A new colorimetric method for the estimation of quinones

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Abstract

A new, rapid, simple and sensitive colorimetric method for the estimation of some quinones in microgram amounts using 8-hydroxyquinoline is described. The method is based on the formation of a colored product between quinones and 8-hydroxyquinoline in the presence of ammonia. This method can also be used for the detection of some quinones on paper and thin layer chromatograms.

Key words : Quinones, color reaction, 8-hydroxyquinoline.

1. Introduction

Several color tests are available for the detection of quinones in biological fluids. These include Kesting's test[1], indole and ethylenediamine tests[2], hydrazone formation[3] and a number of other tests[4]. Some of them have been successfully employed for the quantitative estimation of various quinones. For instance, both Kesting's test[5] and hydrazone test[3] have been used for the estimation of microgram amounts of quinones in urine samples. During our studies on the metabolism of quinones by microorganisms, we observed the formation of a bluish green complex between thymo-1, 4-quinone and 8-hydroxyquinoline in presence of ammonia. This forms the basis for the simple and sensitive colorimetric method for the estimation of thymoquinone, described in this paper. This method can al so be used for the estimation of other quinones.

2. Material and methods

Apparatus : The absorption spectra of the colored complex formed by the reaction of quinones with 8-hydroxyquinoline in the presence of ammonia were recorded in a Cary 14 recording double beam spectrophotometer. Routine absorbance measurements were made with a Coleman Jr. II spectrophotometer.

Reagents : All the chemicals used were of the reagent grade.

1. Standard solution of thymoquinone : Thymo-1, 4-quinone (16·4 mg) was dissolved in 100 ml of distilled ethanol. This solution can be stored in the dark for at least a week. Suitable dilutions of this stock solution were made to obtain working standards of varying concentrations.

2. 8-Hydroxyquinoline : A 2·5% (W/V) solution of 8-hydroxyquinoline in distilled ethanol was used.

3. Ammonium hydroxide : Liquor ammonia (Sp. gr. 0·88) was diluted with equal amount of water and used throughout the studies.

Procedure : To 1 ml of a thymoquinone solution in ethanol were added 0·2 ml of 8-hydroxyquinoline reagent, 0·8 ml of ammonium hydroxide and 1 ml of water in the given order. After mixing, the tubes were heated for 10 min on a water-bath maintained at 60 C. The contents were diluted with water to make up to 6 ml and kept at room temperature (25–30 °C) for 30 min. The absorbance of the blue-green complex developed was measured at 685 nm.

Thin layer chromatography : The test solutions were spotted on a silica gel G plate (1 mm thickness) and air dried. After developing the chromatograms in chloroform-benzene solvent system (3 : 1, V/V), the plates were air dried. The spots corresponding to authentic quinones (Thymo-1, 4-quinone, R_f 0·72; naphtho-1, 4-quinone, R_f 0·01; and phenanthro-9, 10-quinone, R_f 0·46) were scraped and suspended in 1 ml ethanol. After developing the color as outlined above, the tubes were centrifuged at 1000 x *g* and the clear supernatant solution was used for the absorbance measurements.

3. Results

The absorption spectrum of the blue-green colored complex formed by the reaction of thymoquinone with 8-hydroxy-quinoline in the presence of ammonium hydroxide is

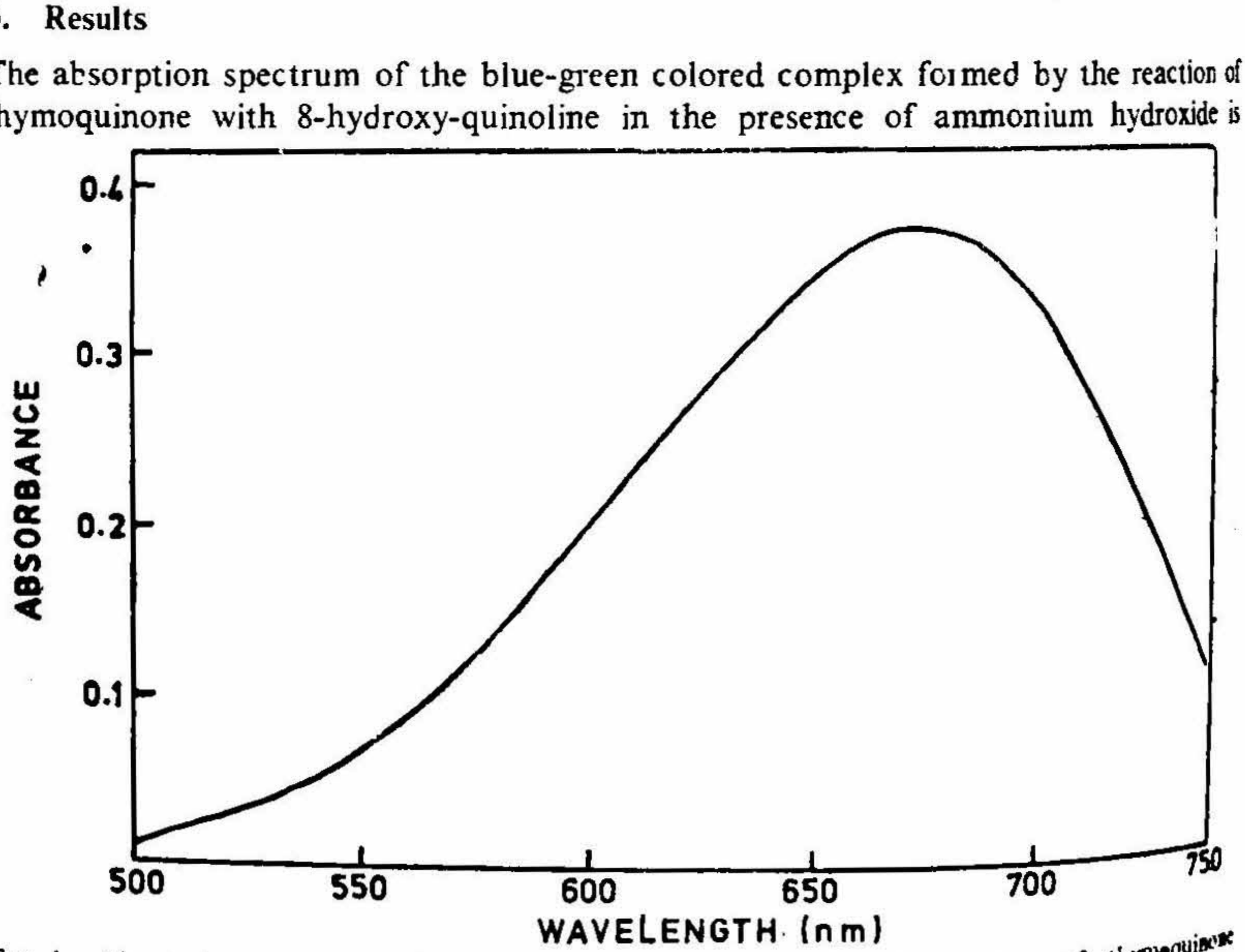

FIG. 1. Absorption spectrum of the colored complex formed by the reaction of thymoquinone with 8-hydroxyquinoline in the presence of ammonium hydroxide.

shown in Fig. 1. The spectrum exhibits an absorption maximum at around 685 nm. After addition of all the reagents, the color developed slowly, reached maximum intensity after 30 min and was stable for about an hr (Fig. 2). The color began to fade slowly after 2 hr.

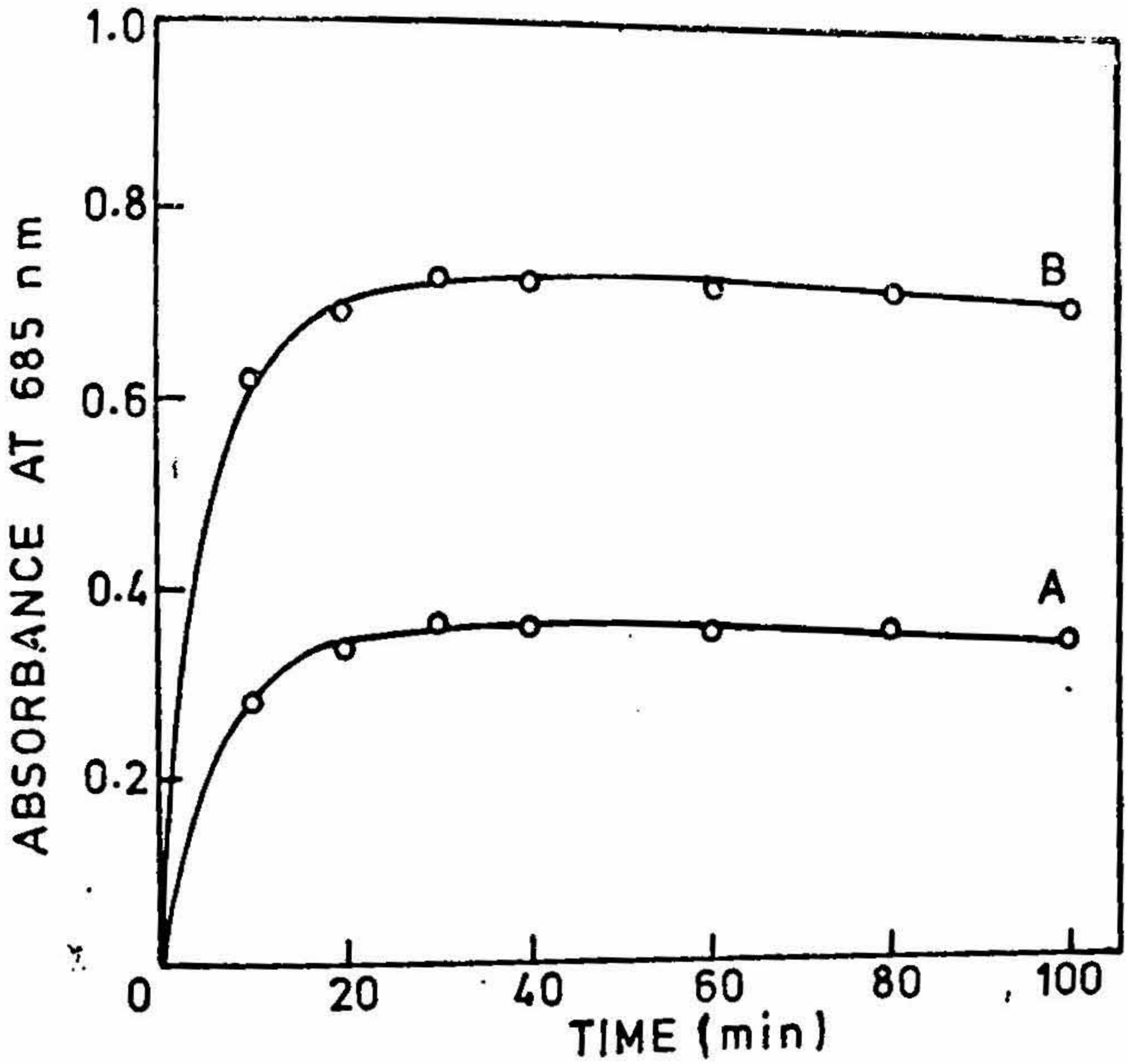

FIG. 2. Influence of time on the development of color. Curve A, with 32·8 μg of thymoquinone. Curve, B, with 65·6 μg of thymoquinone.

Effect of 8-hydroxyquinoline reagent

Effect of increasing amounts of 8-hydroxyquinoline on the intensity of the color produced is shown in Fig. 3. As is evident, a minimum of 5 mg per assay is essential for the maximum color production. Addition of higher amounts of 8-hydroxyquinoline did not have any effect on the intensity of the color produced. Based on these results, 5 mg of 8-hydroxyquinoline per tube were added in all the reactions.

Effect of ammonium hydroxide

Effect of increasing amounts of ammonium hydroxide on the intensity of the color developed is shown in Fig. 4. The optimum amount is found to be 0·8 ml. Higher amounts did not increase the intensity of the color produced.

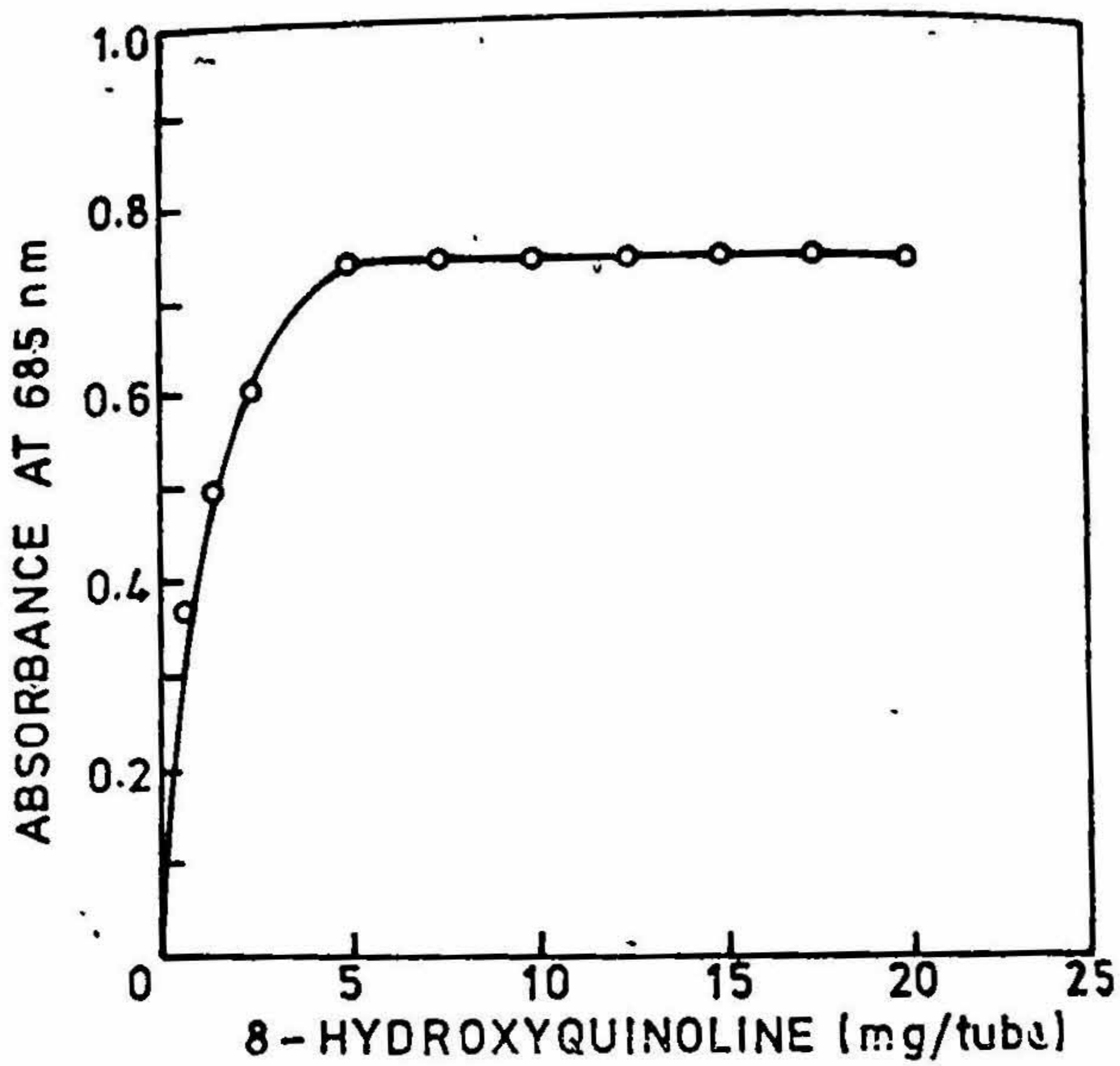

FIG. 3. Effect of increasing amounts of 8-hydroxyquinoline on the intensity of the color produced. About 66 μg of thymoquinone was used and the color developed as outlined in the text.

Standard graph for thymoquinone

The standard graph obtained in the range 0 to 82 μg of thymoquinone is shown in Fig. 5 (curve A). The results obtained after submitting aliquots of known volumes of thymoquinone solution to thin layer chromatography showed that quantitative recovery of thymoquinone could be obtained (Fig. 5, curve B).

Specificity of the color reaction

A number of compounds were tested for their ability to form the colored complex. The following compounds did not give the green complex; Benzene, toluene, naphthalene, benzoic acid, phenylacetic acid, phenylpropionic acid, mandelic acid, benzoylformic acid, phenylpyruvic acid, cinnamic acid, phenol, 2-,3- and 4-hydroxybenzoic acids, 2-,3- and 4-hydroxyphenylacetic acids, 4-hydroxymandelic acid, 4-hydroxyphenylpropionic acid, phenylalanine, tryptophan, *o*-, *m*- and *p*-tyrosines, thymol, vanillin vanillic acid, ferulic acid, isoferulic acid, 4-hydroxydiphenyl, cumic acid, *p*-anisic acid, *p*-toluic acid, *o*-, *m*- and *p*-cresols, 4-dimethylamino benzaldehyde, 4 nitrophenylacetic acid, 4-nitro aniline, benzaldehyde, 2-, 3- and 4-hydroxy benzaldehydes, 2-, 3- and 4-hydroxybenzyl alcohols, anthranilic acid, 3-hydroxyanthranilic acid, indole, phenoxy-

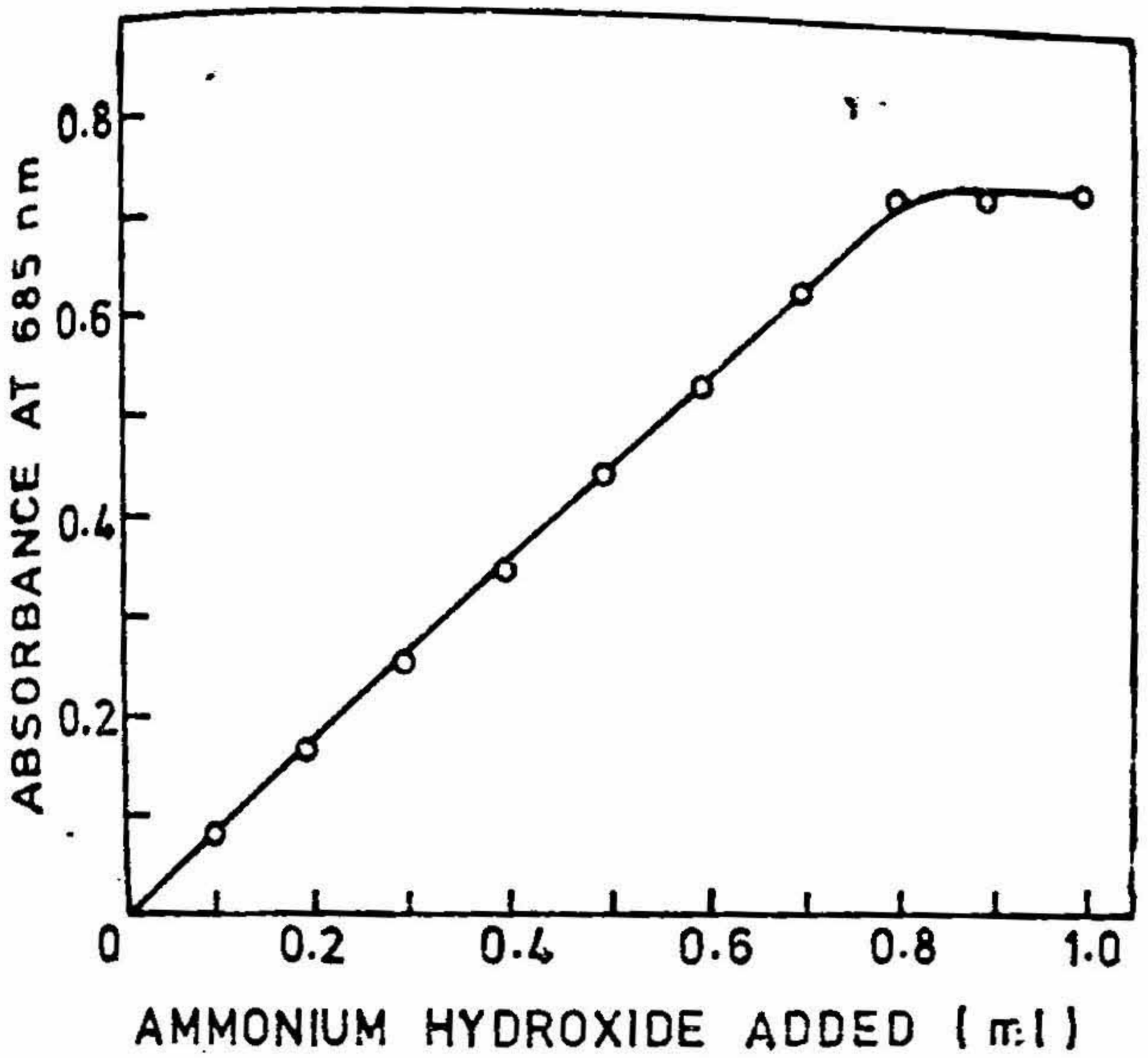

Fig. 4. Effect of increasing amounts of ammonium hydroxide on the intensity of the color produced. About 66 μg of thymoquinone was used and the color developed as outlined in the text.

benzoic acid, 2- and 3-hydroxyphenoxyacetic acids, 4-hydroxy mercuribenzoate, *o*-, *m*- and *p*-coumaric acids, 3, 5-dinitrosalicylic acid, 4-fluorophenylacetic acid, phenyllactic acid, 4-hydroxy phenyllactic acid, coumarin, 3- and 4-amino benzoic acids, 4-chlorobenzoic acid, benzidine, *o*-phenylenediamine, dicumarol, 1- and 2-naphthols, triphenyl tetrazolium chloride, napthoxy acetic acid, indoxylacetic acid, indoleacetic acid, naphthylacetic acid, isophenoxazine, isatin, 2-0-methyl benzoic acid, 2, 4, 5-trichlorophenoxyacetic acid, α-nitroso acetophenone α-nitroso propiophenone, 3-nitrophenol, 3-aminophenol, sulfarilic acid, sulfanilamide, *p*-toluidine, nicotinic acid, nicotinamide, 3-acetyl pyridine, Tween 80, *cis*, *cis*-muconic acid, streptomycin sulfate, tannic acid, glycine, valine, cysteine, ornithine, citrulline, arginine, oxalic acid, citric acid, succinic acid, succinic anhydride, aspartic acid, 2-oxoglutaric acid, 6-amino hexanoic acid, 2-oxo butyric acid, 2-oxoadipic acid, acetone, diacetyl, tris, ethylaceto acetate, diethyl dithiocarbamate, γ-butyrolactone, glucose, sucrose, mannitol, thymine, thiourea, allantion, tricnloro acetic acid, reduced glutathione, NAD, NADH, NADP and NADPH. Dihydroxy aromatic compounds and polyhydroxy aromatic compounds such as catechol, resorcinol, quinol, orcinol, pyrocatechuic acid, protocatechuic acid, resorcylic acid, 2, 3-dihydroxy cumic acid, gentisic acid, homogentisic acid, homoprotocatechuic acid, epinephrine, chlorogenic acid, pyrogallol, hydroxyquinol, gallic acid, phloroglucinol did not answer this test. The following quinones were tested for

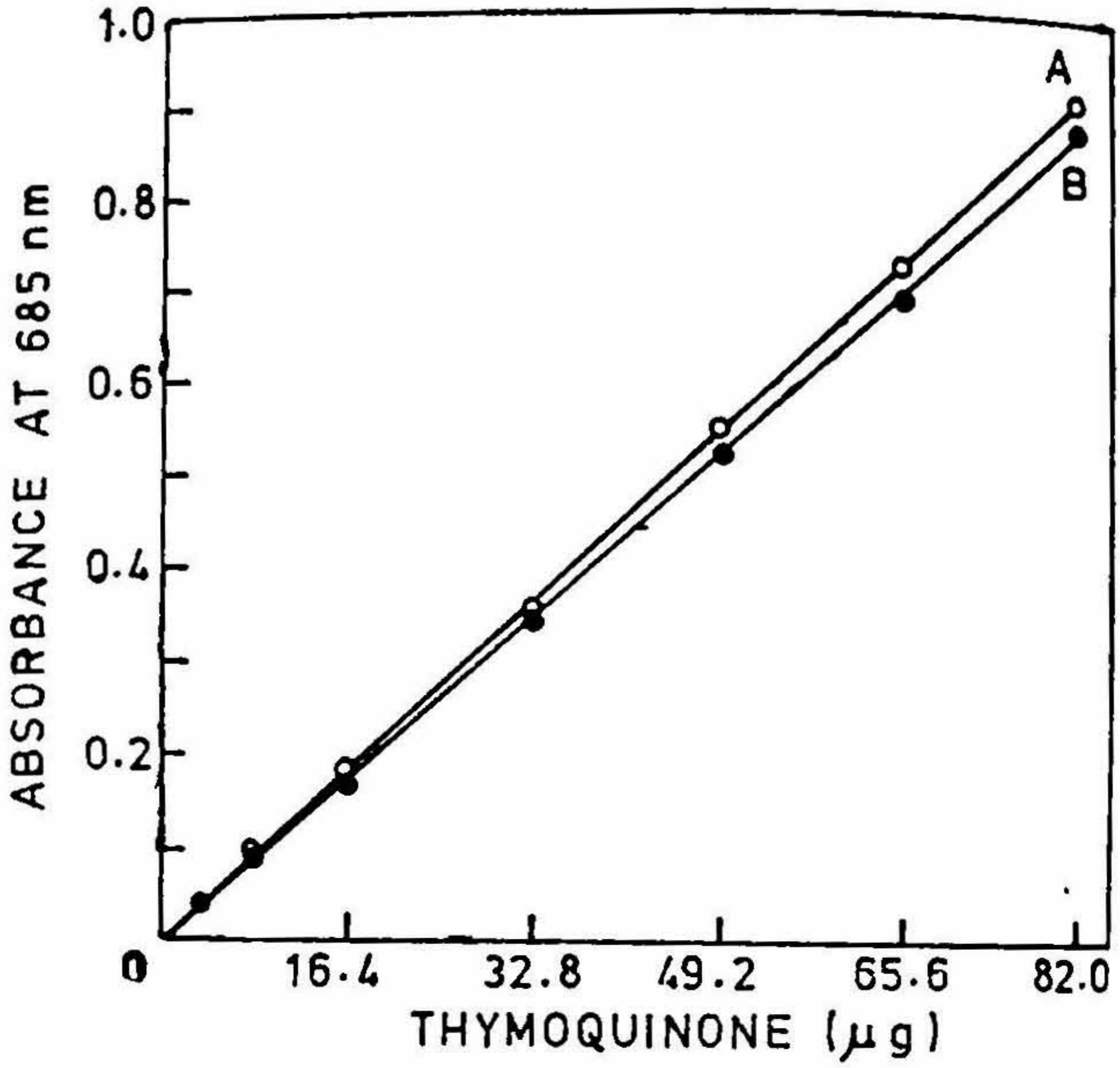

FIG. 5. Standard graph for the estimation of thymoquinone. A, values for untreated thymoquinone. B, values estimated after subjecting to thin layer chromatography.

their ability to form the green colored complex with 8-hydroxyquinoline reagent : Benzo-1, 4-quinone, thymo-1, 4-quinone, naphtho-1, 4-quinone, menadione, 2-bromo naphtho-1, 4-quinone, 8-bromo naphtho-1, 4-quinone, 2-teriatry butyl naphtho-1, 4-quinone, 2, 6-ditertiary butyl napththo-1, 4-quinone, phenanthra-1, 4-quinone, ubiquinone-9 ubiquinone-10, thymo-1, 2-quinone, diphenylenedioxide quinone and phenanthra-9, 10-quinone, With the exception of sterically crowded quinones, *viz.*, 2-tertiary butyl naphtho-1, 4-quinone, 2, 6-ditertiary butyl naphtho-1, 4-quinone, phenanthra-1,4-quinone, ubiquinone-9 and ubiquinone-10, all the other 1, 4-quinones and 1,2-quinones formed the colored complex. As an illustrative example the standard graphs for the estimation of a 1, 4-quinone, *viz.*, naphthoquinone and a 1, 2-quinone, *viz.*, phenanthra 9, 10-quinone are given in Fig. 6.

Effect of interfering substances

The presence of the following compounds affected the intensity of the color produced: trichloroacetic acid, sodium dithionite, hydroxylamine and ammonium sulfate. Hence in the presence of these compounds, quinone estimation gave erroneous results.

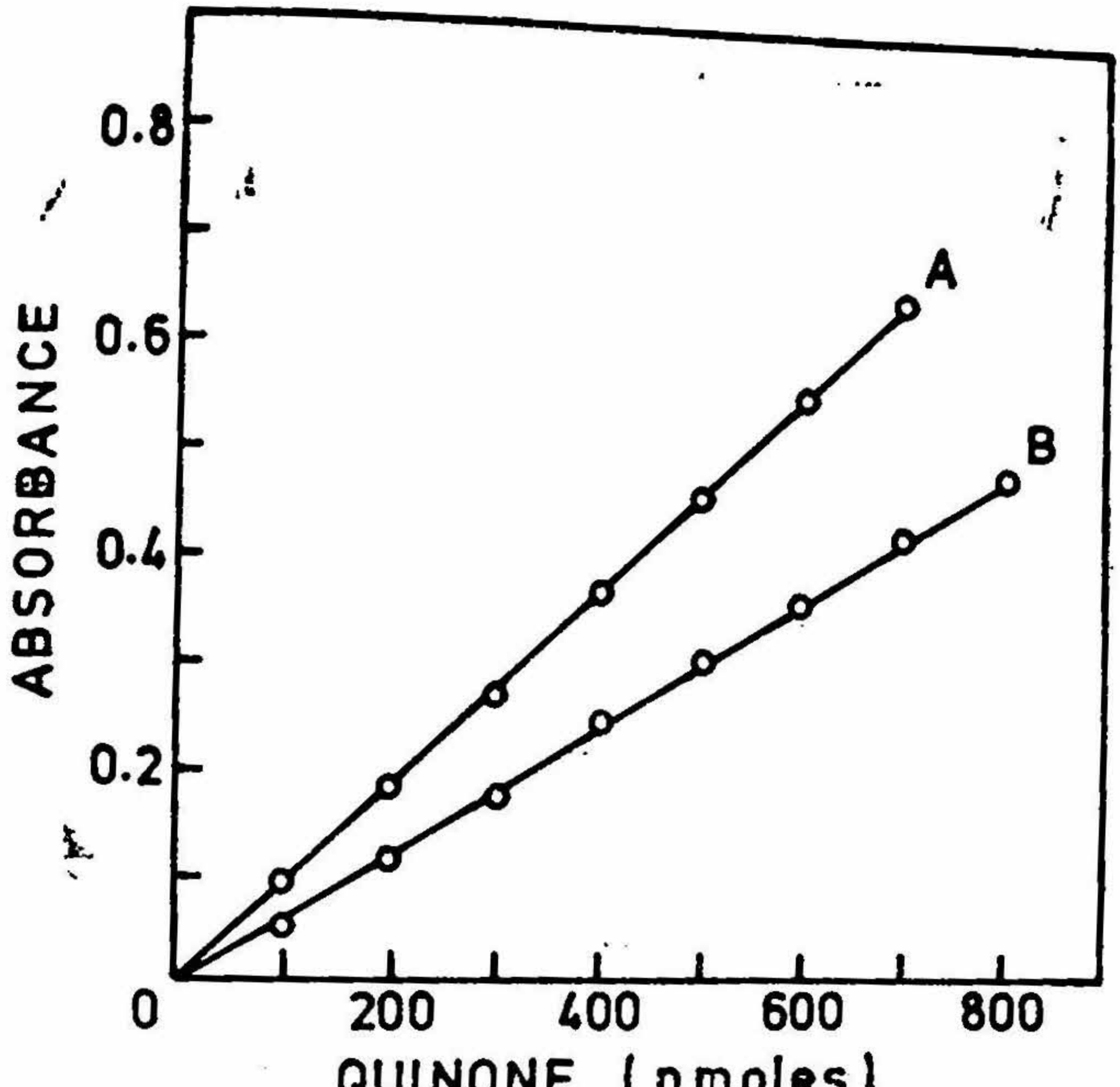

FIG. 6. Standard graph for the estimation of A, naphtho-1, 4-quinone, absorbance measured at 685 nm. B, phenanthra-9, 10-quinone, absorbance measured at 730 nm.

Since metal ions also complex with 8-hydroxyquinoline, it is essential to remove the interfering substances. An ether extraction of the quinone followed by thin layer chromatography aids the separation of these compounds from quinone.

4. Discussion

Based on the finding that thymoquinone reacts with 8-hydroxyquinoline in the presence of ammonia, a simple colorimetric method has been developed for the estimation of both 1, 2 and 1, 4-quinones. The chromogenic reaction seems to be a group specific one. Even in the case of quinones, compounds with bulky substituents and sterically hindered quinones fail to react with 8-hydroxyquinoline reagent. It has been reported that 8-hydroxyquinoline forms a dark red colored 2 : 1 charge transfer complex with chloranil[6]. However, the colored complex formed in the present study seems to be different from the simple charge transfer complex observed by Prout and Wheeler[6]. Characterization and identification of the colored complex is in progress.

This reaction can also be used as a spray test for the identification of quinones on paper and thin layer chromatograms. Quinones give a green color with pale yellow background, while the various compounds mentioned in the text, do not give any color.

The colorimetric method described in this paper is a simple, sensitive and a reliable method. By adjusting the volume of the total solution, the sensitivity of the method can even be increased. Unlike Kesting's reaction which gives an unstable color, the present method produces a comparatively stable colored complex. Another sensitive method which has been used for the estimation of quinones is the method which involves the formation of phenylhydrazone[3]. Though this method is very sensitive, the presence of carbonyl compounds interfere with the estimation of quinones. Such difficulty can be overcome by the use of our method. Though metal ions and some reagents interfere with the color reaction an ether extraction followed by thin layer chromatography enables the separation of quinones from the interfering substances.

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