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SPECTROFLUOROMETRIC AND SPECTROPHOTOMETRIC DETERMINATION OF SOME TERTIARY AMINE DRUGS

I.H.Refaat, M.E.El-Kommos, H.H.Farag, and N.A.El-Rabat

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

ABSTRACT

Seventeen drugs belonging to different Pharmacological groupings have been determined either in pure form or in pharmaceutical formulations by spectrophotometry and spectrofluorometry. The method involves the condensation of malonic acid with acetic anhydride under the catalysis of a tertiary amine group in an aliphatic or a heterocyclic system. The product of condensation reaction is highly fluorescent and allows the spectrofluorometric determination of drug containing such groups in the ng/ml ranges (λ_{ex} = 415 nm, λ_{em} = 455 nm). The product of condensation is also coloured and allows the spectrophotometric determination of these tertiary amine drugs (λ_{max}=333 nm). Results of analyses of pure drugs and their dosage forms by the proposed methods are in good agreement with those of the official B.P. and U.S.P. procedures.

INTRODUCTION

The reaction between a mixed anhydride and tertiary amine has been reported by Feigel[1], Roeder[2] and Groth and Wallerburg[3] to yield a highly coloured product suitable for a spot test for tertiary amines. The mixed anhyride was formed by reacting organic acids, such as malonic or citric acid with anhydride. These coloured products are also known to be fluorescent[2]. Sass et al[4] and Pesez and Bartos[5] reported the use of aconitic acid/acetic anhydride system for the fluorometric determination of tertiary

amines. This system is inferior to the malonic acid/acetic anhydride system in terms of the stability of the fluorescent product and the limit of detection for tertiary amines[6].

Thomas[6] developed the malonic acid/acetic anhydride system for the spectrofluorometric determination of some tertiary amine alkaloids and also for their spectrophotometric determination[7]. Taha et al[8] have made some modification of Thomas method and applied it to the UV-spectrophotometry of some tertiary amine drugs

This work was undertaken to combine the advantages of Thomas method[6,7], Taha et al modification[8] and certaim other modification for the development of sensitive and convenient spectrophotometric and spectrofluorometric methods for the determination of tertiary amine drugs. A novel approach is the application of this method to drugs containing heterocyclic nitrogen e.g. quinoline, pyrimidine and phenanthroline derivatives. Results obtained with the proposed methods for the analysis of pharmaceutical formulations are encouraging in this paper

EXPERIMENTAL

Apparatus:

a- Kontron Spectrofluorometer SFM 23/B, Fonokontron, milano, Italy.

b- Uvidec-320 Spectrophotometer, JASCO, Tokyo, Japan.

Materials :

a- Pharmaceutical compounds : Pharmaceutical grade mepacrine HCl, metronidazole, benzoylmetronidazole, iodoquinol (May and Baker Ltd, England) chloroquine phosphate, levamisole HCl, primaquine phosphate (Imperial Chemical Industries Ltd, England), diethylcarbamazine citrate (Pharmaco-chemical works, Hungary), lucanthone HCl CID Egypt) pyrantel pamoate, tinidazole (Pfizer Inc. U.S.A.), emetine HCl (Misr, Egypt) phanquone, clioquinol, niridazole(Ciba-Geigy, Egypt), pyrimethamine (Wellcome

Foundation Ltd, England) and mebendazole (Janssen Pharmaceutica , Beerse, Belgia).

b- Formulations : the following commercial formulations were subjected to the analytical procedure :

1- Cidoquine tablets (CID, Cairo, Egypt): contain 250 mg chloroquine phosphate per tablet.

2- New Ketrax tablets (Kahira, Cairo-Egypt): contain 40 mg levamisole HCl per tablet.

3- Flagicure tablets (Kahira, Cairo-Egypt): contain 250 mg metronidazole per tablet.

4- Combantrin tablets (Pfizer, U.S.A.): contain 125 mg pyrantel pamoate per tablet.

5- Fasigyn tablets (Pfizer, U.S.A.): contain 500 mg tinidazole per tablet.

6- Daraprim tablets (Wellcome, England): contain 25 mg pyrimethamine per tablet.

7- Vermox tablets (Janssen Pharmaceutica, Belgia):contain 100 mg mebendazole per tablet.

8- Ambilhar tablets (Ciba, Cairo): contain 500 mg niridazole per tablet.

9- Paramibe Tablets (CID, Cairo-Egypt): Contain 250 mg diiodohydroxyquinoline per Tablet.

10- Flagyl suspension (Alexandria Co., Egypt): contains 200 mg benzoyl metronidazole per 5 ml.

11- Antiver suspension (Alexandria, Co., Egypt): contains 100 mg mebendazole/5 ml.

12- Emetine HCl injection (Misr, Cairo-Egypt): contains 60 mg emetine HCl per ml.

Reagents and Chemicals :

Malonic acid/acetic anhydride reagent (MAA reagent): A 10% w/v solution of malonic acid in acetic anhydride was prepared by gentle heating at 60°C with continuous shaking for 5 min. The reagent is stable for 12 hours at 4°C.

Analytical Procedure :

Pipet 1 ml of methanolic solution of the drug in the form of base or its salt into 10-ml volumetric flask, and evaporate till dryness on a water bath. Add to the residue 1 ml of MAA reagent and heat in a boiling water bath for 10 min, cool, and complete to volume with methanol. Mix well and stand for at least 5 min. For spectrophotometric measurements: dilute the solution so obtained 50 times (in cases of chloroquine phosphate, levamisole HCl, metronidazole and bebzoyl metronidazole), 20 times (in case of pyrantel pamoate, emetine HCl, tinidazole and pyrimethamine) or 10 times (in cases of iodoquinol, mebendazole and niridazole) with water. Allow to stand for 20 min and measure the absorbance of the solution at 333 nm against a blank treated concurrently.

For spectrofluorometric measurements:dilute the solution prepared for spectrophotometry 20 times with water. Allow to stand for 5 min and measure the fluorescence intensity at λ_{em} =455 nm, using λ_{ex} =415 nm.

Prepare calibration curves by taking a series of aliquots to produce the required concentration range.

Preparation of Samples :

b- Powdered forms. Dissolve 10 mg of the powder in 50 ml of water (in case of primaquine phosphate)or methanol (in cases of all other compounds). Use gentle heating for complete dissolution (in cases of mepacrine HCl, pyrantel pamoate, phanquone, iodoquinol, clioquinol mebendazole and niridazole). Few drops of HCl may be

required for complete dissolution of mebebdazole and pyrantel pamoate.

b- Tablets. Accurately weigh an amount of the finely powdered tablets equivalent to about 10 mg of the active substance and extract twice with about 15 ml of methanol by heating in a water bath for 5 min. Cool the mixture and filter into a 50-ml volumetric flask. Wash the residue and complete to volume with methanol. Add few drops of HCl in cases of Combantrin and Vermox tablets for clarification of solution.

c- Suspensions. Shake well and introduce a volume of the suspension equivalent to 10 mg of the active drug into a 50-ml volumetric flask. Add about 40 ml of methanol and heat gently (Add few drops of HCl in case of Antiver suspension). Cool the mixture and complete to volume with methanol.

d- Injections. Dilute emetine HCl injection with methanol to contain 0.2 mg per ml

RESULTS AND DISCUSSION

Reaction involved :

The solution resulting from reacting the MAA reagent with a tertiary amine group present in an aliphatic or heterocyclic drug is deep golden orange in colour and is emitting a distinct green fluoroscence. The absorption, excitation and emission spectra for the condensation products of all the seventeen drugs were almost identical suggesting a common condensation product (λ_{max} = 333 nm, λ_{ex} = 415 nm, λ_{em}=455 nm) Fig. 1 shows absorption spectra for some of the investigated compounds and Fig.2 shows the fluorescence spectra for metronidazole.

The mechanism of the overall reaction has not as yet been fully elucidated but it seems apparent that the final product results from the base-catalysed condensation of malonic acid and acetic anhydride[2,3,6] .

Apparent molar absorptivities and fluorescence intensities of the investigated condensation product in presence of various tertiary amine catalysts are presented in table I . Upon correlation of molar absorptivities for some drugs with the reported pk_a values for their protonated species, taking mepacrine HCl[9] , chloroquine phosphate[10] metronidazole[11] , levamisole HCl[12], pyrantel pamoate[12] and emetine HCl[12] as examples it was found that the points (pk_a, log $\mathcal{E}_{max}$) fit the following cubic equation :

$$\log \mathcal{E} = 9.626 - 2.528\ pk_a + 0.403\ pk_a^2 - 0.019\ pk_a^3$$

$$r = 0.9773,\quad n = 6\ ,\ s = 0.04,\ F = 85.23,\quad P < 0.005$$

where r= correlation coefficient, n=number of compounds, s=standard deviation and F= variance ratio of the regression at probability P.

The relation as iluustrated in Fig. 5 indicates that the best tertiary amine to be used as a catalyst in this condensation reaction must have its pk_a in the range 8.5-10.5. This finding confirms the suggestion of Thomas[7] that the rate of condensation reaction is dependent upon the basicity of the tertiary amine catalyst

Optimization of variables :

Investigations were carried out to improve the formation of the condensation product of malonic acid and acetic anhydride under the catalytic effect of tertiary amines. To determine the optimal conditions, different variables were investigated.

Spectrofluorometric and Spectrophotometric Determination of Some Tertiary Amine Drugs

Optimum MAA reagent concentration for maximum fluorescence and absorption intensity was found to be 1 ml of 10% malonic acid solution in acetic anhydride per 10 ml of the original reaction mixture (Fig. 3). Higher concentrations did not affect the absorption or fluorescence intensity. Because of limited solubility of malonic acid in acetic anhydride (1.5 g/ml), higher concentrations were investigated by utilizing volumes of 15 % MAA reagent larger than 1 ml.

The effect of heating time and temperature was studied on levamisole HCl and chloroquine phosphate as representative examples (Fig. 4). The optimum heating time was 15 min at 80°C and 10 min on a boiling water bath. By heating the original reaction mixture for more than 25 min at 80°C or more than 20 min on a boiling water bath caused a decrease in fluorescence intensity probably due to decomposition of the condensation product.

Study of the suitable type of solvent to be used revealed that methanol is necessary for diluting the original reaction mixtures while water can be used for further dilutions prior to spectrophotometric or spectrofluorometric measurements.

The reagent was found to be stable for 12 hours when stored at 4°C.

Quantification :

Under the proposed experimental conditions, a linear response between absorbance or fluorescence intensity and concentration was demonstrated over the concentration ranges shown in table II. Quantitative parameters for some of the investigated drugs are presented in table II.

Application to bulk drugs and dosage forms :

Results of analyses of the widely used tertiary amine drugs in bulk and in the different dosage forms by the proposed method are presented in table III. The recorded data showed a good correlation with those of the B.P. and U.S.P. procedures[13,14]. Student's t-and F-tests show no significant difference between the proposed and pharmacopoeial methods.

Spectrofluorometric and Spectrophotometric Determinatio
of Some Tertiary Amine Drugs

Table I. Apparent molar absorptivities and fluore-
scence intensities for different tertiary amine drugs.

No.	Compound	$\epsilon_{333 \times 10^{-5}}$	Fluorescence intensity*
1	Mepacrine HCl	5.37	74.2
2	Chloroquine phosphate	4.68	66.2
3	Diethylcarbamazine citrate	3.96	71.7
4	Lucanthone HCl	3.34	62.8
5	Levamisole HCl	3.25	97.0
6	Metronidazole	2.77	116.0
7	Benzoyl metronidazole	2.48	63.0
8	Pyrantel pamoate	2.38	28.5
9	Emetine HCl	1.99	25.0
10	Phanquone	1.90	65.0
11	Primaquine phosphate	1.38	21.6
12	Tinidazole	0.98	28.0
13	Pyrimethamine	0.69	19.8
14	Iodoquinol	0.48	8.5
15	Clioquinol	0.44	10.4
16	Mebendazole	0.35	8.4
17	Niridazole	0.26	8.6

* Final concentration is 0.4 mcg/ml.

Table II. Quantitative parameters for some tertiary amine drugs

Compound	Spectrophotometry				Spectrofluorometry			
	linear range mcg/ml	intercept	slope	correlation coefficient	linear range ng/ml	intercept	slope	correlation coefficient
Chloroquine phosphate	0.1-0.8	0.0064	0.8842	0.9998	2-30	0.0387	3.2896	0.9998
Levamisole HCl	0.1-0.8	0.0033	1.3573	0.9999	2-30	0.0796	4.9239	0.9999
Metronidazole	0.1-0.8	0.0054	1.5482	0.9999	2-30	-0.2603	5.8323	0.9997
Benzoyl metronidazole	0.1-0.8	0.0027	0.9493	0.9998				
Pyrantel pamoate	0.2-1.6	0.0004	0.3811	0.9997				
Tinidazole	0.2-1.6	0.0072	0.3930	0.9999				
Pyrimethamine	0.2-1.6	0.0007	0.2689	0.9996				
Emetine HCl					5-80	0.6261	1.2346	0.9999
Iodoquinol					10-160	-0.0208	0.3968	0.9989
Mebendazole					10-160	0.5083	0.4115	0.9992
Niridazole					10-160	0.6833	0.3970	0.9997

Table III: Application of the method to bulk drugs and pharmaceutical formulations.

Sample	Recovery % ± SD [1]						
	Proposed method		Official method	Spectrophotometry		Spectrofluorometry	
	Spectrophotometry	Spectrofluorometry		t^2	F^3	t^2	F^3
Chloroquine phosphate	100.66±1.01	100.56±1.57	99.72±0.75*	1.670	1.81	1.080	4.38
Levamisole HCl	100.02±0.74	100.33±0.53	99.93±1.20*	0.143	2.63	0.681	5.12
Metronidazole	99.95±0.37	99.88±0.85	100.44±0.49@	1.782	1.75	1.276	3.01
Benzoyl metronidazole	97.97±0.96						
Pyrantel pamoate	100.26±0.91						
Tinidazole	99.75±0.88						
Pyrimethamine	98.18±1.56		98.65±0.73@	0.610	4.57		
Emetine HCl		100.99±0.93					
Iodoquinol		101.66±1.43	101.79±0.88**			0.173	2.64
Mebendazole		102.89±1.37	101.89±0.85@			2.774	2.60
Niridazole		101.59±1.43					
Cidoquine tablets	100.37±1.14	100.57±0.87	100.15±0.74*	0.362	2.37	0.822	1.38
New Ketrax tablets	99.28±0.32	98.33±0.59					
Flagicure tablets	100.44±0.37	100.89±0.49	100.72±0.49@	1.018	1.75	0.548	1.00
Combantrin tablets	100.27±0.76						
Fasigyn tablets	100.00±0.76						
Daraprim tablets	98.18±1.09		98.65±0.73*	0.801	2.23		
Vermox tablets		101.65±0.69					
Ambilhar tablets		101.59±1.43					
Paramib tablets		100.07±0.93	99.58±0.50@			1.038	3.46
Flagyl suspension	97.26±0.63						
Antiver suspension		100.45±1.39					
Emetine HCl injection		100.44±1.61					

1 Mean of 5 determinations
2 Tabulated t for 4 degrees of freedom at P0.05=2.776
3 Tabulated F for (4,4)degrees of freedom at P0.05=6.39
* B.P. 1980 ; ** B.P. 1958; @ U.S.P. XXI, 1985

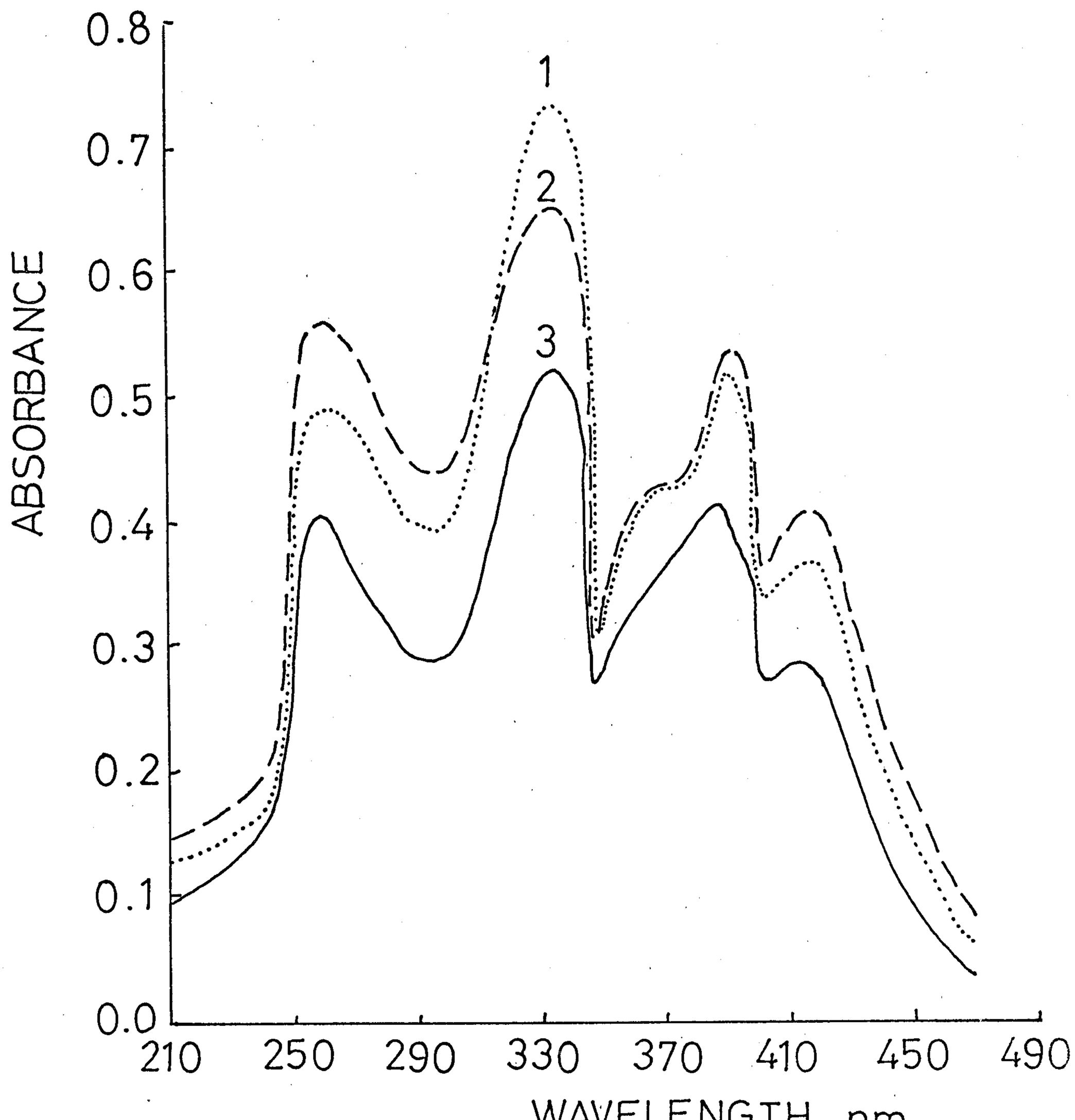

Fig. 1: Absorption spectra of the condensation product using the following basic catalysts: (1) metronidazole-0.47 mcg/ml. (2) phanquone-0.72 mcg/ml and (3) benzoyl metronidazole-0.55 mcg/ml.

Spectrofluorometric and Spectrophotometric Determination of Some Tertiary Amine Drugs

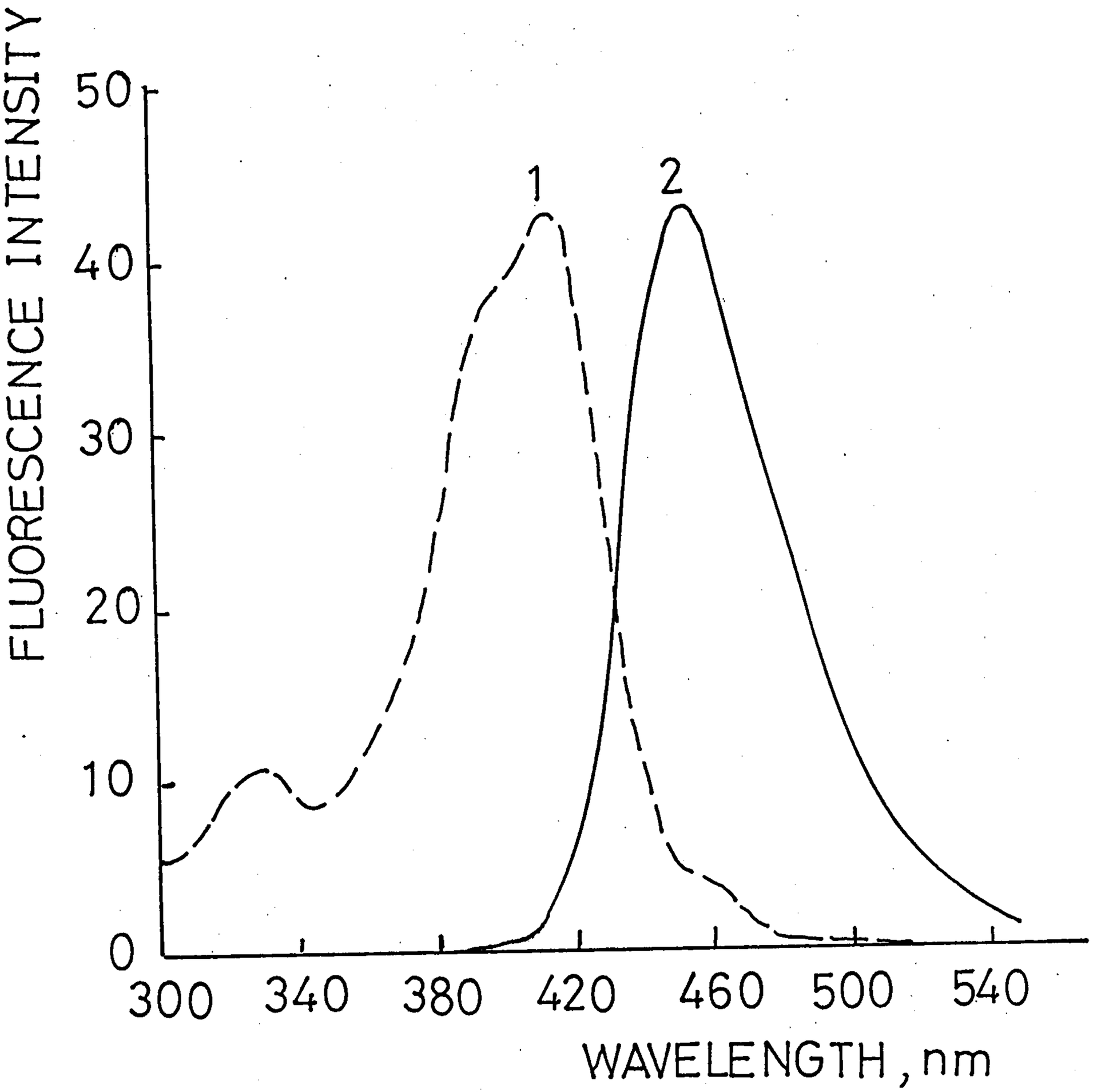

Fig. 2: Excitation (1) and emission (2) spectra of the condensation product using metronidazole as a basic catalyst.

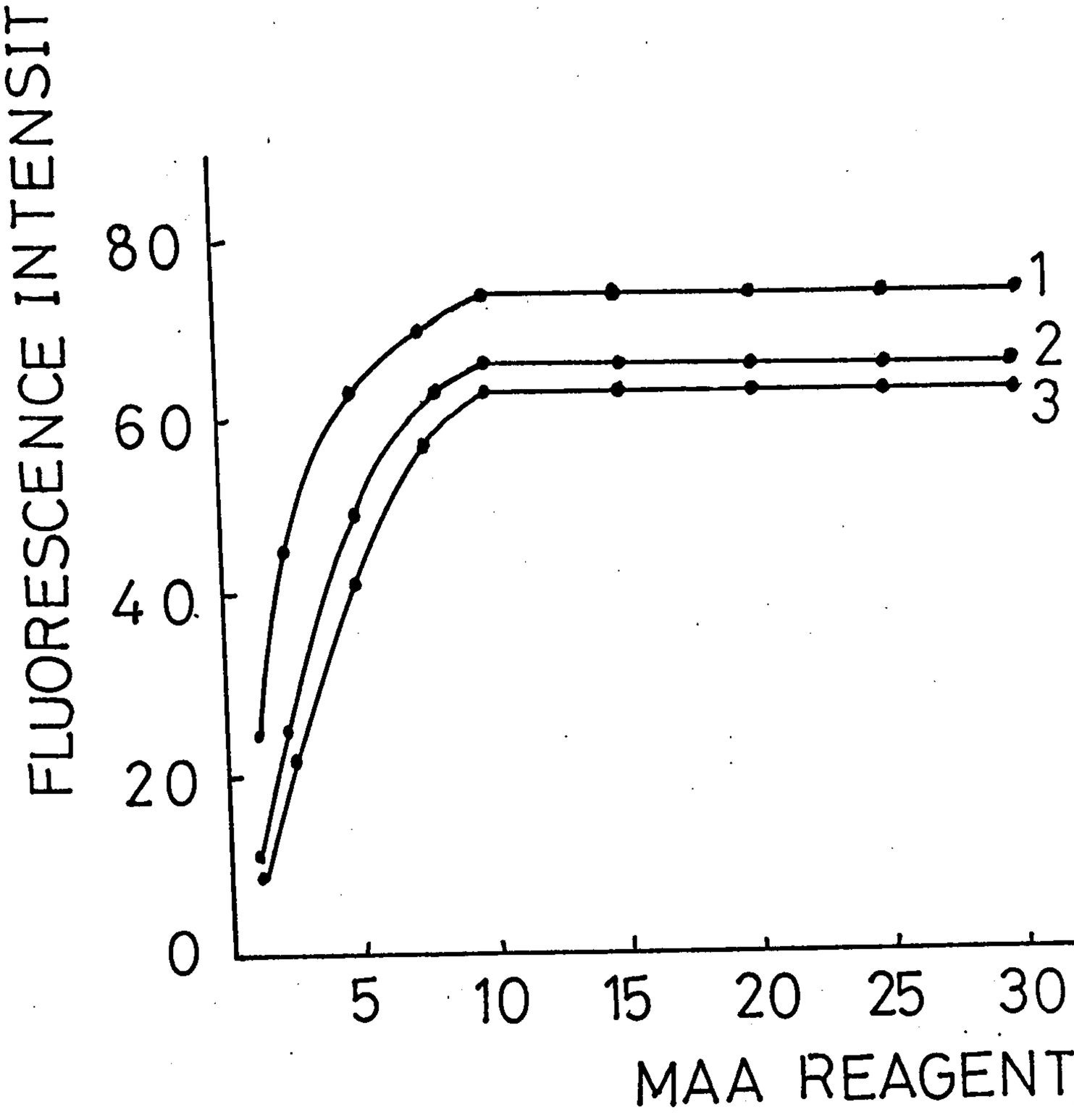

Fig. 3: Relative fluorescence intensity of the condensation product as a function of reagent concentration, using the following basic catalysts: (1) mepacrine HCl, (2) metronidazole and (3) lucanthone HCl.

Spectrofluorometric and Spectrophotometric Determination of Some Tertiary Amine Drugs

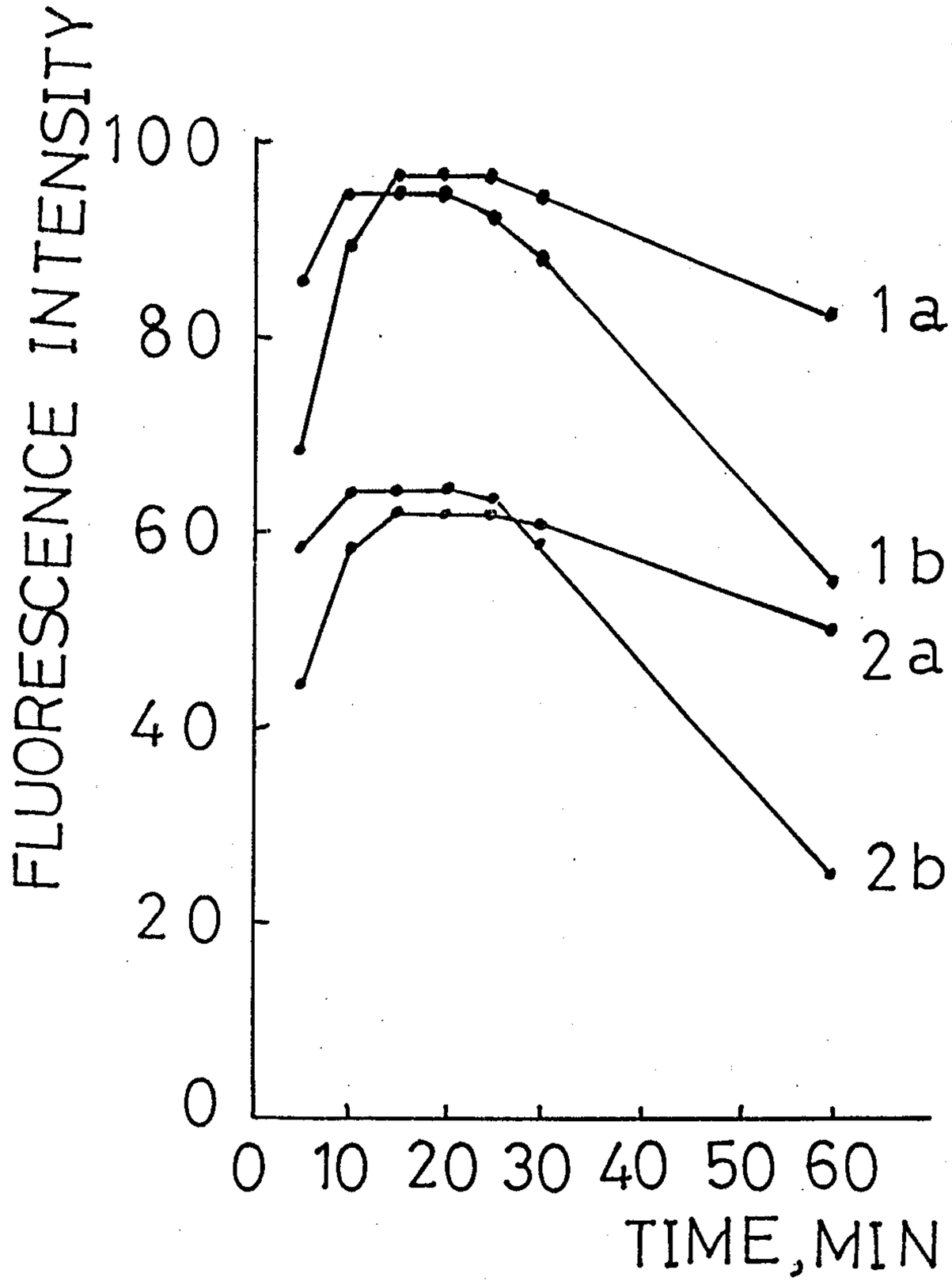

Fig. 4: Effect of heating time and temperature on the development of the condensation product using (1) levamisole HCl and (2) chloroquine phosphate at (a) 80°C and (b) 100°C.

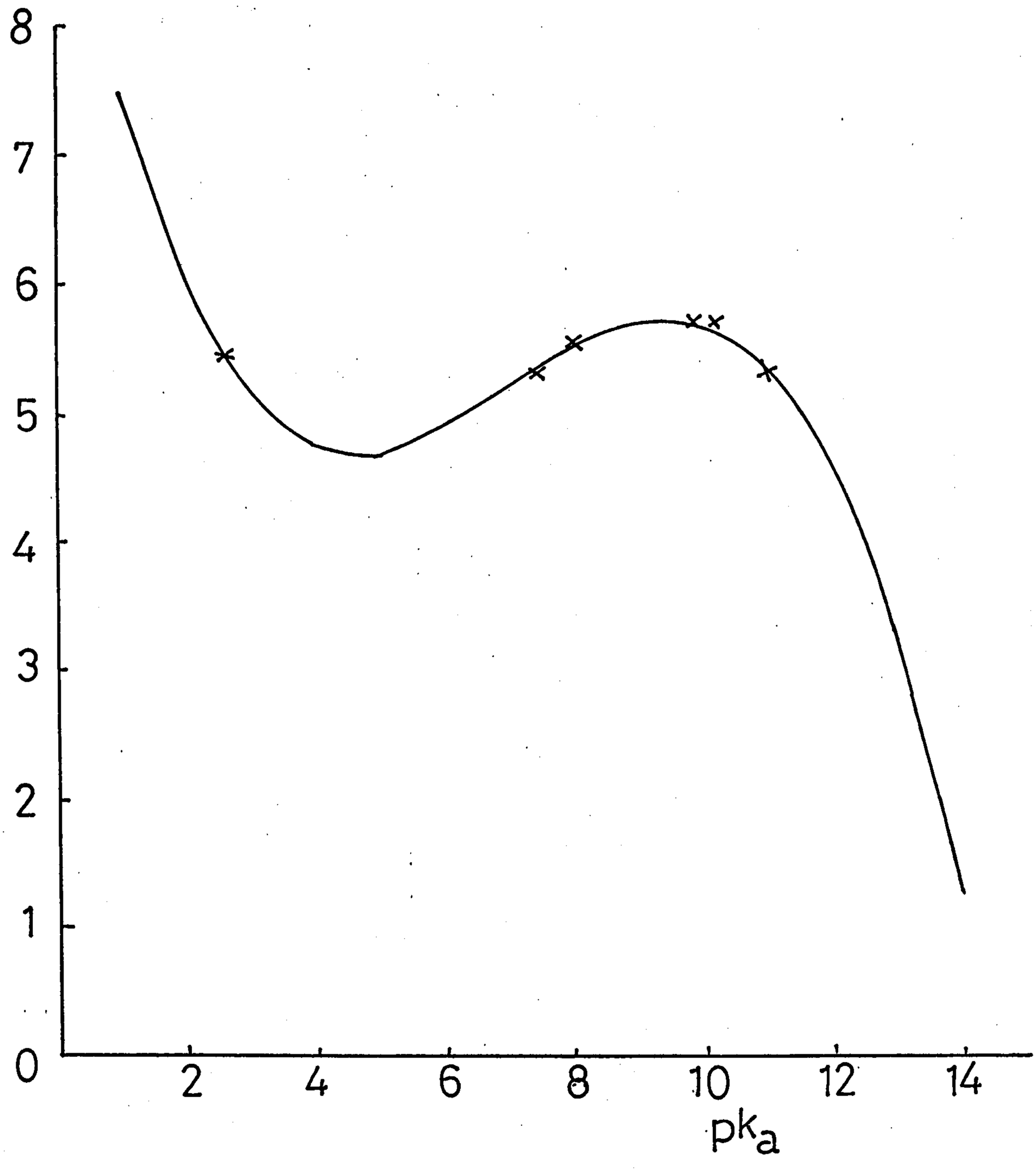

Fig. 5: Log ε_{333} as a function of pk_a

REFERENCES

1) F. Feigel; "Spot Tests in Organic Analysis", 7th Ed., Elsevier, Amsterdam, p. 251 (1966).

2) G.Roeder, J. Amer. Pharm Assoc., 30, 74 (1941).

3) Å.B. Groth, and G. Wallerburg, Acta Chem. Scand., 20, 2628 (1966).

4) S. Sass, J.J. Kaufman, A.A. Cardenas, and J.J.Martin, anal. Chem. 30, 529 (1958).

5) M. Pesez, and J. Bartos, Talanta, 16, 331 (1969).

6) A.D. Thomas, Talanta, 22, 865 (1975).

7) A.D. Thomas, J.Pharm. Pharmacal., 28, 838 (1976).

8) A.M. Taha, S.R. El-Shabouri,and A.I. Rageh, Egypt. J. Pharm. Sci., 21, 363 (1980).

9) D.D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solvents". Butterworth, London (1965).

10) P.D. Hong, "Analytical Profiles of Drug Substances", Vol. 5, Ed. K.Florey, Academic Press, N.Y.,p. 69 (1976).

11) D.E. Schuartz and F. Jeunet, Chemother., 22, 19 (1976).

12) A.C. Moffat, J.V.Jackson, M.S. Moss, and B.Widdop, Editors, "Clarkes Isolation and Identification of Drugs", 2nd Ed., The Pharmaceutical Press, London pp. 701, 947,581 (1986)

13) British Pharmacopoeia 1980, HM Stationery Office, London pp. 103, 254, 746, 817. (1980)

14) United States Pharmacopeia XXI and National Formulary XVI, U.S. Pharmacopeial Convention, Rockville, MD pp. 690,921,622 (1985). 551.

التقدير الطيفى والـلصفى لبعض الادويـــــــة التى تحتـوى على مجموعة امين ثلاثيـــــــــة

ابراهيم حسن رفعت ـ ميشيل ايليا القمص ـ حسن حسن فرج ـ نوال على الربـــــاط
قسم الكيميـــــاء الصيدلية ـ كليـــة الصيدلـــة ـ جامعة أسيوط ـ أســيوط

فى هذا البحث تم التوصل الى طريقة طيفية لصفية لتعيين ١٧ مركبا دوائيـــا ينتمون لمجموعات أقربازينية مختلفة ولكن يشتملون فى تركيبهم على مجموعة أميـــن ثلاثية وذلك بتحرير طريقة ثوماس . وتعتمد الطريقة على تكثيف حمض المالونيك مع أنهيدريد الخليك حيث تعتبر مجموعة الامين الثلاثية سواء فى مركب اليفاتى أو مركب حلقى غير متجانس هى العامل المساعد للتفاعـــل .

ويمكن قياس ناتج التفاعل طيفيا عند أطوال الموجات ٤١٥ ن م، ٤٥٥ ن م أوطيفيا عنـــد ٣٣٣ ن م .

وتتميز الطريقة ببساطتها حيث أن التفاعل من حمض المالونيك وأنهيدريـــــد الخليك فى وجود العامل المساعد لايستغرق أكثر من ١٠ دقائق عند درجة حرارة الحمـــام المائى المغلى وقد تمت دراسة كل العوامل التى تؤثر على التفاعل : تركيز الكاشـــف درجة ووقت التسخين وتأثير المذيبات المختلفـــة .

وقد تم تطبيق الطريقة بنجـــاح للتقييم الكمى للمركبات الاتية فى صورتهـا النقية وأيضـا فى بعض المستحضـــرات الصيدلية : هيدروكلوريد المباكرين ، فوســفات الكلوروكويـــن ، سترات ثنائى أثيـــل الكاربامازين ، هيدروكلوريد اللوكانثـــــــون هيدروكلوريد الليفاميسول ، مترونيدازول بنزويل مترونيدازول ، باموات البيرانتل هيدروكلوريد الاميتين ،فانكون ، فوسفات البريماكين تينيدازول ،بيريميثاميـــــن يودوكوينول ، كليوكوينول ، ميبيندازول ،والنيريدازول . وقد وجد أن نتائج التحليل متطابقـة تماما مع نتائج طرق دساتير الادويـة البريطانية والامريكيـة .

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