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Spectrophotometric Determination Of Some Amino Acids Using 7,7',8,8'-Tetracyanoquinodimethane Reagent

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ABSTRACT

A sensitive and selective spectrophotometric method is described for the determination of some amino acids namely; glycine, glutamine, valine, and lysine. The method is based on the interaction of these amino acids as electron donors with 7,7,8,8-tetracyanoquinodimethane (TCNQ) as a π-acceptor in acetonitrile at 70°C (80°C in the case of valine), to give highly yellowish-green colored charge transfer complexes which are exhibit maximum absorption at 841nm. Beer's law is obeyed over the concentration range 0.1-4, 0.1-8, 0.3-3 and 0.1-3.5 μg/ml with molar absorptivity of 2.2058×10^4, 3.9184×10^4, 2.6999×10^4 and 4.2056×10^4 $L.mol^{-1}.cm^{-1}$for glycine, glutamine, valine, and lysine respectively. The different experimental parameters affecting the development and stability of the color were carefully studied and optimized. A proposal of the reaction pathway has been postulated.

KEYWORDS

Spectrophotometry;
Charge transfer;
Amino acids;
Tetracyanoquinodimethane.

INTRODUCTION

Many spectrophotometric methods have been proposed for determination of amino acids using various reagents such as ninhydrin[1], o-phthalde hyde[2] in the presence of 2-mercaptoethanol, α, β-dinitrostilibene[3], 2,4,6-trinitrobenzene[4], syringal dehyde[5] and indirect method by using cupper (II)phosphate[6]. Charge transfer complex formation reactions have also been used for determination of amino acids using various acceptors such as chloranil[7], fluoranil[8], DDQ[9], bromanil[10] and p-benzoquinone[11]. Also; titrimetric[12], chemilumino metric[13], fluorimetric[14], chromatographic[15] and electroanalytical[16] methods have also been used for determination of various amino acids. However; most of these methods are either insufficiently sensitive or tedious and required an extraction step or

needed of highly sophisticated instruments. TCNQ is strong electron acceptor and applied in the determination of several electron donor drugs containing primary, secondary or tertiary amino group and the review of literature in the last decade had been mainly concentrated on the CT-complexes spectral studies[17–18]. The present research aims chiefly to developing a sensitive spectrophotometric method for the determination of some amino acids namely; glycine, glutamine, valine, and lysine as n-donors with TCNQ reagent as π-acceptor by measuring the absorbance at λ_{max} of new charge-transfer absorption band.

EXPERIMENTAL

Apparatus

Shimadzu UV-210 Double-beam spectrophotometer with 1cm. quartz cells.

Reagents

All reagents used were of analytical grade and obtained from Fluka and BDH companies.

TCNQ solution ($4.9x10^{-3}$M) is prepared freshly by dissolving 0.05g of 7,7',8,8'-tetracyano quino dimethane in acetonitrile solvent and diluted to the mark in 50ml-volumetric flask with the same solvent.

Standard solutions of amino acids (100μg/ml) are prepared individually by dissolving 0.01g of pure amino acids (glycine, glutamine, valine, and lysine) in acetonitrile solvent and diluted to the mark with the same solvent in 100 ml-volumetric flask. These solutions were further diluted with acetonitrile to required concentrations for working solutions.

Triton X-100 surfactant (0.1%) was prepared by dissolving 0.1g of triton X-100 in hot distilled water and diluted to the mark with distilled water in 100ml-volumetric flask.

General procedure

Aliquots of standard amino acid solutions of glycine, glutamine, valine, and lysine were transferred separately into a series of 5ml calibrated flasks. To these were added 2ml of TCNQ for arginine and lysine and 2.5ml of TCNQ for glycine and valine followed by addition of 1.2ml of 0.1% triton X-100 in the case of glycine. The solutions were heated at 70°C (80°C for valine) for 60 min for arginine and lysine and 70 min for glycine and valine, then the solutions were cooled to room temperature and diluted to the mark with acetonitrile. The absorbances of the complexes were measured at 841 nm against corresponding reagent blank.

RESULTS AND DISCUSSION

Absorption spectra

The interaction between the studied amino acids as n-donors (D) and TCNQ π-acceptor (A) in polar solvent of acetonitrile was found to produce yellowish-green colored charge transfer complex and gave absorption bands at 841,740 and 680 nm with λmax of 841nm (Figure 1). The spectrophotometric properties of the colored charge transfer complexes as well as different parameters affecting the color development between the studied amino acids and TCNQ reagent were extensively studied to determine the optimal conditions for the assay procedure.

Effect of solvent

To select the solvent that would give the highest absorbance, different solvents were tested like

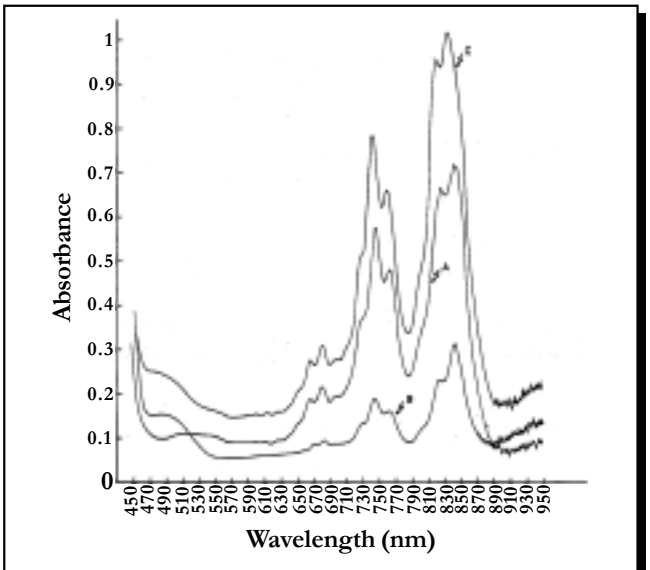

Figure 1: Absorption spectra of (a): arginine (3μg/ml) with TCNQ(2ml of 4.9×10^{-3}M) in acetonitrile versus blank reagent, (b): TCNQ versus acetonitrile and (c): arginine-TCNQ complex versus acetonitrile

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methanol, ethanol, acetone, acetonitrile and dioxane in addition to mixtures of these solvents with water. It was found, as shown in TABLE 1, that acetonitrile was considered as an ideal solvent for arginine, as typical sample for amino acids, and TCNQ reagent because it offered an excellent solvating power for TCNQ reagent to give high absorbance.

TABLE 1: Effect of solvents on the absorption of 5µg/ml arginine

Arginine dissolved in	TCNQ dissolved in	Dilution by	Absorbance
Water	Ethanol	Water	0.108
Ethanol	Ethanol	Ethanol	0.070
Water	Methanol	Water	Negative
Methanol	Methanol	Methanol	Negative
Water	Acetone	Water	0.123
Acetone	Acetone	Acetone	0.012
Water	Acetonitrile	Water	Negative
Acetonitrile	Acetonitrile	Acetonitrile	0.920
Water	Dioxane	Water	Negative
Dioxane	Dioxane	Dioxane	Negative

Effect of pH and buffer solutions

The effect of pH on the absorption of the complexes produced by the reaction of TCNQ and amino acids was studied using different pHs of HCl and NaOH ranged from 2-12. It was found that the absorbances of these complexes were decreased through addition of NaOH or HCl and it was found that the final pH of their reaction solutions were ranged between 4.342 and 5.856 for glycine and arginine with TCNQ respectively, (TABLE 2). Therefore different buffers of pH values, as mentioned in TABLE 2, were prepared by using carbonate, borate, phosphate and citrate buffers to investigate the sensitivity of the amino acid - TCNQ complexes. It was found that sample and blank became deep green colored solutions and the absorbances of these complexes were also decreased.

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in thermostatically controlled water-bath at different temperatures. The absorbance was measured against reagent blank treated similarly. It was observed that the sensitivity reached maximum at 70°C for glycine, arginine, lysine and 80°C for valine and the stability of their absorbances were achieved after cooling to the room temperature, (TABLE 2). These temperatures and reaction time were chosen for color development.

Effect of TCNQ concentration

The effect of changing the TCNQ concentration on the absorbance of solution containing a fixed amount of amino acid was studied. It is evident that the absorbance increases with increasing TCNQ concentration and reached maximum on using 2 ml of 4.9×10^{-3}M TCNQ for arginine and lysine and 2.5 ml for glycine and valine, (TABLE 2). Therefore, these concentrations were used in all subsequent work.

Effect of surfactant

Effect of various surfactants including cetavlon, cetylperydinum chloride (CPC), tween-80 and triton X-100 were tested. It was found decreasing in the sensitivity for the studied amino acids complexes with TCNQ except glycine-TCNQ complex which shows increasing in its sensitivity on using triton X-100 surfactant, and it was found that 1.2 ml of 0.1% is the optimum amount which is recommended in subsequent experiments.

However; the optimum reaction conditions for developing the color intensity of amino acid - TCNQ complexes are summarized in TABLE 2.

Analytical parameters

Under the experimental conditions described in TABLE 2, standard calibration curves of CT complexes for amino acids with TCNQ were constructed by plotting absorbance versus concentration. The correlation coefficients ranged from 0.9978 to 0.9994, indicating good linearity. Beer's law is obeyed in the ranges as cited in TABLE 3, and the molar absorptivity values indicating the high sensitivity of the method.

Precision and accuracy

Six replicate measurements are performed at three different concentrations of each amino acid. The relative standard deviation and recovery % results

TABLE 2: Optimum conditions for the determination of the studied amino acids with TCNQ reagent

Amino acid	λ max (nm)	Temp. (°C)	Development time (min)	Stability period (min) after cooling to RT.	TCNQ 4.9×10^{-3} M (ml)	Surfactant	Final pH
Glycine	841	70	70	5	2.5	Triton ×-100(1.2 ml of 0.1%)	4.34
Arginine	841	70	60	60	2	-	5.85
Valine	841	80	70	180	2.5	-	4.70
Lysine	841	70	60	30	2	-	5.21

TABLE 3: Summary of optical characteristics and statistics for the proposed method

Parameters	Complex of TCNQ with			
	Glycine	Arginine	Valine	Lysine
λ_{max}	841	841	841	841
Linear range(μg/ml)	0.1-4.0	0.1-8.0	0.3-3	0.1-3.5
Limit of detection(ng/ml)	9.12	11.91	11.62	9.31
Slope	0.2941	0.2251	0.2307	0.2880
Intercept	0.0029	0.0402	0.0943	-0.0074
Correlation coefficients	0.9994	0.9989	0.9978	0.9980
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	2.2058×10^4	3.9184×10^4	2.6999×10^4	4.2056×10^4

TABLE 4: Precision and accuracy data for amino acids determination obtained by the proposed method

Amino acid	Amount added (μg/ml)	Recovery* (%)	Average recovery* (%)	RSD* (%)
Glycine	1	100	98.7	0.653
	2.5	100		0.238
	4	96.2		0.696
Arginine	1	100	96.6	0.350
	4	95		1.007
	8	95		3.370
Valine	0.5	100	100	2.130
	2.0	100		0.162
	3.0	100		0.442
Lysine	0.3	100	101.1	2.1
	1.5	100		0.392
	3.0	103.3		0.160

indicated the high precision and accuracy of the proposed method (TABLE 4).

Stoichiometry and stability constant of amino acid -TCNQ complexes

The stoichiometry of the reaction of valine (containing only one amino group) and arginine (containing two amino groups) with TCNQ was studied by the Job and mole ratio methods[19,20], using solutions of equimolar of each amino acid and TCNQ reagent. The results obtained in figure 2 show that 1:1 amino acid to reagent was formed. This indicates that only the nitrogen atom in each amino acid, which has more electron density and less sterically hindered, is responsible for the formation of the n-p charge transfer complexes were formed.

TABLE 5: Association constants of the TCNQ-amine complexes

Amino acid	ml of amino acid / 5 ml	Conc. (M)	Absorbance		α	Average Kst $L.mol^{-1}$
			As	Am		
Glycine	0.05	$(1\times10^{-3}M)$	0.027	0.043	0.372	1.565×10^5
	0.25		0.036	0.215	0.832	
	0.35		0.141	0.445	0.683	
Arginine	0.20	$(1\times10^{-4}M)$	0.048	0.066	0.272	1.238×10^6
	0.40		0.112	0.164	0.317	
	0.60		0.207	0.332	0.376	
Valine	0.05	$(1\times10^{-3}M)$	0.084	0.148	0.432	1.096×10^5
	0.15		0.112	0.503	0.777	
	0.25		0.209	0.731	0.714	
Lysine	0.03	$(1\times10^{-4}M)$	0.015	0.297	0.949	0.480×10^5
	0.15		0.062	0.316	0.803	
	0.30		0.104	0.699	0.851	

The apparent stability constant was estimated

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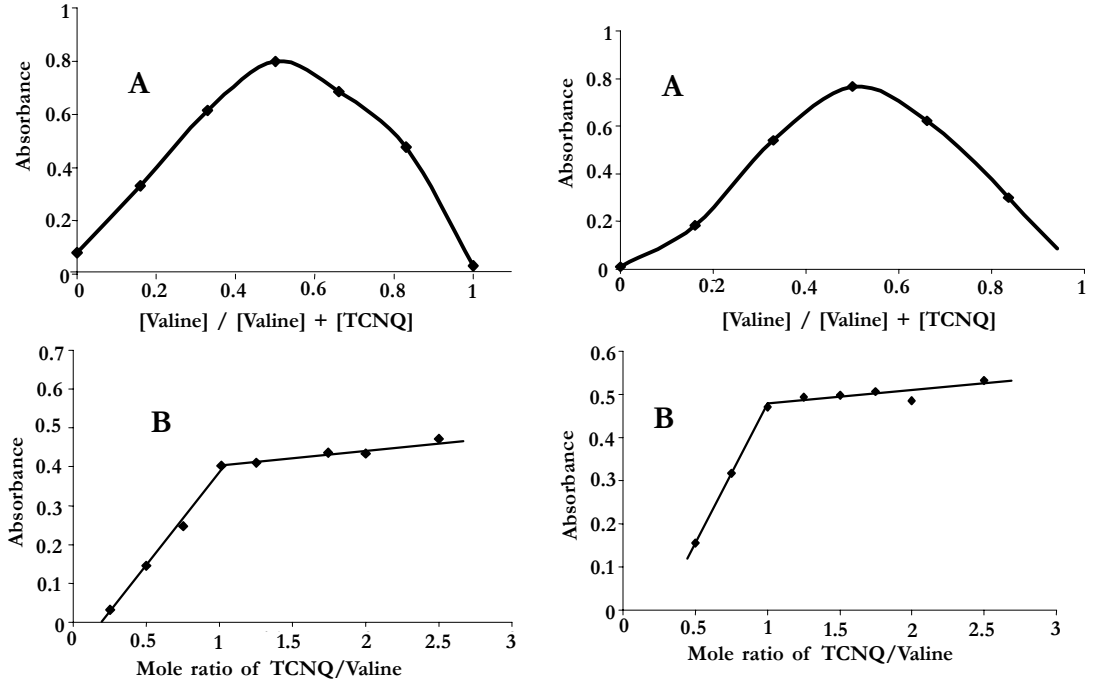
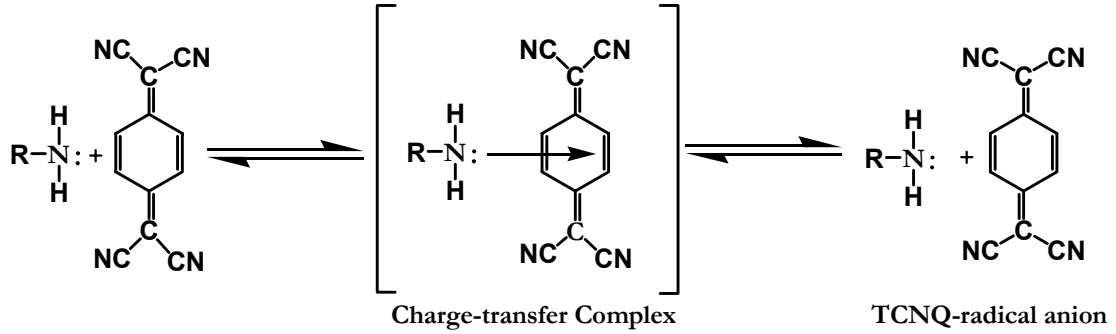


Figure 2: Continuous variation (A) and mole ratio (B) plots for reaction of valine (1×10^{-3} M) and arginine (1×10^{-4} M) with TCNQ



R : H-CH(NH2)-COOH, CH3-CH(CH3)-CH2-COOH, C(=NH)(NH2)-NH-CH2-NH-CH2-CH2-CH2-CH(NH2)-COOH

CH2(NH2)-CH2-CH2-CH2-CH(NH2)-COOH

SCHEME 1: Probable mechanism for the reaction of TCNQ with amino acids

by comparing the absorbance of a solution containing stoichiometric amounts of the amino acid and TCNQ (As) to one containing an excessive amount of TCNQ reagent (Am). The average conditional stability constants of the complexes are calculated by the following equation :

$$Kc = 1 - \alpha / \alpha^2 C$$

$$\alpha = Am - As / Am$$

where Kc is the association constant (l.mol^{-1}), a the dissociation degree and C the concentration of the product which is equal to the concentration of amino acid. The results shown in TABLE 5 indicate that the complexes are relatively stable.

Reaction mechanism

A solution of amino acids and TCNQ yields

an intense yellowish-green color, causing characteristic long-wavelength absorption bands, frequently with numerous vibrational maxima at 841,740 and 680 nm with maximum absorption at 841nm in electronic spectrum (Figure 1). These bands may be attributed to the formation of the radical anion TCNQ, which was probably formed by the dissociation of an original donor-acceptor (D-A) complex with amino acid. The dissociation of the complex was promoted by the high ionizing power of acetonitrile. On the basis of a literature background search and our experimental findings, a reaction mechanism is proposed (SCHEME 1).

CONCLUSION

A simple, rapid, precise and sensitive spectrophotometric method has been developed for the determination of trace amounts of glycine, glutamine, valine, and lysine based on their reaction with TCNQ in acetonitrile medium to form charge transfer complexes. The method do not require any pretreatment of the amino acid or extraction procedure and has a good accuracy and precision.

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