

Separation of levodopa using Nanotubes Carbon modified Methyl Amine in biological samples and determination by UV-Vis Spectrophotometry

Nilofar Tajodini ^{1,2}, Ali Moghimi ^{2*}, Kamelia Karimnezhad ³

¹ Department of Chemistry, Varamin(Pishva) Branch Islamic Azad University, Varamin, Iran. ² Department of Chemistry, Islamic Azad University, Tehran Medical Branch, Tehran, Iran. ³ Department of Chemistry, (UTM) university technology Malaysia, Malaysia.

Correspondence: Ali Moghimi, Department of Chemistry, Islamic Azad University, Tehran Medical Branch, Tehran, Iran. Email: alimoghimi@iauvaramin.ac.ir, kamran9537@yahoo.com

ABSTRACT

A new simple and reliable method for rapid and selective extraction and determination of trace levels of levodopa ion is developed. Thus, drug pharmaceutical control has become a routine technique in many laboratories. This project focuses on the enhancement of a method to determine insignificant amounts of levodopa in aqueous and biological solutions. This study looks at solid phase extraction of insignificant amounts of levodopa using carbon nanotubes modified methyl amine in aqueous samples, and ultraviolet and visible (UV-Vis) spectrophotometry measurement in biological samples. These systems include two phases – the aqueous donor phase and the conjugated carbon nanotubes modified methyl amine acceptor phase. Experiments are performed in two steps – aqueous phase extraction and levodopa resorption using methanol acidic solvent; resorbed samples are provided to UV-Vis spectrophotometry for further analysis. This method is cheap, simple, fast, and compatible with most instrumental analysis methods. Extraction parameters including resorbing organic solvent effect, pH of donor and acceptor phases, duration of extraction, duration of resorption, shaking time, volume of donor phases, and effects of surfactants are optimized and analysed; and measurements are performed under the optimized situation. The mentioned techniques have many advantages including short extraction time, low consumption of organic solvents, removal of effects of prior experiments, low diagnosis threshold, and high concentration factor. For levodopa, concentration factor and diagnosis threshold are 51 and 9.51, respectively. Linear domain and relative standard are 1.32%.

Keywords: levodopa, Solid Phase Extraction, Carbon Nanotubes modified methyl amine, Spectrophotometry.

Introduction

Biological samples or samples from natural sources often contain very complex compounds that interfere in analysis and measurements; sometimes, the amount of pollutant compounds is so low that it cannot be detected even with the strongest detection systems or it is incompatible with analysis processes. It is clear that some pollutants have hazardous biological effects even in low amounts; thus, it is necessary to develop very

sensitive, specific, and accurate methods to measure the amounts of such pollutants. Analytic devices such as chromatography, spectroscopy, and microscopy devices, as well as micro-sensors and devices have advanced, even though very accurate and non-destructive methods are not available in most cases. Thus, to enhance current methods, one or more preparatory steps of the sample are necessary [1].

It is obvious that an inappropriate method of preparation can affect the whole analysis procedure. Despite the high importance of this method, it has not received enough attention in recent years. Of course, sometimes it seems necessary to add additional steps such as derivation. Some of the common steps in an analysis procedure include the preparatory steps. This study intends to measure the interaction of resorbing nanoparticles and drugs using UV-Vis spectrophotometry. This method is based on drug and nano absorbers. Effective interaction parameters and effective measurement parameters are evaluated. Advantages include rapid analysis, feasible procedures, automation

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capability, analysis of low amounts, condensation and purging of samples, transfer and storage of samples, and using small amounts of organic solvents that are mostly hazardous [2]. L-DOPA, also known as levodopa and L-3,4-dihydroxyphenylalanine, is an amino acid that is made and used as part of the normal biology of humans, as well as some animals and plants. High performance of liquid high pressure chromatography is enhanced by the HPLC method for levodopa and three of its metabolites in plasma, serum, and urine analyses. A previously published HPLC method consisted of separating levodopa and the three metabolites in urine samples on a polystyrene column; quantitative analysis was performed using UV detector. The current procedure includes chromatographic separation, which is suitable for plasma and serum levels that are the same as the urine level [3]. A type of conjugated ofloxacin polymer is used for diffusing hard step matrix to determine enrofloxacin and levodopa in birds, and selectively enriching bird tissues; this substance is good enough for further analysis [4]. A solid phase extraction method is used to simultaneously detect enrofloxacin and its active metabolite, levodopa, in milk samples. Conjugated polymer is compatible with water and methanol integration, demonstrating enrofloxacin and levodopa in aqueous medium. Mean recycling of enrofloxacin and levodopa in milk samples with relative standard deviation (RSD) less than 1.9% were 0.52–1.39% and 2.55–5.34%, respectively. This method is easy and sensitive, and can replace available HPLC methods to analyze enrofloxacin and levodopa levels in biological samples [5]. For food safety control, remaining fluoroquinolones in pig meat should be measured; and compatibility of pollution levels with upper acceptable limit should be proved based on commission regulations updated in December 22, 2013. Solid phase extraction is widely used for antibiotic analysis of animal origin foods. In this paper, results of a comparative study using different solid phase extraction columns are presented for comparison of levodopa and enrofloxacin in pig meat. Additionally, a diverse anti-lipid, protein coagulation, removing positive ion and increasing ionic power have been used [1].

In this study, levodopa interaction with amino acids and complex formation constants are studied using spectrophotometry methods; and fluorescence spectrum of levodopa is obtained using a wavelength of 941 nanometers in the presences of each amino acid. Obtained spectrums of amino acids containing levodopa solution show lower intensity in 101–901 nanometers as compared to pure levodopa solution. Silence constant of the interaction of levodopa and amino acids is calculated by the Stern-Volmer equation. Formation constant is obtained using DATAN software, and results show the highest interaction between levodopa and aspartic acid with levodopa acting more selective against this amino acid. Aspartic acid (amino acid) with carboxyl R sub-chain having high acidic power acts as a strong proton donor, thus forming a hydrogen bond with levodopa; this results in lowering fluorescence property and some

hypsochromic movement. Quantitative calculations are performed on aspartic acid-levodopa complex for the estimation of interaction energy. Moreover, solvent causes changes in intensity, position, shape of absorption, and emission spectrums, based on their properties. The relationship between differences in energy level and polarity parameter of the solvent is assessed. Solvent polarity parameters play a role in the interaction between solvent and indicator. Soloautochomatic correlation is used to estimate the dipole moment in basic state (μ_g) and excited state (μ_e). Also, charging dipole moment ($\Delta\mu$) using soloautochomatic method is evaluated [2].

In this study, we are going to measure levodopa in real samples (blood plasma and urine) through interaction between the nano-adsorbent and drug by using a UV-Vis spectrophotometer. This method is based on the interaction between the drug and nano-adsorbent. Parameters affecting the interaction as well as the measurement will be examined.

Experiments

• Chemicals and Reagents

levodopa($C_9H_{11}NO_4$) is prepared according to the method used by Merck in Darmstadt, Germany. It is dried for a week over phosphorus pentoxide in vacuum desiccators before use. Multiwall carbon nanotubes modified methyl amine from Merck are prepared [6]. All solutions are prepared with doubly distilled deionized water, also from Merck. It is conditioned before use by suspending in 4 M nitric acid for 20 minutes and then washed two times with water. Substances and solvents used for the preparation of solutions and standards are highly pure in analytical terms.

• Instruments

Double beam UV-Vis spectrophotometer (model UV1700) is used in the Razi Laboratory, University of Science and Research. Conditions are tabulated in pH measurements using a Sartorius model (PB-11). Digital scale with 0.1 milligram accuracy (AND GR-200 model) is used.

• Synthesis Method of Carbon Nanotubes modified methyl amine

An amount of 0.5233 gram of multiwall raw carbon nanotubes modified methyl amine is added to 4 to 3 nitric acid to sulfuric acid. The solution is kept in an 11 kHz ultrasonic bath for 31 minutes; then, it is refluxed in an agitator for 31 hours. The obtained product is washed with distilled water until its pH reaches 9. Solid phase is separated and dried in vacuum for 43 hours in 61 degrees centigrade. MWCNT-COOH, produced in the second step, is mixed with 31 milliliters of ethylene di amine (mixed for nine hours in an 11k Hz ultrasonic bath). The obtained mixture is agitated for 31 hours at 61 degrees centigrade; 1.33 micron millipore polycarbonate is separated in a membrane filter

and the solid product is washed with water-free methanol. The obtained solid product is dried overnight in vacuum so that MWCNT-NH is produced^[7].

• Method of Preparation

In this study, new solid phase techniques are used to detect insignificant amounts of levodopa using carbon nanotubes modified methyl amine in aqueous samples and UV-Vis spectrophotometry in biological samples. These techniques are part of a biphasic system – the aqueous donor phase and the conjugated carbon nanotubes modified methyl amine acceptor phase. Experiments are performed in two steps – aqueous phase extraction and levodopa resorption using methanol acidic solvent; the resorbed samples are further analyzed by UV-Vis spectrophotometry. This method is cheap, simple, fast, and compatible with most instrumental analysis methods. Extraction parameters including resorbing organic solvent effect, pH of donor and acceptor phases, duration of extraction, duration of resorption, shaking time, volume of donor phases, and surfactant effect are optimized; and analysis and measurements are performed under the optimized situation.

• Primary Experiment: Absorbent Effect of levodopa Extraction

The following steps are performed to assess the effect of amine or carbon nanotubes modified methyl amine. For each experiment tube, buffer (in the range of 10–2) is added. Since the goal is to select an appropriate absorbent, the lower absorption is considered to be the best value. An amount of 5 ml of the drug with 50 pm concentration for levodopa is taken. Then, 1.15 g of amine conjugated carbon nanotubes modified methyl amine and 5 ml of buffer in the range of 2–10 are taken, and added to a 50 ml balloon and delivered to volume with distilled deionized water. Thereafter, the balloon is shaken for 51 minutes, centrifuged for 15 minutes, and passed through syringe filters; finally, a quantitative analysis of levodopa takes place. The same procedure is conducted for carbon nanotubes modified methyl amine. Quantitative analysis of filtered solutions for levodopa is performed using UV-Vis spectrophotometry in a wavelength of 200–500 nm.

After conducting primary experiments and ensuring the efficacy of conjugated carbon nanotubes modified methyl amine in levodopa extraction, efforts are made to enhance the efficacy of this method as described.

• Optimizing Wavelength in Extraction of levodopa

This medication with carbon nanotubes modified methyl amine and 2–10 buffer range undergoes quantitative analysis using a UV-vis spectrophotometer, after being shaken and centrifuged

• Effect of pH on levodopa Extraction

The following steps are initially performed to assess the effect of pH on levodopa extraction. The desired tampon is added for each container since the goal is to determine the appropriate pH. The lowest absorption is considered to be the best value.

An amount of 50 ml of the drug with 500 pm concentration for levodopa is taken. Then, 1.15 g of carboxyl absorbents and 5 ml of buffer in the range of 2–10 are taken, and added to a 50 ml balloon and delivered to volume with distilled deionized water. Thereafter, the balloon is shaken for 15 minutes, centrifuged for 15 minutes, and passed through syringe filters; finally, a quantitative analysis of levodopa takes place.

• Amount of Absorbent in levodopa Extraction

In this step, optimized pH and absorbent are used in the optimized wavelength based on previous experiments; different amounts of absorbent (3, 5, 10, 12, 15, and 20) are used for each medication.

An amount of 5 ml of the drug is taken. Then, 0.15 g of carbon nanotubes modified methyl amine and 5 ml of buffer in the range of 2–10 range are taken, and added to a 50 ml balloon and delivered to volume with distilled deionized water. Thereafter, the balloon is shaken for 15 minutes, centrifuged for 15 minutes, and passed through syringe filters; finally, a quantitative analysis of levodopa takes place.

Quantitative analysis of filtered solutions for levodopa is performed in a wavelength of 272 nm using a UV-Vis spectrophotometer.

• Salt Effect

The other important parameter is salt; this is due to paired ion function between reactants that leads to better reaction between substances. It is also very important in absorption intensity. An amount of 0.20 g is selected as the optimum value for levodopa. In this step of the experiment, optimum pH, absorbent, and wavelength are used. Different amounts of salt are used for the drug.

• Effect of Drug Absorption Time in Solution

Another important parameter of the absorption system and drug measurement based on their extraction is the reaction rate. Five solutions are prepared with optimum properties and shaking is performed at different times. Then, they are centrifuged and passed through a filter, and their absorption rate at maximum wavelength is checked.

• Effect of Type of Elution Solvent

The type of elution solvent is one of the most important parameters affecting the absorption system. In this study, for each medication, methanol, ethanol, acetonitrile, acidic and basic methanol, and acidic and basic ethanol are used; and optimum

solvent is detected for each medication. After choosing the optimum solvent, acidic or basic form of the solvent is assessed. Seven balloons are used (by considering optimum conditions) and the upper water is removed after centrifuging; then, the solvents are added. After shaking for 20 minutes and centrifuging for 15 minutes, solutions are filtered; then, their absorption at maximum wave length is recorded and resorption takes place. Due to resorption, the highest absorption should be selected.

• Effect of Volume of Elution Solvent

The volume of elution solvent is another effective parameter impacting the absorption intensity of the system. In this study, different volumes of selected solvents are assessed. Amounts of 5, 7, 10, 12, 15, and 18 ml of the solvent are added to the absorbent in optimum conditions. Absorption intensity is recorded with an UV-Vis spectrophotometer after shaking for 20 minutes and centrifuging for 15 minutes.

• Determining Limit Volume and Condensation Factor

To determine the limit volume, different solutions of levodopa are prepared with 50, 100, 150, and 200 ml volumes. Then, 100 ml is selected as the limit volume.

• Analytical Properties

After optimizing all parameters affecting absorption intensity, calibration curve of the method is drawn. For this purpose, a 10 ml volumetric flask is filled with different concentrations of the medication. Then, sodium choroid salt is added to 0.12 g levodopa in percentage form and carbon nanotube absorbent is added to the volumetric flask with optimum pH. Finally, it is delivered to volume by adding distilled deionized water. Then elution and ... steps are performed. Finally, absorption intensity of solutions is recorded at laboratory temperature and the calibration curve is drawn.

• Calculation of Limit of Detection (LOD)

Generally, LOD of a laboratory substance is considered to be the concentration at which device response is significantly different from control or background. In analytical chemistry, LOD is commonly defined as the concentration of a substance with a response equal to three times the control standard deviation (S_b) according to the following equation:

$$LOD = \frac{3S_b}{m}$$

To calculate the LOD for levodopa measurement, four control solutions with optimum situations are prepared without adding the medication, and absorption intensity is recorded at the peak wavelength of medication absorption as follows:

$$LOQ = \frac{10S_b}{m}$$

• Accuracy of Percentage of RSD Method

This parameter is used to evaluate experiment accuracy and closeness of study data. To assess the accuracy of this method (based on RSD), absorption intensity is measured in one day for four solutions of levodopa. For this purpose, four standard solutions with optimum concentrations are prepared based exactly on the purposed method.

• Evaluating Disturbing Species and Selectivity

The effect of disturbing species in the measurement of levodopa is evaluated based on biological matrix under optimum conditions. For this purpose, medication sample is mixed with different concentrations of disturbing species; the measurement is performed one hour later and absorption intensity is compared with the pure medication sample. Fluoxetine drug is the disturbing species.

• Preparation of Biological Samples for levodopa Measurement

Blood samples are taken from patients and put into EDTA-containing tubes with a volume of 3.9 ml. The samples are centrifuged at 3000 RPM for 30 minutes. The yellow fluid above the samples (plasma) is taken. To ensure there is no protein in the plasma, 10 ml of acetone is added to 10 ml of plasma and centrifuged at 3000 RPM for 10 minutes so that excess proteins coagulate. For measurement with the proposed method, a definite volume of plasma is taken and various measurement steps are performed.

• Urine Samples for levodopa Measurement

Human urine samples are taken and filtered. They are stored in black glass containers. To perform measurement with the proposed method, a definite volume of urine is taken and measurement steps are performed.

Discussion and Results

• Assessment of Fourier Transform Infrared Spectroscopy (FT-IR) Spectrum Results

This combination of two tensile vibration strips belongs to C-O bond of carboxyl carbon, which is observed in a 1559 cm^{-1} area. On the other hand, two tensile vibration strips belonging to C=O bond in the carboxyl group can be observed in a 1653 cm^{-1} area.

Finally, there is a strong vibration in a 3409 cm^{-1} area belonging to O-H bond tensile vibration (Figures 1 and 2).

Emergence of absorption peaks in 1653 and 1019 cm^{-1} obviously introduce carboxyl groups on carbon nanotubes modified methyl amine.

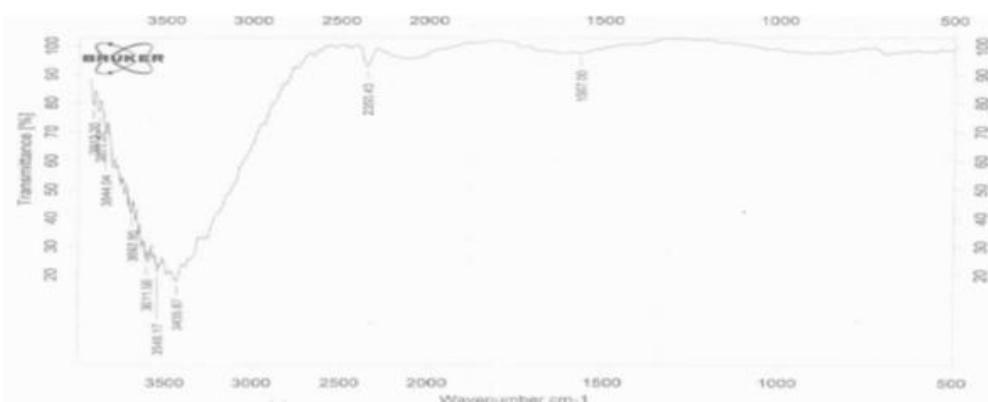


Figure 1- Fourier transformation of infrared spectrum of carbon nanotubes modified methyl amine before absorption

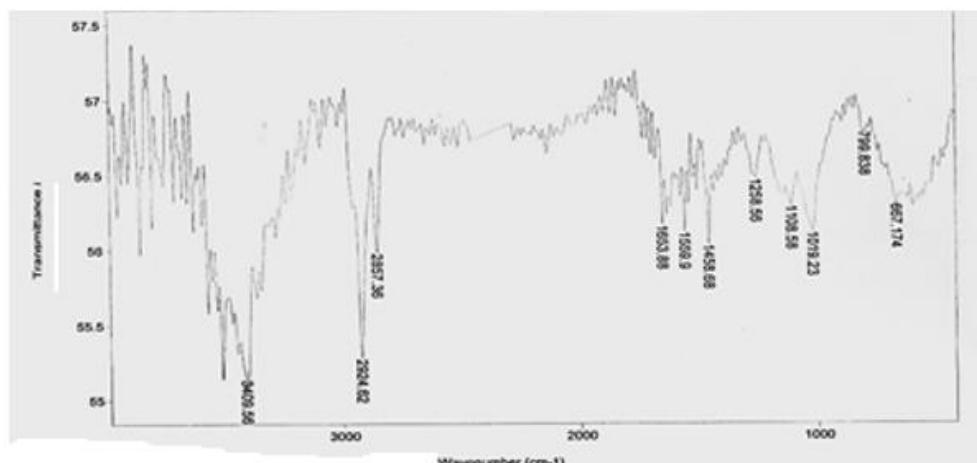


Figure 2- Fourier transformation of infrared spectrum of carbon nanotubes modified methyl amine after absorption

• Assessment of X-Ray Diffraction (XRD) Spectrum Results

In the XRD spectrum below, there is a combination of a very high intensity peak in $\Theta=26.8$ belonging to carboxyl nanotubes

modified methyl amine, and a very low intensity peak in 42.2 region having very sharp and small peaks, respectively (Figures 3 and 4).

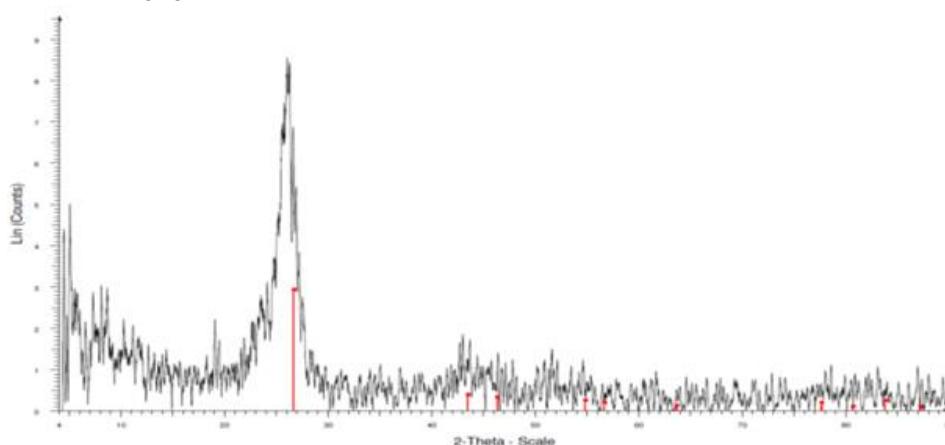


Figure 3- XRD spectrum of carbon nanotubes modified methyl amine before absorption

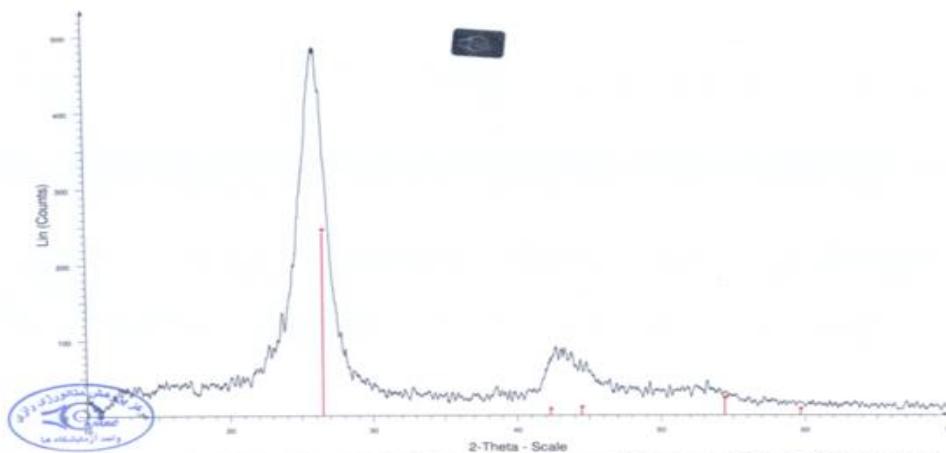


Figure 4- XRD spectrum of carbon nanotubes modified methyl amine after absorption

- **Assessment of Scanning Electron Microscope (SEM) Spectrum Results**

SEM picture of carbon nanotubes modified methyl amine are depicted in the figure below. For carboxyl nanotubes modified methyl amine, particles with 200 nm size are produced.

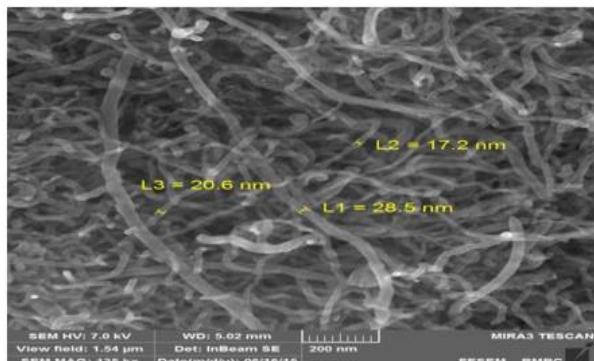


Figure 5- SEM picture of carbon nanotubes modified methyl amine before absorption

Moreover, SEM picture after absorption shows the placement of desired metal on carbon nanotubes modified methyl amine from which it is inferred that the width of sheets increases. As seen in the picture, carboxyl functional groups are shown as the brighter points on the surface of carbon nanotubes modified methyl amine (Figures 5 and 6).

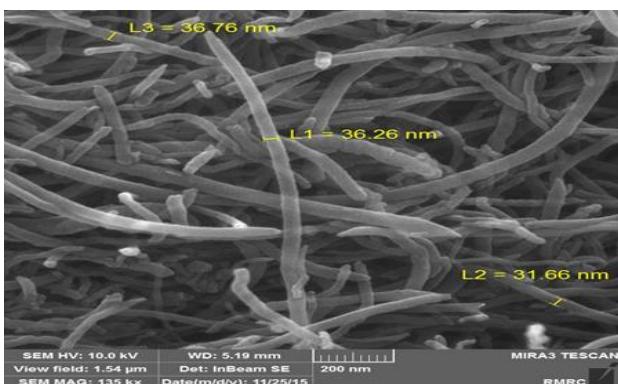


Figure 6- SEM picture of carbon nanotubes modified methyl amine after absorption

- **Primary Experiment: Absorbent Effect on levodopa Extraction**

According to the chart, carbon nanotubes modified methyl amine show better absorption. Consequently, carboxyl absorbent is selected for levodopa extraction.

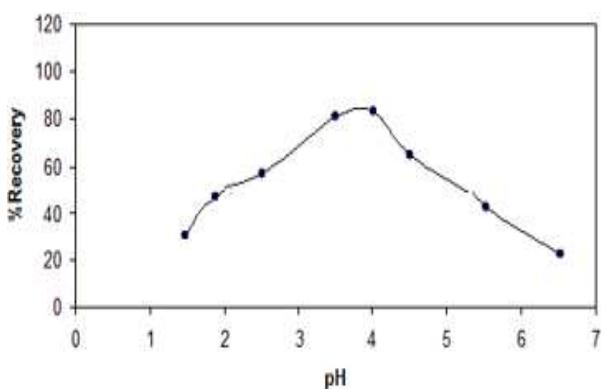


Figure 7- The curve of changes in levodopa absorption with regard to pH

All spectrums are put together in a wavelength of 272 nm. As a result, this wavelength is determined as the optimum absorption wavelength.

• Effect of pH on levodopa Extraction

The chart shows that pH=4.0 is appropriate for protonating carbon nanotubes modified methyl amine, which is associated with higher levodopa absorption on carbon nanotubes modified methyl amine. Thus, the best electrostatic situation for absorption and the drug for surface attraction are present at pH=4.0

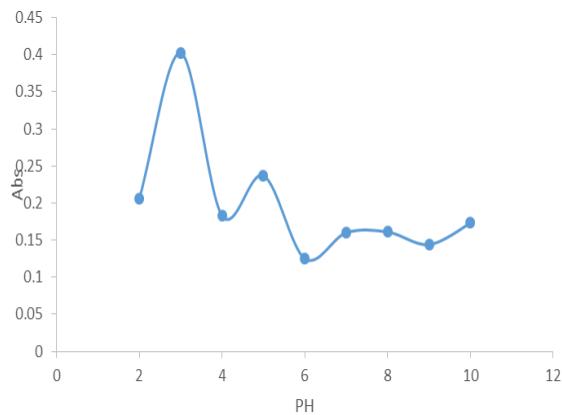


Figure 8- The curve of changes in levodopa absorption with regard to pH

• Amount of Absorbent in levodopa Extraction

Another parameter affecting absorption is the amount of absorbent. For levodopa, an amount of 0.12 g is selected. Reported results indicate that in a lower amount of absorbent, some substances may enter the solution since absorption can take place in the drug at maximum wavelength.

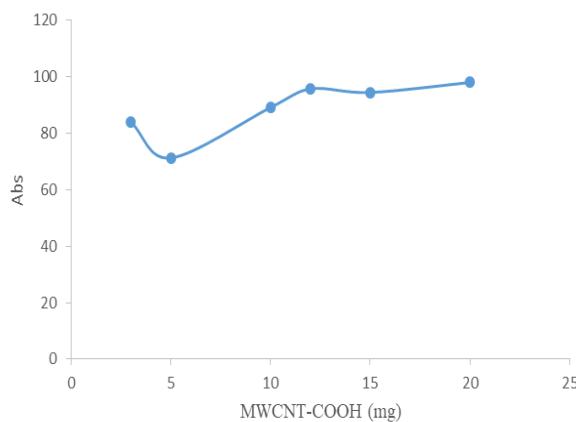


Figure 9- The curve of changes in levodopa with regard to the amount of absorbent

• Salt Effect

The chart shows that adding 0.20 g sodium chloride produces appropriate electrostatic charge on the absorbent and drug

sample for levodopa extraction; and 2% W/V is the optimum salt concentration showing the highest drug absorption.

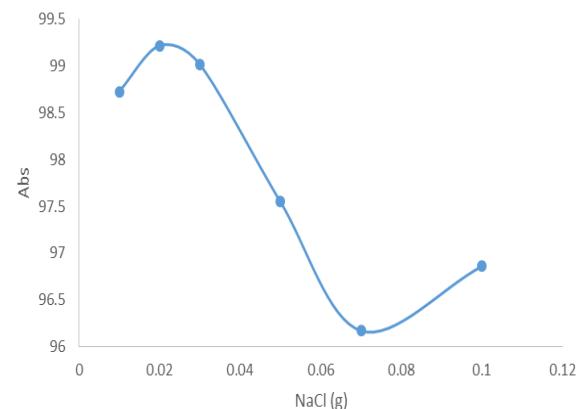


Figure 10- The curve of changes in levodopa with regard to the amount of salt

• Effect of Time of Drug Absorption in Solution

The chart shows that as the duration of exposure of absorbent and drug increases, equilibrium condition gets better; and after that no changes take place in the concentration of the medication. Twenty minutes was selected as the optimum reaction time for levodopa.

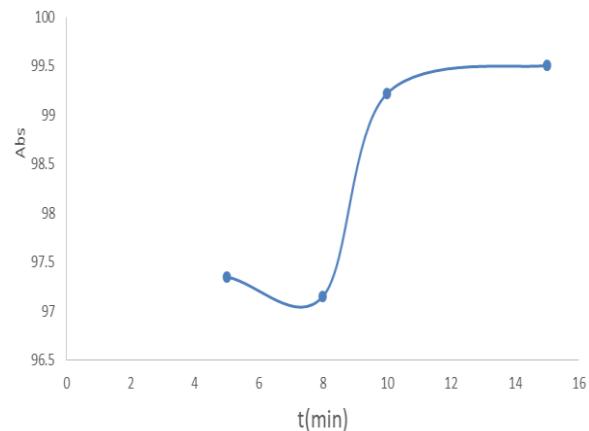


Figure 11- The curve of changes in levodopa with regard to the time of drug absorption

• Effect of Type of Elution Solvent

According to the chart, basic solvents show best conditions in the equilibrium between absorbent and elution solvent. Consequently, basic methanol is selected as the optimum solvent for highest absorption.

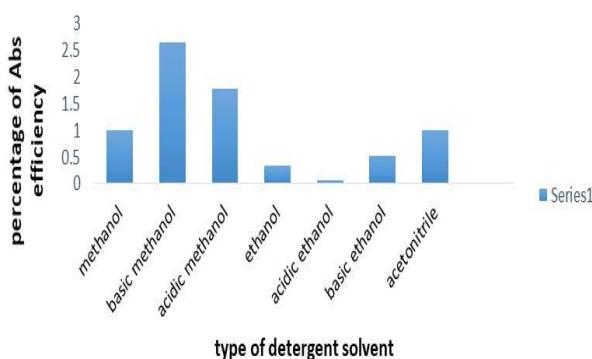


Figure 12- Effect of type of desorption solvent

• Effect of Volume of Elution Solvent

According to the chart, all medication enters the elution solvent, equilibrium goes to the side of the solvent, and complete resorption takes place for volumes above 10 ml. Thus, 10 ml is selected as the optimum volume for levodopa.

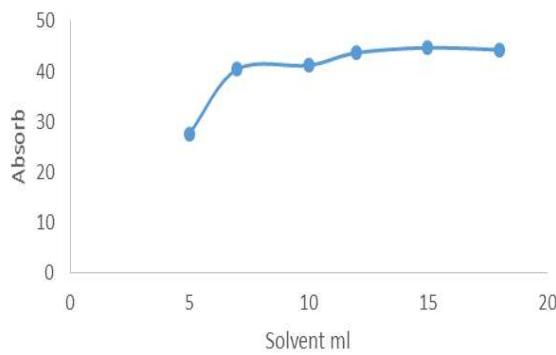


Figure 13- The curve of changes in levodopa with regard to volume of elution solvent

• Determining Limit Volume and Breakthrough Volume

According to the chart, the possibility of complete absorption on the absorbent decreases as the medication is diluted. Thus, 100 ml is selected as the limit volume for levodopa.

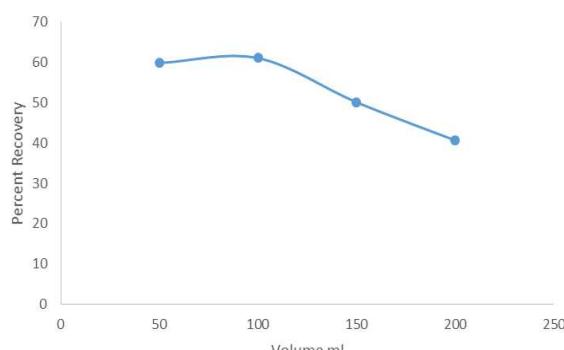


Figure 14- The curve of changes in levodopa with regard to breakthrough volume

• Calibration Curve for levodopa Medication Method

According to the obtained results in optimum conditions, there is a linear relationship between absorption intensity and concentration of the drug.

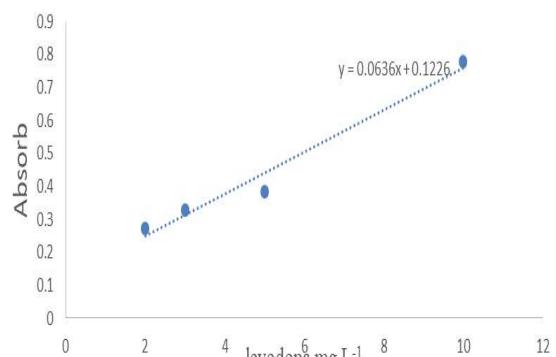


Figure 15: Calibration curve of levodopa

• Calculation of LOD

$$LOD = \frac{3S_b}{m}$$

Since the slope of the calibration diagram is equal to 3/3000, LOD is calculated as follows:

$$S_b = 3/3382; C_{LOD} = 42.0$$

Using the above data, limit of quantification (LOQ) can be calculated as follows:

$$LOQ = \frac{10S_b}{m}$$

$$C_{LOQ} = 3.32 \text{ ppb}$$

• Accuracy of Percentage of RSD Method

This parameter is used to evaluate experiment accuracy and closeness of study data. RSD percentage is calculated by measuring absorption intensity at the maximum wavelength of each drug in one day.

$$S_b = \sqrt{\frac{\sum (A_i - \bar{A})^2}{n-1}}$$

$$\%RSD = \frac{S}{m} \times 100$$

$$\text{Mean} = 3.201; \text{Standard Deviation} = 3.34$$

$$\%RSD = 0.96$$

Fluoxetine is the disturbing species and more disturbance is observed in higher concentrations. The absolute amount of disturbing species is decreased by dilution. In this step, we are reassured that the amount of added drug and the amount of found

drug in plasma and urine are the same, which shows that the accuracy of this method is acceptable.

Table 1- Recovery of levodopa added to 1000mL of different water samples (pH= 10.0).

Sample	levodopa added (µg)	levodopa determined(ng.mL ⁻¹)	ND
urine	0.0	1.76 (2.1) ^a	ND
	10.0	11.60(2.5)	11.6
plasma	0.0	4.48(2.4)	ND
	10.0	14.67(2.4)	14.9

^a Values in parentheses are %RSDs based on five individual replicate analysis

^b Not detected

Conclusion

In the methodology of the conducted research and presented results in previous chapters, diffusing solid phase extraction technique and UV-Vis spectrophotometer, and precondensation and measurement of slight amounts of levodopa in biological samples are used. The intent of this study is to develop a highly efficacious, selective, cheap, and simple method for evaluating the amount of levodopa in biological samples. In recent years, the development of the solid phase extraction method has introduced the need for an effective absorbent^[41-51]. Thus, in this study, carbon nanotubes modified methyl amine are used to enhance the yield of levodopa extraction. Effective parameters are assessed including pH, type and concentration of buffer, amount of absorbent, type and volume of elution solvent, rate (time) of reaction, and salt effect. This method has good repeatability, wide linear range (0.1–10 ppm), and proper condensation factor to determine levodopa. It also has a LOD of 6.6 ppb. According to the results of Table 1, the superiority of this method (as compared to other methods) is that the absorbent used has high characteristic surfaces, which is a key factor in choosing this material, and can be recovered. Another advantage is that the LOD is less than in other methods. It also has a concentration factor better than in many other methods. Moreover, the technique is easy and has high accuracy^[8-40].

Acknowledgements

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