Micelle-mediated cloud point extraction and spectrophotometric determination of rhodamine B using Triton X-100

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**A B S T R A C T**

A new micelle-mediated cloud point extraction method is described for sensitive and selective determination of trace amounts of rhodamine B by spectrophotometry. The method is based on the cloud point extraction of rhodamine B from aqueous solution using Triton X-100 in acidic media. The extracted surfactant rich phase is diluted with water and its absorbance is measured at 563 nm by a spectrophotometer. The effects of different operating parameters such as concentration of surfactant and salt, temperature and pH on the cloud point extraction of rhodamine B were studied in details and a set of optimum conditions were obtained. Under optimum conditions a linear calibration graph in the range of 5–550 ng mL\(^{-1}\) of rhodamine B in the initial solution with \(r = 0.9991\) (\(n = 15\)) was obtained. Detection limit based on three times the standard deviation of the blank (3 \(S_b\)) was 1.3 ng mL\(^{-1}\) (\(n = 10\)) and the relative standard deviation (R.S.D.) for 50 and 350 ng mL\(^{-1}\) of rhodamine B was 2.40 and 0.87% (\(n = 10\)), respectively. The method was applied for the determination of rhodamine B in soft pastel, hand washing liquid soap, matches tip and textile dyes mixture samples.

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1. Introduction

Rhodamine B with the chemical structure shown in Fig. 1, belongs to the class of xanthene dyes, which is highly water-soluble. It is among the oldest and most commonly used synthetic dyes. Initially, it was used as a colorant in textiles and foodstuffs, and is also a well-known water tracer fluorescent. Rhodamine B is harmful if swallowed by human beings and animals, and causes irritation to the skin, eyes and respiratory tract. The carcinogenicity, reproductive and developmental toxicity, neurotoxicity and chronic toxicity towards humans and animals have been experimentally proven [1]. Thus, due to the hazardous nature and harmful effects of rhodamine B, it was considered worthwhile to make efforts to develop a simple method for the determination of rhodamine B in different samples. Only few methods are available for the determination of rhodamine B [2–6]. These methods are based on electrokinetic capillary chromatography [5] and high-pressure liquid chromatography (HPLC) [2–4].

In the last decade, there has been an increasing interest on the use of aqueous micellar solution in the field of separation science. Micellar-mediated cloud point technique is often used as a preconcentration method prior to instrumental analysis, e.g. HPLC. This technique can be also used to recover various organic pollutants and metal cations [7,8], the latter after complexation with hydrophilic reagents [9].

At certain temperature, aqueous solution of a non-ionic surfactant separates into two phases. The first one is a surfactant-rich phase containing a high concentration of surfactant, which has small volume compared to the solution and the second one is the aqueous phase containing a low concentration of surfactant. This temperature is known as cloud point temperature (CPT) of the surfactant [10]. The solute molecule present in aqueous solution of non-ionic surfactant is distributed between the two phases above the cloud point temperature [11]. This phenomenon is known as cloud point extraction (CPE).

In the present paper, a simple and sensitive cloud point extraction procedure has been developed for the spectrophotometric determination of rhodamine B using Triton X-100 as non-ionic surfactant. The effect of temperature, concentrations of surfactant and salt on the extraction of dye has been studied.

2. Experimental

2.1. Instrumentation

A GBC Cintra 101, UV–Visible spectrophotometer was used for recording absorption spectra and absorbance measurements using 1 cm glass cells. A Metrohm digital pH-meter model 632 with a...
combined glass electrode was used for pH adjustments. A Colora thermostat bath maintained at the desired temperature was used for the cloud point temperature experiments.

2.2. Reagents

All chemicals used were of analytical grade and double distilled water was used throughout.

A solution of Triton X-100 (0.5 mol L\(^{-1}\)) was prepared by dissolving 64.88 g of Triton X-100 (Aldrich) in water and diluting to 200 mL in a volumetric flask.

A stock solution of 500 \(\mu\)g mL\(^{-1}\) of rhodamine B was prepared by dissolving 0.05 g of the rhodamine B (Merck) in water and diluting to 100 mL in a volumetric flask. More diluted solutions were prepared daily using this stock solution.

A solution of hydrochloric acid (0.1 mol L\(^{-1}\)) was prepared by diluting 8.3 mL of HCl (Merck, \(d = 1.19\) and 37%) in water and diluting to 1000 mL in a volumetric flask.

A 1.0 mol L\(^{-1}\) of sodium chloride was prepared by dissolving 5.85 g of NaCl (Merck) in water and diluting to 100 mL in a volumetric flask.

2.3. General procedure

In a 50 mL volumetric flask were added: an aliquot of the rhodamine B solution, 3.5 mL of 0.5 mol L\(^{-1}\) of Triton X-100, 5 mL of 1.0 mol L\(^{-1}\) of NaCl and 5 mL of 0.1 mol L\(^{-1}\) HCl. This solution was then diluted to the mark with water and transferred to a 50 mL tube and placed in a thermostat bath at 78 °C for 30 min. After the separation of two phases, the turbid solution was placed in an ice bath for the surfactant rich phase to become viscous. Then the dilute phase was removed by decantation. The surfactant rich phase was diluted with water in a 5 mL volumetric flask. The absorbance of the solution was measured at 563 nm. A blank solution was also run using water instead of rhodamine B.

2.4. Preparation of samples

Appropriate amounts of soft pastel (Mongyu, Korea), hand washing liquid soap (Top Company, Iran), matches tips (Tabriz Company, Iran) or textile dyes mixture (Textile Company, Iran) samples were dissolved in water, filtered if necessary and diluted to 50 mL in a volumetric flask. An aliquot of the above solutions was treated under the general procedure for cloud point extraction and subsequent determination of rhodamin B.

3. Results and discussion

The rhodamine dyes exist in solution as ionized specie, neutral form, lactone and/or molecular aggregates, depending on pH, solvent, temperature and concentration. Each form of rhodamine is characterized by typical absorption spectra, which is further influenced by specific medium effect, i.e. ionic strength and presence of additives [12]. The absorption spectrum of rhodamine B in acidic media shows that maximum absorbance occurs at 563 nm and the presence of surfactant does not have significant effect on its \(\lambda_{max}\). Therefore, all the absorbance measurements were performed at this wavelength. As the extent of cloud point extraction is influenced by the presence of additives, the surfactant concentration and the pH of the medium, these parameters were optimized in order to achieve the highest sensitivity.

3.1. Effect of hydrochloric acid concentration

The absorption band of rhodamine B at 563 nm was observed in acidic media, an increase in the pH of the solution caused a decrease in intensity of this absorption band. At higher pH values, the cationic form is converted to the neutral one and its absorbance at 563 nm is decreased. Thus, the effect of different acids such as nitric, sulfuric and hydrochloric acid in the same concentration was investigated. The results indicated that there is not much difference between them. Therefore, hydrochloric acid was chosen as convenient and the effect of its concentration was studied. Maximum absorbance was observed when acid concentration was in the range of 0.008–0.014 mol L\(^{-1}\). Thus, an acid concentration of 0.010 mol L\(^{-1}\) in the final solution was chosen as the optimum for subsequent experiments.

3.2. Effect of Triton X-100 concentration

For successful cloud point extraction of dye, it is desirable to use minimum amount of surfactant for maximum extraction of dye. Therefore, the effect of the surfactant concentration was investigated in order to ensure maximum extraction efficiency. Quantitative extraction was observed when the Triton X-100 concentration was above 0.030 mol L\(^{-1}\). The surfactant concentration of 0.035 mol L\(^{-1}\) was chosen as optimum.

3.3. Effect of electrolytes

It has been reported that the presence of electrolytes decreases the cloud point temperature (salting-out effect), resulting in a more efficient extraction. The lower cloud point is attributed to electrolytes promoting dehydration of the poly(oxyethylene) chains. The salting-out phenomenon is directly related to the desorption of ions from the hydrophilic parts of the micelles, increasing inter-attraction between micelles and consequently leading to the precipitation of surfactant molecules [13].

In order to study the effect of the addition of electrolytes and additives on micellar solutions of rhodamine B, NaCl, KCl and CaCl\(_2\) solutions were tested. The results indicated that the presence of NaCl, KCl and CaCl\(_2\) provoked the clouding phenomena and decreased the cloud point temperature. In addition, the presence of electrolytes increased the phase separation and enhanced the concentration of the solubilized dyes in coacervate phase. The extraction efficiency of the dye increased similarly in the presence of NaCl, KCl and CaCl\(_2\). The results of this study are shown in Fig. 2.

As can be observed the results were more consistent in the presence of NaCl. Therefore, it was chosen as the electrolyte for this study. An increase in the concentration of NaCl up to 0.06 mol L\(^{-1}\) increased the absorbance and above this value, no significant change was observed. Thus, a concentration of 0.10 mol L\(^{-1}\) was chosen for further work.
3.4. Effects of equilibration temperature and incubation time

Two important parameters in cloud point extraction are incubation time and equilibration temperature. The effect of the equilibration temperature (50–90 °C) on the cloud point extraction was also investigated. Although the solution turns cloudy at room temperature, it was found that better phase separation and thus maximum extraction efficiency is obtained above 78 °C. So, an equilibration temperature of 78 °C was used. The incubation time was also studied. Maximum extraction efficiency was observed at 78 °C after 30 min. Accordingly, an incubation time of 30 min was chosen for use in the next experiments.

3.5. Analytical performance

A linear calibration graph in the range of 5–550 ng mL\(^{-1}\) of rhodamine B in the initial solution was obtained by applying the optimized conditions. The equation for the line was \(A = 1.7 \times 10^{-3} C + 0.0141\) with regression coefficient \(r\) of 0.9991 (n = 15) where \(A\) is the absorbance and \(C\) is the concentration of rhodamine B in ng mL\(^{-1}\). Detection limit based on three times the standard deviation of the blank (3\(\sigma\)) was 1.3 ng mL\(^{-1}\) (n = 10) and the relative standard deviation (R.S.D.) for 50 and 350 ng mL\(^{-1}\) of rhodamine B was 2.40 and 0.87% (\(n = 10\)). The preconcentration factor defined as the ratio of the slopes of calibration curve before and after preconcentration was 8.5.

3.6. Interference studies

The influence of some ions and dyes on the determination of rhodamine B was studied. Various amounts of other species were added to a solution containing 200 ng mL\(^{-1}\) of rhodamine B and the recommended procedure was applied. An error of less than or equal to ±5% in the absorbance reading was considered tolerable. The results presented in Table 1 show the good selectivity of the procedure. Two similar dyes, amaranth and allura red were also tolerable up to 15 and 8 ratios, respectively.

Table 1

<table>
<thead>
<tr>
<th>Foreign ions</th>
<th>Tolerance ratio (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(^{2+}), Cu(^{2+}), Cd(^{2+}), Ca(^{2+}), Mg(^{2+}), and Zn(^{2+})</td>
<td>1000</td>
</tr>
<tr>
<td>Pb(^{2+}), Co(^{2+}), Cr(^{3+}), NH(_4)(^+), Br(^-), Mn(^{2+}), K(^+), F(^-), NO(_3)(^-),</td>
<td>500</td>
</tr>
<tr>
<td>H(_2)PO(_4)(^-), CO(_3)(^2-), HCO(_3)(^-), I(^-), SO(_4)(^2-), and Fe(^{3+})</td>
<td>8</td>
</tr>
<tr>
<td>Allura red</td>
<td>15</td>
</tr>
<tr>
<td>Amaranth</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. The effect of different concentrations of NaCl, KCl and CaCl\(_2\) on the absorbance of 100 ng mL\(^{-1}\) of rhodamine B after cloud point extraction.

![Absorbance vs Concentration of salts](image)

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rhodamine B added (ng mL(^{-1}))</th>
<th>Rhodamine B found (\alpha) (ng mL(^{-1}))</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft pastel 1(^\text{a})</td>
<td>–</td>
<td>31.0 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td>50.0</td>
<td>78.0 ± 1.5</td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td>100.0</td>
<td>135.0 ± 1.6</td>
<td>103.0</td>
<td></td>
</tr>
<tr>
<td>Soft pastel 2(^\text{b})</td>
<td>–</td>
<td>16.5 ± 0.4</td>
<td>–</td>
</tr>
<tr>
<td>20.0</td>
<td>37.0 ± 1.5</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td>40.0</td>
<td>56.5 ± 1.5</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Textile dyes mixture(^\text{c})</td>
<td>–</td>
<td>23.5 ± 0.4</td>
<td>–</td>
</tr>
<tr>
<td>25.0</td>
<td>50.0 ± 1.5</td>
<td>103.0</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>76.0 ± 1.5</td>
<td>103.5</td>
<td></td>
</tr>
<tr>
<td>Hand washing liquid soap(^\text{d})</td>
<td>–</td>
<td>6.5 ± 0.2</td>
<td>–</td>
</tr>
<tr>
<td>10.0</td>
<td>16.0 ± 0.5</td>
<td>97.0</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>27.0 ± 0.9</td>
<td>102.5</td>
<td></td>
</tr>
<tr>
<td>Matches tips(^\text{e})</td>
<td>–</td>
<td>11.0 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td>10.0</td>
<td>21.0 ± 0.6</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>30.0 ± 0.9</td>
<td>97.0</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{a} \times \pm t/\sqrt{n} \text{ at } 95\% \text{ confidence } (n = 3)\).

\(\text{b} \text{ Amount of rhodamine B was } 97.0 \text{ µg g}^{-1} \).

\(\text{c} \text{ Amount of rhodamine B was } 51.0 \text{ µg g}^{-1} \).

\(\text{d} \text{ Amount of rhodamine B was } 112.5 \text{ mg g}^{-1} \).

\(\text{e} \text{ Amount of Rhodamine B in hand washing liquid soap was } 5.3 \text{ µg g}^{-1} \).

\(\text{f} \text{ Amount of Rhodamine B in matches tips was } 770.8 \text{ µg g}^{-1} \).

Fig. 3. The absorption spectrum of (a) soft pastel (b) rhodamine B and (c) rhodamine B and soft pastel.

4. Application to real samples

In order to test the reliability of the proposed methodology for the assay of rhodamine B, it was applied to the determination of its concentrations in soft pastel, hand washing liquid soap, matches tip and textile dyes mixture samples. Since an official or standard method does not exist for the determination of rhodamine B [6], the developed methodology was validated by recovery studies. The results shown in Table 2 confirm the validity of the proposed method. As also shown in Fig. 3, the UV–vis spectrum of the component present in soft pastel (a) corresponded very well with the standard spectrum of rhodamine B (b).

5. Conclusion

Up to our knowledge, only few methods are available for determination of rhodamine B [2–6] and this method offers a simple way for the determination of rhodamine B in different samples.
The detection limit of the method is lower than some of the previously reported methods [3–5] and does not require sophisticated instruments. The proposed cloud point extraction is an easy, safe and inexpensive methodology for the separation and determination of trace amounts of rhodamine B in aqueous solutions using non-ionic surfactant Triton X-100. The analytical results obtained lead to the conclusion that the method developed can be successfully adopted for the separation and determination of rhodamine B with high sensitivity and selectivity.

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References