

DETERMINATION OF ANILINE IN WASTEWATER BY CLOUD POINT EXTRACTION FOLLOWED BY HPLC USING 8-HYDROXYQUINOLINE AS A DERIVATIZATION AGENT

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ABSTRACT

A procedure, depending on the derivatization and determination of aniline was depicted and validated in this study. 8-hydroxyquinoline (8-HQ) was used as the derivatizing agent for the determination of aniline. An optimization study was performed for the derivatization reaction, i.e., the diazonium coupling reaction, the optimum parameters were as follows: 22 mM of hydrochloric acid, 54mM of sodium hydroxide, and 1.8mM of sodium nitrate. The optimization study of the method of cloud point extraction (CPE) revealed that the extraction solvent was 0.5 ml of Triton X-100, the optimum temperature was 90 °C, and the incubation time was 25 min. The linearity, correlation coefficients, molar absorptivities, and limits of detection were improved using the CPE method. The extraction product was injected into HPLC. The chromatographic conditions were as follows: the mobile phase was acetonitrile:water 60:40, the stationary phase was C18 RP column, the detection was at visible wavelength of 420 nm, flow rate 1ml/min. The proposed method was applied for the trace detection in environmental application of wastewater. The concentration of aniline in wastewater was in range of 0.2-7 µg/l.

KEY WORDS : Aniline, Wastewater, Hydroxyquinoline

INTRODUCTION

Aniline is a chemical intermediate for many industrial uses including agricultural chemicals, polymers, rubbers, and pharmaceuticals. Both cancer and non-cancer risks of aniline were estimated by US EPA, which has classified aniline as a probable human carcinogen (Xiao *et al.*, 2007). Aniline can be released into the environment directly as industrial effluent or indirectly as breakdown products of herbicides and pesticides. Due to the high solubility in water, aniline can easily permeate through soil and contaminate groundwater, and they may be present at a certain concentration in the environment (Luongo *et al.*, 2016). Aniline is the most common impurity that can be found in cyclamate samples (Saurina and Hernández-Cassou, 1999).

Major problems of the analytical procedures used in those studies were the poor clean up and the large

amount of solvents and time required. In recent times the use of CPE has increased in order to improve the cleanup of samples with complex matrices rich in natural waste. A number of authors have reviewed the applications of CPE indifferent fields (Arya *et al.*, 2019). Analytical procedures based on CPE followed by HPLC have also shown an improved cleanup efficiency of environmental samples compared to methods based on conventional methods.

Although the spectrophotometric and photometric analyses are the most widely used methods, chromatographic analysis integrates various processes, such as sampling, enrichment, separation and detection, providing a convenient, fast and accurate process, and the purpose of on-line detection can be better achieved (Rahimi *et al.*, 2020).

In this study the potential of using a CPE-HPLC to improve the cleanup of the wastewater samples

extracts was evaluated. A method to determine the aniline by HPLC was developed.

EXPERIMENTAL

Chemicals and equipment

All chemicals used were of analytical reagent grade with high purity, sodium nitrite, 8-hydroxyquinoline, hydrochloric acid, sodium hydroxide, urea, analytical standard and aniline pure purchased from Fisher Scientific (Oslo, Norway). Double distilled water was used throughout the experiments for the preparation of the reagents and samples.

For all absorbance measurements, a double beam dual chopper UV-Vis spectrophotometer, provided with a Czerny-Turner 0.28 m monochromator, Varian, Cary 100, (Mulgrave, Victoria, Australia) was used.

Dionex HPLC system include, Dionex GP40 Gradient Pump, Dionex LC20 LC Column Enclosure, Dionex, dual-beam, programmable wavelength selection, variable wavelength photometer ultraviolet- visible wavelength operation, AD20 absorbance detector.

Mettler digital balance laboratory scale model AE200-200 (Derwood, MD, USA), A PHS-3E pH meter, Ray Magnetic Instrument Factory, (Shanghai, China) and were also used.

General procedures

The primary arylamine, 0.1 ml of 2.5M Maniline was treated with 0.12 ml of 155mM sodium nitrite in the presence of 0.22 ml of 1000mM hydrochloric acid solution at 0 °C –5 °C using a cooling ice bath for 10 minutes to form a diazonium salt (Lu, *et al.*, 2018). Then, 0.1 ml of 660 mM urea was added. The mixture was shaken well and then left to stand for a few minutes, followed by the addition of 0.18 ml of 2.5mM 8-HQ and 0.18 ml of 3000mM sodium hydroxide. The red colour completely developed immediately. The contents of the flasks were diluted to 10.0 ml with distilled water (Narayana and Sunil, 2009). For the CPE procedure, 0.5 ml of 10% Triton X-100 was added to a 10 ml derivatization reaction aliquot in sealed 15 ml centrifuge tubes. The tightly sealed (to avoid evaporation) tubes were transferred to a water bath at 90 °C for 25 min until full separation was achieved. In order to increase the viscosity, the tubes were transferred to an ice bath, and the cloudy layer appeared after 2 min. The

aqueous phase was decanted, and the cloudy layer remained in the bottom of the tubes (Blanchet-Chouinard and Larivière, 2018); the cloudy layer was clarified at 55 °C in a water bath, then 0.5 ml of it was mixed with 0.5 ml absolute ethanol. Detection was achieved at 440 nm for both spectrophotometric detection or HPL-UV-visible separation and detection. For spectrophotometric detection. Measurements were applied against a blank consisting of all components except the analyte.

Analysis of aniline in wastewater samples

Aniline was determined in wastewater samples. The samples were analysed, according to the proposed method. The samples were determined by the CPE-HPLC procedure. Spiked samples were prepared for recovery determination by adding 0.5 ml of 10 µg.ml⁻¹ of aniline.

RESULTS AND DISCUSSION

Different methods were validated and optimized in the determination of aniline, the direct method including the derivatization reaction, i.e., diazonium coupling reaction (Scheme 1), and the extraction method, whereby the analytes of the coupling reaction were concentrated by CPE. The optimal parameters of derivatization reaction included the type of acid, acid concentration, sodium nitrite concentration, base type and base concentration. Further optimization steps for the CPE method included surfactant type, surfactant volume, incubation temperature and incubation time. Three replicates were performed for each experiment to determine the standard error for each result.

Derivatization reaction optimization

Diazonium coupling starts with the formation of nitrous acid by the reaction between sodium nitrite and acid (Narayana and Sunil 2009). The acid concentration are the essential factors in obtaining a high concentration of nitrous acid that leads to a high concentration of the diazonium salt, which was significant in higher light intensity of the coupling product (Lu *et al.*, 2018). The reaction conditions at all stages of the procedure are given in the figure captions. Since the mechanism of diazotization involves attack by the nitrosating species on the free amine and not the amine salt, it is necessary to choose a higher acid strength in order to complete the diazotization of weakly basic amines such as nitroanilines and trihalogenoanilines (Ridd 1959).

For the acid concentration study, the other conditions were as in the preceding study. The increase of the acid concentration was effective in increasing the absorbance; the optimum concentration was 22 mM, Figure 1. At low acid concentrations, the absorbance was low, possibly, because the quantity of acid was not enough to obtain the nitrous acid concentration required to achieve the optimum diazotization, (Al-Majidi and Al-Khuzai, 2019; Huang, *et al.*, 2016).

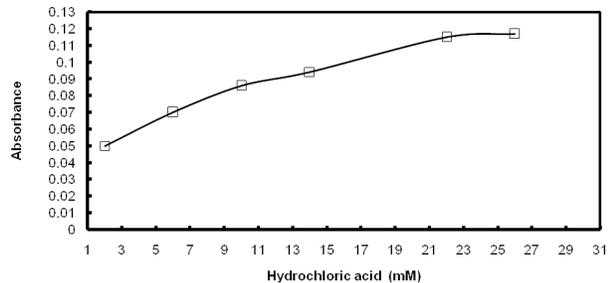


Fig. 1. Hydrochloric acid optimization. Conditions: 0.025 mM aniline, 1.5 mM sodium nitrite, 6.6 mM urea, 0.025 mM 8-HQ and 30 mM KOH.

The effect of sodium nitrite concentration was studied with the previously optimized acid concentration and the same concentrations of aniline, Figure 2, from which a sodium nitrite concentration of 1.8 mM was chosen as the optimum. That absorbance was decreased in the lower ranges may due to the interferences that exist at the insufficient amount of nitrous acid formed at low concentrations of sodium nitrite (Karimi Zarchi and Karimi, 2012). Phenolic compounds were used as the active compounds that react with diazonium salts under alkaline conditions (Huang *et al.*, 2016), 8-HQ in this case. The optimum base was chosen to be sodium hydroxide when compared to potassium hydroxide, sodium carbonate and ammonia (data given in Supplemental Figure A for bases other than NaOH). Sodium hydroxide concentration variation showed the optimum to be 54 mM. The absorbance

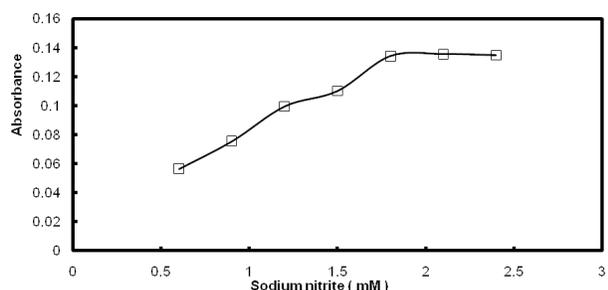


Fig. 2. Sodium nitrite optimization. Conditions: 0.025 mM aniline, 6.6 mM urea, 0.025 mM 8-HQ and 30 mM KOH 22 mM hydrochloric acid.

was directly proportional to the sodium hydroxide concentration until the optimum point, but decreased after the optimum point, which may due to the dilution effect or the interference that comes from diazonium salt alkaline hydrolysis (Figure 3) (Huang *et al.*, 2016; Narayana and Sunil 2009).

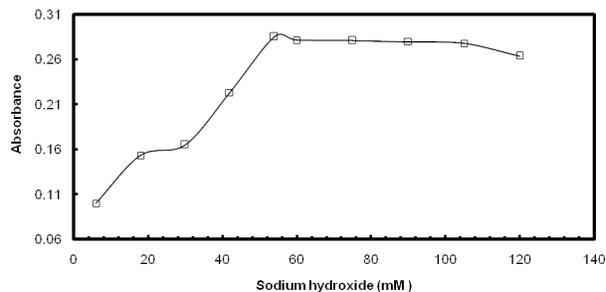


Fig. 3. Sodium hydroxide optimization. Conditions: 0.025 mM aniline, 6.6 mM urea, 0.025 mM 8-HQ, 1.8 mM sodium nitrite and 22 mM hydrochloric acid.

Sample pretreatment and extraction optimization of aniline

Pre-treatment and pre concentration experiments were performed using a Cloud point extraction method to achieve a good selectivity and low detection limit. With the optimized derivatization reaction solution, extraction was carried out. In deciding the optimum surfactant and surfactant volume, the distribution coefficient (D) was the parameter used to evaluate the optimum extraction efficiency. The coefficient of distribution was determined by dividing the aniline concentration in the organic phase by the aniline concentration in the aqueous phase. To improve the extraction and pre-treatment stage, optimization of the surfactant and its volume was required. In the ranked list, Triton X-100 > Tween 20 > Tween 80 > hexadecyl trimethylammonium bromide (CTAB) > Triton X-100 was selected as the best surfactant (Supplementary Figure B). The optimum volume was 0.5 mL, Figure 5 in the optimization analysis for

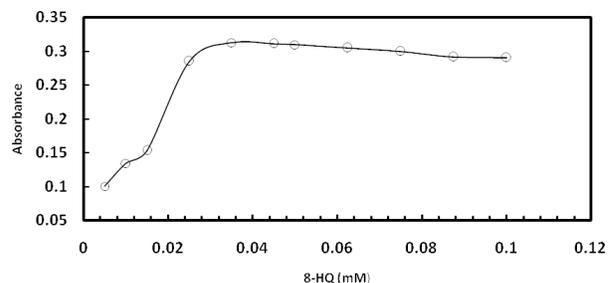


Fig. 4. 8-HQ optimization. Conditions: 0.025 mM aniline, 6.6 mM urea, 1.8 mM sodium nitrite, sodium hydroxide 54 mM and 22 mM hydrochloric acid.

the surfactant volume. The extreme points in Figure 5 display low D values, which may be due to insufficient surfactants to achieve complete extraction at low volumes, while sample dilution may be responsible for high surfactant volumes. Among the surfactants tested, Triton X-100 is soluble in water and the most polar organic solvent (Arya *et al.*, 2019). To reach a good distribution coefficient of extraction (El Hadri and Hackley, 2017), complete equilibrium between the aqueous and organic phases is required (Bezerra *et al.*, 2005). The temperature and incubation period were adjusted to obtain this. The best time was chosen as an incubation period of 25 min, Figure 6. The coefficient

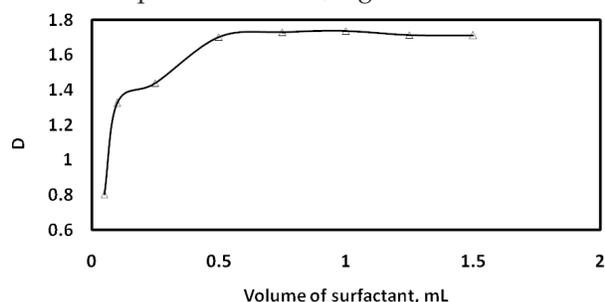


Fig. 5. Volume of surfactant Conditions: 5 mM aniline, Temperature 60 °C, Time 20 min.

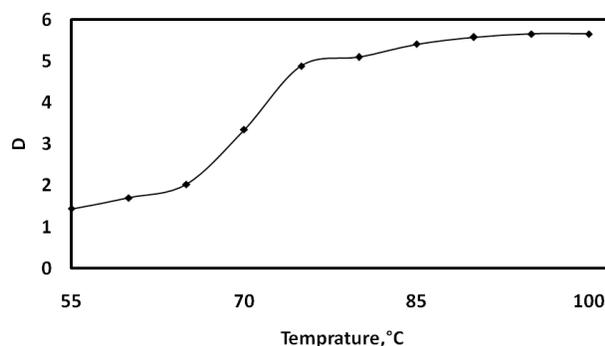


Fig. 6. Temperature study. Conditions: 5 mM aniline, Time 20 min, volume of surfactant 0.5 mL

of distribution decreased at incubation times bigger than the optimum time, which may be due to the decomposition of the azo dye [26]. The temperature variation, Figure 7, showed that 90 °C was the suitable extraction temperature. There was no rise in extraction at temperatures higher than the optimum point, likely due to full extraction capability being achieved (Wang *et al.*, 2013). At the cloud temperature and above the solution with nonionic surfactant becomes cloudy, so that the specimen can be divided into two immiscible phases; one of them is a low volume surfactant phase that contains the analyte's micellar form, and the other phase is the aqueous dilute phase (Bezerra *et al.*, 2005).

HPLC optimization

Hplc peak symmetry approach was optimized. Different parameters were optimized such as organic modifiers percentage, flow rate, column temperature using reversed phase of C18 column. In range of 60-85 % of methanol in water, the methanol percentage of 75 % was chosen the best organic percentage. The flow rate range of 0.6-1.0 ml/min was experimented; the flow rate of 0.8 ml/min was selected the best. The column temperature change in range of 30-50 °C was not gave the sensitive effect on the peak symmetry; however, 35 °C was used for the HPLC working. The dye formed by derivatization reaction and extracted by CPE technique was injected to HPLC.

Calibration curves and validation study

Calibration curves were constructed for aniline for the proposed methods (Table 1; Supplementary Figures C and D).

The molar absorptivities of aniline using the CPE-HPLC method were higher than the molar absorptivities using both the direct method and

Table 1. Regression equation, linear range, correlation coefficient, LOD, LOQ, and molar absorptivity of the proposed methods

Method	Slope/ absorbance/ μgml^{-1}	y-Intercept/ μgml^{-1}	Correlation coefficient	Linear range/ μgml^{-1}	LOD/ μgml^{-1}	LOQ/ μgml^{-1}	molar absorptivity/ $\text{l mol}^{-1} \text{cm}^{-1}$
Derivatization reaction	0.269 ± 0.005	0.078 ± 0.003	0.9969	0.1-1.2	0.0862	0.2613	25136.6 ± 17
DLLME-Spectro	0.746 ± 0.001	-0.027 ± 0.01	0.9981	0.1-1.2	0.051	0.154	69393.5 ± 326
Method	Slope/ μV $\text{min}/\mu\text{g l}^{-1}$	y-Intercept/ μgml^{-1}	Correlation coefficient	Linear range/ $\mu\text{g l}^{-1}$	LOD/ μgml^{-1}	LOQ/ μgml^{-1}	molar absorptivity/ AU l mol^{-1} cm^{-1}
DLLME-HPLC	22862 ± 169.1	1290.6 ± 94	0.9993	0.011-1.6	0.0273	0.082	2128132.4 ± 658.3

CPE-spectrophotometry. The limits of detection (LOD) of aniline using the CPE-HPLC method were lower than those of the direct method and CPE-spectrophotometry indicating the improvement of CPE-HPLC method due to the efficiency of the CPE extraction step and HPLC efficiency (information and data in Table 1). The accuracy and precision were calculated for both methods for aniline. The precision was lower than 0.5 (RSD % < 0.5), while the accuracies were in the range 100.4 to 100.7%.

Analytical Application

Due to its selectivity, the ability to minimize the matrix effect and the high molar extinction coefficient (Table 1), and the implementation of the system for evaluating aniline in wastewater samples was checked (Table 2) using the CPE procedure followed by HPLC.

Even though direct method is simpler and the CPE method is a no longer technique, the superiority of the CPE method followed by HPLC is seen in Table 1; it was therefore selected for actual sample analysis purposes. Recoveries were

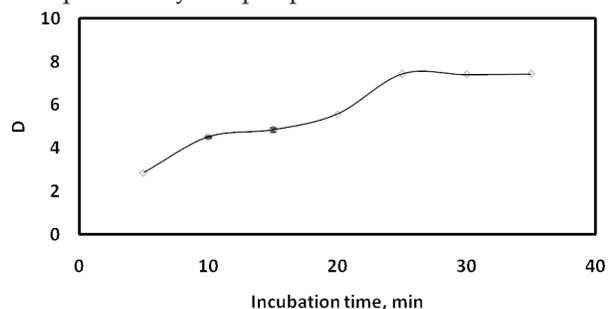


Fig. 7. Time study. Conditions: 5 mM aniline, volume of surfactant 0.5 mL, temperature 90 °C.

computed, good precision and recoveries were achieved.

The sewage treatment plant (Scheme 2) has been selected in the Baghdad governorate (Al Dora - Al Buithah). The sewage treatment plant is located in the Baghdad, near Al-Karkh, to the south of the Dora area at the West Bank of the Tigris River and confined between two circles of latitude 33°13'20.97 north and longitudes 44°27'14.29 east. The treatment plant was operated in 2014 to serve a large area of population estimated 750,000 people, and with a design capacity of 200,000 cubic meters per day of wastewater. The end stream of this station is discharged to the Tigris River from a point at the end of the treatment. This wastewater can be the source of aromatic compound that can enter the river and affect the health of living organisms in the river. Therefore, it is necessary to evaluate the amount of priority pollutant compounds in the stages of the waste water treatment unit. Aniline are among of the priority pollutant compounds that can be found in Al-Bueitha waste water Treatment and therefore, needs to be checked periodically to evaluate their pollution effects on Tigris River. The waste water streams are collected in the main tanks to collect all wastewaters effluents coming from different stages of the different collecting channels. The stream goes down to the first treatment subunit (skimmers and discoil skimmers in which the oily phase is separated from the watery phase. In the second stage, the stream goes down to the physiochemical subunit tank (including filters and centrifuges). Many chemicals are added to this tank, such as poly electrolyte and alum, that can help to aggregate the

Table A HPLC parameters optimization.

No.	Organic modifier percentage %	Temperature °C	Flow rate ml/min	Retention time min	Asymmetric factor
1	60	30	0.6	2.8	1.05
2	65	30	0.6	2.65	1.04
3	70	30	0.6	2.4	1.04
4	75	30	0.6	2.2	1.04
5	85	30	0.6	2.1	1.04
6	75	30	1.0	1.7	1.04
7	75	30	0.8	1.8	1.03
8	75	30	0.6	1.95	1.03
9	75	30	0.4	2.1	1.03
10	75	50	0.8	2.1	1.03
11	75	45	0.8	2.1	1.03
12	75	40	0.8	2.1	1.03
13	75	35	0.8	2.1	1.03
14	75	30	0.8	2.1	1.03

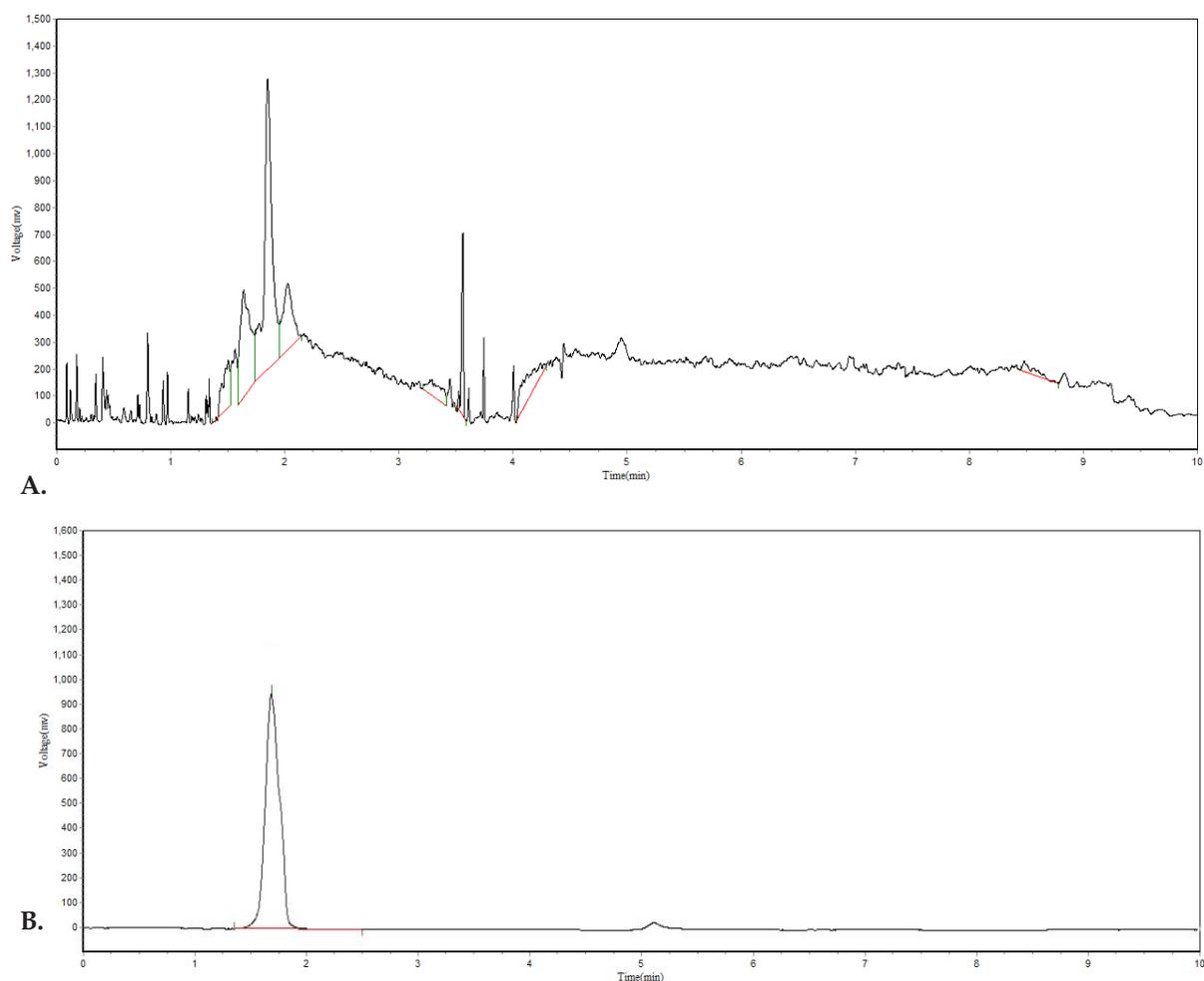


Fig. 8. The chromatogram of A. real sample analysis, B. $0.5 \mu\text{gL}^{-1}$ aniline.

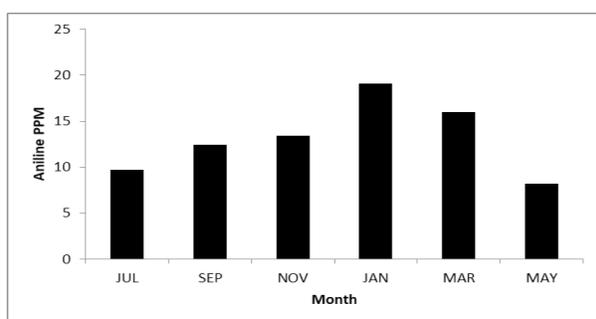


Fig. 9. Aniline concentration at different seasons at site A

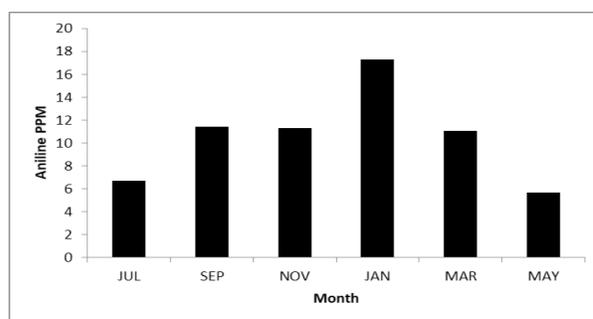
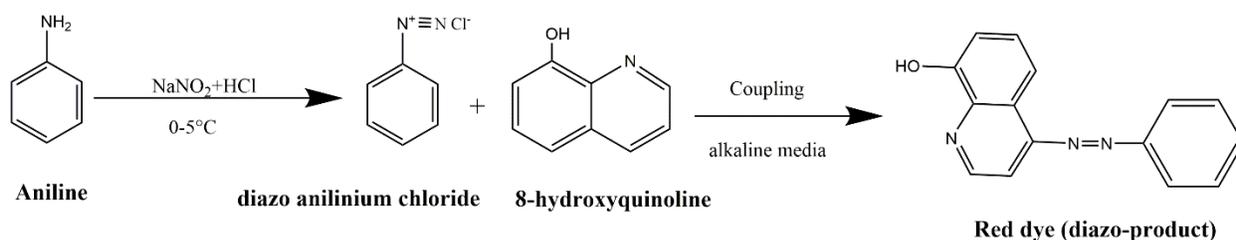


Fig. 10. Aniline concentration at different seasons at site B

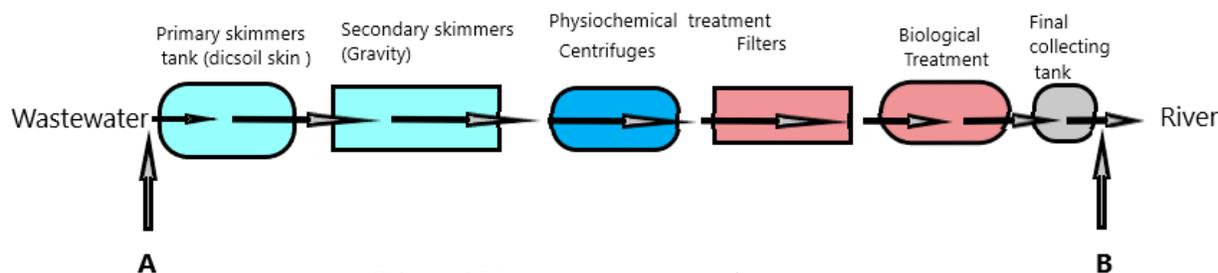
semi soluble materials and help their separation by using special skimmers in the next stage. In the third stage, the stream enters the biological treatment tank, in which bacteria are used for the biodegradation of organic and inorganic molecules. Phosphoric acid, urea and other supplements are added to this tank together with some flotation ventilator which can help in the reoxygenation of the

bacteria. The last tank, called the final UV disinfection tank, the final wastewater is collecting before streaming it out to the river. Two sites have been identified to take samples from this unit, the first site before entering wastewater to the station (site A) and the second site After the final treatment unit (site B) which is UV disinfection.

Aniline (AN) determined and followed for one



Scheme 1. The proposed mechanism of derivatization reaction (Obayes *et al.*, 2016).



year at site A and B. The studied aniline was found in most samples. The samples were pre-filtered, extracted and eluted (CPE) under optimum extraction conditions. The samples were separated, detected, followed by HPLC detection, under the optimum conditions.

The samples collected at site A were turbid. The results are shown in Fig. (9). Generally, the amounts of all aniline selected in this study were lower in summer and spring than other seasons. The highest results were obtained in January with values of 19.1 $\mu\text{g}/\text{l}$. While, in autumn the concentrations were below the detection limits. The variation in the levels of these compounds may be due to by the operating conditions (periodic maintenance). The low volatilization in the winter being the tanks are opened exposed to the air, the increase in bacterial activity in summer and sunlight may increases the breakdown of the aniline.

Site B located after the biological treatment subunit and final precipitation tank. The sampling was from a pump located after the biological treatment and final precipitation tank. The water was more dilute than that in other unit. Most of the aniline were found in most samples. The results are shown in Fig. (10). The levels of all aniline selected in this study were lower in the summer than in the spring and winter. The higher results were 17.3 $\mu\text{g}/\text{l}$ in Jan. The lowest amounts were 5.7 $\mu\text{g}/\text{l}$ in May, The variation in the levels of these compounds may be caused by the previous subunit biological and UV- treatment that As for the decrease of the aniline

Supplementary Materials

Determination of Aniline in wastewater by Cloud Point Extraction Followed by HPLC using 8-hydroxyquinoline as a derivatization agent

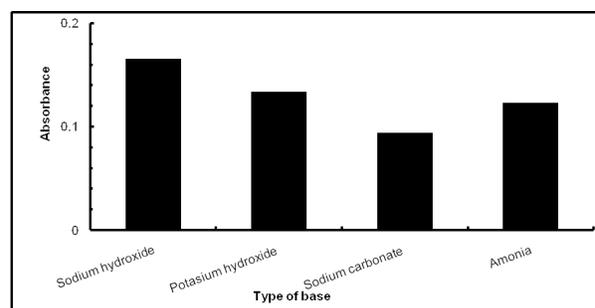


Fig. A. Base optimization. Conditions: 0.025mManiline, 6.6 mM urea, 0.025 mM8-HQ and 30 mM base, 22 mM hydrochloric acid and 1.8mM sodium nitrite.

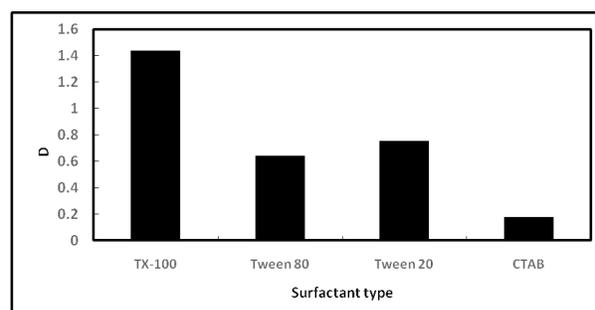


Fig. B. Surfactant type selection Conditions: 5 mM aniline, volume of surfactant 0.25 mL, Temperature 60 °C, Time 20 min.

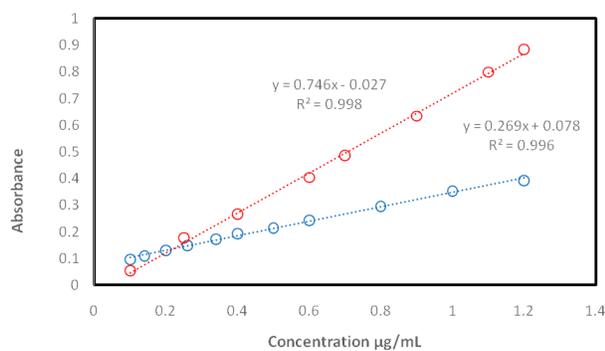


Fig. C. Calibration curves for A. CPE-spectrophotometric (red symbols) B. direct (blue symbols), both under optimal conditions

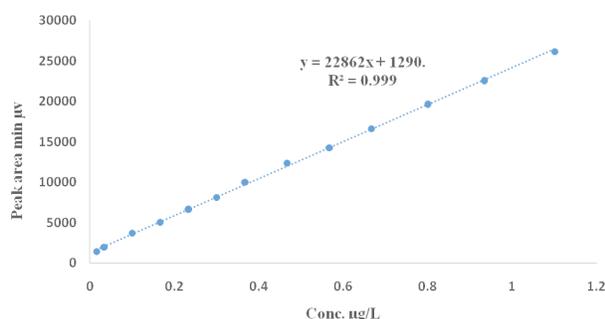


Fig. D. Calibration curves of aniline of CPE-HPLC of extracted dye formed by derivatization reaction.

from the first site, the reason may be dilution organic matter, biological and UV- treatment.

CONCLUSION

In the proposed derivatization procedure, the concentration of the base used and the reagent were the most sensitive factors in the forming the colored product. On the other hand, the surfactant volume and temperature, in addition to the time, were the most critical factors that determined the value of the distribution ratio in the CPE of the colored aniline derivative. CPE method, followed by HPLC measurement, was successfully applied to determine aniline in the wastewater. Because of the method sensitivity and low detection limit, the CPE method could be applied to trace aniline in samples, such as industrial and environmental samples. This method could be readily extended to analytes other than aniline that can be diazomomly coupled to 8-HQ.

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