Bull. Pharm. Sci., Assiut University
Vol. 9, Part 2, pp 1 - 13

UTILITY OF XANTHYDROL FOR THE SPECTROPHOTOMETRIC DETERMINATION OF CERTAIN PHARMACEUTICAL PHENOLS

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ABSTRACT

The reaction of xanthydrol with phenols was applied for the determination of thymol, guaiacol, morin and naringenin, out of 30 phenolic compounds tested. Mixture of methanolic solutions of the phenol and xanthydrol was heated in presence of hydrochloric acid for 10 minutes on a water bath. The λ_{max} *of the coloured chromogen was located in the vicinity of 485 nm (orange) to 540 nm (violet). Molar absorptivities range from* $1.0x10^3$ *to* $4.3x10^3$*. A linear correlation was found between absorbance at* λ_{max} *and concentration in the range of 5-40 mcg* ml^{-1} *(for thymol), 20-100 mcg* ml^{-1} *(for guaiacol) and 20-150 mcg* ml^{-1} *(for morin and naringenin).*

INTRODUCTION

Xanthydrol was synthesized by Mayer and Saul[1] in 1893. In 1958, Iavorski[2-4] described the reaction of this reagent with phenols to give highly coloured products. The reaction was utilized for the colorimetric determination of phenol itself and some other simple phenols[5]. The present work was undertaken to study the response of various pharmaceutical phenols to the above

mentioned reaction and the utility of xanthydrol as a chromogenic reagent for the spectrophotometric determination of pharmaceutical phenols.

EXPERIMENTAL

Materials:

Pure samples of Pharmaceutical phenols were used as working standards.

Reagents:

1- Xanthydrol AR (Fluka AG, Switzerland) 0.8% methanolic solution.

2- Hydrochloric acid AR (Prolabo, France), 10 N.

3- Acetone, methanol, ethanol, n-propanol, isopropanol and n-butanol (Merck, West Germany) were of spectral grade.

Apparatus:

Unicam SP 1750 Spectrophotometer (Pye Unicam Ltd , Cambridge, England) with AR 55 linear recorder (Pye Unicam) and Unicam SP 1805 program controller.

Preparation of Working Standards:

Dissolve 75.0 mg of the appropriate working standard in 50 ml of methanol in a 100-ml volumetric flask and complete with methanol to the mark. Dilute the solution quantitatively and stepwise with the same solvent to obtain a concentration of 0.15 mg ml^{-1} (for thymol), 0.375 mg ml^{-1} (for guaiacol), or 0.5 mg ml^{-1} (for morin and naringenin).

Procedure:

Pipet 2.0 ml of the assay solution into a 10-ml volumetric flask, add 1.0 ml of xanthydrol solution and 0.6 ml of hydrochloric acid and mix thoroughly . Heat the mixture on a boiling water bath for 10 min.(for thymol and guaiacol) or for 15 min (for morin and naringenin). Cool to room temperature,

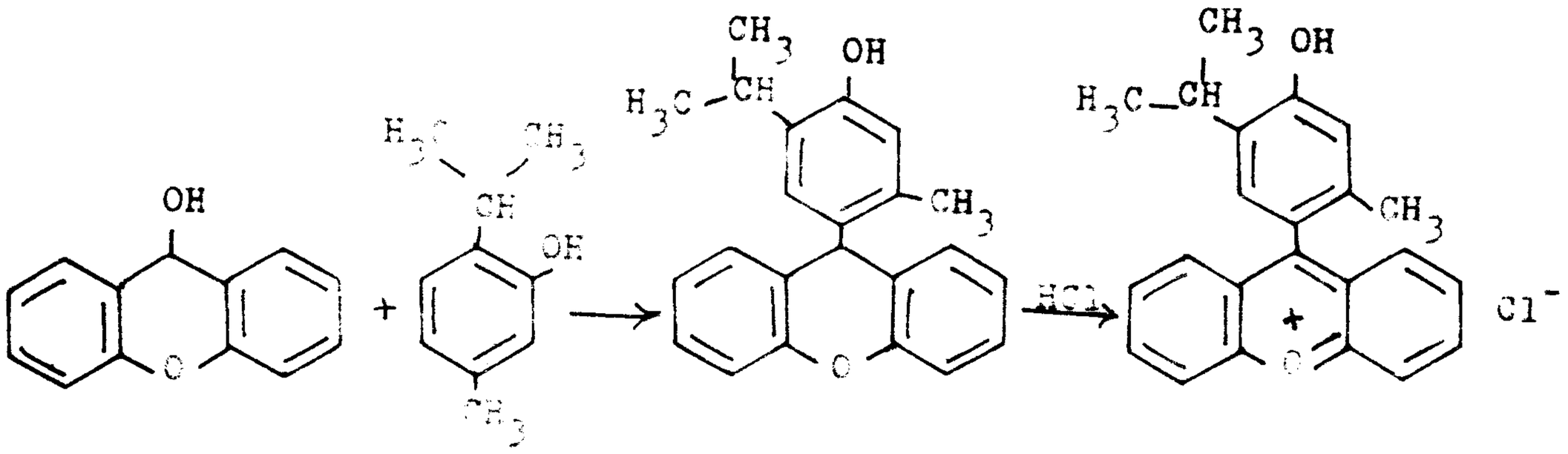
complete with acetone to the mark, and measure the absorbance of the solution at the specified λ_{max} (Table 1) against a blank similarly treated, substituting sample solution with 2.0 ml of methanol. The concentration of the assay solution is found from a properly constructed calibration graph. Covered glass cells have to be used for absorbance measurements to avoid the volatilization of acetone.

Construction of Calibration Graph:

An accurate weight of the working standard (20.0 mg of thymol, 50.0 mg of guaiacol, 75.0 mg of morin or naringenin) was dissolved in methanol and diluted to volume in a 100-ml volumetric flask. The solution was diluted stepwise to give a series of concentrations suitable for construction of the calibration graph in the linear range for each phenol (see Table 1). Two ml of each solution was utilised for colour formation with xanthydrol and hydrochloric acid as described under the Assay Procedure.

RESULTS AND DISCUSSION

The mechanism of the reaction of xanthydrol with phenols is not well-established[6]. However, the following scheme can be suggested for the reaction of thymol with xanthydrol:



xanthydrol thymol colourless coloured product

This suggestion is supported by the reported separation of colourless 9-(p-hydroxyphenyl)xanthan from the reaction mixture of phenol and xanthydrol[6] and by the experimental fact that the resulting colour disappears upon the addition of alkali.

In the present work, 30 pharmaceutical phenols were tested for colour formation with xanthydrol, including monohydric phenols (acetaminophen, phenylephrine HCl, thymol, guaiacol, eugenol, pyridoxine HCl, pyridoxal HCl, chiniofon sodium, oxyphenbutazone, sodium p-aminosalicylate, stilbosterol, salicylic acid, sodium salicylate, methyl salicylate, salicylamide, vanillin and niclosamide), dihydric phenols (epinephrine, norepinephrine, isoprenaline SO_4, methyldopa, orciprenaline sulphate and dobutamine HCl) and polyphenols (quercetin, rutin, morin, naringenin, hesperidin, hesperetin and isosalipurposide).

Among these compounds, only thymol, guaiacol, morin and naringenin gave coloured products with xanthydrol under the reaction conditions used. Absorption spectra of the resulting coloured products are shown in fig. 1 and their spectral characteristics are summarized in Table 1 .

The four factors affecting colour intensity are: (a) reagent concentration, (b) amount of acid added, (c) heating time and (d) solvent used for final dilution. The data presented here illustrate the effect of the above mentioned factors on the determination of thymol as a representative example of monohydric phenols and of morin as a representative example of polyphenols.

The optimum concentration of xanthydrol for maximum colour intensity was 1 ml of 0.8% w/v solution per 10 ml of the reaction mixture (Fig. 2). Optimum amount of 10 N hydrochloric acid in the original reaction mixture was found to be 0.6 $\pm$ 0.1 ml.

Larger amounts of the acid up to 1.0 ml did not affect colour intensity but caused darkening of the blank solution. The optimum heating time in a boiling water bath was found to be 10 min for monohydric phenols and 15 min for polyphenols (Fig. 3). Longer heating times decreased colour intensity in both cases. this is probably due to partial decomposition of the coloured product.

The solvent used for final dilution affected to a great extent the colour intensity and to less extent, the wavelength of maximum absorption. The solvents investigated were methanol, ethanol, n-propanol, isopropanol, n-butanol and acetone. Fig 4 shows that acetone gives the highest absorption intensity of coloured chromogen. These solvent effects can be explained on the basis of tautometric equilibrium of the coloured product[7].

The colours obtained were sufficiently stable for spectrophotometric measurements.

A linear correlation (Fig.5) was found by the proposed method between the absorbance at λ_{max} for each compound and concentration in the range given in Table 1. The molar absorptivities of the resulting coloured products ranged from 1.0×10^3 to 4.3×10^3. The method shows a good precision; the relative standard deviation for thymol, guaiacol, morin and naringenin were in the range of 0.4-1.2% (Table 2).

Table 1: Spectral Characteristics of the Reaction Products of Xanthydrol with some Pharmaceutical Phenols.

Compound	λ_{max}, nm	$\varepsilon_{max} \times 10^{-3}$	Linear concentration range, $\mu g\ ml^{-1}$
Thymol	540	4.32	5- 40
Guaiacol	535	1.00	20-100
Morin	485	1.92	20-150
Naringenin	505	1.46	20-150

Table 2: Spectrophotometric Determination of Phenols using Xanthydrol

Compound	Weight (mg)		Recovery (% + S.D.)
	Taken	Found*	
Thymol	10.00	9.96	99.6 ± 0.43
	20.00	20.04	100.2 ± 0.38
	40.00	39.96	99.9 ± 0.45
Guaiacol	10.25	10.17	99.2 ± 0.70
	20.68	20.62	99.7 ± 0.62
	40.14	40.46	100.8 ± 0.68
Morin	10.00	10.04	100.4 ± 0.98
	20.00	19.84	99.2 ± 1.04
	40.00	40.12	100.3 ± 0.92
Naringenin	10.00	9.98	99.8 ± 1.10
	20.00	20.02	100.1 ± 1.16
	40.00	40.16	100.4 ± 1.09

* Mean of five determinations.

Utility of Xanthydrol for the Spectrophotometric Determination of Certain Pharmaceutical Phenols.

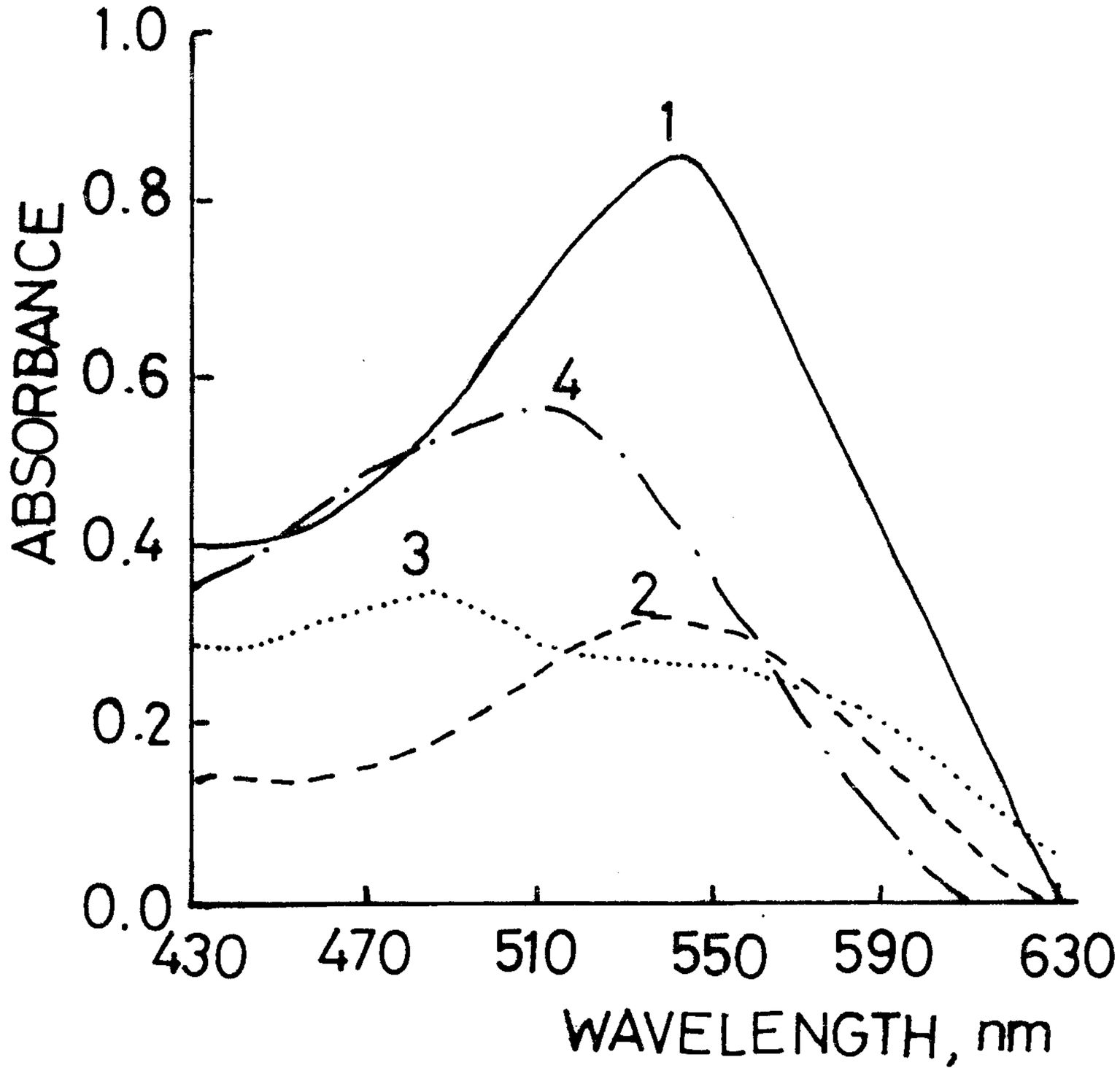


Fig. 1: Absorption spectra of the reaction products of xanthydrol with:
1- Thymol (30 $\mu g/ml^{-1}$)
2- Guaiacol (40 $\mu g/ml^{-1}$)
3- Morin (55 $\mu g/ml^{-1}$)
4- Naringenin (105 $\mu g/ml^{-1}$)

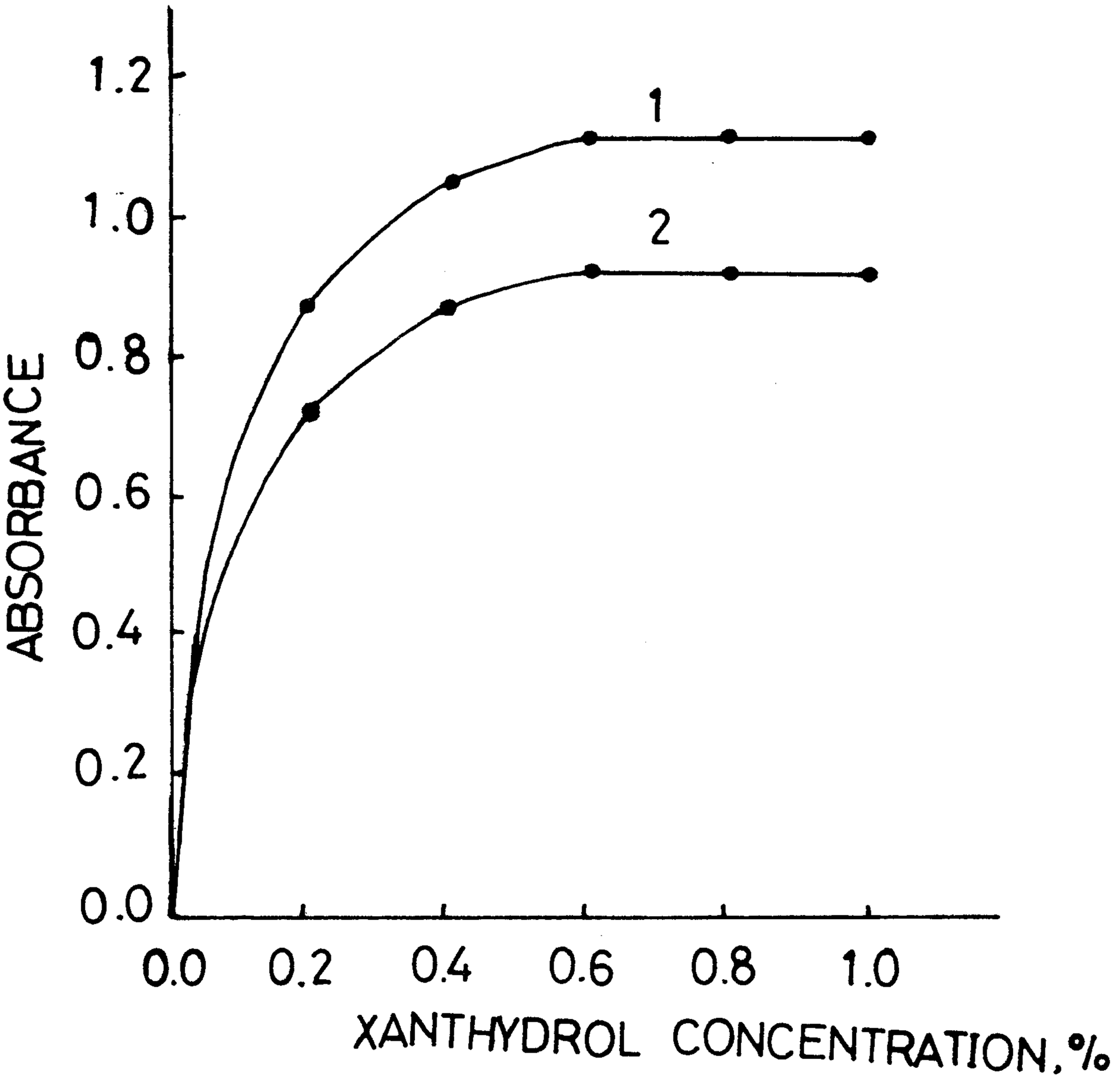


Fig. 2: Effect of concentration of xanthydrol solution with: (1) Thymol, 40 $\mu g/ml^{-1}$ and (2) Morin, 150 $\mu g/ml^{-1}$.

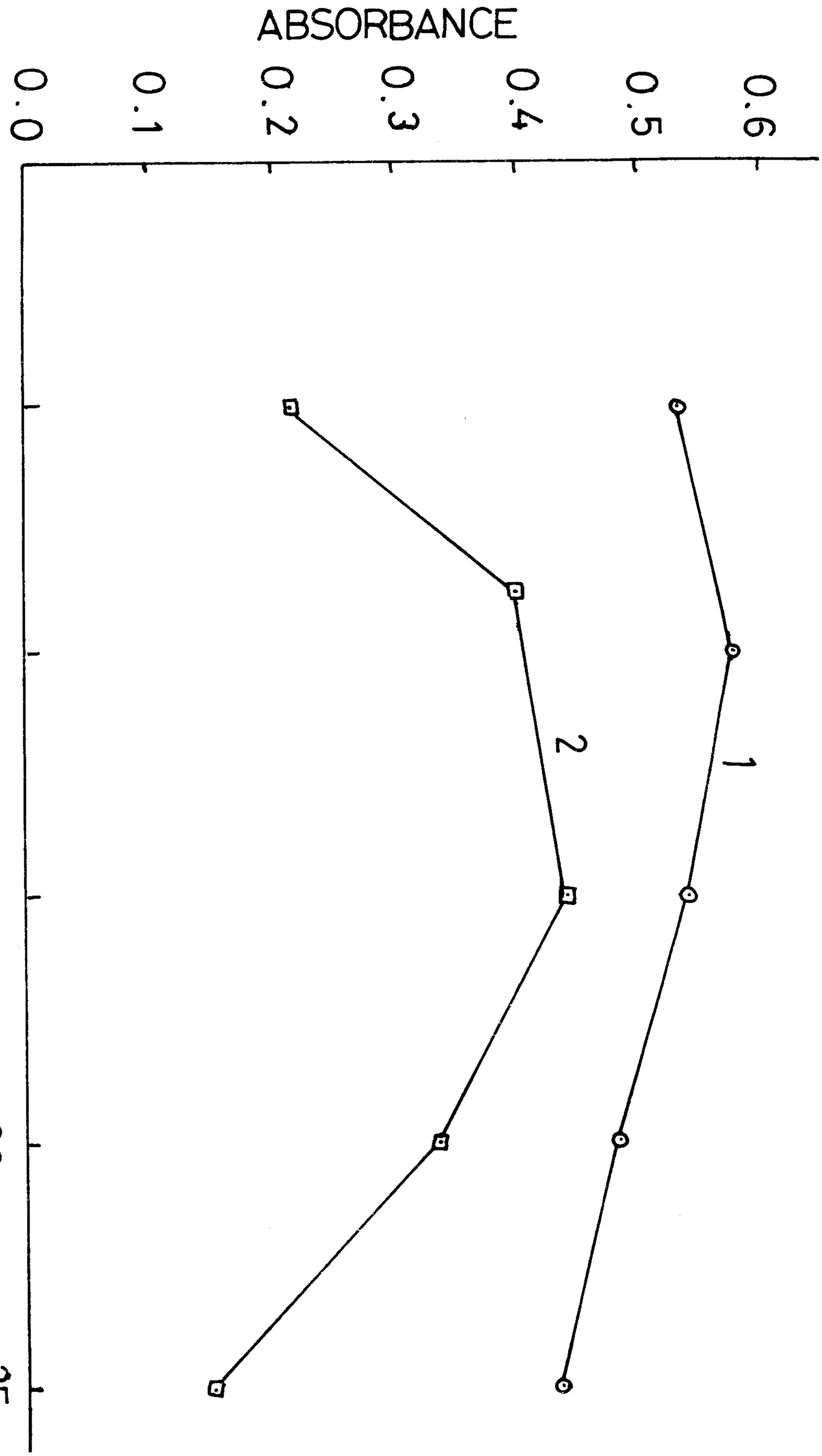


Fig. 3: Effect of heating time on absorption intensity of the reaction products of xanthydrol with:

1. thymol (20 $\mu g/ml^{-1}$) at 540 nm.
2. morin (70 $\mu g/ml^{-1}$) at 485 nm.

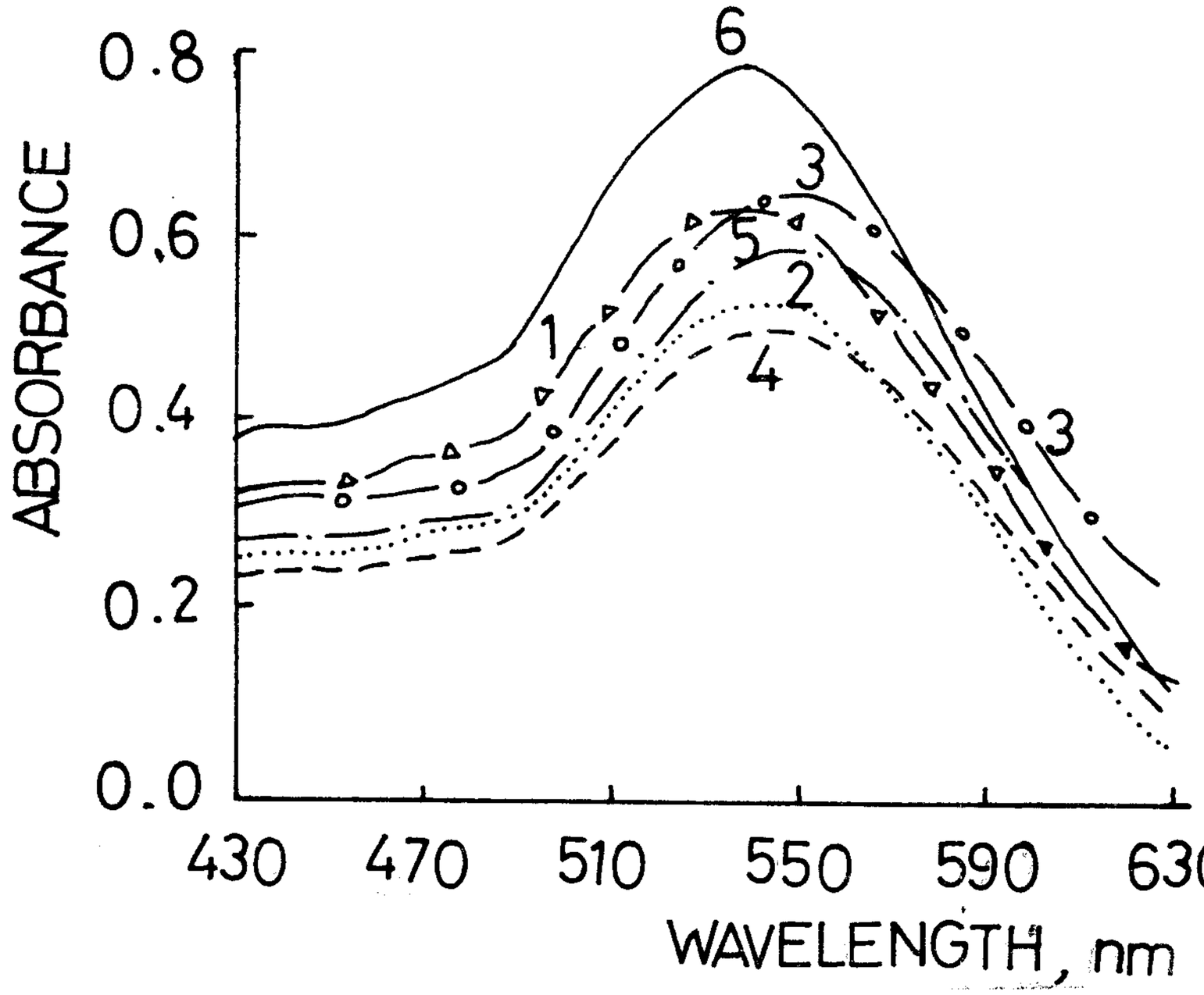


Fig. 4: Absorption spectra of the coloured product of thymol and xanthydrol in (1) methanol, (2) ethanol, (3) n-propanol, (4) isopropanol , (5) n-butanol and (6) acetone.
Concentration of thymol in the final solution is 27.5 µg/ml.

Utility of Xanthydrol for the Spectrophotometric Determination of Certain Pharmaceutical Phenols.

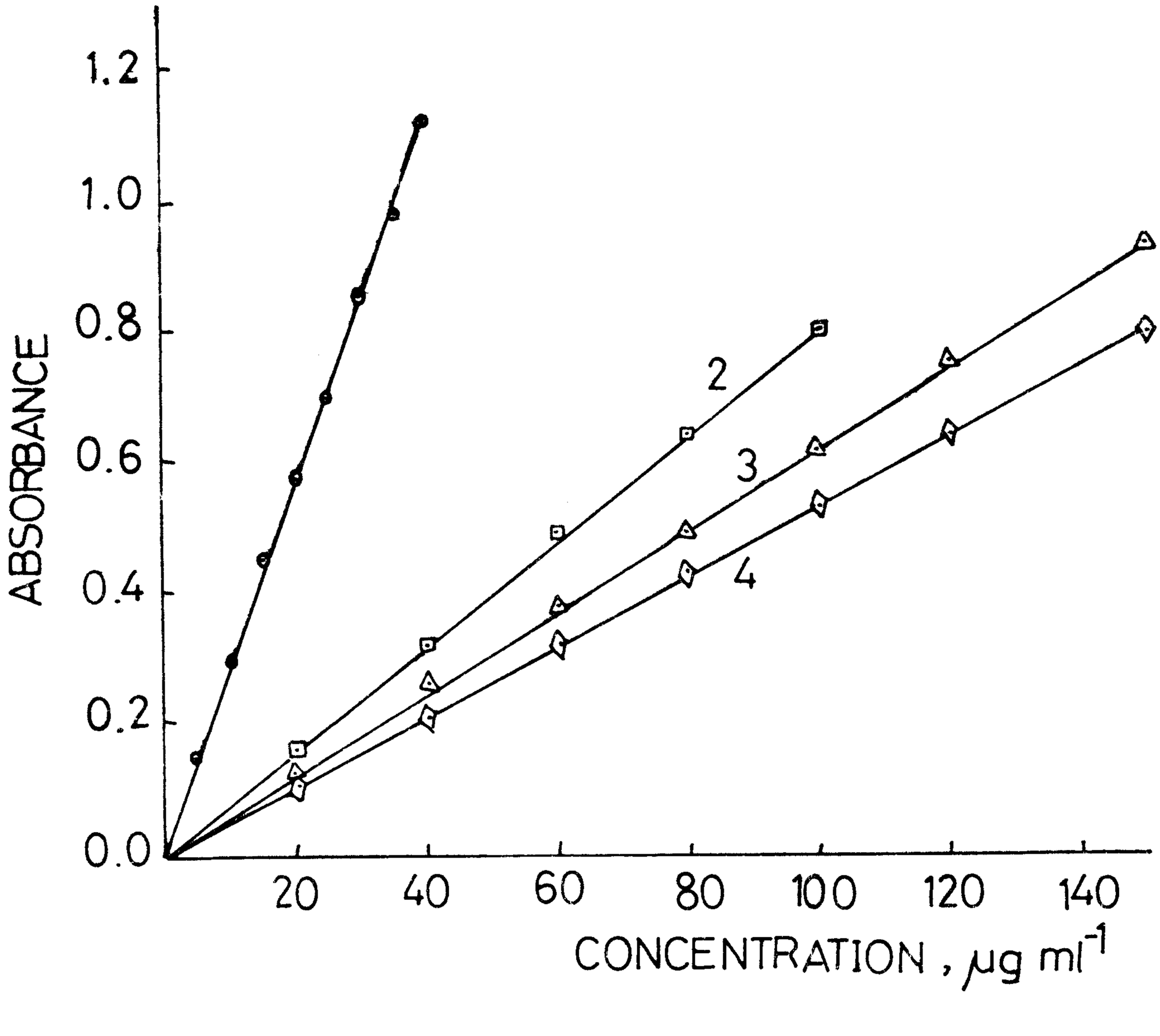


Fig. 5: Calibration graphs for the determination of:
1) thymol, (2) guaiacol, (3) morin and (4) naringenin.

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أستعمال كزانثهيدرول فى التعيين الطيفى لبعض الفينولات ذات الاهمية الصيدلية

ميشيل ايليا القمص ـ قسم الكيمياء الصيدلية ـ كلية الصيدلة
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تم التوصل الى طريقة طيفية للتعيين الكمى لاربعة مركبات فينولية (ثيمول ـ جواياكول ـ مورين ـ نارينجنين) تنتمى الى مجموعتى الفينولات أحادية الهيدروكسيل وعديدة الهيدروكسيل وذلك بتفاعلها مع كزانثهيدرول فى وسط حمضى قوى يحتوى على حمض الهيدروكلوريك لتكون مركبات برتقالية او وردية أو بنفسجية اللون .

وتتراوح اطوال الموجات التى يلاحظ عندها اعلى درجه امتصاص للضوء من ٤٨٥ الى ٥٤٠ نم . وتتراوح قيمة الامتصاص الجزيئى للمركبات الناتجة من هذا التفاعل من ١ × ١٠ ^{٣} الى ٤٫٣ × ١٠ ^{٣} .

وقد وجد ان مثل هذه المركبات اللونية لاتنشأ عن تفاعل عدد كبير من الفينولات الاخرى (٢٦ مركبا) مع الجوهر المذكور . وقد وجدت علاقة خطية بين الامتصاص والتركيز فى الحدود الاتية : من ٥ ـ ٤٠ مكجم/مل (للثيمول) ، ٢٠٠ ـ ١٠٠ مكجم / مل (للجواياكول) ومن ٢٠ ـ ١٥٠ مكجم / مل (للمورين والنارينجنين) وبتطبيق الطريقة وجد ان الانحراف القياسى النسبى يتراوح بين ٠٫٤ و ١٫٢٪

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received in 28/4/1986 & accepted in 1/9/1986