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Ternary composites of nanocellulose, carbonanotubes and ionic liquids as new extractants for direct immersion single drop microextraction

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ABSTRACT

We proposed for the first time the use of Nanocellulose (NC) into a single drop for extracting and preconcentrating a heterocyclic amine (HCA) in fried food. In conventional single-drop microextraction (SDME) techniques, ionic liquids (IL) or other organic solvents cannot extract HCAs due to its polarity. The advantageous combination of nanomaterials and nanohybrids based on NC and multiwalled carbonanotubes (MWCNT) with IL allows the preparation of a stable droplet with an excellent and selective ability for the preconcentration of the mutagenic 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) by the simple direct immersion SDME technique. The main variables involved in the extraction and preconcentration steps have been evaluated and optimized. The developed method was found to achieve a linear calibration curve in the concentration range of 0.1–10 mg L^{-1} (r^2=0.998), with a detection limit (LOD) of 0.29 mg L^{-1}. Recovery of the method, which was studied in quintuplicate in sausage samples, varied from 90.1% to 95.3% for MeIQx.

1. Introduction

The employment of nanomaterials in different areas has increased exponentially in the last decade because of their unusual advantages such as unique thermal, mechanical, electronic and biological properties not found in conventional materials [1–4].

Within the group of nanomaterials we can find the nanocellulose (single individual fibers with nanometric size), which is a natural biopolymer renewable, cheap and abundantly available in nature with fascinating properties such as high specific surface area, high chemical or biological reactivity, and occasionally even high porosity. Their applications in the field of nanocomposites can be summarized as non-caloric food thickeners, emulsion/dispersion, oil recovery and cosmetic/pharmaceutical applications in the electronics sector [5]. However, there is no record of the application of nanocellulose in analytical chemistry. The preparation of nanocellulose can be performed by oxidation and defibrillation of microcellulose with strong acids or 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) radical [6]. Other methodologies for preparing nanofibers involve the use of high-intensity ultrasonication and deacytilation and cationization reactions of cellulose [7,8].

Carbonanotubes (CNTs) are well-known type of nanomaterials characterized for their unusual strength and physical properties that make them very unique and promising sorbent materials for analytical purposes [9]. However, CNTs are highly prone to aggregate, which limits their excellent properties. Last decade ionic liquids have emerged as a green alternative to those common toxic organic solvents (dimethyl formamide and N-methyl pyrrolidone) for the dispersion effectiveness of nanotubes. Fukushima et al. [10] used imidazolium ion-based ILs as a new class of CNT dispersants for the first time. The employment of CNTs in combination with ILs has been described in our group for the determination of pesticides [11], in which the soft material was immobilized on cotton fibers to perform the preconcentration of polycyclic aromatic hydrocarbons (PAHs) from river water.

Heterocyclic aromatic amines are one family of compounds that were shown to be potent geno-toxins. The group of amino-imidazo-azaarenes is formed under mild heating conditions (150–300 °C) during the cooking of food, in particular red meat, but also fish. Imidazoquinoxaline derivatives such as 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline (DiMeIQx) were found to be much more mutagenic than imidazopyridine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [12], which is most prevalent in cooked food [13]. The determination of this family is

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Abbreviations: CNTs, carbon nanotubes; c-MWCNTs, carboxylated multiwalled carbonanotubes; ILs, ionic liquids; BMIM · PF_6, 1-butyl-3-methylimidazolium hexafluorophosphate; NC, nanocellulose; SDME, single-drop microextraction; DI, direct immersion; HS, head space; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy; PAHs, polycyclic aromatic hydrocarbon; HCAs, heterocyclic amines; MeIQx, 2-amino-3, 8-dimethylimidazo[4,5-f]quinoxaline; PHiP, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine; 4,8-DiMeIQx, 2-amino-3,4-8-trimethylimidazo[4,5-f]quinoxaline; Fa, adhesion force; LQ, limit of quantification; LOD, limit of detection; RSD, relative standard deviation

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mainly carried out by chromathographic techniques but also using capillary electrophoresis (CE) [14,15]. Since CE has LOD higher than liquid chromatography–mass spectrometry, it is important to preconcentrate analytes. Preconcentration of HCAs has been carried out by using solid phase extraction techniques [16]; however, no record for the preconcentration of HCAs using SDME has to date been reported.

The use of ILs in SDME [17,18] has been extensively described for being a simple and low cost method of preconcentration analytes from different matrices. Previously, our group has described the innovative combination of quantum dots (QDs) and ILs for the first time in Head Space Single Drop Microextraction [19], with the aims of preconcentrating aliphatic amines into ILs and detecting them *in-situ* using luminescence QDs as nanosensor.

This paper proposes the first introduction of NC and/or CNTs into IL for selectively preconcentrating 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in fried-sausages using the direct immersion SDME technique, owing to the enhancement of the adsorption ability and the stability of the single drop and being possible the introduction of a highly stirring step.

For better comprehension of the paper, we will introduce the terms “hybrid” to name the combination of NC and c-MWCNTs, and “composite” to talk about nanomaterials combined with 1-butyl-3-methylimidazolium hexafluorophophate ($BMIM \cdot PF_6$).

2. Experimental section

2.1. Reagents and materials

Avicel PH-101 cellulose microcrystalline (50 μm of particle size), 2,2,6,6-tetramethylpiperidine-1-oxyl radical (98%), sodium hypochlorite solution (10–15%), sodium chloride (BioXtra, ≥99.5%), sodium bromide (>99%), phosphoric acid (85%), potassium hydroxide (85%), potassium bromide (FTIR grade, ≥99%), ethanol (anhydrous) and immersion oil were purchased from Sigma-Aldrich; nitric acid (69%), hydrochloric acid (37%), sodium hydroxide and methanol from PANREAC; MWCNTs from Baytubes (C150F, Lot no. Z0010AAD07, Drum-no. 040); 1-butyl-3-methylimidazolium hexafluorophophate (99%) from MERK; 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PHiP), and 2-amino-3,4-8-trimethylimidazo[4,5-f] quinoxaline (4,8-DiMeIQx) from Toronto Research Chemicals Inc. All cartridge-type filters were purchased from Análisis Vínicos.

Ultrapure water used throughout all experiments was purified through a Millipore system.

All reagents were used as received without further purification.

2.2. Instrumentation

A P/ACE MDQ Capillary Electrophoresis System from Beckman (Palo Alto, CA, USA) equipped with a DAD and using a fused silica capillary (Beckman Coulter) of 75 μm inner diameter, 70.2 cm total length, and 40 cm effective separation length was used. The applied voltage was 20 kV and the working temperature was 25 °C. The samples were injected into the capillary by hydrodynamic injection for 10 s at 0.5 psi. All buffer solutions were filtered through a nylon membrane of 0.45 μm of pore size before analysis. Prior to first use, the capillary was conditioned by rinsing with 1 M HCl for 5 min, 0.1 M NaOH for 10 min, and water for 5 min using a pressure of 20 psi in all cases. The capillary was prepared for daily use by rinsing with 0.1 M KOH in methanol for 2 min, methanol for 5 min, water for 5 min and separation buffer for 15 min, with a pressure of 20 psi.

Raman spectra were obtained using a frequency doubled Nd-YAG laser with 532 nm excitation with a WITec UHTS 300 spectrometer. Nanomaterials and composite were placed onto a glass and objectives of Eplan 100 × /0.9 EPI and 100 × /1.25 oil 160/0.17 WDO.14 applying oil immersion were used, respectively.

Infrared spectra were recorded with a Tensor 27 FT-MIR spectrophotometer equipped with a Hyperion 2000 microscope, using KBr pellets prepared from the samples.

Using a Q 50 TGA instrument thermogravimetric measurements were performed. Temperature programs for dynamic tests were run from 100 °C to 900 °C at a heating rate of 10 °C/min. These tests were carried out under nitrogen atmosphere (20 ml/min) in order to prevent any thermoxidative degradation.

2.3. Preparation of nanomaterials and composites

In this subsection, the carboxylation of pristine-MWCNTs, the oxidation and defibrillation of microcellulose and the preparation of composites are described.

2.3.1. Preparation of carboxylated MWCNTs (c-MWCNTs)

Pristine MWCNTs (80 mg) were oxidized with HNO_3 3 N (70 ml) under refluxed conditions. Prior to the process, MWCNTs were sonicated in nitric acid for 2 h to avoid agglomeration of nanotubes and anchoring acid solution uniformly on the carbon surface. Thereafter, homogenized carbon solution was oxidized under reflux at 120 °C for 72 h to introduce functional groups. Stirring and decantation were consecutively conducted for five times and finally c-MWCNTs were filtered and washed with plenty of deionized water till the water pH reach approximately 7. A yield of 90.5% was obtained after drying the resulted nanotubes under vacuum. The final product was characterized using TGA and Raman spectroscopy.

2.3.2. Functionalization and defibrillation of microcellulose

Dry microcellulose (2 g) is suspended in water (80 ml) and stirred. Then, NaBr (12.2 mmol, 1.25 g) and TEMPO (0.125 g prepared in 10 ml of water) were added. A pH-probe was used to maintain the pH at 10 with NaOH 1 M during all the reaction period. Next, NaClO (2.5 mmol, 23 ml) was added dropwise from a plastic syringe mounted on a syringe pump to keep constant pH = 10. The end of the reaction is reached when no further changes in pH are observed. Finally, quenching is performed with 15 ml of ethanol and filtration and washing steps with water were followed afterwards. A yield of 80% was obtained after drying under vacuum. This material was characterized using TGA, IR and Raman spectroscopy.

2.3.3. Preparation of the composites

Preparation of the composites based on CNTs, NC and NC–CNT hybrid in $BMIM \cdot PF_6$ follows the same procedure; all components were well mixed manually in different proportions (see Table 1) during 15–20 min in an agate mortar to assure the homogeneity of the resulting material. The final mixtures were stable and homogeneous and were subsequently stored at room temperature for the posterior evaluation of their preconcentration abilities. The selected composite was characterized by IR and Raman spectroscopy with an oil immersion lens.

2.4. Proposed method to determine 2-amino-3, 8-dimethylimidazo [4,5-f]quinoxaline

2.4.1. Extraction procedure

A single drop fixed in a syringe-needle came into direct contact with 3 ml of stock solutions containing different concentrations of analyte. Each vial contains a 7 mm × 2 mm magnetic stirring bar. All vials were tightly sealed with a silicone septum, placed in a stirring plate. During the extraction procedure, vials were kept stirring at different speeds (900–2000 rpm) at 25 °C while the droplet was carefully exposed to the sample at the needle tip for a period of time (0.2–2.5 h). Once the extraction was finished, the drop was retracted into the syringe and deposited in an eppendorf vial for the elution by sonication with 150 μL of methanol, and posterior centrifugation for removal of the nanomaterials. The analyte was detected at 259 nm by capillary electrophoresis using a buffer of 30 mM H_3PO_4 and 20 mM NaCl in 30% (v/v) of methanol at pH 2.

A scheme of the extraction protocol is depicted in Fig. 1.

2.4.2. *Preparation and pretreatment of sausage samples*

Pork sausages were purchased from a local supermarket. The pork product was pan-fried very well. The degree of doneness was based primarily on visual inspection. The meat was minced and stored at −18 °C until use.

The levels of MeIQx were measured in quintuplicate by the analyses of different aliquots of the sample after a clean-up process, in which the minced meat was treated with NaOH 1 M during 2 h under stirring and the solution was then filtered through diatomaceous earth. The preconcentration step was conducted as described in Section 2.4.1.

Table 1
Nanomaterial proportions and extraction efficiencies of prepared composites based on BMIM · PF_6; the optimal proportions for each kind of composite are indicated in bold letters.

% c-MWCNTs (w/w)	% Nanocellulose (w/w)	Extraction efficiency (0.5 h) (%)	Extraction efficiency (2 h) (%)
2.20	–	1.40	37.48
1.70	–	5.89	43.63
1.10	–	**8.97**	**45.33**
0.70	–	2.93	18.90
–	2.10	4.97	9.31
–	1.40	27.46	61.43
–	**1.10**	**31.15**	**68.90**
–	0.70	6.37	47.90
0.75	0.50	32.96	53.20
0.48	0.69	2.15	55.58
0.48	1.37	38.60	56.39
0.50	0.50	39.00	40.61
0.33	**0.33**	**41.36**	**68.76**
0.25	0.25	36.32	64.51
0.25	0.50	25.75	59.55
1.00	0.50	36.66	64.24
0.50	0.25	10.66	59.32
0.50	0.25	10.64	67.48

3. Results and discussion

The selection of an appropriate extraction solvent is a major challenge for the optimization of the SDME. Besides the fact that the solvent must be immiscible with the sample matrix, it should have a great and selective extraction capability for the analyte. For this purpose, the methodology developed in this work consists in the preconcentration of a certain pollutant by the use of a complex matrix formed by different nanomaterials combined with an immiscible ionic liquid. The advantages observed from the combination of CNTs and ILs have been previously described as a way to obtain well-dispersed nanomaterials in such a solvent but no application to SDME has been developed till now. In our group, we had described the existence of a synergic interaction that allows these combinations based on CNTs (different types) and ILs to have novel and tunable properties regarding analytical purposes [20].

Here we described the effect of NC and/or CNTs introduced into imidazolium-based IL for the extraction and preconcentration of MeIQx from meat samples.

3.1. *Oxidation of multiwalled carbonanotubes*

Functional groups attached on CNT surface are found to be responsible for various physicochemical and catalytic properties of the matter [21]. As many researchers have oxidized pure CNTs from different routes, an efficient method using nitric acid under refluxed conditions was followed and the resulting c-MWCNTs were characterized by TGA analyses for identifying the oxygenated acidic surface group as a qualitative technique (depicted in Fig. S1A); detachment of carboxylated groups from CNT surface can be observed from the higher weight lose at around 200–400 °C but no destruction occurred on the sidewalls as Raman spectroscopy (Fig. S2) indicates for the presence of both typical D and G bands.

3.2. *Functionalization and defibrillation of cellulose*

2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) belongs to the highly stable nitroxyl or nitroxide radical class of compounds. Saito et al. [22] recently reported a successful disintegration of cellulose from various sources following TEMPO-mediated oxidation to obtain individualized nanofibers. The TEMPO-oxidized nanofibrils ranged from 3 to 5 nm in width and several hundred nanometers to a few microns in length as measured from TEM images [23]. The oxidation mechanism to obtain 6-carboxycellulose *via* TEMPO is summarized in Fig. S3.

The resulting nanocellulose was characterized using TGA, FTIR and Raman spectroscopy. TGA analyses indicate that the microfiber started decomposing at around 300 °C while nanocellulose did so over 200 °C under N_2 atmosphere (Fig. S1B). FTIR (Fig. S4)

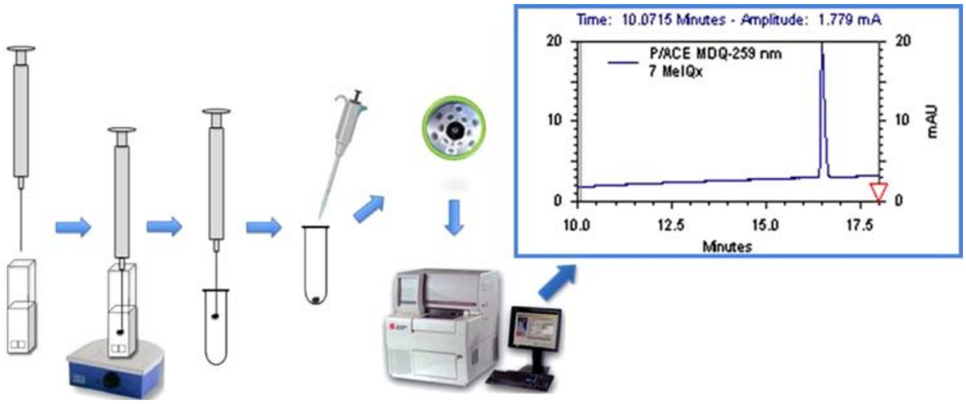

Fig. 1. Methodology for the extraction process.

and RAMAN analyses (Fig. S2) were carried out to analyze the surface chemistry of nanocellulose, in which the resulting peaks indicate the preservation of the cellulosic structure.

3.3. Composites of functionalized nanocellulose and/or carboxylated carbonanotubes

The combination of NC and/or MWCNTs with BMIM · PF_6 gives rise to a very stable system in which both nanomaterials are well dispersed and orientated in a favorable manner inside the IL directed *via* weak interactions such as hydrogen-bonding, π-stacking and other weak electrostatic forces.

The excellent features of each component independently can be transferred and implemented by the formation of the NC–CNT hybrid into IL. Thus, the excellent physical and thermal stability of IL and the large specific surface area of the three-dimensional frameworks of both types of nanomaterials give rise to excellent and promising sorbent materials for certain target molecules.

To assure the homogeneity of the composite, the mixing procedure was carried out manually. We employed 1-butyl-3-methylimidazolium cation despite other ILs because MWCNT dispersability increased with the length of the imidazolium-hydrocarbon chain. Also the selection of PF_6 anions is due to their higher immiscibility with water.

The procedure for the preparation of all composites is summarized in Fig. 2.

Raman spectroscopy was used to evaluate the integrity of NC and MWCNTs into IL and to provide information about the interaction between the nanomaterials employed in the preparation of the composite. The Raman spectrum of the composite depicted in Fig. S2 is the result of the combination of both nanomaterials (0.33% (w/w) each one) into BMIM · PF_6, being possible to distinguish the typical bands of MWCNTs (D and G) and nanocellulose (Csp^3, COC); moreover, a decrease in the intensity of these bands and the presence of a small peak corresponding to the IL anion, PF_6^-, were found. A blue-shift of the D-band was observed. The magnitude of the shift was ~58 cm^{-1}, being an indication of the integrity of the CNTs into the matrix formed by the NC and IL; presumably, the system is ruled by a great number of weak interactions between the ingredients, owing to the big change in surface properties of CNTs when NC is present in IL.

3.4. Preconcentration and detection of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline

Different composites were evaluated for the isolation and preconcentration of MeIQx present in aqueous samples. The method developed consists in the use of the well-known dispersive-SDME technique applying innovative matrices as a supported drop. The stability of the organic drop suspended at the needle tip is correlated to adhesion force (Fa) and both densities (aqueous and organic phase). It is expected that the introduction of nanomaterials into IL increases the viscosity, and thus the Fa.

Therefore, the advantages of this methodology are the higher stability of the drop immersed in the aqueous solution, the use of high stirring process to facilitate a complete contact between the analyte present in the aqueous solution with the extracting drop, and as well the possibility of using higher temperatures to extract non-volatiles analytes from aqueous solution to increase the extraction efficiency and the preconcentration step because of the enhancement of the diffusion rates of the analyte in the phases.

Typical stirring rates in direct immersion SDME are no higher than 1000 rpm unless specially modified needle tips are employed, in which 2000 rpm had been achieved [24]. To allow this stirring speed, a new class of a single drop microextraction technique was proposed by Bagheri et al., in which the microdrop was positioned at the bottom of homemade-conical vial instead of using a needle, while the magnetic bar stirs above the organic droplet.

After evaluating different solvents as the extraction system, no preconcentration was observed by using different types of organic solvents neither ILs as the droplet immersed in the aqueous solution is enriched with the analyte. However, the introduction of carboxylated MWCNTs into IL has improved considerably the extraction efficiency if compared with only BMIM · PF_6 in presence or absence of pristine MWCNTs. This fact indicates that carboxylic groups of CNT surface play an important role on the sorption ability of the composite possibly *via* hydrogen bonding.

After these considerations, the introduction of a cheap, abundant and costless element inside IL that could act as CNTs to improve the extraction efficiency of the target analyte was examined. One material that fixed perfectly with these expectations is NC. Cellulose as microfibers has been employed for isolation of MeIQx without any result; however, a significant improvement on the efficiency of the extraction by the use of oxidized-nanofibers of cellulose was observed, even when compared with CNT composites.

3.4.1. Optimization of key variables

3.4.1.1. Drop volume. The effects of drop size on the preconcentration of the MeIQx were examined in the range of 2–6 μL while keeping constant the volume of spiked sample at 3 ml. The recommended drop volume was 4 μL since higher extraction efficacy was obtained. Bigger sizes produce unstable drops.

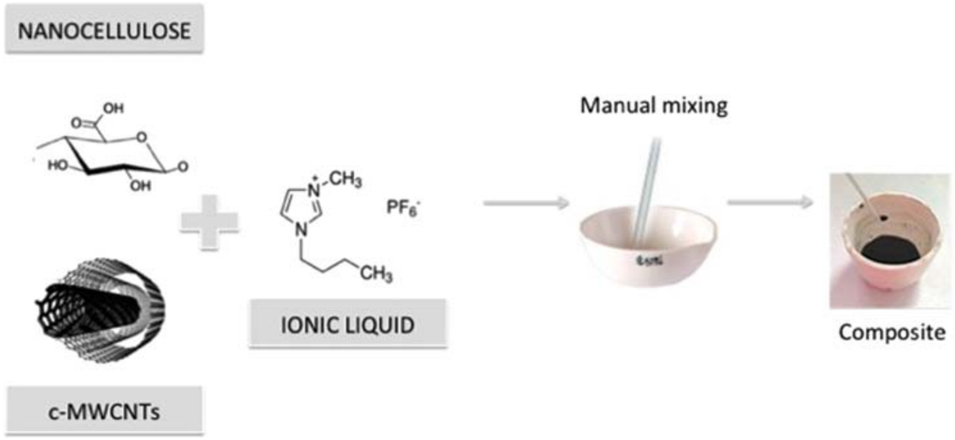

Fig. 2. Scheme for the preparation of composites.

3.4.1.2. Stirring speed. We proceed to evaluate the stirring speed of the sample to improve the extraction efficiency and reduce the extraction time by increasing and facilitating the contact of the analyte present in the aqueous solution with the microdrop. Three sets (900, 1100, and 2000 rpm) of stirring rates were considered. Fig. S5A shows the extraction efficiency as a function of the speed ratio. As we expected, the extraction efficiency increased with the stirring rate up to 2000 rpm; speed of 2000 rpm produces the higher extraction value after 30 min but the instability of the drop under such strong speeds makes it unbearable. From the data we concluded that better efficiency extraction was achieved using a speed of 1100 rpm, and this value was used for subsequent experiments.

3.4.1.3. Concentration of nanomaterials. Composites containing different proportions of NC and/or c-MWCNTs were prepared and evaluated (see Table 1). In general, NC-composites provide a better extraction if compared with CNT-composites. In fact, the highest extraction efficiency was found for 1.10% (w/w) of NC, as depicted in Fig. S5B. At concentrations of NC higher than 2.10% (w/w) no extraction was observed possibly due to a high saturation of the IL with nanomaterial inhibiting their adsorption ability against the analyte. This effect was also observed in CNT-composites and it can be explained for the possibility of bundle formation. On the other hand, those composites with low concentration of nanomaterials induce low extraction efficiencies due to a reduction of nanomaterials in the droplet that affects directly with the sorption features of the matrix. Interestingly, when both NC and CNTs are together into the IL the extraction of the target analyte is slightly improved, possibly due to a favorable and special assembled network of all components in the matrix.

3.4.1.4. Extraction time. Evaluation of extraction profiles by varying the exposure time from 0.5 to 2.5 h was performed. As depicted in Fig. S5C, no significant changes in the effectiveness of extraction after 2 h were observed for NC–CNT composite. In all assays we have compared both extraction time values of 0.5 and 2 h as the most significant ones; however, extraction time was set at 0.5 h as the optimized value.

3.4.2. *Optimized composite materials*

Our goal is to find maximum values of extraction efficiency as the function of several variables over each type of composite.

Table 1 summarizes all composites based on NC and/or c-MWCNTs at different proportions in BMIM · PF_6; the optimal proportions for each kind of composite are indicated in bold letters. Composites based on c-MWCNTs or NC provide the best extraction efficiency with 1.1% (w/w) of the nanomaterial into IL; however, the highest extraction efficiency is reported for the composite containing both NC and c-MWCNTs with proportions of 0.33% (w/w) for each nanomaterial.

As depicted in Fig. 3 the best option is the composite based on NC and c-MWCNTs. Despite having similar extraction efficiency than the composite of NC (1.10% (w/w)) after 2 h of extraction, the combination of all nanomaterials together implement considerably the extraction efficiency in the first half an hour; a special interaction between NC and CNTs into IL implies a synergic effect in the sorption mechanism. This fact evidence the important role of NC by improving selectivity and enrichment factor of the preconcentration step. Thus, the composite containing NC and c-MWCNTs (0.33% (w/w) each) was selected for further experiments. Viscosity at 25 °C of this composite (80.3 mPa s) was found slightly higher than for BMIM · PF_6 (60.9 mPa s) indicating higher Fa; therefore, stabilization of the drop is reinforced.

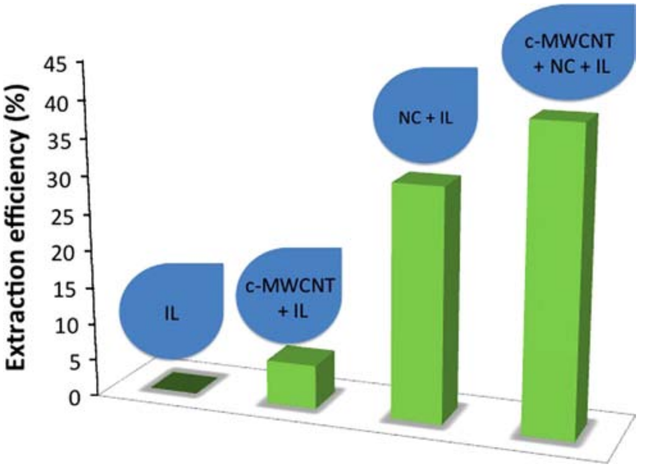

Fig. 3. Extraction efficiencies of the proposed composites and ionic liquid as extractants (values calculated for an extraction time of 0.5 h).

Table 2
Analytical features of merit for the determination of MeIQx.

Figures of merit (MeIQx)	RSD[a] (%) $n=5$	Linearity range ($mg\ L^{-1}$)	r^2[b]	LOD[c] ($mg\ L^{-1}$)	ER[d]
	1.28	0.1–10	0.998	0.29	70.9

[a] RSD: relative standard deviation.
[b] r^2: Correlation coefficient.
[c] LOD: limit of detection.
[d] ER: absolute extraction recovery.

3.4.3. *Determination of 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline*

The method was characterized on the basis of its linearity, sensibility, precision and extraction efficiency (Table 2). A linear calibration graph was constructed from 10 working standard solutions containing MeIQx at different concentrations in the range of 0.1–10 mg L^{-1} ($y=0.1512x–0.115$) with a $r^2=0.998$ and RSD of 1.28%. The standard solutions were subjected to the whole extraction procedure as described in Section 2.4.1.

The sensitivity of the method was evaluated according to the limit of detection (LOD). LOD, calculated as three times the standard deviation of the blank signal divided by the slope of the calibration curve, was 0.29 mg L^{-1}. The limit of quantification (LQ), established for 10 times the standard deviation of the blank signal divided by the slope of the calibration curve, was 0.96 mg L^{-1}.

For the evaluation of the precision, studies of repeatability and reproducibility were performed by quintuplicate at the limit of quantification and were expressed in terms of relative standard deviation (RSD). The repeatability resulted to be 2.52%, while reproducibility in different months was 4.21%.

The absolute extraction recovery, which is referring to the percentage of total analyte that can be extracted efficiently by the composite and eluted with methanol, was 70.9%.

In order to evaluate the applicability of the method, the extraction efficiency of the analyte was examined in the presence of other species of the same family, such as PHiP and 4,8-DiMeIQx. Concentration of MeIQx was kept constant at 2 mg L^{-1} while different concentrations of interferences (5–10 mg L^{-1}) were added. The extraction efficiency of the analyte resulted to be unaltered with concentrations up to 6 mg L^{-1} of 4,8-DiMeIQx and 8 mg L^{-1} of PHiP. The maximum interference levels tolerated were 60% for 4,8-DiMeIQx and 80% for PHiP.

Furthermore, the method was validated by applying to fried pork sausages. No matrix effect was found in the analyses by comparing both calibration curves obtained from spiked sausages and standard solutions.

In order to study the precision of the method, samples were analyzed by quintuplicated obtaining a RSD of 5.36%. With the use of the standard addition method the concentration of analyte in the sausage sample was calculated, being 0.226 mg L^{-1}/g. Samples were spiked with 51 μg/g of analyte, and a relative recovery of 93.3% was found.

Finally, the proposed method was compared with others previously published based on solid phase microextraction procedures (DSC-C18 [25] and propylsulfonic acid silica gel [26]). In our case, the recovery rate was found to be 20% higher than those previously mentioned (see Table S1).

4. Conclusions

This work proposes introducing nanomaterials into ionic liquid as promising extractants to perform direct immersion single-drop microextractions. The presence of nanomaterials into the ionic liquid enhances considerably the sorbent ability of the system due to their high dispersibility, sorbent ability as well as the special non-covalent interactions between them in a synergistic manner. Another advantage of this work is the introduction of a stirring process during the preconcentration step to improve the contact and transference of the analyte present in the aqueous solution to the single-drop. A proposed method was successfully applied to determine 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in meat samples.

Concluding, the fact of introducing nanomaterials into ionic liquid opens new doors for their use as efficient extractants in SDME thanks to their high thermally and mechanical stability of the drop under certain conditions such as stirring or high temperatures.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.02.055.

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