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On-line micellar-enhanced spectrofluorimetric determination of rhodamine dye in cosmetics

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Abstract

A simple FI-fluorimetric analytical methodology for the continuous and sequential determination of rhodamine B (RhB) in cosmetic products has been developed and evaluated in terms of sensibility and selectivity. The influence of several surfactant solutions on RhB fluorescence signal has been studied; particular attention was paid in the aggregation behavior of RhB–SDS system. Linear response has been obtained in the range of 1.6×10^{-9} and 1×10^{-6} mol L^{-1}, with a detection limit of 5×10^{-10} mol L^{-1}. The novel technique provides a simple dissolution of sample, on-line filtration with sampling rate higher than 100 samples h^{-1} and has been satisfactorily applied to the RhB determination in commercial lipsticks.

Keywords: Flow injection spectrofluorimetry; Rhodamine B; Cosmetics; Micelles

1. Introduction

Cosmetic products cover a wide range of preparations that are used for cleansing, beautifying or promoting attractiveness. The economic impact of these products cannot be underestimated, worth possibly billions of dollars per year to the cosmetic industries [1].

As cosmetic products are applied on the skin, direct contact with harmful substance could cause skin disease such as irritation, allergic reactions and could be absorbed and be stored in different organs, manifesting at short or long time their toxicity, reason by which governmental entities have listed prohibit substances for cosmetic use.

Among the list, the Food and Drug Administration (FDA) has now regulated the use of Rhodamine B (RhB, $C_{28}H_{31}N_2O_3Cl$) in the cosmetic industries, because of its carcinogenesis.

RhB is a fluorescent dye derivative of the xanthene dyes class, synthesized from condensation of phthalic anhydride with *m*-dialkylaminophenols; it is between the oldest and most commonly used synthetic dyes applied in cloth and food coloring [2]. As fluorescent dye, it is noted due to its special photochemical and photophysical properties [3,4] being very efficient sensitizer in photography [4–8], bioanalytical chemistry [9,10], laser dyes [11–13] and fluorescence probes [14–16] causing a vast and increasing up applications in chemistry, biochemistry and physics probes. In pharmaceutical industries, it has been used as a drug and cosmetic color additive in aqueous drug solutions, tablets, capsules, toothpaste, soap, hair-waving fluids, bath salts, lipsticks [17], eyes shadows and rouges [18].

It has been demonstrated that the acute exposure to RhB results in mucous membrane and skin irritation [19] and its carcinogenesis has been probed injecting RhB subcutaneously in

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rats, producing local sarcomas [20–21]. Applied on lips, it has the capacity of decreasing the collagen content of the fibroblast cell layer of the human lips, which may result from a non-specific inhibition of protein synthesis without non-specific cell damage [22].

Customary standard methods for cosmetic analysis involve an assessment by microscopy and/or microspectrophotometry [23,24], separation techniques such as electrokinetic capillary chromatography [25], thin layer and high-pressure liquid chromatography [24,26]. Most of techniques are not entirely satisfactory since they have inadequate sensitivity, either involve a subjective opinion, or require a complicated pretreatment to isolate the dyes and pigments from the waxy matrix.

In this paper, a direct quantitative determination method of RhB in real cosmetic samples based on its native fluorescence without extraction or pretreatment step has been developed. Enhancement of sensitivity was attempted employing an anionic surfactant SDS (sodium laurylsulfate); likewise the effect of RhB on the aggregation processes of SDS at the experimental conditions was investigated, which suggests that the formation of micelles involving RhB and SDS occurs at concentrations below the critical micelle concentration (cmc). Under established optimal conditions, the system was adapted to flow injection analysis (FIA), showing a very short sampling time, potentially useful to control in routine analysis.

2. Experimental

2.1. Apparatus

A Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp and 1 cm quartz cells were used for the fluorescent measurements.

Solutions were propelled by Gilson Minipuls 3 peristaltic pumps with PVC pumping tubes. All tubing connecting the different components of the flow system was PVC, 0.8 mm i.d. and a homemade valve was used for FIA configuration.

A pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940 with combined glass electrode was used for monitoring pH adjustment.

2.2. Reagents

Stock standard solution of 50 $\mu g\,mL^{-1}$ was prepared by dissolving RhB (Fluka AG, Chemische Fabrik, Buchs SG, Switzerland) in ultra pure water and stored in a dark bottle at room temperature. In these conditions, RhB was stable at least for 4 weeks. A working standard of 5 $\mu g\,mL^{-1}$ was prepared by adequate dilution of the stock standard solution with ultra pure water. Sodium dodecylsulfate (SDS), Triton® X-100 and hexadecyltrimethyl-ammonium bromide (HTAB) were purchased from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). Sodium tetraborate were purchased from Mallinckrodt Chemical Works (New York, Los Angeles, St. Louis, USA), concentrated chlorhydric acid, sodium hydroxide, sodium chloride and potassium chloride were purchased from Merck (Darmstadt, Germany). Lipstick samples were obtained from several commercial available trademarks.

2.3. Sample preparation

A fraction of 5.0 mg of lipstick sample was accurately weighted and transferred to 50 mL beaker. 20 mL of ultra pure water were added and dissolved by mechanical stirring during 15 min at 333 K before being introduced in the FIA system for its determination.

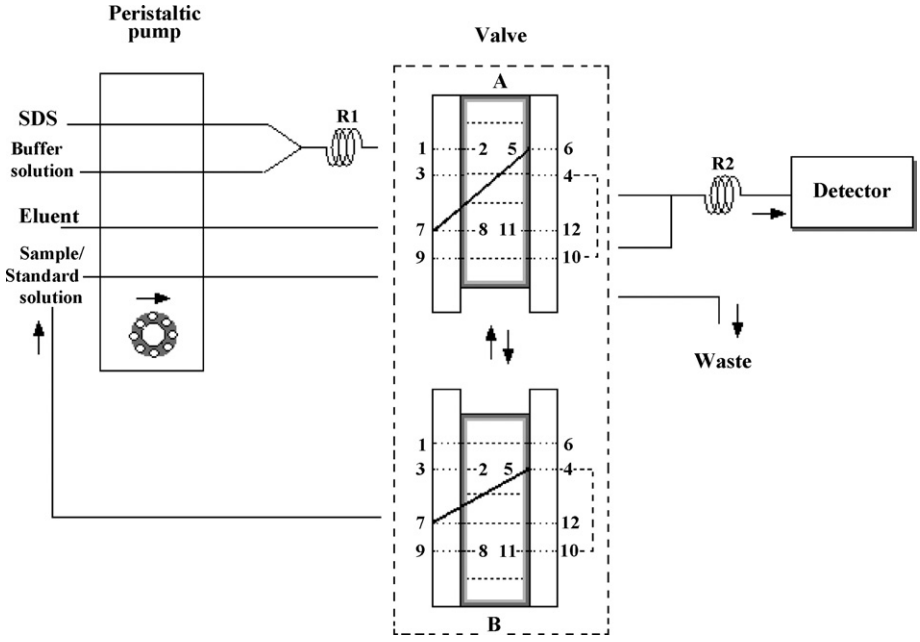

Fig. 1. FIA manifold RhB determination.

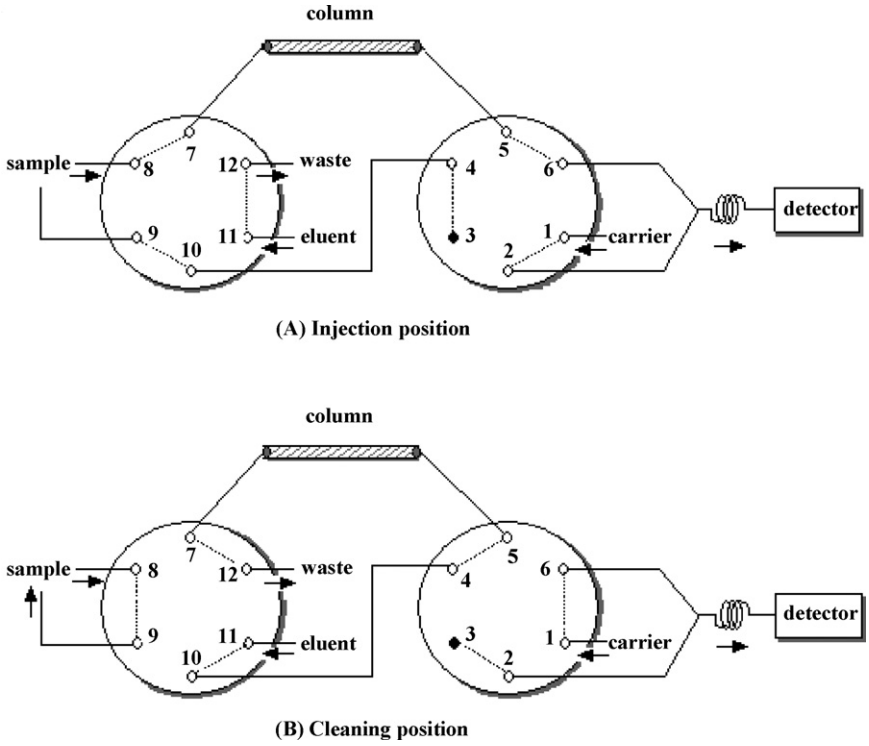

Fig. 2. Detail valve position. Schematic representation of the manifold where the valve could be fit at two positions: (A) at the injection position, the sample or standard solutions are injected to the detector after going through the column and being mixed with carrier solution; (B) at cleaning position, the eluent is washing the mini-column in back-stream, while sample or standard solutions are recycled.

2.4. Experimental procedure

A stream of SDS (2.1×10^{-3} mol L^{-1}) solution was combined with borax (0.1 mol L^{-1}) into reactor R_1, forming the carrier stream (Fig. 1). The sample/standard solution went through to a homemade mini-column packed with cotton wool (CC) to collect the waxy component of the sample solution, and then injected for 15 s into the carrier stream. RhB contained in the sample/standard is interacted with the carrier stream into reactor R_2, and then flowed to the fluorescence detector, measured at λ_{ex} 560 nm and λ_{em} 577 nm. The whole of linearity range was obtained varying the slit widths of 1.5–1.5 nm to 5–5 nm. After injecting, the valve was switched allowing the eluent stream (ultra pure water at 338 K) go through the CC column in the contra-stream, cleaning the retained waxy components and conducting to waste (Fig. 2).

3. Results and discussion

3.1. Spectrum properties of RhB

The RhB exists in solution as ionized species, neutral form, lactone and/or molecular aggregates, depending on pH, solvent, temperature and concentration. Each form of RhB is characterized by typical absorption and emission spectra which are further influenced by specific medium effect, i.e. ionic strength, additive presences, etc. Therefore, spectroscopic properties of RhB are still the subject of numerous research studies and controversies [27–29].

Traditionally Rhs have large molar absorptivity in the visible region of the electromagnetic spectrum, which is attributed to a $\pi \rightarrow \pi^*$ transition [27]. The absorption and fluorescent emission are influenced by the substituents on the nitrogen atom of the amino groups of the xanthene base.

3.2. Influence of surfactant's nature

In order to perform the luminescent emission, the fluorescence properties of RhB in various surfactant media were studied: anionic surfactant (SDS, 0–9×10^{-3} mol L^{-1}), cationic surfactant (HTAB, 0–5×10^{-2} mol L^{-1}) and non-ionic surfactant (TX-100, 0–1×10^{-3} mol L^{-1}). Experimental data showed that the enhancement factor for RhB–SDS system (2.5 folds respect to RhB fluorescence in water medium) was higher than RhB–HTAB (2.0 folds) (Fig. 3). For RhB–TX-100, an important spectrum interference was observed; thus, the anionic surfactant SDS was chosen for further work. At SDS concentration above 1×10^{-2} mol L^{-1}, solubility problems appeared causing errors in the measurement of fluorescence.

The SDS aggregation equilibrium in presence of RhB in our optimal experimental conditions showed a quite different behavior than pure SDS aqueous solution. For RhB–SDS system, the fluorescent response showed an inflection point at [SDS] = 3.1×10^{-4} mol L^{-1} (Fig. 4). The change in the SDS cmc suggests the formation of mixed aggregates at concentrations below the cmc reported (8.1×10^{-3} mol L^{-1}) [30]; in concordance to obtained results, other authors have reported similar behavior for SDS systems [31–34].

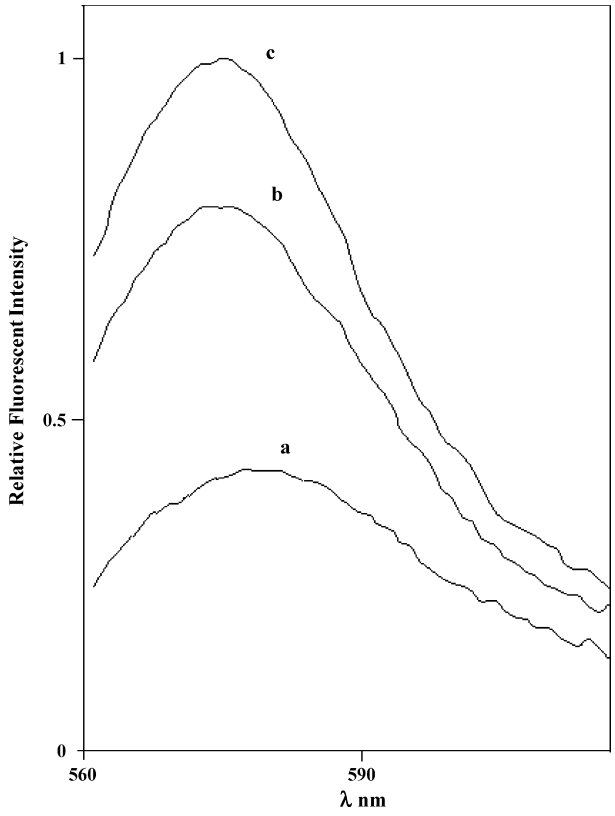

Fig. 3. Emission spectra of RhB in micellar media. $\lambda_{ex} = 560$ nm and $\lambda_{em} = 577$ nm. (a) aqueous solution; (b) $C_{HTAB} = 4 \times 10^{-2}$ M; (c) $C_{SDS} = 2 \times 10^{-3}$ M.

The enhancement of RhB fluorescent intensity by addition of SDS was associated to a slight hypsochromic shift of the maximum λ_{em} (Fig. 3); it reflects that the microenvironment around the dye is quite different from that in aqueous solution. It can be attributed to restrictions imposed on the free rotational motions which are competitive with luminescent emission [35]. In addition, the RhB equilibrium of molecular aggregate formation could be displaced to the monomer species, taking place the disaggregation of the dye.

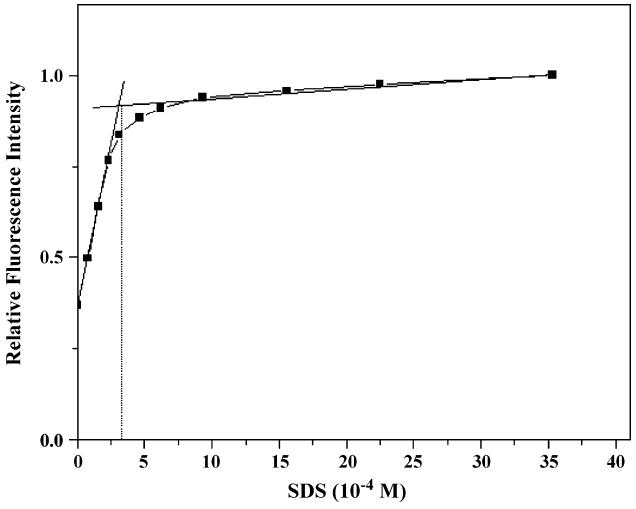

Fig. 4. RhB emission at different SDS concentration levels. $\lambda_{ex} = 560$ nm and $\lambda_{em} = 577$ nm. $C_{RhB} = 1 \times 10^{-7}$ M.

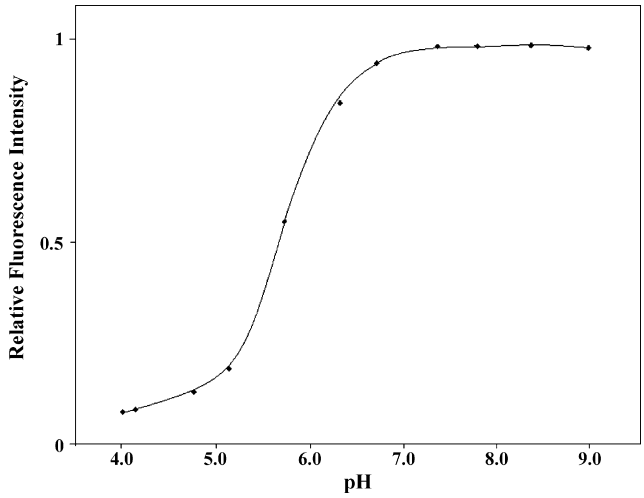

Fig. 5. Luminescent emission of RhB at different pH in SDS micelles. $\lambda_{ex} = 560$ nm and $\lambda_{em} = 577$ nm. $C_{RhB} = 1 \times 10^{-7}$ M; $C_{SDS} = 2 \times 10^{-3}$ M.

3.3. Influence of pH and ionic strength

Fluorescent intensity of RhB in SDS solution reached a maximum value between pH 7.0 and 8.5 (Fig. 5); hence, pH 8.0 was selected as optimum acquired with sodium tetraborate for further assays.

In order to study the effect of the addition of inert salts on micellar solutions of RhB, NaCl and KCl solutions were tested. In all studied cases, an increase of concentration above 6×10^{-2} mol L^{-1} provoked a clouding phenomenon to the system, and below these values no significant effect was observed.

3.4. Sample dissolution parameters

In polar solvents as water, RhB is widely soluble and is found as ionic species. Therefore, ultra pure water was chosen as an adequate solvent for sample dissolution.

The complex matrix of cosmetic products complicates their routine analysis, not only due to the presence of potential dyes interfering, but also for the diverse matrix compounds and additives in such products. A lipstick typically consists of high percents of castor oil, a variable percent of beeswax, carnauba wax, and lanolin, a variable number of soluble and insoluble dyes, pigments and perfume [36].

The waxy composition of lipstick makes difficult its complete dissolution at room temperature; therefore, a heat applied to help this process was necessary during 15 min with mechanic stirring. The influence of temperature for sample dissolution was studied in the range of 298–363 K, being melting range for the waxy component next to 333 K. At these conditions, non-decomposition was observed for the dye.

Although the presence of ethanol accelerates the dissolution process helping the partial dissolution of the waxy components, when the proportion of ethanol is raised, increase of turbidity

Table 1
Optimization of FIA variables for RhB–SDS system

Variables	Studied ranges	Optimum values
SDS 2.0×10^{-3} mol L^{-1} flow rate (ml min^{-1})	0.50–5.00	4.00
Buffer borax 1×10^{-2} mol L^{-1} flow rate (ml min^{-1})	0.50–5.00	4.00
Sample solution flow rate (ml min^{-1})	0.50–5.00	4.20
Eluent flow rate (ml min^{-1})	1.00–8.00	6.00
Loading time (s)	5–30	15
Reactor R_1 length (mm)	100–300	200
Reactor R_2 length (mm)	100–300	200
Cotton wool weight (mg)	10–40	25

of final filtrate was observed due to the emulsification of the system.

3.5. Optimization of the FIA variables

The variables influencing the performance of the method were studied and optimized in order to obtain a high signal and good reproducibility, using univariated method. The studied range of the FIA variables and their optimum values are listed in Table 1.

In order to achieve on-line waxy and/or particulate components retention, a homemade mini-column using a 100 μL conical polypropylene tube packed with different filtering materials was tested (as natural and synthetic fiber). For this hitch, the commercial cotton wool was found to provide satisfactory waxy retention, holding a good reproducibility.

To remove the retained material in the CC and prepare the FIA system for the next assay, ultra pure water at different temperatures was tested (298–353K) as washing solution being efficient at 338 K.

3.6. Analytical performance

Calibration curves of RhB were realized under optimal working conditions according to the procedure described above. It was obtained linearity in the range of 1.6×10^{-9} to 1×10^{-6} mol L^{-1} (concentration before dilution on-line), varying the excitation and emission slit widths of 1.5–1.5 nm to 5.0–5.0 nm.

Fig. 6 shows the fiagram obtained with its corresponding calibration graph for excitation and emission slit widths of 3 and 5 nm, respectively. Data were fitted by standard least-squares treatment giving the regression equations for calibration graphs of $F = 11.867 + 9 \times 10^9$ C ($r^2 = 0.9995$), where F is the fluorescence intensity (average of three measurement for each) and C is the concentration of RhB expressed in mol L^{-1}. The slope of the calibration graph is the calibration sensitivity according to IUPAC definition which supported the validation of the proposed procedure for quantification of RhB.

The detection limit [37] employing excitation and emission slit width of 5.0–5.0 nm, was estimated as the concentration of analyte which produces an analytical signal equal to three times the standard deviation (SD = 1.456) of the background fluorescence giving a value of 5×10^{-10} mol L^{-1} and the quantification limit equal to 10 times SD of the background fluorescence (1.6×10^{-9} mol L^{-1}). These values were 2 orders magnitude lower than others reported [25].

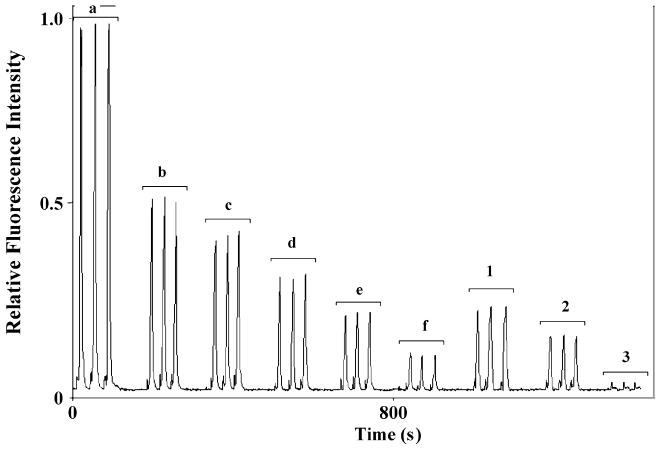

Fig. 6. Calibration curve and sample signals. Standard solution of RhB (a) 1×10^{-7} M; (b) 5.2×10^{-8} M; (c) 4.1×10^{-8} M; (d) 3.1×10^{-8} M; (e) 2.1×10^{-8} M; (f) 1×10^{-8} M. $C_{SDS} = 6.6 \times 10^{-4}$ M. Real lipstick samples of different trademark (1) 2.52×10^{-8} M; (2) 1.74×10^{-8} M; (3) not detectable. $\lambda_{ex} = 560$ nm and $\lambda_{em} = 577$ nm. Slit width exc = 3 nm and em = 5 nm. Injection time: 15 s.

3.7. Application and validation

In order to check the applicability of the proposed methodology for quantitative determination of RhB in cosmetic products, it was successfully applied to different trademark lipsticks. Since there does not exist official or standard methods to this determination, to validate the developed methodology recovery studies were carried out on these samples and the obtained results are shown in Table 2.

Table 2
Determination of RhB in real lipsticks samples

Samples	Base value (mol L^{-1})	RhB added (mol L^{-1})	RhB found[a] (mol L^{-1})	Recovery ± RSD (%)[b]
1	–	–	2.52×10^{-8}	–
	2.52×10^{-8}	1×10^{-8}	3.54×10^{-8}	102.4 ± 1.0
	2.52×10^{-8}	2×10^{-8}	4.45×10^{-8}	98.5 ± 2.1
	2.52×10^{-8}	3×10^{-8}	5.52×10^{-8}	100 ± 1.5
2	–	–	1.74×10^{-8}	–
	1.74×10^{-8}	1×10^{-8}	2.75×10^{-8}	101.2 ± 1.8
	1.74×10^{-8}	2×10^{-8}	3.67×10^{-8}	98.2 ± 1.9
	1.74×10^{-8}	3×10^{-8}	4.64×10^{-8}	98 ± 2.2
3	–	–	Not detectable	–
		1×10^{-8}	9.92×10^{-9}	99.2 ± 2.5
		2×10^{-8}	1.96×10^{-8}	98 ± 1.8
		3×10^{-8}	2.98×10^{-8}	99.5 ± 1.6

$\lambda_{ex} = 560$ nm and $\lambda_{em} = 577$ nm.
[a] Mean value, $n = 6$.
[b] 100 × [(found−base)/added].

The preliminary selection of the sample was made choosing the colors palettes which possibly contained the studied dye; they included the group of strong pink, fuchsia and red lipsticks. The applications were performed taking by triplicate 5 mg of 10 real samples, and after suitable dilution, they were injected by triplicate in the FIA system. In Fig. 6 the results of three samples are presented in which two of them present a significant level of RhB (12.0 and 8.3 $\mu g\,L^{-1}$, respectively), and the third represents the rest of samples with no detectable levels of RhB.

The RhB levels found in our samples could vary greatly with other reported, due to the multiplicity of samples, as well as different dye proportion. Other consideration to be take into account is the politics of the place where lipsticks are made or commercialized. In our sample, the detected levels were similar than others [24–26,38].

The obtained results showed good reproducibility, low dispersion and adequate sensibility for each peak series of the assayed samples. Additionally, high sampling rate was obtained (more than 100 samples h^{-1}) comparing other reported methodologies which involve time-consuming pretreatment sample, indicating the utility of the proposed method for routine analytical control.

4. Conclusions

The FIA spectrofluorimetric method proposed for the determination of RhB in real samples of lipsticks has the advantages of simplicity, speed, accuracy, low detection limit and the use of inexpensive equipment. The use of SDS micellar system provides a simple means to enhance the fluorescence from RhB giving about 2.5-fold increase in sensitivity and improves the limit of detection without further sample manipulation. The recommended procedure was found to be selective enough to tolerate the common additive present in commercial cosmetic forms. Additionally, it can be remarked the wide range linearity obtained in the calibration curve, with high sensitivity resulting adequate for the quality control and routine analysis of lipsticks.

This methodology has demonstrated potentiality in its applications; it could be applied to the determination of RhB present in other cosmetics products as eye shadows, rouge and other types of samples. Likewise, we are working in the development of a new application for the determination of RhB in foods and beverages.

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