SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF AMIKACIN USING P-AMINO ACETOPHENONE

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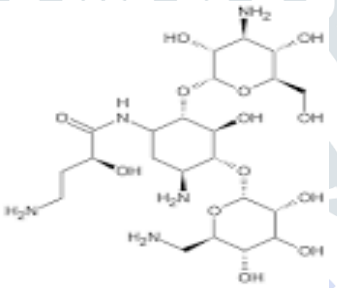
Abstract : The spectrophotometric determination of Amikacin was studied using sodium Meta periodate ($NaIO_4$) as oxidizing agent and p-amino acetophenone (AAP) as coupling agent. This Oxidative coupling reaction was carried out at laboratory temperature (28^0 +) 3^0 C). The influence of time for maximum color development and stability of the colored species formed between amikacin, and $NaIO_4$ –AAP was found to be 5 min. The absorbance of red colored complex formed was measured at 460 nm against reagent blank. The proposed method is simple, rapid, sensitive and specific with reasonable precision and accuracy.

IndexTerms **- Amikacin, Sodium Meta Periodate, p-Amino acetophenone.**

INTRODUCTION

Amikacin is an aminoglycoside antibiotic used to treat different types of bacterial infections. Amikacin works by binding to the bacterial ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth Amikacin is most often used for treating severe, hospital-acquired infections with multidrug resistant gram negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter*, andand *Enterobacter*.



Amikcin

Materials and methods of Preparation of Solutions

Amino Aceto Phenone (AAP): 0.1% solution was prepared by dissolving 0.1 g of p-amini aceto phenone sample (A.R.grade: SDFCL Mumbai) in small quantity of alcohol and diluted to 100 ml distilled water.

Sodium Meta periodate, NaIO4**:** 2.1392 g of NaIO4 (A.R grade: Hi Media laboratories Mumbai-66) was dissolved in distilled water and the total volume was brought to 1 Lt (0.01M).

Standard solution of Amikacin Standard solution of amikacin was prepared by dissolving 100 mg of drug sample [ALFAKIM-Ranbaxy] in 100mL of distilled water. Working solutions of drug sample (100g / mL) were prepared by diluting aliquots of the stock solutions with distilled water. Spectral measurements and absorbance readings were made on Elico SL 177 double beam Spectrophotometer. pH measurements were carried out using Elico pH meter model LI 615.

Aliquots ranging from 1-4 mL of the working standard solution of Amikacin along with 1 mL of AAP solution and 1 mL of sodium per iodate solution were added to a series of 10 mL graduated test tubes and the tubes were kept aside at room temperature for 5 min. Appropriate quantities of distilled water was added to each tube to make the volume. The absorbance of red colored complex formed was measured at 460 nm against reagent blank, prepared in a similar manner. The amount of amikacin was read from calibration curve prepared with its standard solution under identical conditions.

In order to ascertain the optimum wave lengths (λmax) of the colored species formed on mixing amikacin with suitable reagents in appropriate pH medium exhibiting maximum absorbance, the absorption spectra were scanned on a spectrophotometer in the range 400 – 550 nm against the reagent blank using the proposed procedure under experimental conditions (Table – 1.) and the results are graphically presented in Fig-.1.

Optical Characteristics.

Adherence to Beer's Law:

In order to test whether the amikacin--per iodate-AAP system adheres to Beer's, the absorbance at λmax of a set of solutions containing varying amounts of amikacin, specified concentrations of sodium Meta per iodate were measured against reagent blank on a spectrophotometer. The linearity of the plot between absorbance and the concentration range specified in Table-II. shows that the color system obeys Beer's law, Fig-2. Beer's law limits, molar absorptivity, optimum photometric range, and Sandell's Sensitivity values were calculated and the results are incorporated in Table-III.

The precision and accuracy of the methods in the determination of amikacin was tested by measuring the absorbance of six replicates each containing a final concentration value, approximately ¾ of Beer's range. The % relative standard deviations and confidence limits (0.05 and 0.01 levels) are presented in Table-IV.

The accuracy of the method was further tested in injections with proposed and reported methods. The results of these estimations are incorporated in Table-V.

RESULTS AND DISCUSSION

The optimum conditions were established in each case basing on the development of maximum color and stability and results are presented in Table – I. Among the various oxidizing agents tried, IO4- is the best one, followed by H_2O_2. The other oxidizing agents such as IO_3^-, Fe (III), MnO_4^-, ClO^-, Fe $(CN)_6^{3-}$, are inferior. The efficiency of the oxidizing agent depends upon its relative reactive tendency towards reactants, (drug, AAP-NaIO4) products (indo-dyes) and also on the behavior of its reduced form. The formation of colored species of same λmax in the case of amikacin with reagent AAP-IO_4^- suggests that the indo dye formed with compounds is the same.

Amikacin undergoes oxidation in the presence of mild oxidizing agent per iodate to form carbonyl group which undergoes coupling with the – amino group of AAP to form red colored chromogen at a λmax of 460 nm. As AAP contains electron withdrawing group, - $CO\text{-}CH_3$ in para position to aromatic amine, AAP-IO_4^- can successfully be used for the estimation of amikacin.

Based on the results furnished in Tables I -IV reveal that the method proposed for the spectrophotometric determination of amikacin is simple, rapid, sensitive and specific with reasonable precision and accuracy. The proposed method appears to be superior to many of the reported methods and so it can be employed in routine determinations.

The oxidative coupling reaction can be represented as follows.

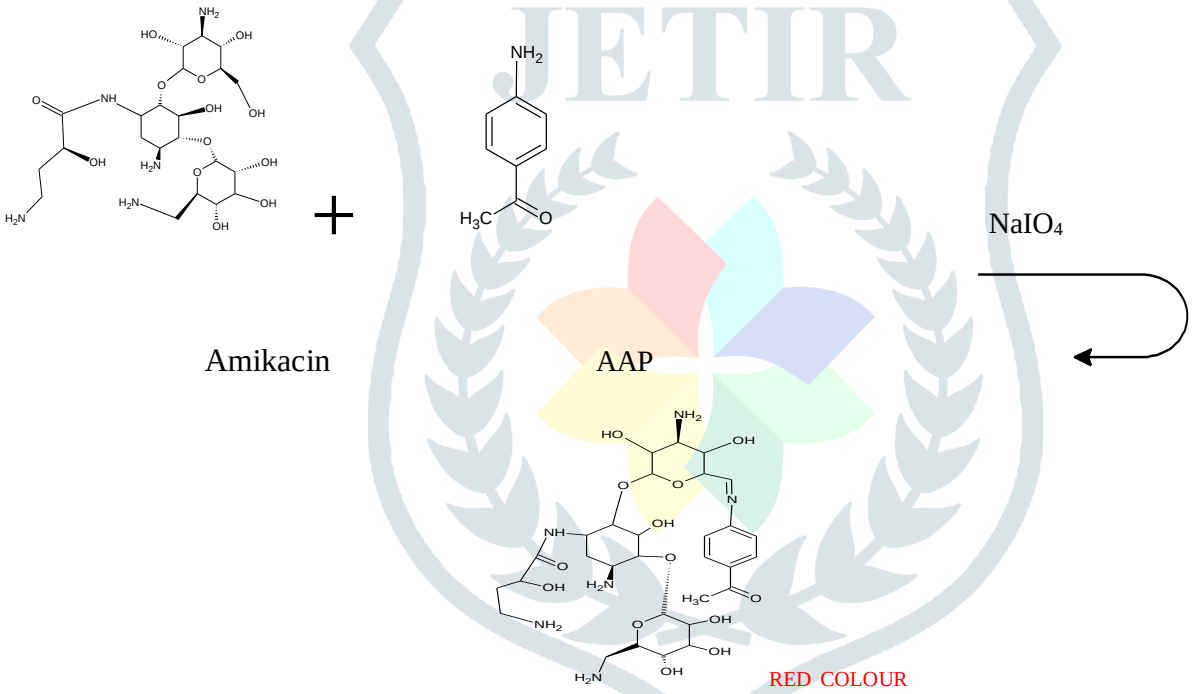


Fig.1 Absorption Spectrum of Amikacin-Periodate-AAP

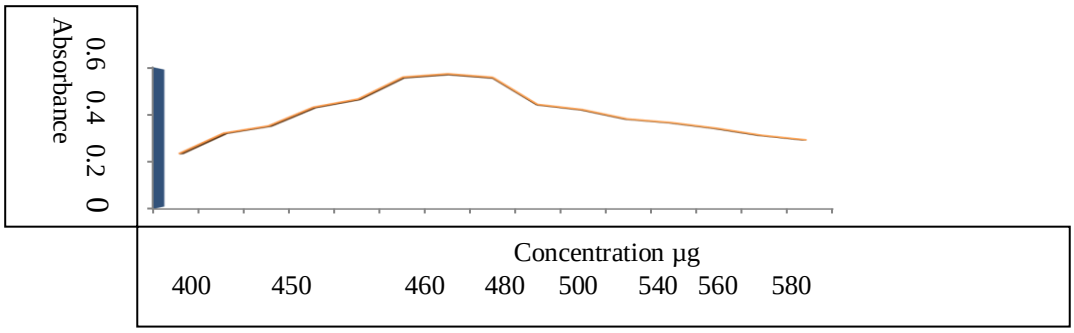


TABLE-I. EXPERIMENTAL CONDITIONS

Optimum conditions				
AAP	**$NaIO_4$**	**Time for max. Color in min**	**Stability of Color in min**	**λ_{max} nm**
1.0 mL	**1.0 mL**	**5**	**70**	**460**

Fig.2 Beers Law Verification

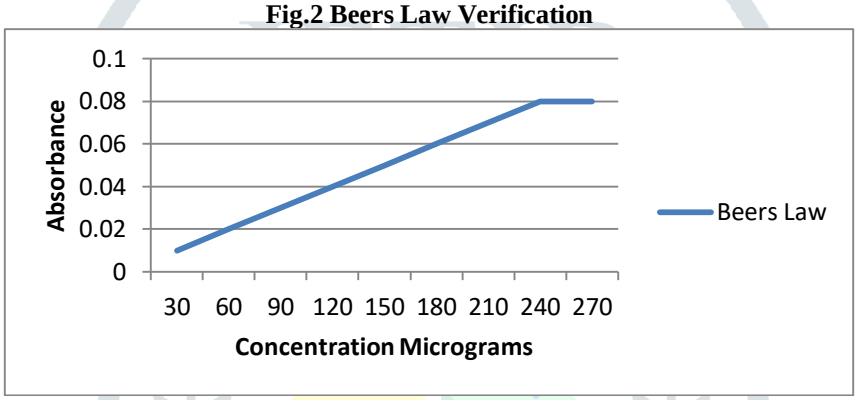


Table – II: Optical Characteristics

Reagent	Beer's Law Range µg/25 ml	Molar Absorptivity Lt/mol/cm	Sandell's Sensitivity $\mu g/cm^2/0.001$ absorbance units	Optimum Photometric Range µg/25 ml
AAP- IO_4^- reagent Amikacin	**30-250**	**4.65×10^3**	**0.026**	**56 - 316**

Table. III .Precesion and Accuracy.

Amikacin	Amount of Drug *		% Error	% R.S.D	% Range of Error	
	Taken mg	Found mg			95% Confidence Limit	99% Confidence Limit
AAP- IO_4^- reagent Amikacin	0.20	0.198	1.0	1.1	±1.25	±1.85
	0.25	0.248	1.2	1.28	±1.32	±1.72

TABLE- IV+ ANALYSIS OF FORMULATIONS-RECOVERY EXPERIMENTS.

Sample	Labeled Amount mg	Mean of % amount found		% Recovery Experiments	
		Reported method	Proposed method	Amount added	% Recovery
Amikacin injection	200	197.2	197.8	0.350	99.3
Amikacin injection	200	196.8	197.2	0.400	98.7

Conclusion

The authors conclude that the proposed spectrophotometric method is sensitive and reproducible for the analysis of Amikacin in pharmaceutical dosage forms with short analysis time.

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