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QUANTITATIVE MOLAR ABSORPTIVITY-STRUCTURE RELATIONSHIPS OF CERTAIN CATECHOLAMINES

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ABSTRACT

Molar absorptivities of six catecholamines (CA) are dertermined from their reactions with hydrochloric acid, boric acid molybdic acid, germanium dioxide, iron (II)- Citrate and zirconyl chloride. Significant linear correlations are obtained between $\mathcal{E}_{max}$ and the number of ligands. $\mathcal{E}_{max}$ of most of the (CA) investigated shows a significant correlation with the molecular connectivity indices of the side chains and pka values. A dimeric state is suggested for (CA) in 0.1 N hydrochloric acid medium.

INTRODUCTION

In studies on quantitative molar absorptivity-structure relationships (QMSR), $\mathcal{E}$ was expressed as a linear combination of parameters that represent the physical properties of a certain function or group in a series of analysed compounds. The electronic parameters (σ_p, $\mathcal{F}$ and/or $\mathcal{R}$) and the connectivity index $^n\chi^v$ were defined as major factors in (QMSR) of seventeen phenothiazines[1,2].

Molar absorptivity is generally determined from the spectrophotometric procedures involving UV or visible measurement at λ_{max}. The (CA) subject of (QMSR) are dopamine, norepinephrine, epinephrine, levodopa, methyldopa and isoprenaline, Table 1. Existing analytical methods for (CA) were recently reviewed[3]. The values of molar absorptivities were taken from reported spectrophotometric methods applied for the analysis of the targeted (CA), like UV measurement of a solution in 0.1 N hydrochloric acid[4], chelates with germanium dioxide[5] and esters of boric acid[6].

Values of molar absorptivities were also available from reported quantitative colorimetric procedures. For example, epinephrine and isoprenaline were determined by iron (II)- citrate reagent[7,8], while epinephrine and methyldopa were estimated by molybdic acid[9,10]. In the present work, both reagents, iron (II)-citrate and molybdic acid, were applied for the colorimetric determination of the (CA) not previously reported. In addition, zirconyl chloride was applied as a chelating agent for the colorimetric determination of the specified (CA).

EXPERIMENTAL

Materials :

Pure samples of dopamine hydrochloride, norepinephrine bitartrate, epinephrine bitartrate, levodopa, methyldopa and isoprenaline sulphate were used as working standards.

Apparatus :

A Uvidec-320 spectrophotometer (JASCO, Tokyo, Japan) was used.

Reagents :

1- Ammonium molybdate solution, 10% in distilled water.
2- Sulphuric acid, 0.1 N.
3- Iron (II) sulphate-citrate solution (B.P. 1980).
4- Glycine buffer (B.P. 1980).
5- Zirconyl chloride solution, 2% in distilled water.
6- Acetate buffer pH 6.0.
7- Hydrochloric acid, 0.01 N and 0.1 N solutions.

All solvents used were spectral grade.

Preparation of Working Standards :

Dissolve 25.0 mg of the appropriate working standard in 50.0 ml of distilled water containing 0.1 % sodium metabisulphite as antioxidant. Free catecholamine bases are dissolved in 0.01 N HCl, containing 0.1 % sodium metabisulphite, freshly dissolved. Dilute the solution quantitatively with the same solvent to obtain the required concentration.

Procedures :

(a) Molybdic acid method was carried out according to the procedure of Cohen[9]. Measurements were made at λ_{max} at 360 nm using ammonium molybdate and 0.1 N sulphuric acid solutions.

(b) Iron (II)-Citrate method was carried out according to the procedure of B.P. 1980[8]. Measurements were made at 530 nm using glycine buffer (pH 8).

(c) Zirconyl chloride method :
Pipette 2.0 ml of the assay solution (5×10^{-4} M) into a graduated test tube, add 1.0 ml of acetate buffer pH 6.0 and 2.0 ml of zirconyl chloride solution and mix thoroughly.
Measure the absorbance of the resulting solution at 294 nm against a reagent blank. The concentration of the assay solutions is found from a properly constructed calibration graph.

Mathematical and Statistical Treatment of Data:

(a) Other absorptivity data were taken from published data[4-10] to complete the picture presented in Table 2.

(b) Molar ratio of the chelates was determined for all investigated (CA) by both the molar ratio and Job's techniques[11]. Results are presented in Table 2.

(c) Calculation of molecular connectivity index ${}^{1}\chi^{v}$ of the side chain (R) followed the method described by Hall and Kier[12], Table 1.

(d) Pka data are taken from the Extrapharmacopoeia, 28th Ed[13].

RESULTS AND DISCUSSION

Among the spectroscopic procedures developed are those reported depending on the catechol group as a target for reaction with germanium dioxide, boric acid, iron (II)-citrate, molybdic acid and zirconyl chloride. Generally, the (CA) react with the given metal reagents to give M : (CA) with different ratios. The number of ligands (N) depends on the chelating properties of the metal as well as the side chain (R).

The analysis of (CA) using germanium dioxide, boric acid and zirconyl chloride allowed the measurement of $\Delta\varepsilon$. Here $\Delta\varepsilon = \varepsilon_2 - \varepsilon_1$, where ε_2 and ε_1 are the molar absorptivities measured at the corresponding λ_{max} after and before the addition of the reagents respectively. With other reagents ε values were determined. Either ε or $\Delta\varepsilon$ is shown in Table 2 according to the reagent used and both are referred to as ε^*. Values of ε^* and molar ratios displayed in Table 2 are obtained from reported or experimentally determined data.

From Table 2, it is clear that each of the first three reagents reacts irrespective of the (CA) in a constant ratio, which is not the case with the last two reagents.

We next examined the relation of ε^* with the number of ligands (N). In all cases, the molar absorptivity increased linearly with the increase in (N) i.e., the larger the number of built up aggregates formed from the ligands with the reagent , the more sensitive the reaction obtained. The linear relations could be expressed by Eq. 1.

$$\varepsilon^* = b(N) - a \qquad (1)$$

For these five reagents, the following relations represented by equations (2-7) were obtained by least-squares calculations. In these equations, n is the number of reagents used, r is the correlation coefficient and S is the standard deviation. These equations are highly significant by the F-test.

For dopamine :

$$\varepsilon^{*} = 1598.652\ (N) - 720.696 \quad (2)$$

r= 0.9941 s=304.40 n=5

F= 253.77 P<0.005

For norepinephrine :

$$\varepsilon^{*} = 1575.735\ (N) - 785.059 \quad (3)$$

r= 0.9970 s=185.50 n=5

F= 490.85 P<0.005

For epinephrine :

$$\varepsilon^{*} = 1972.167\ (N) - 1433.000 \quad (4)$$

r= 0.9816 s=242.70 n=5

F=79.30 P< 0.005

For levodopa :

$$\varepsilon^{*} = 1733.250\ (N) - 1043.150 \quad (5)$$

r= 0.9952 s=196.69 n=5

F= 310.69 P< 0.005

For methyldopa :

$$\varepsilon^{*} = 1669.750\ (N) - 996.650 \quad (6)$$

r= 0.9752 s=437.78 n=5

F= 58.17 P< 0.005

For isoprenaline :

$$\varepsilon^{*} = 1858.500\ (N) - 1155.000 \quad (7)$$

r= 0.9673 s=308.11 n=5

F= 43.68 P< 0.010

The linear equations (2-7) were exploitated for the prediction of the possible number of polymers of the (CA) in 0.1 N hydrochloric acid solution. This was carried out by solving separately the equations (2-7) for (N) as shown by the general Eq. 8.,

$$(N) = (\varepsilon^{*} + a)/b \quad (8)$$

and substituting ε^* values determined at 280 nm of the acidic solution of (CA) for ε^* in each equation. The values of (N) calculated from each equation are listed in table 3, with an average value of 2.23 + 0.11. From this treatment, it can be concluded that (CA) exist in a dimeric state in the acidic milieu.

Now another problem was tried to solve, namely the quantitative relation between ε^* and the side chain (R). To answer this question, two basic requirements are to be considered: (1) matching ε^* values when using the same reaction conditions; (2) description of the side chain in numerical values. With regard to the first consideration, table 2 displays in the horizontal rows the ε^* values of (CA) obtained from reactions with the given reagent. With regard to the second consideration, the molecular volume can be used as a numerical descriptor of (R). Viewed at the molecular level, the molar absorptivity value is governed by the size of the choromophore[14]. Under the same conditions, the only difference in ε^* values of our (CA) is that attributed to variations of (R) and its impact on the probability nature of electron density. The lowest order connectivity index $^1\chi^v$ reflects the general characteristics of molecular volume, like number of atoms and branching[12].

Accordingly, we examined the relation of ε^* with $^1\chi^v$ values and obained the equations (9-11).

For germanium dioxide :

$$\varepsilon^{*} = 3978.589 + 178.655 \ {}^{1}\chi^{v} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

$r = 0.9055$ $\quad s= 61.84 \quad n=6$

$F = 18.36$ $\quad P < 0.025$

For boric acid :

$$\varepsilon^{*} = 2182.905 + 178.286 \ {}^{1}\chi^{v} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

$r= 0.9382$ $\quad s=48.68 \quad n=6$

$F=29.48$ $\quad P < 0.01$

For zirconyl chloride :

$$\varepsilon^{*} = 737.336 + 557.851 \ {}^{*}\chi \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

$r= 0.9375$ $\quad s=687.43 \quad n=6$

$F= 29.01$ $\quad P < 0.01$

Where ${}^{*}\chi$ equals the ${}^{1}\chi^{v}$ value of R after being multiplied by the corresponding number of ligands (N) . Analogous regression analysis using iron (II)-citrate reagent, molybdic acid and hydrochloric acid solutions resulted in very poor correlations.

Addition of the pka value of the first dissociable hydrogen ion of the phenolic groups of (CA) as a factor slightly improved the correlation in addition to a significant improvement of S and the significance level of F . Furthermore, good correlations were obtained for reactions of iron (II)-citrate and for solutions in hydrochloric acid. In all cases, increase of ${}^{1}\chi^{v}$ values increases ε^{*}, i.e., increases the sensitivity of the reaction. This is in agreement with that found from eq. (2-7), since either N or ${}^{1}\chi^{v}$ reflects bulkiness of the chromophore.

For germanium dioxide :

$$\varepsilon^{*} = 5145.196 + 182.663\ {}^{1}\chi^{v} - 133.879\ pk_a \quad \ldots\ldots (12)$$

$r=0.9342$ $s=52.12$ $n=6$

$F=27.46$ $P < 0.01$

For boric acid :

$$\varepsilon^{*} = 945.180 + 174.033\ {}^{1}\chi^{v} + 142.040\ pk_a \quad \ldots\ldots (13)$$

$r= 0.9718$ $s=32.98$ $n=6$

$F= 68.66$ $P < 0.005$

For zirconyl chloride :

$$\varepsilon^{*} = 5218.599 + 578.539\ {}^{*}\chi - 520.021\ pk_a \quad \ldots\ldots (14)$$

$r= 0.9391$ $s=678.16$ $n=6$

$F= 29.92$ $P < 0.01$

For solutions in 0.1 hydrochloric acid :

$$\varepsilon^{*} = 1794.513 + 105.475\ {}^{1}\chi^{v} + 506.372\ pk_a \quad \ldots\ldots (15)$$

$r= 0.9839$ $s=28.52$ $n=6$

$F= 118.22$ $P < 0.005$

For iron (II)-citrate :

$$\varepsilon^{*} = 6406.669 - 35.459\ {}^{1}\chi^{v} - 478.844\ pk_a \quad \ldots\ldots (16)$$

$r= 0.9502$ $s=40.91$ $n=6$

$F= 37.63$ $P < 0.005$

The regression coefficients of the regression equations (12-16) were rescaled to give respectively eq.(17-21).

$$\varepsilon^{*} = 0.9262\ {}^{1}\chi^{v} - 0.2305\ pk_a \quad \ldots\ldots (17)$$

$$\varepsilon^{*} = 0.9162\ {}^{1}\chi^{v} + 0.2540\ pk_a \quad \ldots\ldots (18)$$

$$\varepsilon^{*} = 0.9723\ {}^{*}\chi - 0.0663\ pk_a \quad \ldots\ldots (19)$$

$$\varepsilon^{*} = 0.4952\ {}^{1}\chi^{v} + 0.8074\ pk_a \quad \ldots\ldots (20)$$

$$\varepsilon^{*} = 0.1985\ {}^{1}\chi^{v} - 0.9114\ pk_a \quad \ldots\ldots (21)$$

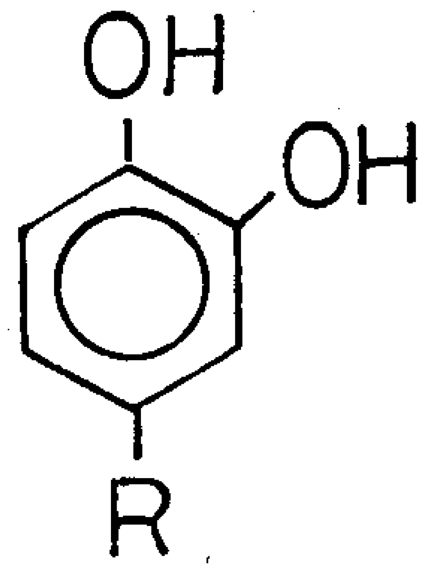
Quantitative Molar Absorptivity-Structure Relationships of Certain Catecholamines.

Equations (17-19) show a predominant weight of $^{1}\chi^{v}$ where pH of the reaction medium is 6-7. Reversed pattern shown by Eq. 20 and 21 can be attributed to pH of the reaction medium in each case. Solutions of (CA) in 0.1 N hydrochloric acid are protonated at the basic center of the side chain while ionization of phenolic OH is highly suppressed. On the other hand, at pH 8 of iron (II)-citrate method the phenolic OH is ionized while ionization of the basic center of the side chain is suppressed. These factors of ionization probably affect the chromophore formation and/or stabilisation more than the $^{1}\chi^{v}$ parameter can influence the molar absorptivity values.

Correlation of $\mathcal{E}$ as $f(\chi$ and $pk_a)$ using molybdic acid in 0.1 N sulphuric acid was very poor. In our work, Mo: (CA) ratios were found to be 1:3, 1:4 and 1:5 in agreement with such higher ratios reported in solution by Weinland and Gaisser[15]. On the other hand, molybdenum chelates of polyphenols with at least two adjacent hydroxyl groups were reported not to exceed 1·2 ratio[16]. Our data with higher ratios may be attributed to an active role of the side chain in chelate formation in addition to the expected role of the phenolic groups. This speculation may explain the deviation of $\mathcal{E}^{*}$ values from correlations tried.

As the most simple conditions are given by measurement at λ_{max} 280 nm in 0.1 N HCl, in addition to the highest significant correlation shown by eq. (15), the latter equation was exploitated for the calculation of pk_a of the (CA), table 4.

Table 1: Catecholamines subject of the quantitative ε-structure relationship.



R	$^{1}\chi^{v}$	Compound
$-CH_2CH_2NH_2$	0.8162	Dopamines
$-CH(OH)CH_2NH_2$	1.3017	Norepinephrine
$-CH(OH)CH_2NH(CH_3)$	1.7489	Epeniphrine
$-CH_2-CH(NH_2)COOH$	1.8086	Levodopa
$-CH_2-C(CH_3)(NH_2)COOH$	2.1806	Methyldopa
$-CH(OH)CH_2N(CH_3)_2$	2.7145	Isoprenaline

Table 2: Effects of reagents and number of ligands on ε^*

Reagent	ε^* of the catecholamines/$(M:L_N)$					
	Dopamine	Norepinphrine	Epinephrine	Levodopa	Methyldopa	isoprenaline
1) GeO_2 +	4077 (1:3)	4211 (1:3)	4366 (1:3)	4358 (1:3)	4306 (1:3)	4442 (1:3)
2) H_3BO_3 +	2331 (1:2)	2360 (1:2)	2506 (1:2)	2544 (1:2)	2622 (1:2)	2619 (1:2)
3) $FeSO_4$	2141 (1:2)	2217 (1:2)	2241 (1:2)	2202 (1:2)	1917 (1:2)	2160 (1:2)
4) H_2MoO_4	7392 (1:5)	7004 (1:5)	4601 (1:3)	5899 (1:4)	5940 (1:4)	4399 (1:3)
5) $ZrOCl_2$ +	1238 (1:1)	2343 (1:2)	2787 (1:2)	5780 (1:4)	5278 (1:4)	2907 (1:2)

+ $\Delta\varepsilon$ values are presented

Table 3: Prediction of (N) for (CA) in 0.1 N HCl

Catecholamine	$\mathcal{E}$ at λ_{max} 280 nm	Number of ligands (N)	Equation No.
1. Dopamine	2727	2.16	2
2. Norepinephrine	2707	2.22	3
3. Epinephrine	2840	2.17	4
4. Levodopa	2781	2.21	5
5. Methyldopa	3097	2.45	6
6. Isoprenaline	2831	2.14	7

Table 4: Reported and calculated pk_a values of catecholamines

Catecholamine	pk_a reported*	pk_a calculated eq.(15)
Dopamine	8.8	8.8
Norepinephrine	8.6	8.6
Epinephrine	8.7	8.8
Levodopa	8.7	8.7
Methyldopa	9.2	9.2
Isoprenaline	8.6	8.6

* Data from reference 13.

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العلاقات الكمية بين شدة الامتصاص الجزيئى والتركيب الكيميائى لبعض الكاتيكولاميـــــنات

عادل فوزى يوســـف ، ميشــيل ايليـا القمــص ، حسن حســـن فرج
قســم الكيميـاء الصيدلية ـ كلية الصـيدلة ـ جامعة أســـــيوط

فى هذا البحث تم تقدير شدة الامتصاص الجزيئى لنواتج تفاعلات ستة عقاقير من مجموعة الكاتيكولامينات (دوبامين ، نورابينفرين ، ابينفرين ، ليفودوبا ، ميثيل دوبا ،وآيزوبرينالين) مع ستة كواشف (حمض الهيدروكلوريك ،حمض البوريك ، حمض الموليبديك ، ثانى آكسيد الجرمانيـــوم ، أيونات الحديدوز فى وجود الســـترات ، كلوريد الزركونيل) .

ولقد تمت دراسة العلاقات الكمية بين شدة الامتصاص الجزيئى لنواتج هــــذة التفاعلات والتركيب الكيميائى وقد أوضحت النتائج أن العلاقة بين شدة الامتصـــاص الجزيئى وعدد جزيئات مركب الكاتيكولامين التى تدخل فى بناء ناتج التفاعل ذات دلالة احصائية عالية فى المركبات الستة التى تمت دراستها.ولقد تم استخـــدام المعادلات المستنبطة فى اقتراح بلمرات ثنائية من الكاتيكولامينات فى محلول عشر عيارى من حامض الهيدروكلوريك .

كما أثبتت الدراسة أن العلاقة بين شدة الامتصاص الجزيئى من ناحيــــة وعامل الترابط الجزيئى للسلسلة الجانبية للكاتيكولامين وثابت التأين لمجموعـــة الفينول الاولى من ناحية أخرى ذات دلالة احصائية عالية بالنسبة لمعظم الكواشـــف التى تمت دراستها .

ولقد تم استخدام المعدلات الخطية المستنبطة فى حساب ثابت التأين لمجموعة الفينول الاولى للكاتيكولامـــنات .

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