

# INVESTIGATION OF SORBATES FOR THE DETERMINATION OF THE $F_A$ VALUES OF CHITIN AND CHITOSAN BY AN ADSORPTION TECHNIQUE

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## 1. Introduction

There are a large number of techniques available for determining the  $F_A$  (the mole fraction of N-acetyl-D-glucosamine residues present) of a sample of chitin or chitosan. The proposed methods vary widely in approach, accuracy, and the cost of equipment necessary [1].

The ideal method should be suitable for application to both chitin and chitosan, be capable of determining the  $F_A$  to within  $\pm 0.015$ , give reproducible results, and not require the use of expensive equipment. These requirements are fulfilled by the dye adsorption method proposed previously by the author [2, 3] and has been used for many years in our research. However the sorbate, C.I. Acid Orange 7 (Orange II), has been found to be somewhat less than the ideal sorbate for use in this technique. One major problem is that a commercial textile dye is not a chemical compound, as this term is normally understood. It is a mixture that has been standardised to give a particular hue when applied under the given conditions and the nature and amounts of other compounds in the mixture may vary from batch to batch of the one manufacturer as well as from manufacturer to manufacturer. Purifying the dye is therefore difficult, particularly to ensure that all anionic surfactants, such as wetting agents, have been removed from the dye as they would compete with the dye anions for the protonated amine groups in the chitosan. It was therefore considered important to find an alternative and improved sorbate.

## 2. Experimental

### 2.1. Materials

Chitosan – two commercial samples of chitosan were used, both of high molecular weight. Three different techniques, IR spectrophotometry [4], metachromatic titration [5], and dye adsorption [3] (with C.I. Acid Orange 7 as sorbate) were used to determine their  $F_A$  values,

which were found to be  $0.253 \pm 0.009$  (Sample 1) and  $0.300 \pm 0.007$  (Sample 2) based on the average values obtained all three techniques.

Sorbates – C.I. Acid Orange 7 was a commercial sample (Sandoz Ltd); 2,4,6-trinitro-toluene (picric acid), naphthalene-1-sulphonic acid ( $\text{Na}^+$  salt) and anthraquinone-2-sulphonic acid ( $\text{Na}^+$  salt) were General Purpose Reagent grade chemicals. All were purified by recrystallisation from 80% aqueous ethanol. The molar extinction coefficients ( $\epsilon$ ) were measured at 355 nm for picric acid and 256 nm for anthraquinone-2-sulphonic acid ( $\text{Na}^+$  salt) and were found to be 14,375 and 46,650 litres.mol<sup>-1</sup>.cm<sup>-1</sup> respectively.

Adsorption studies – these were carried out at 60 °C as previously described [3].

### 3. Discussion

The method is based on established dye uptake techniques for analysing ionic fibres such as polyamide and polyacrylonitrile, and involves treating a sample of chitin or chitosan with a solution of C.I. Acid Orange 7 in 0.1 M  $\text{CH}_3\text{COOH}$  and determining the amount of dye adsorbed at equilibrium:



The sorbate molecule must be of high enough molecular weight to ensure that the equilibrium goes completely to the right hand side and also, in the case of chitosan samples, that the sample does not go into solution in the acid medium. However the technique assumes that only specific site adsorption occurs, that is all adsorption of the anionic dye ions occurs at protonated amine groups and none occurs by diffuse adsorption at other parts of the polymer chain. The higher the molecular weight of the sorbate ion the more likely it is to undergo diffuse adsorption and so the selection of sorbate must be something of a compromise. The tendency for diffuse adsorption to occur can be reduced by raising the temperature of the adsorption process and a temperature of 60 °C was previously found [2, 3] suitable as it reduced diffuse adsorption of C.I. Acid Orange 7 to a minimum and multiple analyses could readily be maintained at this temperature overnight using a simple waterbath.

Three sorbates were examined as possible replacements for C.I. Acid Orange 7. These were:

1. Picric acid (2,4,6-trinitrophenol) – molecular weight = 229,
2. Naphthalene-1-sulphonic acid ( $\text{Na}^+$  salt) – molecular weight = 230,
3. Anthraquinone-2-sulphonic acid ( $\text{Na}^+$  salt) – molecular weight = 286.

All three have lower molecular weights than C.I. Acid Orange 7 (350) and may be considered to have 1, 2 and 2.5 rings respectively compared to 3 rings for the latter.

#### 3.1. Picric acid

Picric acid is an obvious possible alternative to C.I. Acid Orange 7 in the dye adsorption method of analysis being both a ‘normal’ chemical compound, and hence readily purified, and a textile dye, having been used as a dye for wool in the 18<sup>th</sup> and early 19<sup>th</sup> centuries.

A further possible advantage of picric acid is that it will act both as the sorbate and as the acidifying agent, thereby eliminating the need to add acetic acid as another component in the equilibrium depicted in equation (1). It was previously used by Neugebauer to determine  $F_A$  values [6] using a technique in which a sample of chitin or chitosan, packed in a column, is steeped in a solution of picric acid in methanol at room temperature for between 6 - 24 hours. However, unlike the dye adsorption method, where the dye uptake is calculated by determining the decrease in dye concentration in the liquid phase, Neugebauer et al. determined the picric acid uptake by desorbing it from the sample with methanolic DIPEA.

Initial studies with picric acid using Sample 1 followed the general conditions of Neugebauer et al. [6] and used 0.05 M picric acid in methanol at 25 °C as the reaction medium, together with steeping times of up to 4 days. The  $F_A$  value obtained by this method was 0.52 (previously determined value =  $0.253 \pm 0.009$  – see Experimental section) showing that some interaction between the amine groups and picric acid had occurred but that a 1:1 stoichiometry was not achieved, despite having a picric acid:amine group ratio of 10:1. Extending the steeping time to 8 days did not increase the amount of picric acid adsorbed and hence did not give any change in the calculated value of  $F_A$ .

The study was extended to the use of aqueous solutions of picric acid, using ratios of picric acid:amine groups of 2:1 and 8:1.

**Table 1.** *Effects of time, temperature and picric acid:amine group ratio on measured  $F_A$  value.*

Ratio PA:amine group	2:1	2:1	2:1	2:1	2:1	8:1	2:1
Temperature/°C	25	25	25	25	25	25	~100
Reaction time/hours	16	64	120	144	168	144	4
Calculated $F_A$ value	0.58	0.44	0.41	0.32	0.314	0.320	0.392

Although the effects of the variables are as expected, none of the sets of conditions used give a result close to the known  $F_A$  value for Sample 1 ( $0.253 \pm 0.009$ ) although it is possible that extending the time of reaction at reflux temperature to 16-24 hours might have done so, although degradation of the sample would have been extensive.

### **3.2. Naphthalene-1-sulphonic acid ( $\text{Na}^+$ salt)**

The initial study was carried out to test the solubility behaviour of chitosan in solutions of naphthalene-1-sulphonic acid ( $\text{Na}^+$  salt) [NaNSA] in 0.1 M acetic acid. Samples of chitosan (10 mg) were steeped in 0.1 M aqueous acetic acid solutions containing (a) a 5:1 ratio and (b) a 10:1 ratio of NaNSA:amine group. However the chitosan dissolved in both solutions, indicating its unsuitability as a replacement for C.I. Acid Orange 7 in the dye adsorption method for analysing chitosan. Although it is probably possible to prevent the chitosan dissolving by using even higher ratios of NaNSA:amine groups, increasing the ratio would result in much smaller differences in absorbance values between the reference and sample solutions, thus introducing an increasing margin of error. No further studies were carried out using NaNSA as sorbate.

### 3.3. Anthraquinone-2-sulphonic acid (Na<sup>+</sup>)

As with NaNSA the initial study involved testing the solubility behaviour of chitosan in solutions of anthraquinone-2-sulphonic acid (Na<sup>+</sup> salt) [NaASA] in 0.1 M acetic acid; again ratios of 5:1 and 10:1 for NaASA:amine groups were used initially. The chitosan became slightly swollen, but did not dissolve in either system, unlike its behaviour in the NaNSA-containing solutions. Further tests established the minimum practical ratio of NaASA:amine groups to be 3:1 as the chitosan was partially soluble at a ratio of 2:1.

The adsorption of NaASA by chitosan was examined using Sample 2 and conditions similar to those proposed for C.I. Acid Orange [2]. The effect of time at 60 °C on the adsorption was determined at two NaASA:amine group ratios, 3:1 and 5:1.

**Table 2.**  $F_A$  values calculated from adsorption of NaASA at 60 °C and a NaASA:amine group ratio of 3:1.

Steeping time/hours	2	4	6	24	48
Calculated $F_A$ value	0.290	0.298	0.305	0.298	0.305

Table 1 shows that adsorption is complete after approximately 4 hours and that there is no further change on extending the steeping time to 48 hours.

**Table 3.**  $F_A$  values calculated from adsorption of NaASA at 60 °C and a NaASA:amine group ratio of 5:1.

Steeping time/hours	2	4	6	24	48
Calculated $F_A$ value	0.256	0.295	0.295	0.290	0.293

Table 2 shows a similar pattern to that of Table 1 but the  $F_A$  value obtained (0.293) is slightly less than that given in the latter (0.301). This may be attributed to a small amount of diffuse adsorption occurring due to the higher concentration of NaASA used, a 5:1 rather than a 3:1 ratio. This would result in an apparently higher value for the concentration of amine groups and hence give a lower  $F_A$  value.

The repeatability of the technique was then examined, again using Sample 2. The  $F_A$  values obtained in 4 separate runs are: 0.305, 0.302, 0.300, 0.303. These results, together with its ready availability and ease of both recrystallisation and confirmation of purity, demonstrate that anthraquinone-2-sulphonic acid (Na<sup>+</sup> salt) is a suitable sorbate to replace C.I. Acid Orange 7 for use in the dye adsorption method to determine the  $F_A$  value of chitin and chitosan samples.

## 4. Conclusions

Of the three potential sorbates examined for use in the dye adsorption technique for analysing chitin and chitosan only anthraquinone-2-sulphonic acid (Na<sup>+</sup> salt) has been found to give accurate results. Both picric acid and naphthalene-1-sulphonic acid (Na<sup>+</sup> salt) are unsuitable.

## **5. References**

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