Studies on quenching of fluorescence of reagents in aqueous solution leading to an optical chloride-ion sensor

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Abstract

The sensitivity of quinoline- and acridine-type indicators towards the detection of low levels of chloride ions in aqueous and amine-doped media, by fluorescence quenching, is reported in this paper. Solution studies are based on the fluorescence of quinine sulphate, acridine and 3-(6-methoxyquinolino)propanesulphonate (SPQ) and the fluorescence response of these indicators has been examined at different pH values and at various chloride ion concentrations in aqueous media.

Keywords: Chloride ions; Fluorescence quenching; SPQ

1. Introduction

The importance of chloride-ion detection involves a wide area including pure, environmental, bio- and industrial chemistry. Industrial monitoring of chloride ions in feedwater is necessary since chloride ions are a major cause of corrosion in the metal components of steam-generating systems. Certain industrial specifications require the limit of detection for chloride ions in feedwater to be 1.7–57 µM and the feedwater typically contains amines, dissolved oxygen, carbon dioxide and trace levels of copper and iron with a pH range of 5–10. Many industries also requires the detection systems to be inline for real-time process-control applications.

Traditional methods of chloride-ion detection involve titrations using silver nitrate, silver fluoresceinate and silver chromate [1,2]. Titrations can be time consuming with the end-point often difficult to detect. Spectrophotometric methods involving reagents such as mercury thiosalicylate iron (III), mercury chloranilate and mercury diphenylcarbazide can be hazardous due to their toxicity [3–5].

Fluorescence intensity sensing is an established analytical method due to its high sensitivity and selectivity for a wide variety of analytes. Fluorescence quenching is a common technique used in the detection of gases and metal ions [6,7]. Collisional quenching by anions, such as alkyl halides, of quinine- and acridine-type fluorophores has been previously reported [8]. The fluorescence quenching has been related to the halide concentration by the Stern–Volmer equation [9]. This reaction has been adapted for fibre-optic chemical sensing of chloride ions for analytical and clinical purposes [10]. A mechanism involving the fluorescence quenching of N-(6-methoxyquinolino)acetoxyl ester (MQAE) by chloride ions has been utilized in the development of a fibre-optic probe for measuring chloride in aqueous solution [11].

In this paper we describe a comprehensive solution study based on the collisional fluorescence quenching of quinine sulphate, acridine and 3-(6-methoxyquinolinolino)propanesulphonate (SPQ) by low levels of chloride ions in aqueous media. The fluorescence studies in aqueous solution are preliminary and indispensable steps for a later sensor development based on this principle.

2. Experimental

Quinine sulphate (BDH), acridine (BDH) and SPQ (Sigma) were used as purchased. All quinine sulphate and acridine solutions were prepared in 0.05 M sulphuric acid, while SPQ solutions were prepared in deionized water (Elgastat). The concentration of all the fluorophore stock solutions was 10 µM. The stock amine (2-amino-2-methyl-1-propanol, AMP, (97% Aldrich)) solution (200 mg l⁻¹) was prepared in deionized water from which a working solution of AMP (20 mg l⁻¹) was made. Potassium chloride (Aldrich) dissolved in either AMP or deionized water was used to prepare standards of various chloride-ion concentrations.
The solution studies were carried out on the Perkin-Elmer LS-5 Luminescence Spectrometer using 90° excitation and emission alignment. In the solution studies a fixed volume of 3.1 ml was maintained and all measurements were performed at room temperature. pH measurements were made using a hand-held meter (Mettler Toledo-Checkmate 90).

3. Results and discussion

Both quinine sulphate and acridine exhibit fluorescence in ethanol, but to be selectively quenched by halide ions (chloride) they must be in their protonated form. Both indicators were protonated in 0.05 M sulphuric acid as described previously [8,12]. Quinine sulphate will not fluoresce in hydrochloric acid due to its fluorescence quenching effect [13]. SPQ is a neutral fluorophore and is therefore not pH dependent. It can fluoresce in acid, alkali, alcohol or water. Thus SPQ solutions were prepared in deionized water.

To determine the effect of amine-doped feedwater on the fluorescence of the indicator species, excitation and emission spectra were recorded in its presence. These spectra compared favourably with previous work [9,14]. Table 1 shows the excitation and emission wavelengths for the indicators.

The AMP working solution (20 ppm) is alkaline, whereas deionized water is almost neutral (pH 6.30). The pH of 0.05 M sulphuric acid is 1.3. Table 2 shows the change in pH of the indicators in their appropriate solvents when added to AMP and deionized water. The sulphuric acid in which quinine sulphate and acridine are prepared protonates the weak AMP. The pH of AMP was not affected when SPQ was added. No spectral shift or change in peak shape was observed in the spectra, indicating that the presence of AMP in the solvent had no effect on the fluorophores. A shift in wavelength of the emission spectrum observed in different solvents is a common phenomenon when solvent polarity is changed. Water is more polar than alcohol, but in this case AMP is present at very low concentrations (20 ppm) and hence the solvent properties are more characteristic of water.

The fluorescence intensities of fluorophore solutions have been experimentally observed to stabilize and then decrease over a period of time. For fluorescence quenching the fluorophore solution should not be degrading, hence it is important to use the fluorophore solution only when the fluorescence intensity is stable. The aim of this study was to determine experimentally the time it takes for the fluorescence of the solution to stabilize and therefore the optimum time for use. All solutions were stored in the dark to minimize any photobleaching effects due to ambient light. The fluorescence intensities of quinine sulphate, acridine and SPQ prepared in their appropriate solvents were monitored at 24 h intervals over a 96 h period. After 24 h the intensities of all the fluorophores were noted to increase. For acridine, a period of 24 h appeared to be the optimum age. The excitation and emission intensities of SPQ and quinine sulphate remained relatively constant after 24 h. While SPQ appeared to be the most stable fluorophore, acridine was the least stable. It was therefore decided to use only fluorophore solutions that have been stored for a period of 24 h for further studies.

To determine the most sensitive fluorophore towards low levels of chloride ions, a fluorescence quenching study was carried out. By using the Stern–Volmer equation

$$F_0 / F = K_{sv} [Q] + 1$$

where $F_0$ is the fluorescence intensity in the absence of the quencher, $F$ is the fluorescence intensity in the presence of the quencher, $K_{sv}$ is the Stern–Volmer quenching constant and $Q$ is the concentration of the quencher. Calibration graphs for quinine sulphate, acridine and SPQ were obtained (Figs. 1–3). From these calibration graphs the detection limit of chloride ions, defined as the concentration equivalent to $F_0 / (F_0 - 3sd)$ where $sd$ is the standard deviation of the blank ($F_0$), could be calculated. The detection limits, although theoretical values [15,16], are valuable as a comparison of the different detection processes employed. Therefore, the detection limits for the fluorescence quenching process of quinine sulphate, acridine and SPQ with the experimental values of the lowest measurable concentration of chloride ions and the

### Table 1

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Quinine sulphate (0.05 M H₂SO₄)</th>
<th>Acridine (0.05 M H₂SO₄)</th>
<th>SPQ (water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous medium</td>
<td>water</td>
<td>AMP</td>
<td>water</td>
</tr>
<tr>
<td>Excitation wavelength (nm)</td>
<td>362</td>
<td>357</td>
<td>367</td>
</tr>
<tr>
<td>Emission wavelength (nm)</td>
<td>465</td>
<td>495</td>
<td>492</td>
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</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Indicator</th>
<th>QS</th>
<th>Ac</th>
<th>SPQ</th>
<th>QS</th>
<th>Ac</th>
<th>SPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>AMP</td>
<td>H₂O</td>
<td>H₂SO₄</td>
<td>H₂SO₄</td>
<td>H₂O</td>
<td>H₂SO₄</td>
</tr>
<tr>
<td>Aqueous medium</td>
<td>9.79</td>
<td>6.30</td>
<td>1.33</td>
<td>1.31</td>
<td>5.37</td>
<td>2.47</td>
</tr>
</tbody>
</table>

Fig. 1. Collisional quenching of quinine sulphate by chloride ions. (Solid and dashed lines are the regression plots.)

Fig. 2. Collisional quenching of acridine by chloride ions. (Solid and dashed lines are the regression plots.)

Fig. 3. Collisional quenching of SPQ by chloride ions. (Solid and dashed lines are the regression plots.)

$K_{SV}$ values are given in Table 3. The lowest measurable concentration values are higher than the detection limits. The lowest measurable concentrations of chloride ions are real values and hence more useful in comparing the different detection processes employed in the working system. The $K_{SV}$ values indicate the order of sensitivity of the different fluorophores for chloride ions. The larger the $K_{SV}$ value, the more sensitive the fluorophore is to chloride ions. The order of sensitivity was found to be SPQ > quinine sulphate > acridine.

The fluorescence quenching of quinoline and acridine derivatives, such as SPQ, by halide ions is not a novel phenomenon, and has been previously used in solution and sensor applications. Wolfbeis and coworkers [10] applied this quenching system to develop an optical sensor for continuous monitoring of halide ions with a $K_{SV}$ value of 118 M$^{-1}$ for chloride ions and a limit of detection of 10 mM. This $K_{SV}$ value was around 10–20% lower than those of the non-immobilized indicator in solution. This reduction was explained by immobilization limiting the mobility of the fluorophore and therefore lowering the probability of the quenching process occurring. Krapf et al. [17] used SPQ to detect intercellular chloride ion via fluorescence quenching, with a $K_{SV}$ value of 118 M$^{-1}$. Kar and Arnold have determined the $K_{SV}$ for chloride-ion quenching of MQAE as 199 M$^{-1}$ [11]. In our work we have found that the sensitivity of SPQ quenching to chloride ions ($K_{SV}$) was greatly enhanced (717 M$^{-1}$) without the aid of preconcentration techniques, which could be a great advantage for sensing applications.

It is well known that bromide and iodide quench the fluorescence of the dyes investigated in this work much more strongly than the chloride ion [10]. However, interference studies were not carried out in this investigation due to the fact that the feedwater contains only chloride ions.

4. Conclusions

The presence of AMP at low concentrations did not affect the fluorescence quenching of quinine sulphate, acridine and SPQ. While SPQ is a neutral fluorophore, the fluorescence of quinine sulphate and acridine were quenched by chloride ions in their acidified forms. The dilute sulphuric acid in which quinine sulphate and acridine were prepared protonated the AMP solution; hence quenching by chloride ions occurred.

Quinine sulphate and SPQ were found to be photostable, whereas the fluorescence intensity of acridine was found to decrease after 24 h. Subsequently all solutions were stored in the dark for 24 h prior to use, therefore letting the fluorophores diffuse and equilibrate in solution.

Table 3

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Quinine sulphate (0.05 M H$_2$SO$_4$)</th>
<th>Acridine (0.05 M H$_2$SO$_4$)</th>
<th>SPQ (water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous medium</td>
<td>water</td>
<td>AMP</td>
<td>water</td>
</tr>
<tr>
<td>Detection limit (mM)</td>
<td>0.5 0.6</td>
<td>0.9 1</td>
<td>0.1 0.1</td>
</tr>
<tr>
<td>Lowest measurable concentration (mM)</td>
<td>0.7 0.8</td>
<td>1 1.1</td>
<td>0.3 0.3</td>
</tr>
<tr>
<td>$K_{SV}$ (M$^{-1}$)</td>
<td>213 213</td>
<td>112 111</td>
<td>717 717</td>
</tr>
</tbody>
</table>
SPQ was found to be the most sensitive fluorophore with the lowest calculated detection limit and the lowest measurable chloride-ion concentration. Immobilization of a lumiphore onto solid supports can enhance the rigidity of the molecule and improve its luminescence characteristics, such as intensity and quantum yield [18], thus increasing the sensitivity of the lumiphore. This can lead to lower detection limits of the analyte to be determined. Solid surface analysis is most commonly applied to room-temperature phosphorescence but can also be applied for fluorescence. Therefore studies involving the immobilization of SPQ with a view to lowering the detection limit of chloride ions are being currently performed. A detection of chloride ion in the range 1.7–57 μM in feedwater is required for the intended industrial application. Investigations on the physical immobilization of SPQ onto anion-exchange resins and chemical immobilization by covalent bonding to amine gels and entrapment into sol–gels are currently in progress with a view to developing an optical chloride-ion sensor with the above detection limits.

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References


Biographies

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