

Analytical Methods

Analysis of sudan I, sudan II, sudan III, and sudan IV in food by HPLC with electrochemical detection: Comparison of glassy carbon electrode with carbon nanotube-ionic liquid gel modified electrode

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Received 26 February 2007; received in revised form 2 January 2008; accepted 12 January 2008

Abstract

A method was developed for analyzing of sudan I, sudan II, sudan III, and sudan IV by high performance liquid chromatography coupled with an electrochemical detector. The electrochemical oxidation of each compound was investigated with a carbon nanotube-ionic liquid gel modified glassy carbon (MWNTs-IL-Gel/GC) electrode using cyclic voltammetry. The results were compared with those of glassy carbon electrodes. At the MWNTs-IL-Gel/GC electrode, highly reproducible, well-defined cyclic voltammograms for sudan oxidation were obtained. In the flow system, MWNTs-IL-Gel/GC exhibited highly reproducible and well-defined amperometric signals without peak tailing. In addition, the determination of sudan dyes by mean of isocratic reverse-phase HPLC, with amperometric detection at MWNTs-IL-Gel/GC and GC electrode, have been investigated. The chromatograms showed excellent separation. The detection limits ranged from 0.001 to 0.005 ppm. The method was sensitive, selective and could be applicable for the assay of sudan dyes in soft drink samples.

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Keywords: Sudan I; Sudan II; Sudan III; Sudan IV; Sudan azo-dyes; Food analysis; Electrochemical detection; HPLC; Carbon nanotube

1. Introduction

For food industries, color is the first notable characteristic of a food. Colorants are often added to food to enhance its visual aesthetics and promote sales. Color additives reinforce the colors already present in the food and ensure its uniformity. Sudan dyes (Fig. 1) are synthetic industrial azo-dyes, traditionally used in waxes, plastics, oils, and polishes. These dyes are categorized as class 3 carcinogens by the International Agency for Research on Can-

cer (IARC). Although recognized as carcinogens, sudan dyes have been found recently in food products in some European countries. They are added to food such as chili powder to mimic, intensify, and prolong the appearance of natural red hues. In UK, more than six hundred products containing sudan dyes have been recalled such as fish sauce, Worcester sauce, noodle soup, and pizza. Therefore, they are illegal to use as food additives, according to the FAD and EU. The European Commission requires products to have documentation confirming the absence of sudan dyes. The EU has set the detection limit at 0.5–1 mg/kg for sudan dyes, and any food material containing more than those limits should be withdrawn from

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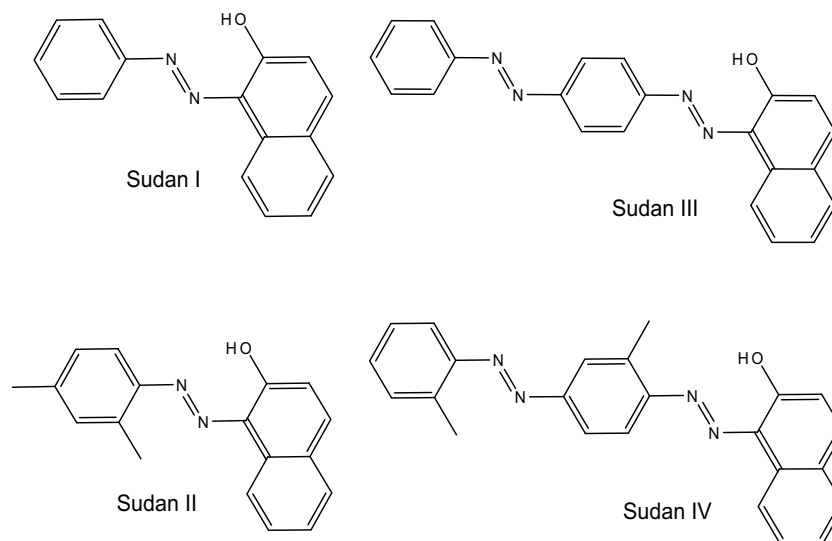


Fig. 1. Chemical structures of four sudan azo dyes analyzed in this study.

the market (http://www.ift.org/news_bin/news/archive-Frames.php?view=5-2005¹). From the toxicological viewpoint, several studies have been carried out in order to check the noxious effects on humans; therefore, there is a pressing need to develop fast and sensitive methods for the determination of sudan dyes that contaminated food.

A scientific literature survey of methods indicated that a large number of techniques have been employed for the analysis of synthetic colors in beverages. The principal problems associated with the determination of color additives in food are the mixtures of colors and the diversity of potential interference present. The analytical techniques frequently employed for the determination of the colors include solid-phase spectrophotometry (Valencia, Uroz, Tafersiti, & Capitan-Vallvey, 2000), thin layer chromatography (Marshall, 1977), capillary electrophoresis (Mejia, Ding, Mora, & Garcia, 2007), HPLC coupled with photodiode array (Cornet, Govaert, Moens, Van Loco, & Degroodt, 2006), UV (Zhang, Zhang, Gong, Gopalan, & Lee, 2005), chemiluminescence (Zhang, Zhang, & Sun, 2006), and MS (Calbiani, Careri, Elviri, Mangia, & Zagnoni, 2004; Ma, Luo, Chen, Su, & Yao, 2006; Mazzetti et al., 2004; Rovellini, 2005; Tateo & Bononi, 2004). In all instances, most of these methods require a highly qualified operator and high cost instrumentation. Thus, a highly sensitive and low cost method is still needed for the development in the field of analytical chemistry.

Electrochemical methods of detection are a more attractive option because they are inexpensive, highly sensitive, and have long-term reliability and reproducibility. From the literature review, there is only one researcher using a polarographic method (De la Cruz Yaguez, Pingarron Carrazon, & Polo Diez, 1986) for the detection of sudan

dyes. However, a method based on mercury has limitations due to its possible toxicity and the rapid deterioration of the electrode response.

Recently, carbon nanotubes have become one of the most interesting materials since their discovery in 1991 (Harris, 1997; Iijima, 1991). Carbon nanotubes were found in two types of structure: the multi-wall carbon nanotubes (MWNTs) and the single-wall carbon nanotubes (SWNTs) (Li, Shi, & Hao, 2006). Besides the nano-size effects common to other nano material, carbon nanotubes also show the characteristics of unique size distribution, a novel hollow-tube structure, high specific surface area, and excellent electronic semi-conductivity and conductivity. These properties of carbon nanotubes confer with a broad range of potential applications such as catalyst (Zhao, Xu, Ma, & Wei, 2007) and biological cell electrodes (Wei, Kvarnstrom, Lindfors, & Ivaska, 2007; Whitten, Spinks, & Wallace, 2005). The possible hazards of carbon nanotubes reported in Material Safety Data Sheet, are irritation to the skin, the eyes, and mucous membranes. Elemental carbon or carbon black of carbon nanotubes can cause conjunctivitis, corneal damage, and inflammation of the eyelids. However, it is very safe to use with caution. Room temperature ionic liquids (RTILs), which are liquid compounds that consist only of ions, are around room temperature. They have great potential as green reaction media, due to the advantages such as no measurable vapor pressure, good thermal and chemical stability, high conductivity, and low toxicity (Zhao et al., 2005). Fukushima et al. have recently demonstrated that pristine single-walled carbon nanotubes can form gels when mixing them with imidazolium ionbased RTILs by grinding (Fukushima et al., 2003). Zhao et al. have reported involvement in the development of chemically modified electrodes, based on CNTs and RTILs to multi-walled carbon nanotube gels of 1-butyl-3-ethylimidazolium hexafluorophosphate on a

¹ www.ift.org/news_bin/news_bin.

glassy carbon electrode, for studying the direct electrochemistry of proteins. The preliminary investigation demonstrated that such a gel electrode is thermally stable with high conductivity, and that the proteins adsorbed on the electrode can still retain their activities (Zhao et al., 2002).

Therefore, the aim of this work was to report the excellent performance and superiority of the MWNTs-ionic liquid gel modified electrode for the investigation of sudan I, sudan II, sudan III, and sudan IV. A preliminary qualitative analysis was carried out using cyclic voltammetry and the results were compared with those of the bare glassy carbon electrode. In addition, the MWNTs-ionic liquid gel modified electrode is coupled with a hydrodynamic system such as that for flow-injection analysis (FIA) and HPLC, in amperometric mode, and it is also used to detect sudan dyes in real samples.

2. Experimental

2.1. Chemicals and reagents

MWNTs and the ionic liquid of 1-octyl-3-methylimidazolium hexafluorophosphate (OMIMPF₆) were obtained as a gift from the Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University.

Sudan I (Solvent Yellow 14), sudan II, and sudan IV (Solvent Red 24) were all obtained from Sigma. Sudan III was purchased from Fluka. Acetonitrile (HPLC grade) was supplied by Merck (Darmstadt, Germany). Gacial acetic acid was purchased from BDH. Ammonium acetate was obtained from Riedel de Haen. Tetrabutylammonium perchlorate was purchased from Sigma. Water was purified by the Milli-Q-gradient system (Millipore, Benford, MA, USA). The stock solutions (1.5 mM) of sudan compounds were prepared daily in 0.05 M tetrabutylammonium perchlorate/acetonitrile. The working standards were prepared in corresponding supporting electrolyte solutions.

The HPLC mobile phase, which consisted of acetonitrile and acetate buffer (20 mM ammonium acetate and 1% acetic acid) (90:10, v/v), was filtered through a Millipore filter (0.2 µm, 47 mm, Whatman) prior to use. A flow rate of 1 mL/min was used throughout the experiment.

2.2. Electrode

The glassy carbon (GC) electrode (0.07 cm², Bioanalytical System, Inc.) was pretreated by sequential polishing with 1 and 0.05 µm of alumina/water slurries on felt pads, followed by rinsing with ultrapure water and then sonicating with ethanol and ultrapure water for 5 min prior to use.

2.3. Fabrication of the modified electrode

The glassy carbon disc working electrode (3 mm diameter, China) was polished before use with 1 µm and 0.05 µm

alumina slurries. After rinsing, the electrode was placed in an ultrasonic cleaner for 5 min, first with ethanol and then with deionized water, to remove any traces of impurities. To dry the surface of the GC electrode, it was flowed with N₂. A mixture of 12 mg MWNTs and 0.2 mL OMIMPF₆ was ground with an agate mortar for about 20 min, and a black gel was formed (Zhao et al., 2005). Then, the GC electrode was spread with carbon nanotube gel and placed on a smooth glass slide, and the gel was mechanically attached to the electrode surface. The gel on the electrode surface was smoothed with a spatula to leave a thin gel film on the GC electrode surface. Finally, the surface was smoothed by polishing on a piece of weighing paper and the gel modified the glassy carbon electrode (denominated as MWNTs-IL-Gel/GC electrode in this paper) was fully fabricated.

2.4. Cyclic voltammetry

Electrochemical measurements were recorded using an Autolab Potentiostat 30 (Metrohm, Switzerland) with a standard three-electrode configuration. The working electrode was a glassy carbon (GC) electrode or modified GC electrode. The auxiliary and reference electrodes were platinum wire and Ag/AgCl, respectively. Cyclic voltammetry was used to probe the electrochemical reaction. The electrochemical measurements were housed in a Faradaic cage to reduce electronic noise. All experiments were done at room temperature.

2.5. Flow injection analysis and HPLC with amperometric detection

The FIA system consisted of a thin layer flow cell (Bioanalytical System, Inc.), an injection port (Rheodyne7725) with a 20 µL injection loop, a HPLC compact pump Model 2250 (Bischoff, Germany), and an electrochemical detector (PG30). The carrier stream, acetonitrile and 20 mM acetate buffer (90:10, v/v), were regulated by a reagent delivery module at a flow rate of 1 mL/min. The thin layer flow cell consisted of a teflon rubber (1 mm) gasket as a spacer, an Ag/AgCl as the reference electrode, and a stainless steel tube as an auxiliary electrode and outlet. The chromatographic column (Inertsil ODS-3 HPLC packed column) was used for the separation of sudan compounds. The experiments were performed in a copper Faradaic cage to reduce electrical noise. A hydrodynamic voltammogram was obtained before the amperometric determination was performed. The peak current after each injection was recorded, together with the corresponding background current. These data were plotted as a function of applied potential to obtain hydrodynamic voltammograms. The amperometric measurements were carried out at the potential giving a maximum signal-to-background (S/B) ratio in the hydrodynamic voltammograms.

2.6. HPLC–EC method

2.6.1. Linearity and regression analysis

Stock solutions of sudan dyes (2 mM) were prepared in mobile phase. Standard curves were constructed by serial dilutions of the stock standard solutions.

2.6.2. Precision

The precision was determined by calculating intra-day repeatability as the relative standard deviation (RSD).

2.6.3. Accuracy

The accuracy of the method was obtained from replicate analysis at three different concentrations of spiked samples containing known amounts (target concentrations) of sudan I, sudan II, sudan III and sudan IV on the same day (Table 1). The concentrations of the analytes were determined from the standard addition curve. Accuracy (bias) was expressed as the percentage difference between the calculated mean concentrations relative to the nominal concentration.

2.7. Sample preparation

Five milliliters of soft drink were collected in beakers and purged by nitrogen gas to allow the water to evaporate and leave 2 mL of the solution. The solution was transferred into a 5 mL volumetric flask, spiked with mixed standard (sudans I–IV), and diluted to the final volume with the mobile phase. Before injection, the prepared solution was filtered through a 0.45 μm PTFE Syringe filter membrane (Chrom Tech), and then degassed for 1 min.

3. Results and discussion

3.1. Electrooxidation of sudan I, sudan II, sudan III, and sudan IV

As shown in Fig. 2, cyclic voltammograms of 100 μM sudan I at the MWNTs-IL-Gel/GC electrode and bare GC electrode were obtained in acetonitrile and 20 mM acetate buffer (90:10, v/v). At both electrodes, the voltammo-

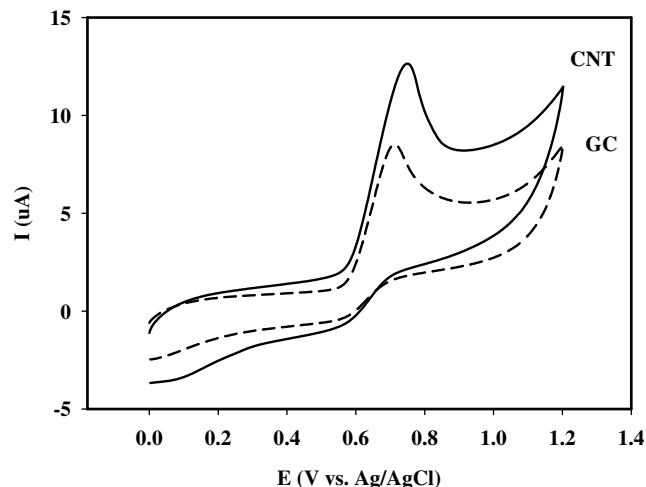


Fig. 2. Cyclic voltammograms of 100 μM sudan I in acetonitrile and 20 mM acetate buffer (90:10; v/v) at the GC electrode (dash line) compared with MWNTs-IL-Gel/GC electrode (solid line). The sweep rate was 50 mV/s; the electrode area, 0.07 cm^2 .

grams exhibited similar irreversible oxidation peaks. However, the charging and the peak currents that increased at the MWNTs-IL-Gel/GC electrode were much larger than those at the bare GC electrode. During the scan of potential toward a positive direction at the MWNTs-IL-Gel/GC electrode, a well-resolved oxidation wave was observed at approximately +0.74 V. Interestingly, it was found that a slight fouling of the product occurred during consecutive scans with the modified electrode in sudan solutions. After rinsing with distilled water and placing in blank solution, the background current was nearly the same as the one before performing the measurement in sudan solutions. These results indicated that the CNT-based detector offers greatly improved performance, with a substantially higher sensitivity. This also means that the electrochemical behavior of sudan I can be improved by this modified electrode. Furthermore, comparing results indicated that CNT is a promising material for fabricating electrochemical electrodes for electroanalysis applications. The results obtained from sudan II, sudan III, and sudan IV provided similar results to those from sudan I.

3.2. Scan rate and concentration dependence

In order to check the adsorption of sudan compounds on the modified electrode surface, the scan rate dependence was performed in the range of 0.01–0.3 V/s (data not shown). The anodic peak currents increased linearly with the square root of the scan rate over the examined range, with the linear regression yield $r > 0.999$. This result showed that sudan compounds are slightly adsorbed at the surface of this modified electrode and the reaction was controlled by the diffusion of sudan compounds in the solution.

The concentration dependence was investigated for all four sudan compounds using a scan rate of 50 mV/s at

Table 1

Linear range (LR), detection limit (DL), and quantification limit (QL) of sudan I, sudan II, sudan III, and sudan IV

	Electrode	SUD I	SUD II	SUD III	SUD IV
LR (ppm)	GC	0.01–15.0	0.01–12.0	0.05–120.0	0.10–150.0
	CNT	0.005–15.0	0.005–20.0	0.05–20.0	0.05–25.0
R^2	GC	0.9994	0.9989	0.9970	0.9997
	CNT	0.9976	0.9989	0.9986	0.9989
DL (ppm)	GC	0.005	0.005	0.005	0.05
	CNT	0.001	0.001	0.005	0.025
QL (ppm)	GC	0.01	0.01	0.05	0.10
	CNT	0.005	0.005	0.05	0.05

MWNTs-IL-Gel/GC electrode. The response was in general higher when the concentration increased. The voltammetric responses were linear in the range from 0.025 to 2 mM. (At concentrations higher than 2 mM, the solubility limit was reached for all four sudans). The linear regression analysis of peak current (mA) versus concentration (mM) was obtained, linearly proportional in these with detection limit of 50 μM . From the cyclic voltammetric results suggest that the determination of sudans for the quantitative analytical applications is possible. However, it was found that the detection limit obtained from this technique was not low enough for legally allowed maximum concentration. Therefore, amperometry coupled with flow injection system was used to solve this problem.

3.3. Hydrodynamic voltammetry

To investigate the optimal detection potential in the flowing system, hydrodynamic voltammetry at the modified and unmodified electrode was employed. The 20 μL injection of submillimolar concentrations of four different sudan compounds at the GC and MWNTs-IL-Gel/GC electrode was investigated. The curves were developed point-wise by applying potential over the 0.0–1.3 V range. All four compounds displayed similar current-potential profiles, with defined waves, starting around 0.7 V, and leveling off above 1.0 V. The maximal peak potentials at GC electrode, were found at 0.95 V for sudan I and II, sudan III, and sudan IV. At MWNTs-IL-Gel/GC electrode, the maximal peak potentials were found at 0.85 V for sudan I, II, sudan III, and sudan IV. More positive potentials exhibited higher signal-to-noise characteristics, due to rising background signals.

3.4. Flow injection analysis

In this work, we obtained excellent FIA results using the GC and MWNTs-IL-Gel/GC electrode for the detection of sudan dyes. Fig. 3 shows a series of repetitive 20 μL injections of various concentrations of sudan II at the GC electrode in acetonitrile and 20 mM acetate buffer (90:10, v/v) at a detection potential of 0.95 V. Well-defined signals without peak tailing were obtained and the current signal decreased linearly with the concentration decreasing from 10 to 0.25 ppm ($r^2 > 0.99$), as shown in the inset. The sensitivity, which is the slope of the plot of peak current vs. concentration, was 31.5 nA/ppm.

3.5. HPLC amperometric detection

HPLC separations were carried out under isocratic reversed-phase conditions on a Inertsil ODS-3 column. The mobile phase consisted of the acetonitrile and 20 mM acetate buffer (90:10, v/v) mixture at a flow rate of 1 mL/min. The analysis was completed within 22 min. The detector response was thus improved by changing the working electrode from GC to MWNTs-IL-Gel/GC.

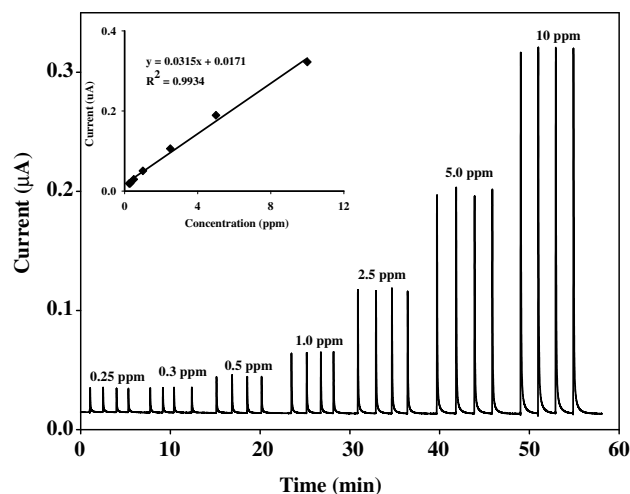


Fig. 3. Flow injection signals of standard sudan II in acetonitrile and 20 mM acetate buffer (90:10; v/v) and using this solution as a carrier stream at the GC electrode. The flow rate was 1 mL/min.

Typical HPLC–ECD chromatograms of standards are shown in Fig. 4. The marked catalytic electrochemical properties of the MWNTs-IL-Gel/GC electrode displayed enhanced sensitivity compared with a bare GC electrode. The sensitivity of sudan detection at the MWNTs-IL-Gel/GC electrode was higher under the same conditions than at the unmodified electrode. The well-defined peaks indicated convenient quantitative analysis of sudan compounds down to the sub-ppm level.

3.6. Method validation

The proposed HPLC–ECD assay, using the MWNTs-IL-Gel/GC electrode for the determination of sudan I,

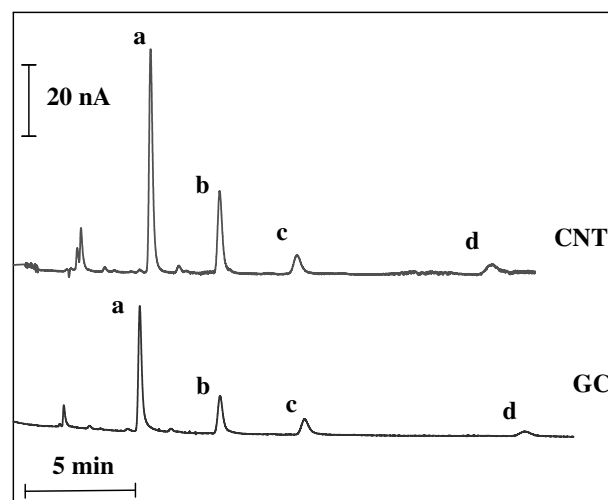


Fig. 4. HPLC-EC chromatogram of a standard mixture containing 1 ppm concentration of (a) sudan I, (b) sudan II, (c) sudan III, and (d) sudan IV at the GC electrode compared with CNT electrode. The mobile phase was acetonitrile and 20 mM acetate buffer (90:10; v/v). The injection volume was 20 μL , and the flow rate 1 mL/min.

Table 2
Precision (relative standard deviation, RSD and accuracy from analysis of four sudan dyes in soft drink samples ($n = 3$))

Type of soft drink	Type of Azo dyes	Spike level (ppm)	GC-electrode ($n = 3$)		CNT-electrode ($n = 3$)			
			Total Measured (mean \pm SD, ppm)	%Recovery	%RSD	Total Measured (mean \pm SD, ppm)	%Recovery	%RSD
F-EC	Sudan I	1.0	99.36 \pm 4.68		4.71	103.05 \pm 2.19		2.12
	Sudan II	1.0	98.74 \pm 1.53		1.54	98.18 \pm 0.91		0.93
	Sudan III	1.0	97.07 \pm 4.80		4.94	104.09 \pm 2.52		2.42
	Sudan IV	1.0	99.85 \pm 0.44		0.44	98.99 \pm 1.65		1.66
G-EC	Sudan I	1.0	97.94 \pm 0.86		0.87	99.39 \pm 4.15		4.18
	Sudan II	1.0	97.17 \pm 1.80		1.80	103.40 \pm 1.63		1.57
	Sudan III	1.0	100.87 \pm 0.17		0.17	95.68 \pm 2.13		2.23
	Sudan IV	1.0	96.39 \pm 2.10		2.18	97.82 \pm 0.69		0.71
M-EC	Sudan I	1.0	93.91 \pm 0.58		0.62	98.47 \pm 2.78		2.82
	Sudan II	1.0	100.22 \pm 3.68		3.67	101.14 \pm 0.94		0.93
	Sudan III	1.0	101.94 \pm 1.36		1.33	99.45 \pm 1.46		1.47
	Sudan IV	1.0	96.77 \pm 1.84		1.90	98.01 \pm 1.62		1.65

sudan II, sudan III and sudan IV, was validated in accordance with the guidelines for validation methods. Typical acceptance criteria for validation of an analytical method such as linearity, limit of detection/quantitation, precision and accuracy were determined.

3.6.1. Linearity and limits of detection and quantitation

Table 1 summarizes data from the experiments. The linearity of response was examined for each compound. The coefficients of linear regression of the standard curves were greater than 0.99. Detection limits were determined using progressively lower concentrations of sudan dyes at a signal/noise ratio of 3:1 ($S/N = 3$), and with an injected volume of 20 μ L. Also, limit of quantitation was calculated at a signal/noise ratio of 10:1. The limit of detection and quantitation obtained from the MWNTs-IL-Gel/GC electrode was less than the GC electrodes by around 2 times (Table 1).

3.6.2. Accuracy and precision

The data for accuracy and precision are shown in Table 2. The accuracy of the assay was determined by repetitive analysis of blank samples spiked with 1 ppm of each sudan dye. The accuracy of the proposed assay was determined by comparing the measured concentration to its true value. Recovery ranged from 93.91–101.94% and 95.68–104.09% at the GC electrode and MWNTs-IL-Gel/GC electrode, respectively. No significant improvement in the recovery was achieved by changing the electrode. The precision of the assay, calculated as a relative standard deviation, was in the range of 0.17–4.71 and 0.71–4.18 for GC electrode and MWNTs-IL-Gel/GC electrode, respectively.

3.7. Analysis of sudan dye samples

The HPLC amperometric method developed in this study was applied to the assay of sudan dyes in soft drink samples. The separation and detection for measurement of sudan dyes in the matrix are demonstrated by spiking the

standard mixture solution. It was found that the amperometric signals resulted in well-defined and separated peaks, with no apparent interference from the sample matrix. The response was higher when comparing the GC electrode with the MWNTs-IL-Gel/GC electrode.

In addition, when we compared the data obtained from the proposed method to the method using an activated glassy carbon electrode for the determination of sudan I (Meiju, Xiaogang, Zihao, & Shouguo, 2007). It was found that using the proposed method gave the wider linear dynamic range (three orders of magnitudes), but using activated glassy carbon electrode provided only one order of magnitude. Interestingly, the proposed method (using carbon nanotube-ionic liquid gel modified electrode) also provided a very low detection limit of 0.001 ppm compared to the detection limit obtained from using activated glassy carbon (0.176 ppm). Therefore, we can conclude that using carbon nanotubes, which are nanomaterials provide the advantage over glassy carbon electrode. This outstanding performance of the MWNTs-IL-Gel/GC electrode made it attractive for using as the working electrode in HPLC system for analysis not only sudan I but also sudan II, sudan III, and sudan IV.

4. Conclusion

The recent trends and challenges in the use of nanomaterial to make electrodes for the electrochemical detection system have been presented. This is the first study to demonstrate the utilization of the MWNTs-IL-Gel/GC electrode for the detection of sudan compounds. Cyclic voltammetry studies were used to investigate the electrochemical properties of sudan compounds. The S/B ratio of all four sudan compounds indicated that higher sensitivity and diffusion control at the MWNTs-IL-Gel/GC electrode was verified by scan rate dependence data. Hydrodynamic voltammetry was employed to obtain the appropriate detection potential applied for FIA and HPLC amperometric detection. The FIA amperometric results

confirmed that the MWNTs-IL-Gel/GC electrode is sensitive and provides stable amperometric measurements of sudan compounds. This is useful in determining low levels of these compounds without blank interference caused by the limited number of substances that could undergo redox reactions under this condition. The chromatographic analysis with electrochemical detection indicated that the method was also applicable to real samples. Due to its selectivity and sensitivity for sudan dyes, the proposed HPLC amperometry of analysis could be applicable in carrying out controls in the field of food analysis, and also the evaluation of sudan dyes in small amounts. In addition the CNT modification procedure is also simpler and quicker than other modification methods (Zhao et al., 2005).

Acknowledgements

This work was supported by the Thailand Research Fund (Research Team Promotion Grant), Chulalongkorn University and The 90th Anniversary of Chulalongkorn University (Ratchadphiseksomphot Endowment Fund). Special thanks are extended to the National Research Council of Thailand (NRCT), NSFC (No. 20305001).

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