Selective Determination of Acidic Drugs in Water Samples Using Online Solid Phase Extraction Liquid Chromatography with Alginate Incorporated Multi-Walled Carbon Nanotubes as Extraction Sorbent

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Abstract: *A rapid and effective method is developed for selective determination of five selected acidic drugs (salicylic acid, naproxen, diclofenac, ibuprofen and mefenamic acid) in water samples by using online solid phase extraction (Online-SPE) prior to liquid chromatography diode array detector (LC-DAD) analysis. In this study, Alginate incorporated multi-walled carbon nanotubes (Alg-MWCNT) beads were prepared and utilized as solid phase extraction sorbent. Optimization of online SPE-LC operating parameters such as valve switching time, composition of acetonitrile and buffer pH was conducted using Box-Behnken Design of Response Surface Methodology (RSM) to evaluate the interactive effects of these three variables. Under the optimized conditions (valve switching time: 1.5 min, composition of acetonitrile: MSA, 60:40 and buffer pH: pH 2), the method showed good linearity (1–500 μg L^{-1}) with coefficient of determination (R^2) of 0.9971–0.9996 and low limits of detection ≤ 0.018 μg L^{-1}. The method showed high relative recoveries in the range of 75–110% for river water and tap water samples, respectively with RSDs of ≤ 7.8 (n = 3). This method was successfully applied to the determination of acidic drugs in river and tap water samples. In addition, Alg-MWCNT sorbent offered high degree of selectivity and efficiency for online SPE-LC-DAD analysis.*

Keywords: *online SPE-LC; alginate; MWCNTs; acidic drugs; water samples*

■ INTRODUCTION

One of the trends in drug analysis nowadays is the development of rapid, highly efficient, as well as sensitive and reliable method for the identification and quantification of analytes in various matrices. Recent developments and improvements in analytical methodologies and advanced instruments have made the detection of pharmaceutical drugs at low concentration level possible. As a result of this advancement, pharmaceutical residues can be detected in various environmental samples and recognized as an 'emerging' contaminant [1].

In the last few decades, pharmaceutical drugs have played an increasingly important role in improving the quality of human life. Thousand tonnes of pharmaceuticals are consumed each year to treat and prevent illnesses [2]. However, the increasing consumption may lead to an accumulation of unused medicines in the household area. Nearly one-third of the pharmaceuticals sold annually are unused [3]. This situation may put human and animal health at risk due to accidental ingestion or exploitation of unused medicine.

Non-steroidal anti-inflammatory drugs (NSAIDs) can be present in water supplies, either from domestic or industrial waste water discharges. The common method used for analyzing these pharmaceuticals in trace level is chromatography, either gas chromatography or liquid chromatography coupled with sensitive detection technique which is mass spectrometry [4-8]. However,

the matrix effects and the selection of internal standards should be taken into consideration [9]. Even though the concentrations of NSAIDs detected in a surface or ground water are in a range of ng L^{-1}, they persist for longer periods of time in soils and sediments [10]. Therefore, the challenge remains in reaching these levels of detection using a method that is affordable, simple and yields reliable results.

In this study, a method of using online solid phase extraction liquid chromatography (online SPE-LC) for the separation of five selected pharmaceutical drugs was proposed. Quantitative, non-destructive and time saving are some criteria of an ideal extraction system [11]. Solid phase extraction (SPE) is the most common method used for the extraction of pharmaceutical drugs in water samples. SPE has been claimed as an effective sample preparation method for removal of interfering compounds and enrichment of analytes [12]. Over time, the SPE technique not only can be performed in the off-line mode but also provides the possibility of online coupling to other analytical steps, such as chromatographic analysis. The application of online coupling with chromatographic analysis may shorten the analysis time, reduce sample contamination and analyte loss, as well as improve the precision and accuracy [13].

Carbon nanotubes (CNTs) possess advantages in adsorbent-adsorbate interactions. On the other hand, this potential sorbent has high production cost, involves non-biodegradable materials and leads to some environmental issues. Meanwhile, alginate is a potential bio-sorbent for the removal of pharmaceutical drugs from water sample. However, it is readily soluble in aqueous media due to its hydrophilic nature which limits its application as an adsorbent.

Hence, this research pursues the potential usage of MWCNTs incorporated with alginate as a promising material to overcome both weaknesses with the aid of online-SPE-LC which is a simpler, more rapid and efficient method compared to the traditional offline SPE.

■ EXPERIMENTAL SECTION

Materials

Ibuprofen, naproxen, diclofenac, mefenamic acid and salicylic acid were purchased from Sigma-Aldrich (purity assay in range of 98–101%). Acetonitrile (ACN) and methanol (MeOH) of high performance liquid chromatography (HPLC) grade was obtained from Merck (Darmstadt, Germany). Calcium chloride ($CaCl_2$) was obtained from HmbG Chemicals (Germany) and methanesulfonic acid (MSA) was procured from Sigma-Aldrich (St. Louis, USA). Ultrapure water was produced by Barnstead Nanopure (Thermo Scientific). Sodium hydroxide and hydrochloric acid were obtained from Merck, Darmstadt, Germany and sodium alginate from Qrec (New Zealand). A multi-walled carbon nanotubes (MWCNTs) with specific surface area > 233 m^2/g, purity of > 95%, 8–15 nm outer diameter × 50 μm in length was purchased from Sun Nanotech (Jiangxi, China).

Procedure

Preparation of standard and sample solutions

The individual stock solution (1000 mg mL^{-1}) of ibuprofen (IBU), naproxen (NAP), diclofenac (DIC), mefenamic acid (MEF) and salicylic acid (SAL) were prepared separately in methanol. All standard solutions were stored in the amber glass bottle at 4 °C when not in use. A series of working standard solutions were prepared in methanol by dilution prior to analysis to prevent from decomposition of analytes.

The water samples were collected in bottles, pre-cleaned with acetone and filtered through a nylon membrane filter to remove colloidal particles and stored in a freezer at 4 °C until analysis commenced. The tap water and river water samples (10 mL, pH 3) were spiked with the standard solution of five pharmaceuticals NSAIDs mixture to give a final concentration of 0.1 μg mL^{-1} for each analyte.

Preparation of Alg-MWCNT sorbent

The preparation method for composite beads was adapted and modified from literature [14-15]. The composite beads were formed by suspension technique. Initially, 3% (w/v) of sodium alginate solution was prepared under stirring at 60 °C. Meanwhile, 0.3 g of MWCNT was dispersed in 30 mL deionized water under sonication for 30 min. Then, MWCNT was added into the sodium alginate solution and sonicated for another

1 h. The mixture solution was dripped through an injection needle into 1000 mL of 4% (w/v) of calcium chloride solution. The alginate-MWCNT beads were formed upon contact with calcium ions. After removal from the calcium chloride bath, the beads were rinsed thoroughly with deionized water using 11 µm filter paper and dried in an oven at 50 °C for 24 h. The dried Alg-MWCNT beads (0.3 g) were packed in an online SPE empty column, ready to be used for analysis.

The alginate-MWCNT beads were then characterized using Fourier Transformed Infrared (FTIR) spectroscopy (Thermo Fischer Scientific) in the range of 4000–400 cm^{-1} using Attenuated Total Reflection (ATR) technique with diamond as the ATR crystal. This method was used to determine the surface chemistry and components of the biopolymer composite beads. The functional groups were determined based on specific broad bands observed. Surface morphology of the Alg-MWCNT sorbent was analyzed using an ultra-high resolution field emission electron microscope (FESEM) model Zeiss Supra 40VP with 10K magnification. Nitrogen adsorption and desorption isotherm of the adsorbent were measured at -196 °C using BELSORP-mini II instrument (BEL. Japan Inc.). Prior to measurements, the samples were degassed at 70 °C for 24 h in a vacuum oven. The isotherm was further analyzed using Brunauer-Emmett-Teller (BET) method to determine the specific surface area, total pore volume and average pore diameter of the sorbent.

Online solid phase extraction system

All analyses were performed using an automated high performance liquid chromatography Dionex Ultimate 3000 (Sunnyvale, CA, USA) system. The system comprises of a large volume loop (10.2 mL) autosampler, dual gradient pump, left and right, a solvent rack with an integrated vacuum degasser, a thermostat column compartment, two columns (online SPE column and analytical column) and a diode array detector (DAD).

The analytical column was an Acclaim Polar Advantage II (5 µm, 120 Å, 4.6 × 150 mm) (Thermo Scientific USA) and the online SPE clean-up was performed using alginate incorporated multi-walled carbon nanotube biopolymer sorbent packed in an empty online SPE column. The system was equipped with a programmable 6 port/2 position switching valve for several modes (loading, clean-up, elution and separation). Data were processed by the Chromeleon™ Software v.6.8 (Dionex). Fig. 1 shows the schematics of the online SPE-LC system.

The method comprises three steps which are sample loading, clean-up, elution and LC separation. Both pumps ran simultaneously. The flow rate was set at 1 mL min^{-1} throughout the analysis with a temperature of 40 °C.

At equilibrium mode, 10 µL of spiked water sample was loaded onto the flow system using auto-sampler (fitted with a 100 µL syringe). SPE column was then positioned into the loading mode using the switching valve. The left pump was used to load sample from the sample loop onto the SPE column at 1 mL min^{-1} and simultaneously the analytical column was equilibrated with the right pump. Next, the co-retained sample matrix was flushed out using a washing composition of 10 mM MSA and ACN (95:5) while analytes were retained on the SPE column. During the cleanup process, the choice of mobile phases and time of switching valve are important factors that influence the extraction recovery [16]. After sample loading, the valve was switched to clean-up using the clean-up mobile phase (ACN: 10 mM MSA, 5:95 (v/v) and kept for 1.0 min to remove any possible impurities retained together with analytes.

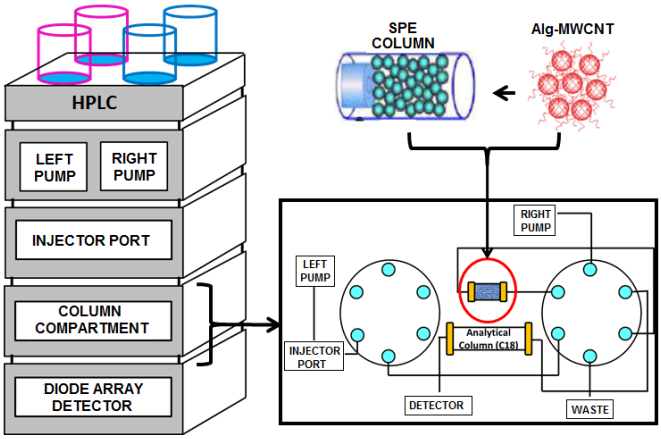

Fig 1. Schematic Diagram for Alg-MWCNT-Online-SPE-LC

In the elution step, the switching valve was switched into the elution position which couples the SPE column with the analytical column. The analytes were transferred using gradient elution mobile phase composition. Lastly, in the separation step the switching valve was switched back into equilibration mode and disconnected the SPE column from the analytical column. In this mode, analytes continued separating in the analytical column using the right pump. In the left pump, the SPE column was equilibrated prior to the next sample being loaded into the sample loop.

Detection method

All analytes were simultaneously analyzed using diode array detector (DAD) at various wavelengths. The DAD was set at 230 nm. Identification of NSAIDs was based on retention time and ultraviolet (UV) spectrum of each analyte.

■ RESULTS AND DISCUSSION

Physical Properties of the Prepared Alg-MWCNT Beads

Before the drying process, the shape of the composite beads formed were spherical with the turgidity coming from the formation of bivalent ions when alginate comes in contact with calcium ions [17]. Approximated size of the bead was ~2 mm in diameter. After the drying process, the composite beads shrunk by half (~1 mm in diameter) from the original size of the beads before drying as the water content in the beads had been removed through evaporation.

Characterization of Alg-MWCNT Sorbent by FESEM Analysis

Fig. 2(a-c) show the FESEM images of alginate, MWCNTs and Alg-MWCNT. Surface structure of alginate (Fig. 2(a)) under the magnification of 10 K shows a very smooth and clear surface. Meanwhile, the structure of MWCNT (Fig. 2(b)) under the same magnification shows some ball-of-string-like structure clumping together in random manner. It was also noticed that there were numerous entangled carbon nanotubes observed. The structure of the synthesized Alg-MWCNT (Fig. 2(c)) under the same magnification showed that the clumps of MWCNTs have been deposited onto the surface of the alginate. From the micrograph, it can be suggested that the MWCNTs particles had bound around the alginate surface [18].

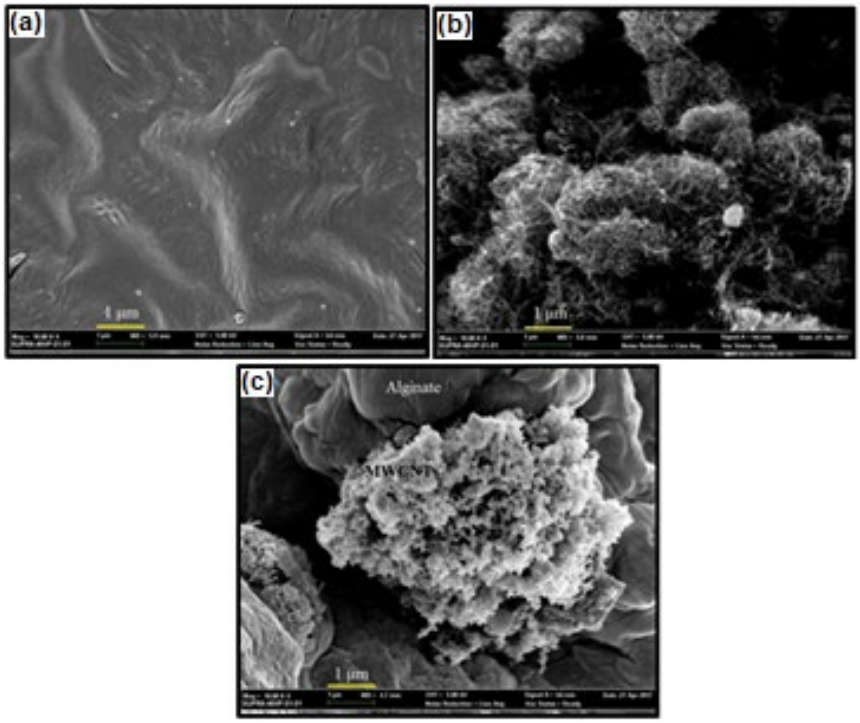

Fig 2. FESEM Micrographs of (a) Alginate (b) MWCNTs (c) Alg-MWCNT under 10 K Magnification

Characterization of Alg-MWCNT Sorbent by FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to investigate functionalized spectra of sodium alginate, multi-walled carbon nanotubes (MWCNTs) and alginate-MWCNTs. The results for FTIR spectroscopy were recorded and compared. Fig. 3(a) shows the spectra for the three compounds.

Spectrum of sodium alginate showed important absorption bands corresponding to hydroxyl, ether, and carboxylic functional groups. Stretching vibrations of O–H bonds of alginate appeared in a very broad band at 3258 cm^{-1}. Observed bands in 1594 and 1407 cm^{-1} were attributed to the stretching vibrations of carbon-oxygen double bond and carbon-carbon double bond, respectively. Meanwhile, the band at 1026 cm^{-1} could be attributed to the C-O stretching vibration.

On the other hand, the IR spectrum of carboxylated multi-walled carbon nanotubes (MWCNTs) displays some unresolved peaks due to the low transparency of CNT that caused it difficult for IR ray to penetrate and be absorbed into the sample. A broad peak appeared at 3305 cm^{-1} corresponding to the O–H bond stretch of carboxylic acid functional group of MWCNTs. A peak appeared at 1772 cm^{-1} which represents C=O from carboxylate group. Peaks at 1645 and 1405 cm^{-1} suggest that the compound has a benzene ring which is the main structure of MWCNTs.

The FTIR spectrum of alginate incorporated multi-walled carbon nanotube shows slightly different peaks as compared to the alginate spectrum. It is suggested that the hydroxyl group present at 3258 cm^{-1} in the alginate spectra had been shifted to 3353 cm^{-1} in the Alg-MWCNT spectra and the peak is broader than in the alginate spectra, indicating that the carboxyl group of MWCNT and the carboxyl group from sodium alginate were cross-linked synchronously with calcium ions [19]. Apart from that, this also shows that the alginate was cross-linked by the aid of calcium chloride solution [20].

The adsorption bands for carbon-oxygen double bond and carbon-carbon double bond were shifted from 1594 and 1407 cm^{-1} to 1601 and 1423 cm^{-1}, respectively. It was also observed that the carbon-oxygen single bond band at 1026 cm^{-1} in the alginate spectra was less intense in the Alg-MWCNT spectra, and the peak was shifted to 1010 cm^{-1}. This suggests that there could be substitution reaction that may have occurred during the formation of Alg-MWCNT [21].

Isotherm and Surface Area Analysis of Alg-MWCNT Sorbent

Nitrogen adsorption and desorption isotherm of the adsorbent were measured at -196 °C using BELSORP-mini II instrument (BEL. Japan Inc.). Prior to these measurements, the samples were degassed at 70 °C for 24 h in a vacuum oven. The isotherm was further analyzed using Brunauer-Emmett-Teller (BET) method

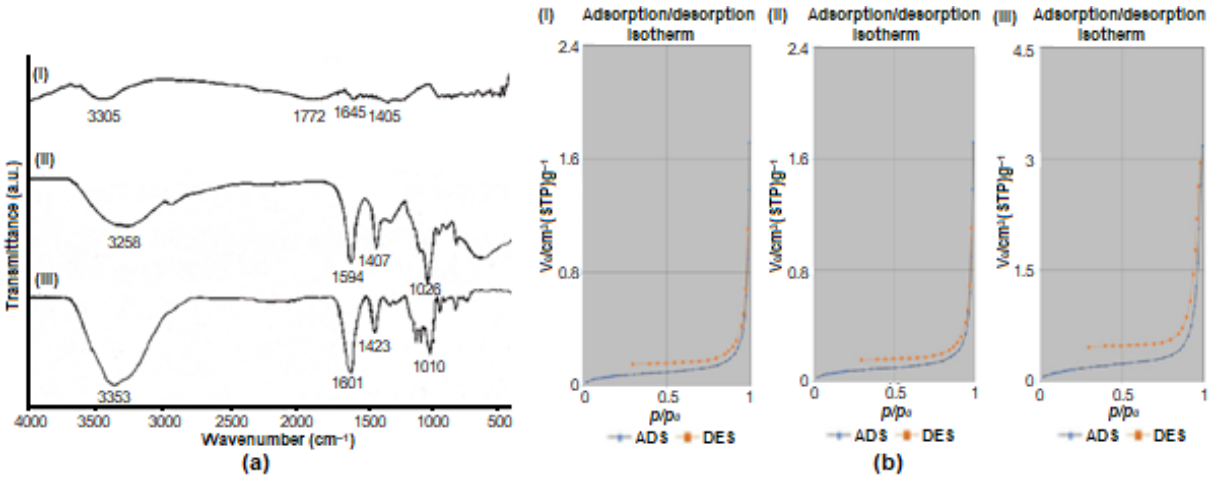

Fig 3. (a) Infrared spectra of (i) MWCNTs, (ii) Alginate and (iii) Alg-MWCNT and (b) Brunauer Emmet Teller (BET) analysis of (i) Alginate, (ii) MWCNTs and (iii) Alg-MWCNT bead

to identify the specific surface area, total pore volume, and average pore diameter of the adsorbent. The porosity and surface area of pristine alginate, MWCNTs and Alg-MWCNT beads were determined by BET. The BET analysis provides the value of specific surface area, total pore volume and average pore diameter while pore analysis provides information on the pore distribution of the adsorbents. Nitrogen adsorption-desorption isotherm of pristine alginate, MWCNTs and Alg-MWCNT (Fig. 3(b)) at 77 K shows that they possess Types IV hysteresis loop which indicates a mesoporous to macroporous adsorbent with strong affinities towards adsorbate [22].

Table 1 shows the specific surface area, total pore volume and average pore diameter obtained from BET analysis. The results show that the pristine alginate has lower surface value than MWCNTs. The results show that the incorporation of MWCNTs in alginate matrix increases the surface area, and total pore volume of the Alg-MWCNT beads. The low surface area for Alg-MWCNT is probably due to the low amount of MWCNTs used in the composition of beads (3%).

Sorbent-Sorbate Interactions between Alg-MWCNT with Target Analytes

The possible interaction between the sorbents (alginate and MWCNTs) and the interaction between the sorbent with the five target analytes were shown in Fig. 4. Strong hydrogen bonding interactions can occur between side chains of biopolymers alginate with other molecules either with the multi-walled carbon nanotubes or with the analytes. The highly associated interaction between blended Alg-MWCNT through hydrogen bonding helped improve swelling and gelation properties of alginate while imparting mechanical strength to agar. Meanwhile, the hydrogen bonding between alginate and target analytes can contribute to the extraction efficiency. On the other hand, π-π interaction may occur between the MWCNTs and the target analytes. At lower pH, strong interactions between the non-polar aromatic bonds of targeted analytes towards the hexagonal array of graphene sheets of MWCNTs could be attributed to π-π and hydrophobic interactions [23-25].

Optimization of Online SPE-LC Parameters Using Box-Behnken Design (BBD)

Optimization for the extraction of five types of NSAIDs using online solid phase extraction yielded three optimized conditions, which are 1.5 min for valve switching time, 60% composition of acetonitrile in elution solvent composed of ACN: MSA and pH 2 for buffer with desirability 0.974. The regression equation of the fitted model is as shown in Eq. (1), where Y is the response (total peak area) of target analytes, A is the valve switching time, B is acetonitrile composition and C is buffer pH.

$$Y = 9.62 - 0.037A - 0.009B + 0.30C - 0.31A^2 - 0.30B^2 - 0.35C^2 - 0.062AB + 0.081AC - 0.060BC \quad (1)$$

The statistical significance of the terms used in the model was defined via ANOVA analysis. A multi-linear regression analysis was applied to the results of Box-Behnken design. Composition of mobile phase and valve

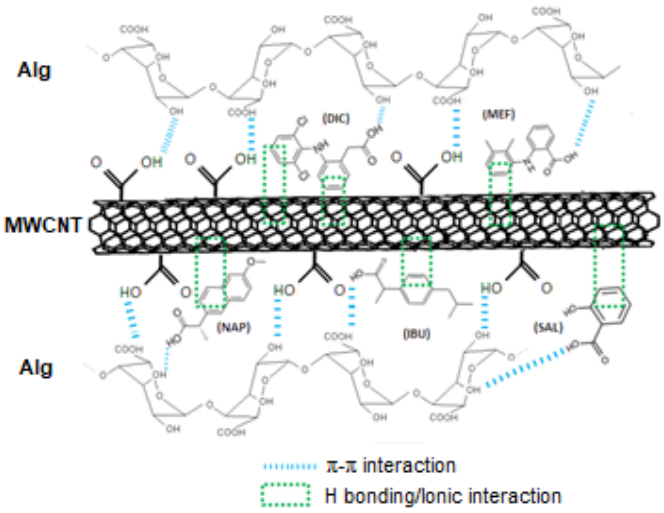

Fig 4. Sorbent-sorbate interactions between Alg-MWCNT with target analytes

Table 1. Results for BET analysis

Sample	Pristine alginate	MWCNTs	Alg-MWCNT
sBET (m^2g^{-1})	0.28363	134.14	0.62756
Total pore volume ($cm^3\ g^{-1}$)	0.0024974	1.4358	0.0048269
Average pore diameter (nm)	35.221	42.817	30.766

switching time are two significant variables for the clean-up step [26]. The effect of independent variables, which are valve switching time, acetonitrile composition, and buffer pH were evaluated by second order (quadratic). Table 2 shows the analysis of variance (ANOVA) regression model for response quadratic model for salicylic acid, naproxen, ibuprofen, diclofenac and mefenamic acid. The reliability of the fitted model was proven by the high F-values and the low P-values.

The quality of fit on the quadratic polynomial model was presented by the coefficient of determination, R^2 [27]. The value of R^2 shows that there is an acceptable relationship between the predicted and actual values (Fig. 5(a)). The closer the value of R^2 to unity, the better is the empirical model fit to the actual data. Criteria for a good fit of a model, the R^2 should be at least 0.80 [28]. The R^2 value calculated for the extraction of five NSAIDs was 0.9052, indicating a significantly good fitted model.

The main effects of the variables were visualized by Pareto chart (Fig. 5(b)). According to this figure, the acetonitrile composition affects the extraction the most. This is because, among the three parameters being investigated, acetonitrile composition is very crucial to ensure that the analyte will be well-eluted. The most commonly used organic solvents in HPLC are acetonitrile as it results in the lowest system backpressure in water mixtures and has a very low UV cut off for better UV/Vis detection sensitivity [29].

Effect of valve switching time on the peak of analytes

The right time to switch the valve is very important to ensure all the analytes have been fully extracted by the sorbent and ready for separation step. Effect of valve switching time depends on the step that has been done in the SPE column. For example, if the valve has been switched too early, the analyte would not have been fully extracted by the SPE column. Some analytes would have skipped the SPE column and directly flowed into the analytical column during the separation step. The result will either be low in recoveries or some analytes may not be eluted (Fig. 6(a)).

Effect of ACN:MSA composition on the peak of analytes

In optimizing solvent composition, various isocratic elution of acetonitrile and 10 mM MSA were studied. Each analyte were eluted by increasing the strength of elution solvent. The increase in acetonitrile composition means that the elution strength of the

Table 2. Summary of ANOVA analysis of NSAIDs

Transform	Model	Lack of fit	DF	R-square	Equation
Square Root	Quadratic Significant	Not significant	9	0.9052	Sqrt (Total Peak Area) = $9.62 - 0.037A - 0.009B + 0.30C - 0.31A^2 - 0.30B^2$ $- 0.35C^2 - 0.062AB + 0.081AC - 0.060BC$

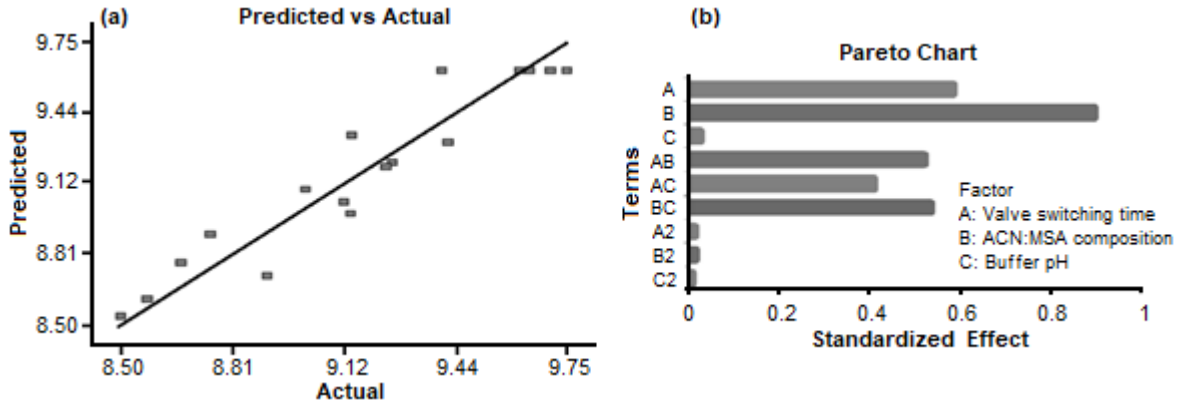

Fig 5. (a) The parity plot between predicted and actual (experimental) values for all analytes, (b) Pareto chart of the standardized effects in Alg-MWCNT online SPE-LC

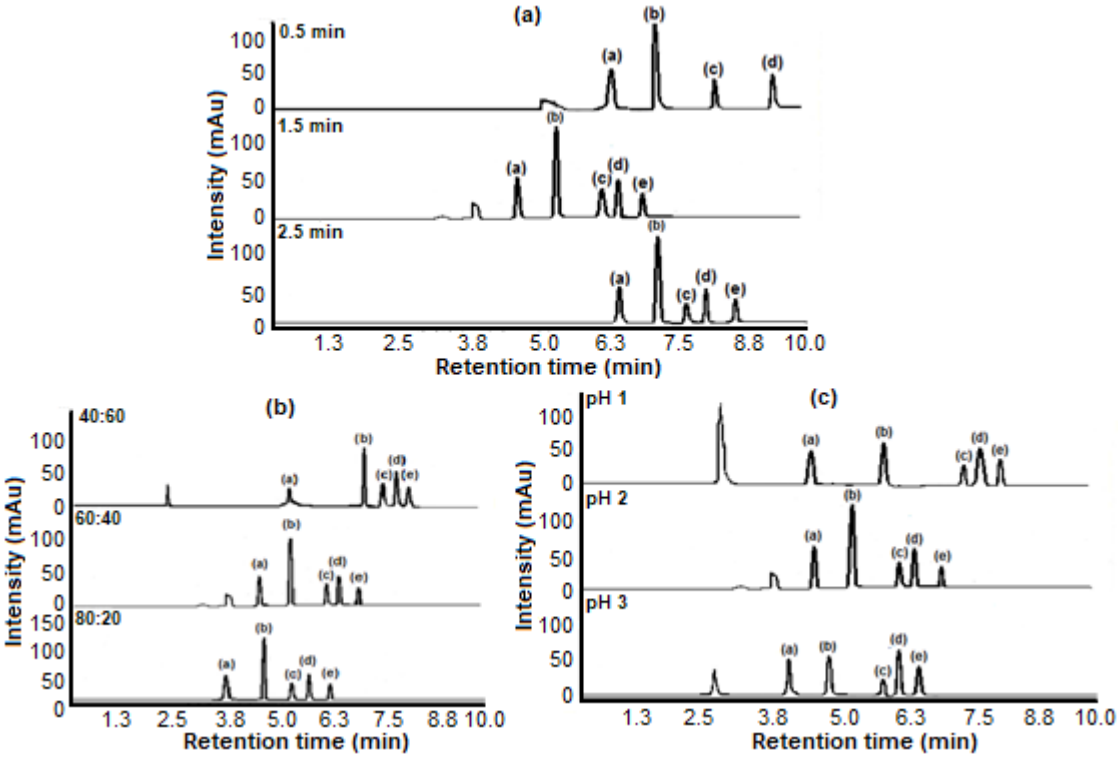

Fig 6. Effect of (a) Valve switching time, (b) ACN:MSA composition and (c) Buffer pH on the peak of analytes, (a) salicylic acid, (b) naproxen, (c) diclofenac, (d) ibuprofen and (e) mefenamic acid

retained analyte on the sorbent will be increased. All of the analytes were eluted from the column within 60–70% of ACN. A decrease in composition of 10 mM MSA with increasing ACN composition, improved the separation of the NSAIDs. Fig. 6(b) shows that the increase in the strength of the elution solvent (ACN:MSA) is proportional to the total peak area of the analytes.

Effect of buffer pH on the peak of analytes

Buffering is commonly needed when analyzing ionizable analytes with reversed phase LC. The ionized species always elute from the column earlier. Sometimes buffer is needed because some impurities are ionizable. The most suitable buffer pH for acidic analyte is at least two units below the pKa of the analyte. As shown in Fig. 6(c), at pH 2, the peak area of analytes were higher compared to pH 1. However, the peak area of analytes at pH 3 decreased as there could be some analytes that were still in ionized form and combined with the impurities [30].

Response Contour Plot

Fig. 7(a) shows the three-dimensional contour plot for the valve switching time and the buffer pH against the total peak area of the analytes. Meanwhile, the third parameter, which is composition of acetonitrile were kept at a mid-constant level: 60%. As observed, the hyperbolic contour plot shows that both buffer pH and valve switching time were correlated to one another. The optimal extraction efficiencies were observed near the middle of the contour plot at medium buffer pH and valve switching time which was located within the experimental region.

Fig. 7(b) shows the three-dimensional graph plot for the composition of acetonitrile and the buffer pH against the total peak area of the analytes. The third parameter which was valve switching time was kept at a mid-constant level: 1.5 min. At lower pH, strong interaction between the non-polar aromatic bonds of the targeted analytes toward the hexagonal arrays of graphene sheets of MWCNT could be attributed to π-π and van hydrophobic interactions [31].

A three-dimensional graph was plotted for the valve switching time and acetonitrile composition against

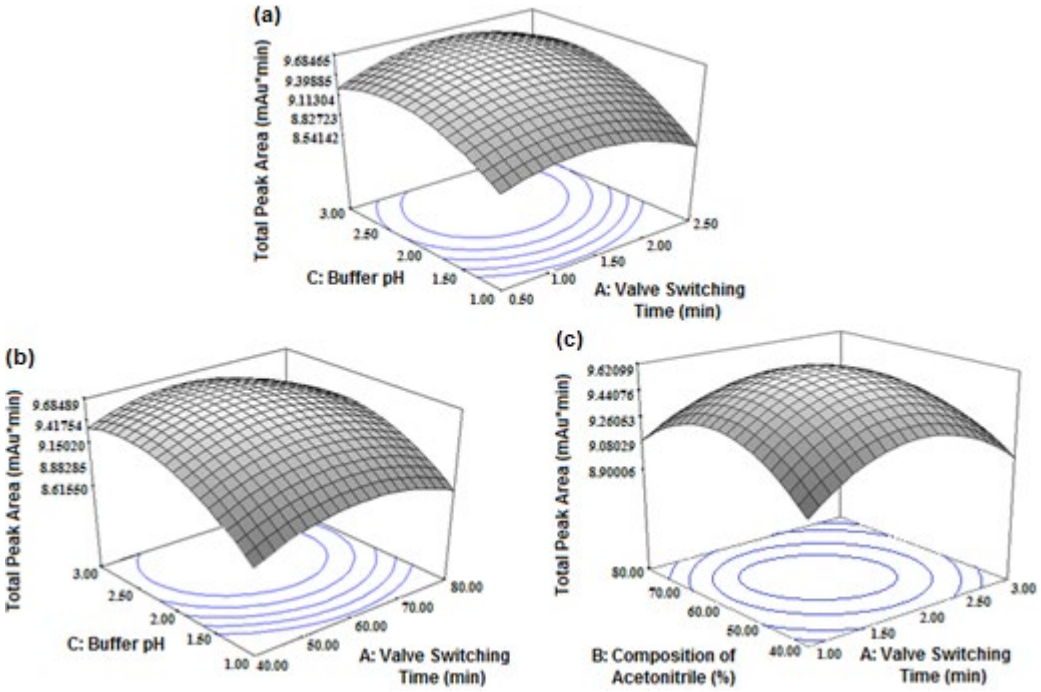

Fig 7. RSM 3-D contour plots for Alg-MWCNT-online SPE-LC. Legends: (a) pH buffer and valve switching time (b) pH buffer and ACN composition (c) ACN composition and valve switching time against peak area of analytes

the total peak area of the analytes, as shown in Fig. 7(c). The third parameter which was pH buffer was kept at a mid-constant level that is pH 2.0. It suggests that the optimum valve switching time is at 1.5 min and optimum acetonitrile composition is at 60%. With increase in the strength of the elution solvent, there is an increase in the total peak area of analytes. At one level, the high acetonitrile composition and the total peak area was maintained and gradually started to decrease. It could be due to the 60% acetonitrile composition which provides the most suitable polarity strength for the analyte to be eluted.

Method Validation and Analytical Performance of Online SPE-LC

The optimization of online SPE-LC method was then validated for relative recoveries, sample calibration, and Limit of Detection (LOD). A calibration curve was generated using five concentrations of standard mixture in the range of 1 to 500 μg L^{-1} with three replicates. Linear curves for each analyte were obtained with a good

Table 3. Validation data of Alg-MWCNT-online SPE-LC method of NSAIDs in tap and river water samples

Sample	Analytes	Linear range (μg L^{-1})	Coefficient of determination, R^2	LOD (μg L^{-1})	Precision (RSD, %) (n = 3)
Tap water	Salicylic acid	1–500	0.9993	0.0183	4.9
	Naproxen	1–500	0.9975	0.0118	5.0
	Diclofenac	1–500	0.9991	0.0062	1.4
	Ibuprofen	1–500	0.9996	0.0075	3.4
	Mefenamic acid	1–500	0.9971	0.0141	3.8
River water	Salicylic acid	1–500	0.9984	0.0152	0.9
	Naproxen	1–500	0.9992	0.0159	6.8
	Diclofenac	1–500	0.9978	0.0176	1.7
	Ibuprofen	1–500	0.9995	0.0144	2.5
	Mefenamic acid	1–500	0.9983	0.0095	2.1

correlation coefficient (R^2 = 0.9971–0.9996). The sensitivity of the method expressed as Limit of Detection (LOD) was calculated at a signal to noise ratio of 3 based on linear regression method and the results were in the range of 0.0062–0.0183 μg L^{-1} for LODs. This proved that LOD for online SPE-LC method is much lower than traditional SPE method [32]. Table 3 shows the validation data for online SPE-LC of NSAIDs from water sample.

Application of Online SPE-LC on River Water and Tap Water Samples

To investigate the practicality of the proposed method for the analysis of acidic drugs in real samples, the method was applied to analyze salicylic acid, ibuprofen, naproxen, diclofenac and mefenamic acid in the tap and river water samples (Fig. 8). However, there was no peak detected for naproxen, ibuprofen, diclofenac and mefenamic acid in the samples. Percentage recovery study was conducted by spiking river water samples to give final concentration of 50 and 100 μg L^{-1}. The results showed that good percentage recoveries were obtained in the range of 75 to 110% (Table 4) [33]. Thus, the online solid phase extraction method proved to be a simple, sensitive, and selective extraction method that complies to green chemistry, which could potentially be used in the chemical laboratory for routine analysis of water samples.

■ CONCLUSION

In this study, an online SPE-LC method by using Alg-MWCNT as sorbent has been successfully developed, for rapid analysis of five selected acidic drugs in water samples. Alg-MWCNT sorbent is expected to be an alternative green sorbent material that has a higher performance of extraction efficiency and higher selectivity rather than common SPE sorbent. An alternative online

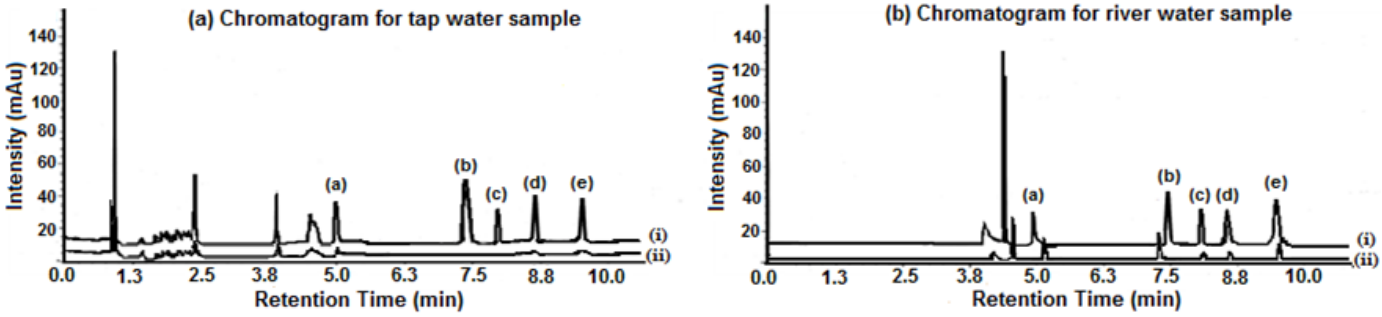

Fig 8. Chromatogram of online-SPE-LC of five NSAIDs in tap and river water samples; (i) spiked sample (ii) blank sample; (a) salicylic acid, (b) naproxen, (c) diclofenac, (d) ibuprofen and (e) mefenamic acid

Table 4. Relative recoveries (%) and method precisions (RSD %, n = 3) at two different concentrations of Alg-MWCNT-online SPE-LC in tap water and river water samples

Sample	Analyte	Average relative recovery, % (RSD, %)	
		Spiking level (n = 3)	
		50 μg L^{-1}	100 μg L^{-1}
Tap water	Salicylic acid	75 (4.5)	79 (4.6)
	Naproxen	91 (4.1)	96 (7.8)
	Diclofenac	76 (3.7)	78 (5.5)
	Ibuprofen	79 (4.1)	76 (3.8)
	Mefenamic acid	85 (3.2)	82 (5.3)
River water	Salicylic acid	91 (3.2)	104 (4.8)
	Naproxen	83 (3.9)	86 (2.7)
	Diclofenac	104 (4.3)	110 (0.9)
	Ibuprofen	79 (5.3)	83 (2.0)
	Mefenamic acid	87 (3.6)	103 (4.7)

solid phase extraction combined with liquid chromatography (SPE-LC) has shown to have improved the extraction efficiency as it shortens the extraction and analysis time. Several parameters were optimized in the online SPE method. The optimum conditions were as follows: buffer pH at pH 2, ACN: MSA composition at 60:40 and suitable valve switching time at 1.5 min. The optimum parameters were used in the analysis of real samples. All five analytes were successfully extracted using the same conditions in online SPE-LC. Good linearities were achieved for the analytes with coefficients of determination, R^2, in the range of 0.9971–0.9996. The method was successfully applied for the analysis of river water and tap water samples, with good relative recoveries in the range of 75–110%. Online SPE-LC method by using Alg-MWCNT as sorbent proved to be a rapid, selective and efficient technique for the extraction and separation of acidic drugs in aqueous matrices.

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