Chapter-IV

MICROPROCEDURE FOR SOME ACRIDINE DERIVATIVES WITH BROMAMINE-B

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ACRIDINES:

Acridine is tertiary base, occus in the anthracene traction of coal-tar . It is the parent substance of a number of dyes and antiseptics. The parent compound has following structure:

Acridine dyes are all yellow to orange and brown, basic dyes, They are used in calico printing, dyeing cotton, silk and particularly leather. Some acridine dyes have medicinal and antiseptic properties. Acriflavine possessess trypanocidal action (i.e. the power to kill trypanosomes which are micro organisms causing sleeping sickness and other diseases.) It has now been replaced by more potent trypanocides. But it is still used as an antiseptic. Pentaflavine which has acriflavine as active constituent has been used as mouth and throat disinfectant. The structure of some important acridines are given below.

Due to the great commercial values, the assay of acridine need prime attention. A survey of literature reveals that some methods have been reported so far, for the determination of acridines.

Reimers introduced a photometric tiration method based on the titration of a alcoholic solution of acridine with sodium hydroxide. Hall and Powell[2] criticised this method for the difficulty in determining the end point shaw and wilkinson[3] suggested colorimetric method for the estimation of proflavine hemisulphate with nitrous acid. Nitrous acid develops an unstable purple colour which was stablished by removal of excess of nitrous acid, quinone -imine thus formed was coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride.

A spectrophotometric method[4] has been reported for the determination of purine and acridine in the presence of other analogous compounds.

A fluorometric method[5] using ultraviolet light has been suggested for the determination of ethacridine lactate in drugs.

Thielemann[6] separated mixture of acriflavine, ethacridine and 3, 6-diamino acridine hydrogen chloride by thin layer chromatography. The plates when sprayed with Ehrlich's reagent showed yellow spot for ethacridine and red spot for acriflavine and 3.6 diamino acridive hydragen chloride.

Udall[7] suggested a method depending on the estimation of aminor group via diazotisation with standard sodium nitrite solution using starch-iodine paper as external indicator.

Gaillot[8] has shown that commercial acriflavine actually consists of a mixture of hydrochloride of 2;8. diamino-10-methyl acridinium chloride and diamino acrodome hydrochloride, Hall and Powell[9] introduced a procedure for the determination of unmethylated compounds in acriflavine and culflavine depending on the difference in behaviour on treatment with alkali. Collins and Stasiak[10] suggested a method for determining the total chlorine in acriflavine.

Powell and Hall[11] suggested a method depending on the insolubility of femicyaride compound of acridine.

Zora Blagojeuic[12] et al. proposed a conductometric titration procedure for the determination of acridine derivatives using allicolungetic acid , sodium hydroxide and picric acid as titrants. Acheson and Adcock[13] have reported a method based on oxidation of acridine with peroxybenzoic acid.

Veleviu and Adolkert[14] suggested an amperometric titration method for the determination of acriflavine and trypaflavine with sodium nitrite. Acriflaving was dissolved in hydrochloric acetic acid mixture and titrated amperometrically at 400mv in presence of potassium bromide with sodium nitrite solution. Rao[15] et al letermined acridine by titrating it with chlorosulphonic acid in acetone acetic acid solution.

Singh, Gopal and Pandey[16] developed a method for small scale determination of acridine compounds using N-bromosuccinimide as direct titrant.

Chauhan[17] has described a method for microscale determination of acridine compounds using bromine monochloride as brominating agent in glacial acetic acid medium in ice cooled bath.

Most of the methods described above involve sophisticated instrumentation or elaborate experimental procedures. Some methods described above, are for individual compounds. Some methods also suffer from such drawback as filtration of precipitate, use of unstable reagent and uncertainty in location of exact and point. Further most of the methods lack general applicability.

Present Work:

In the present work a simple and convenient method has been developed for microestimation of some acridine and puring derivatives viz. Acriflavine, Proflavine, Acridine orange and Acridine yellow, using Bromamine-B (Sodium salt of N-bromo) benzenesulphonamide in acidic medium at room temperature. The developed method provides a method of choice for milligram determination of acridine and purine derivatives.

reaction time, was prescribed as reaction time for stoichiometry determination of sample. Stocichiometry of reaction of sample with BAB determined at this reaction time was taken to be real value. Table-1 describe stoichiometry determination of reaction of acriflavine (taken as test sample from acridines with BAB .The stoichiometry of reaction of acridine derivatives viz. Acriflavine, proflavine, Acridine orange and Acridine yellow, theophylline, with BAB were determined by the above recommended method, the restuls obtained for acridines are shown in Table-2. In this table for the sake of convenience onlyh the constant molar ratio of BAB with sample at a particular reaction time are reported.

Study of Variables:

In order to develop a suitable reaction condition for microestimation of acridine derivatives with Bromamine-B reagent, the effect of following variables were studied. Acriflaving (acridines) was taken as test sample.

Effect of Reaction Time:

Keeping the amount of acridines, concentration of Bromamine-B and sulphuric acid as constant, the reaction time was varied from 0-10 minutes.

Aliquots containing 2 mg of sample solution was taken in a 100 mL Erlenmmeyer flask and 5mL of BAB (o.1N) solution

was added to it, followed by 2mL of $2NH_2SO_4$. The flask was stoppered and contents were shaken thoroughly. The reaction was carried out for varying range of time 0-40 minutes in different sets of experiment. After different intervals of time, the unconsumed Bromamine-B was titrated iodometrically. A blank was also carried out, similarly using all the reagents except the sample. The recover of the sample for each sets of experiment was calculated. Similar experiments were performed with all acridine derivatives. It was observed that acridine derivatives required 20 minutes for completion of reaction. The recover of the sample become constant within the prescribed reaction time (Table 3)

Effect of Sulphuric Acid:

Keeping the amount of sample, reaction time and concentration of Bromamine-B reagfent as constant, the effect of concerntratikon of sulphuric acid on the recover of the sample was studied. Aliquots containing 2 mg of test sample (Acriflavine) were taken in 100ml Erlenmmeyer flasks and 5 mL of 0.1N Bromamine-B reagent was added, followed by 2mL of sulphuric acid of concentration ranging from 0.5 to 4N in different sets of experiment. The reaction mixture was allowed to stand at room temperature for prescribed reaction time (20 minutes for acriflavine. The unconsumed Bromamine-B was

titrated iodometrically. A blank experiment was also run under identical conditions using all the reagents except the sample and the recovery of the sample was calculated for each sets of experiment (Table 4). The results obtained show that the best recovery of the sample was obtained with 2N concentration of sulphuric acid.

The effect of volume of 2N-sulphuric acid on the revoery of test sample (Acriflavine) was also studied. The varying volume of 2N sulphuric acid 0-4 mL were added to reaction mixture containing 2mg of test sample and 5mL of 0.1N Bromamine-B reagent in different sets of experiment. The reaction mixture was allowed to stand at room temperature for prescribed reaction time of the test sample. After the readion time the unconsumed bromamine-B reagent was titrated iodometrically. A blank was also run under identical condition.

The recovery of the sample was calculated for each sets of experiment (Table 5). The best recovery of the sample was obtained with 2mL of 2N sulphric acid.

Thus for general procedure 2mL of 2N sulphuric acid is recommended.

Effect of Bromamine-B concentration:

Keeping amount of test sample 2mg (acridine derivatives-acriflavine as test sample, sulphric acid (2mL of 2N-H_2SO_4), and reaction time (20 minutes for acriflavine and 30 minutes for xanthine) as constant, the effect of concentration of Bromamine-B reagent on estimation of sample was studied. The concentration of the reagent was varied from 0.01 to 1.0N in different sets of experiment. After completion of reaction the unconsumed Bromamine-T was titrated iodometrically. A blank was also carried out similarly using all the reagents except the sample. The recovery of sample for each sets of experiment was calculated. Results obtained are shown in Table 6. It was noticed that the best recovery of sample was obtained with 0.05 to 0.10N concentration of reagent.

However, for present work 5 mL of 0.1N reagent is recommended.

Effect of Temperature:

Keeping amount of test sample (acriflavine)- 2mg. concentration of Bromamine-B (5ml of 0.1N) sulphuric acid (2mL of 2N-H_2SO_4) and reaction time (20 minutes for Acriflavin as constant, the temperature is varied from room temperature to boiling water bath temperature in different sets of experiement. After the prescribed reaction time the

unconsumed reagent was titrated iodometrically. A blank was also carried out similarly using all the reagents except the sample. The recovery of sample was calculated for each sets of experiment. The results obtained are shown in Table 7. It was observed that the best recovery of the sample was obtained at room temperature. Thus the suitable reaction temperature for microestimation of acridine and purine derivatives is room temperature.

After studying various vaiable following suitable reaction condition was recommended for microestimation of some acridine derivatives.

Recommended Procedure:

Aliquots containing 1-5 mg of sample solution was taken in a 100ml Erelenmeyer flask and 5mL of BAB (0.1N) solution was added to it followed by 2mL of 2N-H_2SO_4. The flask was stoppered and the reaction mixture was shaken thoroughly. Contents were allow3ed to stand at room temperature for prescribed reaction time. After the completion of reaction the stopper was washed with 5mL of distilled water and 5mL of potassium iodide (10%) was added to reaction mixture, the contents were shaken thoroughly and kept for one minute. The liberated iodine was titrated with standardized 0.5N sodium thiosulphate solution using starch indication. A blank

experiment was also run under identical conditions using all the reagents except the sample. Recovery of the sample was calculated from difference in titre value of hypo with blank and sample (i.e. amount of reagent consumed for sample).

Table -1

Determination of stoichiometry of reaction of acriflavine with Bromamine-B (0.1N) in acidic medium

Aliquots taken (ml)	Amount* present (mg)	Reaction time (Min)	Titre value of sodium thiosulphate (hypo) with sample (S) (ml)	Molar ratio of BAB with per mole of acriflavine
2.0	1.982	0	-	-
2.0	1.982	5	8.02	2.553
2.0	1.982	10	7.88	3.011
2.0	1.982	15	7.78	3.338
2.0	1.982	17	7.68	3.666
2.0	1.982	20	7.58	3.993
2.0	1.982	25	7.58	3.993
2.0	1.982	30	7.58	3.993
2.0	1.982	40	7.58	3.993

*For each sample three determination were done

Table -2

Determination of stoichiometry of some acridine derivatives with Bromamine -B (0.1N) reagent in acidic medium

Sample	Aliquots taken (ml)	Amount* present (mg)	Amount obtained by calculation (mg)	Reaction time (Min)	Molar ratio of bromamine-Twith sample
1	2	3	4	5	6
1.Acriflavine	1.0	0.991	0.995	20	3.989
	3.0	2.973	2.985		4.003
	5.0	4.955	4.941		4.004
2. Proflavine	1.0	1.008	1.004	20	4.007
	3.0	3.024	3.032		4.005
	5.0	5.040	5.062		3.997
3. Acridine Orange	1.0	1.011	1.008	20	4.002
	3.0	3.033	3.048		4.004
	5.0	5.055	5.036		4.005
4. Acridine Yellow	1.0	0.986	0.991	20	2.003
	3.0	2.958	2.949		1.995
	5.0	4.930	4.945		1.992

*For each sample three determination were done

Table -3

Effect of reaction time on the estimation of acriflavin with Bromamine-B (0.1N) reagent in acidic medium

Aliquots taken (mL)	Amount* present (mg)	Reaction time (Min)	Amount obtained by calculation (mg)	Error %
2.0	1.982	0	-	-
2.0	1.982	2	1.895	-4.39
2.0	1.982	5	1.929	-2.67
2.0	1.982	10	1.960	-1.11
2.0	1.982	15	1.964	-0.91
2.0	1.982	17	1.966	-0.81
2.0	1.982	20	1.993	+0.55
2.0	1.982	22	1.993	+0.55
2.0	1.982	25	1.993	+0.55
2.0	1.982	30	1.993	+0.55
2.0	1.982	40	1.993	+0.55

*Average of three determinations.

Table -4

Effect of concentration of H_2SO_4 (2 ml) on t he estimation of acriflavine with Bromamine-B (0.1N) reagent

Aliquots taken (mL)	Amount* present (mg)	Concentration of H_2SO_4 (N)	Amount obtained by calculation (mg)	Error %
2.0	1.982	0	1.896	-4.34
2.0	1.982	0.5	1.910	-3.63
2.0	1.982	1.0	1.924	-2.93
2.0	1.982	1.5	1.956	-1.31
2.0	1.982	2.0	1.971	-0.55
2.0	1.982	2.5	1.971	-0.55
2.0	1.982	3.0	1.971	-0.55
2.0	1.982	3.5	1.971	-0.55
2.0	1.982	4.0	1.971	-0.55

*For each sample three determination were done

Table -5

Effect of volume of 2N-H_2SO_4 on the estimation of acriflavine with Bromamine-B (0.1N) reagent

Aliquots taken (mL)	Amount* present (mg)	Volume of 2N-H_2SO_4	Amount obtained by calculation(mg)	Error %
2.0	1.982	0	1.905	-3.88
2.0	1.982	0.5	1.913	-3.48
2.0	1.982	1.0	1.958	-1.21
2.0	1.982	1.5	1.974	-0.40
2.0	1.982	2.0	1.974	-0.40
2.0	1.982	2.5	1.974	-0.40
2.0	1.982	30.	1.974	-0.40
2.0	1.982	4.0	1.974	-0.40

*For each sample three determination were done

Table -6

Effect of Bromamine-B concentration on estimation of acriflavine in acidic medium

Aliquots taken (ml)	Amount* present (mg)	Concentration of Bromamine-T (N)	Amount obtained by calculation (mg)	Error %
2.0	1.982	0.01	**1.947**	-1.77
2.0	1.982	0.03	1.963	-0.86
2.0	1.982	0.05	1.971	-0.40
2.0	1.982	0.08	1.976	-0.30
2.0	1.982	0.10	1.976	-0.30
2.0	1.982	0.15	2.00	+0.91
2.0	1.982	0.20	2.007	+1.26
2.0	1.982	0.50	2.013	+1.56
2.0	1.982	1.00	2.022	+2.02

*Average of three determinations.

Table -7

Effect of reaction temperature on the estimation of acriflavine with Bromamine -B(0.1N) reagent in acidic medium

Aliquots taken (ml)	Amount* present (mg)	Temperature (°C)	Amount recovered (mg)	Error %
2.0	1.982	25	1.974	-0.40
2.0	1.982	40	2.002	+0.91
2.0	1.982	50	2.012	+1.51
2.0	1.982	70	2.035	+2.67
2.0	1.982	80	2.058	+3.53
2.0	1.982	90	2.062	+4.04
2.0	1.982	Boiling water bath	2.099	+5.90

*Average of three determinations.

MICROESTIMATION OF SOME ACRIDINE DERIVATIVES

With the recommended procedure micro estimation of some acridine derivatives viz. acriflavine, proflavine, acridine orange and acridine yellow, were carried out (Table 8 to 12). The consolidated table-24 describe the results obtained with all of the acridine and purine derivatives taken in present study.

Calculation:

For testing the quantitative validity of the recommended procedure, percentage error, standard deviation and coefficient of variation were calculated for each sample and sample size (as in Chapter II pp-45-46).

Table-8: Microestimation of acriflavine with Bromamine -B (0.1N) regent in acidic medium

Aliquots teken (ml)	Amount* present (mg)	Reaction time (min)	Molecularity	Amount** obtained by Calculation (mg)	Error %	SD	CV %
1.0	1.006	20	4	1.001	-0.50	.0060	.5994
2.0	2.012	20	4	2.006	-0.30	.0104	.5184
3.0	3.018	20	4	3.026	+0.27	.0121	.3999
4.0	4.024	20	4	4.033	+0.22	.0180	.4463
5.0	5.030	20	4	5.048	+0.36	.0097	.1913

Table-9

Microestimation of proflavine with Bromamine-B (0.1N) reagent in acidic medium

Aliquots teken (ml)	Amount* present (mg)	Reaction time (min)	Molecularity	Amount** obtained by Calculation (mg)	Error %	SD	CV %
1.0	1.014	20	4	1.018	+0.39	.0020	.1965
2.0	2.028	20	4	2.030	+0.10	.0137	.6749
3.0	3.042	20	4	3.051	+0.30	.0127	.4164
4.0	4.056	20	4	4.068	+0.30	.0219	.5383
5.0	5.070	20	4	5.075	+0.10	.0.222	.4374

Table-10

Microestimation of acridine orange with Bromamine-B (0.1 N) reegent in acidic medium

Aliquots teken (ml)	Amount* present (mg)	Reaction time (min)	Molecularity	Amount** obtained by Calculation (mg)	Error %	SD	CV %
1.0	1.009	20	4	1.012	+0.30	.0045	.4447
2.0	2.018	20	4	2.022	+0.20	.0067	.3314
3.0	3.027	20	4	3.018	-0.30	.0150	.4970
4.0	4.036	20	4	4.046	+0.25	.0035	.0865
5.0	5.045	20	4	5.055	+0.20	.0168	.3323

Table-11

Microestimation of acridine yellow with Bromamine-B (0.1 N) regent in acidic medium

Aliquots teken (ml)	Amount* present (mg)	Reaction time (min)	Molecularity	Amount** obtained by Calculation (mg)	Error %	SD	CV %
1.0	1.003	20	2	1.000	-.30	.0040	-4000
2.0	2.006	20	2	2.010	+0.20	.0085	-4229
3.0	3.009	20	2	3.016	+0.23	.0092	-3050
4.0	4.012	20	2	4.025	+0.32	.0075	-1865
5.0	5.015	20	2	5.029	+0.28	.0289	-5747

RESULTS AND DISCUSSION:

As described earlier first of all stoichiometry is established. Acriflavine as representative of acridines is taken as test samples. The reaction time is varied from 0-40 minutes, (Table 1) and the consumption of hypo solution is noted. With the amount of hypo consumed the amount of BAB consumed for the sample is calculated. It is found that the titre value of hypo with sample decreases with increases in reaction time meaning thereby that the consumption of BAB reagent increases with reaction time. The consumption of VAT reagent become constant after 20 minutes in the case of acriflavine (Table1). Beyond this reaction time, there is no increase in the consumption of the reagent. It proves that the reaction of BAB with acriflavine complete within 20 minutes of reaction time. Similar experiments are performed with other acridines and purines and the molar ratio of BAB with acridines are established (Tables 2).

As described in the study of variables a particular reaction time is necessary for completing the reaction giving concordant and accurate results. Table-3 shows that the reaction time of 20 minutes with acriflavine gives minimum error. The percentage error cannot be minimised by increasing reaction time. It indicates that the consumption of reagent under prescribed

reaction time is constant and the increase in reaction time is meaningless. However, if a hasty experiment is done to save reaction time the percentage error is deplorable. The reason for higher values of negative error is due to incomplete reaction. Starting from 5 minutesx and onwards consumption of the reagent increases and percentage error shows an improvement. It shows that the estimation of acriflavine cannot be done accurately at a time lesser than 20 minutes. The same phenomenon is observed in the case of other acridines and purines.

The use of BAB reagent in a proper ionising medium has been studied. As deswcribed in chapter II the reagent needs a proper ionising medium for improvement in its reactivity. In acidinc medium it works as oxidising as well as brominating agent with considerable improvement of reactivity. In the estimation of acridines also the sulphuric acid has been used as reaction medium. Aliquots 2mL of 2N-H_2SO_4 is sufficinet to give accurate results (Tables 4&5). The significance of using this amount and strength of acid is as described earlier. Brom amine-B is the main reactive species in the reaction process. its concentration and the volume has got an important effect. As required for analytical procedure a 2 to 4 fold excess of the reagent as compared to sample is essential for completing ther

reaction and getting a constant stoichiometry. Keeping this view the concentratikon of BAB was varied (Tables 11-12). It is observed that weith about 2mg of test samples 0.1N concentration of BAB (5ml) is sufficient for constant resul;ts. This concentration of BAB may work up to 5mg of acridine derivatives. However, if the amount of sample is increased a proportional increase in the amount of BAB is to be adjusted. Depending upon the stoichiometry of the compound the concentration of the reagent is varied, it means that before the determination of an unknown compound, the knowledge ofthe stoichiometry is essential. The procedure recommended for estimation in my work applies well for acriflavine, proflavine, acridine orange and acridine yellow, from 1 to 5 mg sample size. From Tables 6 it is also evident that a less concentration of the reagent does not give appreciable results perhaps it is due to insufficient excess of the reagent. However, a very large excess of the reagent is unnecessary as shown in the last three readings of the Table 6, I have noticed a loss of accuracy with a very high concentration of the reagent. In my opinion this is due to loss of active bromine in a concentrated solution. Thus it is concluded that 5mL of 0.1N concentration of reagent is suitable for a quantitative reactions. It is also possible that the reagent concentration may be increased and the volume may be

decreased to maintain the amount of available reagent constant. It will decrease the ionisation of the reagent and will not be convenient for titration.

As described in previous chapters the BAB reagent is thermo-unstable, looking this the estimation of acridines are carried out at a reaction temperature of 25-30°C i.e. room temperature. Tables 13 and 14 show the effect of temperature on estimation of test samples acriflavine. it is noted that at higher temperatures there is considerable loss of accuracy of estimation.

Possible course of reaction:

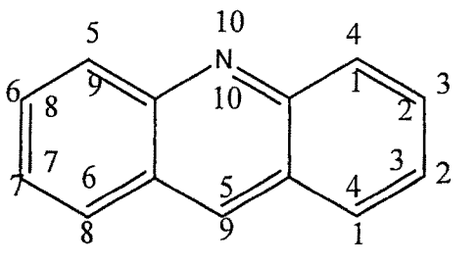
Based on the stoichiometry of reaction of acridine derivatives with Bromamine-B and literature available, possible course of reaction for every sample has been suggested.

Acridines:

Singh et al 16 have reported a method for determination of acridines with N-bromosuccinimide and a possible reaction mechanism has been also discussed. The authors have reported that the N-bromosuccinimide brominates acridine at the active sites of the benzene ring, giving rise to the corresponding bromoderivatives.

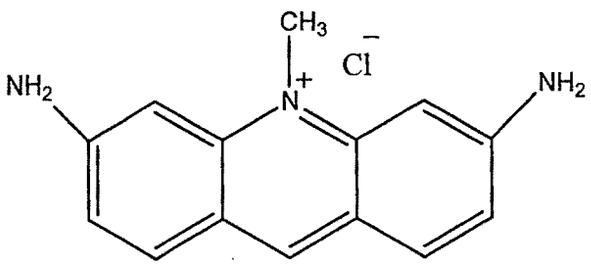
The acriflavine molecule contains two 0-fused benzene rings A and B having one amine group at positions 2 and B in

each of the rings respectively, Since the amino groups are activating and 0-, p- directing , the positions1,3,7 and 9 are activated and these positions are sites for electrophilic substitution. Thus theoretically we may conclude a tetravalent derivative of acriflavine, Acriflavine consumes four moles of BAB reagent. Based on the stoichiometry, reference discussed above and keeping in view the brominating potentialities of reagent, the stoichiometric reaction of acriflavine with BAB can be represented as follows-



$+ 4C_6H_5SO_2N.NaBr + 4H_2O \longrightarrow$

Acriflavine



$+4C_6H_5SO_2NH_2 + 4NaOH$

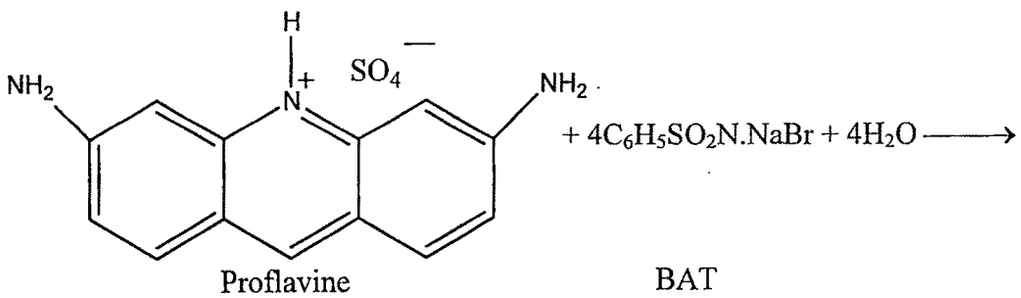
Tetrabromo acriflavine

The coruse of reaction finds support from previous work[16,17].

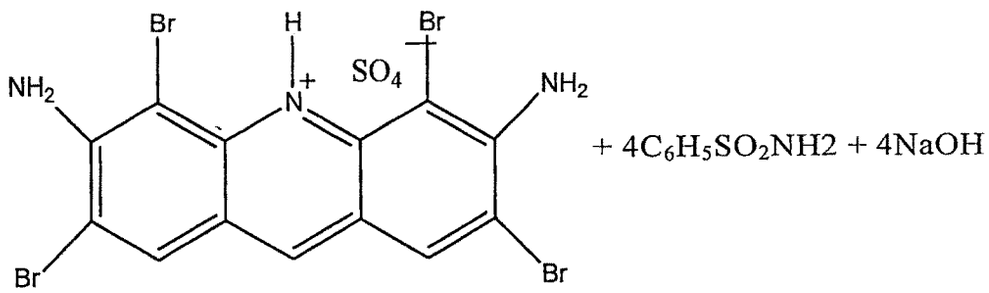
The basic structure of other acridines viz proflavine, acridine orange and acridine yellow resembles to one another and to acriflavine to a great extent with minor differences. The

differences liesd only in substitutents present in ring A and B of basic structural unit acridine. Keeping in view the structural similarities with acriflavine and potentialities of BAT as brominatig agent, it can be anticipated that proflavine, acridine orange and acridine yellow follow similar course of reaction with BAB reagent as acriflavine does.

The stoichiometry determination shows the molar ratio of proflavine with BAB reagent is 1:4 .This undergoes bromination to give tetrabromo derivatives. The positions of incoming substitutents (Br+) are same as in acriflavine. The course of reaction can be represented as



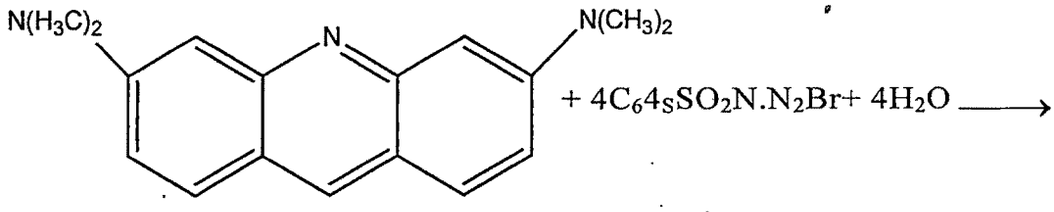
Proflavine BAT



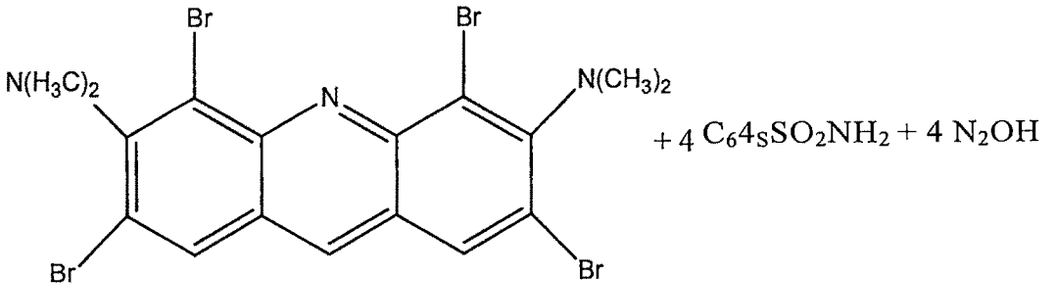
Tetrabromo proflavine

The stoichiometry determination shows the molar ratio of acridine orange with BAT reagent as 1:4. The position1,3,7 and

9 are liable to attack electrophile bromonium ion. This is due to these positions are ortho of dimethyl aming group which activates both A and B benzene rings towards electrophilic substitution. Thus the stocihiometric reaction of acridine orange with BAB can be represented as

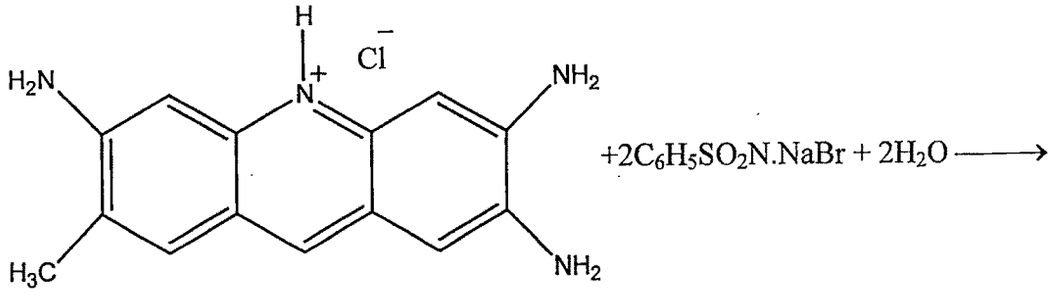


Acridine orange

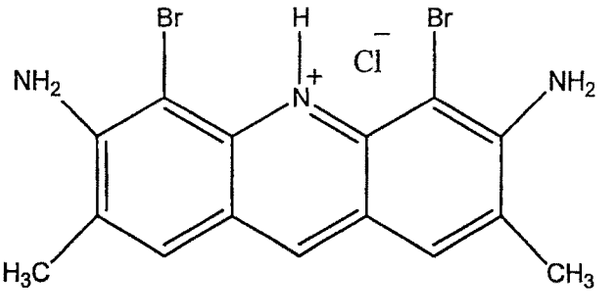


Tetrabromo acridine orange

Acrdine yellow behaves in similar manner. The stoichimetry determination shows the molar ratio of acridine yellow with BAB reagent is 1:2 .The substitutions takes place at available activated position. The course of reaction can be given as:-



Acridine Yellow



$+ 2C_6H_5SO_2NH_2 + 2NaOH$

Dibromo Acridine Yellow

Interferences:

It is observed that the presence of easily oxidisable substances such as phenols, amines, aromatic amines, thioureas, hydroxy acids and aminoacids interfere in the determination thus in order to get accurate results the presence of such compounds should be avoided.

EXPERIMENTAL

REAGENT AND SOLUTIONS:

Bromamine-B-0.1N (0.05M) Solution-

Approx. 0.1N(o.05M) stock solution was prepared by dissolving 4gm of BAt in distilled watger in a 250 mL volumetric flask and made up to the mark with distilled water. The solution was standardised iodometrically.

Sodium Thiosulphate-0.05N Solution (BDH)-

Stock solution of sodium thiosulphate was preapred as in Chapter II.

Copper Sulphate-0.05N Solution (GR):

Stock solution was prepared as in Chapter II.,

2N-Sulphuric Acid:

2N solution of sulphuric acid was prepared by diluting concentrated sulphuric acid (Analar, sp.grav.-1.84)v/v with distilled water.

Potassium Indide (Baker analysod reagent):

10% w/v aqueous solution was prepared.

Starch Solution-

1% w/v aqueous solution was prepared.

Sample solution-

Stock solutions of acridine derivatives were prepared by dissolving accurately weighted 100mg of the samples in

distilled water in 100ml calibrated flask to give a concentration of 1 mg/mL. The acridine derivatives used were of analytical grade (E. Merck).

General Procedure:

Aliquots containing 1-5 mg of the sample solution was taken in 100ml Erlenammeyer flask and 5mL of 0.1N BAB solution was added to it followed by mL of 2N sulphuric acid. The flask was stoppered and the reaction mixture was shaken thorughly. Contents were allowed to stand at room temperature for prescribed reaction time. After the completion of reaction the stopper was washed with 5mL of distilled water and 5mL of potassium iodide (10%) was added to reaction mixture, contents were shaken thoroughly and kept for one minute. The liberated iodine was titrated with standarised 0.5N sodium thiosulphate solution using starch indicator. A blank was also run under identical condition using all the reagents except the sample.

Calculation:

The amount (in mg) of the sample obtained by the proposed method was calculated as in Chapter II (pp.79).

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