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O-PHTHALALDEHYDE AS A DERIVATISING REAGENT FOR THE SPECTROPHOTOMETRIC DETERMINATION OF AMINES AND AMINO ACIDS

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O-Phthalaldehyde has been used as a derivatising reagent for compounds containing an amino functional group. The reaction product is an isoindole which absorbs at 335 nm. This property has been utilized for spectrophotometric determination of amines and amino acids. The spectrophotometric method is found to be suitable for quantities as low as 4.0×10^{-5} mol/l. The method is simple, rapid and reproducible.

Key words: *O*-Phthalaldehydes, Spectrophotometric determination, Amines and amino acids.

Introduction

The OPA-derivatisation of amines and amino acids in the presence of a mercaptan is a well known reaction [1]. The 1-alkylthio-2-alkylsubstituted isoindole formed is highly fluorescent [2] and forms the basis for the determination of primary amines. Roth [3] and Svedas *et al.* [4] developed an analytical procedure for the spectrophotometric estimation of these products. *O*-phthalaldehyde reacts with some amino acids [5-9], e.g. glycine, arginine and tryptophan, to produce coloured products in strongly acidic or organic media. The nature of organic solvent affects colouration. This property has been utilized for the selective determination of tryptophan and other amino acids such as glycine in paper chromatography [8]. The reaction of amino acids with *O*-phthalaldehydye has been extended to a kinetic study and spectrophotometric assay [10 of the reaction product. Previous studies [11,12] have indicated that the oxidative degradation of the indole could be lessened by the introduction of steric bulk on or near the isoindole ring. *O*-phthalaldehyde-like reagents, *O*-dicarbonylaryl compounds, have been used [13] to improve the stability of the absorbing molecules. The isoindoles offer high sensitivity detection [14] via fluorimetric techniques. However, the spectrophotometric method [10,15] has also been very useful for the estimation of a number of organic compounds including proteins [16]. This paper describes: (a) an improvement of the OPA-based spectrophotometric assay procedure for amino acids, (b) factors affecting the determination and stability of the reaction product, (c) concentration effect of the analyte and (d) an application of the assay for detection of drugs containing amino groups.

Experimental

Chemicals. Ortho-phthalaldehyde was obtained from BDH. A number of L-amino acids (e.g. alanine, arginine glycine and glutamic acid) from Merck were used without further purification. Ethanethiol, 2-methyl-2-propane-thiol and 2-mercapto-ethanol were also obtained from Merck. All other chemicals were of analytical reagent grade and were obtained from Fluka.

Sample and other solutions. Amino acid solutions (10^{-4} - 10^{-3} M) were made in distilled water. Thiol solutions (0.1% v/v) were prepared in water and stored in dark vessels for a week. Ortho-phthalaldehyde solution (10^{-3} M) was obtained by dissolving 0.134 g in 5 ml of ethanol and making up the volume to 1000 ml with either distilled water or 0.25 M NaOH-Borax buffer. This was diluted with water to obtain a 10^{-4} M solution.

Borate buffer (pH 10) was obtained by dissolving 4.8 g borax and 6.8 g sodium hydroxide in 900 ml of water, adjusting the pH with sodium hydroxide or hydrochloric acid and finally making up the volume to 1000 ml with water. The other buffers, from pH 5.0 to 7.0 and 8.0 to 8.6, were made up from citric acid (0.1M)- dibasic sodium phosphate solution (0.2M) and tris acid maleate (0.2M) - sodium hydroxide (0.2M) solutions, respectively.

General experimental procedure for derivatisation. A small aliquot (1-5 ml) of the sample solution (10^{-4}M) was added into a 25 ml measuring flask. To this was added 3 ml of borate buffer (pH 10.0) and 0.5 ml of 0.1% v/v thiol solution. For each 1 ml of sample solution 1 ml of OPA solution (10^{-4}M) was added and the volume made up to the mark with distilled water. The absorbance of the solution was measured at 330 nm against reagent blank. This procedure of derivatisation was followed for all compounds containing an amino group unless stated otherwise. The order of addition of the reagents remained the same for all other studies including the effect of pH and concentration on absorbance of the derivatives.

Spectrophotometric instrumentation. The following instruments were used:

(1) Hitachi U-2000 double beam UV-visible spectrophotometer.

(2) Milton Roy Spectronic-20, suitable for absorption analysis of liquids in the visible portion of light.

Results and Discussion

It has been shown that the reaction products, isoindoles, are highly fluorescent [17,18]. We have chosen to investigate the amino acid derivatives of OPA as absorbing molecules which have absroption maxima in the region 330-335 nm.

Influence of pH on absorbance. To the aqueous solution containing amino acid (glycine) and thiol (ethanethiol, 2-methyl-2-propanethiol or 2-mercapto-ethanol) was added a fixed quantity of buffer solution, the pH of which was varied in the range of 5-10. The absorption was measured at 335 nm. As illustrated in Fig. 1, the absorbance gradually increases with increasing pH, reaching a maximum in the range pH 8-10. A similar effect of pH on absorbance has been reported [15] for the ethylenediamine derivative with OPA/2-mercaptoethanol. The absorbance value seems to be affected by the structure of the thiol. This, however, needs confirmation by a separate study.

Effect of time delay and reaction temperature. Aqueous solutions of the amino acid derivatives were held in a thermostat at temperatures varying between 20-95°C, during which the absorbances were measured at regular time intervals. The results are shown in Tables 1 and 2. For the OPA-derivative of glycine (1.0 - 4.0 x 10^{-5} mol/1), the reaction is quick with maximum absorbances obtained within 5 min. and remaining unchanged for about 1 hr at room temperature (Table 1).

TABLE 1. EFFECT OF TIME ON ABSORBANCE.

Glycine (mol/1)x10^{-3}	Absorbance (Gly-OPA derivative) interval (min)								
	0	20	40	60	80	100	120	140	200
1.0	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.04
2.0	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.12
3.0	0.21	0.21	0.21	0.20	0.19	0.19	0.19	0.18	0.17
4.0	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.25	0.19

TABLE 2. EFFECT OF TEMPERATURE ON ABSORBANCE.

Glycine (mol/1)x10^{-3}	Absorbance (Gly-OPA derivative) at temperatures (°C)				
	0°	20°	30°	70°	95°
1.0	0.05	0.085	0.06	0.02	0.02
2.0	0.13	0.15	0.14	0.07	0.07
3.0	0.19	0.21	0.19	0.1	0.1
4.0	0.25	0.26	0.25	0.18	0.18
5.0	0.30	0.32	0.30	0.24	0.24

The absorbance of the derivatives containing a low concentration of glycine were, however, much reduced over 3.5 hr. However, the OPA-derivative is stable for all concentrations of glycine studied over a period of 2 hr.

The OPA-derivatives were found to be least affected between 20-30°C, whereas the absorbance decreased very sharply beyond 30°C (Table 2).

Concentration versus absorbance relationship. In order to establish that there exists a linear relationship between the amount of glycine and absorbance, the reactions were performed using different concentrations of glycine. Glycine solutions from 10^{-7} to 10^{-5} M were employed, and as illustrated in Fig.2, a linear relationship was found. Predictably, the absorbance decreased as the molar concentration of glycine was decreased.

The absorption spectra (200-400 nm) for the glycine derivative of OPA for a series of glycine concentrations are shown in Fig. 3. The absorbance maxima which are similar

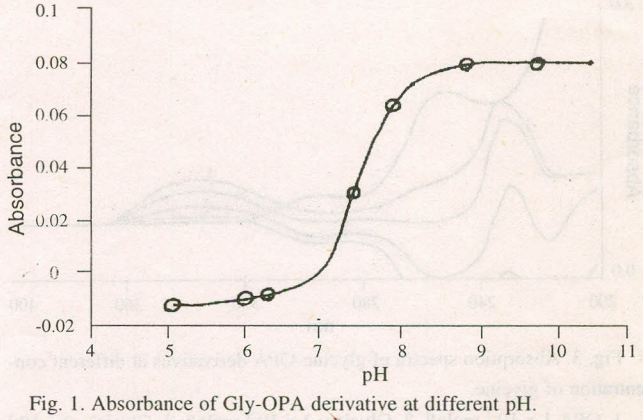

Fig. 1. Absorbance of Gly-OPA derivative at different pH.

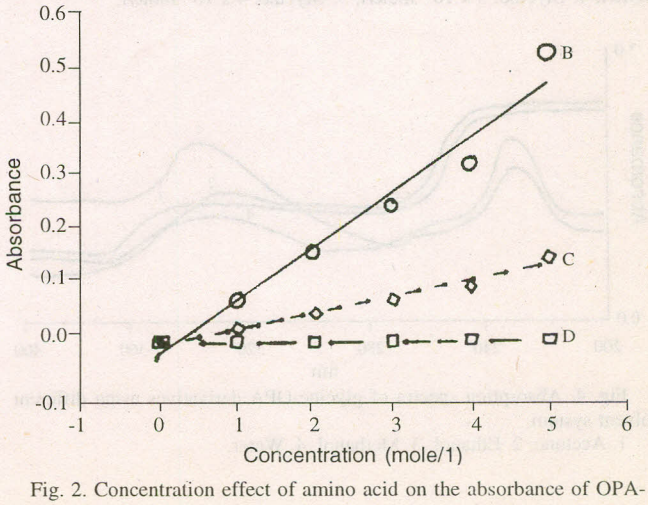

Fig. 2. Concentration effect of amino acid on the absorbance of OPA-adduct.

Absorbances of OPA-adduct of: **B**=Glycine concentration (mole/1) x 10^{-4}. **C**=Glycine concentration (mole/1) x 10^{-3}. **D**=Glycine concentration (mole/1) x 10^{-2}.

to alanine, arginine and glutamic acid derivatives are at 335 nm. The absorbance at 335 nm was found to increase gradually with increasing concentration of glycine from 1.0 x 10^{-3} to 4.0 x 10^{-5} mol/l in agreement with the linear relationship.

Influence of different solvents on absorbance. Organic solvents miscible with water were tested for their effect on absorbance values (Fig. 4). For this investigation, a 5 ml solution of glycine (1 x 10^{-3} mol/l) was reacted in borate buffer, adjusted to appropriate pH. The mixture was diluted to 25 ml with the appropriate solvent and the absorption spectra were obtained as a function of wavelength.

The spectra show that the absorbance at the absorption maximum was markedly enhanced by the addition of acetone.

Methanol and ethanol increased the absorbance slightly and also slightly altered the wavelength maximum.

OPA reaction and the spectra of OPA complexes. The OPA/ethanethiol-glycine is a fluorescent product (absorbs at 330 nm) and its absorption spectral properties are clearly distinct from those of the OPA/ethanethiol and OPA- glycine

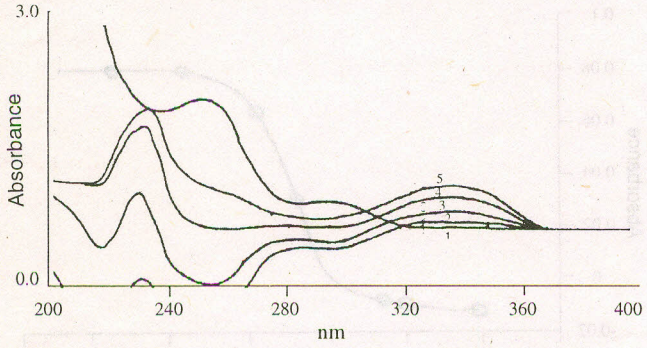

Fig. 3. Absorption spectra of glycine-OPA derivatives at different concentration of glycine.

1. OPA 1 x 10^{-3} mole/l, 2. Glycine: 1 x 10^{-3} mole/l, 3. Glycine: 2 x 10^{-3} mole/l, 4. Glycine: 3 x 10^{-3} mole/l, 5. Glycine: 4 x 10^{-3} mole/l.

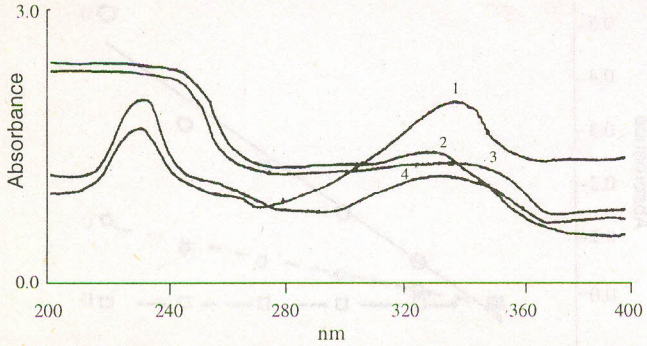

Fig. 4. Absorption spectra of glycine-OPA derivatives using different solvent system.

1. Acetone, 2. Ethanol, 3. Methanol, 4. Water.

complexes. OPA forms complexes by interacting with thiol which is demonstrated by the band diminishing behaviour of OPA peak at 299 nm in the presence of either thiol or an amino acid [19].

In conclusion, the ortho-phthalaldehyde (OPA) is a useful reagent for producing derivatives of compounds containing a primary amino group, and is suitable for spectrometric analysis at levels down to 4.0 x 10^{-5} mol/l.

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