Experiment 5: Aspirin in pills and urine by fluorescence

1.0 Introduction:
Aspirin is a common over the counter drug. You will develop an analytical method to monitor this in standard capsules and in urine using fluorescence. The analysis of aspirin by spectroscopic means is a common introductory lab. However, modifying this for use with biological materials requires a deeper understanding. One specific problem arises from the fact that many biological materials are considered biological waste and therefore require documented and approved protocols to deal with these materials. Given this the analytical team needing to conduct proof of concept studies face either a long process to get appropriate approval or the task of developing an appropriate synthetic matrix.

2.0 Brief Procedure Summary:
There are a couple of experimental approaches for the analysis of aspirin attached to this document. You will review these and determine the most practical approach to take. You will also need to come up with a viable way to deal with the issue of biohazards.

3.0 Report Requirements:
You are tasked with developing a viable fluorescence based method that can handle a diverse range of matrix types, including that from filler material commonly used in pill formulations and also urine.
Introduction

Fluorescence is the emission of light by a molecule in an excited electronic state. The phenomenon of fluorescence in most cases represents the energy emitted when a substance returns from an excited or higher energy state to its normal or lower energy state.

Two types of information may be obtained when the fluorescence emission is studied:

1. The intensity-wavelength distribution, which is an indication of the electronic structures of the molecule.
2. The intensity at any wavelength where emission occurs which is an indication of the concentration of the fluorescent substance in the solution.

It is this second aspect of fluorescence that we are mainly concerned with when using the total fluorescence method of analysis.

In the total fluorescence measurement, the sample is irradiated with radiation of a known wavelength and the fluorescence emission which occurs over the complete wavelength range is measured. The emission is measured at 90° to the excitation direction to minimize scattering effects as shown in figure 1. The amount of radiation from the fluorescent substance in the sample is then used as an indication of the sample concentration.

This at work describes one of the many pharmaceutical applications of fluorescence spectroscopy. Compounds with an intrinsic fluorescence that normally appear in drug preparations can be easily analyzed for conformance with label specifications. Fluorometric determination of acetylsalicylic acid (ASA) as found in aspirin tablets, can be performed in 1% acetic acid v/v in chloroform solvent. In this solvent the fluorescence excitation wavelength for ASA is approximately 290 nm, and the emission band approximately 300-420 nm.

Equipment

Cary 1/3E UV-Vis spectrophotometer
Cary 1/3E Fluorescence accessory
Secondary (emission) filter UG5
Stoppered quartz fluorescence cells
Filter paper (Whatman, #1 qualitative)
Cary 1/3E Concentration Application software
Printer

Reagents

Chloroform, Spectroscopy grade (BDH)
Acetic acid, Reagent grade (BDH)
Acetylsalicylic acid (ASA), pure compound (AJAX Chemicals)
Aspirin tablets

Standard preparation

10 mg of pure acetylsalicylic acid was prepared in a 100 ml standard flask with 1% acetic acid v/v in chloroform and diluted to appropriate concentrations with the same solvent.

ASA Standards: 0.0, 2.0, 4.0, 6.0, 15.0, 50.0 mg/L.

A calibration curve for acetylsalicylic acid was then obtained using the Concentration Application software.
**Note:** Use the maximum SBW when optimizing the parameters and adjust the gain so that the highest standard reads the maximum %T, this will ensure that you obtain the maximum fluorescence intensity for each of your standards.

**Sample preparation**

The aspirin tablets for the sample analysis were crushed and dissolved using the 1% acetic acid/chloroform solution, and made up to volume in a 100 ml standard flask. The solution was then rapidly filtered through filter paper. Dilutions of 1 to 100, and then 1 to 1000 were performed. The fluorescence intensity for each sample was compared with a standard of acetylsalicylic acid using the calibration curve obtained previously.

**Note:** For accurate analysis, fluorescence measurements of the standards and sample aspirin tablet should be taken within 45 minutes of dissolving the solid, thereby avoiding significant hydrolysis of ASA to salicylic acid.

**Results and discussion**

Fluorescence intensity is proportional to the number of fluorescing molecules in the sample, which would assume that a linear relationship exists between fluorescence intensity and concentration of the sample. In practice however, this linearity is often absent and curvature is observed. A process called ‘self absorption’ is largely responsible for this curvature which occurs when the excitation and emission spectral envelopes overlap to a small extent. When this happens some of the emitted radiation is able to re-excite ground state molecules and is lost. The amount of self absorption increases with increasing concentration. Figure 2 shows how the emission intensity increases and decreases in a typical fluorescence calibration graph. This phenomenon can also be seen in the acetylsalicylic acid calibration curve shown in figure 3.

![Figure 2: Emission intensity in a typical fluorescence calibration graph](image)

**Table 1:** Results for acetylsalicylic acid in 1% acetic acid/chloroform solution

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc (mg/L)</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>0.00000</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>30.8025</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>36.4756</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>44.3820</td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>61.9050</td>
</tr>
<tr>
<td>6</td>
<td>50.0</td>
<td>91.8025</td>
</tr>
</tbody>
</table>

**Table 2:** The level of acetylsalicylic acid found in the samples

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Level of ASA (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>309</td>
</tr>
<tr>
<td>2</td>
<td>307</td>
</tr>
<tr>
<td>3</td>
<td>298</td>
</tr>
<tr>
<td>4</td>
<td>302</td>
</tr>
<tr>
<td>5</td>
<td>304</td>
</tr>
</tbody>
</table>

According to the manufacturer’s specifications, the commercially available aspirin tablets contained 300 mg aspirin. For the tablets to comply with the requirements of the British Pharmacopeia, the content of aspirin needed to be between 95% and 105% of the prescribed or stated amount. The results obtained indicate that all the tablets meet the requirements.

**Conclusion**

The Cary 1/3E Fluorescence accessory together with the Cary Concentration Application software can be used to successfully determine the amount of acetylsalicylic acid in a commercially available aspirin tablet.

**References**

Fluorescence Determination of Aspirin in APC Tablets

The determination of aspirin, phenacetin, and caffeine in APC tablets according to the procedure of Jones and Thatcher (1) is popular as an undergraduate laboratory experiment because it illustrates several principles, including separation by solvent extraction and spectrophotometric analysis of a two-component system. In the original procedure the tablet is dissolved in chloroform and the aspirin is extraction into aqueous Na₂CO₃ solution. The aqueous solution is then acidified so that the aspirin can be back-extracted into chloroform and determined by spectrophotometry. We have modified the procedure so that the aspirin is hydrolyzed to salicylate and determined by fluorescence. This enables us to introduce students to fluorescence methods without adding a separate experiment to our usual sequence. It also reduces the cost of the experiment since the back-extraction is eliminated and less chloroform is required. We have also scaled down the procedure to reduce chloroform consumption.

In the modified procedure about 0.1 g of a crushed APC tablet is accurately weighed and dissolved in 20 ml of chloroform. The chloroform is extracted with two 10-ml portions of 1% Na₂CO₃ and one 5-ml portion of water. The Na₂CO₃ solution is then poured into a 50-ml volumetric flask and 20 ml of 0.5 M NaOH is added. The high base concentration causes the aspirin to rapidly hydrolyze to salicylate. The solution in the 50-ml volumetric is diluted to the mark with deionized water. One milliliter of this solution is added to a 250-ml volumetric flask and diluted to the mark with 1% Na₂CO₃. A 10 ppm salicylate standard solution is prepared by weighing and diluting salicylic acid. The pH of the standard solution is brought to 11 by adding KOH. The analysis was performed on a Perkin-Elmer Model 204 spectrophotometer. The salicylate standard is used to set 100% emission and deionized water to set 0% emission. The spectrophotometer was set for excitation at 310 nm and emission at 435 nm. These are the maximum excitation and emission wavelengths reported in the literature. 2 On the Perkin-Elmer 204, the maxima are shifted to 305 and 405 nm respectively, because spectra are not corrected for variations in source intensity and detector sensitivity with wavelength. The analysis for phenacetin and caffeine was performed as in the original procedure.

Four different Empirina® (APC) tablets analyzed by the above procedure were found to contain 0.254, 0.222, 0.211, and 0.217 g of aspirin per tablet, respectively. These values are all within the manufacturer's specifications (0.226 ± 15%). A separate portion of the fourth tablet was analyzed and found to contain 0.218 g. Thus we feel confident that the above procedure yields satisfactory results. In preliminary experiments with acetylsalicylic acid it was verified that hydrolysis to salicylate is complete within seconds when the 0.5 M NaOH is added to the aspirin containing extracts.