/L HCl, and strip: 150 mL of 0.1 mol/L NaOH)



a) b)

Figure-2.20: Transport of metabolite mixtures in SL-FM, undecane with 10 % (w/w) of TOPO as liquid membrane, a) Forward-extraction b) back-extraction (Extraction conditions: see Figure-2.19)



a) b)

Figure-2.21: Transport of metabolite mixtures in SL-FM, decane with 10 % (w/w) of TOPO as liquid membrane, a) Forward-extraction b) back-extraction (Extraction conditions: see Figure-2.19)

2.2.2.3 Influence of extraction time

SL-FM is a three-phase extraction system with two liquid-membrane interfaces; as a result, the metabolite molecules need time to diffuse through each phase and cross all interfaces to get into strip phase. Hence, the influence of extraction time on the extraction efficiency of the

metabolites in SL-FM was studied. SFM-Glu and CBZ-DiOH remain in the membrane phase as shown in (Figure-2.22 **a** and **b**), while IBU-2OH and SFM-Ac were simultaneously extracted from the feed and back-extracted into the strip phase see (Figure-2-22 **c** and **d**) and the extracted amounts of both compounds increase with extended extraction time. The maximum extraction efficiencies of IBU-2OH and SFM-Ac were obtained 8 hours and was chosen for further experiments. The extraction time in this regard is relatively long, but it is possible (as the membrane is more stable) to achieve the best extraction efficiency.

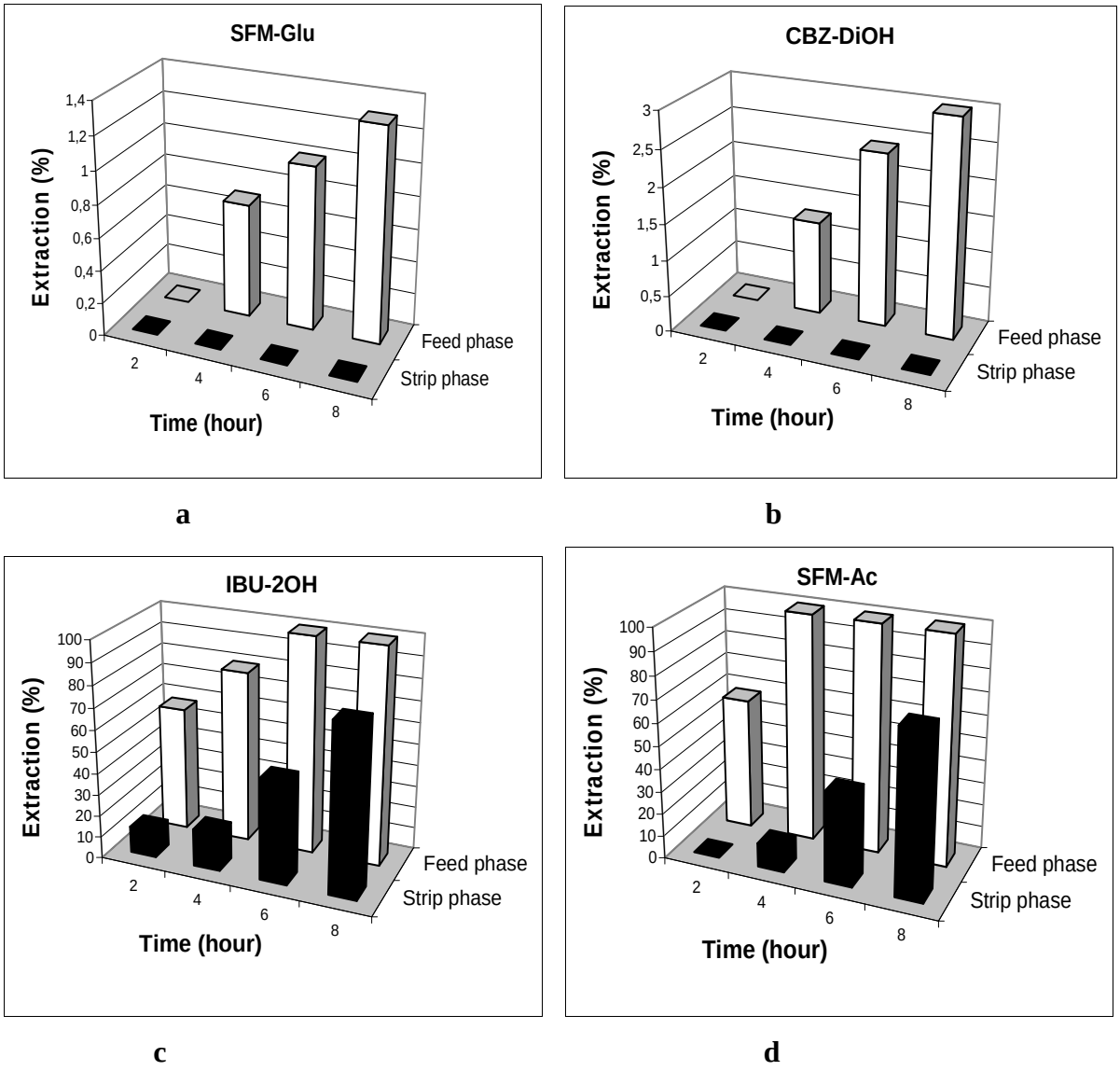


Figure-2.22: Time effect on the extraction efficiencies of metabolites by using SL-FM system and DHE with 5 % (w/w) of TOPO as liquid membrane, a) SFM-Glu, b) CBZ-DiOH c) IBU-2OH, d) SFM-Ac (Extraction conditions: see Figure-2.19)

2.2.2.4 Influence of TOPO concentration on the stripping process

The effect of the TOPO concentration in the membrane phase (DHE, undecane and decane) on the re/ back-extraction/ stripping of IBU-2OH and SFM-Ac can be seen in Figures-2.23 and 2.24. For a low carrier concentration (≤10 % w/w) the extraction efficiency of IBU-2OH and SFM-Ac increase with increase of TOPO concentration in the liquid membrane. These observations are the result of the influence of two factors on the mass transfer of analyte through the liquid membrane, namely the concentration gradient of the analyte-carrier complex and the viscosity of the organic liquid membrane phase. If it is assumed, that the flux of the compound through the membrane is (J_M) related to the concentration gradient (ΔC) and the membrane thickness (h_M) through Fick's first law, where D_M is the diffusion coefficient

$$J_M = \frac{D_M}{h_M} \times \Delta C \tag{23}$$

In this case, high fluxes can be achieved when the high diffusion coefficient is maintained. From the other side, the diffusion is dependent on the viscosity of the organic phase (η) and the radius (r) of the species according to the Stokes-Einstein relationship [142]:

$$D_M = \frac{kT}{6\pi\eta r} \tag{24}$$

Therefore, as an increase of the carrier concentration generally increases the driving force as well as the viscosity of the liquid membrane (see Tables-2.1 and 2.2).

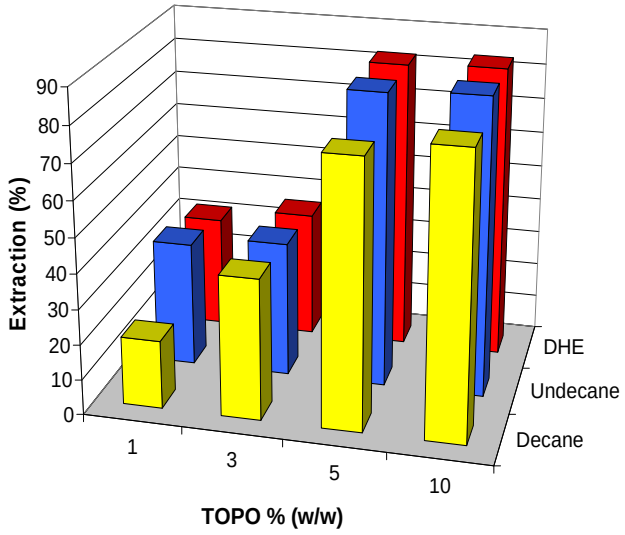


Figure-2.23: Stripping of IBU-OH as a function of TOPO concentration in DHE, undecane and decane (Extraction conditions: see Figure-2.19)

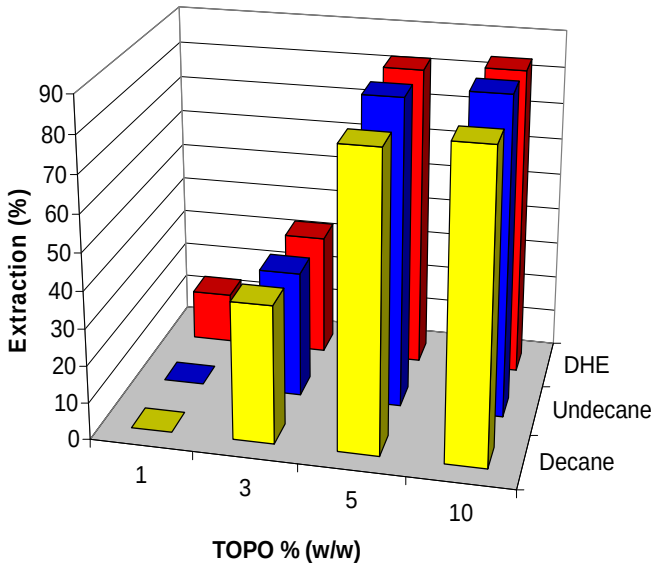


Figure-2.24: Stripping of SFM-Ac as a function of TOPO concentration in DHE, undecane and decane (Extraction conditions: see Figure-2.19)

2.2.3 Conclusion

The metabolites 10,11-dihydroxycarbamazepine (CBZ-DiOH), 4-hydroxyibuprofen (IBU-2OH), N-4-acetylsulfamethoxazole (SFM-Ac) and sulfamethoxazol-N1-glucuronide (SFM-Glu) were synthesized and characterized by IR, ^{1}H-, ^{13}C-NMR and mass spectroscopy see section 3.1. The synthesis steps of 4`-hydroxydiclofenac (DCF-4OH) have faced problems, leading at the end to incapability to synthesize this compound (section 3.1.2).

In order to carry out experiments with BLM and SL-FM systems a HPLC-UV method was developed and validated as shown in section 3.2.2.

The mass transfer of the drug metabolites through the liquid membranes was carried out in a three-compartment transport cell and supported liquid membrane-chamber. The three-phase bulk liquid membrane system (BLM) consisted of an aqueous feed solution, an organic solvent (1-pentanol, DHE, undecane, and decane) with and without a dissolved tri-*n*-octylphosphine oxide as a liquid bulk membrane and an aqueous stripping solution. The BLM employed offers a tool to separate selectively and efficiently various metabolites from aqueous solution. It extracts most efficiently IBU-2OH and SFM-Ac by combining TOPO as carrier, the appropriate solvent and a pH-gradient between feed and strip. In general the extractability of SFM-Glu and CBZ-DiOH by the liquid membrane is lower. Maximum extraction yield were obtained with 1-pentanol as a polar membrane.

The supported liquid flat-membrane (SL-FM) system 150:150 (v/v) based on a porous polypropylene (PP) membrane (pore size 0.1 μm, total thickness 90 μm) was impregnated with an appropriate water-immiscible organic membrane phase. It contained the solvent with or without a dissolved carrier held by capillary forces placed between two aqueous phases, feed and strip.

Certain three-phase compositions were tested in SL-FM. Different factors have been studied to find the best extraction conditions for these metabolites:

- strip phase: adjusted to pH >13 by using 0.1 mol/L NaOH;
- liquid membrane: DHE with 10 % (w/w) TOPO or undecane with 10% (w/w) TOPO, and decane with 10 % (w/w) TOPO;
- Time of extraction: 8 hours.

This condition gave ~ 90 % of IBU-2OH and ~ 85 % of SFM-Ac transported from the feed into the strip solution at a concentration of 1 mg/L.

By contrast, SFM-Glu and CBZ-DiOH which have a very lower partition coefficient (octanol/ water) value can not be transported to the strip phase by using both BLM and SL-FM systems.

High enrichment factors are possible by employing certain SLM-cells, so that even traces of metabolites residues or drugs can be separated and enriched. Therefore, the investigations are continued to develop particular SLM devices which should be suitable for trace analysis.

2.3 Supported liquid bag membrane (SL-BM) systems

In SLM extractions, a high enrichment is a result of high solubility of the analytes in the membrane and complete trapping into the stripping phase. Theoretically infinitely high enrichment factors can be obtained. In practice, however, enrichment factors are limited by several factors such as the processing time, the adjustable volume ratio of sample and strip phase, and affectivity of extraction and stripping.

The objectives of the present experiments are two-fold. The first one is to develop a method to extract drug traces from water samples. The second one is to compare the efficiency and applicability of this method with common solid-phase extraction.

The original version of SL-bag membrane was developed at the University of Paderborn [112]. Special polypropylene-bags (PP-bags) were produced from PP-sheets by a special technique used as a support for the liquid membrane. The method provides high enrichment factors (up to 1100) for a limited number of analytes employing a DHE/ OcSA-based SLM-system [112].

The SL-BM device is shown in Figure-2.25. The PP-bag soaked with the liquid membrane and filled with 0.3 - 0.6 mL of 0.1 mol/L NaOH as the strip phase was placed in 500 mL of the aqueous, acidified (pH 1) sample, containing the analytes of drug traces dissolved. A magnetic stir bar was used to agitate the sample solution during the extraction. At the end of the extraction process (4 h), the strip solution was drawn into a syringe and transferred into a vial insert for HPLC-UV or LC-MS analysis.

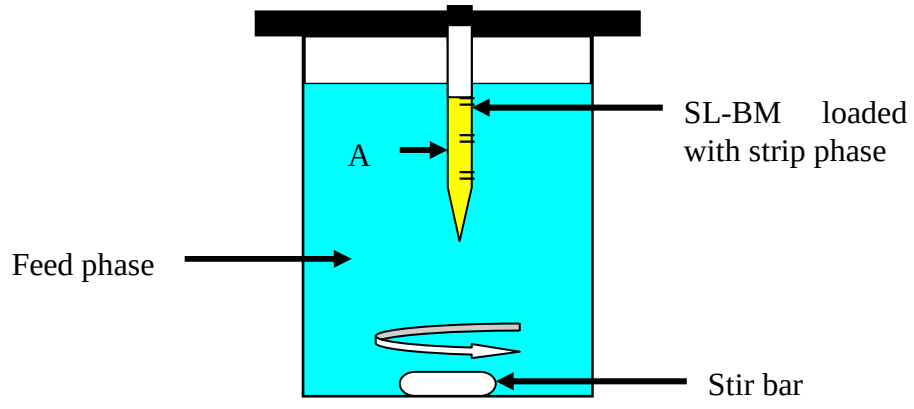


Figure-2.25: Schematic diagram of SL-BM; A represents the analyte

2.3.1 Extraction efficiency and enrichment factor: Theoretical approach

As shown in Figure-2.26, the analytes (IBU-2OH, SFM-Ac, IBU and DCF) in their undissociated form first diffuse from the bulk feed solution to the surface of the membrane and then partitioned into the membrane liquid. After migration across the membrane, they were extracted into the strip via deprotonation. In the ionized form they can not re-enter the membrane. The two processes occurred simultaneously, and the overall extraction can be highly efficient [143].

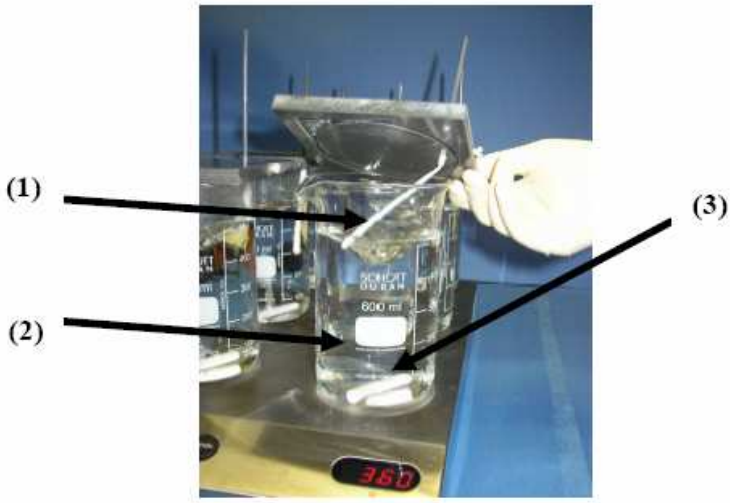


Figure-2.26: SL-BM device; (1) bag membrane filled with strip phase 0.3 - 0.6 mL; (2) sample solution with 500 mL of feed phase; (3) stir bar [112]

At equilibrium, the mass balance in the SL-BM system can be written as [144]:

$$C_F V_F = C_{F,i} V_F + C_M V_M + C_S V_S \quad (25)$$

C_F is the initial analyte concentration in the feed phase, $C_{F,i}$, C_M, and C_S are the equilibrium analyte concentrations in the feed, membrane, and strip respectively. V_F, V_M, and V_S are the volumes of the feed, membrane, and strip, respectively.

K_F is the partition coefficient between the membrane and the feed, and K_S is the partition coefficient between the membrane and the strip [143]:

$$K_F = \frac{C_M}{C_F \alpha_F} \quad (26)$$

$$K_S = \frac{C_M}{C_S \alpha_S} \quad (27)$$

The extraction efficiency, *E*, is defined as the fraction of the analytes extracted, and is given as:

$$E = \frac{C_S V_S}{C_F V_F} \tag{28}$$

The enrichment factor, E_e, is defined as the ratio of the analyte concentration in the strip and to that in feed phase:

$$E_e = \frac{C_S}{C_F} = E(\frac{V_F}{V_S}) \tag{29}$$

When (V_F/V_S) is fixed, according to Eq. (29), E_e is proportional to E. Combining Eqs. (25) - (29), the E at equilibrium can be written as:

$$\mathrm{E} = \frac{1}{(\alpha_S V_F K_S)/(\alpha_F V_S K_F) + (\alpha_S K_S V_M)/V_S + 1} \tag{30}$$

Combining Eqs. (25)- (27) and (29), the E_e at equilibrium can be written as:

$$E_e = \frac{1}{(\alpha_S K_S/(\alpha_F K_F) + (\alpha_S K_S V_M)/V_F + V_S/V_F} \tag{31}$$

Because α_F is approximately 1, and if K_S and K_F are assumed to be similar, Eqs. (30) and (31) can be simplified to calculate maximum enrichment $E_{(max)}$ and extraction efficiency $E_{e(max)}$ as:

$$E_{(\max)} = \frac{1}{(\alpha_S V_F)/V_S + (\alpha_S K_S V_M)/V_S + 1} \tag{32}$$

$$E_{e(\max)} = \frac{1}{\alpha_S + (\alpha_S K_S V_M)/V_F + V_S/V_F} \tag{33}$$

Eq. (32) indicates that in order to achieve a high E, α_S should be low. Theoretically the maximum possible E is 1 corresponding to an extraction yield of 100 %. According to Eq. (33), a small α_S is also necessary for a high E_e-value. E_e decreases with increase of V_S, and increase with the increase in V_F. The maximum possible E_e can be calculated by:

$$E_{e(\max)} = \frac{V_F}{V_S} \tag{34}$$

A high E_e-value is desired to obtain lower detection limits. In case of the SL-BM used the theoretically highest E_e-value is 1250, in case of quantitative extraction ($E_{(max)} = 1$)

2.3.2 Extraction of the metabolites IBU-2OH and SFM-Ac

For the efficient enrichment of metabolites, factors that control the transfer of the analytes from the feed phase to the strip phase across the bag membrane and the entrapment of the analytes to the strip phase were optimized. For an efficient enrichment analytes in the feed phase need to be non-ionic or in an uncharged form before they diffuse across the membrane. The partition coefficient K_F (see Eq.26) of the analyte molecules between the organic solvent

and the aqueous feed phase has to be as high as possible for the target molecules IBU-2OH and SFM-Ac. For the interfering compounds it has to be low. Also an efficient trapping or conversion of analytes into the inactive form which in turn prevents back-diffusion into the feed phase, should take place from the stripping phase. Therefore, a number of parameters were optimized in order to achieve the objective of efficient trapping of the analyte.

2.3.2.1 Influence of composition of the liquid membrane

The compositions (solvent/carrier) used in the SL-FM (section 2.2.2) which provided best extraction results (see Table-2.7) were selected to test their applicability in SL-BM systems.

Table-2.7: Composition of liquid membranes used for SL-BM systems

Solvent	Carrier
DHE	1, 3, 5 and 10 % (w/w) of TOPO
Undecane	1, 3, 5 and 10 % (w/w) of TOPO
Decane	1, 3, 5 and 10 % (w/w) of TOPO

Liquid membranes consisting of undecane or decane and higher concentrations of TOPO were not stable; the bag membrane leaked the strip solution into the feed phase after 1 hour of extraction. By contrast, DHE with concentration in the range of 1- 10 % (w/w) of TOPO was more stable. Consequently, DHE with dissolved TOPO as carrier was qualified for further experiments with the SL-BM system.

The low drug concentrations ($\leq$ 1 mg/L) chosen, do not allow the determination of the extraction yield from the feed into the SL-BM. Therefore, the judgment of the membrane performance is exclusively based on the determination of the trapped pharmaceuticals in the strip.

2.3.2.2 Influence of TOPO concentration

The influence of the carrier on the extraction efficiency for metabolites was studied by varying the TOPO concentration in DHE from 1 to 15 % (w/w). As demonstrated in Figure-2.27, the re-extraction for both compounds increased with increasing TOPO-concentration and reaches a maximum at 10 % (w/w). At a higher carrier concentration, the higher stripping yield determined could be attributed to an increased formation of aggregate complexes with low diffusion constants [145]. As a consequence, for further experiment, the TOPO concentration in the membrane was adjusted to 10 % (w/w).

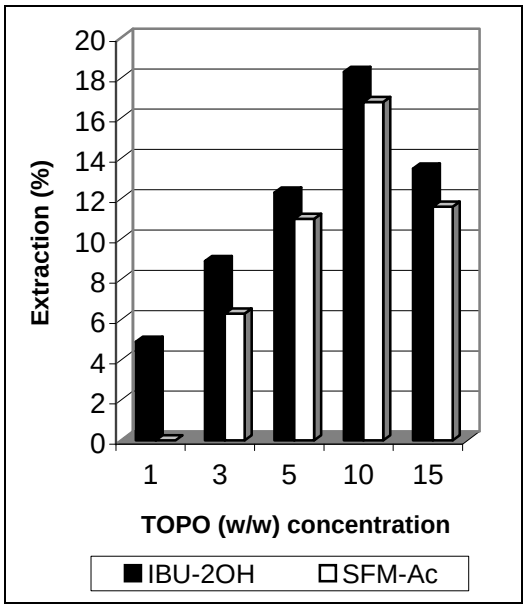


Figure-2.27: Stripping of IBU-2OH and SFM-Ac as a function of TOPO concentration in DHE (Extraction conditions: feed 0.01 mg/L of metabolites in 500 mL of 0.1 mol/L HCl, strip: 0.4 mL of 0.1 mol/L NaOH, 4 h of extraction)

2.3.2.3 Influence of extraction time

Figure-2.28 shows effect of time on the stripping process. Stripped amounts increased over a period of 4 h and then they declined. This effect may be attributed to the influence of the pH on the stripping reaction. As IBU-2OH and SFM-Ac continue to be trapped, the pH value adjusted decreased slightly from pH 13 to pH 11 during the process and this may cause the analyte to back extract. In comparison to SL-FM systems, the pH of the strip phase is more stable (as the volume is comparatively bigger) thus, the analyte is continuously trapped into the strip phase and the extraction efficiency increased with increasing time.

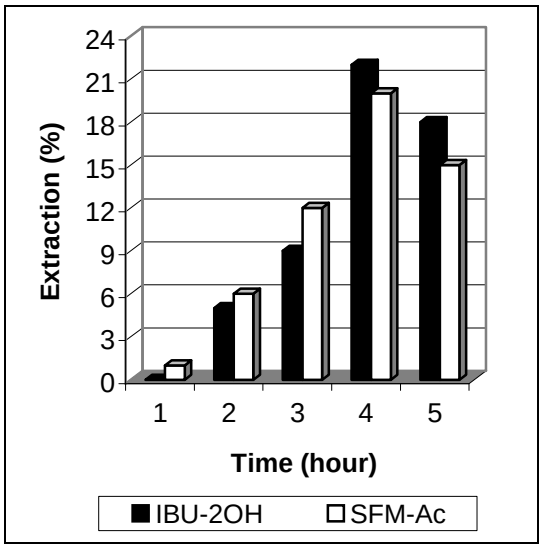


Figure-2.28: Stripping of IBU-2OH and SFM-Ac as a function of extraction time (Extraction conditions: see Figure-2.27)

2.3.2.4 Influence of the pH of the strip phase

The pH of the strip phase also plays an important role on the extraction efficiency. Theoretically, for a nearly complete trapping of IBU-2OH and SFM-Ac, the pH on the strip side should be at least 3.3 pH units higher than the pK_a of these compounds (Figure-2.18). Different buffer solutions (Figure-2.29) and different concentrations of NaOH in the strip phase (Figure-2.30) were used. Figure-2.30 shows that optimal stripping of IBU-2OH and SFM-Ac occurred at pH ~ 13 by using 0.1 mol/L NaOH.

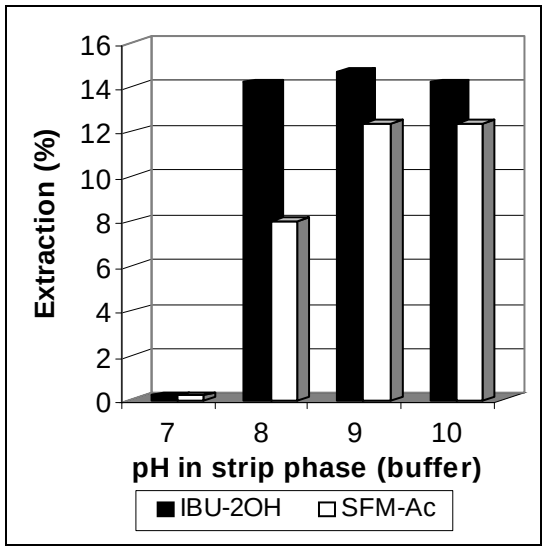


Figure-2.29: Stripping of IBU-2OH and SFM-Ac as a function of pH of buffer in strip phase, (Extraction conditions: see Figure-2.27)

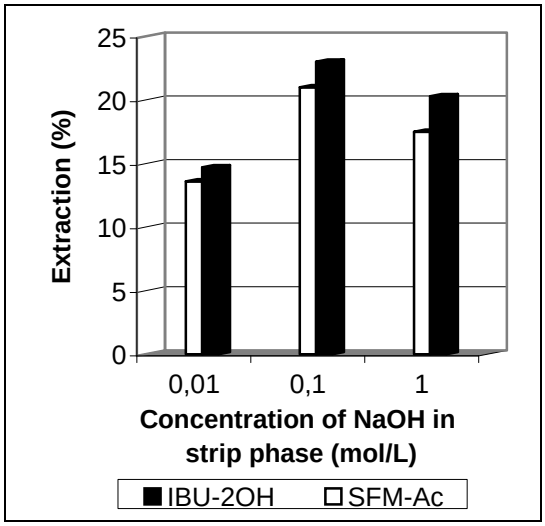


Figure-2.30: Stripping of IBU-2OH and SFM-Ac as a function of the concentration of NaOH in strip phase, (Extraction conditions: see Figure-2.27)

2.3.2.5 Influence of strip phase volume

In the present work, the volume of the strip phase was varied in the range of 0.3- 0.6 mL, while the concentration of metabolites in the feed phase was kept at 0.01 mg/L. The volume of the feed phase was 500 mL. The results show that the maximum extraction efficiencies for IBU-2OH and SFM-Ac were obtained at a strip phase volume of 0.4 mL (Figure-2.31).

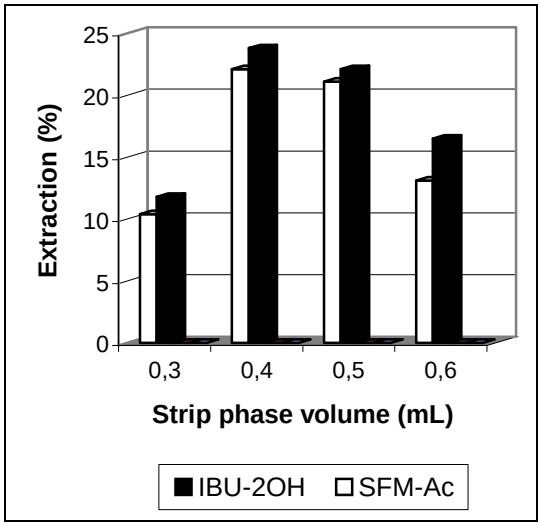


Figure-2.31: Stripping of IBU-2OH and SFM-Ac as a function of the volume of strip phase (0.1 mol/L NaOH), (Extraction conditions: see Figure-2.27)

2.3.2.6 Influence of metabolites concentration

Three concentrations 1, 10, and 100 µg/L of metabolites were investigated. In this range of concentration the effect on the extraction efficiency is negligible for IBU-2OH and SFM-Ac (Table-2.8).

Table-2.8: Extraction efficiency of SL-BM for IBU-2OH and SFM-Ac as function of concentration (Extraction conditions: feed phase 500 mL of 0.1 mol/L HCl; membrane: DHE with 10 % (w/w) TOPO; strip phase 0.4 mL 0.1 mol/L NaOH; n = 3)

Metabolite	Concentration of feed (µg/L)	*Cs* (mg/L)	*E* (%)	E_e ($E_{e(max)}$ =1250)
IBU-2OH	1	0.228	18.3	228.7
	10	2.325	18.6	232.5
	100	23.875	19.1	238.8
SFM-Ac	1	0.212	16.96	212.5
	10	2.105	16.84	210.0
	100	22.050	17.64	220.5

2.3.3 Extraction ability of the active drugs CBZ, DCF, IBU and SFM

The SL-BM system was also employed to extract the active drugs SFM, CBZ, DCF, and IBU from spiked water samples. Different liquid membranes with and without carrier, e.g. DHE, DHE with 0.025 g/L OcSA, and DHE with 10 % (w/w) TOPO were tested. As shown in Table-2.9, the maximum extraction yield for DCF (~ 64.3 %) and for IBU (~ 70.1 %) were

obtained by using DHE with OcSA as liquid membrane, whereas SFM and CBZ remain not extractable.

Table-2.9: Extraction efficiency of SL-BM for DCF and IBU as function of liquid membrane composition, (Extraction conditions: feed phase 0.01 mg/L of active drugs in 500 mL of 0.1 mol/L HCl; strip phase 0.4 mL 0.1 mol/L NaOH; n = 3)

Active drug	Membrane	*Cs* (mg/L)	*E* (%)	E_e ($E_{e(max)}$ =1250)
DCF	DHE	5.10	40.8	510
	DHE with 0.025 g/L OcSA	8.03	64.3	803
	DHE with 10 % (w/w) of TOPO	3.425	27.4	342
IBU	DHE	5.71	45.7	571
	DHE with 0.025 g/L OcSA	8.76	70.1	876
	DHE with 10 % (w/w) of TOPO	5.812	46.5	581

2.3.4 Comparison between the extractability of active drugs and metabolites by using SL-BM systems

The extraction of active drugs and metabolites were compared to find out the different behaviour of these compounds in SL-BM extraction systems. For this purpose the same extraction conditions with three different liquid membrane systems (DHE, DHE with 0.025 g/L OcSA and DHE with 10 % (w/w) TOPO) were applied in a SL-BM cell.

As shown in Figure-2.32, a co-relation between the extraction efficiency and partition coefficient of the *acidic compounds* is obvious. DHE as a liquid membrane gave a satisfactory extraction efficiency only for the compounds with a high partition coefficient like DCF and IBU. By using DHE admixed with OcSA, the extraction efficiency for DCF and IBU increased, however, the membrane is not able to extract the compounds with lower partition coefficient. DHE with TOPO as a carrier extracts DCF, IBU and to some extend, also the compounds having lower partition coefficients such as IBU-2OH and SFM-Ac. It is to assume that TOPO forms hydrogen–bonded associates with the carboxyl and/or hydroxyl substituents of DCF, IBU, IBU-2OH and SFM-Ac, thus enhancing the analyte-DHE interactions so that transport of these compounds across the liquid membrane occurred. On the other hand, adding of TOPO to DHE facilitates the transport into the membrane (see Table-2.1). However, the release of these compounds (DCF, IBU, IBU-2OH, and SFM-Ac) into the strip phase is negligible. Therefore, the overall extraction efficiency is low. A similar behavior was found for SFM-Glu and SFM which can not be extracted at all.

Also the *basic compounds* CBZ and CBZ-DiOH were not transported into the strip phase of the different liquid membranes employed.

Furthermore, the extraction of active drugs and metabolites depend in a different way on the pH of the feed phase. For the metabolites, the extraction efficiency of IBU-2OH and SFM-Ac decrease with a decreasing acidity of the feed phase in the range from acidic (pH ~1, by 0.1 mol/L HCl) to neutral water sample. The pH value of the feed phase has no effect on the extraction efficiency of the active drugs DCF and IBU.

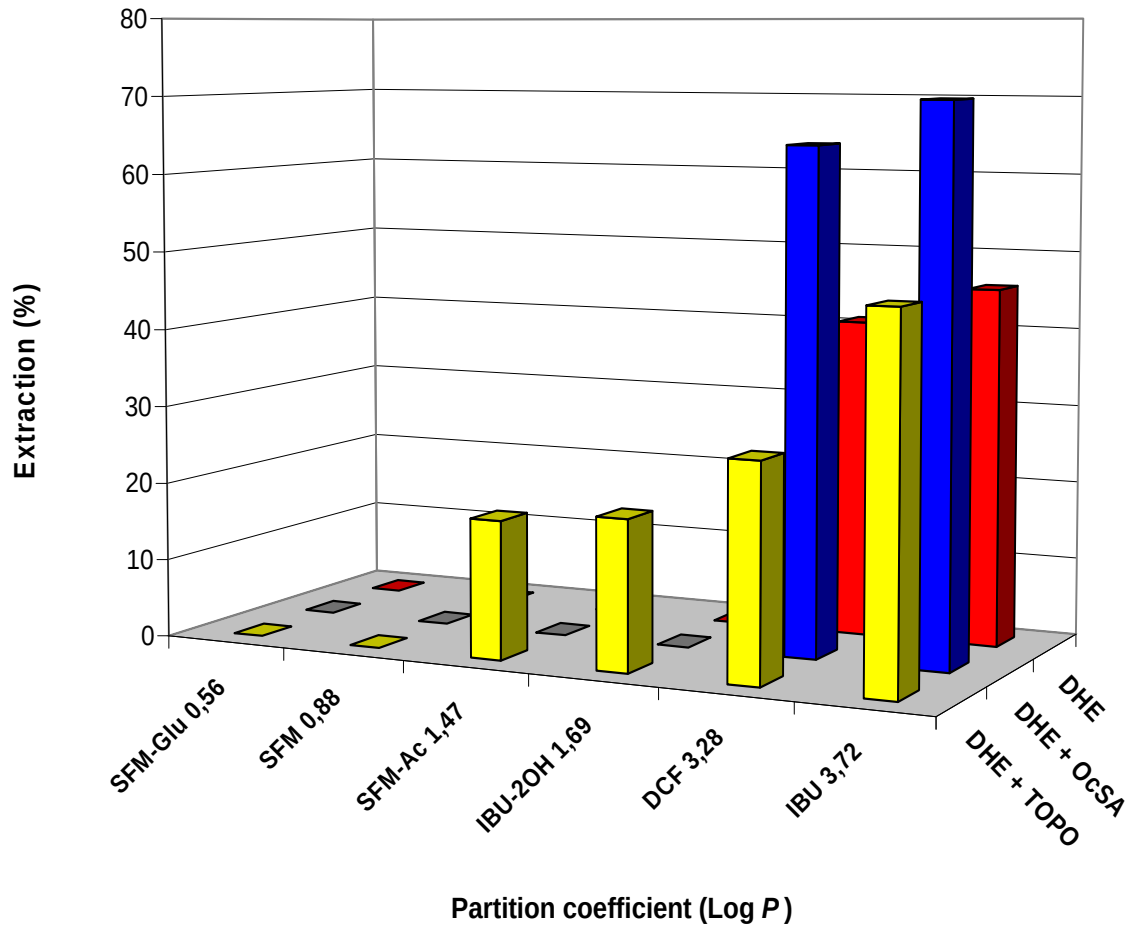


Figure-2.32: Stripping of SFM-Glu, SFM, SFM-Ac, IBU-2OH, DCF, and IBU as a function of the extraction efficiency with Log *P* of these compounds, membrane: DHE, DHE with 0.025 g/L OcSA, and DHE with 10 % (w/w) TOPO, (Extraction conditions: feed 0.01 mg/L of selected pharmaceuticals in 500 mL of 0.1 mol/L HCl, strip: 0.4 mL of 0.1 mol/L NaOH, and 4 hours of extraction)

2.3.5 Extraction of IBU-2OH, SFM-Ac, DCF and IBU by using a SL-4-bag membrane (SL-4-BM) system

In order to improve the extraction yields and the extraction efficiencies, the number of bag membranes in the same extraction device was raised. It was found that a higher number of bags in the same extraction device increased the extraction efficiencies markedly. As presented by the data in Table-2.10: ~ 44 % of IBU-2OH, ~ 58 % of SFM-Ac, ~ 30 % of DCF and ~ 52 % of IBU were trapped in to the strip phase by using four bags as SLM-support (Figure-2.33).

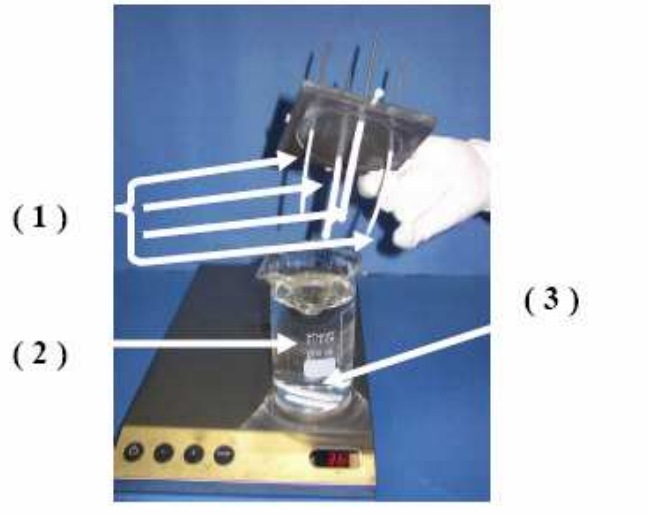


Figure-2.33: SL-4-BM device; (1) bag membrane filled with strip phase with volume 0.3 - 0.6 mL for every bag; (2) sample solution with volume 500 mL of feed phase; (3) stir bar

Table-2.10: Extraction efficiency of SL-BM systems as a function of number of bag membranes (Feed phase: 0.01 mg/L of IBU-2OH, SFM-Ac, DCF, and IBU in 500 mL of 0.1 mol/L HCl; strip phase: 0.4 mL of 0.1 mol/L NaOH for every bag; liquid membrane: DHE with 10 % TOPO (w/w); n = 3)

Number of bags	IBU-2OH		SFM-Ac		DCF		IBU	
	Cs **(mg/L)**	***E*** **(%)**	***Cs*** **(mg/L)**	***E*** **(%)**	***Cs*** **(mg/L)**	***E*** **(%)**	***Cs*** **(mg/L)**	***E*** **(%)**
1	2.28	18.3	2.1	16.8	0.71	5.7	1.83	14.7
2	3.46	27.7	2.9	23.2	1.08	8.7	2.81	22.5
3	4.98	39.9	4.82	38.6	2.38	19.1	4.56	36.5
4	5.58	44.7	7.27	58.2	3.8	30.4	6.52	52.2

The data of table Table-2.10 clearly reveal that the increased number of bags increases the overall extraction yield drastically.

2.3.5.1 Reproducibility of extraction with the SL-4-BM system

The reproducibility of the SL-4-BM system was tested by a series of tests under the same conditions. The total relative standard deviations (S_{rel}) were found to range from 0.3 to 1.2 for IBU-2OH, SFM-Ac, DCF, and IBU as listed in Tables-2.11 -2.14. The methods show reasonable reproducibility of the extraction of low concentration of analytes (0.01 mg/L).

Table-2.11: Reproducibility for SL-4-BM system for extraction of IBU-2OH

4 Bags number	**Bag I**	**Bag II**	**Bag III**	**Bag IV**	**Total**
***Cs* (mg/L)**	1.17	1.65	1.13	1.72	**5.67**
***E* (%)**	9.4	13.2	9.1	13.8	**45.5**
***Cs* (mg/L)**	1.0	1.5	1.47	1.61	**5.58**
***E* (%)**	8.0	12.0	11.8	12.9	**44.7**
***Cs* (mg/L)**	1.08	1.56	1.43	1.53	**5.6**
***E* (%)**	8.7	12.5	11.5	12.3	**45**
***Cs* (mg/L)**	0.88	1.58	1.4	1.86	**5.72**
***E* (%)**	7.1	12.7	11.2	14.9	**45.9**
***Cs* (mg/L)**	0.9	1.61	1.3	1.8	**5.61**
***E* (%)**	7.2	12.9	10.4	14.4	**44.9**
S_{rel}	**0.9**	**0.4**	**1.0**	**1.0**	**0.5**

Feed phase 0.01 mg/L of IBU-2OH in 500 mL of 0.1 mol/L HCl, strip phase: (4 x 0.4 mL) of 0.1 mol/L NaOH, liquid membrane: DHE with 10 % (w/w) TOPO, extraction time: 4 hours, n = 5,S_{rel}: standard deviation.

Table-2.12: Reproducibility for SL-4-BM system for extraction of SFM-Ac (extraction conditions see Table-2.11)

4 Bags number	**Bag I**	**Bag II**	**Bag III**	**Bag IV**	**Total**
***Cs* (mg/L)**	1.7	2.02	1.53	2.15	**7.41**
***E* (%)**	13.6	16.2	12.3	17.2	**59.3**
***Cs* (mg/L)**	1.71	2.11	1.3	2.15	**7.27**
***E* (%)**	13.7	16.9	10.4	17.2	**58.2**
***Cs* (mg/L)**	1.73	2.11	1.46	2.15	**7.45**
***E* (%)**	13.9	16.9	11.7	15.8	**58.3**
***Cs* (mg/L)**	1.78	2.03	1.48	1.97	**7.26**
***E* (%)**	14.3	16.3	11.9	16.5	**59**
***Cs* (mg/L)**	1.68	2.01	1.45	2.13	**7.27**
***E* (%)**	13.5	16.1	11.6	17.1	**58.3**
S_{rel}	**0.3**	**0.3**	**0.7**	**0.6**	**0.5**

Table-2.13: Reproducibility for SL-4-BM system for extraction of DCF (extraction conditions see Table-2.11)

4 Bags number	Bag I	Bag II	Bag III	Bag IV	Total
***Cs* (mg/L)**	1.0	1.08	0.8	0.71	**3.59**
***E* (%)**	8.0	8.7	6.4	5.7	**28.8**
***Cs* (mg/L)**	0.93	1.07	0.82	0.73	**3.55**
***E* (%)**	7.5	8.6	6.6	5.9	**28.9**
***Cs* (mg/L)**	1.08	1.02	0.87	0.72	**3.69**
***E* (%)**	8.7	8.2	7.0	5.8	**29.7**
***Cs* (mg/L)**	1.03	1.02	0.85	0.72	**3.62**
***E* (%)**	8.3	8.2	6.8	5.8	**29.1**
***Cs* (mg/L)**	0.97	1.11	0.76	0.77	**3.61**
***E* (%)**	7.8	8.9	6.1	6.2	**29**
S_{rel}	**0.4**	**0.3**	**0.3**	**0.1**	**0.3**

Table-2.14: Reproducibility for SL-4-BM system for extraction of IBU (extraction conditions see Table-2.11)

4 Bags number	Bag I	Bag II	Bag III	Bag IV	Total
***Cs* (mg/L)**	1.61	2.81	0.47	1.83	**6.72**
***E* (%)**	12.9	22.5	3.8	14.7	**53.9**
***Cs* (mg/L)**	1.36	2.72	0.55	1.81	**6.44**
***E* (%)**	10.9	21.8	4.4	14.5	**51.6**
***Cs* (mg/L)**	1.46	2.56	0.77	1.72	**6.51**
***E* (%)**	11.7	20.5	6.2	13.8	**52.2**
***Cs* (mg/L)**	1.26	2.82	0.52	1.83	**6.43**
***E* (%)**	10.1	22.6	4.2	14.7	**51.6**
***Cs* (mg/L)**	1.56	2.65	0.7	1.83	**6.74**
***E* (%)**	12.5	21.2	5.6	14.7	**54**
S_{rel}	**1.1**	**0.8**	**1.0**	**0.3**	**1.2**

It is remarkable, that the overall standard deviation (S_{rel}) of the extraction data of the SL-4-BM system were found to be relatively lower (IBU-2OH) or at a similar level compared to the individual bags (DCF, IBU and SFM-Ac).

2.3.5.2 Influence of humic acids

Humic substances are widespread in the aquatic environment, i. e., natural waters, lakes and sediments, in both soluble and insoluble forms. These macromolecular substances are formed as a product of chemical and biological transformations of animal and plant residues. The

concentration of humic acids in groundwater is in the range of 10 ng/L -100 μg/L. The principal properties of humic acids and their subsequent potential application depend strongly on their origin and the isolation procedure [146].

Humic acids are considered natural polyelectrolytic organic compounds of complex structures including condensed aromatic rings with a large number of attached carboxylic and hydroxyl groups. The structure of these biopolymers is still not clearly defined. Humic acids are organic ligands and play a crucial role in speciation, transport and deposition of a variety of compounds ranging from metal ions to lipophilic compounds [147].

It is very common that surface waters contain humic acids. Therefore, the effect of the presence of humic acids on the extraction efficiency of IBU-2OH, SFM-Ac, DCF, and IBU were studied by adding 18 mg/L of humic acids (HUS-Standard Hohlohsee 13) [112] to the sample solution (feed phase). Figure-2.34 shows, that the extraction efficiency of IBU-2OH and SFM-Ac were slightly increased in present of humic acid in the feed phase. By contrast, the extraction efficiency of DCF and IBU decreased after adding 18 mg/L humic acids in feed phase as shown in Figure-2.34.

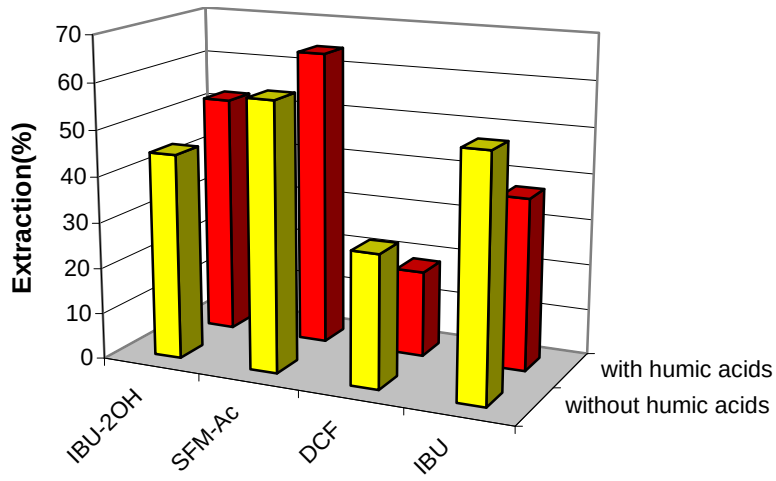


Figure-2.34: Stripping of IBU-OH , SFM-Ac, DCF, and IBU in SL-4-BM as a function with humic acids adding in feed phase, [Extraction conditions: feed: 0.01 mg/L of drugs with or without 18 mg/L humic acids (HUS-Standard Hohlohsee 13) in 500 mL of 0.1 mol/L HCl, liquid membrane: DHE with 10 % (w/w) TOPO, 4 bag membranes filled with (4 x 0.4 mL) 0.1 mol/L NaOH as strip phase, after 4 hours of extraction]

As pointed out in section 2.4, the second object is to compare the analytical applicability of SL-4-BM with another standard method such as SPE that proved to be useful to extract the selected analytes from water samples.

2.3.6 Solid phase extraction (SPE)

Solid-phase extraction (SPE) is widely used in the determination of pharmaceuticals from environmental water samples [148, 149]. SPE is essentially a three-step process. A sample is initially passed through the sorbent bed, and analytes present in the sample are exhaustively extracted from the sample matrix to the solid sorbent. In the second step, unwanted analytes or matrix components are selectively desorbed from the solid sorbent by washing with a solution or appropriate solvent. In the final step, an eluting solvent is able to desorb analytes of interest. The resulting eluent may then be concentrated by evaporation to the desired volume [150, 151]. Depending on the choice of sorbent, a wide range in polarity and chemical class may be covered. For the extraction of high polar analytes SPE with polymeric sorbents often proved to be superior to alkyl-bonded silica (e.g., octadecasilane). A variety of hyper-crosslinked sorbents are commercially available, differing in the degree of linkage, porosity and surface area. They are either co-polymerisates of styrene or a polar component (e.g., methacrylate or N-vinylpyrrolidone) or the function groups are introduced after polymerization (e.g., by sulfonation). This functionalisation results in mainly two effects: improved wetting characteristics for better mass transfer and additional possibilities for interaction with functional groups of the analytes and thus a higher degree of retention.

At the first stage of this study, a variety of cartridges were investigated such as polystyrol-DVE-copolymer (Isolute ENV) [152, 153], octadecasilane (Bakerbond C-18) [152, 153], [poly(divinylbenzene-co-N-vinylpyrrolidone)] (Oasis HLB) [152, 153] and C18-material endcapped (Strata C18-E) [154] with the extraction procedure presented schematically in Figure-2.35.

Table-2.15 shows the results of a comparative study on the recovery efficiencies of the analytes in question obtained by means of the four cartridges applied to prepared water samples. At sample volume 500 mL with 0.01 mg/L the concentration of analytes, the higher recoveries were obtained with Oasis-HLB cartridge.

Table-2.15: Analyte recoveries obtained with various SPE cartridges (sample volume: 500 mL, concentration of analytes: 10 µg/L, n = 3)

SPE-Material	Recovery (%)			
	IBU-2OH	**SFM-Ac**	**DCF**	**IBU**
Bakerbond	56.7 %	60.7 %	92.8 %	80.3 %
Isolute	~ 0 %	~ 0 %	5.2 %	23.7 %
Strata	73.5 %	71.4 %	76.3 %	80.0 %
Oasis	74.6 %	77.4 %	76.6 %	82.4 %

The Oasis-HLB cartridge is classified as a mixed mode; it features two retention mechanism mainly strong cation exchange and reversed-phase. The most attractive features of Oasis-HLB cartridge are its hydrophilic-lipophilic-balanced composition that is responsible for both strong reversed-phase retention and water-wetability. In addition, these sorbent are stable from pH 1 to 14 due to the use of pH wash systems [155]. Therefore Oasis-HLB cartridge was finally chosen as the best cartridge for extraction of selected analytes.

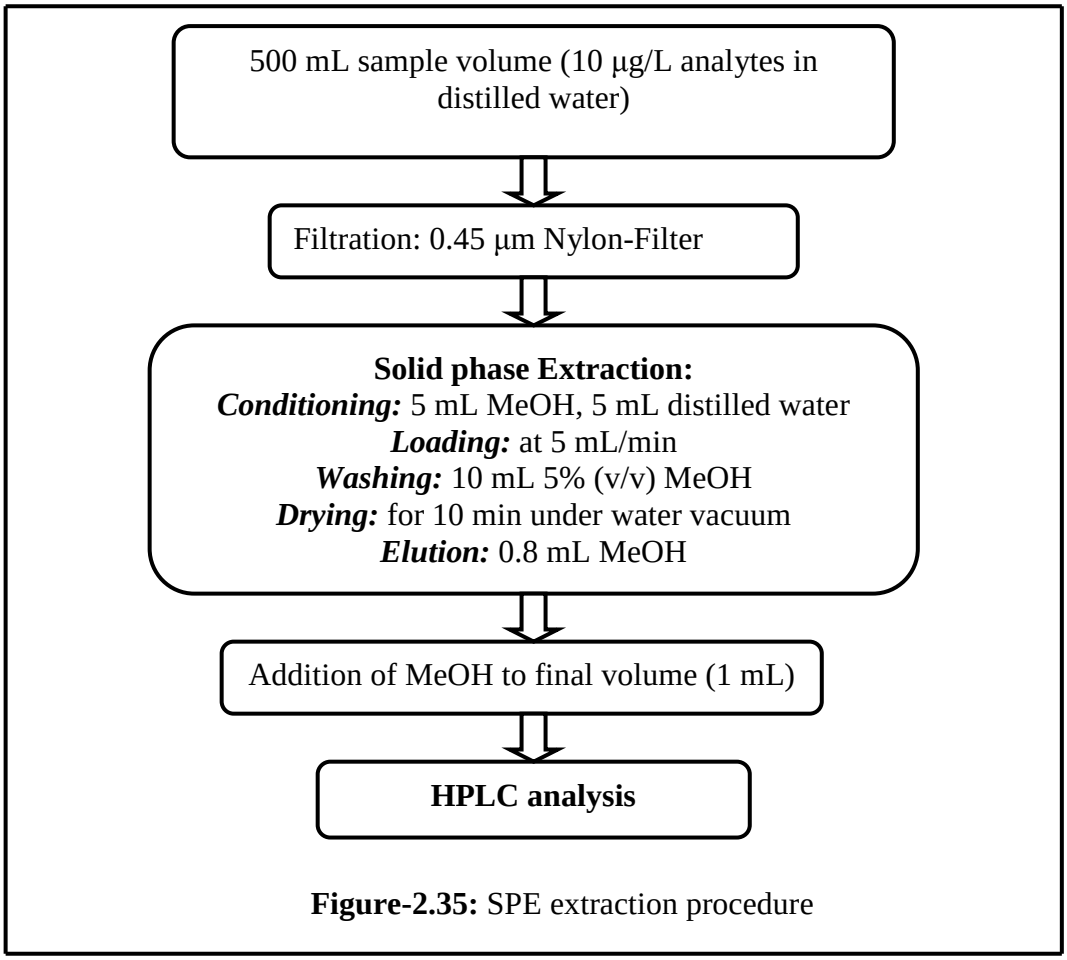


Figure-2.35: SPE extraction procedure

2.3.7 Comparative study of SL-4-BM and SPE extraction techniques

The comparison between Oasis-HLB of SPE cartridge (Figure-2.35) and SL-4-BM system (Figure-2.36) in extraction of IBU-2OH, SFM-Ac, DCF and IBU compounds from prepared water samples has the following results:

- **Recovery:** the recovery obtained with SPE (70% for IBU-2OH, 71.7% for SFM-Ac, 75.1% for DCF and 81.5% for IBU) were higher than with SL-4-BM (44.7 for IBU-2OH, 58.2 % for SFM-Ac, 30.6 % for DCF and 52.7 % for IBU) as listed in Table-2.16.
- **Enrichment factor:** the enrichment factor obtained with SL-4-BM (558 for IBU-2OH, 727 for SFM-Ac, 383 for DCF and 658 for IBU) were higher than with SPE (350 for IBU-2OH, 355 for SFM-Ac, 375 for DCF and 407 for IBU) (see Table-2.16).
- **Solvent consumption:** SL-4-BM has a very low consumption of organic solvent (only organic solvent needed is used to fill the pores of porous support) comparing to SPE (more than 15 mL); as a result the former is more environmentally friendly.
- **Extraction time:** the extraction time for SL-4-BM is 4 hours, while SPE need more than 6 hours.
- **Clean up:** SL-BM gave a considerably more efficient clean-up than SPE [112].
- **Selectivity:** there are many examples of analytical applications to aquatic environment system which show nearly complete selectivity of SLM systems. For example, triazine herbicides were extracted from spiked river water both with SLM and with SPE [14]. The results show that in SLM extract virtually only the expected compounds are found, while a typical "humic hump" from humic acids together with disturbing from unknown compounds is seen in the SPE extract.

Table-2.16: Comparison of performance between SL-4-BM and SPE, (concentration of metabolites: 1 µg/L, and n = 3)

Extraction technique	IBU-2OH			SFM-Ac			DCF			IBU			$E_{e(max)}$
	R (%)	C_S µg/L	E_e	R (%)	C_S µg/L	E_e	R (%)	C_S µg/L	E_e	R (%)	C_S µg/L	E_e	
SPE	70	350	350	71.7	355	355	75.1	375	375	81.5	407	407	500
SL-4-BM	44.7	558.7	**558**	58.2	727.5	**727**	30.6	383.7	**383**	52.7	658.7	**658**	1250

Step-1

Preparation of SLM-system

4 bag membranes (PP)

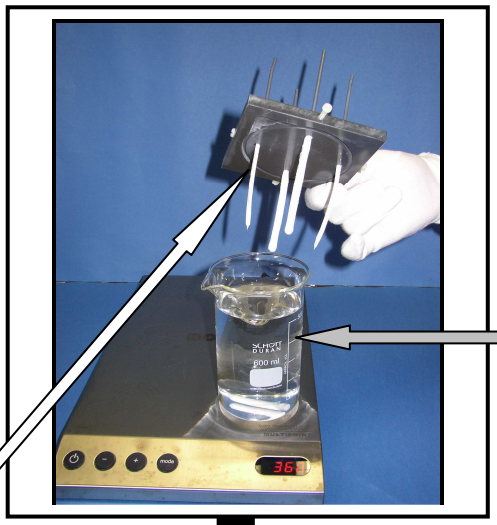
Washing: 3 times with water

Drying: for 30 min at room temperature

Soaking: 2 h in liquid membrane (dihexyl ether with 10 % (w/w) TOPO)

Filling: 0.4 mL (0.1 mol/L NaOH) every bag

Mounting of 4 bag membranes to holding device



Step-2

500 mL water acidified with 6.5 mL of 25% HCl

Filtration: 0.45 µm Nylon-Filter

Step-3

Extraction

. Dipping of SL-4-BM device in feed at room temperature

. ***Stirrer setting:*** at 360 rpm

. ***Extraction time:*** 4 h

Neutralizing the strip phase of every bag with 0.4 mL of 0.1 mol/L HCl

HPLC analysis of each strip

Figure-2.36: Extraction procedure with the SL-4-BM system

2.3.8 Conclusion

SLM bag membrane systems were developed as a sample enrichment device to extract and enrich metabolites and drugs from spiked water samples. The liquid membrane used to trap these compounds consisted of DHE with TOPO and OcSA as carrier.

Several factors affecting the extraction efficiency during SL-BM enrichment were studied. These factors can be *structure* or *operator dependent*.

In the first case, it can be assumed that the transport of all investigated compounds is influenced by its acid-base (pK_a, pK_b) properties and partition coefficient (octanol/water) Log *P*.

In the second case, it can be concluded that from the obtained results, the most effective conditions for membrane transport are: 10 % (w/w) TOPO as carrier dissolved in DHE, low pH of the feed phase (0.1 mol/L HCl), high pH of the strip phase (0.1 mol/L NaOH), a 4-bag membrane system, and 4 hours of extraction to create a driving force of the process which sufficient by produces a high mass transfer of IBU-2OH and SFM-Ac.

SL-4-BM system was used to enrich and determine the target metabolites from prepared water sample by means of HPLC-UV. High enrichment factors were achieved for both acidic metabolites IBU-2OH and SFM-Ac (Table-2.16). Extraction efficiency was 44 % for IBU-2OH and 58 % for SFM-Ac.

DCF and IBU were also separated and enriched under the same conditions. The extraction efficiency of these compounds was 27 % for DCF and 50 % for IBU.

Solid phase extraction (SPE) was used to extract the target drug metabolites from prepared water samples. The achieved recoveries with SPE (80 % IBU-2OH, 71 % SFM-Ac, 96.8 % DCF, and 91.5 % IBU) were higher than SL-4-BM (44 % IBU-2OH, 58 % SFM-Ac, 27 % DCF, and 50 % IBU), but the novel SL-4-BM offer more advantages due to higher enrichment factors, low consume of organic solvents and time.

2.4 Application: determination of drug traces in water by means of SL-4-BM system and HPLC-UV

2.4.1 Influence of analyte concentration and matrix

To judge to what extent the developed method can be affected by parameters having direct influence on the extraction recoveries and signal response, e.g. sample concentration and matrix effects were studied.

Three sample concentrations (300, 500, and 1000 ng/L) were chosen to study the concentration effects at a constant sample volume of 500 mL. To insure the robustness of the developed method, spiked tap water of Paderborn (see Table-3.14) was analyzed.

The percentage recovery of the analytes spiked into each type of tested sample was calculated as follows:

$$R(\%) = \frac{AS - OC}{S} \times 100 \qquad (35)$$

Where *AS* is the peak area of spiked sample, *OC* is the peak area of unspiked sample and *S* is the peak area of non enriched standard [160, 161].

Recoveries obtained by varying the sample concentrations in spiked distilled water and tap water showed no significant differences (Table-2.17 and 2.18).

Table-2.17: Extraction recoveries (*R*) of selected analytes from **distilled water**

Compound	Concentration Spiked	C_S µg/L	C_F µg/L	*R* (%)	S_{rel}
IBU-2OH	0.3 µg/L	155.2	0.124	40.6	8.1
	0.5 µg/L	269.3	0.215	43.1	6.1
	1 µg/L	528.7	0.422	42.3	6.5
SFM-Ac	0.3 µg/L	199.5	0.159	53.2	7.7
	0.5 µg/L	343.7	0.274	55.0	6.5
	1 µg/L	718.7	0.574	57.5	6.8
DCF	0.3 µg/L	96	0.076	25.6	4.5
	0.5 µg/L	185	0.148	29.6	2.5
	1 µg/L	392	0.313	31.4	4.5
IBU	0.3 µg/L	177.3	0.141	47.3	7.1
	0.5 µg/L	323.1	0.258	51.7	8.7
	1 µg/L	685	0.548	54.8	8.0

Table-2.18: Extraction recoveries (*R*) of selected analytes from **tap water** (city of Paderborn)

Compound	Concentration spiked	C_S µg/L	C_F µg/L	R (%)	S_{rel}
IBU-2OH	0.5 µg/L	266.8	0.213	42.7	4.2
	1 µg/L	543.7	0.434	43.5	6.2
	1.5 µg/L	860.6	0.688	45.9	5.2
SFM-Ac	0.5 µg/L	341.8	0.272	54.7	7.2
	1 µg/L	692.5	0.554	55.4	6.9
	1.5 µg/L	1038.7	0.830	55.4	6.6
DCF	0.5 µg/L	183.1	0.146	29.3	2.5
	1 µg/L	380	0.304	30.4	4.1
	1.5 µg/L	613.1	0.490	32.7	2.9
IBU	0.5 µg/L	309.3	0.247	49.5	8.9
	1 µg/L	632.5	0.506	50.6	6.2
	1.5 µg/L	997.5	0.798	53.2	5.8

2.4.2 Application of the developed method for real surface water samples

The developed methods were tested for applicability to real water samples by analyzing the surface water from river Ruhr affected by effluent STP. The water samples were spiked and non-spiked with the metabolites synthesized and the parent drugs to determine recoveries. The samples were treated by the developed separation procedure. The strip phase contained the enriched analytes separated from the surface water matrix.

For trace analysis of real samples the extraction procedure was modified and the chromatographic conditions were adjusted to HPLC-UV analysis. The method detection limits (MDLs) were determined by spiking the analytes into 500 mL of surface water in the concentration range 0.3-1 µg/L. MDLs are distributed between 0.071- 0.185 µg/L. Recoveries obtained by varying the sample concentrations in surface water and distilled water showed no significant differences (Table-2.17 and 2.19). As a result, no target analytes were found in the surface water sample and the water was evidently not polluted.

Table-2.19: Extraction recoveries (*R*) of selected analytes from **surface water** (river Ruhr)

Compound	***OC****	**Concentration spiked**	C_S **µg/L**	C_F **µg/L**	***R*** **(%)**	***MDL*** **µg/L**
IBU-2OH	< 0.001	0.3 µg/L	171.6	0.137	44.8	0.185
		0.5 µg/L	261.1	0.208	41.7	
		1 µg/L	484.1	0.387	38.7	
SFM-Ac	< 0.001	0.3 µg/L	202	0.161	53.8	0.094
		0.5 µg/L	355.4	0.284	56.8	
		1 µg/L	591.4	0.473	47.3	
DCF	< 0.001	0.3 µg/L	125	0.100	33.3	0.071
		0.5 µg/L	190	0.152	30.4	
		1 µg/L	335	0.268	26.8	
IBU	< 0.001	0.3 µg/L	197.4	0.157	52.6	0.122
		0.5 µg/L	304.2	0.243	48.6	
		1 µg/L	640.2	0.512	51.2	

***OC**:** the peak area of unspiked surface water sample

2.5 Development method based on LC/MS

Mass spectrometry (MS) is widely used detection technique that provides quantitative and qualitative information about the components in mixture. In qualitative analysis it is very important to determine the molecular weight of an unknown compound and MS is a technique capable for that. MS is also generally more sensitive than an UV-detector for quantification.

An MS detector consist of three main parts: the ionization source (interface) where the ions are generated, the mass analyzer (separation), which separates the ions according to their mass-to-charge ration (m/z), and the electron multiplier (detector). There are several types of ion sources, which utilize different ionization techniques for creating species.

Three popular ionization techniques are: electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and matrix-assisted laser desorption (MALDI). Electrospray is the most widely used ionization technique when performing LC-MS, and has proved to be a most versatile tool for soft ionization of a large variety of analytes [156, 157].

Common mass analyzers that are commercially available are: quadrupole, ion-trap, time-of-flight (TOF) and magnetic sector analyzers. Ion trap MS is especially useful for advanced qualitative analysis due to the ability of technique to provide scans up to the MS^5 range. The power of ion trap MS stems from the fact, that several generations of daughter ions can be generated providing MS^n spectra. This capability provides a rich source of structural information [157].

Previously published methods for analysis of pharmaceuticals in natural water samples commonly used gas chromatography coupled to mass spectrometry (GC-MS). However, recently, the application of liquid chromatography/ mass spectrometry (LC-MS) rapid growth, in part due to the instrumental developments afforded in this field. With the development of atmospheric-pressure ionization techniques, the combination of liquid chromatography and tandem mass spectrometry (LC-MS/MS) has become the method of choice for high-sensitivity quantitative analysis of drugs and metabolites in water samples. This because LC-MS/MS with ESI and APCI offers [158]:

- versatility: most drugs and metabolites can be effectively analyzed by LC-MS/MS;
- specificity: the combination of liquid chromatography and tandem mass spectrometric detection can provide unparalleled specificity;
- sensitivity: ESI and APCI often permit accurate measurement of analyte at sub-ng/L;

- rapid analysis: no derivatisation of analyte is required, and run times are often a few minutes long;
- quantitative accuracy and precision: by the use of stable isotope-labeled analogs as internal standards one can avoid problems resulting from differences in extraction and chromatographic behavior between the analyte and the internal standard, and can correct for analyte losses due to chemical instability;
- Ease of operation: LC-MS/MS analyses are often easier to set up and perform than GC-MS analysis.

Therefore, LC-MS was chosen for this study. The LC-MS/MS system used was a LCQ Advantage in ESI-mode (Thermo Finnigan, electron, Egelsbach, Germany), connected with a gradient pump (Spectra SYSTEM P4000) was employed.

2.5.1 Mass spectrometer parameters

Several mass spectrometric parameters must be optimized in order to obtain the highest possible abundance of the analytes in MS.

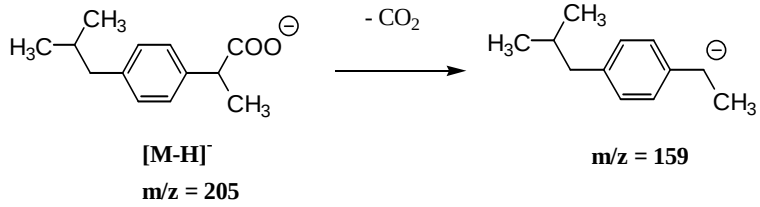
***Electrospray operation*:** electrospray operation parameters were optimized by direct infusion of the analytes by means of a syringe pump at flow rate of 10 µg/min into the ESI source. Nitrogen was used as sheath gas at a flow rate of approximately 47.0 L/min, the spray voltage was set to 5 kV in the positive ionization mode and to 4.5 kV in the negative ionization mode, and the transfer capillary was set to 250 ^{o}C.

Capillary voltage: the properties of the functional groups define the ionization mode; IBU-2OH, DCF and IBU contain carboxylic acid groups, so they will favor the negative mode while, SFM-Ac prefer the positive mode because of its amine functionality. The capillary voltage, ion optics parameters were optimized for all compounds based on mass peak intensity. The optimal collision energy was between 5 and 10 V in negative ion mode.

Analyte fragmentation pattern: all analytes were measured in full scan mode under MS/MS conditions. The chemical structure and typical mass spectra of the analytes are shown in (Figures-2.37- 2.40) and the selected ions are listed in Table-2.20.

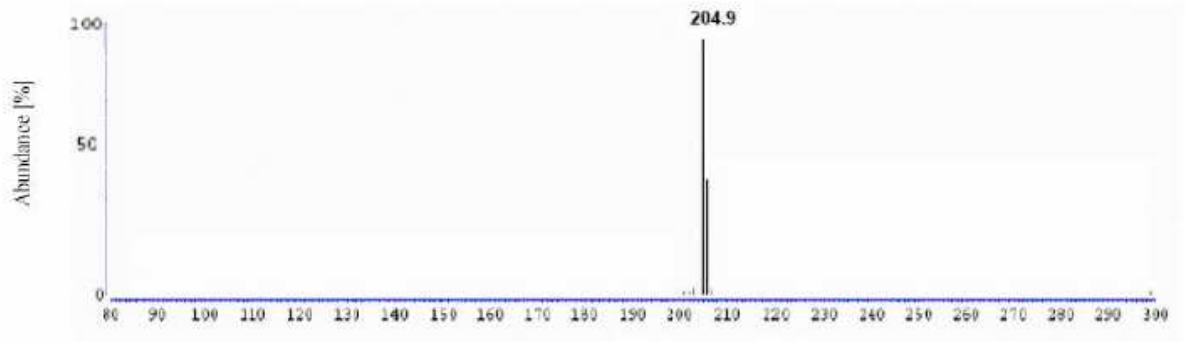
Table-2.20: LC-ESI-MS/MS parameters for analysis of the selected drugs

Analyte	Mode	Collision energy [V]	Normalized collision energy [%]	Fragment ion (m/z)
IBU-2OH	-ve	5	30	221/177
SFM-Ac	+ve	10	35	296/197.9
DCF	-ve	5	30	293.8/250
IBU	-ve	5	28	204.9/159

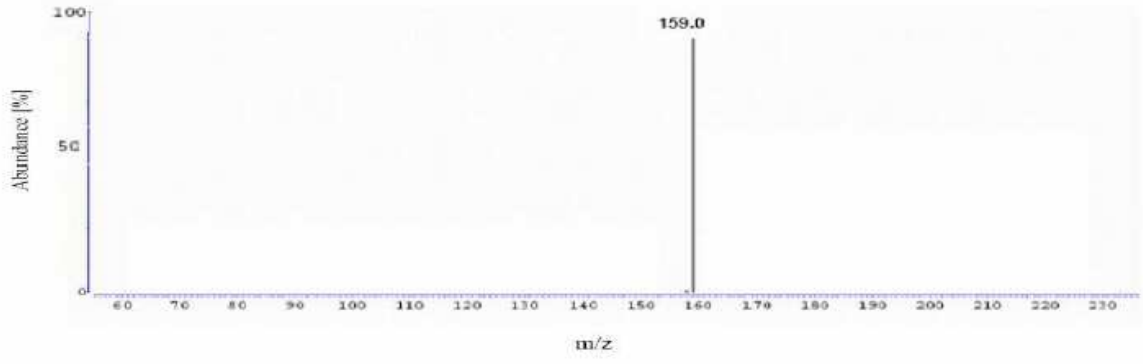


[M-H]$^-$
m/z = 205

m/z = 159

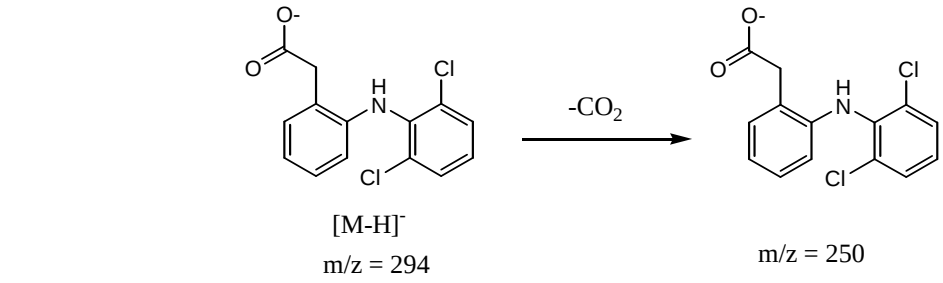


a)



b)

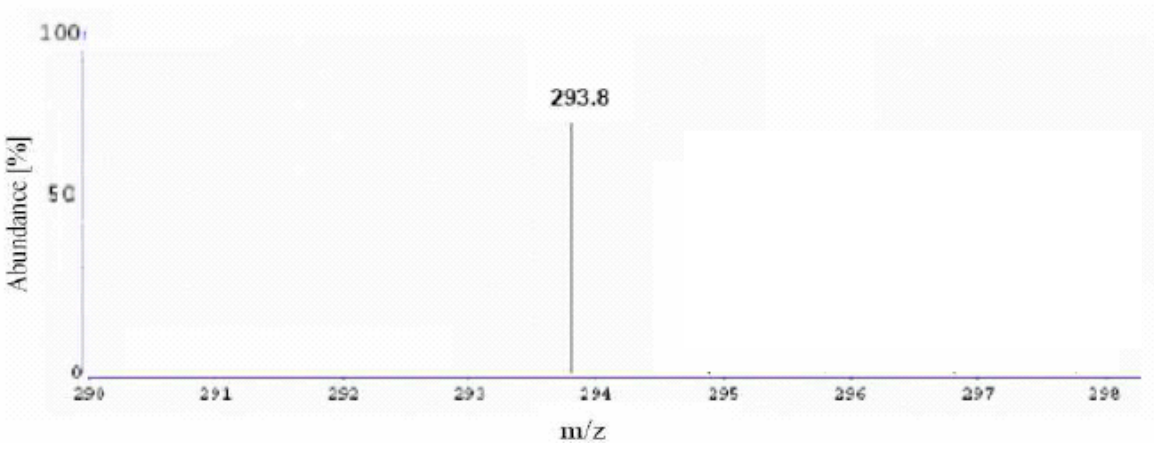
Figure-2.37: (a) LC-ESI/MS spectrum of IBU and (b) the MS/MS product ion spectrum of the [M-H]$^-$ at m/z 205 (Concentration of analyte: 1 mg/L in methanol)



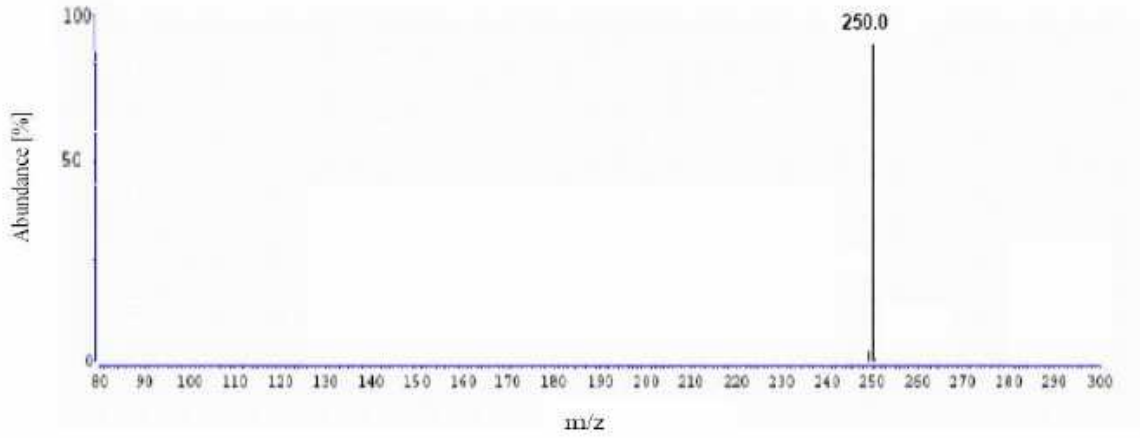
$[M-H]^-$

m/z = 294

m/z = 250

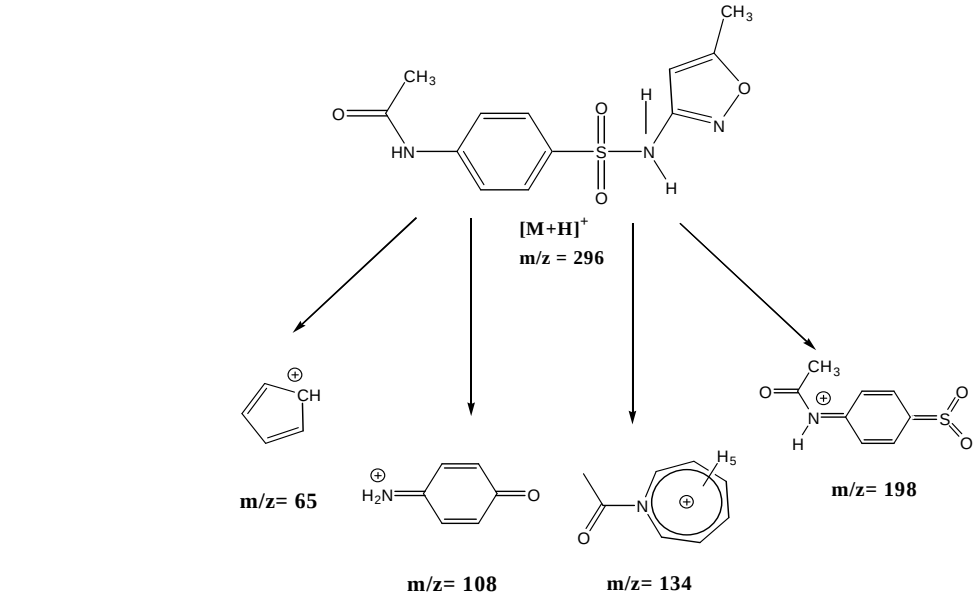


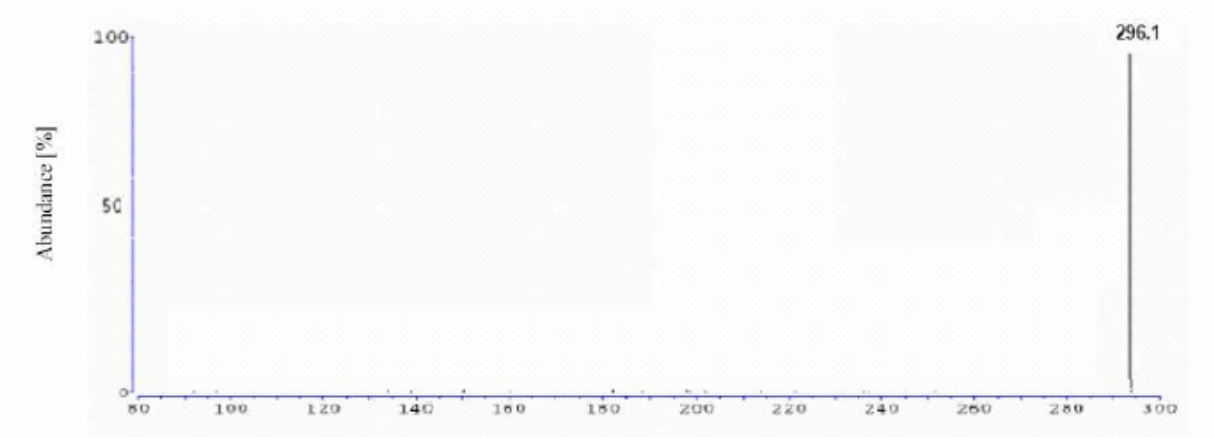
a)



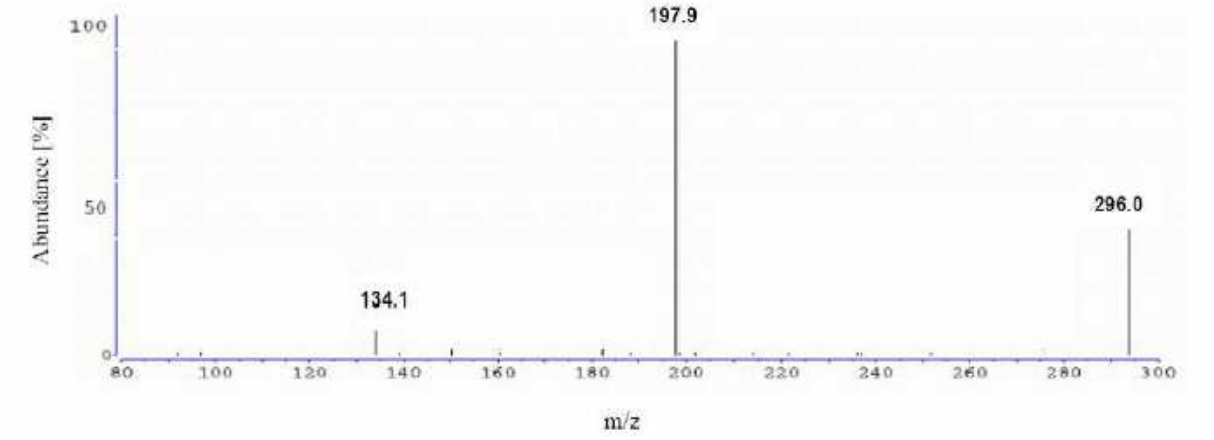
b)

Figure-2.38: (a) LC-ESI/MS spectrum of DCF and (b) the MS/MS product ion spectrum of the $[M-H]^-$ at m/z 294 (Concentration of analyte: 1 mg/L in methanol)





a)



b)

Figure-2.39: (a) LC-ESI/MS spectrum of SFM-Ac and (b) the MS/MS product ion spectrum of the $[M+H]^+$ at m/z 296 (Concentration of analyte: 1 mg/L in methanol)

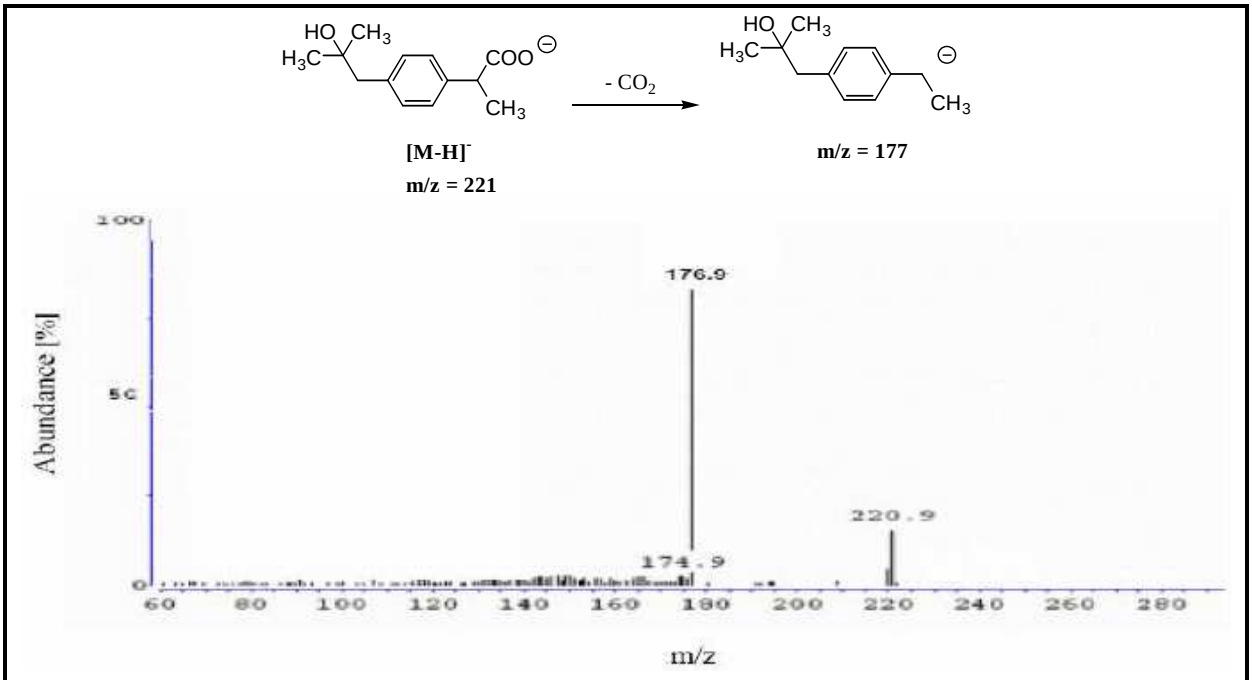


Figure-2.40: (a) LC-ESI/MS spectrum of IBU and the MS/MS product ion spectrum of the $[M-H]^-$ at m/z 221 (Concentration of analyte: 1 mg/L in methanol)

The extraction steps by SL-4-BM end up in a solution of 0.1 mol/L NaCl. NaCl is reported potentially to cause signal suppression during the ESI process [159]. It was experienced that a salt film built up quickly on the spray needle and curtain plate, resulting in a decrease in signal intensity and stability of the mass spectra, when the analyte sample contained NaCl. Therefore, it was decided to avoid NaCl (see section 3.2.5.3).

Mobile phase: in HPLC, the pH-value of the samples or the mobile phases can have a great influence in the retention time for the analytes having an acidic group (IBU-OH, SFM-Ac, DCF and IBU). In all cases, a comparison has to be made between the improvement of separation and deterioration of the ionization process. Therefore, many mobile phase compositions with different pH values were studies to achieve suitable chromatographic separation and a stable ionization spray. Methanol is a better protic solvent and generally produces more ions than acetonitrile. Whereas, acetonitril lead to better HPLC separation and a lower column back pressure. Initially, both acetonitrile and methanol were tested as organic mobile phase for LC separation. Different methods have been tested to find out the better separation and resolution of IBU-2OH, SFM-Ac, DCF and IBU. Due to the widely varying properties of these pharmaceuticals, no single method was capable of measuring all of these

compounds. Hence, further work is needed to optimize the mass spectrometer parameters and to acquire best separation and resolution of the selected analytes by LC-ESI-MS.

2.6 Conclusions and outlook

Residues of pharmaceutical products reaches wastewater treatment plants via human and veterinary urinary or fecal excretion and from pharmaceutical manufacturing discharges. Wastewater treatment plants influent constituents have to face a complex mixture of various organic and inorganic substances and detailed information on potential wastewater composition are often scarce. Pharmaceutical compounds are not totally eliminated in the wastewater treatment plants and due to this fact, variable concentrations of pharmaceutical drugs and their metabolites can reach surface, ground water and exceptionally drinking water.

The concentrations of drugs and metabolites, which have been detected in aquatic environment, are in the range from ng to µg/L and this level would be sufficient to induce estrogenic response and cause reproductive and development effects in wildlife. Consequently, there is an urgent need to improve the techniques for water and wastewater and to develop sensitive analytical methods for monitoring the drugs and metabolites input into the aquatic environment. The analytical techniques usually used such as HPLC-UV-MS or GC-MS still afford an efficient sample pretreatment to enrich and separate the analytes from complex matrix. The aim of this study was to investigate the applicability of certain types of liquid membranes to extract efficiently selected drugs of environmental concern and some of their metabolites from water samples.

For this purpose certain types of liquid membranes were used such as: bulk liquid membranes (BLM) and supported liquid membranes (SLM).

The metabolites 10,11-dihydroxycarbamazepine (CBZ-DiOH), 4′-hydroxydiclofenac (DCF-4OH), 4-hydroxyibuprofen, (IBU-2OH), N-4-acetylsulfamethoxazole, (SFM-Ac), sulfamethoxazol-N1-glucuronide (SFM-Glu) and their parent drugs carbamazepine (CBZ), diclofenac (DCF), ibuprofen, (IBU) and sulfamethoxazole (SFM) were selected due to their high quantity applied in medicine and their relative high concentrations found in the aquatic environment in previous studies. Except 4′-hydroxydiclofenac (DCF-4OH), the metabolites could be synthesized and characterized by IR, ^{1}H-, ^{13}C-NMR and mass spectroscopy as they are not commercially available.

The transport of the analytes in membrane chamber was monitored by HPLC-UV detection. Aliquots were taken from the liquid phases (feed or strip) at intervals by means of a micro-

liter syringe. The validation was achieved by analyzing the detection system parameters: linearity, sensitivity, limit of detection, and limit of quantitation

The mass transfer of metabolites through the liquid membranes was carried out in a three-compartment transport cell and various supported liquid membrane-chambers. The three-phase liquid bulk membrane system (BLM) consisted of an aqueous feed solution, an organic solvent like: 1-pentanol, dihexyl ether (DHE), undecane and decane with and without dissolved tri-*n*-octylphosphine oxide (TOPO) as a liquid bulk membrane and an aqueous stripping solution. The transport of metabolites shows some differences, which can be attributed to their acid/basic-behavior and their partition coefficients Log *P* (octanol/ water).

The supported liquid flat-membrane system (SL-FM) has been also used to extract the metabolites. SL-FM based on a porous polypropylene membrane (PP) flat sheet impregnated with a water-immiscible organic membrane phase placed between two PTFE-chambers of equal size, one filled with feed sample solution and another filled with the strip phase. The feed solution was stirred by a magnetic stirrer. Certain membrane conditions used for bulk liquid membrane were selected to test their applicability in SL-FM system, with different concentrations of TOPO as a carrier.

By means of the SL-FM-chamber 4-hydroxyibuprofen, (IBU-2OH), and N-4-acetylsulfamethoxazole, (SFM-Ac) were efficiently extracted by combining an organic solvent (DHE, decane, undecane with TOPO as a carrier), and a pH-gradient between feed and strip phases. Maximum extraction yields (~ 90 %) of IBU-2OH and (~ 85 %) of SFM-Ac were obtained by using DHE with 10 % (w/w) of TOPO.

A SL-bag membrane (SL-BM) has been developed as a miniaturized sample enrichment tool for analytical purposes. The SL-BM device consists of PP-bag soaked with the liquid membrane and filled with 0.3 - 0.6 mL of 0.1 mol/L NaOH, as the strip phase placed in 500 mL of the aqueous, acidified sample, containing the analytes of drug traces dissolved. A magnetic stir bar used to agitate the sample solution during the extraction. Several factors affecting the extraction efficiency during SL-BM enrichment were studied. These factors can be *structure* or *operator dependent*. It can be concluded that from the obtained results, the most effective conditions for membrane transport are: 10 % (w/w) TOPO as carrier dissolved in DHE, low pH of the feed phase (0.1 mol/L HCl), high pH of the strip phase (0.1 mol/L NaOH), a 4-bag membrane system, and 4 hours of extraction to create a driving force of the process which sufficient by produces a high mass transfer of IBU-2OH and SFM-Ac.

SL-4-BM system was used to enrich and determine the target metabolites from water samples by means of HPLC-UV. High enrichment factors (558 for IBU-2OH and 727 for SFM-Ac) with an extraction efficiency of 45 % for IBU-2OH and 58 % for SFM-Ac were achieved in a concentration range from 1-100 µg/L.

DCF and IBU were also separated and enriched by using the SL-4-BM device with DHE and 10 % (w/w) of TOPO as a liquid membrane. The extraction efficiency of these compounds was 27 % for DCF and 46 % for IBU with an enrichment factor of 383 for DCF and 658 for IBU at concentration range from 1-100 µg/L in 500 mL sample volume.

Solid phase extraction (SPE) was applied to extract IBU-2OH, SFM-Ac, DCF and IBU from water samples. The achieved recoveries with SPE (70 % IBU-2OH, 71 % SFM-Ac, 75 % DCF and 81 % IBU) were higher than SL-4-BM (45 % IBU-2OH, 58 % SFM-Ac, 30 % DCF and 52 % IBU), but the novel SL-4-BM offer more advantages due to higher enrichment factors, efficient clean-up-effects, low consume of organic solvents and time.

To prove if the SL-4-BM is robust against interferences, parameters**,** e.g. sample concentration and matrix effect were studied. It can be recognized that neither an increasing sample concentration nor the matrixes loaded (distilled or tap water) had a noticeable influence in the extraction recoveries of selected analytes.

Finally the SL-4-BM method was applied to real aqueous samples from the river Ruhr. No target analytes have been detected and the surface water sample was evidently not polluted.

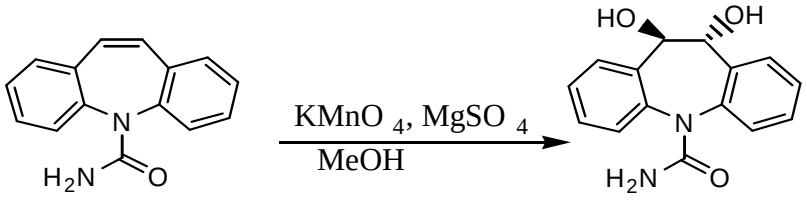
These results are encouraging in terms of improving the capability of the SL-4-BM system to extract polar pharmaceuticals from water samples, by choosing adequate liquid membrane and operating parameters. More work is required to fully understanding the extraction process of the analytes cross the membrane, and with this, the process may be important in future analytical chemistry for isolation or pre-concentration. Especially in miniaturized analytical systems, this new concept may have future.

3 Experimental

3.1 Synthesis of drug metabolites

3.1.1 CBZ-DiOH

CBZ-DiOH was successfully prepared by using the oxidation procedure of carbamazepine with potassium permanganate as shown in Scheme-3.1. The product of CBZ-DiOH purified by column chromatography gave white crystals with yield 20 % and m.p. 244-246 ^{o}C [117, 118].



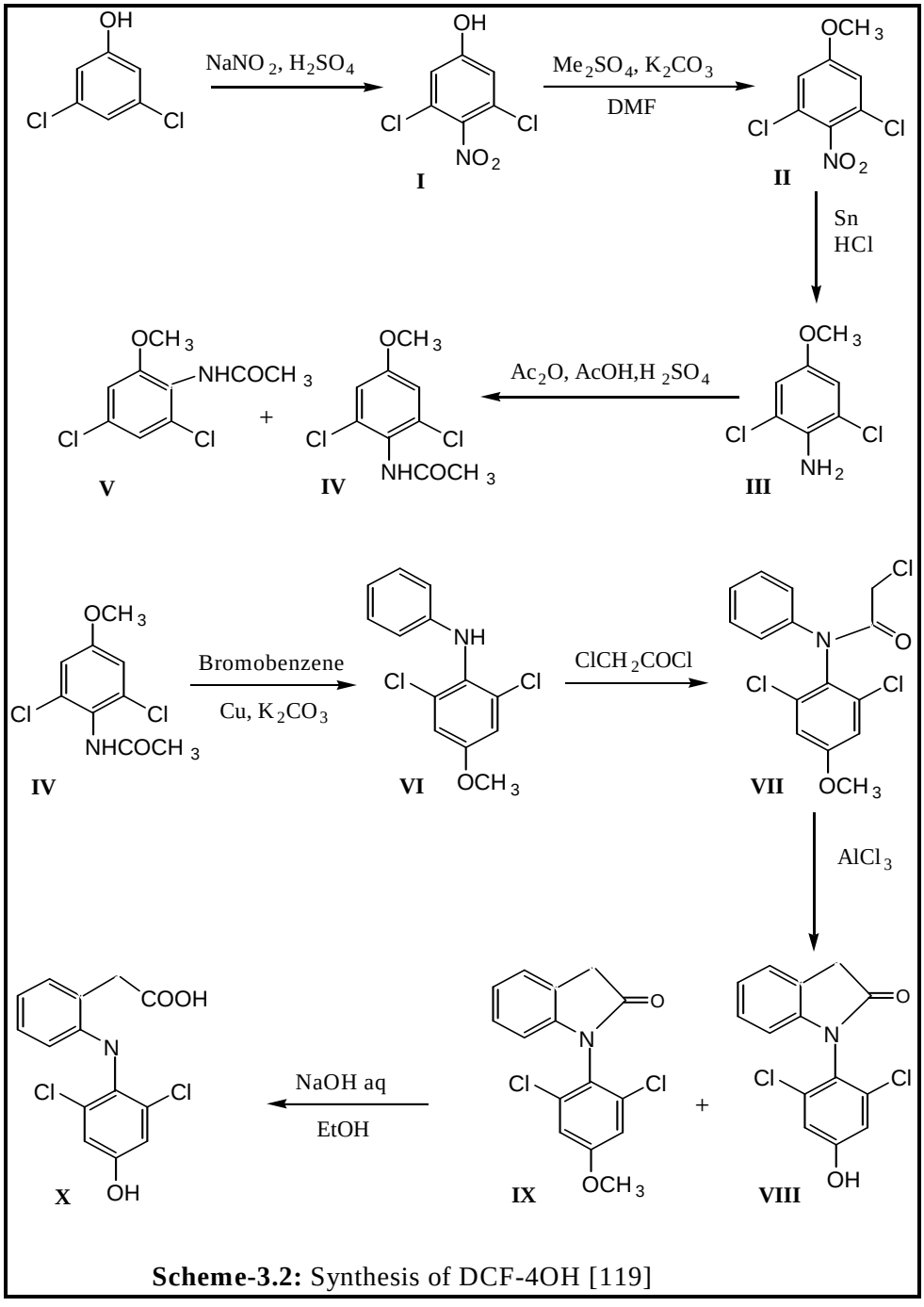
Scheme-3.1: Synthesis of CBZ-DiOH

(0.004 mole) of carbamazepine was dissolved in dry methanol (15.2 mL). The mixture was stirred and cooled at (-2 to 0 ^{o}C). A solution of $KMnO_4$ (0.003 mole), $MgSO_4$ (0.5 g) and (15 mL) of water was added drop wise to the main solution of carbamazepine with methanol. The resulting mixture was stirred at (5 ^{o}C) for (3 hours), then leaved to be at room temperature and filtered under vacuo. The product was collected on a filter and washed with MeOH, acetone, and CH_2Cl_2, then dried under vacuo at (40 ^{o}C) to constant weight. Finally the product was purified by chromatography over silica gel eluted with CH_2Cl_2 MeOH (9:1). The 10,11-dihydroxy-carbamazepine was obtained as a white solid with yield (20 %) and m.p. (244- 246 ^{o}C) (literature data [134]: m.p. 247 ^{o}C)

3.1.2 DCF-4OH

The procedure was contained a multistep as shown in Scheme-3.2 [119]. Starting with 3,5-dichlorophenol which treated with sodium nitrite in sulphuric acid gave the required nitro compound (I) together with the *o*-nitro product. The isomeric nitro compounds were not easily separate hence; the mixture was methylated with metallic tin and then acetylated. The regioisomeric acetanilides were readily separated chromatographically to give the desired isomer of acetamide compound (IV) as a mauve solid, m.p. 179-182 ^{o}C, in 12% overall yield from first compound (3,5-dichlorophenol). Copper-mediated N-arylation of acetamide with bromobenzene proceeded smoothly to give, after basic hydrolysis, an excellent yield of

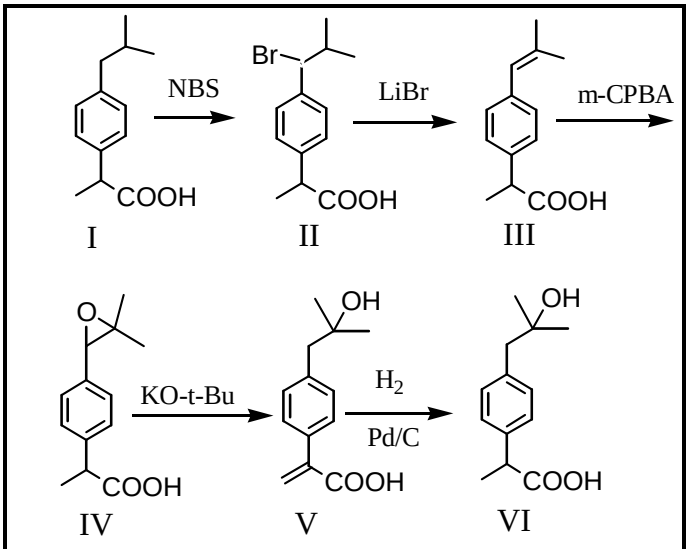
diphenylamine (VI). Acylation of amine with chloroacetyl chloride yielded crude chloroamide (VII) as a purple solid, which was then cyclised with alumimium chloride. The reaction conditions for this cyclisation were so critical; and gave a mixture of indolone (VIII) and ether form (IX) together with a more polar impurity. The mixture was not separable. Repeating of these steps gave the same problem; therefore we decided to stop the synthesis steps for this compound.



Scheme-3.2: Synthesis of DCF-4OH [119]

3.1.3 IBU-2OH

The compound was prepared in a multistep procedure starting with IBU, which was converted to 2-(*p*-(1-bromo-2-methylpropyl)-phenyl)propionic acid (I) with *N*-bromosuccinimide. Then lithium bromide dissolved in dimethylformamide was reacted with (I) (see Scheme-3.3) to achieve 2-(p-(2-methylprop-l-ene)phenyl)propionic acid (II). Compound (II) was converted with m-chloroperbenzoic acid to 2-(*p*-(2-methyl-1,2-epoxypropyl)phenyl)propionic acid (III). In the next step, product (III) gave 2-(p-(2-methyl-2-hydroxypropyl)phenyl)propenoic acid (IV) by the action of potassium *tert*-butoxide in tetrahydrofuran under nitrogen and final quenching by dilute HCl. Finally, compound (IV) was hydrogenated in tetrahydrofuran under 50 psi of H_2 at room temperature in the presence of 10 % (w/w) Pd/C, yielding the final 2-(p-(2-methyl-2-hydroxypropyl)phenyl)propionic acid (IBU-2OH) as shown in scheme-6.3. The solid product of IBU-2OH was filtered with fresh cyclohexane, and dried to constant weight at 50 ^{o}C in vacuo which gave white crystal with yield 45 % and m.p. 122-124 ^{o}C [120-122].



Scheme-3.3: The synthesis steps of IBU-2OH [120]

2-(*p*-(1-Bromo-2-methylpropyl)-phenyl)propionic acid (II)

A solution of (1.16 mole) of ibuprofen (I), and (2.5 L) of CCl_4 was refluxed for (10 min) under nitrogen, cooled to room temperature, and the treated with (1.06 mole) of *N*-bromosuccinimide and 300 mg of benzoyl peroxide. The mixture was refluxed for (6 hours), stirred overnight at room temperature, and filtered. The filtrate was concentrated to a reddish-brown oil which was diluted with (1.5 L) of hexane to give crystals. The product was collected on a filter and washed four times with (200 mL) portions of hexane and then dried to constant weight to afford 185 g of (II), m.p. 112-117 ^{0}C.

2-(*p*-(2-Methylprop-l-ene)phenyl)propionic acid (III)

A solution of (0.35 mole) of 2-(*p*-(1-Bromo-2-methylpropyl)-phenyl)propionic Acid (II), (0.83 mole) of lithium bromide, and (1.5 L) of DMF was heated under nitrogen between (80-100 ^{0}C) for (5 hours). The solution was cooled to room temperature, diluted with (5 L) of water and extracted with three (1 L) portions of ether. The ether extracts were combined and washed with two (1 L) portions of water and (500 mL) of brine. After the solution was dried over $MgSO_4$, the ether was removed in vacuo to give 73 g (100 %) as yellow oil.

2-(*p*-(2-Methyl-1,2-epoxypropyl)phenyl)propionic acid (IV)

To a well-stirred solution of (0.35 mole) of 2-(*p*-(2-Methylprop-l-ene)phenyl)propionic Acid (III), in (750 mL) of CH_2Cl_2 at room temperature under nitrogen was added a slurry of (0.37 mole, 85 % quality) of *m*-chloroperbenzoic acid in (700 mL) of CH_2Cl_2. The reaction was slightly exothermic and was maintained below (35 ^{o}C) with a water bath. The mixture was reduced to (500 mL) in vacuo. Upon dilution with an equal volume of hexane, *m*-chlorobenzoic acid (*m*-CBA) precipitated. The solids were filtered and washed with hexane to dissolve any 2-(*p*-(2-Methyl-1,2-epoxypropyl)phenyl)propionic Acid (IV). The filtrate was concentrated to an oil and again diluted with (500 mL) of hexane. More *m*-chlorobenzoic acid was removed by filtration. The filtrate was concentrated to oil which was used in the next step without further purification.

2-(*p*-(2-Methyl-2-hydroxypropyl)phenyl)propenoic acid (V)

To a solution of 2-(*p*-(2-Methyl-1,2-epoxypropyl)phenyl)propionic Acid (IV), obtained in the previous steo(0,3 mole) in (1 L) THF at (15 ^{0}C) under nitrogen was added dropwise over (2 hours) (550 mL) of (20 %) potassium *tert*-butoxid in THF. As the pH became neutral, a milky slurry resulted, and as the pH became basic an orange mixture was observed. After disappearance of starting material the reaction was quenched by the dropwise addition of (1 N) HCl over a (20 min) period at (15 ^{o}C). The two-phase, acidic system was diluted with (700 ml) of the aqueous phase was removed. The organic phase was washed twice with (1 L) of water and once with (500 mL) of saturated sodium chloride and dried over $MgSO_4$.The mixture was filtered, and the filtrate concentrated to a yellow oil. The oil was azeotroped with (500 mL) of cyclohexane and then slurried with another (500 mL) of cyclohexane until crystallization occurred. The solids were filtered, washed twice with fresh cyclohexane, and dried to constant weight to give (52.4 g) as a first crop and (7.3 g) as a second crop. The yield

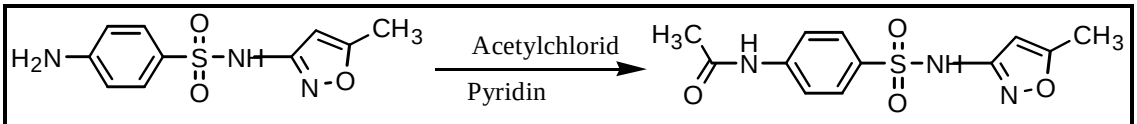
from (III) to (V) was (78 %). Recrystallization of (50 g) of (V) from acetone/cyclohexane afforded (30.9 g) of material, m.p. (110-114 ^{o}C).

2-(*p*-(2-Methyl-2-hydroxypropyl)phenyl)propionic acid (VI)

A mixture of (0.12 mole) of 2-(*p*-(2-Methyl-2-hydroxypropyl)phenyl)propenoic Acid (V), (250 mL) of THF, and (1 g) of 10 % Pd/C was placed in a Parr hydrogenation apparatus and reduced under 50 psi of H_2 at room temperature for (1.5 hour). The resulting mixture was filtered through a Celite pad, and the filtrate concentrated to yellow oil. The oil was slurried in (400 mL) of cyclohexane for a few minutes and crystallization of a white solid was observed. The solids were filtered, washed with fresh cyclohexane, and dried to constant weight at (50 ^{o}C) in vacuo to give (27.2 g) with yield was (80 %) of 2-(*p*-(2-Methyl-2-hydroxypropyl)phenyl)propionic acid (VI), and m.p. (122-124 ^{o}C) (literature data [128]: m.p. 122 ^{o}C).

3.1.4 SFM-Ac

SFM was reacted with acetylchloride in pyridine as described in Scheme-3.4. Recrystallisation from acetonitrile yield the title compound (65 %) as a yellow amorphous solid m.p. (205-210 ^{o}C) [123-125].



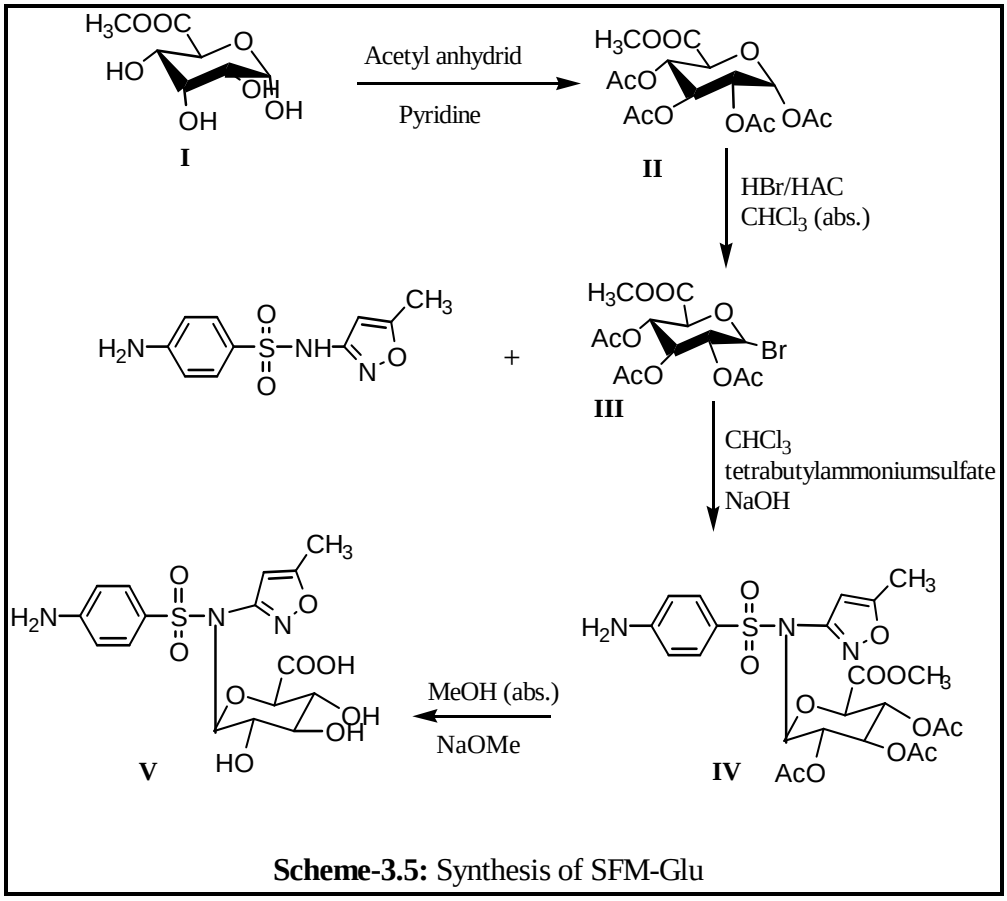
Scheme-3.4: Synthesis of SFM-Ac

To a stirred solution of sulfamethoxazole (0.1 mole) in pyridine (300 mL) was added dropwise acetylchloride (0.1 mole) at (0-5 ^{o}C). The reaction mixture was stirred for 8 hours at room temperature; the solution was concentrated to about (40 mL) and poured into excessive water. The precipitate was washed with (1 M) hydrochloric acid and water successively, and then dried to constant weight. Recrystallisation from acetonitrile yield the title compound (65 %) as a yellow amorphous solid m.p. (205-210 ^{o}C) (literature data [133]: m.p. 207 ^{o}C).

3.1.5 SFM-Glu

Initially, SFM was coupled in a 2-phase system (aqueous dilute NaOH, chloroform) with methyl-2,3,4-tri-O-acetyl-α-bromoglucuronate in presence of tetrabutylammonium

hydrogensulfate. As shown in Scheme-3.5, the resulting sulfamethoxazole-N-(methyl-tri-O-acetyl-β-D-glucuronide) was converted to the final SFM-Glu by the action of sodium methoxide and careful neutralization by means of carbon dioxide [125, 126-129].



Scheme-3.5: Synthesis of SFM-Glu

β-Tetraacetylglucuronic acid methyl ester (II)

(3.2 g) of the methyl ester glucuronic acid (I) were dissolved in (12 mL) of pyridine and (8 mL) of acetic anhydride at (0 ^{o}C). The mixture was allowed to stand for (3 hours) in ice bath. The solvent was evaporated by distillation in vacuo until crystals of β-tetraacetylglucuronic acid methyl ester separated. The mixture was cooled to (0 ^{o}C) and filtered. The crystals were washed with cold absolute ethanol and ether [136].

Methyl 2,3,4-tri-O-acetyl-α-bromoglucuronate (III)

(0.133 mole) of methyl β-tetraacetylglucuronic acid methyl ester (II) was dissolved in (200 mL) of (30 %) hydrobronic acid in acetic acid and the mixture, after solution, allowed to stand in the refrigerator overnight. Solvent was removed under reduced pressure and the residue dissolved in (100 mL) of chloroform The chloroform layer was separated and washed twice with aqueous sodium hydroxide (1.25 M, 3 mL) and dried (with sodium sulphate). The

solvent was removed under reduced pressure. Recrystallization from ethanol yielded the product (85 %) [137, 138].

Sulfamethoxazol-N-(methyl-tri-O-acetyl-β-D-glucuronide) (IV)

By dissolving sulphamethoxazole (0.002 mole) and benzyltriethylammonium bromide (1 mole) in aqueous sodium hydroxide (1.25 M, 2 mL). The resulting solution was added to a solution of methyl 2,3,4-tri-O-acetyl-α-bromoglucuronate(0.001 mole) in chloroform (5 mL). The resulting mixture was stirred vigorously and heated under reflux (3 hours). After cooling water (5 mL) was added. The chloroform layer was separated and washed twice with aqueous sodium hydroxide (1.25 M, 3 mL) and dried (with sodium sulphate). The solvent was removed yielding a yellow amorphous solid. Recrystallization from ethanol yielded the product (64 %) [139].

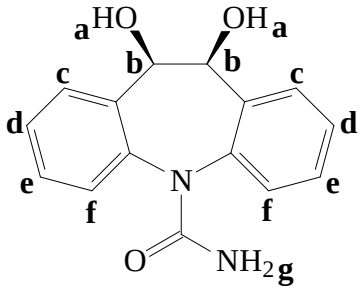
Sulfamethoxazol-N1-glucuronide (V)

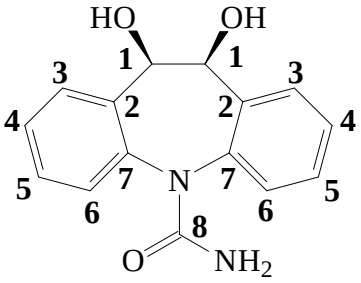
(0.001 mole) of sulphamethoxazol-N-(methyl-tri-O-acetyl-β-D-glucuronide) (IV) was dissolved in dry methanol (5 mL) and sodium methoxide. The mixture stirred at room temperature for (2 hours) and then condensed to (1 mL). To the solution was added aqueous sodium hydroxide (1 M, 2 mL) and the resulting mixture was stirred at room temperature for (2 hours). The solution was adjusted to (pH 3). After filtration, the solution was removed yielding a pale yellow solid. Recrystallization from ethanol yielded the product (15 %) [139] and m.p. (112-114 ^{o}C) (literature data [140]: m.p. 109 ^{o}C).

3.1.6 Characteristic data of drug metabolites

3.1.6.1 CBZ-DiOH

1- Molecular Formula: $C_{15}H_{14}N_2O_3$





2- ^{1}H-NMR, and ^{13}C-NMR data:

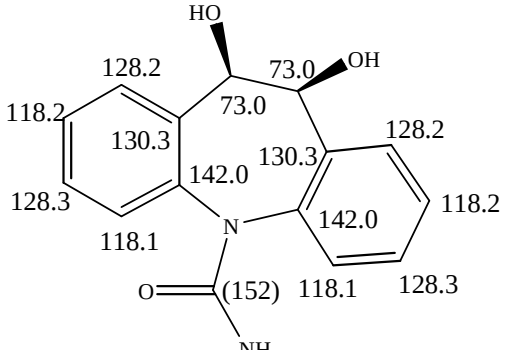
Table-3.1:^{1}H-NMR (D_6-DMSO)

H-Atom	cis-Diol (Lit. [01]) δ [ppm]	cis-Diol (Synthese) δ [ppm]	trans-Diol (Lit. [01]) δ [ppm]
B	5.01 (d, J= 4.3Hz, 2H)^{A}	5.01 (d, J= 4.8, 2H)	4.02 – 5.02 (very br., 2H)
A	5.37 (d, J= 4.3Hz, 2H)^{B}	5.43 (d, J= 5.3^{C}, 2H)	5.74 (d, J= 4.2, 2H)^{B}
G	5.73 (br, s, 2H)^{B}	5.76 (br, s, 2H)	5.81 (br, s, 2H)^{B}
C – f	7.20 – 7.60 (m, 8H)	7.32 – 7.23 (m, 6H$_{c-e}$)	7.15 – 7.60 (m, 8H)
		7.49 (dd, 2H$_f$)	

^{A}~Becomes s after exchange with D_2O
^{B}~Exchangeable with D_2O
^{C}~Resolution of the NMR-Device 0.5 Hz

Table-3.2: ^{13}C-NMR (D_6-DMSO)

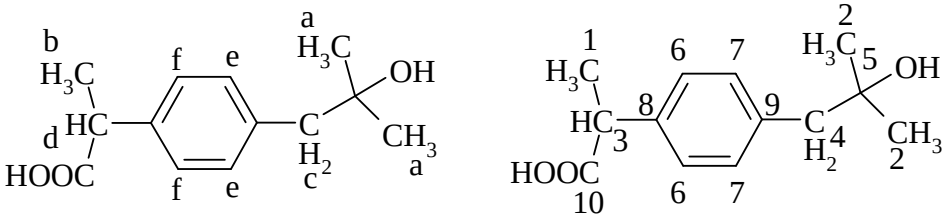
C-Atom	cis-Diol δ [ppm]
1	71.06
2 – 7	127.15 – 130.3
8	So difficult to detected



3- IR-data:
3550 – 3200 cm^{-1} (s, OH, NH_2), 1690cm^{-1} (s, C=O)

3.1.6.2 IBU-2OH

1- Molecular Formula: $C_{13}H_{18}O_3$



2- ^{1}H-NMR, and ^{13}C-NMR data:

Table- 3.3: ^{1}H-NMR and ^{13}C-NMR data:

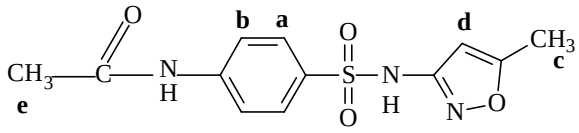
H-Atom	Δ [ppm]	δ* [ppm]	Multiplicity	number H	C-Atom	δ [ppm]	Number C
A	1.25	1.2	S	6	1	18.13	1
B	1.53	1.5	D	3	2	29.13	2
C	2.77	2.7	S	2	3	44.95	1
D	3.74	3.7	Q	1	4	49.26	1
E	7.19	7.1-7.3	d/m (4 H)*	2	5	70.99	1
F	7.28		D	2	6	127.43	2
					7	130.78	2
					8	136.82	1
					9	138.04	1
					10	180.10	1

3- IR-data:

3402 cm^{-1} s, (OH) 2978 cm^{-1}, 2923 cm^{-1} s, (C-H) 1694 cm^{-1} s, (C=O)

3.1.6.3 SFM-Ac

1- Molecular Formula: $C_{12}H_{13}N_3O_4S$



2- ^{1}H-NMR, and ^{13}C-NMR data:

Table-3.4: ^{1}H-NMR data

^{1}H-Atome	δ [ppm]	J [Hz]	J [Hz]*	Δ [ppm]*
2H, d, H_a-$H_{a'}$	7.53	J_{ab}= 8.9	7.52	J_{ab}= 8.8
2H, d, H_b-$H_{b'}$	6.76	J_{ab}= 8.9	6.61	J_{ab}= 8.8
1H, s, H_d	6.19		6.08	
3H, s, H_c	2.40		2.39	
3H, s, H_e	2.1		2.15	

- Literature data

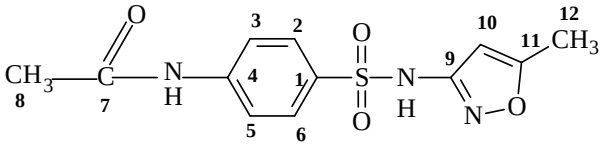


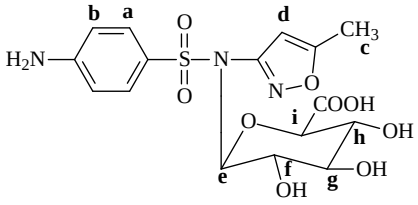
Table-3.5: ^{13}C-NMR data

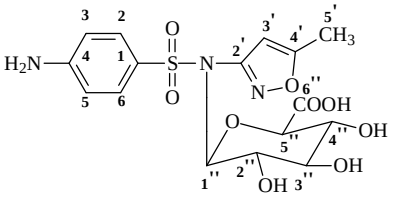
^{13}C-Atome	Δ [ppm]	δ [ppm]*
Aromat		
C_1	156.7	156.8
C_4	171.0	170.3
C_2+C_6	130.3	129.9
C_3+C_5	114.5	112.7
Isoxazol		
C_9	123.7	123.7
C_{10}	86.6	86.9
C_{11}	153.2	152.6
C_{12}	11.9	11.8
Acetyl		
C_7	158	160
C_8	24.5	27.9

* Literature data

3.1.6.4 SFM-Glu

1- Molecular Formula: $C_{16}H_{19}N_3O_9S$





2- ^{1}H-NMR, and ^{13}C-NMR data:

Table-3.6: ^{1}H-NMR data

^{1}H-Atome	**δ [ppm]**	**J [Hz]**	**J [Hz]***	**δ [ppm]***
2H, d, H_a-$H_{a'}$	7.53	J_{ab}= 8.9	7.52	J_{ab}= 8.8
2H, d, H_b-$H_{b'}$	6.76	J_{ab}= 8.9	6.61	J_{ab}= 8.8
1H, s, H_d	6.19		6.08	
1H, d, H_i	5.25	J_{hi}= 9.3	5.25	J_{hi}= 9.2
1H, d, H_e	3.76	J_{ef}= 9.9	3.91	J_{ef}= 9.4
1H, t*, H_g	3.55	J_{fg}= 9.1, J_{gh}=9.1	3.54	J_{fg}= 9.0, J_{gh}=9.0
1H, t*, H_f	3.36	J_{fg}= 9.4, J_{ef}= 9.6	3.47	J_{fg}= 9.0, J_{ef}= 9.4
1H, t*, H_h	3.21	J_{gh}= 9.1, J_{hi}= 9.1	3.21	J_{gh}= 9.0, J_{hi}= 9.2
3H, s, H_c	2.37		2.39	

Table-3.7: ^{13}H-NMR data

^{13}C-Atome	**Δ [ppm]**	**δ [ppm]***
Aromat		
C_1	156.7	156.8
C_4	171.0	170.3
C_2+C_6	130.3	129.9
C_3+C_5	114.5	112.7
Isoxazol		
$C_{2'}$	123.7	123.7
$C_{3'}$	86.6	86.9
$C_{4'}$	153.2	152.6
$C_{5'}$	11.9	11.8
Glucuronid		
$C_{1''}$	102.5	102.3
$C_{2''}$	76.7	76.4
$C_{3''}$	76.1	76.3
$C_{4''}$	71.4	70.6
$C_{5''}$	69.6	69.4
$C_{6''}$	173.2	170.9

Further information by GC/MS and LC/MS data for all these metabolites see [80].

3.2 Liquid membrane Extraction

3.2.1 Development of HPLC-UV method

The transport of analytes was monitored by HPLC and UV-detection. Aliquots were taken from the liquid phase (feed or strip) at intervals by means of a micro-liter syringe. The HPLC-UV development methods for metabolites and active drugs are described in chromatogram (Figure-7.1). The stock solutions of metabolites and active drugs were prepared by dissolving an appropriate amount of the drugs in methanol. The external calibration curve were built from 8 concentrations (n = 3) in a concentration range 0.5- 10 mg/L obtained by diluting of aliquot of appropriate stock solution with 0.1 mol/L NaCl.

The analytes were introduced into the chromatographic system by an autosampler connected with UV-Vis Detector. Detection was performed at 225 nm. The analytical column used was LichroCART RP 18. Deferent mobile phases were tested to determine the calibration curve of metabolites. The best mobile phase was consisted a mixture of 8.295 mmol/L KH_2PO_4: acetonitrile 80:20 (v/v). The flow rate of the mobile phase was 1.0 mL/min. Retention data (R_t) of analytes: SFM-Glu.:3.57 min, CBZ-DiOH: 5.33 min, IBU-2OH: 17.88 min, SFM-Ac: 20.43 min.

For the active drug, the best mobile phase was consisted a mixture of 0.6 mmol/L NaH_2PO_4: acetonitrile 50:50 (v/v). The flow rate of the mobile phase was 1.0 mL/min. Retention data (R_t) of analytes: SFM: 3.55 min, CBZ: 4.60 min, DCF: 6.34 min, IBU: 7.73 min.

In addition the LC-MS/MS system LCQ Advantage in ESI-mode (Thermo Finnigan, Egelsbach, Germany), connected with a gradient pump (SpectraSYSTEM P4000) was employed for metabolite trace analysis. The separation was performed on a Nucleosil 100 C_{18} ec-column (5 µm, 250 x 5 mm i.d., Macherey-Nagel, Düren, Germany).

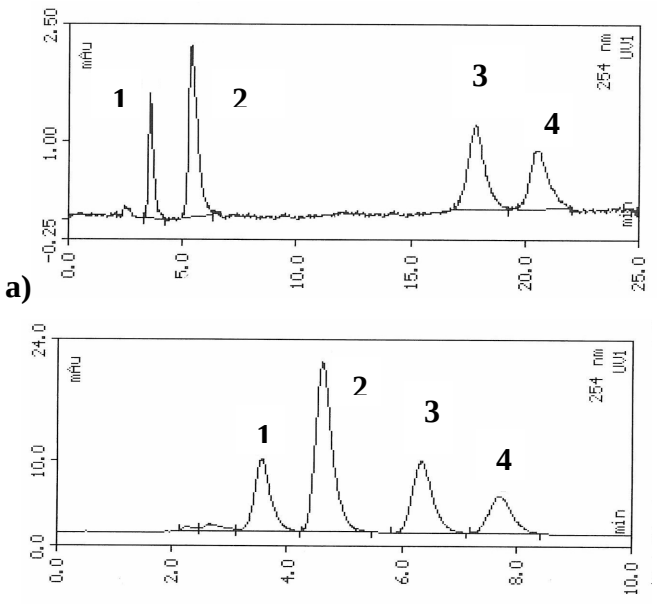


Figure-3.1: HPLC-UV chromatogram for the drug metabolites and active drugs
a) 1. SFM-Glu, 2. CBZ-DiOH, 3. IBU-2OH and 4. SFM-Ac,
b) 1. SFM, 2. CBZ, 3. DCF and 4. IBU
The stock solutions of active and metabolite drugs were prepared by dissolving an appropriate amount of the drugs in methanol. HPLC separation was achieved with LichroCART RP 18 (5µm, 250 x 4 mm; Merk) column at wavelength of 225 nm

3.2.2 Validation of HPLC-UV method

Method validation has received considerable attention in literature, from industrial committees and regulatory agencies. In recent years an increasing number of laboratories have applied for accreditation according to EN 45000 series. Quality management and quality assurance systems according to ISO 9000ff are now certified in analytical laboratories; therefore, the validation of HPLC-UV was based on the methods of analytical quality assurance (AQA), which have many possible solutions for routine practice analysis by providing quality data. The validation characteristics of analytical quality assurance methods are:

- The range tested
- The coefficients of the calibration function:
- in the case of a first order calibration function ($y = a + bx$): axis intercept a and slop b (characteristic of sensitivity of the analytical procedure);

- in the case of a second order calibration function($y = a + bx + cx^2$): axis intercept a, coefficient b of the linear term, as the coefficient c of the quadratic term, the sensitivity E of the analytical process determined from the function;
- the standard deviation of the procedure S_{xo} as an absolute measure of precision for the calibration and;
- the process variation coefficient V_{xo} as a relative measure of precision.
- In addition, the general evaluation of the analytical process also documents the:
- Limit of detection LOD
- Limit of quantitation LOQ

1. Sample preparation

During the preparation of the standard samples we tried to concern that:

- The precision of the balance and the volumetric equipment have been taken into account.
- No successive dilution since during the preparation of standard samples

The stock solutions of drug metabolites (SFM-Glu, CBZ-2OH, IBU-OH, and SFM-Ac) were prepared at 1 mg/mL in methanol. Appropriate dilutions of stock were made with 0.1 mol/L NaCl to prepare of standard solutions for calibration at concentrations of 0.025, 0.05, 0.1, 0.5, 1, 2, 3, 4 and 5 mg/L.

2. Fundamental calibration

The preliminary first and second-degree calibration functions are calculated from the measured of these standard samples and listed in Tables-3.9 and 3.9.

Table-3.8: First order calibration function

Compound	Calibration function first order	A	B	R^2	S_y
SFM-Glu	0.4446x + 0.026	0.026	0.4446	0.9986	0.0341
CBZ-DiOH	1.1363x – 0.0275	- 0.0275	1.1363	0.9993	0.0645
IBU-2OH	1.0307x - 0,0358	- 0.0358	1.0307	0.9995	0.0490
SFM-Ac	-0.0105 + 0.6806x	- 0.0038	0.6806	0.9997	0.0310

Table-3.9: Second order calibration function

Compound	Calibration function second order	A	b	C	R^2	S_y
SFM-Glu	$0.0069x^2 + 0.4148x + 0.0341$	0.0341	0.4148	0.0069	0.999	0.0361
CBZ-DiOH	$0.0066x^2 + 1.1054x + 0.0179$	- 0.0179	1.1054	0.0066	0.999	0.0680
IBU-2OH	$0.0025x^2 + 1.0264x + 0.0309$	- 0.0309	1.0264	0.0025	0.999	0.0532
SFM-Ac	$0.0044x^2 + 0.6598x + 0.0065$	0.0065	0.6598	0.0044	0.999	0.0316

3. Sensitivity

The measure of sensitivity results from the change in the measured value caused by a change in the concentration values. If the calibration function for an analytical procedure is linear, then the sensitivity is constant over the entire range and is equivalent to the regression coefficient *b*. From the sensitivity the process of standard deviation S_{xo}, and relative process of standard deviation V_{xo} can be calculate, and the results for the drug metabolites are listed in Table-3.10.

Table-3.10: The standard deviation and the coefficient of variation for HPLC-UV method

	SFM-Glu	CBZ-DiOH	IBU-2OH	SFM-Ac
S_{xo}	0.0715	0.0601	0.0523	0.0456
V_{xo} [%]	3.650	3.07	2.67	2.33

4. Linearity

a) Mandel's fitting test: The Mandel's fitting test is recommend for the mathematical verification of linearity. For this, the first order and second- order calibration function including their respective residual standards deviation S_y are used. The testing value TV is compared with the value obtained from the table $F(f_1 = 1, f_2 = N\text{-}3, P = 99\%)$ as shown in Table-3.11.

Table-3.11: Mandel's fitting test for HPLC-UV method

	SFM-Glu (N = 9)	CBZ-DiOH (N= 9)	IBU-2OH (N=9)	SFM-Ac (N=9)
DS^2	1.8×10^{-4}	9.14×10^{-4}	-6.036×10^{-4}	6.981×10^{-4}
TV	0.1381215	0.1976643	- 0.2132711	0.6990485
$F(f_1 = 1, f_2 = N\text{-}3, P = 99\%)$	3.7047	3.7047	3.7047	3.7047

b) Variance homogeneity test

The linear regression calculations described assume a constant (homogeneous) imprecision (variance of measured values) over the range. Inhomogeneity leads not only to a higher imprecision, but also to a higher inaccuracy through possible change in the linear slope. In order to verify the homogeneity of variances, n = 10 standard samples of each of the lowest (S^2_1) and the highest (S^2_N) concentrations of the preliminary range are analyzed separately. The variances of both of measurement are checked for homogeneity using an F-test. As listed in Table-3.12, the TV for the metabolites are so bigger than F , and to solve this problem we choose narrow range of concentrations for all drug metabolites from 0.5 to 5 mg/L .

Table-3.12: The homogeneity test for HPLC-UV method

	SFM-Glu	**CBZ-DiOH**	**IBU-2OH**	**SFM-Ac**
S^2_1	3.131789×10^{-5}	3.222964×10^{-5}	$4.070281746 \times 10^{-6}$	5.725999×10^{-5}
S^2_N	0.01010725	0.00625666	0.0014724	0.006018844
TV	322.7315	194.1275	361.7440	105.1143
$F(f_1 = 1, f_2 = N\text{-}3, P = 99\%)$	5.35	5.35	5.35	5.35

5. The limit of detection and limit of quantitation

The limit of detection LOD is the lowest of substance concentration that produces a response detectable above the noise level of the system. The limit of quantitation LOQ for a basic analytical process is defined as the smaller concentration of substance that can be determined using a given analytical precision. The values of LOD and LOQ for metabolites are listed in Table-3.13.

Table-3.13: The capability of detection and limit of quantitation for HPLC-UV method

	SFM-Glu	**CBZ-DiOH**	**IBU-2OH**	**SFM-Ac**
LOD [mg/L]	0.13	0.11	0.09	0.08
LOQ [mg/L]	0.35	0.33	0.27	0.24

3.2.3 BLM

Extraction procedure

As shown Figure-2.4, the three-phase system was established in a home-made glass cell equipped with an agitator (PTFE) which allows extraction and back-extraction in one unit. The cell consists of two concentric chambers dividing it into separate compartments. Thus, the feed is allowed to contact the bulk-membrane while the strip solution contacts the membrane. The three liquid phases stirred at a frequency of 60 rpm. The whole cell is covered by a fitting glass lid in order to minimize the loss of solvent by evaporation.

3.2.4 SL-FM

Extraction procedure

The SL-FM is based on a porous polypropylene membrane (pore size 0.1 μm, total thickness 90 μm, Membrana GmbH, Wuppertal, Germany) impregnated with a water-immiscible organic membrane phase containing neutral carrier (tri-*n*-octylphosphinoxid) or acidic carrier (octane sulfonic acid) dissolved in an appropriate solvent (1-pentanol, DHE, undecane and decane), which is held by capillary forces placed between two aqueous phases, the feed and the strip. The SL-FM as shown in Figure-2.15 was mounted in a window between two PTFE-chambers, one for the sample input (feed phase), one for the permeate uptake (strip phase). Both chambers were filled up to 150 ml. The size of membrane which used in every batch of extraction was (5 x 5 cm), and the time of soaking was (2 hours) in liquid membrane. The feed solution was stirred by magnetic stirrer at 360 rpm.

3.2.5 SL-BM systems

3.2.5.1 Preparation of bag membrane

Bag membranes were prepared by using the procedure developed in Paderborn University [112]. The procedure steps are:

1- Two layers (10 x 17 cm) of polypropylene (PP) membrane (pore size 0.1 μm, total thickness 90 μm) were held between two aluminum layers which designed to be identical to each other and to prepare simultaneously several as shown in Figure-3.2.

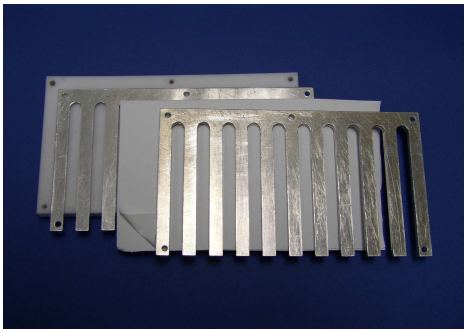


Figure-3.2: Two aluminum plates which used to prepare the bag membrane [112]

2- The aluminum plates with PP-membrane were heated and pressed by using iron plate sees Figure-3.3.

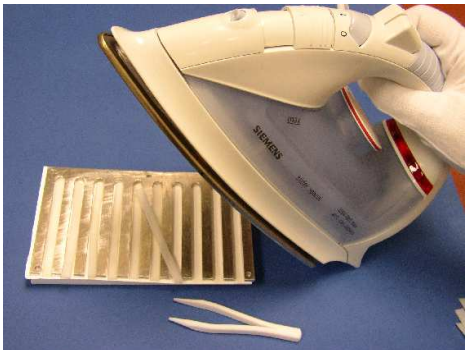


Figure-3.3: Heating and pressing with iron plate to prepare the bag membrane

3- Heating and pressing with iron plate for at least 2 minutes, the two PP-membrane layers will be melted, bended and take the shape of aluminum plate then the bag membrane can be removed from the aluminum plates.

4- After checking and washing the bag membranes were soaked in the liquid membrane for 2 hours then used for SL-BM extraction

3.2.5.2 SL-BM

Extraction procedure

Figure-2.26 illustrates the SL-BM system. The bag membrane (1) was filled with volume between 0.3- 0.6 mL of strip phase, and the beaker down (2) was filled with volume 500 ml of feed phase. The feed solution was stirred by magnetic stirrer at 360 rpm.

3.2.5.3 SL-4-BM

Extraction procedure

After preparation of bag membranes (see section 3.2.5.1), the bags were washed 3 times by distilled water and methanol and dried for 30 min at room temperature. 4 bags were soaked in liquid membrane for 2 hours. After soaking, 0.4 mL of strip solution 0.1 mol/L NaOH was injected into every bag with a microlitre syringe and the bags were subsequently placed in the sample solution (feed phase) as shown in Figure-2.36. Sample solution contained the analytes with 500 mL of 0.1 mol/L HCl or 6.5 mL of 25 % (w/w) HCl in 500 mL of real water samples: tap water from Paderborn city (see Table-3.14) and surface water from river Ruhr. A magnetic stir plate was used to agitate the sample solution during the extraction. After 4 hours of extraction, the strip solution from every bag was drawn separately and neutralized with the same volume of 0.1 mol/L HCl, and then the neutralized samples were combined in one test tub. To remove the NaCl, the sample was dried under reducing pressure and the solid residue was washed with 3 mL of absolute diethyl ether. After decantation the sample solution was transported to another test tub and dried under reducing pressure. The residue was dissolved in 0.4 mL of methanol. After filtration, the filtrate was transferred into a vial then injected to HPLC-UV analysis.

Table-3.14: Tap water quality of Paderborn city (Wasserwerk Aabach) [162]

Parameter	Dimension	Limit value	Analysis value
pH-value	….	6.50 – 9.50	7.72
Electrical conductivity (20°C)	µS/cm	2.500	315
Calcium	mg/L	…	60.0
Magnesium	mg/L	…	5.70
Sodium	mg/L	200	5.90
Potassium	mg/L	…	1.15
Iron	mg/L	0.20	< 0.01
Manganese	mg/L	0.05	< 0.005
Ammonium	mg/L	0.50	< 0.05
Nitrite	mg/L	0.50	< 0.005
Nitrate	mg/L	50.00	7.00
Chloride	mg/L	250.00	10.00
Sulfate	mg/L	240.00	31.00
Fluoride	mg/L	1.50	< 0.1
Hydrogencarbonate	mg/L	…	160
Acid capacity up to pH 4,3	mmol/L	…	2.68
Carbonate hardness	°dH	…	7.50

Parameter	Dimension	Limit value	Analysis value
Basic capacity up to pH 8,2	mmol/L	…	0.10
Free carbonic acid	mg/L	…	4.40
Water temperature	°C	…	13.7
Color (SAK 436)	1/m	0.50	0.07
Turbidity	FTU	1.0	0.05
Oxygen	mg/L	…	10
Oxidizability	mg/L	5.00	0.90
Phosphate	mg/L	6.70	< 0.01
Aluminum	mg/L	0.20	< 0.01
Lead	mg/L	0.025	< 0.001
Copper	mg/L	2.00	0.01
Total organic carbon (TOC)	…	…	1.48

3.3 SPE

Extraction procedure

As shown in Figure-2.35, the SPE cartridges were conditioned with methanol (5 mL) and distilled water (5 mL), at a flow rate of 5 mL/min. 500 mL of prepare water sample was filtrated by 0.45 μm (Nylon-Filter). Sample loading was also performed at 5 mL/min. Afterwards the cartridge was washed with 10 mL/min of water containing 5 % methanol, at 5 mL/min, and dried by pumping air through the cartridge for 15 min. Elution was performed with 0.8 mL of methanol. After one fold dilution with methanol to complete the total volume to 1 mL, the extract was injected to HPLC-UV system.

3.4 Materials, equipments and chemicals

The materials, equipments and chemicals which have been used in the present work are listed in Tables-3.15- 3.17.

Table-3.15: Materials used in this work

Material	Supplier
Polypropylene membrane (pore size 0.1 μm, total thickness 90 μm)	Membrana GmbH Wuppertal Germany
Bakerbond SPE cartridge	Merck
Isolute ENV SPE cartridge	Merck
Strata C18-E SPE cartridge	Merck
Oasis-HLB SPE cartridge	Merck
Cellulose membrane filter (0.45 μm)	Merck

Table-3.16: Equipments used in this work

Equipment	Supplier
Autosampler GINA 50	Gynkotek/ Dionex Idsten
Isocratic pump P580	Gynkotek/ Dionex Idsten
UV-Vis Detector 655 A	Merk-Hitachi
Analytical column LichroCART RP 18 (5 µm, 250 x 4 mm)	Merk
Digital-pH-Meter 766 Calimatic	Knick/Berlin
Vortex-Mixer VXR	IKA VIBRAX
Magnetic stirrers	H+P Labortechnik AG

Table-3.17: Chemicals used in the present work

Chemical	Supplier
Pyridine	Aldrich
Acetic anhydride	Fluka
Methyl ester glucuronic acid	Fluka
Hydrochloric acide	Fluka
Sodium hydroxide	Fluka
Sodium sulphate	Merck
Benzyltriethylammonium bromide	Aldrich
Sodium methoxide	Merck
Acetyl chloride	Fluka
N-bromosuccinimide	Fluka
Benzyl peroxide	Fluka
Lithium bromide	Fluka
Magnesium sulphate	Fluka
m-chloroperbenzoic acid	Merck
Potassium *tetr*-butoxid	Merck
Sodium chloride	Fluka
Potassium permanganate	Merck
Silica gel	Aldrich
Methanol	Aldrich
Chloroform	Merck
Ethanol	Merck
Acetone	Aldrich
Carbon tetrachloride	Fluka
Hexane	Fluka
Dimethylformamide	Fluka
Diethylether	Merck
Dichloromethane	Merck
Tetrahydrofuran	Fluka
Cyclohexane	Merck
Acetone	Fluka
Heptane	Fluka

Chemical	Supplier
1-pentanol	Fluka
Decane	Fluka
Undecane	Fluka
DHE	Fluka
Potassium dihydrogen phosphate	Fluka
Diclofenac sodium salte	Fluka
Sulfamethoxazole	Fluka
Carbamazepine	Fluka
Ibuprofen	Fluka
Tri-n- octylphosine oxide	Merck
1-octanesulfonic acid Sodium salt monohydrate	Merck
Buffer solution Titrisol pH 7	Merck
Buffer solution Titrisol pH 8	Merck
Buffer solution Titrisol pH 9	Merck
Buffer solution Titrisol pH 10	Merck

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