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Simultaneous spectrophotometric and volumetric determinations of amoxycillin, ampicillin and cloxacillin in drug formulations: reaction mechanism in the base catalysed hydrolysis followed by oxidation with iodate in dilute acid solution

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Abstract

A method for the analysis of degraded products of amoxycillin, ampicillin and cloxacillin in drug formulations, obtained as a result of their base hydrolysis is described. Simultaneous spectrophotometric and volumetric determinations of the antibiotic is based on the neutralization of the degraded product by dilute hydrochloric acid to get a pH ~ 2 to be conducive for redox titration using potassium iodate as titrant. A red purple colour is developed in carbon tetrachloride at the end point. Spectrophotometry is done after separating the organic layer and measuring the absorbance of red-purple colour at λ_{max} 520 nm. The pathways of different degraded products and their oxidation mechanism is described on IR, TLC and UV spectroscopic studies.

Keywords: Amoxycillin; Ampicillin; Cloxacillin; Spectrophotometric and volumetric determination; Simultaneous determination; Pure and drug formulations

1. Introduction

A large number of methods for the analysis of ampicillin, amoxycillin and cloxacillin and related antibiotics are described [1–3]. Emphasis has been given on spectrophotometric and titrimetric methods, because they are simple and easily manageable to the third world. The volumetric titrations are carried out by some oxidants prior to a base or acid catalysed reaction of antibiotics. The degraded hydrolysed products of antibiotics are titrated quantitatively against oxidants such as Hg^{2+} [4] and Iodate solutions [5]. It is observed that when hydrolytic conditions are changed from moderate concentration of alkali or acid to a weak or using the selective pH regions [6], a different pathway of antibiotics mechanism results. The titrimetric method [4] described by alkaline or enzymic hydrolysis followed by titration

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with Hg(II) might suffer a setback. The use of nitric acid to neutralize the excess alkali before performing the titration may oxidize thiazolidine ring of the penicillin molecule and therefore could give erroneous results. The authors themselves reported better results with enzymic hydrolysis than alkaline hydrolysis.

In the direct titration of antibiotics [5], with potassium iodate solution, strong acidic conditions are prevailed. The acid plays a dual role, firstly it brings about acid hydrolysis, secondly it makes quantitative oxidation titration feasible. Two procedures are adopted [5]; in the first, the end point is detected by a colour change in the carbon tetrachloride layer from colourless to brown or deep red and in the second one, dye Amaranth is used to detect change in colour from deep red to pale yellow, as the dye is destroyed by an excess of iodate in aqueous medium at the neutralization point.

The present manuscript describes the base catalysed hydrolysis of antibiotics in dilute sodium hydroxide solution. The hydrolysis product of antibiotics which contains sulphur derivatives, eg. -C-S-C groups, -S-H groups are quantitatively oxidized to sulphonic acid by titrating them against potassium iodate solution as oxidant, in dilute hydrochloric acid to prevail conducive atmosphere of pH ~ 2. The chemistry of this reductant system is described which shows the observed stoichiometric value of thiol group to iodate 1:2 which is consistent with theoretical value. The point of difference of our findings to that of previously described by Andrews titration [12] have been highlighted. The volumetric results are compared to spectrophotometric determination. The pathway and the oxidation mechanism of hydrolysis products of antibiotics have been proposed in the light of IR, TLC and spectrophotometric studies.

2. Experimental

2.1. Reagents

All the experiments were performed with analytical reagent grade chemicals using double distilled water. Stock solution of antibiotics, 1 mg ml^{-1} were prepared by dissolving the pure and standard drugs (Himedia laboratories Bombay India) in water and stored in a well-dark and closed container to avoid direct contact with light. Hydrochloric acid standard solution (1.0 M), potassium iodate and sodium hydroxide of 0.1 M each were prepared in distilled water.

2.2. Apparatus

A Bauch and Lomb Spectonic -20D^{+} and Spectronic-1001 split beam spectrophotometer (Milton Roy Co.) with 1 cm path length quartz cells and a controlled water bath (NSW-133 India) were used. IR spectra of the isolated products were recorded on a spectrometer (Shimadzu IR-408, Japan) with KBr disc.

2.3. General procedure

2.3.1. Procedure I: simultaneous determination of penicillins by-volumetric titration against potassium iodate and spectrophotometry of coloured complex in organic layer

Aliquots of 2.5 ml of 0.1 M sodium hydroxide were added into conical flasks followed by suitable volumes of each of the standard penicillin solutions, containing between 0.2–2.0 mg ml^{-1} of amoxycillin and ampicillin and 0.1–2.5 mg ml^{-1} of cloxacillin. The contents (pH ~ 13) were shaken and placed in a water bath at 80°C for 10–15 min. After completion of the heat treatment, the mixture was cooled to room temperature, followed by 0.5 ml of 1.0 M hydrochloric acid and 5 ml carbon tetrachloride. The mixture contents were titrated against potassium iodate with intermittent shaking. The end point was detected by a colour change from colourless to deep red colour in non-aqueous layer. For spectrophotometric determination, the non-aqueous layer was removed using a 50 ml separating funnel, dried over anhydrous sodium sulphate and measured absorbance at 520 nm against the blank, containing all the species except drug.

2.3.2. Procedure II: determination of penicillins by spectrophotometric method

To a known volume of amoxycillin solution ranging between 0.4–3.5 ml, ampicillin 0.4–2.8 ml and cloxacillin 0.1–2.5 ml, 2.5 ml of sodium hydroxide were added in a conical flask. The reaction mixture was kept for 10–15 min on a water bath at 80°C. After cooling to room temperature, 1 ml of potassium iodate, 0.5 ml of hydrochloric acid and 5 ml carbon tetrachloride were added to the reaction mixture. The solution was shaken vigorously till a deep red colour was obtained in the organic layer. It was determined spectrophotometrically by the recommended procedure I.

2.4. Assay of formations

For the determination of penicillins in tablets and capsules, the above methods were used with no modification. About twenty tablets were grinded into finely divided powder. 100 mg of powder was accurately weighed and transferred into a 100 ml standard flask. Similarly the contents of the capsules were weighed and dissolved equivalent to 100 mg in 0.1 l. The solution was well shaken for about 30 min to ensure a homogeneous solution. The residue was removed through Whatman No. 1 filter paper and the washings were taken into a final volume of 0.1 l followed by the recommended procedures I and II.

3. Results and discussion

The mechanism of penicillins in 0.1 M sodium hydroxide resulting in different degraded products can be rationalized in the light of IR, UV spectroscopic studies, TLC and molar stoichiometries molar ratio of iodate to penicillin titration. The possible degradation pathways are illustrated in Fig. 1.

3.1. TLC and spectrophotometric observations

The presence of Ia has been suggested on the basis of the results obtained by TLC ($Rf = 0.46$ in a solvent system made v/v and comprising, 66% n-butanol, 17% glacial acetic acid, 17% water). This observation is in agreement with previous studies [6], which confirms the existence of 4-hydroxymethylene oxazolone Ia, ($Rf = 0.42$ in the same solvent). Our studies of hydrolysis in 0.1M NaOH at pH ~ 13 involving a single step reaction using water bath at 80°C, show one λ_{max} at 240 nm, at reaction time of 15 min. This attributes the existence of one of the three species viz; Ib (i) or Ib (ii) or Ib (iii). Moreover the formation of penicilloic acid Ib (i) from basic hydrolysis of penicillin is well established [7]Tables 1–3. On extending the time interval to 12 h, another λ_{max} at 270 nm is obtained, which further confirms the establishment of I(a). The previous studies show the existence of I(a) as degraded product of antibiotics in the pH range 13–14 with UV spectroscopic analysis: λ_{max} 297.5. The slight difference in λ_{max} value may be attributed to different experimental conditions of hydrolysis, such as temperature, time of hydrolysis and pH etc.

3.2. IR studies

Three different spectra were taken, (i) pure amoxycillins, spectrum-A (Fig. 2) (ii) degraded in base catalysed hydrolysis, spectrum-B (Fig. 3) (iii) degraded base hydrolysed product treated with 0.1 M hydrochloric acid followed by titration against KIO3, spectrum-C (Fig. 4).

The spectrum (B), compared with spectrum (A), revealed that the characteristic bands for different groups at specified frequencies in spectrum (A) are found, (i) missing, (ii) the formation at different frequencies or (iii) remained unchanged. A broad flat portion (3450–2600 cm^{-1}) same as it was found in spectrum (A), attributed to primary aliphatic amine N-H (3450–3250 cm^{-1}), carboxylic (3300–2500 cm^{-1}) and S-H (2600–2500 cm^{-1}) groupings.

3.3. Missing and formation of new bands

The bands are found missing in the range (1800–1500 cm^{-1}) and (1350–l000 cm^{-1}) resulting the formation of bands at 860 cm^{-1}, 680cm^{-1} and in the range (1500–1350 cm^{-1}).

Table 1
Volumetric determination of amoxycillin, ampicillin and cloxacillin after separating the extracted coloured complex from organic layer formed at equilibrium point during titration using potassium iodate as titrant[a]

Table A(1): amoxycillin				Table B(1): ampicillin				Table C(1): cloxacillin			
Taken mg ml^{-1}	Volume of titrant KIO_3 (ml)	Found mg ml^{-1}	% Recovery ± S.D. mg ml^{-1}	Taken mg ml^{-1}	Volume of titrant KIO_3 (ml)	Found mg ml^{-1}	% Recovery ± S.D. mg ml^{-1}	Taken mg ml^{-1}	Volume of titrant KIO_3 (ml)	Found mg ml^{-1}	% Recovery ± S.D. mg ml^{-1}
0.1	0.02, 0.02, 0.022	0.10	100.0 ± 0.025	0.1	0.022, 0.02, 0.02	0.098	92.8 ± 0.05	0.1	0.01, 0.01, 0.0	0.095	95.2 ± 0.000
0.2	0.04, 0.04	0.0191	99.5 ± 0.006	0.2	0.04, 0.045, 0.04	0.193	96.5 ± 0.013	0.2	0.02, 0.02, 0.02	0.197	98.5 ± 0.012
0.5	0.1, 0.095, 0.1	0.48	96.0 ± 0.020	0.5	0.1, 0.11, 0.1	0.480	96.0 ± 0.027	0.5	0.05, 0.05, 0.052	0.49	98.0 ± 0.009
0.8	0.17, 0.165, 0.17	0.82	102.5 ± 0.014	0.8	0.17, 0.165, 0.17	0.812	101.5 ± 1.09	1.0	0.11, 0.015, 0.11	0.030	103.2 ± 0.030
1.0	0.20, 0.20. 0.205	1.02	102.0 ± 0.070	1.0	0.215, 0.215, 0.215	0.985	98.5 ± 0.120	1.5	0.165, 0.165, 0.16	1.540	102.7 ± 0.007
1.5	0.25, 0.30, 0.30	1.38	92.0 ± 0.140	1.5	0.30, 0.31, 0.31	1.422	94.8 ± 0.070	2.0	0.2, 0.2, 0.205	1.920	96.6 ± 0.030
2.0	0.39, 0.38, 0.40	1.90	95.0 ± 0.050	2.0	0.425, 0.42, 0.425	1.96	98.0 ± 0.120	2.5	0.265, 0.265	2.490	99.6 ± 0.030

[a] Performed simultaneously with spectrophotometric determination.

Table 2
Spectrophotometric determination of amoxycillin, ampicillin and cloxacillin after separating the extracted coloured complex from organic layer formed at equilibrium point during titration using potassium iodate as titrant[a]

Table A(II): amoxycillin			Table B(II): ampicillin			Table C(II): cloxacillin		
Taken mg ml^{-1}	Found mg ml^{-1}	% Recovery $\pm$ S.D. mg ml^{-1}	Taken mg ml^{-1}	Found mg ml^{-1}	% Recovery $\pm$ S.D. mg ml^{-1}	Taken mg ml^{-1}	Found mg ml^{-1}	% Recovery $\pm$ S.D. mg ml^{-1}
40.0	39.90	99.8 ± 0.16	40.0	39.90	99.80 ± 0.22	20.0	20.20	100.0 ± 0.45
100.0	100.08	100.1 ± 0.20	100.0	99.96	99.96 ± 0.05	40.0	40.30	100.8 ± 0.38
160.0	160.12	100.1 ± 0.16	160.0	160.30	100.20 ± 0.68	100.0	99.4	99.94 ± 0.050
200.0	199.64	99.82 ± 0.36	200.0	200.60	100.30 ± 0.83	200.0	204.0	100.2 ± 0.900
300.0	300.52	100.2 ± 1.39	300.0	299.4	99.98 ± 1.04	400.0	399.90	99.98 ± 0.110
500.0	398.80	99.7 ± 0.95	400.0	400.30	100.10 ± 0.69	500.0	499.96	99.99 ± 0.011

[a] Performed simultaneously with volumetric determination.

Table 3
Spectrophotometric determinations of amoxycillin, ampicillin and cloxacillin after separating the extracted coloured complex in organic layer formed by adding excess of potassium iodate

Amoxycillin			Ampicillin			Cloxacillin		
Taken mg ml^{-1}	Found mg ml^{-1}	% Recovery ± S.D. mg ml^{-1}	Taken mg ml^{-1}	Found mg ml^{-1}	% Recovery ± S.D. mg ml^{-1}	Taken mg ml^{-1}	Found mg ml^{-1}	% Recovery ± S.D. mg ml^{-1}
80.0	80.02	100.03 ± 0.033	40.0	39.99	99.98 ± 0.011	20.0	19.98	99.9 ± 0.02
160.0	159.60	99.75 ± 0.380	100.0	100.20	100.2 ± 0.0220	100.0	100.24	100.24 ± 0.33
200.0	199.50	99.75 ± 0.500	160.0	159.98	99.99 ± 0.022	160.0	160.04	100.03 ± 0.044
300.0	299.20	99.73 ± 0.450	200.0	200.12	100.1 ± 0.180	200.0	199.5	99.75 ± 0.5
400.0	399.68	99.92 ± 0.410	300.0	299.97	99.99 ± 0.040	300.0	300.03	100.01 ± 0.027
500.0	499.98	99.99 ± 0.084	400.0	400.40	100.1 ± 0.550	400.0	400.24	100.06 ± 0.24
700.0	699.84	99.98 ± 0.081	560.64	559.64	100.1 ± 0.490	500.0	499.88	99.98 ± 0.14

The identities of thiozolidine and secondary amine groups with different group frequencies lying in the range (1800–1510 cm^{-1}) are completely swept out. The second range of missing band (1350–1000 cm^{-1}) includes the groups e.g. CH_3-S, ester etc. The two missing ranges are revealed by a descending and ascending portion of spectra-B respectively. The formation of a broad band (1500–1350 cm^{-1}) is attributed to the presence of 4-hydroxy-methylene oxazolone (Ia), which is revealed by the groups frequencies C-O stretching and O-H deformation vibration (1440–1395cm^{-1}). The ascending portion (1350 cm^{-1} onwards) of spectrum B further describes the hydrolysis of the rest of the groups present. A strong peak at 860 cm^{-1} may be attributed to S-O (870–810 cm^{-1}) of sulphonic acid. The weak peak at 680 cm^{-1} suggests to disulphide -S-S, C-S

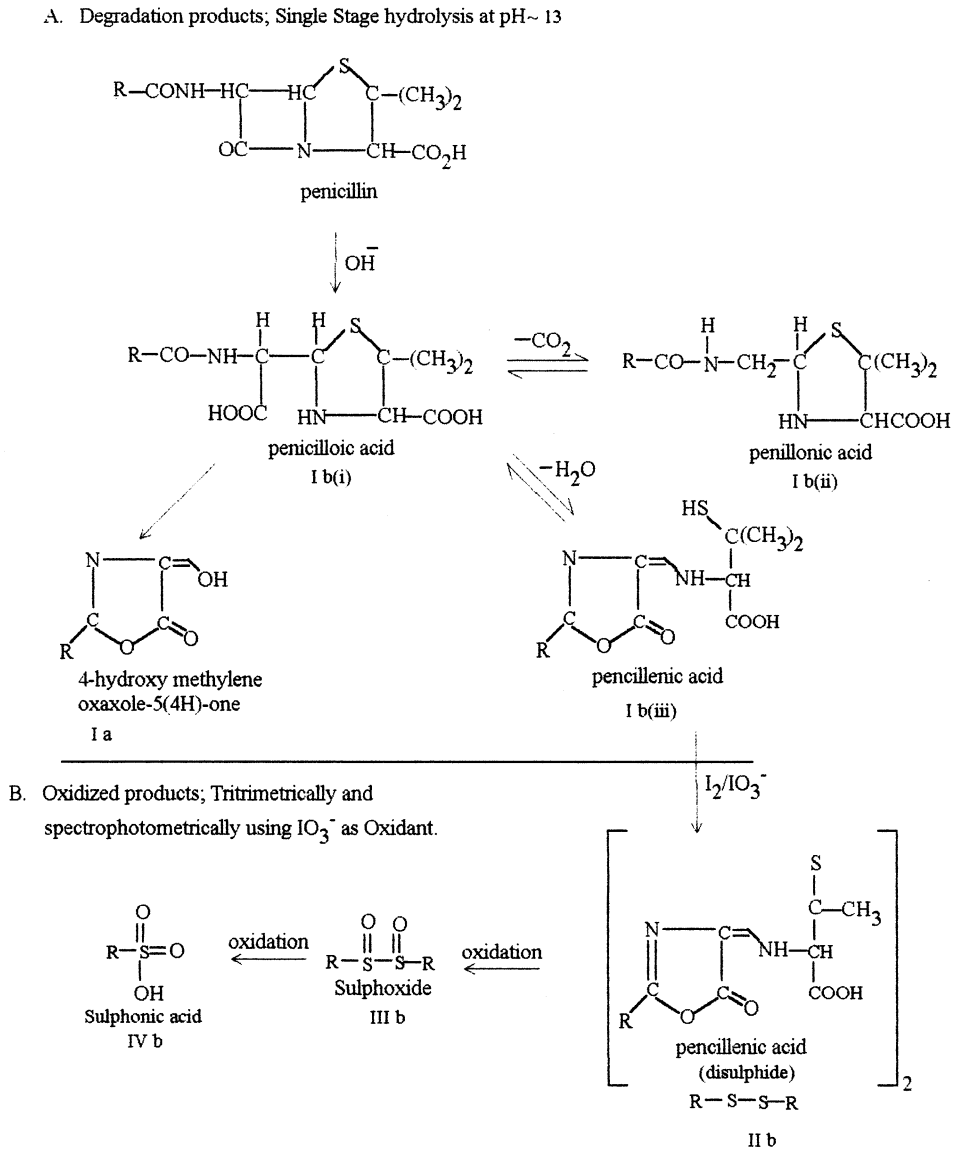

Fig. 1. The possible degradation pathways of pencilline; followed by oxidation with iodate ions.

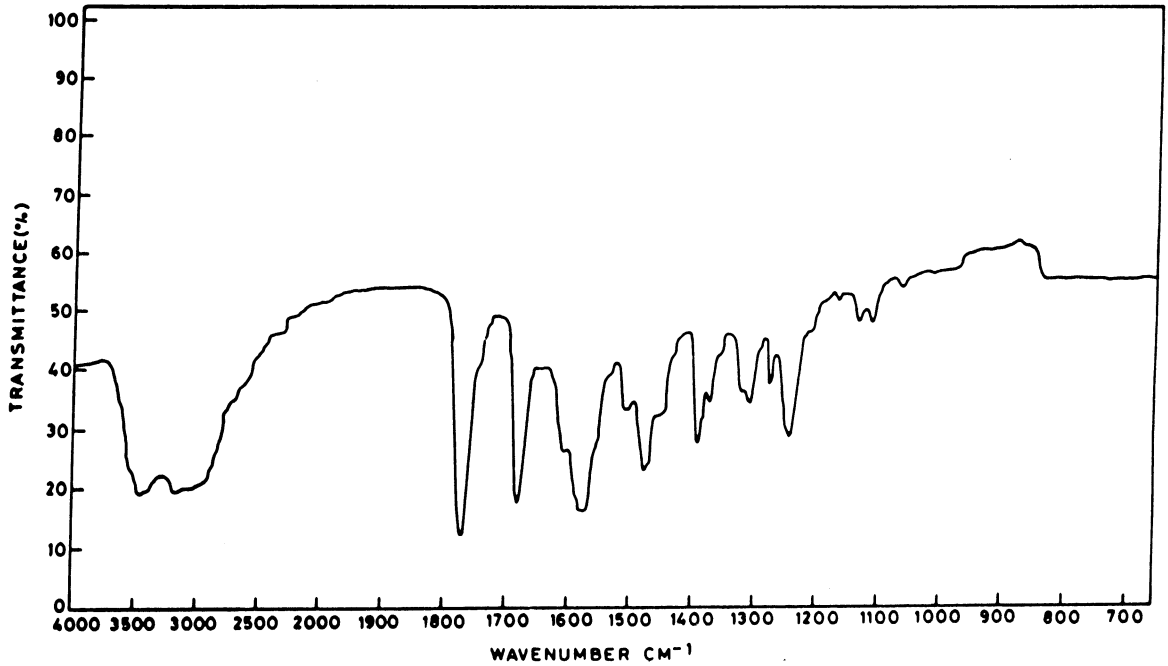

Fig. 2. (i) Pure amoxycillin, spectrum-(A).

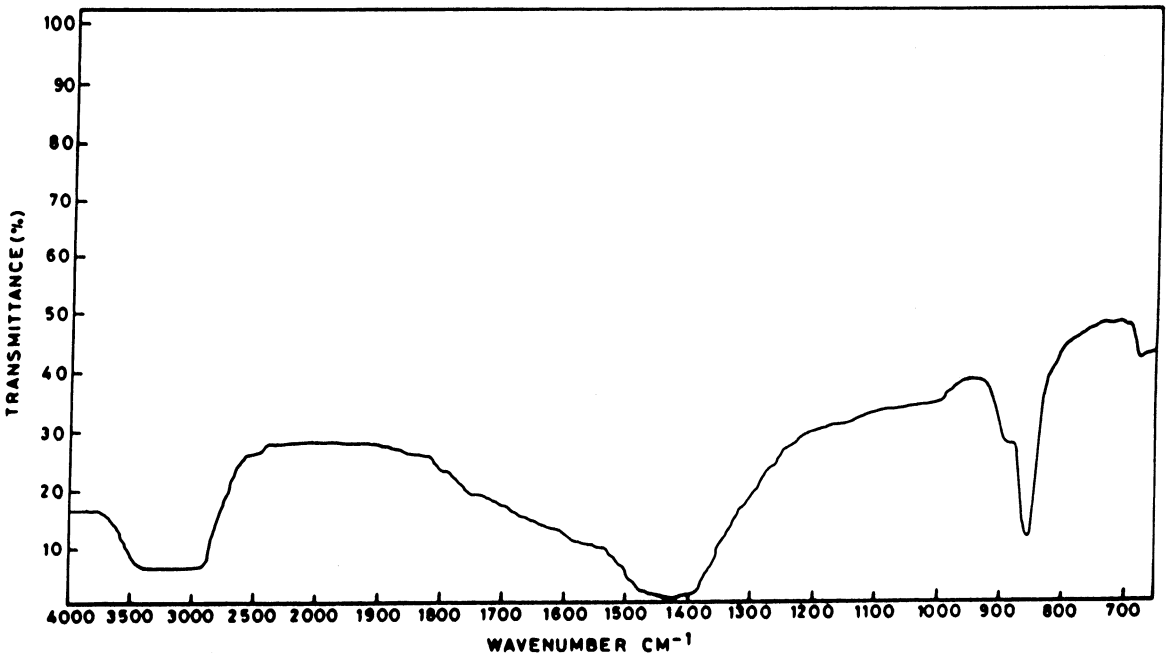

Fig. 3. (ii) Degraded in base catalysed hydrolysis, spectrum-(B).

(705–570 cm^{-1}) and sulphoxides (> S = O, C-S-O at 730–690 cm^{-1}) after their partial oxidation to sulphonic acid in a semiquantitative manner as the sulphur compounds especially containing the group S-H, S-S are oxidized by mild oxidant or even atmospheric oxygen [8]. The spectrum -C, which is obtained after completing the titration against iodate in dilute acid conditions, gave the final product corresponding to strong peak at 835 cm^{-1}, attributed to the presence of sulphonic acid quantitatively.

The recommended procedure I describes simultaneous volumetric determination of penicillin against potassium iodate as oxidant in dilute acid medium. The deep-violet colour extracted in carbon tetrachloride at the equivalence point is stable for 2 h and has been studied spectrophotometrically. The hydrolysis product of penicillins (amoxycillin, ampicillin and cloxacillin) which contained sulphur derivatives, i.e. -C-S-C (Ib), -S-H groups are quantitatively oxidized to some intermediate species and finally to sulphonic acid whose identities have already been discussed in IR studies. The hydrolysis by dilute sodium hydroxide solution has been carried out slowly at a constant temperature of 80°C with intermittent shaking. The excess of base is neutralized by adding hydrochloric acid (0.5 mmoles) so that the reaction mixture attains a pH ~ 2. This provides a conducive environment of H_3O^+ to concentration for carrying out quantitative titration of degraded products against potassium iodate. The reaction is summarized as follows;

$$[HS\text{-}R + 3H_2O \xrightarrow{6e^-} RSO_3H + 6H^+ 6e] \times 2 \quad \text{Step I}$$

$$[IO_3^- + Cl^- + 6H^+ + 4e^- \rightarrow ICl + 3H_3O^+] \times 3$$

In the first step reaction a quantitative oxidation of thiol to sulphonic acid followed by reduction of IO_3^- to to ICl takes place which may be regarded as the reaction at equivalence point, but without any colour change. This shows that the stoichiometric value calculated theoretically should be of 2 mol of thiol group to 3 mol of iodate (1:1.5) which is inconsistent with experimental value 1:2 [9]. However, the system still involves more iodate ions to make the end point visible due to its reduction to iodine monochlo-

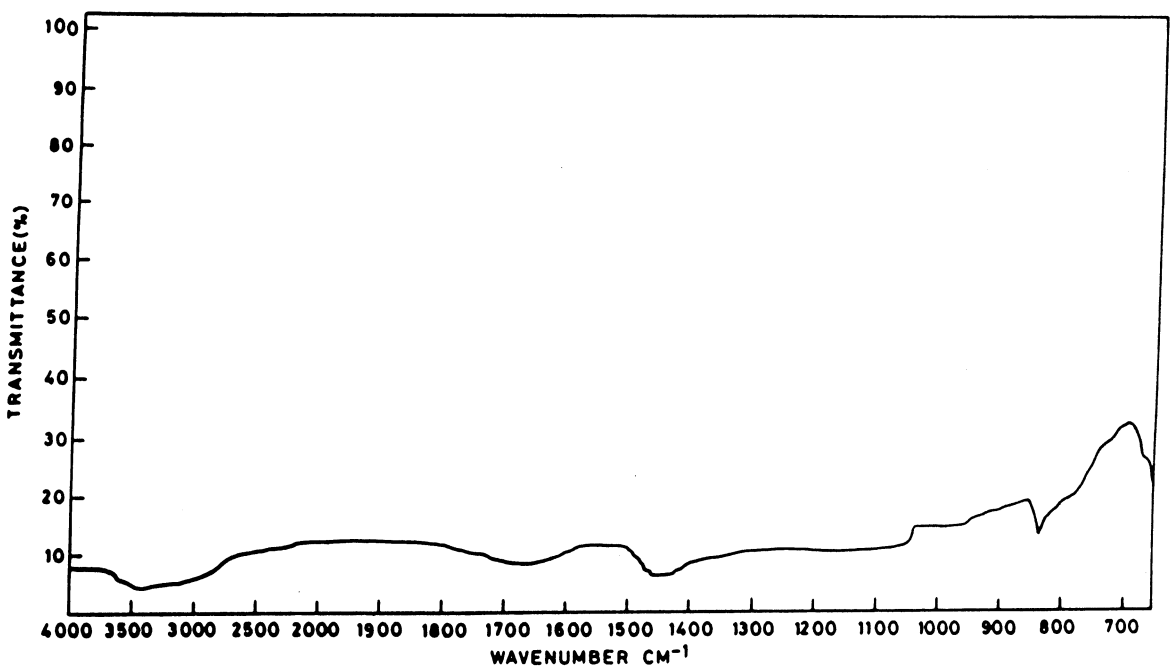

Fig. 4. (iii) Degraded base hydrolyzed treated with 1.0 M hydrochloric acid, followed by titration against KIO_3, spectrum-(C).

ride and finally to free iodine. This is described in step II reaction which is given below:

$$IO_3^- + 6H^+ + Cl^- + 4e^- \xrightarrow{\text{reduction}} ICl + 3H_2O$$

$$ICl \xrightarrow{\text{dissociation}} I^+ + Cl^-$$

$$I^+ + H_2O \xrightarrow{\text{hydrolysis}} HOI + H^+$$

$$5HOI \xrightarrow[\text{of iodate}]{\text{over consumption}} 2I_2 + IO_3^- + H^+ + 2H_2O$$

In the step II, an over consumption of iodate takes place [10], thereby increasing the mole ratio of iodate from 1.5 to 2 mol which is consistent with the experimentally observed stoichiometry of 1 mol thiol to 2 mol iodate. A stable red violet colour of free iodine in organic layer indicates the equivalence point. It is observed that further addition of iodate solution beyond the end point results no decolourization in mixture content. This means that a quantitative oxidation-reduction in dilute hydrochloric acid has taken place in the system. The titrant iodate is reduced first to iodine monochloride and finally to free iodine to show a visible end point. The E° of iodate in dilute hydrochloric acid (1.19 V) is less than the E° in Andrew titration (1.23 V). The E° 1.19 V is high enough to oxidize the degraded products of antibiotics containing derivatives.

In Andrews titration a strong hydrochloric acid (3–9 M) solution is required for reduction of iodate to iodine monochloride (ICl_2^-) complex.

$$IO_3 + 6H^+ + 2Cl^- + 4e^- \rightarrow ICl_2^- + 3H_2O$$

$$E° = 1.23 \text{ V}$$

During the course of titration with reducing agent, the presence of free iodine in the system also results from the further reduction of ICl_2^- complex as given below:

$$2ICl_2^- + 2e^- \rightarrow I_2 + 4Cl^- \qquad E° = 1.06 \text{ V}$$

When the reducing agent has been consumed, the free iodine is titrated to form iodine monochloride complex. The end point is marked by the disappearance of the last free iodine. (A point of difference from our observation given in Step II).

$$2I_2 + IO_3^- + 6H^+ + 10Cl^- \rightarrow 5ICl_2^- + 3H_2O$$

The above conditions prevail if the concentration of the hydrochloric acid at the end point is at least 3 M. The iodate reacts quantitatively with reducing agents such as KI, I_2, AS_2O_3, N_2H_4 and SO_2 to iodine monochloride [11]. In many other cases the concentration of hydrochloric acid is not critical, but for Sb(III), it is 2.5–3.5 M; that is, the optimum acidity for reasonably rapid reaction varies from one reactant to another reactant [12].

The recommended procedure I, which describes the use of dilute hydrochloric acid is applied successfully to the simultaneous quantitative reduction of iodate to free iodine at the equivalence point, especially in a system which contains the degraded products of antibiotics with sulphur derivatives which can be oxidized by very mild oxidizing agents [10]. The half reaction of iodate in dilute acid solution with E° [11] can be proposed as under.

$$2IO_3^- + 12H^+ + 10e^- \rightarrow I^2 + 6H_2O$$

$$E° = 1.19 \text{ V}$$

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References

[1] A.G. Davidson, J.B. Stenlake, Analyst 99 (1974) 476.
[2] J. Talegaonkar, K.S. Boparai, Talanta 29 (1982) 525.
[3] U. Forsman, Anal. Chim. Acta. 93 (1977) 153.
[4] B. Karlberg, U. Forsman, Anal. Chim. Acta. 83 (1976) 309.
[5] J.K. Grime, B. Tan, Anal. Chim. Acta. 105 (1979) 361.
[6] J.L. Longridge, D. Timms, J. Chem. Soc. B (1971) 852.
[7] M.A. Carroill, J.E. Zarembo, J. Antibiot. 30 (1977) 811.
[8] D.R. John, M.C. Caserio, Basic Principles of Organic Chemistry, 2nd edn, 1977, p. 745.
[9] El-Sebai A. Ibrahim, S.M. Rida, Y.A. Baltagy, M.M. Abd El-Khalek, J. Drug. Res. (Egypt) 6 (1973) 13.
[10] I.M. Kolthoff, R. Belcher, Volumetric Analysis, vol. III, 1957, p. 450.
[11] I.M. Kolthoff, E.B. Sandell, E.J. Merhan, S. Bruckenstein, Quantitative Chemical Analysis, 4th edn, 1969, pp. 819–1159.
[12] L.W. Andrews, J. Amer. Chem. Soc. 25 (1903) 756.