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Rapid Determination of Non-steroidal Anti-inflammatory Drugs in Aquatic Matrices by Two-phase Micro-electrodriven Membrane Extraction Combined with Liquid Chromatography

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Abstract

Two-phase micro-electrodriven membrane extraction (EME) procedure for the pre-concentration of selected non-steroidal anti-inflammatory drugs (NSAIDs) in aquatic matrices was investigated. Agarose film was used as interface between donor and acceptor phase in EME which allowed for selective extraction of the analytes prior to high performance liquid chromatography-ultraviolet detection. Charged analytes were transported from basic aqueous sample solution through agarose film into 1-octanol as an acceptor phase at 9 V potential. Response surface methodology in conjunction with the central composite design showed good correlations between extraction time and applied voltage ($R^2 > 0.9358$). Under optimized extraction conditions, the method showed good linearity in the concentration range of 0.5–500 μg L^{-1} with coefficients of determination, $r^2 \geq 0.9942$ and good limits of detection (0.14–0.42 μg L^{-1}) and limits of quantification (0.52–1.21 μg L^{-1}). The results also showed high enrichment factors (62–86) and good relative recoveries (72–114%) with acceptable reproducibilities (RSDs $\leq 7.5\%$ $n = 3$). The method was successfully applied to the determination of NSAIDs from tap water and river water samples. The proposed method proved to be rapid, simple and requires low voltage and minute amounts of organic solvent, thus environmentally friendly.

Introduction

In recent years, pharmaceutical wastes have been recognized as a key source of emerging contaminants and became a serious environmental health issue (1). These emerging contaminants enter the ecosystem via a number of pathways such as disposal of unused drugs, patient excretion, pharmaceutical factories and hospitals (2, 3). The effluent of pharmaceutical waste is released into the environment either as parent compounds or as active/inactive metabolites (4). The occurrence of pharmaceutical residues must be taken seriously as these drugs are present at low concentrations in aqueous matrices (5).

Non-steroidal anti-inflammatory drugs (NSAIDs) are among pharmaceutical drug groups most widely used by humans for major relief of inflammatory, chronic and acute pain (6). NSAIDs have been commonly found in very low concentrations in water samples such as wastewater (7) and also treated drinking water (8). Several methods have been used for the determination NSAIDs including dispersive liquid–liquid microextraction (DLLME) (9) and hollow-fiber liquid phase microextraction (HF-LPME) (10). However, DLLME is not suitable for complex matrices due to the high potential interferences (11) while HF-LPME suffered from longer extraction times (20–40 min) that has been claimed as a drawback (12).

Electrodriven membrane extraction (EME) is a well-established and promising membrane-based extraction technique based on the application of electrical forces for driving analytes from sample solution across supported liquid membrane (SLM) into acceptor phase (13, 14). It has been shown that EME provides fast and selective sample clean-up with minute amount of organic solvent consumption (15). Due to its desirable and excellent features, EME has been employed in various sample preparations for many applications like metals (16), peptides (17) water (18–20), organic acid compound (21) and pharmaceutical compounds (22, 23). Recently, this technique was successfully combined with chromatographic analysis (14) and capillary electrophoresis (CE) (15). The extraction technique provided a faster extraction time, acceptable recoveries and low of limit detection were obtained (14, 24).

Lee and co-workers described the EME of organic compounds from wastewater. With this method, low limits of detection (LODs) (>0.005 µg/L), good linearity and acceptable relative recoveries (74%) were achieved (25). The group of Alhooshani *et al.* proposed EME for the extraction of organic compounds from wastewater using toluene as SLM with 200 V driving force and the recovery values obtained were higher compared to those of solid phase extraction (SPE) (26).

Several reported studies proposed the use of three-phase EME prior to liquid chromatography (LC) (22, 27) and capillary electrophoresis (CE) (28). Three-phase EME combined with high performance liquid chromatography-ultraviolet (HPLC-UV) has been proposed for the determination of acidic compounds from several complex matrices. With this approach, acceptable recovery and good linearities and LODs were obtained (29). Nevertheless, in three-phase EME, the final concentration is in aqueous acceptor phase that leads to limited applicability of the methods of detection.

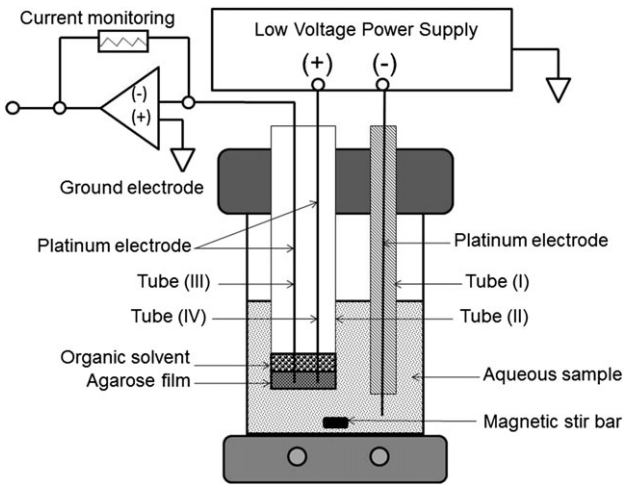


Figure 1. Schematic of the two-phase agarose film EME (AF-EME).

A challenge to broaden the applicability of EME in many instrumental analyses is that the final extraction phase must in organic solvent. Davarani and co-workers have demonstrated two-phase EME using gas chromatography–mass spectrometry (GC-MS) for basic pharmaceutical compounds determination. In this method, organic solvent was used as final extraction phase which can broaden the applicability of EME in combination with many instruments. In addition, two-phase EME has proved that the extraction was simple by skipping the SLM-acceptor phase interface (30).

In our laboratory, two-phase EME has proven to be a technique for extraction of tricyclic antidepressants (TCAs) in aquatic samples using HPLC-UV (31). More recently, we have developed an innovative method based on fast kinetic two-phase micro-EME in utilizing agarose film (AF) impregnated with nitrophenyl octyl ether (NPOE) as SLM for basic drugs extraction (32). Agarose exhibits a number of desirable properties including flexibility, solubity, thermal stability and high mechanical strength which leads for commercial application (33).

To the best of our knowledge, no work has been reported on two-phase EME combined HPLC-UV for the determination of acidic compounds from the aqueous matrices. Therefore, this work was set out to develop and apply two-phase EME that is a simple, fast and green chemistry extraction combined with HPLC-UV for the preconcentration and determination of four selected NSAIDs (ketoprofen, diclofenac, ibuprofen and mefenamic acid) in aquatic matrices. Additionally, this method applied an experimental design using response surface methodology (RSM) in conjunction with the central composite design (CCD) approach for the optimization and evaluation of the interactive effects of extraction time and applied voltage. From the RSM-CCD results, the most significant EME parameter was successfully identified.

Experimental

Chemicals and reagents

Ibuprofen (IBU), diclofenac sodium salt (DIC), ketoprofen (KET) and mefenamic acid (MEF) standards were purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade organic solvents (acetonitrile, methanol, heptanol and 1-octanol) were obtained from J.T. Baker (Pennsylvania, USA). Reagent grade sodium hydroxide (NaOH) and hydrochloric acid (HCl) were obtained from Merck (Darmstadt, Germany). Sodium

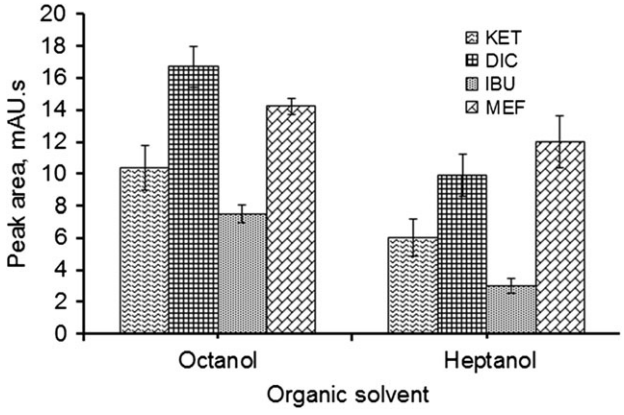
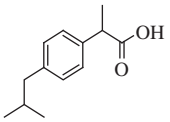
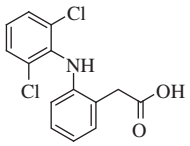
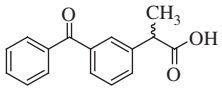
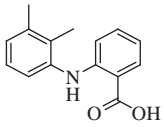


Figure 2. Effect of organic solvent of µ-EME of selected NSAIDs drugs from spiked distilled water. Legends: KET = ketoprofen; DIC = diclofenac; IBU = ibuprofen; MEF = mefenamic acid. AF-EME conditions: 500 µg L^{-1} of spiked solution; extraction time, 10 min; applied voltage, 10 V; stirring speed, 600 rpm. (Error bars represent standard deviations of results, $n = 3$).

Table I. Chemical Structure of the Studied NSAIDs

Analytes	Chemical structure	pKa	Log P
Ibuprofen		4.60	3.79
Diclofenac		4.15	1.56
Ketoprofen		4.0	3.00
Mefenamic acid		4.2	5.28

Source: 'The Drugbank Database' http://www.drugnbank.ca (Accessed on 15 Jun 2015).

acetate anhydrous (CH_3COONa), were obtained from HmbG Chemicals. Agarose (Analytical Grade) was obtained from Promega (Madison, USA). Double-distilled deionized water of 18.2 MΩ was purified using Nano ultrapure water system (Barnstead, USA).

Preparation of standard and sample solutions

Standard solutions of KET, DIC, IBU and MEF (1,000 µg mL^{-1}) were prepared separately in HPLC grade methanol. Water samples were prepared by spiking analytes at a known concentration (0.5 µg mL^{-1}). Tap water and river water samples were collected from Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia Johor Bahru and Skudai river, Johor Bahru, respectively. The samples were filtered through a Whatman™ nylon membrane filter 0.45 µm (Gelman Sciences, Ann Arbor, MI, USA). All the standard and sample solutions were stored in a refrigerator at 4°C until use.

Chromatographic conditions

HPLC separations were performed using a Zorbax Eclipse plus C_{18} column (2.1 × 100 mm, 3.5 µm) using an Agilent Technology 1220 LC system (California, USA) equipped with ultraviolet detector and a 20-µL sample loop. Analytes peaks were detected at 230 nm and processed using Agilent Chemstation software. Acetonitrile-acetate buffer (pH 3.2, 25 mM) (60:40) (v/v) was used as eluent and the flow rate was set at 0.2 mL min^{-1}.

Preparation of AF

The AF was prepared according to the procedure reported by Sanagi and co-workers (32). In the procedure, a solution of 0.8% (w/v) agarose gel was dehydrated to form a thin nano-pore film (12–18 nm pores and 0.02–0.04 mm film thickness).

AF-EME procedure

The experimental setup used for the extraction procedure is shown in Figure 1. A basic aquatic sample (10 mL) with adjusted pH of 7.5 was introduced into a 12-mL sample vial. In this study, the anode (positive electrode) was placed in acceptor phase solution and the cathode (negative electrode) was dipped directly into the sample solution. Next, 1-octanol was immobilized in the pores of porous AF to serve as SLM by dipping the film into the solvent for 5 s. The film was cut into small pieces (ca. 2.5 cm × 2. 5 cm) and attached at the lower end of the glass tube. A minute amount of 1-octanol (20 µL) as an acceptor phase was introduced using a micro-syringe into the glass tube attached with AF and the assembly was directly dipped into the sample solution. The surface area of the AF exposed to the aquatic sample was approximately 0.15 cm^2. The sample solution was agitated at 600 rpm using a magnetic stirrer. Applied voltage (9 V) was applied and the extraction was performed for a certain length of time (e.g., 10 min 36 s). After completion of extraction, 2 µL of extract was withdrawn for LC separation and quantification. The AF was discarded after single use in order to eliminate carry-over effect.

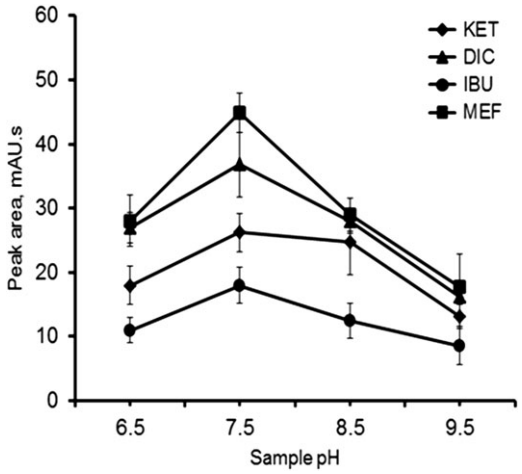


Figure 3. Effect of sample pH on AF-EME of NSAIDs of selected NSAIDs from spiked distilled water. Legends, AF-EME and HPLC-UV conditions are as in Figure 2 with 1-octanol as organic liquid membrane. (Error bars represent standard deviations of results, $n = 3$).

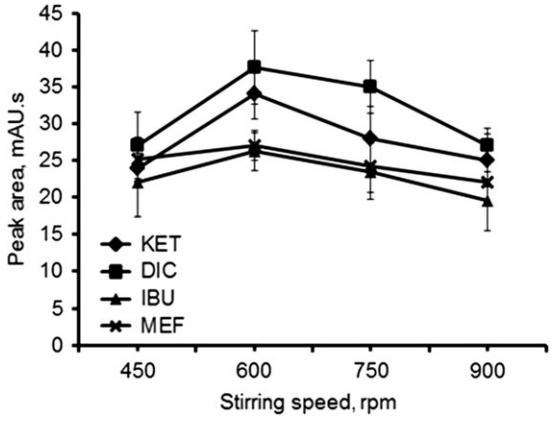


Figure 4. Effect of stirring speed on AF-EME of selected NSAIDs from spiked distilled water. Legends, AF-EME and HPLC-UV conditions are as in Figure 2 with sample pH 7.5. (Error bars represent standard deviations of results, $n = 3$).

Experimental design

The experimental design was generated using Design-Expert version 6.0.4 (Stat Ease Software) for regression analysis of the experimental data to fit the equations.

Validation of AF-EME

The validation of AF-EME was carried out in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), enrichment factor (EF), precision and recovery. LOD was calculated based on three times the signal-to-noise ratio ($S/N = 3$) while LOQ was calculated based on 10 times the signal-to-noise ratio ($S/N = 10$). Precisions were expressed in relative standard deviation (RSD) for inter-day precision. Inter-day precision was assessed by performing three replicates ($n = 3$) analyses at two different concentrations of spiked tap water and river water samples at three different days ($n = 3$). Relative recovery was calculated as the percentage of mean concentration of target analytes found after extraction (derived from the plotted matrix-match calibration curve) against the concentration spiked in the sample. The EF of the proposed method was calculated according to the following equation:

$$EF = C_{final}/C_{initial}, \quad (1)$$

where C_{final} is the final concentration of analyte and $C_{initial}$ is the initial concentration of analyte in samples solution,

Data and statistical analysis

In order to obtain the optimum conditions for the simultaneous extraction of NSAIDs, RSM and CCD were used to optimize two independent variables (applied voltage and extraction time). The experimental design was generated using Design-Expert version 6.0.4 (Stat Ease Software) for regression analysis of the experimental data to fit the equations. The quality of the developed method can be determined from the value of correlation coefficient (R^2). Analysis of variance (ANOVA) was used to evaluate the significance of the equations developed.

Table II. Independent Variables and their Coded Level for the CCD Design

Parameters (factors)	Code	Code variable levels				
		−α	−1	0	+1	+α
Applied voltage	A	1.51	4	10	16	18.49
Extraction time	B	4.34	6	10	14	15.66

Table III. CCD Consisting of Experiments for the Study of Two Experimental Factors in Coded Level and Experimental Results

Run order	Coded level value of variable		Sum of peak areas
	Extraction time	Applied voltage	
1	0	0	239.83
2	+1	+α	29.06
3	0	0	200.16
4	−1	+α	79.86
5	−1	−1	52.38
6	+1	−1	34.79
7	0	0	233.48
8	−1	0	74.83
9	+α	0	101.13
10	0	0	223.42
11	0	+α	22.02
12	0	−α	28.28
13	0	0	230.34
14	0	0	269.82

Results

Optimization of AF-EME extraction

In a preliminary investigation, two parameters (Sample pH, and stirring speed) were evaluated and optimized separately using traditional optimization procedure (one variable at-a-time). Subsequently, the effects of two other independent main parameters in AF-EME (voltage and extraction time) were investigated and evaluated using the RSM and CCD. The optimization was carried out by using deionized water samples spiked with each selected NSAID at a concentration of 0.5 μg mL^{-1}.

Variation in the organic liquid membrane (organic solvent)

Organic liquid membrane is one of the most important factors in AF-μ-EME. In this work, two solvents (1-octanol and heptanol) were evaluated as organic liquid membrane (Figure 2). The results showed that 1-octanol gave the highest extraction efficiencies compared to heptanol. 1-octanol has proved to be a very efficient organic solvent for extraction of acidic substances (34). Therefore, 1-octanol was chosen for subsequent experiments.

Sample pH

Suitable pH value of sample solution can help improve the extraction efficiency and reduce matrix interferences. Since the pKa values of the acidic drugs studied (IBU, KET, DIC and MEF) are in the range of 4.0–4.6 (Table I), these compounds mainly exist as electrically neutral molecules at low pH and ionized species at high pH. In EME, the ionization is more efficient if the pH of the acceptor solution is higher than the pKa of the analyte (26) and thus, extraction of the analyte is more efficient under alkaline condition (35). In this study, the effect of sample pH was evaluated by varying the pH in the range 6.5–9.5 and the results are presented in Figure 3. From the results, it was evident that the best extraction efficiency was achieved at pH 7.5. A further increase of sample pH to 8.5 resulted in gradual decrease of the peak areas as the analytes were transformed into molecular forms. At lower pH, the ionization is hindered and poorer extraction efficiency is obtained (26). Therefore, pH 7.5 was selected and used in subsequent experiments.

Stirring speed

Different stirring speeds in the range of 450–900 rpm on the extraction efficiency were investigated. In general, higher stirring speed enhances the diffusion of the analytes into the acceptor phase (36). Results showed that the highest extraction efficiency was obtained at 600 rpm and the peak areas slightly decreased at stirring speeds of 600 rpm and beyond (Figure 4). This might be due to the possibility of the increase of organic solvent loss at higher stirring speeds (37). Thus, 600 rpm was selected as the optimum stirring speed and used in subsequent experiments.

Experimental design using RSM with CCD

RSM is a useful method for studying the effects of several variables influencing the responses by varying them simultaneously (38). In general, the CCD is an effective design used to reduce the number of experimental trials needed, maximize efficiencies and to investigate

Table IV. ANOVA Regression Model for Response Quadratic Model for Four Selected of NSAIDs Analytes

Model	Source of variation	DF	Sum of squares	Mean square	*F*-value	*P*-value	Comment
KET	Regression	5	7219.10	1443.82	45.99	<0.0001	Significance
	A	1	50.61	50.61	1.61	0.2448	
	B	1	120.92	120.92	3.85	0.0905	
	AA	1	2178.79	2178.79	69.40	<0.0001	
	BB	1	5052.42	5052.42	160.94	<0.0001	
	AB	1	286.79	286.79	9.14	0.0193	
	Residual	7	219.75	31.39			
	Lack of fit	3	127.91	42.64	1.86	0.2775	
	Pure error	4	91.84	22.96			Significance
DIC	Regression	5	21489.05	4297.81	31.19	0.0001	Significance
	A	1	80.35	80.35	0.58	0.4700	
	B	1	77.85	77.85	0.57	0.0002	
	AA	1	7219.33	7219.33	52.40	<0.0001	
	BB	1	14990.01	599.27	108.80	0.0755	
	AB	1	599.27	137.77	4.35		
	Residual	7	964.42				
	Lack of fit	3	189.12	63.04	0.33	0.8084	
	Pure error	4	775.30	193.82			Significance
IBU	Regression	5	2568.16	513.63	20.37	0.0005	Significance
	A	1	16.08	16.08	0.64	0.4508	
	B	1	43.90	43.90	1.74	0.2285	
	AA	1	773.42	773.42	30.68	0.0009	
	BB	1	1709.28	1709.28	67.80	<0.0001	
	AB	1	188.65	188.65	7.48	0.0291	
	Residual	7	176.46	25.21			
	Lack of fit	3	119.27	39.76	2.78	0.1742	
	Pure error	4	57.19	14.30			Significance
MEF	Regression	5	1948.84	389.77	22.16	0.0004	Significance
	A	1	17.97	17.97	1.02	0.3458	
	B	1	18.88	18.88	1.07	0.3347	
	AA	1	651.0	651.03	37.01	0.0005	
	BB	1	1258.46	1258.46	71.54	<0.0001	
	AB	1	131.22	131.22	7.46	0.0293	
	Residual	7	123.13	17.59			
	Lack of fit	3	38.08	12.69	0.60	0.6498	
	Pure error	4	85.05	21.26			Significance

F-value: Variance of the group means/mean of the within group variances.
P-value: The probability of obtaining a result at least as extreme as the one that was actually observed, given that the null hypothesis is true.

the relationship between variables (39). The equation below reveals the number of experiments that should be run:

$$N = 2^n + 2n + n_c, \quad (2)$$

where n is the factor number and (n_c) is the replicate number of the central point.

In this study, two selected variables (extraction time and applied voltage) were investigated. According to Equation 1, 14 experiments were generated with the design matrix consisting of five levels of two factors. The coded level of selected factors ($-\alpha$, -1, 0, $+\alpha$, $+1$). The coded/actual values and the result of experiments are summarized in Table II. CCD consisting of experiments for the study of two experimental factors in coded levels and experimental results are shown in Table III.

For an experimental design with two factors, the quadratic model can be expressed by the following equation:

$$Y = a_o + a_1A_1 + a_2B_1 + a_3A^2 + a_4B^2 + a_5A_1B_1, \quad (3)$$

where Y is the predicted percentage value of peak area/response; A, extraction time; B, applied voltage; $a_o - a_5$ are the coefficient values obtained through multiple linear regression using Design-Expert software. The predicted response (Y) for each four analyse was obtained using Equation 4–7:

For ketoprofen (KET) recovery,

$$Y = 62.40 + 2.52A_1 - 3.89B^2 - 17.18A^2 - 26.16B^2 - 8.47\,A \cdot B. \quad (4)$$

For ibuprofen (IBU) recovery,

$$Y = 36.22 + 1.42A_1 - 2.34B^2 - 10.23A^2 - 15.21B^2 - 6.87\,A \cdot B. \quad (5)$$

For mefinamic acid (MEF) recovery,

$$Y = 30.09 + 1.50A_1 - 1.54B^2 - 9.39A^2 - 13.05B^2 - 5.73\,A \cdot B. \quad (6)$$

For diclofenac (DIC) recovery,

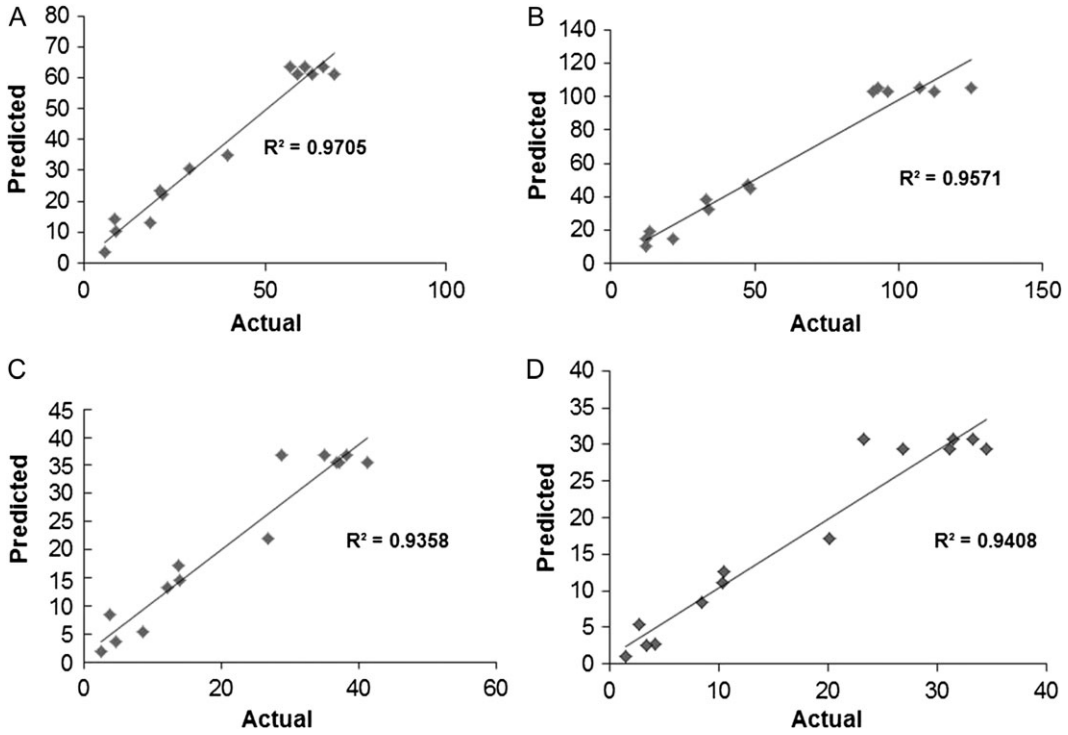


Figure 5. Relationship between predicted and actual (experimental) values for (A) ketoprofen, (B) diclofenac, (C) ibuprofen and (D) mefenamic acid.

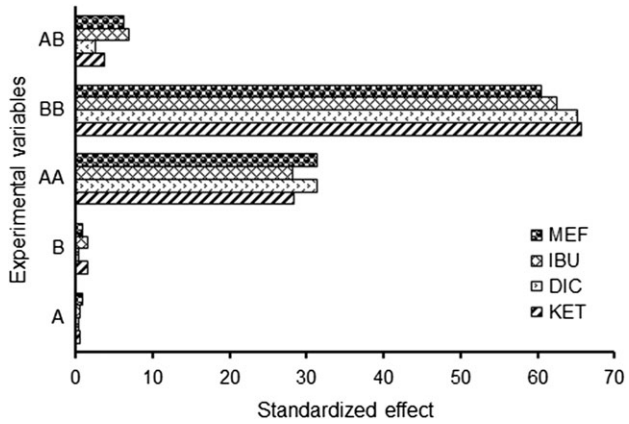


Figure 6. Pareto chart of the main effects in AF-EME.

$$Y = 104.14 + 3.17A_1 - 3.124B^2 - 31.27A^2 - 45.05B^2 - 12.24\,A \cdot B. \quad (7)$$

Analysis of variance

ANOVA and regression analysis was used to assess significance of variables which presented *P*-value, sum of squares, mean square, *F*-value and degree of freedom (DF). As shown in Table IV, the statistical significance of the second-order equation revealed that the regression was statistically significant ($P < 0.0005$). The result revealed that the statistical significance of the second-order equation revealed that the regression was statistically significant ($P < 0.0005$) for all NSAIDs drugs. The quality of fit of the quadratic polynomial model was expressed by the coefficient of determination, $R^{2.}$ The value of R^2 presented whether there is an acceptable relationship between the predicted and actual values (40).

As shown in Figure 5, the coefficient of determination, R^2 was found to be 0.9358 for IBU, 0.9705 for KET, 0.9408 for MEF and 0.9571 for DIC. These results showed that all of the values were close to 1.0, which advocates a high correlation between predicted and observed values. The results indicate that the regression model provides excellent relationship between two variables and the peak area response. The model is considered a good fit model if the value of the coefficient of determination, R^2 is ≥0.80 (41).

The main effects of variables were visualized by the use of Pareto chart (Figure 6). According to this figure, applied voltage, BB has the largest influence on the normalized peak area which affect the extraction efficiencies of NSAIDs in EME. In EME, applied voltage was an important parameter for the efficient extraction of analytes (42, 43). Nevertheless, the peak area decreased when higher voltages were applied. The maximum normalized peak area would be at 10 min 36 s and 9 V.

Response contour plot

The results of a CCD experiments visualized in the form of a response through three-dimensional (3D) surface and contour plots were constructed. RSM was used to investigate the integrated effect of extraction time and applied voltage in the form 3D plots. As illustrated in Figure 7, the extraction time and applied voltage variables were acted on parallel ways which have considerable influence in the response or peak area. The peak area increased with increasing extraction time and applied voltage where the maximum point is located inside the experimental region.

Extraction time could affect the flux of target analytes in the electrokinetic across SLM into acceptor phase (44). From the results, it is apparent that the peak area of all analytes increased

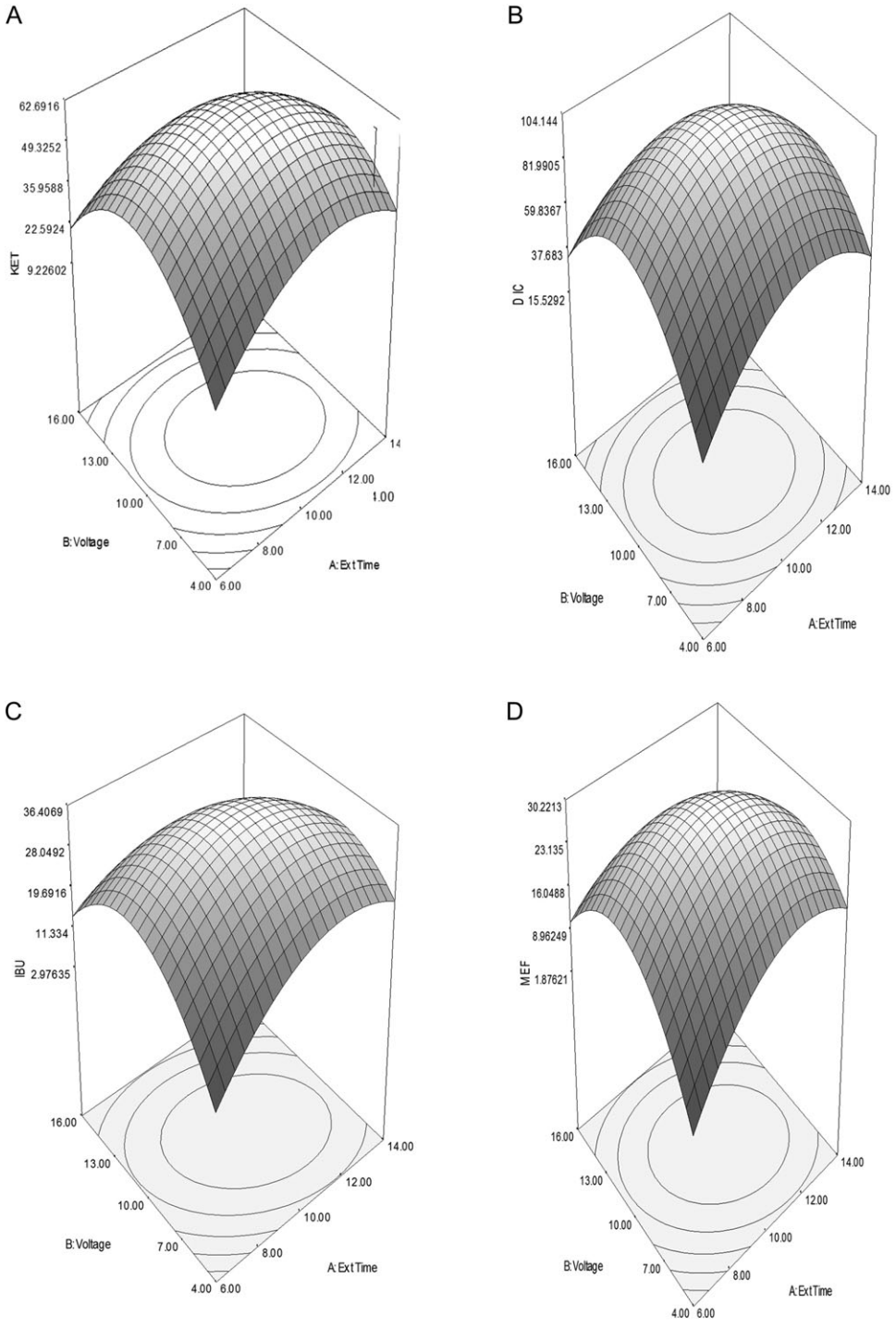


Figure 7. RSM obtained by plotting voltage vs. the extraction time for NSAIDs: (A) KET, (B) DIC, (C) IBU and (D) MEF using CCD on AF-EME extraction.

with extraction time up to a point and decreased thereafter. It should be noted that EME was a non-exhaustive process and over-saturation of analytes in the acceptor phase might occur which lead to back-diffusion into sample solution (45, 46).

In μEME, the number of ions crossing the membrane can be increased by increasing the applied voltage. The application of the higher potential leads the system further from equilibrium and thus creates a strong force for target compounds to migrate from sample solution across membrane into acceptor phase (47). Again, as EME is a non-exhaustive process, the duration of membrane stability is reduced by increasing the voltage. Electrolysis occured at cathode and anode where bubbles were formed at the cathode according to the H_2 formation *via* the following reactions (Equations 7 and 8):

$$\text{Donor solution (negative electrode): } 2H^+ + 2e^- \rightarrow H_2. \quad (8)$$

Table V. Validation Data of Two-phase AF-μ-EME of NSAIDs from Spiked Tap and River Water Samples

Sample	Analyte	Linear range, (μg L^{-1})	Coefficient of determination, r^2	LOD, (μg L^{-1})	LOQ (μg L^{-1})	EF	Precision (RSD, %) ($n = 3$)
Tap water	KET	0.5–500	0.9984	0.21	0.63	71	7.5
	DIC	0.5–500	0.9979	0.18	0.58	82	8.8
	IBU	0.5–500	0.9953	0.30	0.89	73	9.2
	MEF	0.5–500	0.9989	0.14	0.52	86	6.5
River water	KET	0.5–500	0.9954	0.28	0.84	62	4.1
	DIC	0.5–500	0.9942	0.22	0.65	74	6.6
	IBU	0.5–500	0.9976	0.42	1.21	65	9.4
	MEF	0.5–500	0.9991	0.15	0.52	81	3.6

Analytes: KET = ketoprofen, DIC = diclofenac, IBU = Ibuprofen, MEF = mefenamic acid.

Table VI. Relative Recoveries (%) and Method Precisions (RSD %, $n = 3$) at Two Different Concentrations for Two-phase AF-μ-EME in Tap Water and River Water Samples

Sample	Analyte	Average relative recovery, % (RSD, %) Spiking level ($n = 3$)	
		10 μg L^{-1}	100 μg L^{-1}
Tap water	KET	86 (4.1)	108 (2.7)
	DIC	91 (3.7)	114 (3.2)
	IBU	79 (4.1)	102 (7.4)
	MEF	95 (3.2)	106 (4.4)
River water	KET	78 (5.6)	88 (2.9)
	DIC	82 (3.8)	97 (4.7)
	IBU	72 (7.1)	91 (3.8)
	MEF	92 (2.5)	101 (3.2)

$$\text{Acceptor solution (positive electrode): } H_2O \rightarrow 2H^+ + 1/2O_2 + 2e. \quad (9)$$

As a summary, the optimized EME conditions were as follows: agarose concentration, 1.0% (w/v); organic liquid membrane, 1-octanol; sample pH, 7.5; stirring speed, 600 rpm; extraction time, 10 min 36 s and applied voltage, 9 V.

Discussion

Experiments were carried to validate the applicability of two-phase AF-EME by using optimum extraction conditions (1-octanol as an organic solvent, sample solution at pH 7.5, 600 rpm as stirring speed, extraction time of 10 min 36 s and applied voltage of 9 V). In this study, the method was validated in terms of its linearity, LODs, EFs, accuracy and precision under the above-mentioned optimum extraction condition. The calibration curves were constructed by plotting the peak area of analytes vs. the concentration, and every concentration was performed in triplicate. The linearity of the method was evaluated using water samples spiked with the four selected of NSAIDs. Good linearity of response (peak area) for each analyte was observed (Table V) in the concentration range of 0.5–500 μg L^{-1} with coefficients of determination, $r^2 \geq 0.9942$. The proposed method showed good LODs and LOQs for the targeted anaytes in the range of 0.14–0.42 μg L^{-1} and 0.52–1.21 μg L^{-1}, respectively. The results also showed high EFs in the range of 62–86. Method accuracy (or RR %) and precisions (expressed as RSD %) were evaluated on spiked water samples at two different concentration levels: 10 and 100 μg L^{-1}. The results (Table VI) showed excellent relative recoveries in the range of 72–114% and good reproducibility with RSDs of <7.5%. Figure 8 shows that AF-EME coupled with HPLC-UV is suitable for the determination of NSAIDS in water samples.

Comparison of AF-EME with other reported EME methods

The comparison of analytical method between two-phase AF-EME and other reported EME methods is tabulated in Table VII. A few EME methods have been applied in the analysis of NSAIDs. In general, each method has its own advantages and disadvantages. Apparently, most of the EME is based on a three-phase system. Three-phase EME combined with HPLC resulted in good sensitivity with short extraction time for the quantification of six NSAIDs in wastewater samples (48). However, the EFs were not satisfactory. Very short extraction times were achieved in three-phase EME combined with HPLC-UV detection (29) but the EF and low sensitivity recoveries were not satisfactory as compared to the rest of the methods. Three-phase EME method assisted by carbon nanotubes (CNTs) provided excellent pre-concentration factor and high recoveries due to high adsorption capacity offered by the CNTs (49). However, this method utilized hollow fiber made of polypropylene impregnated with 1-octanol as SLM which might leak into the sample under high agitation speed and application of electrical field. As compared to other methods, the proposed two-phase AF-EME method revealed good performance in terms of extraction efficiency and showed excellent LODs, high EFs and high relative recoveries. A two-phase mode system is simple as it reduces the extraction procedure and is compatible into wider range of instrumental analysis. In addition, this proposed method provides short extraction time, minute amounts of organic solvent consumption and utilizes biopolymer AF impregnated with organic solvent as SLM thus supporting the green chemistry concept.

Conclusion

A two-phase AF-EME combined with LC has been successfully applied for rapid, sensitive and efficient determination of four selected NSAIDs in water samples. Agarose films impregnated with 1-octanol have been used as SLM for EME procedure using a low voltage system. The application of two-phase system on this proposed method would reduce the extraction procedure and it is compatible with many analytical instruments. Under the optimized conditions, good correlations were obtained for the two dependent variables (extraction time and applied voltage) in which the applied

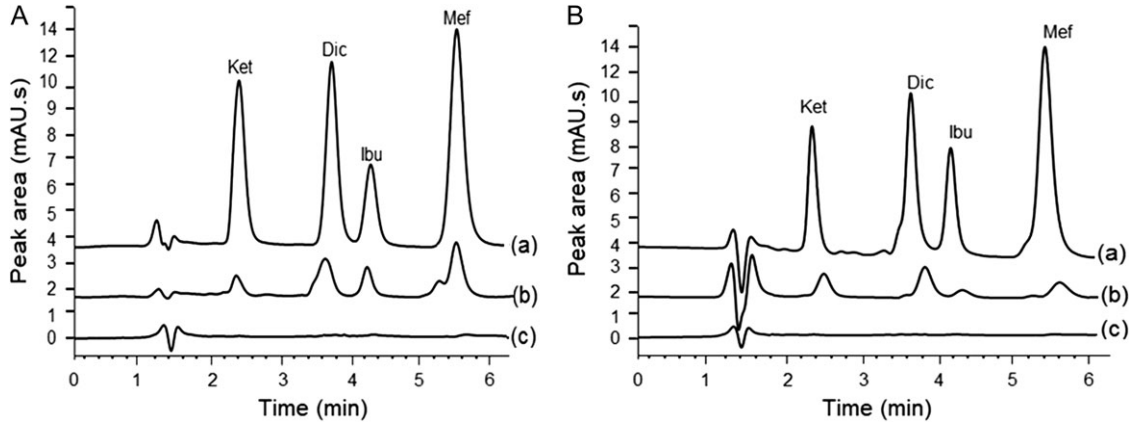


Figure 8. HPLC-UV chromatograms of NSAIDs extracted using two-phase AF-EME. (A) (a) tap water sample spiked with analytes at concentrations of 100 μg L^{-1}; (b) tap water sample spiked with analytes at concentrations of 10 μg L^{-1}; (c) non-spiked tap water sample. (B) (a) river water sample spiked with analytes at concentrations of 100 μg L^{-1} of each analyte; (b) river water sample with analytes at concentrations of 10 μg L^{-1}; (c) non-spiked river water sample. HPLC conditions: column, Agilent Zorbax Eclipse plus C_{18} column (2.1 × 100 mm, 3.5 μm); mobile phase, acetonitrile-acetate buffer (pH 3.2, 25 mM) (60:40, v/v) at a flow rate of 0.2 mL min^{-1}; injection volume, 2 μL and detector wavelength, 230 nm. Peak identities: ketoprofen (KET), diclofenac (DIC), ibuprofen (IBU) and mefenamic acid (MEF).

Table VII. Comparison of the Proposed Method with Other EME Methods Applied for the Determination of NSAIDs in Water and Biological Samples

Analysis	Detection	Linear range (μg L^{-1})	LOD (μg L^{-1})	Extraction time (min)	Applied voltage (V)	EF	Recoveries, %	References
Three-phase EME	HPLC (FLD-DAD)	0.29–100	0.0009–9	10	10	28–49	60–100	(48)
Three-phase EME	HPLC-UV	8–500	2.7–5.0	5	20	44–95	–	(29)
Three-phase CNTs/EME	CE	3–500	1–3	10	10	–	90–94	(49)
Two-phase EME	HPLC-UV	0.5–500	0.14–0.42	10.36	9	62–86	–	Present work

Abbreviations: EME = Electromembrane extraction; HPLC = High performance liquid chromatography; FLD = Fluorescence detection; DAD = Diode array detection; EF = Enrichment factor; UV = Ultraviolet; CE = Capillary electrophoresis, CNTs = Carbon nanotubes; LOD= Limit of detection.

voltage gave highest influence in AF-EME. The optimized extraction conditions obtained were 10 min 36 s of operation with 9 V driving force, this method providing excellent performance extraction in terms of sensitivity and selectivity. The new support biopolymer material (agarose film) utilized in two-phase EME showed excellent extraction efficiencies and is advantageous as it is biodegradable and found abundant from natural sources. This developed method provides rapid extraction, simple and utilizes biopolymer as interface to support the liquid membrane, thus meets the green chemistry concept.

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