Spectrophotometric determination of sulphacetamide sodium via diazotization and coupling reaction

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ABSTRACT

A simple spectrophotometric method has been suggested for the assay of sulphacetamide sodium (SAAMS) through diazotization and coupling. The method include the reaction of SAAMS with sodium nitrite in acidic medium of hydrochloric acid to produce the corresponding diazonium salt-SAAMS(D-SAAMS), which is coupled with 2,4-dihydroxybenzophenone (2,4-DHPB) in presence of sodium hydroxide to produce orange azo dye, water-soluble and stable which shows maximum absorbance at 497.5 nm. The absorbance was found to increase linearly with the increasing amounts of SAAMS in the solution from 10 to 250 µg in 10 ml (1-25 µg.ml⁻¹). Two important factors were calculated to show the sensitivity of the method: molar absorptivity and Sandell’s sensitivity index, have the values 2.27×10⁴ l.mol⁻¹.cm⁻¹ and of 0.011198 µg.cm⁻² respectively. Both values demonstrated good sensitivity to the proposed method. SAAMS has been estimated successfully in eyes drops.

Keywords: Sulphacetamide, 2,4-Dihydroxybenzophenone ; Spectrophotometric ; sodium; Diazotization; coupling.

INTRODUCTION

SAAM is broadly used in medicine according of its excellent inhibitory effect on the growth in many types of bacteria. SAAM is sodium derivative of N-[(4-aminophenyl) sulphonyl]acetamide. SAAMS has the chemical structure as illustrate in scheme 1: Its molecular weight is 254.2 g mol⁻¹. [1].
Scheme 1: The chemical structure of SAAMS.

SAAMS has been determined by several analytical methods or techniques such as volumetric – method[2,3], HPLC[4,5],HPLC,TLC [6], SPE-UPLC[7], SPE/LC/MS[8],spectrophotometric [9,10,11,12,13,14,15,16],solid phase extraction–spectrophotometric [17]; micellar electrokinetic chromatography[18],electro-determination using graphite sensor[19] and potentiometric determination[20]. The objective of the current work to provide an optimized spectrophotometric procedure for determination of SAAMS in its pharmaceutical preparation based on diazotization of SAAMS and coupling reaction with 2,4-DHBP in alkaline medium to form a highly colored azo dye that has been proved successfully for the assay of SAAMS in pharmaceutical formulation(eyes drops).

EXPERIMENTAL
Apparatus

CECELL Recording Spectrophotometric ,within 1cm quartz cells have been used in all measurements.

Materials and there reagents

All chemicals used are of analytical rank. SAAMS was obtained from the State Establishment for Drug Productions and Medical Appliances(SDI).

SAAMS solution,50 µg.ml⁻¹

SAAMS solution was prepared by dissolving 0.0100 g of SAAMS (Sigma company) in 100 ml DW in a volumetric flask and 50 ml of above standard solution was diluted to 100 ml with DW in a volumetric flask.

Solution of 2,4-DHBP, 0.4% (w/v%)

A 0.4g of 2,4-DHBP was dissolved in 50 ml ethanol and then dilution of solution to 100 ml with distilled water in a volumetric flask.

Aqueous solutions of sodium nitrite (0.1%), sulpha mic acid (3%), sodium hydroxide (1M) and 1MHCl also have been prepared and used in the present work.

Drug solution(eyes drops),50 µg.ml⁻¹

1-Abeselpha-10
The solution was prepared by mixing 3 containers of abeselpha-10 eyes drop (100 mg SAAMS/ ml), 1 ml of mixture was diluted to 100 ml with DW in a volumetric flask, only 5 ml of above solution was needed to be diluted to 100 ml with DW in a volumetric flask to get eyes drops solution of 50 µg.ml⁻¹.

2-Abeselpha-20

The procedure for abeselpha-20 (200 mg SAAMS/ml) is the same as in abeselpha-10 eyes drops but with only 2.5 ml instead of 5 ml in the second dilution was diluted.

Analytical procedure

Into a series of 10 ml volumetric flasks an increasing volumes of SAAMS (50 µg.ml⁻¹) were transferred to cover the range of the calibration graph 1 to 25 µg.ml⁻¹, then 1 ml of 1 M HCl and 1 ml of 0.1% NaNO₂ solutions were added and lifted for 3 minutes, only 0.2 ml of 3% sulphamic acid solution was added and shake continuously for 2 minutes to remove the excess of HNO₂, then 1 ml of 0.4% 2,4-DHBP reagent and 1.5 ml of sodium hydroxide (1 M) solutions were added before dilution with DW to the mark. Absorbance of orange azo dye at 497.5 nm versus the reagent blank has been measured. The linear relationships between 1 to 25 µg.ml⁻¹ (10 to 250 µg.10 ml⁻¹ (Fig.1) with good value of determination coefficient (R²).

![Figure 1: Calibration curve of SAAMS determination.](image)

By applying the following mathematical relationships[21], the value of the detection limit (LOD) and the value of the quantitative estimate limit (LOQ) are calculated as follows:

\[
\text{LOD} = \frac{3\sigma_B}{S}
\]

\[
\text{LOQ} = \frac{10\sigma_B}{S}
\]

S = Slope for the standard curve.

\(\sigma_B\) = Standard deviation of 10 blank solutions.
The results obtained for the values of LOD and LOQ equal to 0.028085 μg.ml⁻¹ and 0.093617 μg.ml⁻¹ respectively.

RESULTS AND DISCUSSION

All parameters affected the absorption of colored dye were investigated and the optimum conditions of the reaction have been selected.

**Principle of the method**

SAAMS treatment with nitrous acid (prepared in the bulk solution) to form D-SAAMS.

\[
\text{HNO}_2 + \text{H}_2\text{NSO}_3\text{H} \rightarrow \text{N}_2\uparrow + \text{H}_2\text{SO}_4 + \text{H}_2\text{O}
\]

The last step of the proposed method include coupling reaction of D-SAAMS with 2,4-DHBP in alkaline medium to yield an orange azo dye, which shows maximum absorption at 497.5nm (Scheme 2).

**Study of the optimum reaction conditions**

**Choice of diazotized agent**
Several aromatic coupling agents have been tested for optimum condition. The results in Table (1) show that 2,4-DHBP gave the most sensitive ($\varepsilon = 2.234 \times 10^4$ l.mol$^{-1}$.cm$^{-1}$) in alkaline medium. Therefore, it has been fixed as an optimum reagent.

| Coupling agent, (0.4%) soln. | Structure | Absorbance | $\lambda_{max}$ (nm) | Color of azo dye | $\varepsilon$ (l.mol$^{-1}$.cm$^{-1}$) |
|-----------------------------|-----------|------------|----------------------|-------------------|-------------------------------|
| 4,4-Dihydroxy diphenyl sulphate | ![Structure](image) | 0.191 | 333.5 | Yellow | $0.97 \times 10^4$ |
| 2,4-dihydroxy benzophenone | ![Structure](image) | 0.458 | 497.5 | Orange | $2.234 \times 10^4$ |
| 1,2-phenylene diamine | ![Structure](image) | 0.305 | 415.5 | Yellow | $1.556 \times 10^4$ |

**Type of acid and its quantity**

The obtained results by adding various amounts of different type of acids HCl, HNO$_3$, H$_2$SO$_4$ and CH$_3$COOH showed that HCl gave the highest absorbance with a highest stability of color and also the amount of HCl has been studied, the optimum amount 1ml has been recommended in the subsequence experiments.

**Effect of sodium nitrite quantity and time needed on absorbance**

Different quantities of sodium nitrite have been added to show which of them had given a complete diazotation of SAAMS, the results showed that 1ml of 0.1% sodium nitrite with 3 minutes standing time gave the highest absorbance, so that it has been recommended in the subsequent experiments.

**Effect the quantity of sulphamic acid and the time needed.**

The excess amounts of HNO$_2$ were unwanted affording to its side reactions, so that it is eliminated via adding various amount of sulphamic acid [22]. The results obtained that 0.2 ml of 3% of sulphamic acid with 2 minute shaking gave the highest absorbance for azo dye and it was fixed in the subsequence experiments.

**Effect of 2,4-dihydroxy benzophenone amount:**
Various amounts of 2,4-DHBP (0.2-1.2 ml) has been added, the highest absorbance of the orange azo dye achieved by adding of 1 ml with the best determination coefficient ($R^2$) over a range of determination concentration from 25 to 100 µg.10 ml$^{-1}$ (Table 2).

### Table 2: Effect of the amount of 2,4-dihydroxybenzoic acid reagent on absorbance.

| 2,4- DHBP soln. (ml) | SAAMS (µg) in 10 ml | R$^2$ |
|----------------------|---------------------|-------|
|                      | 25                  | 50    | 75    | 100   |       |
| 0.2                  | 0.188               | 0.384 | 0.432 | 0.455 | 0.9021|
| 0.5                  | 0.192               | 0.390 | 0.662 | 0.823 | 0.9956|
| 1.0                  | 0.210               | 0.455 | 0.699 | 0.932 | 0.9999|
| 1.2                  | 0.198               | 0.450 | 0.623 | 0.928 | 0.9942|

**Effect of base**

The preliminary experiments indicated that the orange formed azo dye was made in alkaline medium. Different base strong and weak solutions have been used and their impact on absorbance of forming dye has been studied. The result indicated that the coupling reaction needs strong alkaline medium and NaOH gave the highest value of absorbance than other weak bases used, and its choose (Table 3), although the sodium bicarbonate gave the highest colour contrast but the azo dye is not stable.

### Table 3: The effect of various base on $A$ and $\Delta \lambda$.

| Base*Solution (1M) | $A$    | $\lambda_{max}$ (nm) | $\Delta \lambda$ (nm)** |
|-------------------|--------|-----------------------|--------------------------|
| NaOH              | 0.460  | 497.5                 | 145                      |
| KOH               | 0.454  | 500                   | 147                      |
| NaCO3             | 0.434  | 447                   | 162                      |
| NaHCO3            | 0.452  | 496                   | 194                      |
| NH4OH             | 0.432  | 445                   | 125                      |

*1.5 ml added. ** $\Delta \lambda$ = Colour contrast = $\lambda_{max}$ for azo dye - $\lambda_{max}$ for blank.
The amount of NaOH has been studied by another experiment. The results illustrated in Table 4, indicated that 1.5 ml of NaOH was the optimum volume.

Table 4: The optimum amount of NaOH.

| NaOH soln. (ml) | Absorbance of µg SAAMS/10 ml |
|----------------|-----------------------------|
|                | 25  | 50  | 75  | 100 |
| 0.5            | 0.08| 0.232| 0.463| 0.612|
| 1.0            | 0.122| 0.385| 0.544| 0.703|
| 1.5            | 0.192| 0.458| 0.663| 0.873|
| 2              | 0.181| 0.432| 0.612| 0.860|

The stability of the dye

Absorbance was measured after the completion of all components of reaction and dilution to mark with distilled water directly and after every five minutes for one hour. The results in Table 5 show very good stability of the formed azo dye.

Table 5: Effect of time on absorbance of azo dye.

| Time (min.) | Abs. / µg SAS in 10 ml |
|-------------|-----------------------|
|             | 50 µg  | 75 µg  | 100 µg |
| After dilution |       |       |        |
| 5           | 0.468  | 0.639  | 0.884  |
| 10          | 0.465  | 0.634  | 0.876  |
| 15          | 0.467  | 0.630  | 0.878  |
| 20          | 0.465  | 0.631  | 0.869  |
| 25          | 0.465  | 0.632  | 0.869  |
| 30          | 0.465  | 0.632  | 0.869  |
| 35          | 0.465  | 0.632  | 0.869  |
| 40          | 0.465  | 0.632  | 0.869  |
| 45          | 0.465  | 0.629  | 0.869  |
| 50          | 0.460  | 0.629  | 0.869  |
| 55          | 0.460  | 0.629  | 0.866  |
| 60 (1hr)    | 0.460  | 0.629  | 0.866  |
Final Absorption Spectra

Absorption spectra of orange azo dye made from coupling of D-SAAMS with 2,4-DHBP in presence of sodium hydroxide, against its corresponding reagent blank shows extreme absorption at 497.5 nm (Figure 2).

![Absorption Spectra](image)

**Figure 2.** The absorption spectra for 60 µg/10 ml of SAAMS measured against blank(A), distilled water(B) and blank against distilled water(C).

The nature of the dye

For the purpose of knowing the correlation ratio of SAAMS with reagent 2,4-DHBP, a continuous variation method (Job’s method) [23] has been applied according to the steps. Following. A number of solutions containing different volumes (0.5-2.5) ml of SAAMS and (2.5-0.5) ml of reagent 2,4-DHBP were prepared at a concentration of $4.520 \times 10^{-3}$ M for each, with the addition of the rest of the solutions under optimum conditions, and the absorption of these solutions was measured against their blank solutions at the wavelength of 497.5 nm. Figure (3) shows the ratio of the SAAMS coupling with reagent 2,4-DHBP is 1:1.
Figure 3. Job's method curve for azo dye resulting from coupling of SAAMS with 2,4-DHBP reagent.

The 1:1 reaction ratio was confirmed by the molar ratio method[23], and Figure 4 confirms that the 1:1 reaction ratio is the same result that obtained by Job's method.

Figure 4. Mole ratio curve for azo dye resulting from coupling of SAAMS with 2,4-DHBP reagent.

The azo dye stoichiometric(1:1) resulted from Fig.3 and Fig.4, so that the proposed chemical structure for the orange azo dye should be as in Scheme 3.

Scheme 3. Orange azo dye.

Estimation of SAAMS in its formulations.
The strength of suggested procedure for spectrophotometric determination of SAAMS was checked by the analysis of SAAMS in different two eyes drops formulations. Table 6 contained the results by using of the linear relationship for calibration curve.

Table 6 : The results of application part.

| Pharmaceutical Preparation | Amount taken , µg | Recovery*, % | Relative error*, % | Relative standard deviation*, % |
|----------------------------|-------------------|--------------|---------------------|-----------------------------|
| Apsolpha-10                | 50                | 99.8         | -0.21               | ±0.22                       |
|                            | 150               | 100.1        | +0.03               | ±0.05                       |
|                            | 200               | 99.5         | -0.05               | ±0.04                       |
| Apsulpha-20                | 50                | 99.8         | -0.44               | ±0.22                       |
|                            | 150               | 99.9         | -0.06               | ±0.06                       |
|                            | 200               | 99.5         | -0.05               | ±0.04                       |

For the purpose of proving that the proposed method is of high accuracy and control, and given the inability to use the standard method [1] fixed in the British Pharmacopoeia, the standard addition method [24] was used to estimate SAAMS in its pharmaceutical preparations and the results are shown in Figures (5) and (6) which are represented. The standard addition curves for the determination of SAAMS in pharmaceutical preparations (eyes drops) for concentrations 100 and 150 micrograms / 10 ml). Table (7) represents the recovery of SAAMS.

Figure 5. The curve of estimation of SAAMS in pharmaceutical preparation [APSULPHA-10(API)] is represented by the standard addition method.
Figure 6. The curve of estimation of SAAMS in pharmaceutical preparation [APSULPHA-20(API)] is represented by the standard addition method.

Table 7: Recovery of SSAAMS from pharmaceutical preparation (eyes drops) by the standard addition method.

| Drug          | Drug solution, μg taken | Drug solution, μg founded | Recovery% |
|---------------|-------------------------|---------------------------|-----------|
| APSULPHA-10(API) | 100                    | 100.14                    | 100.14    |
|               | 150                    | 147.29                    | 98.19     |
| APSULPHA-20(API) | 100                    | 100.83                    | 100.83    |
|               | 150                    | 151.04                    | 100.69    |

The results above indicated the validity of applying this method in estimating SAAMS in the drug preparation (eye drops).

Comparison spectrophotometric methods

A comparison was made between main analytical spectral variables of the proposed method and two methods in literature using in estimating SAAMS (Table 8).

Table 8: Comparison of some analytical variables of the proposed method with other spectral methods

| Analytical parameter | Present method | Literature method(16) | Literature method(13) |
|---------------------|----------------|-----------------------|-----------------------|
| Reagent used        | 2,4-DHBP       | p-Chloranilic acid    | 8-hydroxy-7-iodoquinoline-5-sulfonic acid |
The results obtained indicate that there is no doubt that the present method is sensitive and of wide application to estimate the compound under study.

**CONCLUSIONS**

The proposed method is accurate and sensitive spectrophotometric procedure and suitable for the estimation of SAAMS in its drug formulation (eyes drops) without excipients interference.

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