Bio-Nanohybrid Composite Sorbent-Based Microextraction Combined with High-Performance Liquid Chromatography for NSAIDs in Aqueous Samples

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Chitosan was hybridised with tetraethoxysilane via hydrolysis to obtain a bio-nanohybrid composite sorbent for dispersive solid-phase extraction (DSPE) of three non-steroidal anti-inflammatory drugs in aqueous samples. Field Emission Scanning Electron Microscope (FESEM) micrographs showed that the bio-nanohybrid composite was composed of spherical-shaped nanoscale particles with sizes ranging from 41.1 - 231.5 nm. The effects of three significant parameters, namely extraction time, sample pH, and desorption time, on the determination of naproxen (NAP), diclofenac sodium (DIC), and mefenamic acid (MEF), were evaluated systematically using the Box Behnken design (BBD). R^2 was found to be 0.942 for NAP, 0.966 for DIC and 0.960 for MEF. These results were all close to 1.0, indicating good correlation between predicted and observed values. Under optimized conditions, the DSPE showed good linearity over the range of 0.1 - 500 μgL^{-1}, low detection limits (0.04-0.67 µg/L), excellent limits of quantification (0.15-2.78 μgL^{-1}), excellent relative recoveries (93.06-101.48%) and acceptable precision with relative standard deviation (RSD) values < 7.75%. These results indicate that this bio-nanohybrid composite is a promising sorbent for sorbent-based microextraction and a good alternative to synthetic polymer sorbents.

Key words: Chitosan; nanohybrid; dispersive solid phase extraction; Box Behnken design; non-steroidal anti-inflammatory

Natural biodegradable polymers such as poly-saccharides can be extracted from marine plants and animals. They are naturally abundant and rich in native functional groups that can be tailored to sorption requirements. The extraction efficiency of the sorption process is influenced by functional groups present in the sorbent material. Variation in functional groups contributes to an increase in active sites that allow interactions with analytes and enhance adsorption activity. Chitosan is extracted from the exoskeleton of marine organisms with large amounts of primary amino (-NH₂) and hydroxyl (-OH) groups in their backbone polymer matrix. The presence of these groups allow chemical and electrostatic interactions, thus creating possibilities for chemical modification [1,2]. Theoretically, these native functional groups could turn chitosan into a promising biopolymer-based sorbent with an excellent capacity to entrap trace level residues [3]. However, it is quite challenging to directly use this biomass in the form of flakes or powder in wastewater treatment due to its disadvantages such as swelling, unsatisfying mechanical properties, and mass transfer resistance. Chitosan suffers from pH sensitivity because the amino groups in the chitosan backbone are easily protonated and completely soluble under acidic conditions [1,4-5]. Chitosan partially dissolves at pH 3.0 and instantaneously dissolves at pH 1.2 [6].

A practical way to circumvent these problems is to hybridize chitosan with other materials such as porous silica nanoparticles to form bio-hybrid composite materials. Porous silica has excellent physicochemical properties such as high surface area, high pore volume, chemical inertness, good thermal stability, excellent mechanical resistance, and low manufacturing cost. This material is rich in a number of surface functional groups with excellent selectivity towards specific pollutants [7]. Thus, by hybridizing silica nanoparticles with a high-density biopolymer,

132   Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

the resulting chitosan matrix would be an effective alternative to improve the sorbent's mechanical strength and increase mass density [8-10].

In this work, a bio-nanohybrid chitosan-tetraethoxysilane (Ch-TEOS) composite was prepared as a composite sorbent to determine selected non-steroidal anti-inflammatory drugs (NSAIDs). The selected drugs, naproxen (NAP), diclofenac sodium salt (DIC), and mefenamic acid (MEF), were chosen as models based on the most consumed pharmaceuticals frequently used for suppressing inflammatory processes. These drugs were analyzed via a dispersive solid-phase extraction (DSPE) prior to high-performance liquid chromatography/Ultraviolet-Visible detector (HPLC/UV) analysis. This study used Response surface methodology (RSM) with the Box-Behnken Design (BBD) to improve the DSPE performance, focusing on factors *viz.* extraction time, sample pH, and desorption time for the determination of NAP, DIC, and MEF. Optimization was necessary to minimize the use of resources and better evaluate the interactions of variables that profoundly affected the DSPE method. Therefore, this study aimed to enrich the body of knowledge on nanohybrid biocomposites for potential use in DSPE for drug analysis.

MATERIALS AND METHODS

Reagents, Materials and Samples

NSAID standards were purchased from Sigma-Aldrich (St. Louis, USA). HPLC-grade organic solvents acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), and isopropanol (IPA) were obtained from J.T. Baker (USA) and Merck (Germany). Reagent grade sodium chloride (NaCl) was purchased from Bendosen (Malaysia). Sodium hydroxide 99% (NaOH) and hydrochloric acid 97% (HCl) were obtained from Merck (Germany). Acetic acid glacial 100% was purchased from Qrec (New Zealand). Ammonium hydroxide, chitosan, and tetraethoxy-silane (TEOS) were obtained from Sigma-Aldrich (USA). Deionized water of at least 18 mΩ was obtained from a Nano ultra-pure water system (USA). The standard solutions were prepared by dissolving in HPLC grade MeOH, and were stored in the dark at 4ºC when not in use. The water samples were collected in bottles pre-cleaned with acetone, filtered through a nylon membrane filter to remove colloidal particles, and stored in a refrigerator at 4ºC for further use.

Instrumentation

A HPLC/UV system was used, consisting of a JASCO PU-980 pump (Japan) and a Shimadzu UV-Visible detector (Japan). A Rheodyne 7725 valve with a 20 μL sample loop (USA) was used for sample introduction. Separations were performed on an Agilent Technology Zorbax Eclipse Plus C_{18} column (3.5 μm, 100 mm length × 2.1 mm I.D.) (USA). A 2 μL sample was injected using a Hamilton HPLC syringe (USA). The measured ultraviolet wavelength was 230 nm, as in previous reports [11-12], and the analyte peaks were recorded using a Powerchrom data recording system (eDAQ, Australia). The mobile phase consisted of acetonitrile-acetate buffer (pH 3.2, 25 mM) (50:50, v/v), and the flow rate was 0.2 mL/min in isocratic mode. Each sample was filtered through a 0.45 μm syringe filter (Membrane Solution Nylon) before injection.

The extraction procedure involved using a Heidolph Reax 2000 vortex agitator (Germany) to assist the extraction process and a Bransonic CPX ultrasonic bath (USA) to assist the desorption of analytes from the composite sorbent.

Characterization of the Ch-TEOS bionano-hybrid composite sorbent was performed using a Perkin Elmer Frontier Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectro-photometer (USA) in the range of 4000 - 650 cm^{-1} with a resolution of 4 cm^{-1} averaging 24 scans. The morphology study of the composite was performed on a JEOL JSM-7600F Ultra High-Resolution Field Emission Scanning Electron Microscope (FESEM) with a magnification of 1000X to 25000X, while the Brunauer–Emmett–Teller (BET) pore analysis used the Micromeritics 3Flex Surface Characterization Analyzer (USA).

Preparation of Bio-Nanohybrid Composite Sorbent

The Ch-TEOS bio-nanohybrid composite was synthesized via a hydrolysis technique with some modifications [13]. Silica solution was prepared by mixing 5 mL of tetraethoxysilane with 15 mL of ethanol, 1 mL of deionized water and 0.5 mL of concentrated hydrochloric acid (37%). The mixture was stirred using a magnetic stirrer for 10 min. This solution was slowly added to the previously prepared chitosan solution (0.25 g of chitosan dissolved in 50 mL of 2% acetic acid) and continuously stirred for 24 h. The combined solution was then added dropwise into concentrated ammonium (38%) to form a white, cloudy solution, and this mixture was stirred for 2 h. The white solid product was separated using 11 μm filter paper, rinsed thoroughly with deionized water, and dried in the oven at 50 °C for 24 h.

Dispersive Solid-Phase Extraction (DSPE)

50 mg of Ch-TEOS bio-nanohybrid composite sorbent was added into a 50-mL centrifuge tube containing 10 mL of the aqueous sample at pH 4.13. The sample was vortexed for 14.05 min at 450 rpm. The sorbent was then separated from the solution by centrifugation at a speed of 4000 rpm for 5 min, and the supernatant was discarded. The desorption of analytes was performed with 300 μL of ACN under ultrasonication for 5 min, and the solution was filtered through a 0.45μm

133   Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

polypropylene syringe filter. The solution was then pre-concentrated to 100 µL under a gentle stream of nitrogen gas for analyte enrichment. A 2 µL aliquot of the final extract was analyzed by HPLC/UV at 230 nm. Figure 1 shows the schematics of this method, termed as Ch-TEOS-DSPE-HPLC/UV.

Experimental Design and Statistical Analysis

We have previously developed analytical methods for similar drugs and identified the five most significant parameters [14]. Thus, preliminary experiments were carried out using a classical approach by varying one-variable-at-a-time (OVAT) to identify the significant parameters that affected the adsorption process due to the different types of sorbents and techniques applied. In this study, the parameters assessed were: the addition of salt, sample pH, extraction time, type of desorption solvent, and desorption time. In the BBD optimization experiment, the study only selected three significant parameters from the OVAT study. For the BBD, Design-Expert software version 13 (Stat Ease Software, USA) was used to optimize the DSPE process for: extraction time (A), pH of sample (B), and desorption time (C), for the response

extraction efficiency (mV.s) of NAP, DIC and MEF. The ranges and coded levels of the BBD variables are listed in Table 1. The lower, central and upper ranges and levels were obtained from previous OVAT studies. In this study, the quality of fit of the generated polynomial model equation was gauged using the coefficient of determination (R^2), and the responses were analyzed using analysis of variance (ANOVA). A p value < 0.05 indicates a significant model term. Other main indicators to illustrate the significance and adequacy of the generated model include the model's lack of fit, F-value and signal-to-noise ratio.

Validation of Analytical Method

The DSPE method was assessed for linearity (R^2), limits of detection (LOD), limits of quantification (LOQ), precision and accuracy to ensure that the analytical procedure was reliable and fit for the intended purpose. LOD and LOQ values were calculated based on linear regression of the calibration curve. Precision was expressed in terms of relative standard deviation (RSD %) and accuracy (% relative recovery).
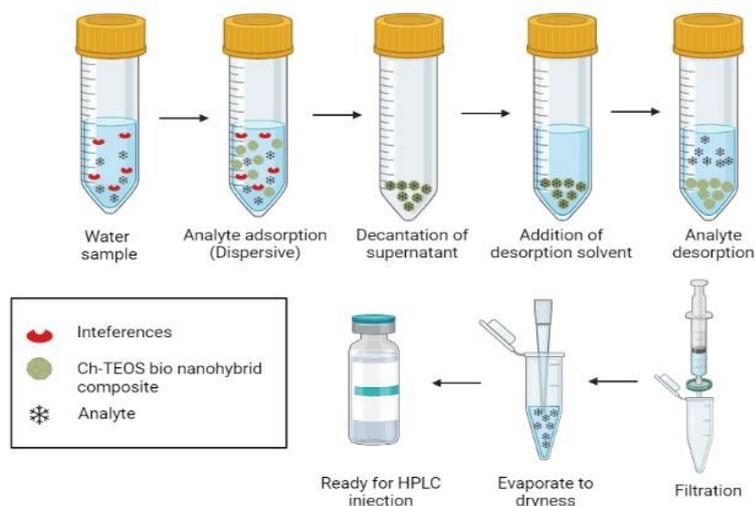
Figure 1. Schematic diagram of the method termed as Ch-TEOS-DSPE-HPLC/UV

Table 1. The BBD independent variables and their levels in the DSPE optimization experiment

Variable	Unit	Key	Type	Range and levels		
				Lower	Central	Upper
Extraction time	min	A	Numeric	5	10	15
pH of sample	pH	B	Numeric	2	4	6
Desorption time	min	C	Numeric	5	10	15

134 Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

RESULTS AND DISCUSSION

Characterization of Bio-Nanohybrid Ch-TEOS Composite Sorbent

The Ch-TEOS bio-nanohybrid composite was obtained as a white powder in non-crystalline form. Table 2 summarizes the significant adsorption bands of functional groups present in pristine chitosan, TEOS, and the Ch-TEOS bio-nanohybrid composite. The broad absorption band of hydroxyl (O-H) stretching vibrations appeared notably weaker and shifted from 3649 cm^{-1} in pristine chitosan to 3607 cm^{-1} in Ch-TEOS, indicating that the H atom in O-H had been partly grafted to the silica group [15]. The amino groups (-NH$_2$) of chitosan, as expected, facilitate the hydrolysis of the silanolate (Si-OR) groups to form a linkage with the polymer chain of chitosan. Thus, the absorption band at 1686 cm^{-1} corresponds to vibrations of amino groups in chitosan that shifted to 1678 cm^{-1} in Ch-TEOS, suggesting weak interactions between the amino groups and the silica network. An intense absorbance at 1072 cm^{-1} represents the Si-O stretching vibration in TEOS that deformed into a wide band at 1065 cm^{-1} in Ch-TEOS, confirming the covalent Si-O-C bonds in the hybrid composite [13].

FESEM analysis of the Ch-TEOS hybrid composite was essential to determine the surface interactions of TEOS and the homogeneity of the chitosan matrix. The surface morphology of the granule form, pristine chitosan, resembled dense fibres [16-17]. However, the micrograph of Ch-TEOS at high magnification (25000X) (Figure 2) revealed spherical-shaped particles in the bio-nanohybrid composite. The structure seen here confirmed that the inorganic silica moiety was trapped and condensed into the chitosan network which led to the observed morphological change. Likewise, similar observations were reported by earlier studies [18-19]. Pertinently, the FESEM measurements revealed that the bio-nanohybrid composite had particles of 41.1-231.5 nm, verifying the presence of nanoparticles in the sorbent.
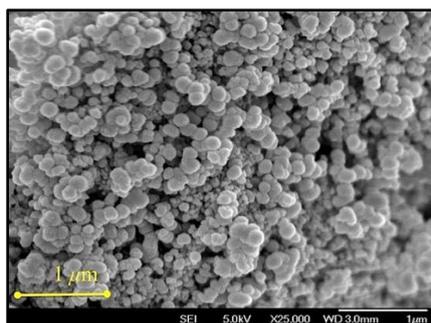
Data from the Brunauer-Emmett-Teller (BET) and nitrogen adsorption analysis showed that introducing large-structured TEOS into pristine chitosan profoundly influenced the surface properties of the resultant composite. The surface area of Ch-TEOS increased to 148.94 m^{2}g^{-1} compared to 3.27 m^{2}g^{-1} for pristine chitosan [15]. The average pore diameter was 92.99 Å, with a corresponding total pore area of 56.05 m^{2}g^{-1}. The data thus indicated that high surface area mesoporous structures existed in the bio-nanohybrid composite [13, 20]. It should be pointed out that particles with a large surface area are better choices for sorbent materials, allowing more active sites for adsorption.

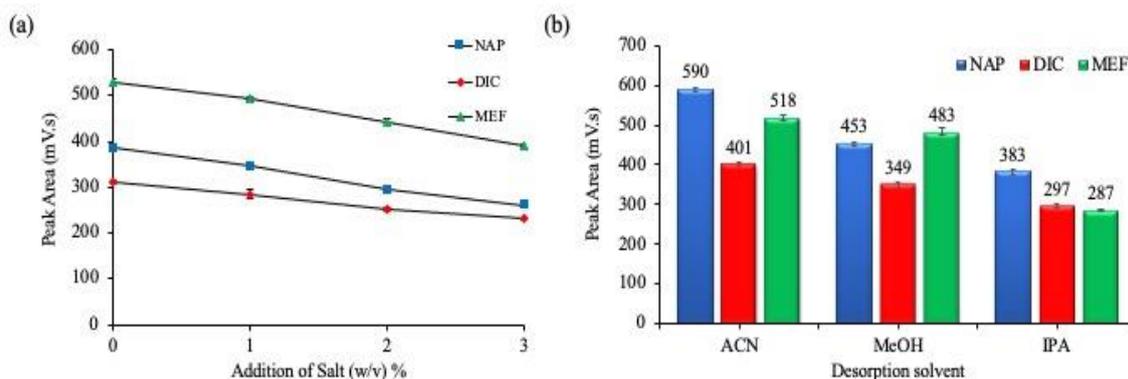### Optimization of the Ch-TEOS-DSPE -HPLC/UV Method

Based on a similar drug analysis in previous studies [21-24], five parameters that significantly affected the microextraction process were identified. A preliminary study using the classical approach by OVAT was undertaken to investigate the effect of salt addition and desorption time. The presence of salt in water samples can disrupt the solvation cage of the water-soluble analytes (salting-out effect) and alter the diffusion rate of analytes toward the sorbent surface.

Table 2. Comparison of FTIR absorption bands in Chitosan, TEOS, and the Ch-TEOS bio-nanohybrid composite

Vibration	Chitosan (cm^{-1})	TEOS (cm^{-1})	Ch-TEOS (cm^{-1})
O-H stretching	3649	-	3607
N-H bending	1686	-	1678
Si-O stretching	-	1072	1065

Figure 2. FESEM images of the Ch-TEOS bio-nanohybrid composite

135   Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

Figure 3. (a) Effect of salt addition; (b) Effect of desorption solvent

In this study 0 - 3 % (w/v) of salt was added, but the extraction efficiency was not improved (Figure 3 (a)). In a prior investigation, a similar phenomenon of diminishing extraction efficiency after the addition of salts was noted, as the salt counter ion in the sample solution had outcompeted the target analytes [25]. Consequently, the subsequent experiments were done in the absence of salt.

The study was then performed on the desorption of extracted analytes from the sorbent using three HPLC-compatible organic solvents of different polarity indices, namely acetonitrile (5.8), methanol (5.1) and isopropanol (3.9). Figure 3(b) showed that the highest extraction performance based on measured peak area was obtained for the relatively polar acetonitrile, followed by methanol and iso-propanol. The outcome correlated well with the relatively polar NSAIDs, in which polar desorption solvents could better desorb these acidic substances compared to less polar solvents [26]. Hence, based on this finding, the subsequent desorption experiment utilized acetonitrile as the solvent.

In the next part of the study, three independent parameters, namely extraction time (A), pH of sample (B), and desorption time (C), were optimized by the BBD approach for the simultaneous microextraction of Ch-TEOS-HPLC-UV using Design-Expert version 13 software. This design produced a total of 16 experiments in which each extracted water sample was spiked with 100 µg L^{-1} NAP, and 500 µg L^{-1} DIC and MEF, respectively. The R^2 value is presented to determine whether there was a good relationship between the predicted and actual values. As shown in Table 3, R^2 was found to be 0.942 for NAP, 0.966 for DIC and 0.960 for MEF. All values were close to 1.0, which indicates a high correlation between predicted and observed values. A very high R^2 value implies excellent correlation and a satisfactory model for predicting the best conditions for the highest extraction efficiency. The model is considered a good fit if the value of the coefficient of determination,
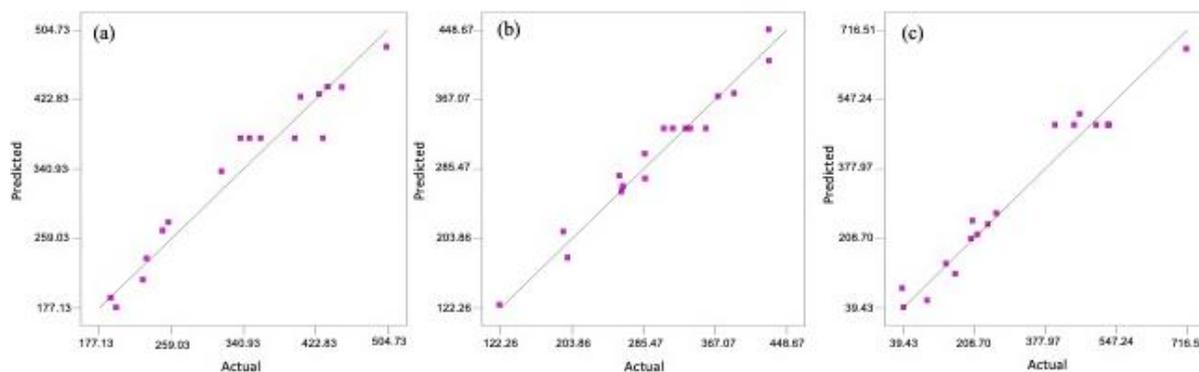
R^2 is ≥ 0.80 [27].

ANOVA analysis of the BBD showed relatively high F values of 12.70, 22.24, and 18.85 for NAP, DIC, and MEF, respectively (Table 3), indicating the variation between the data was sufficient to be used for model construction. The experimental data also closely agreed with the predicted values, as they were scattered closely along the trend line (Figure 4). Also, the F-values of the model, 12.7, 22.24 and 18.85 for NAP, DIC and MEF, respectively, were greater than the tabulated $F_{0.05}(9,7)$ = 3.6767, indicating a highly significant degree of freedom relative to the residual at the 95% confidence level.

Finally, the model's lack-of-fit F-values of 0.64 for NAP, 2.31 for DIC and 1.34 for MEF respectively, were considerably lower than the tabulated $F_{0.05}(3,4)$ = 6.5914, indicating that the lack of fit was insignificant relative to the pure error (Table 3). As for 'adequate precision', this was measured by the signal to noise ratio. A ratio greater than 4 is desirable. Thus, the relatively high ratios of 11.120 (NAP), 18.246 (DIC) and 13.819 (MEF) indicated adequate signals for the model. Therefore, these models could be used to navigate the design space.
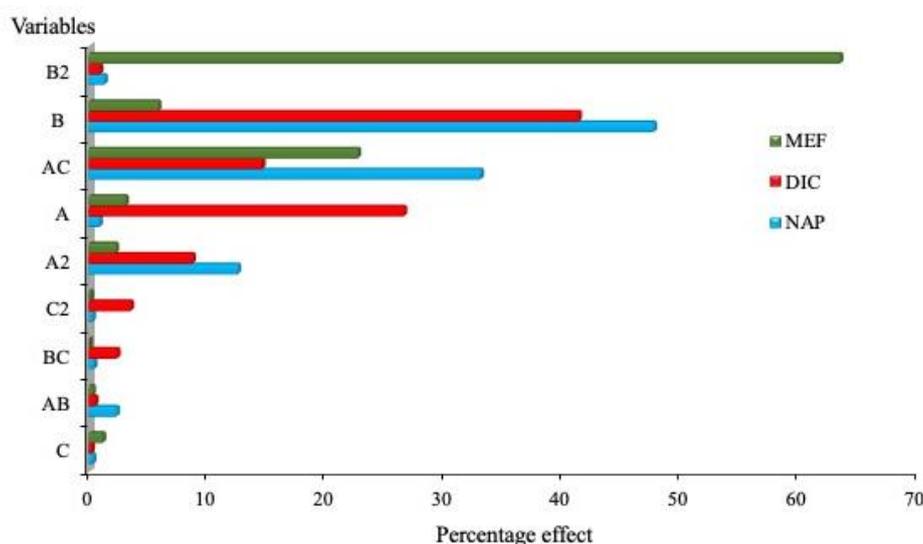
For the DSPE systems, ANOVA indicated that the linear terms A (extraction time) and B (pH of the sample) were significant (p value < 0.05), but not C (desorption time). Only the mutually interactive term, AC (extraction time versus desorption time) was significant, with p values of 0.0005 for NAP, 0.001 for DIC and 0.005 for MEF, while the interactions of extraction time versus pH of sample (AB) and pH of sample versus desorption time (BC) were borderline insignificant. As the constructed plots exhibited the presence of a saddle point, the study resorted to using ridge maximum and canonical analyses to establish the critical levels of the design variables which maximized the extraction efficiencies of the NSAIDs.

136 Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
     Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
     Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
     Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

Table 3. ANOVA of the response surface quadratic model for three selected NSAID analytes

Analyte	Source	Sum of Squares	DF	Mean Square	F Value	p-value	
NAP	Model	147900	9	16437	12.7	0.0015	significant
	A	1516	1	1516	1.17	0.3151	
	B	70519	1	70519	54.47	0.0002	
	C	704	1	704	0.54	0.4848	
	A2	18719	1	18719	14.46	0.0067	
	B2	2125	1	2125	1.64	0.2409	
	C2	646	1	646	0.5	0.5029	
	AB	3628	1	3628	2.8	0.138	
	AC	48949	1	48949	37.81	0.0005	
	BC	819	1	819	0.63	0.4526	
	Residual	9062	7	1295			
	Lack of Fit	2948	3	983	0.64	0.6266	not significant
	Pure Error	6114	4	1529			
	Cor Total	157000	16				
	R^2	0.942		Adeq. Precision	11.120		
DIC	Model	106900	9	11873	22.24	0.0002	significant
	A	28483	1	28483	53.35	0.0002	
	B	44206	1	44206	82.8	< 0.0001	
	C	371	1	371	0.7	0.4319	
	A2	9460	1	9460	17.72	0.004	
	B2	1137	1	1137	2.13	0.1878	
	C2	3901	1	3901	7.31	0.0305	
	AB	705	1	705	1.32	0.2882	
	AC	15738	1	15738	29.48	0.001	
	BC	2697	1	2697	5.05	0.0594	
	Residual	3737	7	534			
	Lack of Fit	2370	3	790	2.31	0.2179	not significant
	Pure Error	1367	4	342			
	Cor Total	110600	16				
	R^2	0.966		Adeq. Precision	18.246		
MEF	Model	598900	9	66544	18.85	0.0004	significant
	A	18832	1	18832	5.33	0.0542	
	B	34975	1	34975	9.91	0.0162	
	C	7619	1	7619	2.16	0.1853	
	A2	13998	1	13998	3.97	0.0867	
	B2	371600	1	371600	105.26	< 0.0001	
	C2	1492	1	1492	0.42	0.5364	
	AB	2552	1	2552	0.72	0.4234	
	AC	133500	1	133500	37.82	0.0005	
	BC	787	1	787	0.22	0.6512	
	Residual	24713	7	3530			
	Lack of Fit	12382	3	4127	1.34	0.3799	not significant
	Pure Error	12331	4	3083			
	Cor Total	623600	16				
	R^2	0.960		Adeq. Precision	13.819		

137   Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

Figure 4. Relationship between predicted vs actual values for (a) NAP, (b) DIC and (c) MEF

Figure 5. Pareto chart of percentage effects of parameters of single factors, squared factors and interaction factors for the optimization of Ch-TEOS-DSP-HPLC/UV analysis of NSAIDs.
A: Extraction time, B: pH of sample, C: desorption time

A comparison of single factors (Figure 5) showed that the sample pH, B, had the most impact on the extraction efficiencies for all NSAIDs at 1.4-63.5%, followed by extraction time, A (1.0-26.7%), and finally desorption time, C (0.3-1.3%). This behaviour was expected because previous studies have shown that the sample pH influences the ionization of analytes, allowing their effective adsorption onto the adsorbent. Furthermore, the low pKa values of NSAIDs cause them to adopt a predominantly neutral form at low pH and become protonated at relatively higher pH [11].

In this work, the hyperbolic contour plots for NAP and DIC extraction efficiencies (Figure 6(a) and (b)) indicate a slight interaction between extraction time and sample pH. Higher extraction times and lower pH values yielded the highest extraction efficiencies. However, extraction time affected the outcome more than pH, which is

consistent with a previous report that showed how extraction time had an appreciable role in controlling mass transfer between phases [28]. Although extraction efficiency tends to increase with prolonged extraction time, extending the extraction time is not always practical. An appropriate length of time must be chosen to maximize the merits of the microextraction technique. In contrast, the half-cylindrical contour plot of MEF (Figure 6 (c)) implied that only pH significantly (p value < 0.05) affected the extraction efficiency. The variation in sample pH results in the variation of surface charge, thus affecting the adsorption efficiencies of the analytes on the sorbent. From this study, it was found that a better absorption of NSAIDs by the sorbent could be achieved at pH 4, which is probably due to the surface of Ch-TEOS being protonated under acidic conditions, allowing for electronic interactions while maintaining hydrophobic interactions towards analytes. [29].

138   Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
      Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
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Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples
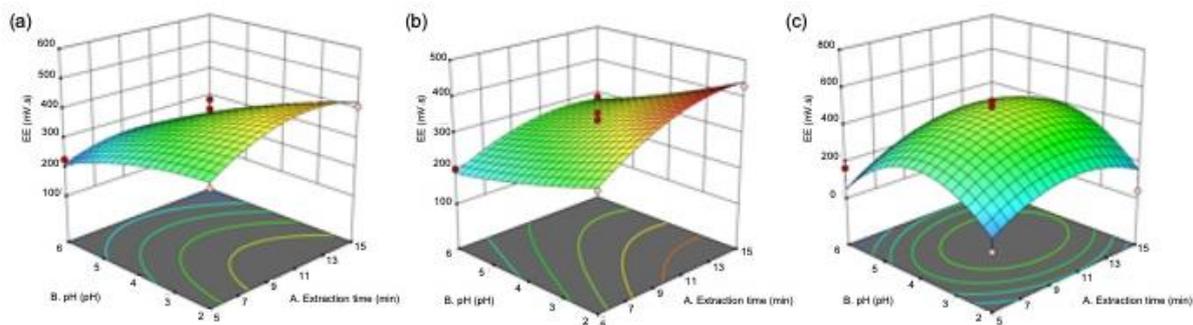
Figure 6. Response surface and contour plots of the effect of pH and extraction time on the Ch-TEOS-DSPE-HPLC/UV extraction efficiency (EE) for (a) NAP, (b) DIC, and (c) MEF
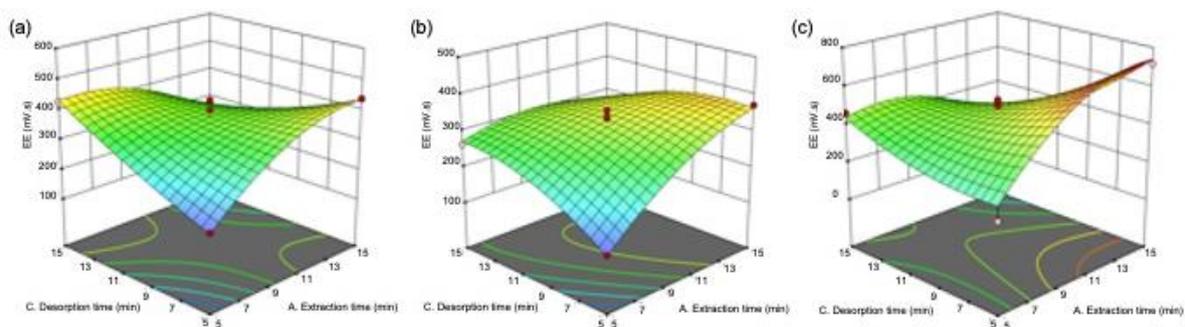
Figure 7 shows contour plots of the interactions between (C) desorption time versus (B) pH of the sample for the three analytes. The slanting planar contour plots for NAP and DIC extraction efficiencies showed slight interactions between factors. Meanwhile, the effect of desorption time (F value = 0.7) was appreciably less dominant than sample pH (F value = 82.8), based on the larger F value of the latter. The peak areas of NAP and DIC significantly increased with desorption time and decreased with the increase in sample pH, which suggests that, in the latter, the optimal threshold of the two parameters has been exceeded. Conversely, the half-cylindrical plot of MEF extraction efficiency showed that only sample pH influenced extraction efficiency.

For the comparison of interaction factors (Figure 5), the highest effect on overall extraction efficiency (12.7%) occurred when extraction time (A) mutually interacted with desorption time (C).

Meanwhile, the interactions between sample pH (B) and extraction time (A) or desorption time (C) only mildly affected the extraction efficiency of NSAIDs (up to 2.5%). Figure 8 illustrates the curved planar-shaped plot for the mutual interaction between extraction time (A) and desorption time (C), being crucial to maximizing the NSAIDs extraction efficiency. The extraction efficiency increased proportionally with increasing extraction time (A) and decreasing desorption time (C). It slightly declined for extraction times of > 13 min, which correlates well with their significant interaction as shown in the ANOVA table (p value 0.001) (Table 3). The extended extraction time had likely elevated the system's temperature during the ultrasonication desorption and partially degraded some analytes [21]. From the ANOVA table and contour plot obtained from this study, the order in which the three tested parameters affected the extraction efficiency of NSAIDs is as follows: pH of sample > extraction time > desorption time.
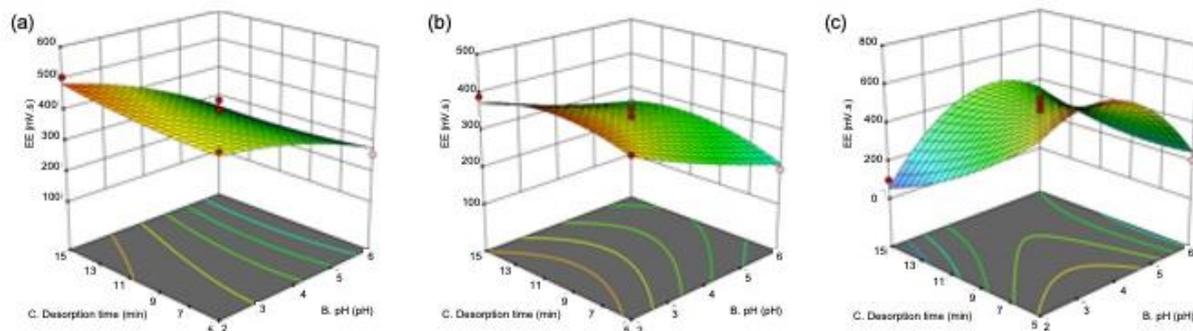
Figure 7. Response surface and contour plots of the effect of pH and desorption time on the Ch-TEOS-DSPE-HPLC/UV extraction efficiency (EE) for (a) NAP, (b) DIC, and (c) MEF

139 Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

Figure 8. Response surface and contour plots of the effect of extraction time and desorption time on the Ch-TEOS-DSPE-HPLC/UV extraction efficiency (EE) for (a) NAP, (b) DIC and (c) MEF

Validation of Ch-TEOS-DSPE-HPLC/UV Method

The DSPE optimization experiment was validated to check for the generated model's reliability to establish the best conditions. Based on the BBD study, the optimum conditions for Ch-TEOS-DSPE-HPLC/UV were 14.05 min extraction time at pH 4.13 with a 5 min desorption time, while holding other variables constant (0.05 g sorbent loading, vortex speed at 450 rpm, and 100 µL acetonitrile as desorption solvent). The chromatogram revealed that all analytes were successfully extracted and separated from both water samples with retention times < 13 min (Figure 9).
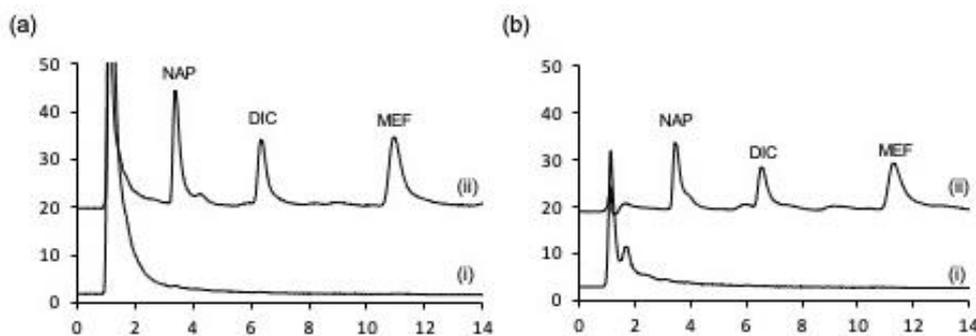
Figure 9. Chromatograms of (i) blank and (ii) spiked analytes in (a) tap water and (b) lake water under optimal conditions.

Table 4. Validation of Ch-TEOS-DSPE-HPLC/UV method

Samples	Analyte	Linearity range ($\mu g L^{-1}$)	r^2	LOD ($\mu g L^{-1}$)	LOQ ($\mu g L^{-1}$)	Spike concentration ($\mu g L^{-1}$)	Average relative recovery %	RSD %
Tap water	NAP	0.1-100	0.998	0.04	0.15	1	97.94	2.82
						80	97.24	4.86
	DIC	1-500	0.999	0.52	2.07	10	98.10	2.27
						400	100.23	3.20
	MEF	1-500	0.998	0.41	1.62	10	96.16	5.65
						400	101.40	2.42
Lake water	NAP	0.1-100	0.999	0.05	0.20	1	93.06	7.75
						80	101.48	3.35
	DIC	1-500	0.998	0.67	2.78	10	97.24	7.47
						400	97.77	4.85
	MEF	1-500	0.998	0.59	2.44	10	94.05	5.61
						400	97.66	2.27

140   Nor Suhaila Mohamad Hanapi, Nur Husna Zainal
Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada
Mohamad Tahiruddin, Nurul Auni Zainal Abidin,
Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based
Microextraction Combined with High-
Performance Liquid Chromatography for
NSAIDs in Aqueous Samples

Table 5. Comparison of the Ch-TEOS- DSPE-HPLC/UV method with other published methods for the analysis of NSAIDs in aqueous samples

Method	Instrument	Type of sorbent	Sorbent amount (mg)	LOD (μgL^{-1})	Extraction time (min)	Ref.
SPE	LC/MS	MCM-41	100.0	0.10 - 3.85	-	[30]
MA-µ-SPE	HPLC/DAD	UPS	20.0	0.48 - 1.79	60	[31]
MSPE	HPLC/UV	Fe_3O_4-polypyrole	20.0	0.9 – 3.5	2.4	[11]
DSPE	HPLC/UV	Ch-TEOS	50.0	0.04 – 0.67	14	This work

Abbreviations: SPE = solid phase extraction, MA-µ-SPE = membrane assisted micro-solid phase extraction, DSPE = dispersive solid phase extraction, MCM-41 = mesoporous material 41, UPS = ureidopropyl-grafted silica gel, Ch-TEOS = chitosan-tetraethoxysilane hybrid composite.

The plotted calibration curves based on peak area response vs analyte concentration were also linear ($r^2 \geq 0.998$), ranging between 0.1-100 µg L^{-1} (NAP) and 1–500 µg L^{-1}(DIC and MEF) (Table 4). The limit of detection (calculated based on a signal-to-noise ratio of 3:1) ranged between 0.04-0.67 μgL^{-1} and yielded excellent LOQs (0.15-2.78 μgL^{-1}). The subsequent recovery study performed using spiked water samples (n = 3) gave final concentrations of 1 and 80 μgL^{-1} for NAP, and 10 and 400 μgL^{-1} for DIC and MEF. Excellent recovery values of 93.06 to 101.48% were obtained at an acceptable reproducibility (relative standard deviation of < 7.75%). The results revealed that this method could be applied in therapeutic drug monitoring and environmental water analysis.

Table 5 summarizes the comparisons between the chromatographic method, types of silica-based sorbent used, and LODs to determine NSAIDs in aqueous samples. The LOD value obtained from this work (0.04 – 0.67 μgL^{-1}) was found to be comparable with the SPE technique using the most precise LC/MS detection [30], and was even lower than MSPE technique using similar LC/UV detector [11]. In addition, this method required a lower extraction time (14 min) compared to the membrane assisted micro-solid phase extraction (MA-µ-SPE) technique [31] with a silica-based sorbent. Therefore, the developed Ch-TEOS-DSPE-HPLC/UV extraction method with the aid of Box Behnken design optimization has excellent potential for analysing non-steroidal anti-inflammatory drugs in aqueous samples.

CONCLUSION

The Box Benkhen design was successfully utilized with Ch-TEOS-DSPE-HPLC/UV to determine the maximum extraction efficiency of three selected non-steroidal anti-inflammatory drugs in water samples. The most significant parameters involved were sample pH > extraction time > desorption time. Optimum conditions were successfully applied to extract

naproxen, diclofenac and mefenamic acid from tap water and lake water. Good detection values at parts per billion levels (0.04-0.67 µgL^{-1}), good reproducibility and excellent recoveries (93.06 - 101.48%) were achieved using the proposed method. It can be concluded that the hybridizing high surface area nanoparticles of silica and biodegradable chitosan with high-loading functional groups increased the sorbent's surface properties and active sites. Thus, the proposed method presents several advantages such as simplicity, minimal use of sorbent, ease of operation, and low consumption of solvent, and thus can be a valuable tool to determine NSAIDs in aqueous matrices.

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Bio-Nanohybrid Composite Sorbent-Based Microextraction Combined with High-Performance Liquid Chromatography for NSAIDs in Aqueous Samples

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142 Nor Suhaila Mohamad Hanapi, Nur Husna Zainal Abidin, Wan Nazihah Wan Ibrahim, Nordiana Suhada Mohamad Tahiruddin, Nurul Auni Zainal Abidin, Roswanira Abdul Wahab and Sheela Chandren

Bio-Nanohybrid Composite Sorbent-Based Microextraction Combined with High-Performance Liquid Chromatography for NSAIDs in Aqueous Samples

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