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Simultaneous ultrasound-assisted emulsification–derivatization as a simple and miniaturized sample preparation method for determination of nitrite in cosmetic samples by microvolume UV–vis spectrophotomety

N. Cabaleiro, I. De La Calle, S. Gil, F.J. Pena, M. Costas, C. Bendicho, I. Lavilla*

Departamento de Química Analítica y Alimentaria, Área de Química Analítica, Facultad de Química, Universidad de Vigo, As Lagoas-Marcosende s/n, 36310 Vigo, Spain

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ABSTRACT

A simple and miniaturized approach based on ultrasound-assisted emulsification–derivatization is proposed for the determination of nitrite in cosmetic samples by UV–vis micro-spectrophotometry. Oil/water emulsions were formed using 15 mg of cosmetic sample and 1 mL of an aqueous medium containing 0.5% w/v SDS and 1% v/v acetic acid. When powerful sonication systems were used to make emulsions, i.e. probe or cup-horn sonoreactor, stable and transparent emulsions were obtained in one or half minute per sample, respectively. The Griess reaction in these special conditions (i.e. sonication and the presence of an organized medium) was investigated. The absence of matrix effects allows external calibration with aqueous standards for nitrite quantification. Analytical features were compared to those of the European official method 82/434/EEC. Detection limit, sample throughput and reagent consumption were significantly improved.

1. Introduction

Nowadays, cosmetics are subjected to many quality controls since many substances used in their formulation are regulated by legislation. In general, the analytical methods used in the cosmetic industry are laborious, time-consuming and they have high requirements of reagents [1]. In order to improve the analytical methodology used in the cosmetic industry, implementation of recent developments is necessary, especially in the sample preparation stage. Some improvements in sample preparation proposed so far for cosmetic analysis are based on supercritical fluid extraction [2–6], pressurized liquid extraction [7], solid phase microextraction [2,8–12], use of new extractant solid phases such as nanoparticles [13,14], extraction with hollow fibre-supported liquid membranes [15], microdialysis [16,17] and ultrasonic-assisted emulsification [18].

Ultrasonic-assisted emulsification is an interesting approach for the formation of transparent and stable emulsions from cosmetics [18]. Emulsification of viscous samples, such as cosmetics, can provide important advantages over classical separation and preconcentration procedures for most of the spectrophotometric, spectrofluorometric or chemiluminiscent detection techniques [19]. The low interfacial tension, low viscosity and high solubility of these emulsions make analytical procedures simpler and shorter [19]. In addition, small scale emulsification procedures can be implemented using suitable ultrasonic processors based on probes or cup-horns that allow the use of small sample volumes (i.e. below 1.5 mL) [18,20].

Sodium nitrite must not be present in cosmetic products above 0.2% w/w. [21]. This compound must not be used as rust inhibitor in cosmetics when secondary or tertiary amines are present because nitrosamines can be formed [21,22]. For this reason, the level of adventitious nitrite must be determined in cosmetics and raw materials. Most of the regulations include methods for nitrite determination based on the Griess reaction such as the European directive 82/434/EEC [1]. Different steps of dilution and clarification are necessary when this methodology is used, so the procedure poses several drawbacks such as little sensitivity and tedious operation. Only methodologies based on ion chromatography have been published as an alternative [23–26]. In these cases, simultaneous determination of several anions is carried out in cosmetics. Samples must be pretreated using, e.g. solid-phase extraction [25], and post-column derivatization can be also necessary [25]. Therefore, spectrophotometric based methodologies result faster and simpler.

In this work, simultaneous ultrasound-assisted emulsification–derivatization was proposed as a simple and miniaturized methodology for the determination of nitrite in different cosmetic samples by micro-spectrophotometry following the Griess reac-

* Corresponding author. Tel.: +34 986 812291; fax: +34 986 812556.
E-mail address: isela@uvigo.es (I. Lavilla).

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tion. Method development was focused on the achievement of an optically transparent emulsion, while performing the Griess derivatization reaction in an organized medium with sodium docecyl sulphate. Analytical characteristics of the ultrasound-assisted emulsification–derivatization method were compared with those of the European official method 82/434/EEC for nitrite determination in cosmetics [1].

2. Experimental

2.1. Instrumentation

A Nanodrop® (Thermo Scientific Wilmington, USA) Model ND-1000 spectrophotometer (optical path length 1 mm) was used to carry out determinations in micro-volumes (2 μL). The absorption measurements were carried out at 540 nm.

An UVIKON XS UV/VIS spectrophotometer (Secoman, Domont, France) equipped with conventional cells (optical path length 1 cm) was used for nitrite determination using the official method 82/434/EEC [1].

A 100 W, 20 KHz high intensity ultrasonic processor model VC 50-1 (Sonics and Materials, Inc., Danbury, CT, USA) equipped with a titanium tip of 3 mm diameter and a 200 W, 24 KHz powerful cup-horn sonoreactor UTR200® (Dr. Hielscher Company, Germany) were employed for simultaneous ultrasound-assisted emulsification–derivatization.

2.2. Reagents and samples

A stock standard solution of nitrite (1000 mg L^{-1}) was prepared from sodium nitrite (Panreac, Barcelona, Spain). Working standard solutions were daily prepared by appropriate dilution of the stock solution.

For Griess derivatization, 0.2 g of sulfanilic acid (Probus, Badalona, Spain) were dissolved in 20 mL of ultrapure water and 0.175 g of α-naphthylamine (Carlo Erba, Milan, Italy) were dissolved in 10 mL of glacial acetic acid (Prolabo, París, France). Both solutions were made up to 25 mL volume with ultrapure water and stored at 4 °C in the dark.

Surfactants used in this work for trying different emulsification media were: sodium dodecyl sulphate (SDS, Fluka, Steinheim, Germany), hexadecyl trimethylammonium bromide (CTAB, Sigma–Aldrich, Milwaukee, USA), Tween 80 (Sigma–Aldrich) and Triton X-100 (Merck, Darmstadt, Germany). These surfactants were selected due to their high solubility in water. All of them have an hydrophilic–lipophilic balance (HLB) higher than 10 (dimensionless scale), i.e. 40 SDS; 15 Tween 80; 13.5 Triton X-100; 10 CTAB [27] and can form o/w emulsions.

1 M zinc acetate (Panreac) prepared in 30% (v/v) acetic acid and 0.25 M potassium cyanoferrate (II) (Panreac) were used as clarification reagents in the official method of analysis for nitrite determination. 1 M sodium hydroxide (Sigma) was used for pH adjustment.

High-purity deionised water was obtained from a PETLAB ultrapure water production system (Peter Taboada, Vigo, Spain). All chemicals were of analytical reagent grade. Different cosmetic samples without nitrite as rust inhibitor were analyzed: conditioner, baby shampoo, hair gel and body milk. Some substances in the cosmetic composition were surfactants (i.e. ceteareth-20 or polyquartenium), stabilizers of emulsions (i.e. cetearyl alcohol), preservatives (i.e. methylparaben, propylparaben or methylisothiazolinone), fragrances (i.e. linalool, limonene or coumarin), skin protectants (i.e. dimethicone) or stabilizers (EDTA tetrasodium). Neither inorganic oxides nor light absorbing species were declared in the cosmetic labels.

2.3. Proposed procedures

Two different miniaturized ultrasound-assisted emulsification–derivatization procedures were proposed using the microvolume UV–vis spectrophotometer for measurement.

2.3.1. Procedure I

15 mg of sample were accurately weighed in a 1.5 mL vial and 0.92 mL of dispersing medium (0.5% w/v SDS and 1% v/v acetic acid), 40 μL of 0.01 M sulfanilic acid and 40 μL of 0.012 M α-naphthylamine were added. Then, samples were sonicated for 1 min at 20% amplitude by means of the 3 mm ultrasonic probe. Blanks were treated in the same way.

2.3.2. Procedure II

15 mg of sample were accurately weighed into a 1.5 mL Eppendorf vial and 0.92 mL of dispersing medium, 40 μL of 0.01 M sulfanilic acid and 40 μL of 0.012 M α-naphthylamine were added. Six vials were simultaneously sonicated for 3 min at 50% amplitude in the sonoreactor.

2.4. Adaptation of the European official method (directive 82/434/EEC)

Since the Griess reaction can be carried out with different reagents, the official method involving sulphanilamide and 1-N-naphthylethylenediamine was adapted using sulfanilic acid and α-naphthylamine as derivatizing reagents for comparative purposes. Then, 500 mg of sample were weighed and diluted with hot water up to a volume of 150 mL into a beaker. This was placed in a water bath at 80 °C about 30 min and shaken occasionally. The mixture was cooled at room temperature and 2 mL of both potassium cyanoferrate and zinc acetate solutions were added while stirring. The pH was adjusted to 8.3 with 1 M NaOH. The content of the beaker was quantitatively transferred to a 250 mL volumetric flask and made up to volume with ultrapure water. The mixture was filtered through a fluted filter paper. An aliquot of 25 mL was pipetted into a 100 mL volumetric flask and ultrapure water was added up to 60 mL. After mixing, 4 mL of sulphanilic acid solution and 1 mL of glacial acetic acid were added. The content was mixed again and allowed to stand for 5 min. Then, 4 mL of the α-naphthylamine solution was added and the resulting solution was allowed to stand for 3 min. Finally, the solution was made up to 100 mL volume with ultrapure water. The conventional UV–vis spectrophotometer was used for measurement.

3. Results and discussion

3.1. Formation of optically transparent emulsions

Turbidity must be considered as an important interference when UV–vis spectrophotometry is used as analytical technique. Then, the transparency of the emulsion must be assured. Transparent oil-in-water emulsions (o/w) can be obtained when small droplets are formed since light can pass through these emulsions without undergoing scattering. On the contrary, emulsions with relatively large droplets scatter light and turbidity is observed [19]. In order to obtain a transparent emulsion, different surfactants, acids and sonication systems were tried.

SDS (0.05–1% w/v), CTAB (0.1–1% w/v), Tween 80 (0.005–0.5% w/v) and Triton X-100 (0.001–0.5% w/v) were used for preparation of emulsions. UV–vis absorption spectra of formed emulsions were obtained using the conditioner sample and 3% v/v acetic acid as aqueous phase. 0.5% w/v SDS was selected for making emulsions on the basis of its lack of turbidity and good stability, as can be observed in Fig. 1. Addition of a co-surfactant (usually an aliphatic

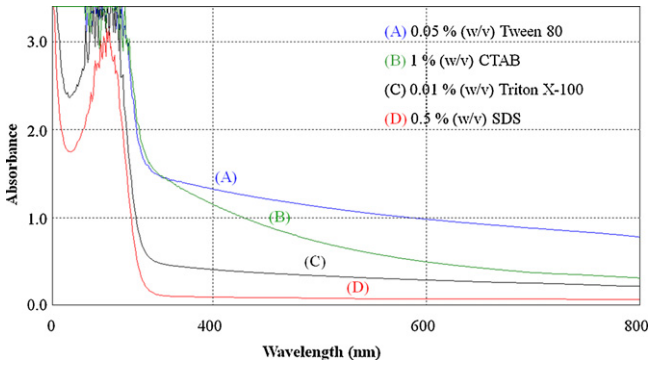

Fig. 1. UV–vis absorption spectra of emulsions formed with the conditioner sample and different surfactants in 3% v/v acetic acid as aqueous medium.

alcohol) was not necessary. CTAB, Triton X-100 and Tween 80 were rejected because emulsions displayed some turbidity.

Turbidity and foams were observed when SDS was used without acid in the aqueous medium. Then, an acid medium seems to be essential in order to reach transparent emulsions. Acetic acid was tried in a concentration range of 0.1–5% v/v. This acid was selected because it could contribute to neutralize oxidant free radicals [28,29] and it is compatible with the Griess reaction [30]. Emulsions were found to be transparent when an acetic acid concentration larger than 0.5% v/v was used.

Three sonication systems were applied: (i) sonication probe; (ii) cup-horn sonoreactor and (iii) sonication bath. When powerful systems (probe or sonoreactor) are used, cavitation is easily achieved and then transparent emulsions are obtained in few minutes. 1 min of sonication at 20% amplitude provides a transparent emulsion with the probe system. With the cup-horn sonoreactor, 3 min of sonication at 50% amplitude were necessary for achieving six transparent emulsions prepared simultaneously. On the contrary, when a sonication bath was used, 15 min of sonication were necessary for achieving a transparent emulsion.

Though the sensitivity of analytical methods increases with sample mass, a high ratio oil/water (i.e. sample/aqueous medium) in the emulsion can contribute to increase turbidity. Then, the maximum ratio sample/aqueous medium was established in 15 mg/mL.

Transparent emulsions were obtained for all cosmetic samples tried in this work. However, bearing in mind that droplet size and then turbidity in emulsions can increase with time due to droplet fusion [31], monitoring of emulsions at 540 nm was carried out in order to verify emulsion transparency for several days. The results are shown in Fig. 2 as a CUSUM chart. This represents the cumulative sum from each successive absorption measurement and a

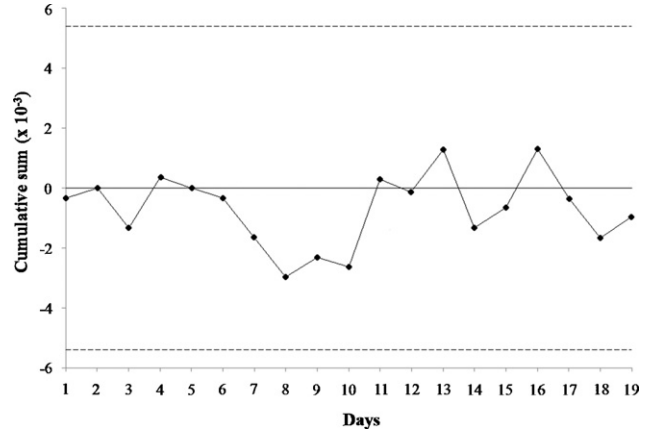

Fig. 2. CUSUM chart for verifying the transparency of the emulsion for 19 days.

reference value corresponding to the initial measurement (emulsion newly formed). This kind of control chart was selected owing to its large sensitivity to small changes. These results guarantee the transparency and stability of cosmetic emulsions for 19 days. As checked by visual observation, the emulsion was kept stable for three months.

3.2. Development of the Griess reaction in special conditions

Sonication and emulsification can affect the Griess reaction. The chemical effects of ultrasound can include the enhancement of reaction rates and the generation of free radicals that can promote redox reactions thereby degrading the analyte [32]. In addition, if an o/w emulsion is used as a medium for a chemical reaction, the special microenvironment existing may change thermodynamic and kinetic reaction constants [19]. Then, the influence of sonication and emulsification on the Griess reaction was studied.

Sonication of an aqueous medium causes the formation of free radicals that can give place to oxidations and, with more difficulty, to reductions. Formation of nitrite and nitrate from N-containing aqueous solutions by ultrasound irradiation has been reported [33,34]. In order to verify that nitrites are not degraded by sonication, standards prepared in 0.5% w/v SDS and 1% v/v acetic acid were used. The Griess reaction with and without sonication was monitored for these standards. Differences were not observed. Probably, the presence of acetic acid in the medium provides additional reducing radicals from ultrasonic degradation of this acid, hence neutralizing in this way the oxidant free radicals and avoiding the degradation of nitrites [28,29].

In addition, the effect of sonication parameters, time and amplitude, on the Griess reaction was investigated using emulsions prepared from the conditioner sample in the sonoreactor. Sonication time was studied up to 6 min and sonication amplitude up to 80% (conditions that assure the emulsion transparency). Results showed that absorbance is comparable in all the cases and then, the use of stronger conditions for sonication does not give rise to the degradation of the azo dye derivative, analyte or reagents.

Reaction kinetics were also studied using a standard without sonication (reference), a standard with sonication and an emulsion prepared with sonication. Pseudo zero order kinetics were observed in all cases. No significant differences in kinetic behaviour were observed (i.e. differences between rate constants were below ±5%). In homogeneous uncatalysed reactions as the Griess reaction, the increase in the reaction rate caused by ultrasound is less marked than in heterogeneous or/and catalysed reactions [35]. Usually, when the Griess reaction is applied for nitrite determination, a rest time of 5 min is necessary after the addition of sulphanilic reagent, then naphthyl reagent is added, a new rest time of 3 min being necessary. In this work, derivatization reagents were added simultaneously and the reaction reaches the equilibrium in 4 min.

The effect of reagent concentrations needed to accomplish the Griess reaction is shown in Fig. 3. 0.01 M, 0.012 M and 1% v/v for sulphanilic acid, α-naphthylamine, and acetic acid, respectively, were found to be optimal in the proposed procedure.

Finally, the azo dye derivative was demonstrated to be stable at least for 1 h following analysis of samples and standards prepared in 0.5% w/v SDS containing 1% v/v acetic acid. Similar times have been established when studying the stability of the derivative in non-micellar media (i.e. water analysis) [36,37].

3.3. Analytical features

Validation of the ultrasound-assisted emulsification–derivatization procedure in order to demonstrate its applicability to nitrite determination in cosmetic samples was performed.

Table 1
Comparison of slopes from external calibration and standard addition methods.

Parameter	Aqueous standards	Standard addition			
		Conditioner	Hair gel	Body milk	Baby Shampoo
Slope	0.4925	0.4785	0.4745	0.4727	0.4691
S.d.[a]	0.0042	0.0011	0.0037	0.0043	0.0039
Change in slope (%)	–	−2.93	−3.79	−4.17	−4.98

[a] S.d.: standard deviation of the slope.

Potential matrix effects were studied by comparison of slopes obtained with external calibration and the standard addition calibration method. The calculated calibration parameters are shown in Table 1. The comparison of slopes is presented in terms of percentage of change in slope (aqueous standard/standard addition). Percentages of change were less than 5% in all cases. Therefore, external calibration with aqueous standards can be made. Calibration graph was linear in the range 0.08–1.6 mg L^{-1}.

Instrumental limits of detection (LOD) and quantification (LOQ) were determined following the 3σ and 10σ criteria, respectively. Procedural LOD and LOQ values in cosmetic samples were determined considering the instrumental LOD and LOQ values and the sample treatment (0.015 g of sample in 1 mL). The precision of the method, expressed as relative standard deviation (RSD), was evaluated in terms of repeatability. RSDs from five independent sample preparations were lower than 2–3% in all cases. Reproducibility was also evaluated during three consecutive days and was found to be lower than 3–4%. No differences in precision of both proposed procedures (probe and sonoreactor) were found.

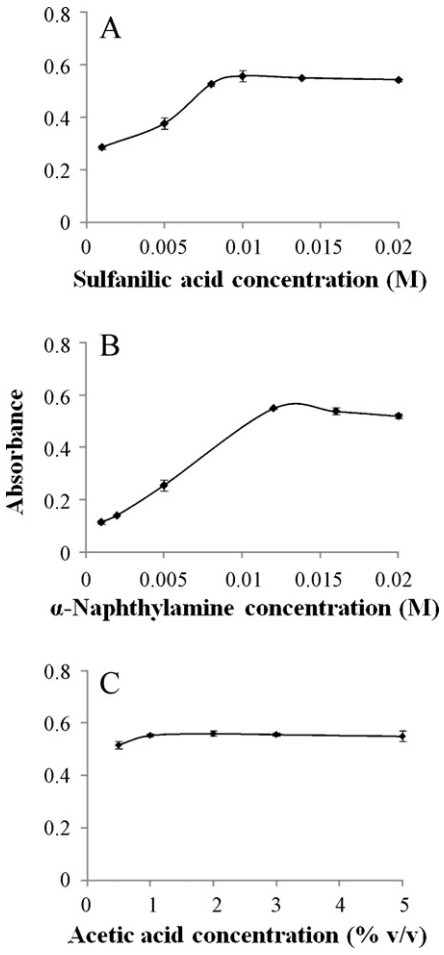

Fig. 3. Effect of reagent concentrations over the Griess reaction using the conditioner sample spiked with 0.8 mg L^{-1} nitrite.

Table 2
Comparison of analytical features obtained with the proposed method and the European official method for the determination of nitrite in cosmetics.

Parameter	Ultrasound-assisted emulsification derivatization	Official method 82/434/EEC
Procedural LOD (μg g^{-1})	0.12	3.54
Procedural LOQ (μg g^{-1})	0.24	7.08
Repeatability (RSD, %)	2–3	2
Reproducibility (RSD, %)	3–5	4
Sample preparation time (min)	1 with procedure I 0.5 with procedure II	38
Final volume of the solution (mL)	1	100
Reagent volume (mL)		
Sulfanilic acid	0.04	4
α-Naphthylamine	0.04	4
Glacial acetic acid	ca. 0.01	1

A comparison of analytical characteristics of the proposed method and the official method are shown in Table 2. As can be seen, an improvement in the detection limit is reached when the proposed method is used. In addition, the reduction in sample preparation time and reagent volumes can be emphasized.

Nitrite concentration was below the detection limit in all samples under study, which was consistent with the labels of the cosmetic products tested. Accuracy was demonstrated by comparison of slopes obtained for aqueous standards and standard addition calibration methods.

4. Conclusions

An ultrasound-assisted emulsification procedure with simultaneous derivatization was developed for nitrite determination in cosmetic samples following the Griess reaction and micro UV–vis spectrophotometry. The use of a cup-horn sonoreactor or a probe sonication system allows obtaining a transparent and highly stable emulsion within a short time. The proposed method entails a high sample throughput, minimal consumption of reagents and an improved detection limit and can be considered as a simple and fast alternative to the European official method for nitrite determination, which could make this method suitable for routine analysis in cosmetic laboratories. In addition, matrix effects are not present and external calibration can be carried out in all cases.

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