Talanta 208 (2020) 120360

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Dispersive liquid-liquid microextraction coupled with surface enhanced Raman scattering for the rapid detection of sodium benzoate

Linke Xue[a,b], Linke Chen[a], Jing Dong[a], Lemei Cai[a], Yiru Wang[a,*], Xi Chen[a,c]

[a] Department of Chemistry and the MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China
[b] Analysis and Testing Center, Gansu Medical College, Pingliang, 744000, China
[c] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian, 361005, China

ARTICLE INFO

Keywords:
Dispersive liquid-liquid microextraction
Surface enhanced Raman scattering
Sodium benzoate
Medicine

ABSTRACT

Medicine safety has become a large concern and prompts an urgent need to develop a rapid, simple and sensitive analytical method, which can monitor excessive preservatives in medicine. In this work, dispersive liquid-liquid microextraction (DLLME) was combined with surface enhanced Raman scattering (SERS) for a quick analysis of a kind of preservatives, sodium benzoate, in ibuprofen oral solution. The experimental parameters affecting DLLME were systematically investigated. Under the optimal conditions, the whole procedure, including DLLME and the SERS analysis, could be carried out within 10 min. A good linearity between the concentration of sodium benzoate ranging from 10 to 500 mg L^{-1} and the SERS signal intensity could be obtained, and the correlation coefficient (R^2) 0.9986. The method detection limit was 0.56 mg L^{-1}. The relative standard deviation was less than 6.33% for ten replicates at the same sample concentrations. The analytical results prove that the method is suitable for rapid determination of sodium benzoate in ibuprofen oral samples.

1. Introduction

Preservatives are a type of additive added to perishable products to maintain quality and prolong shelf life. Benzoic acid (BA) and its salt, the most commonly used additives, have been widely used as preservatives in foods, beverages, cosmetics, personal care products and oral or parenteral medicines [1–5]. Benzoic acid is used in oral medicines up to 0.15% while sodium benzoate is permitted in oral medicines up to 0.5% [6]. Although sodium benzoate is generally recognized as safe additive, the excessive use could lead to metabolic acidosis, convulsions and hyperpnoea in humans [7]. Moreover, these preservative residues can be considered as environmental contaminants, which increases the cost of wastewater treatment [8,9]. Compared with adults, children are at higher risks due to high quantity intake per kg body weight. A study funded by Britain's Food Standards Agency has reported that artificial preservatives are causes of hyperactivity in children [10,11]. Thus, it is necessary to control strictly the content of sodium benzoate in children's medicines, such as ibuprofen oral solution and so on.

Sample preparation procedures are usually necessary before an instrumental analysis, especially for the quantifying trace level components in complex samples. Conventional techniques, such as liquid-liquid extraction (LLE) [12] and solid phase extraction (SPE) [13], are time-consuming and often involve the use of large volumes of organic solvents, which are considered expensive, time-consuming, labour-intensive and harmful to human health and the environment. Recent research activities are oriented towards the development of efficient, economical, and miniaturized sample preparation methods. As a result, solid-phase microextraction (SPME) [14], liquid phase microextraction (LPME) [15] and hollow fiber liquid phase microextraction (HF-LPME) [16] have been developed. However, most of these methods require considerable extraction time to obtain satisfactory extraction efficiency. Disperse liquid-liquid microextraction (DLLME) has been introduced by Rezaee and co-workers in 2006 [17,18], it has become a very popular environmentally benign sample-preparation technique for a wide range of organic compounds and metal ions in different liquid samples due to its simple, time-saving, environmental-friendly, and low-cost [19]. DLLME is an efficient LPME technique that involves the use of extraction solvent and disperser solvent. In a typical DLLME process, when a mixture of extraction solvent and disperser solvent is rapidly injected into an aqueous sample solution, a cloudy solution is formed due to the dispersion of the extraction solvent into fine microdroplets throughout the aqueous sample, which results in a significant increase in the surface area between the extraction solvent and the aqueous sample. This

* Corresponding author.
E-mail address: yrwang@xmu.edu.cn (Y. Wang).

https://doi.org/10.1016/j.talanta.2019.120360
Received 24 June 2019; Received in revised form 3 September 2019; Accepted 14 September 2019
Available online 16 September 2019
0039-9140/

enhanced contact surface area allows for improved extraction efficiency, accelerated mass transfer of analytes, and very short equilibration time [20]. After centrifugation, the sedimented phase containing the extraction solvent and extracted analytes is analyzed using gas chromatography-mass spectrometry (GC-MS) [21], high performance liquid chromatography (HPLC) [22], or HPLC-mass spectrometry (HPLC-MS) [23].

Instrumental strategies have been reported to detect these benzoic acid or sodium benzoate such as GC [24,25], capillary electrophoresis (CE) [26,27], micellar electrokinetic chromatography [28] and HPLC [29]. These assays usually require expensive apparatus, skilled operators and time-consuming pretreatments. Therefore, they are not suitable for rapid screening of preservatives in a variety of drugs. On the contrary, surface-enhanced Raman scattering (SERS) is a rapid vibrational spectroscopic technique with the advantages of high sensitivity and fingerprint recognition, which make it an ideal method for the quick detection of low-concentration analytes [30]. SERS is based upon conventional Raman spectroscopy, but overcomes its low sensitivity by utilizing tremendous surface enhancement effects of Raman scattering signals for analyte adsorbed onto specially treated nanostructured metallic substrate [31]. The surface plasmon resonance excited between the nanoparticles, such as silver or gold nanoparticles (Ag-NPs or Au-NPs), results in strong enhancement of the SERS signal. In general, these SERS active substrates can be easily synthesized using a chemical reduction method.

Previous work from our laboratory dealt with determination of benzoic acid in carbonated beverages, using thin film microextraction (TFME) followed by SERS. In this work, a rapid, convenient and sensitive method has been developed for the preconcentration and determination of sodium benzoate in ibuprofen oral solution using DLLME combined with SERS. Tributyl phosphate (TBP) was used as the extraction solvent for DLLME of sodium benzoate in a homemade glass conical test tube. After centrifuge, the organic extractant was directly detected by SERS.

2. Experimental

2.1. Chemicals and materials

Analytical grade sodium benzoate (C_6H_5COONa), chloroform, carbon tetrachloride, chlorobenzene were bought from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Different concentration stock solutions of C_6H_5COONa were prepared by dissolving it in pure water and gradually diluting to the final concentration in the range of 10–500 mg L^{-1}. Tributyl phosphate (TBP, AR. ≥99%) was purchased from Aladdin Chemistry Co., Ltd. HPLC-grade methanol, ethanol, acetonitrile and acetone were obtained from Sinopharm Chemical Reagent Co., Ltd. Pure water was obtained from a Simplicity Water Purification Systems (Millipore, Molsheim, France).

2.2. Instrumentation

Transmission electron microscope (TEM) images of the silver nanoparticles (Ag-NPs) were acquired using a JEM-1400 microscopy system (JEOL, Japan). The centrifugation of the cloudy solutions was performed on a TD6 centrifuge (Changsha, China). SERS spectra were recorded using a commercial portable Raman spectrometer (DeltaNu Inspector Raman, USA).

HPLC experiments were performed using a prominence-I LC-2030C 3D machine (Shimadzu, Kyoto, Japan) equipped with a LC-2030C degasser, LC-2030C autosampler, LC-2030C column oven and SPD-M20A diode array detector. An HPLC Shim-pack VP-ODS-C_{18} column (150 × 4.6 mm, 5 μm) from Shimadzu Japan was selected for the separation, UH5300 Double Beam Spectrophotometer (Hitachi, Japan) was determined the maximum of absorption wavelength.

2.3. Preparation of Ag-NPs

The Ag-NPs for SERS was synthesized by the reduction of $AgNO_3$ with sodium citrate. The size of Ag-NPs can be controlled by the ratio of $AgNO_3$ to sodium citrate. Briefly, 250 mL of aqueous solution containing 90 mg $AgNO_3$ was first heated to boil, and then 10 mL of 1% sodium citrate was quickly injected into the above boiling solution. After refluxing for 30 min, the resultant yellow-green colloid was cooled to room temperature and then transferred for further experiments. The concentration of the resultant silver colloid was 0.3 mmol L^{-1}. The Ag-NPs was concentrated before used as following: 1.0 mL of silver hydrosol was centrifuged at 4000 rpm for 15 min to obtain 30 μL of silver colloid by removing the supernatant.

2.4. Measurement of sodium benzoate by DLLME and SERS

All SERS measurements were performed using a commercial portable spectrometer at room temperature. The system resolution was 8 cm^{-1} and the Raman signal acquisition integration time was set as 1s. The laser wavelength was selected at 785 nm with a spot size of approximately 3 mm^2 and the signal collection was through a 10× objective lens. Unless otherwise specified, all the SERS measurements in this study were carried out under the same condition. Schematic representation of the DLLME-SERS detection process is shown in Fig. 1, Five-folds diluted samples was prepared by the addition of pure water, 10 mL diluted sample was added into the homemade glass conical test tube and then, a mixture of 30 μL TBP and 500 μL ethanol was rapidly injected into the diluted sample by a syringe. The tube was placed on a vortex mixer and shaken for 2 min to form a homogeneously suspension for better extraction. Subsequently, the solution was centrifuged at 3500 rpm for 5 min, and then the upper organic phase was collected with a micro-syringe. 10 μL extraction solution was injected on a clean silicon wafer, and then 10 μL of concentrated Ag-NPs was dropped onto its surface and well-mixed. SERS signals were collected 3–4 times randomly within the SERS-active area and the data were averaged.

2.5. Measurement of sodium benzoate by HPLC

The HPLC measurement of sodium benzoate was carried out as following: 2 mL ibuprofen oral solution sample was diluted 15 times with pure water in a beaker, after filtered with a 0.22 μm membrane, 10 μL sample was directly injected into the HPLC system for detection. A binary mobile phase at a flow rate of 1.0 mL min^{-1} was used in the HPLC system. The mobile phase (A) was 0.02 mol/L ammonium acetate solution, and the mobile phase (B) was methanol. The elution program was set as follows: 0–6 min, 85% (A), 15% (B). The detection wavelength was set at 280 nm.

3. Results and discussion

3.1. Characterization of Ag-NPs

The synthesized silver colloid displayed a maximum absorbance being at 417 nm (as shown in Fig. S1), Generally, the absorbance maximum of silver colloids appears between 390 and 420 nm [32], indicating that the synthesized colloid fits with this condition.

The morphology and size distribution of Ag-NPs could be observed by TEM. Most of Ag-NPs synthesized are in round or spherical shape and the size of Ag-NPs is around 56–138 nm. Although some reported have indicated that the optimal size of Ag-NPs for SERS is around 50 nm [33], the Ag-NPs synthesized in this work fulfill the experiment requirements.

3.2. SERS characteristics

The SERS spectra obtained using DLLME-SERS were compared with

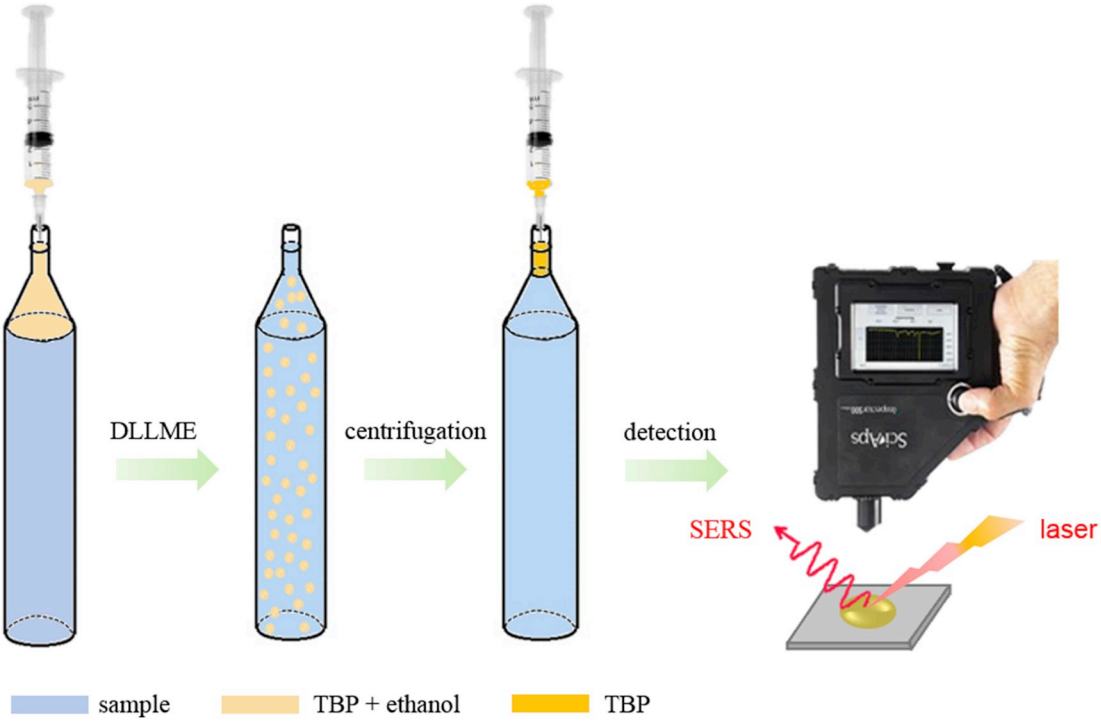

Fig. 1. Schematic representation of the DLLME-SERS detection process.

Table 1
Comparison of SERS shifts of sodium benzoate obtained from experiments and references.

Experimental shift (cm^{-1})	Reported shift (cm^{-1})	Proposed assignment	Reference
832–836	836	$\delta(COO^-)+\beta(C–H)$	[34,35]
1001	1000–1003	Ring breathing	[34,35]
1134	1138	β(C–H)	[35]
–	1373	$v_s(COO^-)$	[35]
1590	1595–1599	v(C–C)	[34,35]

the direct detection of sodium benzoate (200 mg L^{-1}) in aqueous solution. 30 μL concentrated colloidal silver and 200 μL sodium benzoate solutions were mixed in a glass tube and the SERS signal was then measured directly. As shown in Fig. S2, no SERS signal was observed in the liquid phase detection because of the low concentration of sodium benzoate. Conversely, several Raman shifts appeared using DLLME-SERS since TBP was favorable for the enrichment of sodium benzoate from the matrix before the detection. As shown in Fig. S3, sodium benzoate ion adsorbed on two Ag atoms through O atoms on carboxyl groups to form a bidentate bridging structure when the Ag-NPs interacted with sodium benzoate [34,35]. Comparison of the main Raman shifts obtained using the DLLME-SERS and the related references are given in Table 1. Most of the Raman shifts in our experiment were consistent with the previous reports. The slight difference may be caused by the interaction of sodium benzoate and Ag-NPs, owing to the discrepancy in the size of the Ag clusters, and electronic effects. Sodium benzoate bonded to Ag-NPs as $C_6H_5COO^-$. The characteristic bands at 836 cm^{-1} and 1373 cm^{-1} were assigned to COO^- in-plane scissoring ($\delta(COO^-)$) and the COO^- symmetric stretching mode ($v_s(COO^-)$). The Raman shift at 1631 cm^{-1} caused by the C=O stretching (v(C=O)) and the O–H in-plane bending (β(O–H)) in the normal Raman spectrum (Fig. S4a) could hardly be observed (Fig. S4b) because of the $C_6H_5COO^-$ form. In addition, the most obvious vibration frequency of the Raman shift at 1001 cm^{-1} was assigned to ring breathing. Thus, the intensity of Raman signal at 1001 cm^{-1} was selected for the quantitative analysis in the subsequent experiment.

3.3. Optimization of the extraction conditions

There are different factors that affect extraction efficiency such as extraction solvent type and volume, disperser solvent type and volume, ionic strength, pH, and extraction time. Optimization strategy provides a higher efficiency of the methodology.

3.3.1. Selection of the extraction solvent

Selection of appropriate extraction solvents is the first key procedure for the DLLME process. Solvents differ in their extraction capabilities depending on their own and the solute's physical and chemical properties. The desired properties of extraction solvents are a high distribution coefficient, good selectivity toward solute, and little or no miscibility with aqueous solution. Organic extraction solvents are selected on the basis of having a different density relative to water, extraction capability of target compounds, low solubility in water and miscibility with disperser solvent, and also compatibility with final analytical instruments [36]. Traditionally, organic solvents such as halogenated hydrocarbons have been widely used as extraction solvents in the DLLME due to their water immiscibility, higher density compared with water, and their extraction capability toward various target analytes. Therefore, chloroform, carbon tetrachloride, chlorobenzene and tributyl phosphate as extraction solvents were examined in order to find the suitable solvent for sodium benzoate in DLLME. In fact, no emulsion was observed when $CHCl_3$ was selected as extraction solvent. Furthermore, there were no SERS signals observed when CCl_4 and C_6H_5Cl were selected as extraction solvents. Although stable cloudy solutions could be formed in these two extraction system and organic extraction solvents could be separated from sample matrix after centrifugation, few sodium benzoate might be partitioned into CCl_4 or C_6H_5Cl. TBP has a better extraction effect on sodium benzoate due to complexation reaction [37] between sodium benzoate and TBP, therefore, TBP was chosen as the extraction solvent for the following experiments.

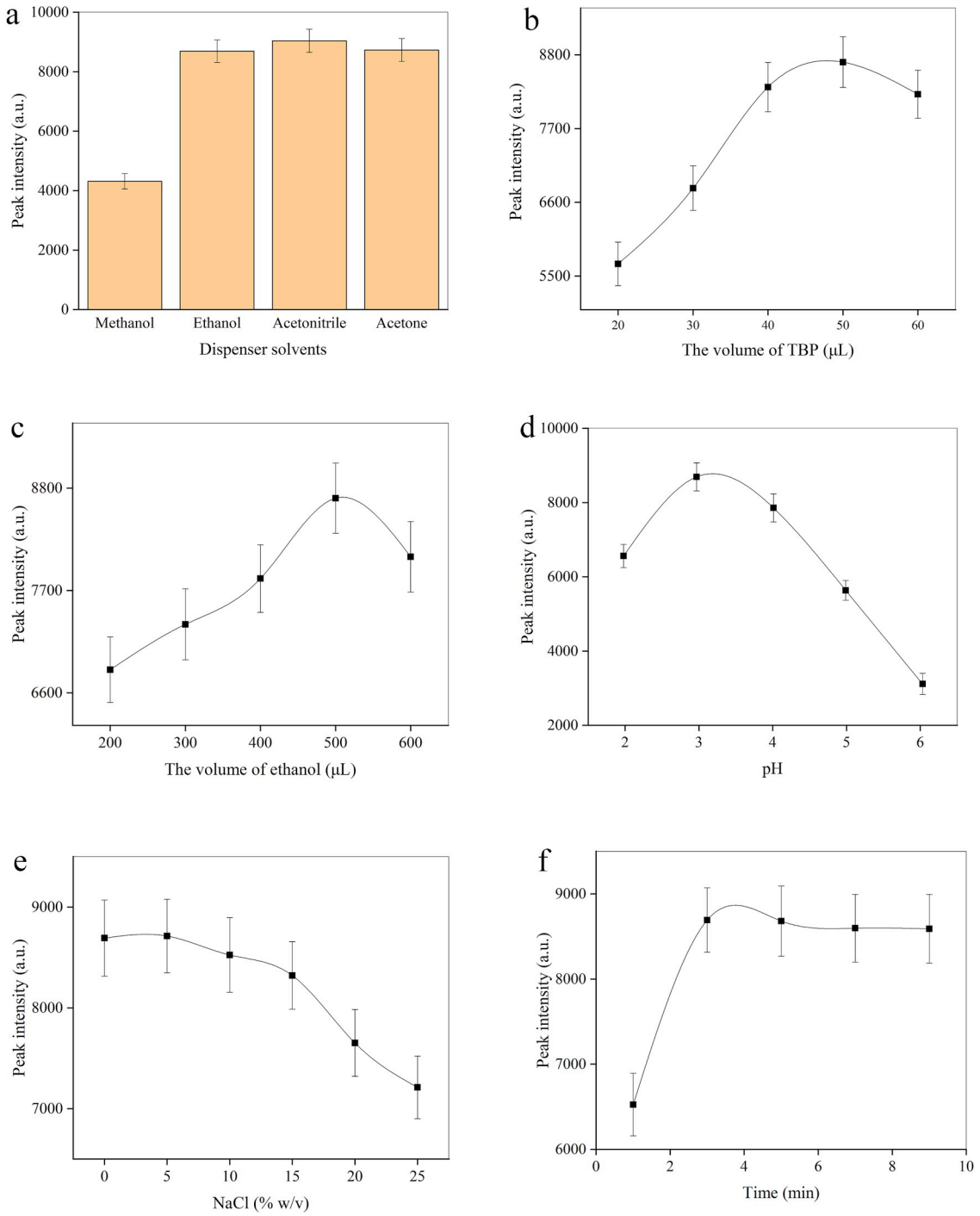

Fig. 2. Optimization of the DLLME extraction conditions: (a) SERS signals of sodium benzoate at 1001 cm^{-1} using different disperser solvents in the DLLME procedure; (b) effects of the volume of TBP; (c) effects of the volume of ethanol; (d) effects of pH; (e) effects of NaCl; (f) effects of extraction time; DLLME conditions: sodium benzoate concentration, 200 mg L^{-1}; sample volume, 10 mL; vibration time, 2 min; centrifugation time, 5 min.

3.3.2. Selection of the disperser solvent

The disperser solvent plays an important part in DLLME on the basis of its miscibility in the aqueous phase and organic phase. Their addition decreases the interfacial tension between the two phases and facilitates the formation of fine droplets in the aqueous phase. As a result, this phenomenon speeds up the mass-transfer process of the analytes from the aquatic phase to the organic phase and thereby overcomes the problem of the time taken [38]. For the sake of acquiring the most suitable dispersive solvent, four kinds of dispersive solvents, namely, acetonitrile, acetone, ethanol, and methanol were studied (Fig. 2a). Ethanol, acetonitrile and acetone could give good extraction efficiency of the target analyte in the presence of 50 μL TBP, which might be due to their higher solubility for analytes and served to facilitate mass transfer of the analytes to the organic extraction solvent. Therefore, ethanol was chosen as the disperser solvent because of its lower toxicity, low-cost and environmentally friendly.

3.3.3. Effect of the extraction solvent volume

The volume of the extraction solvent directly affects the extraction efficiency of the DLLME. As shown in Fig. 2b, the effect of different volumes of TBP, ranging from 20 μL to 60 μL, has been studied. SERS signals increased gradually from 20 to 50 μL due to the increasing of

extraction efficiency. After that, the addition of more TBP gave rise to a more settled phase, resulting in a negative effect due to the decreasing of the concentration of target compound in the settled phase. Thereby, 50 μL TBP was used as extraction solvent in the subsequent experiments.

3.3.4. Effect of the disperser solvent volume

The volume of the disperser solvent directly affects the formation of the cloudy solution (water/disperser solvent/extraction solvent) and the degree of dispersion of the extraction solvent in the aqueous phase, thus affecting the extraction efficiency [39]. At low volume, ethanol cannot disperse extraction solvent properly and cloudy solution is not formed completely. However, at high volume, the solubility of sodium benzoate in water increases, which will result in the decrease of the extraction efficiency. Thus, different volumes of ethanol were investigated to obtain the best results in terms of extraction efficiency. As can be seen from Fig. 2c, the results indicated that the SERS signal increased first then decreased gradually with the increasing volume of ethanol from 200 to 600 μL for the reason mentioned above. Therefore, 500 μL of ethanol was selected as the optimal volume as the dispersive solvent.

3.3.5. Effect of pH

Sample pH has a significant influence on the extraction of analytes in the aqueous phase, because the analytes will be present at different forms (as ions or neutral form) due to acid or base dissociation. The extraction efficiency is related to the existing form of analytes [40]. In addition, the organic extraction solvent, tributyl phosphate, will be hydrolyzed in a strong acid solution or alkaline solution, therefore, the effect of pH on the DLLME performance was investigated within the range of 2.0–6.0. As shown in Fig. 2d, the SERS signals rapidly increased from pH 2.0 to 3.0 and gradually decreased from pH 3.0 to 6.0. The highest SERS signals were achieved at pH 3.0. The functional group of sodium benzoate is –COOH in a strong acid solution, which could decrease polarity of sodium benzoate and contributed to the extraction. On the contrary, the functional group of sodium benzoate is carboxylate radical in alkaline solution, which is not beneficial for sodium benzoate from aqueous phase to organic phase. By adjusting the pH below pK_a 4.21, the number of benzoic acid could increase, and then, solubility increases in organic phase, resulting in better extraction efficiency. Therefore, pH 3.0 was used for the extraction.

3.3.6. Effect of ionic strength

Salt addition influences the partition coefficient of the analyte. With the increase of ionic strength by adding salt into the sample solution, the aqueous solubility of both the non-polar analytes and the organic extraction solvents will decrease. This salting-out effect causes the analytes more easily to transfer from the sample into the organic phase [41]. Thus, effect of the salt concentration from 0 to 25% (NaCl, w/v) was investigated. As shown in Fig. 2e, the SERS signal declined as the concentration of NaCl increased. By increasing the amount of salt, the volume of the settled phase would increase slightly due to salting-out, resulting in the reduction of the SERS signal. Therefore, the DLLME process was carried out with no salt addition.

3.3.7. Effect of extraction time

Mass-transfer of analytes from the sample solution to the extraction phase is a time-dependent process. For this reason, extraction time is one of the most important factors in most of the extraction procedures, especially in microextraction methods such as DLLME [42]. The effect of extraction time on extraction efficiency was investigated in the range of 1–10 min. The SERS signal of sodium benzoate increased when the extraction time increased to around 3 min, and then slightly declined and remained constant over the remaining time (Fig. 2f). The result showed that the equilibrium state was achieved after 3 min, because the large contact surface area between the extraction solvent and aqueous phase causes a very rapid transport of analytes from the aqueous phase to extraction solvent. Consequently, the extraction time of 3 min was selected in the subsequent experiments.

3.4. Repeatability of the SERS signal

The repeatability of the SERS signal is an important factor in the quantitative analysis using DLLME-SERS. In the DLLME, under the optimal condition, 10 mL standard solution of sodium benzoate (200 mg L^{-1}) was added into the homemade glass conical test tube. After the DLLME process, 10 μL extraction phase containing sodium benzoate was extruded on a clean silicon wafer and the same volume of concentrated Ag-NPs was dropped onto it. The SERS spectra were then collected and recorded from 10 randomly selected positions within the SERS-active area. As shown in Fig. 3a, the SERS signal intensities of sodium benzoate at 1001 cm^{-1} were highly uniform, and the relative standard deviations of the SERS signal intensity were found to be 8.9%, suggesting identical capabilities for the different sites in the extraction of sodium benzoate and Raman signal enhancement by the SERS-active substrate.

The repeatability of the SERS signals was obtained from the results of five parallel DLLME experiments. As shown in Fig. 3b, the RSD of the SERS signal at 1001 cm^{-1} of different SERS-active substrates was 12.9%, indicating the acceptable repeatability. These results illustrated that the DLLME-SERS was suitable to be used as the method for sodium benzoate detection.

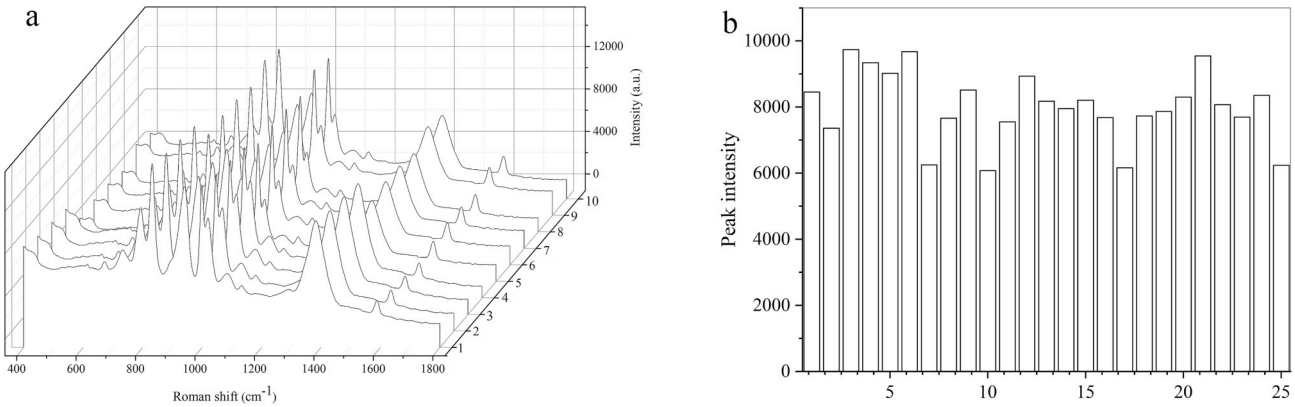

Fig. 3. (a) A series of sodium benzoate SERS spectra acquired from 10 randomly selected positions on the silicon wafer within the Ag-NPs area after DLLME. (b) Peak intensity of sodium benzoate at 1001 cm^{-1}. The data was obtained from silicon wafer at 5 randomly-chosen positions after DLLME.

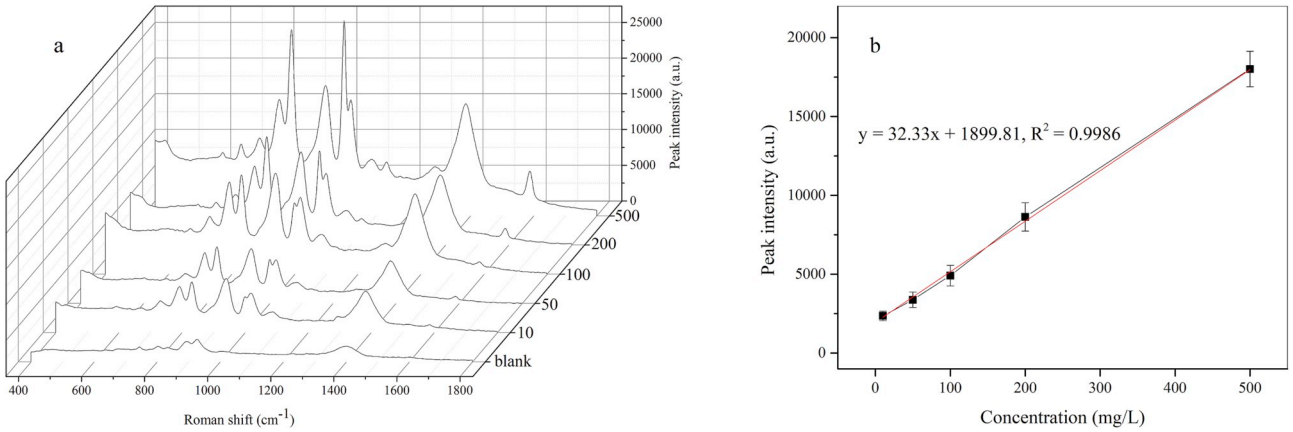

Fig. 4. (a) SERS spectra of sodium benzoate obtained at different concentrations using DLLME-SERS; and (b) simulation curve of the SERS peak intensities at 1001 cm^{-1} using different concentrations of sodium benzoate.

Table 2
Recoveries of sodium benzoate spiked at different levels in ibuprofen suspension samples using the DLLME-SERS method.

Spiking level(mg/L)	Intra day (n = 5)			Inter day (n = 5)		
	Found ($mg{\cdot}L^{-1}$)	Recovery %	RSD %	Found ($mg{\cdot}L^{-1}$)	Recovery %	RSD %
150	120.2 ± 10.0	80.1	8.5	138.2 ± 12.4	92.1	8.8
300	299.1 ± 13.7	99.7	4.6	292.1 ± 24.9	99.3	8.1
500	611.1 ± 38.1	122.2	6.2	611.1 ± 34.0	112.7	6.1

3.5. Analytical performance of the DLLME-SERS for sodium benzoate

3.5.1. Linear range, detection limits and recoveries

The linearity, precision, accuracy and sensitivity were examined to evaluate the DLLME-SERS under the optimal experimental conditions. Generally, 10.0 mL solution containing different sodium benzoate concentrations from 10 to 500 mg L^{-1} was added into the homemade centrifuge tube. The SERS signals of the different sodium benzoate concentrations are shown in Fig. 4. The SERS signal increased with the increase of sodium benzoate concentration. A SERS signal of 10 mg L^{-1} sodium benzoate could still be observed, indicating its high sensitivity. The linear range of sodium benzoate concentration and the SERS signal was in the range of 10–500 mg L^{-1} with the corresponding correlation of 0.9986. The recoveries were achieved in the range of 97.3–105%. RSDs was 6.33% (n = 9) and the LOD was 0.56 mg L^{-1} calculated based on a signal to-noise ratio of 3, suggesting the satisfactory accuracy and application prospects of the method.

The precision of the proposed method was evaluated using ibuprofen suspension samples spiked with three different concentrations of sodium benzoate (150, 300, and 500 mg L^{-1}). As seen from Table 2, the recoveries of the spiked sodium benzoate were in the range 80.1–122.2% and the RSDs were between 4.6% and 8.8%, suggesting the satisfactory accuracy and application prospects of the method.

3.5.2. Determination of sodium benzoate in ibuprofen oral solution

The DLLME-SERS was applied to the preconcentration and determination of sodium benzoate in ibuprofen oral solution samples bought from local drugstore under the optimized conditions. Ibuprofen

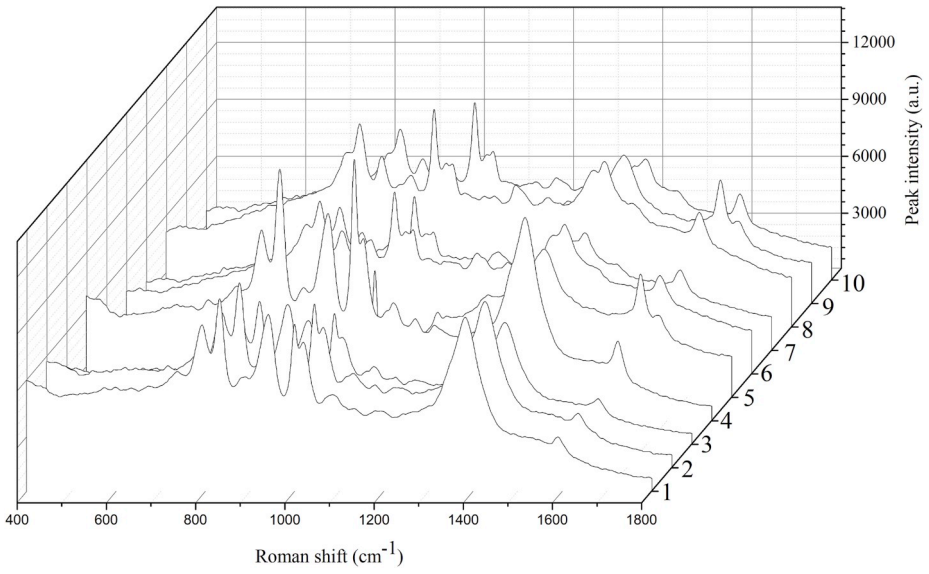

Fig. 5. SERS spectra of sodium benzoate detected in ibuprofen oral solution samples.

Table 3
Amounts of sodium benzoate in different ibuprofen oral solution samples analyzed using the HPLC method and DLLME.SERS.

Sample	HPLC		DLLME-SERS		Analysis of variance		Student's t-test	
	Detected ($mg{\cdot}L^{-1}$)	RSD (n = 2) (%)	Detected ($mg{\cdot}L^{-1}$)	RSD (n = 9) (%)	$F_{calculating}$	$F_{0.05}$	$t_{calculating}$	$t_{0.05}$ (8)
Sample 1	289.5 ± 3.2	3.1	285.7 ± 11.5	8.9	4.2	238.9	0.89	2.31
Sample 2	285.5 ± 2.2	2.5	279.1 ± 8.9	10.2	6.6		1.16	
Sample 3	288.3 ± 4.1	2.7	282.7 ± 9.7	8.6	5.1		1.07	

oral solution is too viscous to transfer analytes more easily than the normal solution from the aqueous phase to the organic phase, therefore, 2 mL ibuprofen oral solution was diluted 5 times with pure water for further extraction. 10 mL of diluted sample was directly added into the homemade centrifuge tube. The DLLME was carried out after the mixture of extraction solvent and disperser solvent was rapidly injected into the homemade centrifuge tube and whirled at 1000 rpm for 2 min. After the mixed solution was centrifuged for 5 min, organic phase contained sodium benzoate was then obtained. 10 μL organic phase and the same volume of concentrated Ag-NPs were placed successively on a silicon wafer for the SERS analysis. As shown in the SERS spectra of sodium benzoate in Fig. 5, all samples revealed the characteristic SERS signals of sodium benzoate and no extra intensive signals could be found, suggesting that the DLLME process possessed the excellent function of eliminating impurities. The results show that sodium benzoate was found in the three samples, although the content of sodium benzoate was not exceed 0.3% (Pharmacopoeia of the People's Republic of China, 2015).

The HPLC method was applied for further quantification and the relevant results (calibration curve, correlation coefficient and chromatograms) are shown in Figs. S5a and b The comparison results of the two methods are shown in Table 3. Although there were some discrepancies between the two methods, the deviation was within a permissible range. Analysis of variance and Student's t-test of the detection results of the SERS and HPLC method shown in Table 3 indicate that there is no significant difference of precision between the SERS and HPLC methods (All $F_{calculating} < F_{0.05}$) as we can see from the variance analysis results. In the Student's t-test, the average value in sodium benzoate detection using the two methods showed no significant differences (All $t_{calculating} < t_{0.05}$), revealing that the accuracy of the SERS method is credible. These results confirmed the capability of the proposed DLLME-SERS method for sodium benzoate quantification in ibuprofen oral solution.

Compared to the traditional methods that generally consist of time-consuming extraction using aether and titration steps, this method is simpler and more time-saving since a single DLLME-SERS run takes only no more than 10 min and the data acquisition takes only 1 s. Furthermore, only one sample at a time can be processed for the analysis of sodium benzoate using the traditional methods, but multi-samples can be simultaneously analyzed in the DLLME-SERS, which greatly reduces the overall analysis time. In addition, only a 2 mL sample was used, insuring a more economical approach in practical applications for routine analysis. The high sensitivity, selectivity and noninvasive nature of the method provides an alternative tool for the fast analysis of sodium benzoate in various liquid medicine sample. The comparison of the SERS spectra of sodium benzoate standard solutions and real samples is shown in Fig. S6. These results revealed that the DLLME-SERS is a convenient and effective method in the determination of sodium benzoate in ibuprofen oral solution.

4. Conclusions

In this study, we developed a rapid, convenient and sensitive method combining DLLME with SERS to analyze sodium benzoate, a widely used and the most versatile preservative in food and medicine. TBP was used as the extraction solvent to separate and enrich sodium benzoate from the samples in the DLLME. Coupled with SERS, the method was successfully applied in the sensitive determination of sodium benzoate in ibuprofen oral solution samples. Sodium benzoate could be detected in ibuprofen oral solution samples in the range 10–500 $mg\,L^{-1}$, and the results were closely consistent with those of HPLC. The characteristics of simpler operation, shorter analysis time and less sample consumption make it a fast, convenient and more economical approach in routine applications. Moreover, since SERS provides an alternative coupling detection tool for the DLLME, DLLME-SERS may provide a promising approach for the fast analysis of sodium benzoate in medicine in order to prevent abuse of sodium benzoate.

Acknowledgements

This work was supported by Scientific Research Foundation of the Higher Education Institutions of Gansu Province (No. 2017A-150) and National Foundation for Fostering Talents in Basic Science (NFFTBS, No. J1310024), which are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.120360.

References

[1] C.Z. Dong, W.F. Wang, Headspace solid-phase microextraction applied to the simultaneous determination of sorbic and benzoic acids in beverages, Anal. Chim. Acta 562 (2006) 23–29.
[2] C.M. Lino, A. Pena, Occurrence of caffeine, saccharin, benzoic acid and sorbic acid in soft drinks and nectars in Portugal and subsequent exposure assessment, Food Chem. 121 (2010) 503–508.
[3] H.M. Pylypiw, M.T. Grether, Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods, J. Chromatogr. A 883 (2000) 299–304.
[4] L.M. Cai, J. Dong, Y.R. Wang, X. Chen, Thin-film microextraction coupled to surface enhanced Raman scattering for the rapid detection of benzoic acid in carbonated beverages, Talanta 178 (2018) 268–273.
[5] A. Del Olmo, J. Calzada, M. Nuñez, Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: uses, exposure, and controversy, Crit. Rev. Food Sci. Nutr. 57 (2017) 3084–3103.
[6] SCCNFP (Scientific Committee on Cosmetic Products and Non-food Products Cosmetic Ingredients), Amended Final Safety Assessment: Benzyl Alcohol, and Benzoic Acid and its Salts and Benzyl Ester, (2002), pp. 1–20 4 June.
[7] S.A.V. Tfouni, M.C.F. Toledo, Determination of benzoic and sorbic acids in Brazilian food, Food Control 13 (2002) 117–123.
[8] H. Farahani, M.R. Ganjali, R. Dinarvand, P. Norouzi, Study on the performance of the headspace liquid-phase microextraction, gas chromatography-mass spectrometry in the determination of sorbic and benzoic acids in soft drinks and environmental water samples, J. Agric. Food Chem. 57 (2009) 2633–2639.
[9] A. Sepehri, M.H. Sarrafzadeh, Activity enhancement of ammonia oxidizing bacteria and nitrite oxidizing bacteria in activated sludge process: metabolite reduction and CO_2 mitigation intensifcation process, Appl. Water Sci. 9 (2019) 131.
[10] C.K. Conners, C.H. Goyette, D.A. Southwick, Food additives and hyperkinesis: preliminary report of a double-blind crossover experiment, Psychopharmacol. Bull. (1976) 10–11.
[11] B. Weiss, J.H. Williams, S. Margen, B. Abrams, B. Caan, L.J. Citron, Behavioral responses to artificial food colors, Science 207 (1980) 1487–1489.
[12] K. Holadová, J. HajšlováA, Comparison of different ways of sample preparation for the determination of phthalic acid esters in water and plant matrices, Int. J. Environ. Anal. Chem. 59 (1995) 43–57.
[13] A. Yasuhara, H. Shiraishi, M. Nishikawa, T. Yamamoto, T. Uehiro, O. Nakasugi, T. Okumura, K. Kenmotsu, H. Fukui, M. Nagase, Y. Ono, Y. Kawagoshi, K. Baba, Y. Noma, Determination of organic components in leachates from hazardous waste disposal sites in Japan by gas chromatography-mass spectrometry, J. Chromatogr. A

774 (1997) 321–332.
[14] N. Negreira, I. Rodríguez, M. Ramil, E. Rubí, R. Cela, Sensitive determination of salicylate and benzophenone type UV filters in water samples using solid-phase microextraction, derivatization and gas chromatography tandem mass spectrometry, Anal. Chim. Acta 638 (2009) 36–44.
[15] L. Vidal, A. Chisvert, A. Canals, A. Salvador, Ionic liquid-based single-drop microextraction followed by liquid chromatography-ultraviolet spectrophotometry detection to determine typical UV filters in surface water samples, Talanta 81 (2010) 549–555.
[16] D.D. Ge, H.K. Lee, Ionic liquid based hollow fiber supported liquid phase microextraction of ultraviolet filters, J. Chromatogr. A 1229 (2012) 1–5.
[17] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid-liquid microextraction, J. Chromatogr. A 1116 (2006) 1–9.
[18] A.B. Tabrizi, Development of a dispersive liquid-liquid microextraction method for iron speciation and determination in different water samples, J. Hazard Mater. 183 (2010) 688–693.
[19] H.A. Mashayekhi, P. Abroomand-azar, M. Saber-tehrani, S.W. Husain, Rapid determination of carbamazepine in human urine, plasma samples and water using DLLME followed by RP-LC, Chromatographia 71 (2010) 517–521.
[20] M. Rezaee, Y. Yamini, M. Faraji, Evolution of dispersive liquid-liquid microextraction method, J. Chromatogr. A 1217 (2010) 2342–2357.
[21] M.A. Gardner, S. Sampsel, W.W. Jenkins, Analysis of fentanyl in urine by DLLME-GC-MS, J. Anal. Toxicol. 39 (2015) 118–125.
[22] D.M. Souza, J.F. Reichert, A.F. Martins, A simultaneous determination of anticancer drugs in hospital effluent by DLLME HPLC-FLD, together with a risk assessment, Chemosphere 201 (2018) 178–188.
[23] N. Wei, X.E. Zhao, S.Y. Zhu, Y.R. He, L.F. Zheng, G. Chen, J.M. You, S. Liu, Z.Q. Liu, Determination of dopamine, serotonin, biosynthesis precursors and metabolites in rat brain microdialysates by ultrasonic-assisted in situ derivatization-dispersive liquid-liquid microextraction coupled with UHPLC-MS/MS, Talanta 161 (2016) 253–264.
[24] C. De Luca, S. Passi, E. Quattrucci, Simultaneous determination of sorbic acid, benzoic acid and parabens in foods: a new gas chromatography-mass spectrometry technique adopted in a survey on Italian foods and beverages, Food Addit. Contam. 12 (1995) 1–7.
[25] Z. Pan, L. Wang, W. Mo, C. Wang, W. Hu, J. Zhang, Determination of benzoic acid in soft drinks by gas chromatography with on-line pyrolytic methylation technique, Anal. Chim. Acta 545 (2005) 218–223.
[26] A.C.O. Costa, L. da Silva Perfeito, M.F.M. Tavares, G.A. Micke, Determination of sorbate and benzoate in beverage samples by capillary electrophoresis-optimization of the method with inspection of ionic mobilities, J. Chromatogr. A 1204 (2008) 123–127.
[27] R. Wei, W. Li, L. Yang, Y. Jiang, T. Xie, Online preconcentration in capillary electrophoresis with contactless conductivity detection for sensitive determination of sorbic and benzoic acids in soy sauce, Talanta 83 (2011) 1487–1490.
[28] M.C. Boyce, Simultaneous determination of antioxidants, preservatives and sweeteners permitted as additives in food by mixed micellar electrokinetic chromatography, J. Chromatogr. A 847 (1999) 369–375.
[29] G.F. Mihyar, A.K. Yousif, M.I. Yamani, Determination of benzoic and sorbic acids in labaneh by high-performance liquid chromatography, J. Food Compos. Anal. 12 (1999) 53–61.
[30] C.K. Muro, K.C. Doty, J. Bueno, L. Halámková, I.K. Lednev, Vibrational spectroscopy: recent developments to revolutionize forensic science, Anal. Chem. 87 (2015) 306–327.
[31] S.M. Nie, S.R. Emery, Probing single molecules and single nanoparticles by surface-enhanced Raman scattering, Science 275 (1997) 1102–1106.
[32] A. Panáček, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V.K. Sharma, T. Nevěčná, R. Zbořil, Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity, J. Phys. Chem. B 110 (2006) 16248–16253.
[33] K.G. Stamplecoskie, J.C. Scaiano, V.S. Tiwari, H. Anis, Optimal size of silver nanoparticles for surface-enhanced Raman spectroscopy, J. Phys. Chem. C 115 (2011) 1403–1409.
[34] X.F. Zhao, Y. Fang, Raman experimental and DFT theoretical studies on the adsorption behavior of benzoic acid on silver nanoparticles, J. Mol. Struct. 789 (2006) 157–161.
[35] J. Gao, Y. Hu, S. Li, Y. Zhang, X. Chen, Adsorption of benzoic acid, phthalic acid ongold substrates studied by surface-enhanced Raman scattering spectroscopy and density functional theory calculations, Spectrochim. Acta A Mol. Biomol. Spectrosc. 104 (2013) 41–47.
[36] Y. Assadi, M.A. Farajzadeh, A. Bidari, 2.10-Dispersive liquid-liquid microextraction, in: Janusz Pawliszyn (Ed.), Comprehensive Sampling and Sample Preparation, 2012, pp. 181–212.
[37] C.J. King, Separation Process Based on Reversible Chemical Complexation, Handbook of Separation Process Technology, John Wiley & Sons, New York, 1987, pp. 760–774 (Chapter 15).
[38] M. Moradi, Y. Yamini, T. Baheri, Analysis of abuse drugs in urine using surfactant-assisted dispersive liquid-liquid microextraction, J. Sep. Sci. 34 (2011) 1722–1729.
[39] M. Saraji, A.A. Hajialiakbari Bidgoli, Dispersive liquid-liquid microextraction using a surfactant as disperser agent, Anal. Bioanal. Chem. 397 (2010) 3107–3115.
[40] M. Zeeb, M. R Ganjali, P. Norouzi, Dispersive liquid-liquid microextraction followed by spectrofluorimetry as a simple and accurate technique for determination of thiamine (vitamin B1), Microchim Acta 168 (2010) 317–324.
[41] S.R. Yousefi, F. Shemirani, Development of a robust ionic liquid-based dispersive liquid-liquid microextraction against high concentration of salt for preconcentration of trace metals in saline aqueous samples: application to the determination of pb and cd, Anal. Chim. Acta 669 (2010) 25–31.
[42] H. Abdolmohammad-zadeh, G.H. Sadeghi, Combination of ionic liquid-based dispersive liquid-liquid microextraction with stopped-flow spectrofluorometry for the preconcentration and determination of aluminum in natural waters, fruit juice and food samples, Talanta 81 (2010) 778–785.