Analytical Methods

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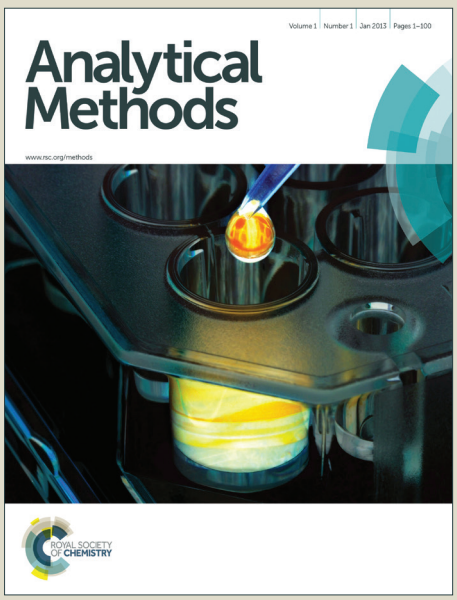

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preparation of Carboxymethyl-α-Cyclodextrin Polymer Grafted onto Nano TiO_2 as a Novel Solid Phase Extraction Sorbent based on Host-Guest Mechanism

Nafiseh, Khoddami, IKIU*, Qazvin, Iran

Farzaneh, Shemirani, TU, Tehran, Iran

*Faculty of Science, IKIU, Qazvin

E-mail: nafiseh.khoddami@yahoo.com

Tel: +98 28 3378 0021

Fax: +98 2833780021

ABSTRACT

Carboxymethyl-α-Cyclodextrin polymer grafted onto nano TiO_2 (CM-α-CD PG/nano TiO_2) is fabricated and its feasibility for determination of Levodopa (L-DOPA) was investigated. The synthesized sorbent characterized by Fourier Transform Infrared (FT-IR) Spectroscopy, Scanning Electron Microscopy (SEM) and Transmittiom Electron Microscopy (TEM). The adsorption mechanism is based on the host–guest effect between L-DOPA and CM-α-CD PG reserved on nano TiO_2. The selectivity is due to the size of L-DOPA and also, the hydrophobic interaction of CM-α-CD polymer with this compound. The experimental conditions were optimized for the separation/determination of L-DOPA. Under the optimum conditions, the adsorption capacity of CM-α-CD PG/nano TiO_2 for L-DOPA and preconcentration factor were 63.8 µg g^{-1} and 20, respectively. The limit of detection (LOD), relative standard deviation (RSD), and linear range were 0.016 µg mL^{-1}, 2.3%, and 0.05-1.3 µg mL^{-1}, respectively.

Keywords: L-DOPA, Carboxymethyl-α-Cyclodextrin Polymer, Titanium Dioxide, Solid Phase Extraction

INTRODUCTION

Solid Phase Extraction (SPE) is an approach to overcome the interference problems in complex systems. The advantages of this method include high recovery, rapid phase separation, low cost without excess consumption of organic solvents, and the capability to be combined with different detection techniques in on-line or off-line mode.[1]

Recently, some novel functional materials such as bio[2], ion-imprinted[3], mesoporous, magnetic, and nano-sized reagents[4,5] have been extensively used in SPE. Among different nano structures, nano TiO_2 has great analytical potential in SPE. However, nano TiO_2 is not selective and therefore not suitable for samples with complicated matrices[6]. In order to improve the selectivity, a modification of the adsorption material is required, such as surface imprinting chlorogenic acid (CGA)[7], ionic liquid-coated[8], and nano B_2O_3/TiO_2[9]. Cyclodextrins (CDs) are macrocyclic carbohydrate, toroid-like shape, consisting of glucopyranose units linked by 1,4-glucosidic bonds. The most common cyclodextrins α, β and γ contain 6, 7, and 8 glucopyranoside units, respectively. The internal cavity exhibits hydrophobic properties, which enable complication of a variety of hydrophobic guest molecules. Mentioned properties make these componds promising for applications in drug carrier systems, nanoreactors, bioactive supramolecular assemblies, molecular recognition, and catalysis[10,11,12]. The size of the guests is the most important factor in host-gust mechanism. Also, the charge and polarity of the guest molecules would influence the complex formation. Highly water-soluble, hydrophilic and hydratable guests are not suitable for this mechanism[13,14].

L-DOPA, beta-(3,4-dihydroxyphenyl)-l-alanine is the precursor of dopamine, which is an important neurotransmitter in the brains and bodies of mammalian[15,16,17]. L-DOPA is widely used as a source of dopamine in the treatment of Parkinson's disease[18,19]. Parkinson's is a chronic, progressive neuro-degenerative movement disorder that occurs when the substantia nigra dies and fails to produce enough dopamine. However, long-term use of L-DOPA can produce serious side

effects such as gastritis, paranoia, and dyskinesia. Therefore, an accurate analysis is necessary in both pharmaceutical formulations and biological fluids[20,21,22].

Different approaches, including titration, spectrometry and HPLC have been reported for L-DOPA determination. All these methods are complicated and time consuming sample pretreatment. Also, as other catecholamine compounds, L-DOPA is determined by electrochemical routes[23]. The electrochemical oxidation of L-DOPA has been studied mostly on the carbon electrode with different chemical modification, e.g. gold nanoparticles and nafion[24], modified carbon nanotube paste electrode[25], electrode modification with trinuclear ruthenium amine complex (Ru-red) supported on Y-type zeolite[26], and poly (xylenol orange) film[27].

The main problem for electrochemical analysis of L -DOPA is the interference of uric acid (UA) and ascorbic acid (AA). L-DOPA, UA and AA are oxidized at nearly the same potential and cause a serious interference in the voltammetric L-DOPA determination[28,29,30,31].

In the current work, a novel selective nano carboxymethyl-α-cyclodextrin grafted onto nano TiO_2 were synthesized and used to investigate the adsorption characteristics of L-DOPA. The selectivity is due to the size and hydrophobic characteristics of L-DOPA in comparison with other interferences. Finally, simple and convenient spectrophotometric method used for L-DOPA determination.

MATERIAL AND METHOD

Apparatus

A pH-meter Model 692 from Metrohm equipped with a glass combination electrode was used for the pH measurements. A Field emission scanning electron microscope (FESEM), model S-4160 was used for preparation of SEM images. Fourier transform infrared spectra (FT-IR) were recorded from a KBr disk using an Equinox 55 Bruker with the ATR method over the wavelength

range of 400-4000 cm^{-1}. The Hitachi transmittiom electron microscopy (TEM), Model HT7700 120kV was used for preparation of TEM images. UV/Visible spectra were measured by means of Perkin Elmer UV/Visible spectrophotometer lambda 25 with 10 mm quartz cells.

Reagents and solutions

All chemicals and reagents used in this work were analytical grade. Alpha-cyclodextrin was purchased from Sigma–Aldrich. (St. Louis, USA). Nano TiO_2 was purchased from Sigma–Aldrich. Sodium hydroxide and monochloroacetic acid were Merck. Also, K_2HPO_4 and KH_2PO_4 for preparation of buffer solutions were Merck.

A 300 µg mL^{-1} of L-DOPA was prepared as stock to be used in preparation of other standard solutions 100 mL volumetric flasks.

Preparation of CM- α–CD polymer

First, CM-α-CD polymer was prepared following the procedure as described in literature 11. α-CD (7g) was dissolved in 30 mL of 10% (w v^{-1}) NaOH and 8 mL of epichlorohydrin were added. The system was vigorously stirred for 8 h, another 3 mL of epichlorohydrin added with stirring and the mixture kept overnight at room temperature. The solution was concentrated and precipitated by addition of cold methanol (100 mL). White gummy precipitate was then washed with ethanol and acetone and dried under high vacuum overnight.

Then, 3 grams of the above polymer were further dissolved in 60 mL 5% (w v^{-1}) NaOH and 2.37 g of monochloroacetic acid were added. The system was vigorously stirred for 5h at 50 ◦C, the reaction mixture was cooled to the room temperature, and pH was adjusted about 6.5 with 2 M HCl. This solution was then poured into 100 mL methanol. The gummy precipitate washed with ethanol and acetone and dried under vacuum overnight.

Modification of nanoTiO_2 with CM-α–CD polymer

0.8 g of nano TiO_2 and 15 mL distilled water were mixed and stirred for 15 minutes. Then, the pH of the mixture was adjusted in 3.0 by HNO_3 and sonicated for 10 min. 1.2 g of CM-α-CD polymer was added to the mixture and stirred for 12 h. Then, it was centrifuged and washed with distilled water and dried.

The scheme of these consecutive reactions is shown in Figure 1.

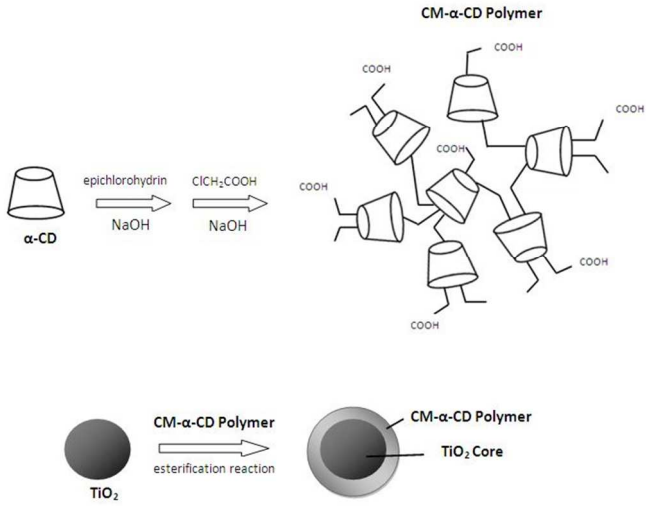

Figure 1. Scheme for preparation Carboxymethyl-α-Cyclodextrin polymer grafted onto nano TiO_2 (CM-α-CD PG/nano TiO_2)

Adsorption of L-DOPA on modified nano TiO_2

An analytical solution containing appropriate amounts of L-DOPA and 0.3 g CM-α-CD PG/TiO_2 was mixed. The mixture was shaken on a timing multifunctional oscillator for about 45 minutes and then placed in a centrifuge with 4000 r/min for 20 minutes. L-DOPA in the water phase was detected by UV-Visible spectrophotometry (λ max=280 nm) a typical spectrum is shown in Figure 2. For elution of adsorbed L-DOPA, CM- α –CD PG/nano TiO_2 was eluted by 3.0 mL of NaOH. The amount of L-DOPA in effluent solution was detected by UV-Visible spectrophotometry.

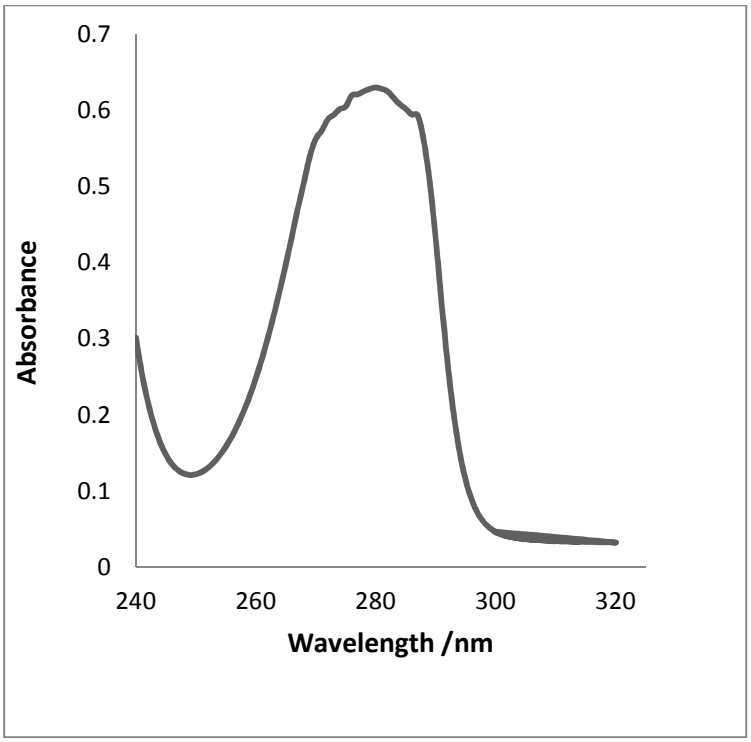

Figure 2. UV spectrum of L-DOPA

Sample preparation

Fresh urine samples obtained from healthy volunteers were kept in sterile and clean containers. Urine samples were stored in the fridge before the analysis. These samples were diluted with phosphate buffer solution prior to investigation. Consent form was obtained from the volunteer prior to the sample collection and it is in compliance with relevant laws and guidelines of Tehran University of Medical Sciences (TUMS).

RESULT AND DISCUSSION

Sorbent characterization

IR spectra

The structural properties of nano TiO_2 was investigated by mid-IR spectra before and after modification by CM- α -CD PG/nano TiO_2 in 400–4000 cm^{-1} spectral region.

The transmittance FT-IR spectrum of pure nano TiO_2 and its modified type are shown in Figure 3. There are some main important regions in these two spectra:

In modified nano TiO_2, esteric band in 1725 refers to C=O stretching band and 1055 and 1250 refers to C-O stretching band which proves the formation of the ester bond in modified nano TiO_2. In about 3400 cm^{-1}, the broadband assigned to stretching vibration of OH groups in TiO_2 is present. The corresponding bending vibration and molecular water band can be observed at 1637 cm^{-1}. These two bands are observed in both pure and modified nano TiO_2. Between 3000 and 2850 cm^{-1}, 2927cm^{-1}, the C-H stretching bands are observed only in modified nano TiO_2. In CM-α-CD nano TiO_2, an additional band is observed at 1325 cm^{-1} which is due to asymmetric bending vibrations of C–H bonds. Within 750 and 450 cm^{-1} region, the O-H deformation overlaps with the asymmetric Ti-O stretching vibrations which appears in modified nano TiO_2 and proves the successful modification.

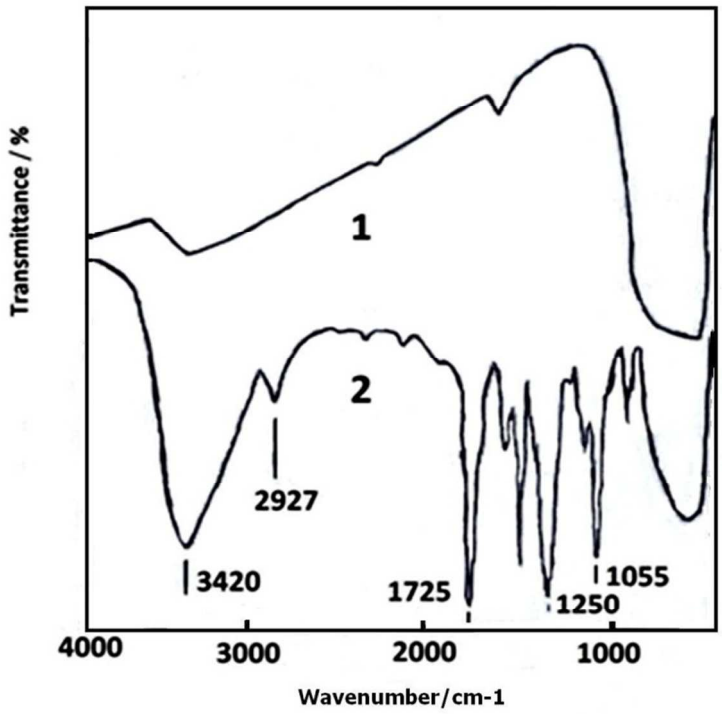

Figure 3. IR spectrum (1, nano TiO_2; 2, CM-α-CD PG/TiO_2)

SEM Analysis

Pure and modified nano TiO_2 samples were studied by SEM to determine the structural situation and morphology. In order to have a more reliable data, 60 points of sample were analyzed and the particle size range was also reported. The obtained results are shown in Figure 4, which which (a) refers to pure nanoTiO_2 and (b) refers to CM-α-CD PG/nano TiO_2. Comparison between these two graphs shows an increase in particle diameter, which is a strong proof for this modification.

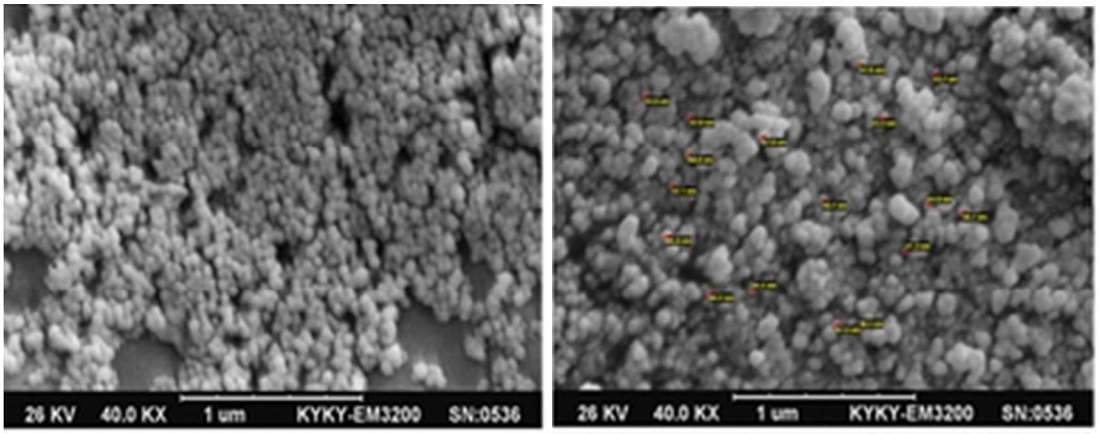

Figure 4. SEM image of pureTiO_2 (left) and CM-α-CD PG/nano TiO_2 (right)

TEM Analysis

The morphology and structure of pure nano TiO_2 and modified nano TiO_2 were studied by TEM. The TEM image is shown in Figure 5. In image (a) the center part of the particle with deeper color is nano TiO_2. In image (b), the particles with deeper color are TiO_2 and the particle surroundings with lighter color are CM-α-CDs.

Comparison between these two images shows successful modification.

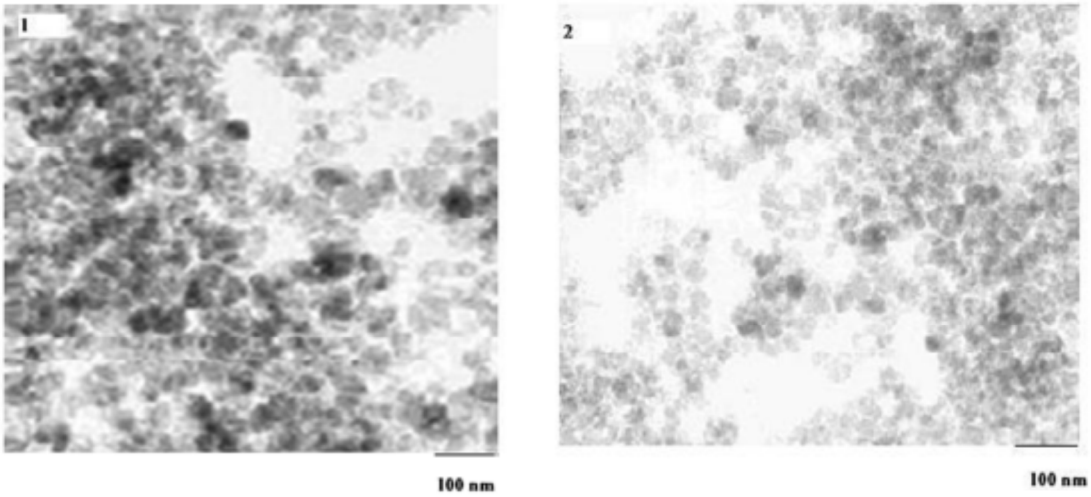

Figure 5. TEM image (1, nano TiO_2; 2, CM-α-CD PG/nano TiO_2)

Thermal Gravimetric Analysis

The structural changes and thermal degradation of pure and modified nanoTiO_2 as a function of temperature were studied by the TGA from ambient temperature to 800 °C (Figure 6). Curve (a) refers to pure nano TiO_2 and Curve (b) refers to modified nano TiO_2 by CM-α-CD polymer. In Curve (a), there is only a small endothermic peak, which refers to adsorbed water on nano TiO_2 surface, but two distinct peaks in Curve (b) refer to H_2O and CO_2, respectively. Increasing temperature from ambient to 350 °C causes loss of compound hydration water and second endothermic peak refers to CO_2 loss that confirms CM- α -CD polymer degradation.

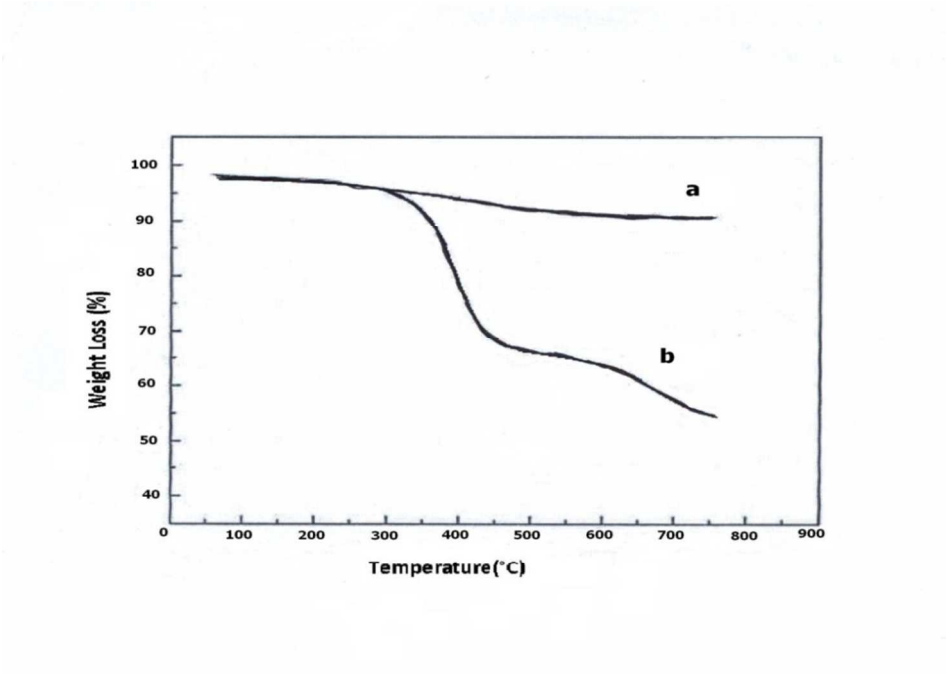

Figure 6.TGA (a, nano TiO_2; b, CM-α-CD PG/TiO_2

Optimization studies

The effect of pH, the amount of sorbent, contact time, the nature of the eluent and eluent volume were studied.

Effect of pH on the adsorption efficiency of L-DOPA

One of the most important factors in SPE procedure is the pH of the media. Considering an appropriate pH value, not only improve the adsorption efficiency, but also decreases the matrix interference. In this work, the pH of each sample solution was adjusted to values ranging from 4 to 9. According to the results (Figure 7), the best recovery for analyte were obtained at pH 6.5. The pH of the medium changes the charge of ionisable groups of L-DOPA. This effect will obviously affect the separation based on hydrophobic interactions. It is found that, the adsorption of L-DOPA changes dramatically below pH 6 and above pH 8. Thus, the states close to the zwitterionic state are advantageous for hydrophobic interaction[32].

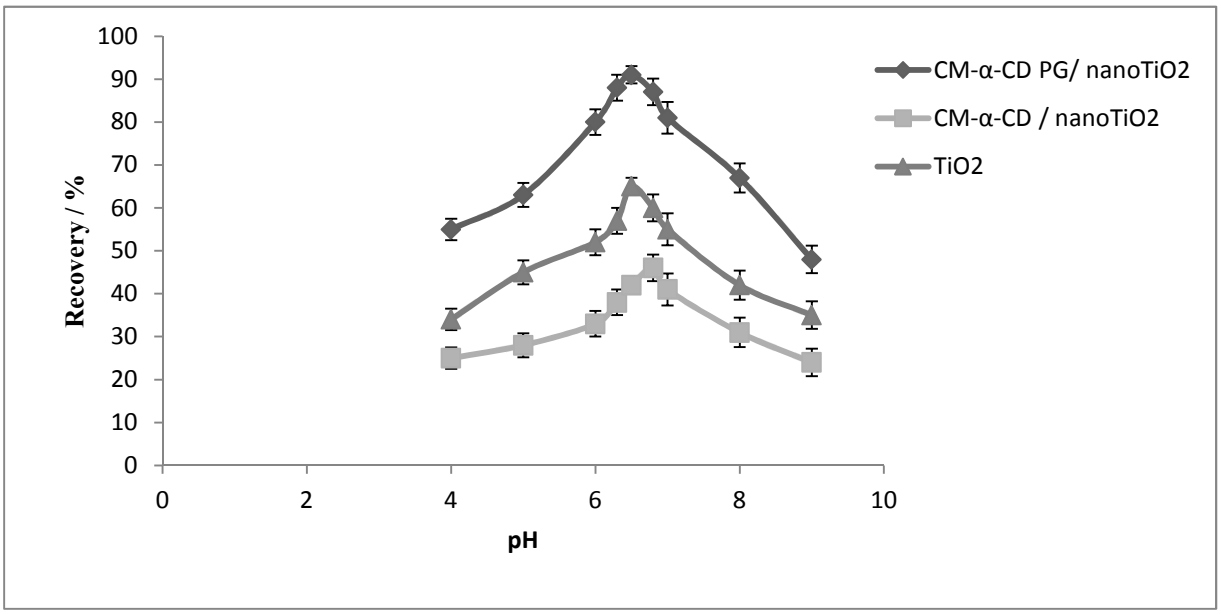

Figure 7. Effect of pH on adsorption efficiency (1, TiO_2; 2, CM-α-CD / nanoTiO_2; 3, CM-α-CD PG/ nanoTiO_2)(sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, volume of eluent: 3 mL, amount of sorbent: 0.3 g, adsorption time: 15 min, temperature: 25^0C)

Effect of the amount of sorbent on the adsorption efficiency

Different amounts of sorbent (0.05 to 0.5 g) were added to sample solution and the recovery were calculated. As shown in Figure 8, by increasing the sorbent amount from 0.05 to 0.3 g, recovery has improved from 65 to about 90%, but the excess sorbent does not make any significant changes in the recovery. Then, 0.3 g of nano CM-α–CD PG/nano TiO_2 was chosen as the optimum amount of sorbent.

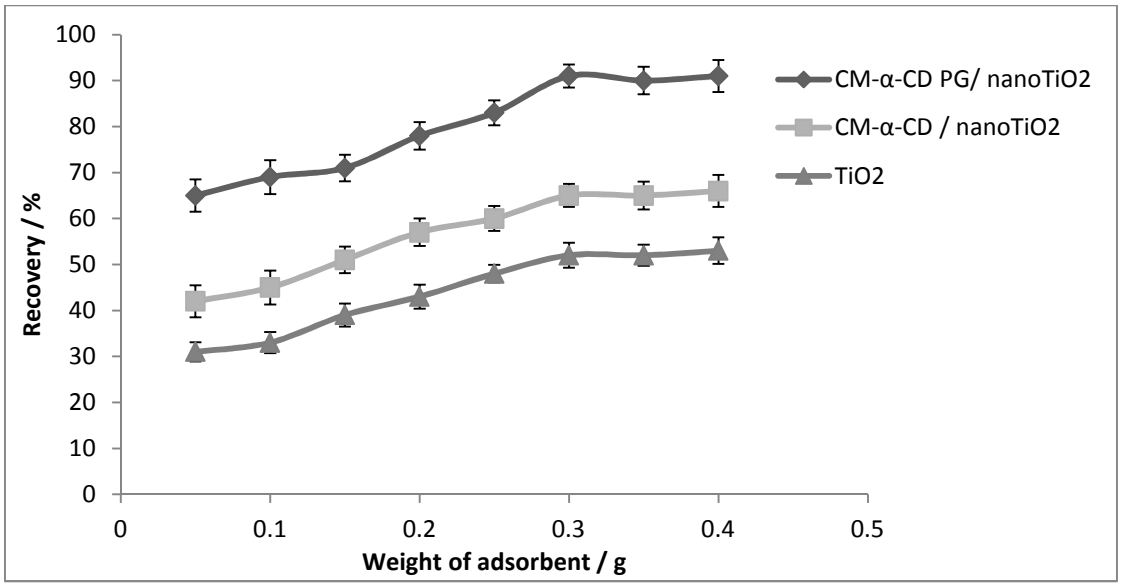

Figure 8. Effect of weight of adsorbent on adsorption efficiency (1, TiO_2; 2, CM-α-CD / nanoTiO_2; 3, CM-α-CD PG/ nanoTiO_2)(pH: 6.5, sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, volume of eluent: 3 mL, amount of sorbent: 0.3 g, adsorption time: 15 min, temperature: 25^0C)

Effect of contact time on the adsorption efficiency

The adsorption efficiency at different contact time (10–30 min) was studied for both pure and modified nano TiO_2. The results are shown in Figure 9. Increasing contact time from 10 to 15 minutes improves recovery from 70 to about 90%, but excess time does not provide any significant changes in the recovery. Therefore, 15 minutes was chosen as the optimum contact time.

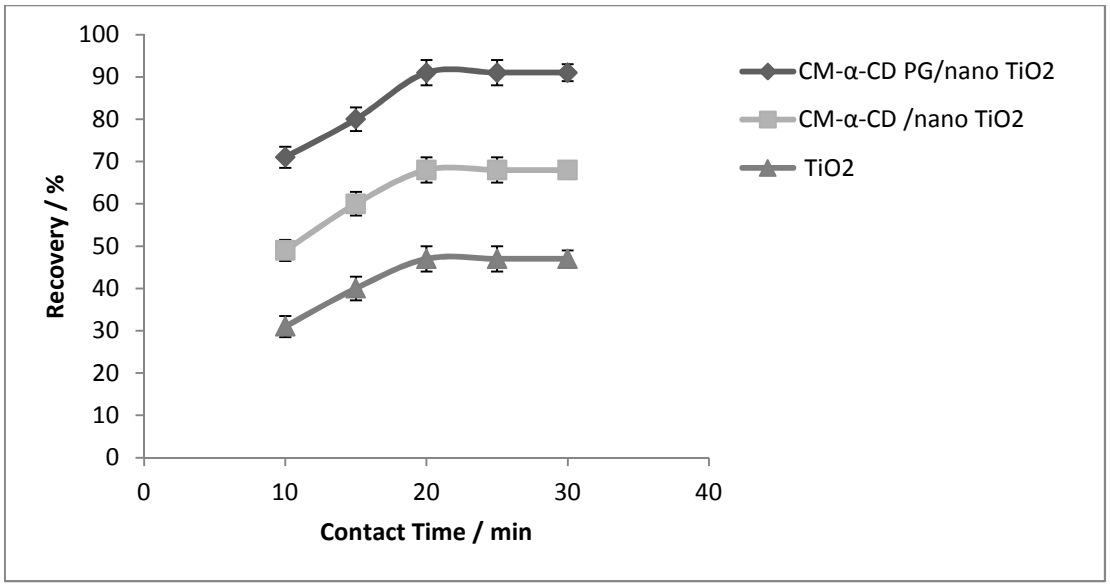

Figure 9. Effect of contact time on adsorption efficiency (1, TiO_2; 2, CM-α-CD /nano TiO_2; 3, CM-α-CD PG/nano TiO_2))(pH: 6.5, sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, volume of eluent: 3 mL, amount of sorbent: 0.3 g, temperature: 25^0C)

Effect of type and volume of eluent

Studies were carried out to investigate the influence of different solvents as eluent for desorption of L-DOPA from the sorbent. Results showed NaOH is an effective eluent for L-DOPA (Table1). Thus, this eluent was selected for further studies. The effect of the volume of eluent solution was also studied. The recovery of L-DOPA increased by increasing the volume of NaOH from 1 up to 3.0 mL and remained constant afterwards (Figure 10). Therefore, the optimum volume of the eluent was chosen 3.0 mL**.** The decline in absorption at lower volume of eluent could be attributed to lack of eluent for maximum adsorption efficiency.

Table1. Effect of type of eluent on L-DOPA recovery

Recovery /%	Eluent
5.5	Acetone
32	HCl /0.01 mol lit^{-1}

48	HNO_3/0.01 mol lit^{-1}
91	NaOH/0.01 mol lit^{-1}

To calculate preconcentration factor, 60 mL of 1 μg mL^{-1} L-DOPA solutions were shaken with 0.3 g of sorbent. The Amount of L-DOPA eluted into the 3 mL of NaOH was measured using UV-Vis spectrophotometry.

The preconcentration factor (P.F) was 20.

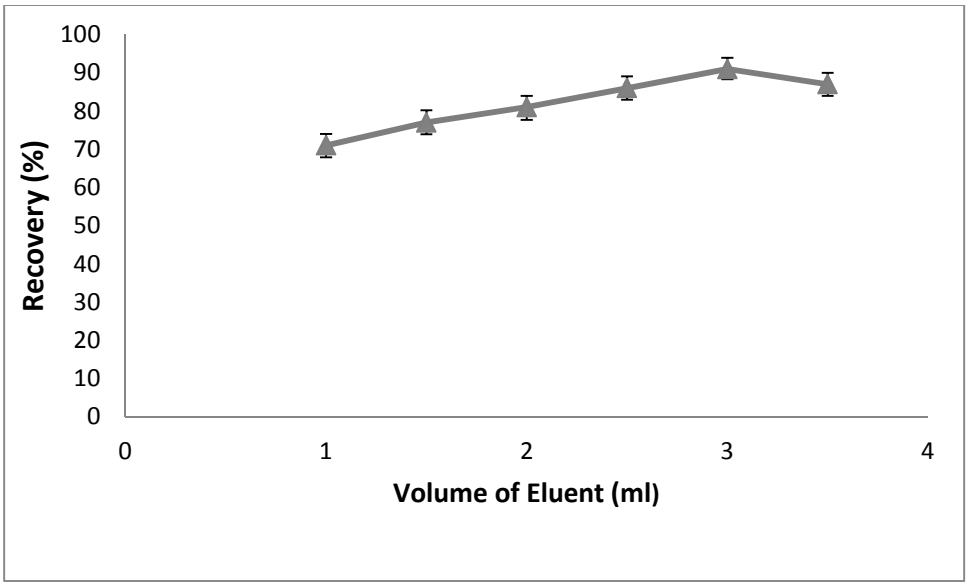

Figure 10. The effect of volume of elutes on desorption of L-DOPA)(pH: 6.5, sample concentration:1 μg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, amount of sorbent: 0.3 g, adsorption time: 15 min, temperature: 25^0C)

Adsorption capacity

The capacity of the sorbent is an important factor that determines how much sorbent is required to quantitatively remove a specific amount of analyte from the solution [25]. To determine the retention capacity (or sorption capacity) of the sorbent (maximum amount of the analyte on 1 g of

sorbent), 300 mg of sorbent with L-DOPA under optimum conditions was saturated and shaken. Then, the L-DOPA content in elutes was measured by UV-Vis spectrophotometry. The adsorption capacity of the sorbent was 63.8 μg g^{-1}. Also, the adsorption capacity of carboxymethyl-α-cyclodextrin polymer grafted onto nano TiO_2 was compared with carboxymethyl-α-cyclodextrin Grafted onto nano TiO_2. Based on experimental results, the adsorption capacity of carboxymethyl-α-cyclodextrin polymer grafted onto nano TiO_2 (63.8 μg g^{-1}) is more than 36.4 μg g^{-1} for carboxymethyl-α-cyclodextrin grafted onto nano TiO_2 which showed polymerization of is satisfied reason for increasing adsorption capacity of sorbent.

Reusability

The reusability is one of the important advantages of a novel sorbent. To show the reusability of this sorbent, the adsorption–desorption cycle of L-DOPA was repeated 6 times by using the same adsorbents. As seen from the cycle experiments, there was no remarkable reduction in the adsorption capacity of this sorbent. The L-DOPA adsorption capacity decreased only 6.8% after 6 cycles.

Figure of merit

The analytical features of the presented method such as linear range of the calibration curve and limit of detection were also examined. The calibration graph was linear in the range of 0.05-1.3 μg mL^{-1} of L-DOPA. The corresponding coefficient of correlation (r^2) was 0.998. The limit of detection (LOD) was 0.016 μg mL^{-1} (n=5). The relative standard deviation (RSD) for L-DOPA was 2.3%.

Interferences

The interference of other coexisting ions and compounds in determination of L-DOPA was examined under the optimized conditions. For this purpose, by spiking appropriate amounts of

potentially interferences in the range of 15-50 μg mL^{-1} solution containing 2 μg L^{-1} of L-DOPA evaluation was done. The obtained results with a relative standard deviation (RSD) less than ±5% were summarized in Table 2. The main interference compounds in L-DOPA determination are ascorbic acid (AA) and uric acid (UA). Also, the interference of urea and creatinine was studied because they are the main components of human urine. From gained experimental results, it is concluded that the developed method has a good tolerance for interferences in L-DOPA determination. With respect to the polarity of urea and creatinine, which are polar compounds, they do not have tendency to enter the hydrophobic cavity of this sorbent. In addition, for the same reason, uric acid and ascorbic acid do not have interference in L-DOPA determination in this method.

Table 2. The effect of interferences on the determination of 1 μg mL^{-1} L-DOPA

Coexistence	Concentration (μg mL^{-1})	RSD(%)
urea	25	3.18
creatinine	30	3.35

Uric acid	15	4.27
Ascorbic acid	20	3.81
Cl^-	25	-3.45
Na^+	45	-4.21
K^+	50	2.78
Ca^{+2}	30	-3.57
Mg^{+2}	40	-2.42
SO_4^{+2}	25	2.28
PO_4^{+2}	30	2.50

Real sample analysis

Determination of L-DOPA in biological fluids like blood and urine is important. Urine is more readily obtainable than blood; therefore, it is source for earning useful data about human body condition. However, due to its approximately complicated matrix, it needs a preconcentraion step prior to analyze.

In this study, urine was chosen as real sample and CM-α-CD PG/nano TiO_2 as the sorbent of L-DOPA in urine samples. L-DOPA was added into the urine since the concentration of L-DOPA is too low in normal human urine. This experiment would be suitable to determine L-DOPA in urine samples. For this purpose, 5-10 mL urine samples were diluted with 15-20 mL phosphate buffer and pH fixed at 6.5, then this solution was used as the adsorption medium instead of pure L-DOPA solution. After the elution of adsorbed L-DOPA by 3 mL of NaOH, the eluent was analyzed by UV-Vis spectrophotometry (Table3 Showed the results).

Table 3. Determination of L-DOPA in urine samples

Experimental conditions: pH=6.5, eluent=3 mL NaOH, the amount of sorbent:300 mg

1:Non Detected

Sample	Total Volume	Added / μg mL^{-1}	Found/ μg mL^{-1}	Recovery /%	RSD/% N=3
5	15	0	Nd[1]	-	-
		0.5	0.41	82	3.45
		1.0	0.9	90	4.03

6	15	0	Nd[i]	-	-
		0.5	0.42	84	3.75
		1.0	0.91	91	3.97
7	18	0	Nd[i]	-	-
		0.32	0.29	91	3.79
		1.2	1.3	108	4.12
8	18	0	Nd[i]	-	-
		0.88	0.91	103	4.17
		1.07	1.03	96	3.62
9	20	0	Nd[i]	-	-
		0.5	0.41	82	3.95
		1.07	1.05	98	4.12
10	20	0	Nd[i]	-	-
		0.36	0.29	81	4.12
		1.04	1.02	98	3.64

CONCLUSION

A new method based on host-guest mechanism is established and CM-α-CD PG/nano TiO_2 with inclusion/complexation properties were successfully synthesized. This selective sorbent was coupled with UV-Visible spectrophotometry for L-DOPA determination. The analytical results showed this method is reasonable for this purpose.

In fact, The inner cores of CM-**α**-CD molecules, with their hydrophobic cavities, easily adsorb L-DOPA through host–guest interactions. Consequently, this nano composite can be used as a reusable sorbent for fast, convenient, and highly efficient sorbent for L-DOPA determination.

As it was mentioned, a reasonable agreement was obtained between the added and measured values (high recoveries) of spiked urine samples. On the other hand, validation of the method was performed using the comparison of the results obtained by the proposed method. Thus, this novel chemically modified sorbent is applicable to the detection of L-DOPA in urine samples.

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list of figure captions:

Figure 1. Scheme for preparation Carboxymethyl-α-Cyclodextrin polymer grafted onto nano TiO_2 (CM-α-CD PG/nano TiO_2)

Figure 2. UV spectrum of L-DOPA

Figure 3. IR spectrum (1, nano TiO_2; 2, CM-α-CD PG/TiO_2)

Figure 4. SEM image of pureTiO_2 (left) and CM-α-CD PG/nano TiO_2 (right)

Figure 5. TEM image (1, nano TiO_2; 2, CM-α-CD PG/nano TiO_2)

Figure 6. TGA (a, nano TiO_2; b, CM-α-CD PG/TiO_2)

Figure 7. Effect of pH on adsorption efficiency (1, TiO_2; 2, CM-α-CD / nanoTiO_2; 3, CM-α-CD PG/ nanoTiO_2)(sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, volume of eluent: 3 mL, amount of sorbent: 0.3 g, adsorption time: 15 min, temperature: 25^0C)

Figure 8. Effect of weight of adsorbent on adsorption efficiency (1, TiO_2; 2, CM-α-CD / nanoTiO_2; 3, CM-α-CD PG/ nanoTiO_2)(pH: 6.5, sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, volume of eluent: 3 mL, amount of sorbent: 0.3 g, adsorption time: 15 min, temperature: 25^0C)

Figure 9. Effect of contact time on adsorption efficiency (1, TiO_2; 2, CM-α-CD /nano TiO_2; 3, CM-α-CD PG/nano TiO_2))(pH: 6.5, sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, volume of eluent: 3 mL, amount of sorbent: 0.3 g, temperature: 25^0C)

Figure 10. The effect of volume of elutes on desorption of L-DOPA)(pH: 6.5, sample concentration:1 µg mL^{-1} of L-DOPA, eluent: 0.01 mol L^{-1} NaOH, amount of sorbent: 0.3 g, adsorption time: 15 min, temperature: 25^0C)