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# Colorimetric Determination Of Catechol Drugs L-Dopa, D-Dopa, And Carbidopa In Aqueous And Solid Samples Utilizing Complexation With A Transition Metal Cation

**Ronald Bartzatt**
**University of Nebraska College of Arts & Sciences**
**Chemistry Department Durham Science Center 6001**
**Dodge Street Omaha, Nebraska 68182 (U.S.A.)**
**Phone: 402 554-3612 FAX: 402 554-3888**
**E-mail: bartzatt@mail.unomaha.edu**

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## ABSTRACT

The compound L-dopa is an important therapeutic tool for the clinical treatment of Parkinsons disease. L-dopa and D-dopa drug structures include a primary amine ($-NH_2$), a carboxylic acid group (-C(O)OH), and an aromatic ring having two adjacent hydroxyl (-OH) substituents, a structure referred to as a catechol. The D- and L- forms of dopa are soluble in water and form an iron(III)-catechol complex seen in solution as a brown to dark-brown mixture. The complex is stable in aqueous solution with a strong absorbance peak at 400 nm which was utilized to identify the presence and quantitate the concentration of dopa. The extinction coefficient of a mixture having 0.167% $FeCl_3 \bullet 6H_2O$ with 6.76E-04 molar catechol drug results in $\varepsilon_{400\,nm}$ =2910.38 Liter/(mole/cm). The methods described here produce a colored endpoint that indicates the presence of these catechol drugs. The colored endpoint is readily seen by eye to an amount of drug < 53.0 μg/ml (53 parts per million) and can be utilized to determine the concentration from 53.0 μg/ml to 533.0 μg/ml. To assay multiple samples a standard curve may be constructed utilizing UV-Visible spectrometers that monitor a strong absorbance peak at 400 nm. As little as 1.50 μg/ml (parts per million) can be detected by utilizing a standard curve. Many inorganic salts such as LiBr and NaCl do not interfere with the colorimetric results. Many medicinal compounds such as aspirin, ampicillin, tetracycline, erythromycin, and quinine sulfate do not interfere with the determination. A Jobs Plot was accomplished to determine the ratio of iron(III) to catechol drugs within the complex to be 3:2, respectively.


## KEYWORDS

L-Dopa;
Dopamine;
Iron complexation;
Colorimetric assay

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## INTRODUCTION

Parkinsons disease was initially recognized in 1817 by an English physician James Parkinson. It is a movement disorder that affects primarily areas of the brain called basal ganglia. Cells in the substantia nigra portion of the ganglia produce dopamine and it is the loss of these cells that results in the tremors and difficulty associated with the disease[1]. Dopamine will not cross the blood-brain-barrier (BBB) so the precursor L-dopa (Levodopa) is administered. L-dopa is analogous to amino acids which are transported across the BBB, therefore it penetrates the brain where it is decarboxylated (loss of -C(O)OH) by DOPA decarboxylase to form dopamine[2]. The steps of L-dopa metabolism may be represented as follows[2]:

**L-dopa → Dopamine → Norepinephrine → Adrenaline**

L-dopa is administered as part of the medicinal treatment of Parkinsons disease and is referred to as L-dopa or Levodopa (having brand names such as Sinemet). The disease continues to increase in severity as time progresses and the amounts of L-dopa are also increased[3,4]. Other therapeutic agents utilized include monoamine oxidase inhibitors, amantadine, and catechol-O-Me transferase inhibitors[5]. Carbidopa is co-administered with L-dopa to inhibit the enzyme L-aromatic amino acid decarboxylase (AAAD) in the peripheral systems. The carbidopa structure also has a catechol portion and it resembles L-dopa and D-dopa. Carbidopa helps avoid the massive decarboxylation of L-dopa that would occur in the peripheral systems[6].

Iron(III) is a transition metal cation which forms a large number of complexes in aqueous solution and it is this tendency to form complexes which is a conspicuous trait of iron(III)[7]. Iron(III) complexes are predominantly octahedral, with the octahedron considered the characteristic polyhedron of iron[7]. It has its greatest affinity for ligands that coordinate by oxygen, such as phosphate ions, polyphosphates, polyols of glycerol and sugars[7]. In water, iron(III) cation hydrolyzes to form coordination layers of $H_2O$ and $OH^-$ (primarily octahedral)[7]. The characteristic yellow color of iron(III) results due to the hydrolysis of the $Fe(H_2O)_6^{3+}$ cation, which can be represented as follows[8]:

$$Fe(H_2O)_6^{3+}(aq) + H_2O \rightarrow Fe(H_2O)_5OH^{2+}(aq) + H_3O^{1+}(aq)$$

These characteristics of hydrolysis and complexation are applied here to produce a colorimetric assay and highly sensitive determination by standard curve of catechol agents D-dopa, L-dopa, and carbidopa. L-dopa and D-dopa may be referred to as catecholamines (carbidopa is a hydrazine). To determine the ratio of the iron(III) species to catechol drug within the complex analysis by Jobs method has been shown to be effective (also referred to as the method of Continuous Variation)[9,10]. This method requires a series of solutions, that each contain the same total number of moles of reactant A (iron(III)) and B (catechol agent), but at different ratios of A to B. The maximum amount of the product (the iron-catechol complex for this study) is obtained at a ratio of A to B that is referred to as the stoichiometric ratio R[9,10]. The ratio R represents the elementary stoichiometry of the atoms comprising the complex.

The methods presented here are simple, produce rapid color development, are reproducible, and highly sensitive.

## MATERIALS AND METHODS

### Chemicals, software, and instruments

Reagents were obtained from Sigma-Aldrich, P.O. Box 14508, St. Louis, MO 63178 U.S.A.. Molecular modeling and calculation of molecular properties utilized ChemSketch (ACD, 90 Adelaide Street West, Suite 702, Toronto Ontario M5H 3V9, Canada) and ChemDraw, Cambridge Soft, 100 Cambridge Park Dr., Cambridge, MA 02140, U.S.A.. A Spectronic 21D and Perkin Elmer Lambda 6 were the utilized UV-Visible spectrophotometers.

### Colorimetric assay

Stock solution of catechol agent (L-dopa, D-dopa, or carbidopa) is made in dimethylsulfoxide at 4.0 μg/μLiter. Stock reagent of 1.00 g/100 ml $FeCl_3 6H_2O$ is made in double distilled water (made

fresh each day). The test solution has total volume of 300 μliters comprised of 60 μliters ferric chloride reagent and liquid or solid samples dissolved in water up to 240 μliters. Mix thoroughly and allow four minutes for the reaction. Compare to Positive Controls comprised of known amounts of catechol analyte.

For dust samples either L-dopa and D-dopa was scattered over a flat dry surface and then collected by clean spatula into a sterile pre-weighed vessel. Sufficient water was added to make a solution of 2 μgrams/μL. Take 15 μL for sample 7 (D-dopa) and 22 μL (L-dopa) for sample 8 in figure 6 (repeated with similar results).

**Standard curve development**

Stock reagent of ferric chloride and stock solution of catechol agent is prepared as described in colorimetric assay. The final total volume of standards will be 4.00 ml in double distilled water. Volume of ferric chloride used is 800 μliters, with the remaining 3200 μliters comprised of added stock catechols (to obtain the desired concentrations at μg/ml) and/or the clear aqueous or solid samples dissolved in double distilled water. Mix well then read absorbances in suitable spectrometer at 400 nm wavelength after allowing four minutes for the complex formation. Convenient concentrations for standards range from 5.00 μg/ml to 200.0 μg/ml (a 40x range). If unknown samples are not colorless than prepare a control solution with an identical amount to the test mixture to determine absorbance at 400 nm prior to adding ferric chloride (the absorbance not due to iron(III) complexation is subtracted from total absorbance).

**Absorbance spectra of catechol-iron(III) complex**

In 3.00 ml total volume, 400 μg of L-dopa was dissolved with 500 μliters of 1.00 g/100 ml of $FeCl_3 \cdot 6H_2O$ made fresh and sufficient double distilled water for total volume of 3.00 ml. Absorbance spectra was obtained from 350 nm to 740 nm (absorbance goes quickly off scale at < 385 nm).

**Jobs plot development**

Total volume of all solutions is 3.00 ml, read absorbances at 400 nm utilizing water as blank, and use double distilled water throughout. The total moles of iron(III) and catechol agent in each solution will be 5.324E-07 moles. Amounts of catechol agent added can range from 4 μg to >100 μg, however the iron(III) must be varied for summation of the total moles to be 5.324E-07.

**Assay of sinemet tablets**

Obtain tablets (or other solid samples) and weigh to obtain the total mass per tablet (or other solid sample), then grind to fine powder in mortor/pestle. Commercial sinemet will have relative amounts of carbidopa and L-dopa listed as 25/100 (meaning 25 mg of carbidopa and 100 mg of L-dopa making the total catechol mass to be 82.78% of the total mass). Use <10% of total mass of ground tablet for Colorimetric Assay or analysis by standard curve. The ground tablets are handled as described above.

## RESULTS AND DISCUSSION

Dopamine along with norepenephrine and epinephrine falls within the category of neurotransmitters, known as monoamines or catecholamines, as a result of the major structural components that include a catechol and amine groups. The loss of dopamine production is associated with Parkinsons disease. Carbidopa is a hydrazine compound which is co-administered with L-dopa to inhibit the decarboxylation of L-dopa that would occur in the peripheral systems prior to penetrating the central nervous system.

The molecular structures of L-dopa, D-dopa, and carbidopa drugs are shown in figure 1 and their scientific nomenclature. The distinction of the L- and D- forms, also considered S- and R- respectively, is depicted in the positioning of the amine and hydrogen shown in the broken circles. The adjacent aromatic hydroxyl groups (or catechol) are indicated on all three molecules by inset arrows. The catecholic structure within the molecules is utilized for detection and determination of these therapeutic drugs.

Iron(III) is a transition metal cation which forms a large number of complexes in aqueous solution. Iron(III) complexes are octahedral and the octahe-

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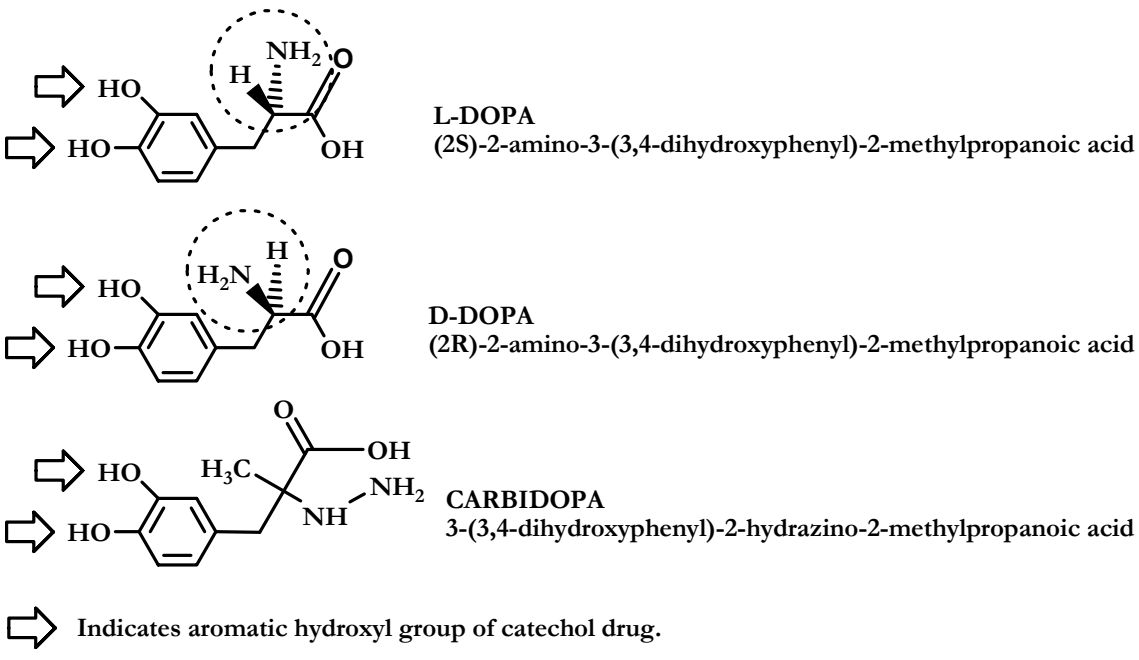


**Figure 1: Structures of L-dopa, D-dopa and carbidopa catechol drugs.**

**The molecular structures of catechol drugs are shown here with scientific notation and indication of the aromatic hydroxyl groups that form the catecholic substituent (see inset block arrows). L-dopa and D-dopa are members of the catecholamines, however carbidopa is a hydrazine compound.**

dron considered the characteristic polyhedron. The catecholic hydroxyl groups of dopa will coordinate as ligands with iron(III) into an octahedral species that generate a brownish mixture which follows Beers law. The absorbance spectra of the complex is presented in figure 2 from 740 nm to 360 nm. At about 370 nm the absorbance quickly increases (see arrow inset at 370 nm) off scale. The absorbance peak at 400 nm is sufficiently resolved to be effective as the wavelength for determining these drugs by standard curve. The extinction coefficient of a mixture having 0.167% $FeCl_3 \cdot 6H_2O$ with 6.76E-04 molar catechol results in $\varepsilon_{400\ nm}$=2910.38 liter/(mole/cm).

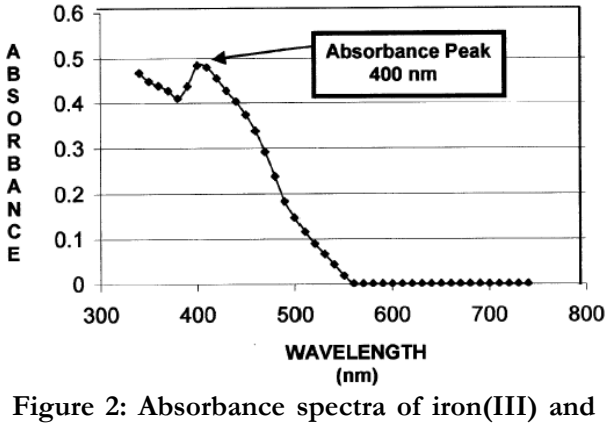


**Figure 2: Absorbance spectra of iron(III) and catechol complex.**

**This plot presents the absorbance spectra of the iron(III)-catechol complex which forms in aqueous solution. The absorbance peak is at 400 nm wavelength. The absorbances below 385 nm rapidly climbs off scale. Spectra was taken in 1 cm glass cells.**

The colorimetric assay can be accomplished in plastic or glass vessels. There is no gas evolution or heat generated. The iron(III) chloride stock reagent has no toxicity of significance and should be made fresh each day. If allowed to set undisturbed iron undergoes significant hydrolysis[8] and ultimately will form hydrous ferric oxide as a precipitate that appears as a red-brown gelatinous mass. Many compounds were found not to interfere with the formation of the distinctive brownish color which develops with these assays. A categorized list of non-intervening compounds is presented in TABLE 1, but is not exhaustive. Categories include medicinal agents, (Erythromycin, Streptomycin, Ampicillin, Aspirin and Quinine sulfate), organic compounds, and inorganic salts. This includes organic functional groups such as carboxylic acids, amides, amines, aromatic rings, and sulfonamides. Many inorganic salts

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**TABLE 1: Compounds which do not interfere with colorimetric development.**

| Medicinal Agents | Inorganic Salts | |
|---|---|---|
| Erythromycin | $CaCO_3$ | $K_2HPO_4$ |
| Streptomycin | $Na2CO_3$ | $Na_2B4O_7$-$10H_2O$ |
| Ampicillin | NaCl | $NaN_3$ |
| Aspirin | $NaH_2PO_4$ | LiBr |
| Quinine sulfate | | |
| **Organic compounds** | | |
| Benzenesulfonamide | Maleic Acid | |
| Sulfanilamide | Myristic Acid | |
| Caffeine | p-Tartaric Acid | |
| p-Nitrobenzoic Acid | Sulfamic Acid | |

do not interfere with detection including carbonate, halogenated, and phosphate salts ($CaCO_3$, $K_2HPO_4$, $Na_2CO_3$, $Na_2B_4O_7$-$10H_2O$, NaCl, $NaN_3$, $NaH_2PO_4$, LiBr). These findings supports the potential of these methods for the detection and measuring of L-dopa, D-dopa, and carbidopa; and for broad applications.

Following the steps of the colorimetric assay described in Materials and Methods a blind study was accomplished to determine the ability of detecting catecholic drugs in unknown aqueous solutions. A series of mixtures of L-dopa were prepared as described in TABLE 2, which remained unknown to an examiner. The examiner without knowledge of the amount and presence of analyte in the vessels then utilized the assay to detect positive samples (containing L-dopa) from the negative samples (no L-dopa). Results are shown in TABLE 2 which indicate the correct identification of L-dopa samples from the negative (no L-dopa) samples. This blind study was repeated with D-dopa and carbidopa with similar results.

Typical results observed by applying the colorimetric assay are presented figure 3. The distinct brownish colorimetric result is clearly seen with this group of test mixtures. The mixtures presented in micro-reaction tubes in figure 3 were constructed utilizing a stock reagent made by combining L-dopa and D-dopa ground to a fine powder (by mortor/pestle) at the ratio of 1:1 (each comprises 50% of mass). Tube A is a positive control having 200 μg dopa and tube B is a negative control having no dopa compound.

**TABLE 2: Results of blind study for the detection of catechol agents L-dopa, D-dopa and carbidopa by [1]colorimetric assay.**

| Amount of drug (micrograms) | Result |
|---|---|
| 28 | Positive |
| 60 | Positive |
| 0 | Negative |
| 100 | Positive |
| 80 | Positive |
| 40 | Positive |
| 0 | Negative |
| 0 | Negative |
| 40 | Positive |
| 80 | Positive |
| 120 | Positive |
| 28 | Positive |
| 32 | Positive |
| 64 | Positive |
| 28 | Positive |
| 0 | Negative |
| 48 | Positive |

[1]This study was repeated utilizing D-dopa, L-dopa and carbidopa with similar results.

Tubes C, E, G, and H are known controls having 16 μg, 36 μg, 100 μg, and 160 μg. The increase in brown colorimetric result as quantities increase is clearly seen. The color is easily seen by eye and dif-

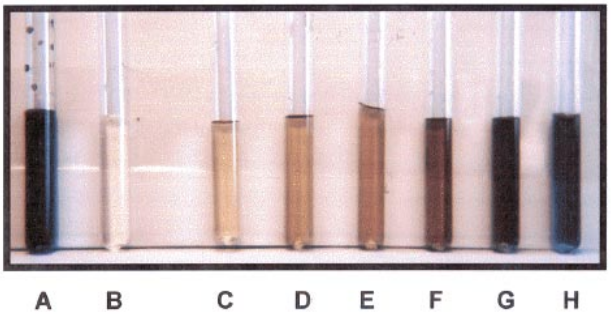


**Figure 3: Results of colorimetric assay for catechol drugs which indicate drug presence and approximates amount of drug by interpolative comparison.**
**Tube A is positive control having 200 μg dopa and tube B is a negative control having no dopa compound. Tubes C, E, G, and H are known controls having 16 μg, 36 μg, 100 μg, and 160 μg. Tubes D and F are unknowns having 28 μg and 60 μg, respectively.**

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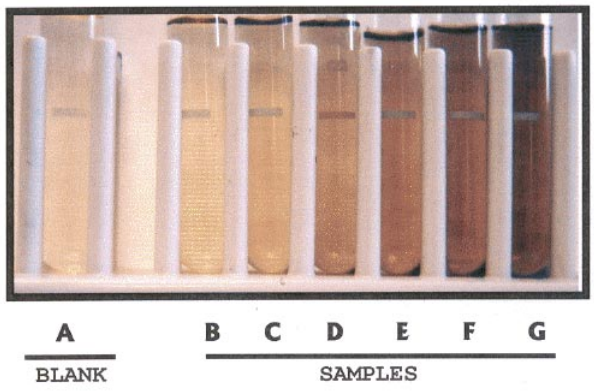



**Figure 4: Representative examples of solutions to be utilized for standard curve development.**
**Standard solutions are shown and arranged in order of increasing concentration of L-dopa. Tube A is a negative blank for zero of spectrometer (no L-dopa). Tubes B, C, D, E, F, and G have the concentrations of L-dopa in μg/ml, respectively: 15, 30, 45, 60, 75, and 90. Color increase is proportional to the amount of catechol (dopa) agent present.**

ferentiation is excellent, which allows quantitative estimation of unknowns by interpolative comparison. Tubes D and F are representative examples of dopa unknowns having 28 μg and 60 μg, respectively. Tubes D and F were readily identified by comparison with the known controls C, E, G, H, and in addition their amounts of catechol approximated by interpolation. Tube D clearly lies between tubes C and E having 16 μg and 36 μg, respectively. Similarly, tube F clearly lies between tubes E and G having 36 μg and 100 μg, respectively. In this manner unknown samples can be identified and estimated quite accurately if a larger number of known controls are utilized for comparison.

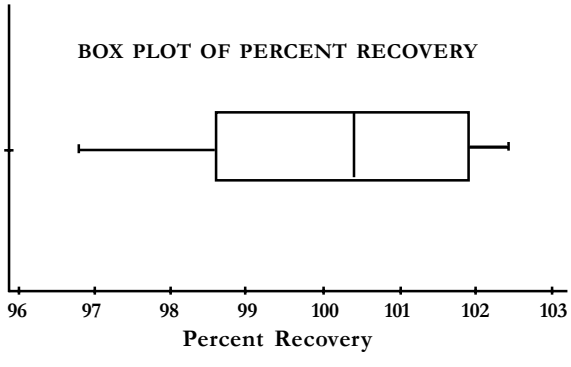
The development of a standard curve is accomplished for the purpose of analyzing multiple samples for these catechol agents. A representative group of standard solutions are shown in figure 4 and arranged in order of increasing concentration of L-dopa. Tube A is a negative blank for zero of spectrometer (no L-dopa). Tubes B, C, D, E, F, and G have the following concentrations of L-dopa (μg/ml), respectively: 15, 30, 45, 60, 75, and 90. Color increase is proportional to the amount of catechol agent present and clearly visible. Figure 5 shows an actual standard curve utilized to determine the catechol drugs accurately. The line is highly linear and reproducible. The correlation coefficient for the line in figure 5 is 0.9963, and the equation of the line is $y = 0.002(\mu g/ml)^{-1} x + 0.0315$ (slope = $0.002(\mu g/ml)^{-1}$ and y-intercept= 0.0315.

A representative group of mixtures quantified by standard curve are presented in TABLE 3, showing the mean experimental values from three attempts at each concentration level. The mean for experimental values obtained at many levels of concentration are compared to the actual values and percent recovery. Percent recovery ranged from 96.8% to

**TABLE 3: Concentration values of catechol drugs obtained by utilizing a standard curve**

| [1]Average Experimental Values (μg/ml) | Actual Values (μg/ml) | [2]Percent Recovered | Residuals |
|---|---|---|---|
| 18.2 | 18.8 | 96.8 | -0.659 |
| 21.9 | 21.5 | 101.9 | 0.353 |
| 24.3 | 25.0 | 97.2 | -0.754 |
| 30.6 | 30.0 | 102.0 | 0.55 |
| 38.9 | 38.0 | 102.4 | 0.857 |
| 43.4 | 44.0 | 98.6 | -0.638 |
| 50.7 | 50.0 | 101.4 | 0.667 |
| 59.1 | 58.0 | 101.9 | 1.074 |
| 63.8 | 65.0 | 98.2 | -1.22 |
| 72.3 | 72.0 | 100.4 | 0.286 |
| 79.3 | 80.0 | 99.1 | -0.707 |
| 84.1 | 85.0 | 98.9 | -0.903 |
| 91.1 | 90.0 | 101.2 | 1.101 |

**[1]Mean value of three attempts with errors ranging from <1% to 3.5% of actual value (ie. Standard deviation=0.61, Variance= 0.372). Correlation of average experimental values with actual values: r=0.999, $r^2$=0.999, standard error of estimate = 0.867, degrees of freedom = 11, t= 99.383, (P, one-tailed<0.0001, two-tailed <0.0001). Equation of best-fit line from ordinary least squares: y= a+bx, where a = -0.0171, b=1.00.**
**[2]Mean value of percent recoveries is 100.0, standard deviation=1.954. For 90% confidence interval = 99.03 to 101.0. For 95% confidence interval = 98.82 to 101.2.**



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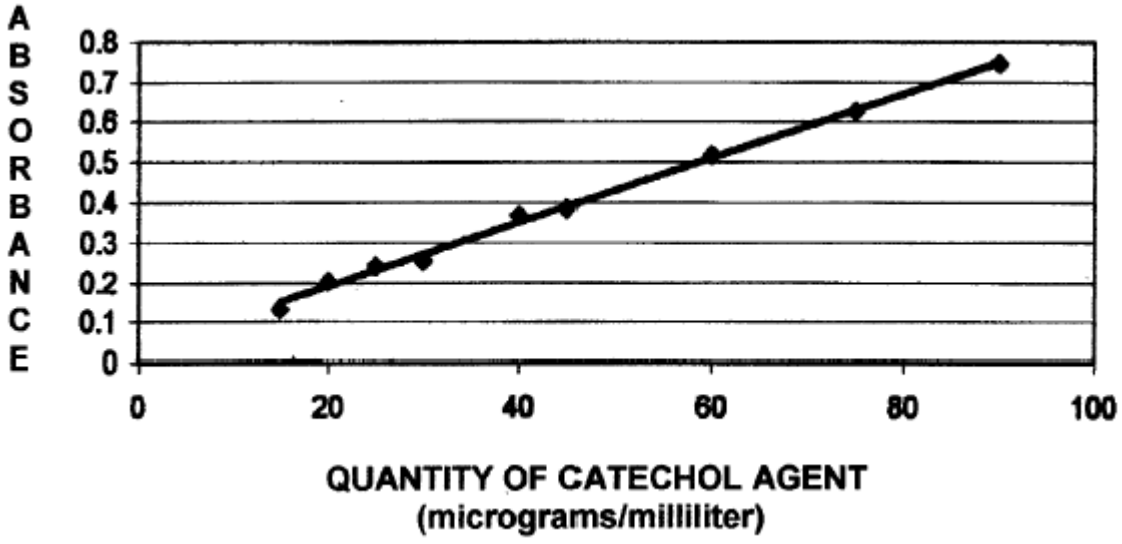


**Figure 5: Standard curve of absorbance versus quantity of total L-dopa, D-dopa and carbidopa present.**

**Standard curve is highly linear and reproducible. The correlation coefficient for the line is 0.9963, and the equation of the line is: $y = 0.002(\mu g/ml)^{-1} x + 0.0315$ (slope=$0.002(\mu g/ml)^{-1}$ and y-intercept= 0.0315).**

102.4%, indicative of good precision and accuracy. Mean value of three attempts having errors ranging from < 1% to 3.5% of actual value (i.e. Standard deviation= 0.61, Variance= 0.372). Correlation of mean experimental values with actual values ordinarily were high as in the data set shown in TABLE 3. $r = 0.999$, $r^2 = 0.999$ (Standard error of estimate= 0.867, degrees of freedom=11, t=99.383). Residual values for the linear regression analysis of Experimental values versus actual Values are presented in TABLE 3. The residuals indicate that the experimental results correspond to a normally distributed set of numerical values based on the following three observations: 1) There is a strong tendency for the variable to take a central value; 2) Positive and negative deviations from the central value are equally likely; and 3) The frequency of deviations falls off rapidly as the deviations become larger. For the percent recoveries a mean value of 100.0 was obtained for experimental values, a standard deviation of 1.954, and a 90% confidence interval of 99.03 to 101.0. Plotting experimental values (column 1) versus actual values (column 2) gave a best fit line of y=a+bx, with a=-0.0171 and b=1.00. Statistical analysis establishes that these drugs may be determined with a high level of accuracy and precision. A box plot provides an excellent visual summary of many important aspects of a distribution. A box plot of the raw percent recovery data (see TABLE 3) shows the tight clustering around the 100% recovery zone. Solid samples that are collected as particulate dust from an on site inspection (as surveys in an industrial manufacturing facility) or from preformed pharmaceutical preparations may be assayed by use of a similar standard curve.

Figure 6 demonstrates the application of the colorimetric assay for L-dopa and carbidopa found in CR sinemet tablets that are administered to Parkinsons patients. Total weight of a tablet of CR sinemet was 0.151 grams with 0.125 grams being the catechol agents of L-dopa (0.100 grams) and carbidopa (0.025 grams), by the manufacturers analysis. The percent of the tablet that is catechol agent is (0.125 g/0.151 g) or 82.78%. One tablet was ground finely in mortor/pestle and 0.013 grams dissolved into water to 2.00 ml total volume (giving 0.0108 g of catechol agent) with a 10 μL aliquot utilized in the colorimetric assay (see Materials and Methods). The amount of catechol drug in this test sample will then be 54 μg, which was assayed correctly and shown in figure 6 as tube 4 which lies clearly between 36 μg and 60 μg controls. In addi-

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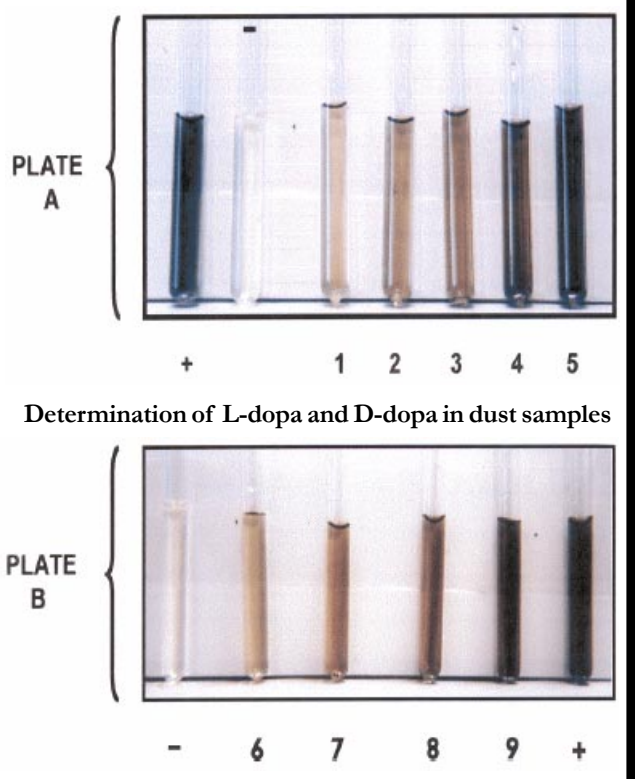


**Figure 6**

**PLATE A:(+) Positive control 80 μg L-dopa;(B) Negative control, no dopa; (1) 20 μg L-dopa; (2) 28 μg L-dopa; (3) 36 μg L-dopa; (4) Sinemet test at about 50 μg L-dopa; (5) 60 μg L-dopa.**

**PLATE B: (B) Negative control, no dopa; (6) 24 μg L-dopa; (7) Dustsample D-dopa about 30 μg D-dopa; (8) Dust sample L-dopa about 45 μg L-dopa; (9) 60 μg L-dopa; (+) Positive control 80 μg L-dopa.**

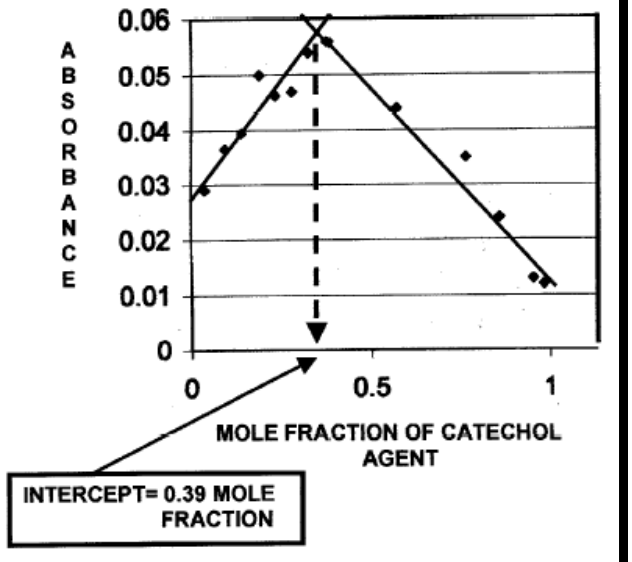


**Figure 7: JOB'S PLOT**

**Plot of absorbance versus mole fraction of catechol drug.**

**The intercept of the two lines that follow the course of the bisected data indicates the mole fraction of the catechol (dopa) agent in the complex. The x-axis intercept indicates a catechol mole fraction of 0.39, then the mole fraction for iron(III) is 0.61 or essentially a ratio of 1:1.5 (or 2:3) for catechol to iron(III), respectively.**

tion, a 20 μL aliquot (which is 108 μg of catechol agent) was taken from the 2.00 ml mixture above and measured by standard curve (see Figure 5) to an absorbance of 0.246, which indicates 107.3 μg catechol agent and a recovery 99.4% (an accuracy to within 1% of the actual value). This was repeated on another CR sinemet tablet with similar results.

These results demonstrate the utility of these colorimetric assays, which give high sensitivity and accuracy. Plate B of figure 6 shows the results of colorimetric assay of dust samples obtained as D-dopa or L-dopa and the approximation of amount catechol agent present by utilizing the colorimetric assay and applying interpolative comparison with known controls. Samples in Plate B are as follows: (**B**) Negative control, no dopa; (6) 24 μg L-dopa; (7) Dust sample D-dopa about 30 μg D-dopa; (8) Dust sample L-dopa about 45 μg L-dopa; (9) 60 μg L-dopa; (+) Positive control 80 μg L-dopa. The dust samples (7) and (8) clearly lie between known control (6) (24 μg L-dopa) and control (9) (60 μg L-dopa). The use of additional known controls would allow even greater accuracy and resolution of test samples.

To determine the stoichiometry of the iron(III)-catechol complex a Jobs plot was constructed and shown in figure 7. In a Jobs plot the ratio of reactants A and B are varied, but the total number of moles reactants remains constant. The intercept of the two lines that follow the course of the bisected data indicates the mole fraction of the catechol agent in the complex. The x-axis intercept indicates a catechol mole fraction of 0.39, which is followed then by a mole fraction for iron(III) to be 0.61 or essentially a ratio of 1:1.5 or 2:3 for catechol to iron(III), respectively. Positions of the polyhedron having iron(III) have been shown in previous work to be filled by coordinated $H_2O$[7,8]. Other methods of determining L-dopa

include a chemiluminogenic oxidation with acidic potassium permanganate to obtain a sensitivity to <1 μg/ml[11]. L-dopa is found in some vegetable material, which can be assayed by rapid reversed-phase HPLC to a sensitivity of percent of total mass injected (ie. mg per gram vegetable material)[12]. Electrospray LC/MS/MS has been shown to detect L-dopa with high sensitivity, depending on the overall instrument resolution, and shown to have limit of quantitation to approximately 3 ng/ml[13]. Chemireaction methods for determining L-dopa for flow analysis-spectrometry can has been accomplished in an alkaline mixture utilizing an imine for of p-aminophenol. Limit of L-dopa detection for this method was approximately 52 ng/ml. Previous studies have applied photochemical detection of L-dopa based on its strong inhibition of the photochemical reaction between phloxin and EDTA. By this method, L-dopa was detected to about 2 μg/ml[15]. As little as 1.00E-04 molar L-dopa is detectable by a kinetic potentiometric method with reaction to 2,4-dinitrofluorobenzene[16].

## CONCLUSION

In summation, colorimetric techniques for the determination of catechol agents L-dopa, D-dopa, and carbidopa include a rapid micro-reaction test and standard curve development. Both techniques are highly sensitive with detection limits of <15 μg/ml and <1.5 μg/ml, respectively. The brownish colorimetric endpoint is easily discerned by eye. The total catechol content of pharmaceutical drugs (ie. CR sinemet) can be determined with a high degree of accuracy when utilizing a standard curve and excellent level of sensitivity accompanying an approximation of amount when using the rapid colorimetric micro-reaction procedure. Solid or liquid samples may be analyzed. Many organic compounds such as aspirin, streptomycin, ampicillin, erythromycin, caffeine, and quinine sulfate do not interfere with the colorimetric endpoint. Many inorganic compounds such as carbonates and phosphate salts do not interfere with the identification and determination of catechol drugs. A Jobs plot indicated that the ratio of iron(III) and catechol drug is 1.5:1 or 3:2, respectively.

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