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Modification of multi-walled carbon nanotube by p-amino acetanilide for extraction of buspirone drug

Sh. Reshad[1], Z. Azizi[2], E. Moniri[3]*

[1,2] Department of Chemistry, Karaj Branch, Islamic Azad University, Karaj, Iran
[3] Department of Chemistry, Varamin Branch, Islamic Azad University, Varamin, Iran

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ABSTRACT: The improvement of medication techniques that causes the reduction of toxicity and enhancement of drug effectiveness enjoys a special significance. Hence, in this research, many attempts have been made to use factored-in carbon nanotube for measurement and determination of the dose of anti-anxiety disorder drugs in human body's liquids. In the first place, in order to possess more active sites, the multi-walled carbon nanotube (MWCNT) was factored-in by cyanuric chloride. Then, p-amino acetanilide was placed on the absorbent, as a ligand that has an exclusive suitable interaction with the buspirone drug. In order to confirm the synthesized nanostructure, different techniques, including infrared spectrometry (FT-IR), thermogravimetric analysis (TGA), scanning electron microscope (SEM), and energy dispersive spectroscopy (EDAX) were used and the results were analyzed. In order to determine the optimum conditions, the absorption of the drug under pH conditions and optimum time was studied. Finally, under optimum conditions, the absorption of the drug in blood plasma and urine were carried out by high performance liquid chromatography (HPLC).

Keywords: *Buspirone; Carbon nanotube, Drug; Functionalized; HPLC; P-amino acetanilide*

INTRODUCTION

Nanotechnology is a new approach in most fields, and what it plays a role in comprehensiveness epidemic is high surface area to volume ratio of materials. This is one of the most important properties of materials produced in nanoscale Carbon compound have been taken an important role in this field (Aliev, *et al.*, 2009). After the discovery of the third allotropic form of carbon fullerene in 1991, Sumio Iijima identified a new structural form of this allotrope, the cylindrical fullerene and named them as carbon nanotubes (CNTs) (Iijima, 1991). There are two groups of carbon nanotubes: multi-walled carbon nanotubes (MWCNT) and single-walled carbon nanotubes. Carbon nanotubes (CNTs) have drawn considerable attention for many years due to their excellent electrical, mechanical, thermal, and optical properties. The unique structure and excellent properties allow carbon nanotubes to be suitable for many applications (Amelinckx, *et al.*, 1995; Elhissi, *et al.*, 2012; Dementev, *et al.*, 2012). They can be used in many fields such as nanoelectronic devices, sensor, energy storage, nanocomposite (NC) materials and

(*) Corresponding Author - e-mail: zahra.azizi@kiau.ac.ir

drug delivery (Tahermansouri, *et al.*, 2013). Many research groups have reported different functional reactions on carbon nanotubes. According to studies, these reactions are divided into two categories which are non-covalent functionalization (hydrophobic interaction between molecules and nanotubes; π-π interactions between the non-resident electrons. electrostatic forces between non-resident electrons of nanotubes with positive charge of surfactant etc.) and covalent functionalization (direct connection of the functional groups to surface; direct covalent binding of carboxylic acid groups). Covalent bonds between functional groups with carbon nanotubes are very promising, since they create a very sturdy connection. According to the reported results, carbon nanotubes containing mixture of H_2SO_4/H_2O_2, H_2SO_4/HNO_3, H_2O_2/HNO_3, $KMnO_4$ or using of superoxide at room temperature or heat results in opening the closed-end of materials, and they are functionalized at the end and on the surface (Cenacchi, *et al.*, 2000; Yubing, *et al.*, 2005;Giuseppe, *et al.*, 2009; Bhirde, *et al.*, 2009; Fan, *et al.*, 2009). In this article, carboxylate multivalued carbon nanotubes were functionalized with cyanuric chloride then modified by P-amino acetanilide.

Carboxylated-multiwalled carbon nanotubes functionalized were used to measurement and determine the amount of buspirone drug in human body fluids. Buspirone with chemical name 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl) butyl]-8-azaspiro [4.5] decane-7,9-dione; hydrochloride is an anxiolytic agent and serotonin receptor agonist belonging to the azaspirodecanedione class of compounds (Fig. 1).

Multi-walled carbon nanotubes modified was characterized by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), scanning electron microscope (SEM) and energy dispersive spectroscopy (EDAX) were used to confirm the nanostructure synthesized. In order to determine of the optimum conditions, the effects of varying parameters such as pH, contact time, concentration and desorption were examined in various solutions. Later on, through applying the optimum condition, efficiency of the drug adsorption and desorption were evaluated in plasma. Spectroscopy ultraviolet (UV) and high performance liquid chromatography (HPLC) were used for determining of drug adsorption value and verification of drug desorption in plasma respectively.

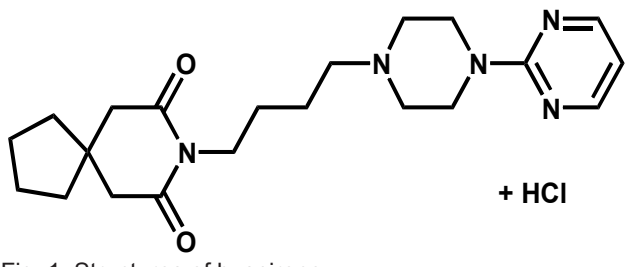

Fig. 1. Structures of buspirone

EXPERIMENTAL

Instruments

Infrared spectra were recorded on Fourier transform infrared spectroscopy (Spectrum100, PerkinElmer, Baesweiler, Germany). Thermogravimetric analysis was carried out using a TGA/SPTA851 (Metter Toledo, Germany). The scanning electron microscopy (SEM) micrographs were obtained on a MIRA3TESCAN of RMRC (USA) scanning electron microscopy. Elemental analysis was carried out on a Thermo-Finniganmodel Flash EA elemental analyzer.

Reagents and solutions

MWCNT-COOH (purity >95 wt.%, inner diameter of 3–5 nm, outer diameter of 15–20 nm, length and ~50 μm carboxyl content of 1.56 wt.%), was obtained from US Research Nanomaterials, Houston, Texas , USA. Buspirone was purchased from Tehran drug co, Iran. In addition, cyanuric chloride, p- amino acetanilide, 1,4-dioxane, methanol, Potassium di hydrogen phosphate, Xylene, acetic acid and all the inorganic acid and salt were products of Merck (Darmstadt, Germany). The stock solution of buspirone was prepared in water (500 mg L^{-1}). All solutions were made by stock solution and their pH was adjusted by acetate buffer. For preparing the buffer used in the mobile phase, 10 mM of buffer phosphate solution (KH_2PO_4) was solved in the pure distilled water and then the solution's pH was adjusted 5.0±0.01 using the concentrated phosphoric acid solution. For preparing a standard sample of 1000 μg mL^{-1} of buspirone drug, 0.01 g of this drug was weighted and reached a desired volume using H_2O in a 10 ml volumetric flask. For making more dilute samples, including 1, 2, 5, 10,

15, 20, 25 and 30 μg mL^{-1} (8 points for calibration), a certain amount of the reference solution reached a 10 ml volume.

Preparation of MWCNT- COOH/ P-amino acetanilide

First, a sample of acetylated carboxylic carbon nanotube (CNT-COOH) was purchased and forwarded for FT-IR, TGA tests, observed in Fig. 2, and 5 respectively. 1.5 g of cyanuric chloride was solved by a 25:25 mixture of xylene/dioxane in a beaker at 25°C for 1h through mixing (250 rpm). Then, 2 g of Carboxylated carbon nanotube was added to the beakers solution. The reaction continued at 25°C for 24 h (250 rpm). The resulted compound was smoothed by nanopaper and washed by 20 mL of petroleum ether several times in order to remove pollutants. Then, it was dried under the temperature of 40°C for 24 h in oven and consequently a sample of the resulted powder was forwarded for IR test, as observed in Fig. 3. For the synthesis of the final carbon nanotubes functionalized by P-amino acetanilide,100 mL of sodium acetate buffer (0.01 M) was poured into an reflux system and 1g of P-amino acetanilide was added in order to be solved under the temperature of 65-70°C through mixing (250 rpm) for 2 h. Finally, the compound resulted from the previous step (CNT-Group) was added to the solution and reflux was continued for 12 hours. The resulted powder was washed by the deionized water and 0.1 molar sodium chloride dried at the temperature of 40°C for 24 h in oven. A sample of nanoadsorbent was forwarded for FT-IR, TGA and SEM tests, observed in Figs. 4, 6 and 7, respectively.

Chromatographic conditions

In order to analyze the drug, HPLC apparatus equipped with a UV-VIS detector and the column C18 (250 mm × 4.6 mm id, 5 μm) was used. The Buffer used in the solution system 10 mM has been of the phosphate buffer (KH_2PO_4) which was arranged by the phosphoric acid solution with pH= 7.5. The mobile phase consisted of phosphate buffer solution of 10 mM with pH= 3 and Acetonitrile in the ratio (40:60) was used. Flow rate was 1 mL / min and the injection volume of 20 micro-liters well as the column temperature was set to 35 degrees Celsius. The Wavelength set for the detector equals to 238nm.

Sorption & recovery of buspirone drug

Solutions of buspirone drug with concentrations of 1 and 20 μg mL^{-1} (concentration 20 for determining optimum parameters and concentration 1 for sorption of drug in plasma) and pH= 3 (adjustment of pH by buffer) were prepared in a 10 mL volumetric flask and 2 mL of them was added to the microtube. Then, 0.05 g of the adsorbent was added to microtubes containing solutions in order to be mixed for 15 minutes. It led to the adsorption of buspirone drug on the adsorbent. After the end of mixing, samples were centrifuged and supernatant was filtered by the syringe tip filter and finally injected to HPLC for determining the volume. Then, the drug adsorbed on the adsorbent was

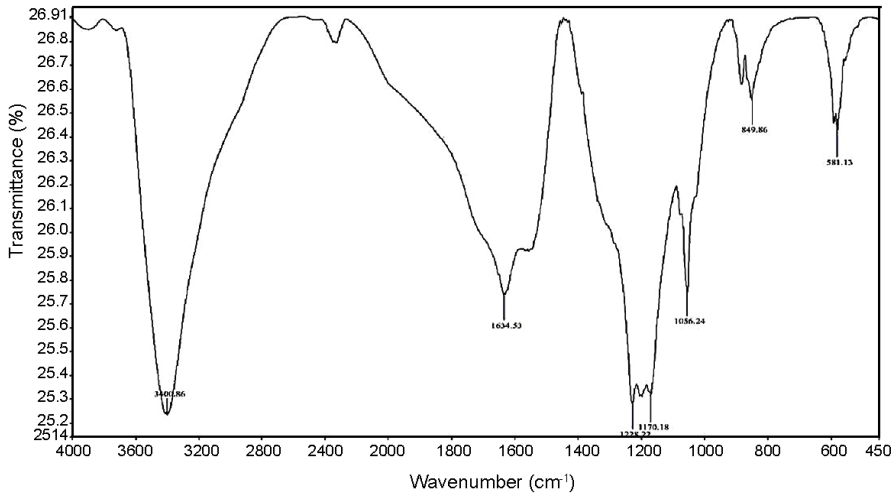

Fig. 2. FT-IR spectrum of the carboxylated multi-walled carbon nanotube

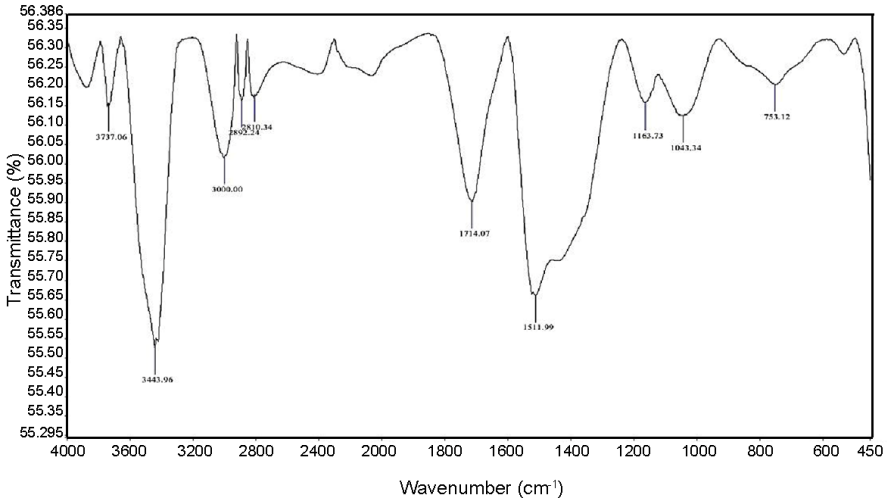

Fig. 3. FT-IR spectrum of the multi-walled carbon nanotube functionalized by cyanuric chloride

recovered by the optimum desorption solvent. HPLC was used for determining the concentration. After the determination and adoption of optimum conditions of parameters, the sorption and recovery percentage of drug (with the concentration of 1 μg mL^{-1}) relative to a standard similar to its environment was quantified by HPLC.

RESULTS AND DISCUSSION

Characterization of structures

Review of spectrum (FT-IR)

In order to verify the structure of samples in each step, their IR spectrum was reviewed. Results of FT-IR, MWCNT-COOH, MWCNT-COOH/$C_3Cl_3N_3$ and MWCNT- COOH/ P-amino acetanilide are observed in Figs. 2, 3 and 4, respectively. In Fig. 2, the peak observed in the area 1634 cm^{-1} is related to carboxyl C=O existing in –COOH. Furthermore, the wide peak band observed in the area 3400 cm^{-1} is related to OH stretching vibration. In a spectrum provided in Figs. 3 and 4, the peak observed in the area 1714 cm^{-1} is related to C=O carboxyl group existing. In addition, stretching vibrations between carbon and chlorine C-Cl and C-H Stretching vibration was observed.

Review of TGA

According to the spectrum provided in Fig. 5, the initial nanotube has a stable structure which maintains its structural skeleton to the temperature of 600°C. Of course, there are impurities in the compound which

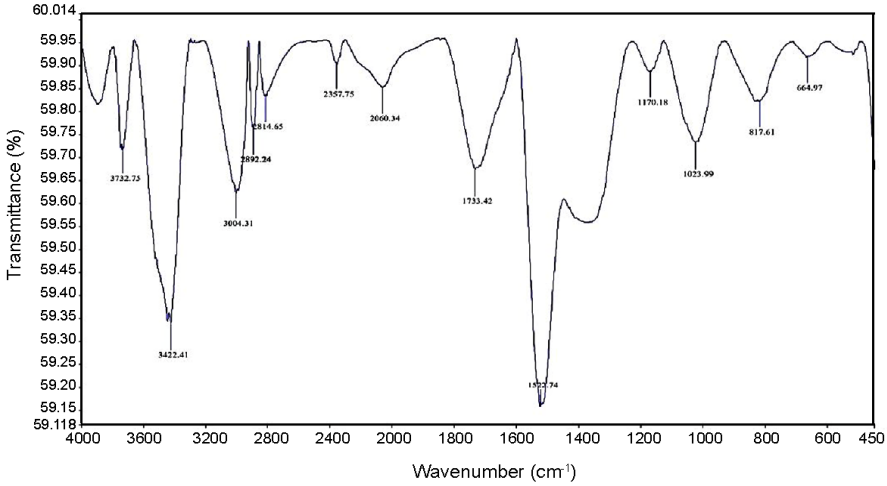

Fig. 4. FT-IR spectrum of the multi-walled carbon nanotube modified by P-amino acetanilide

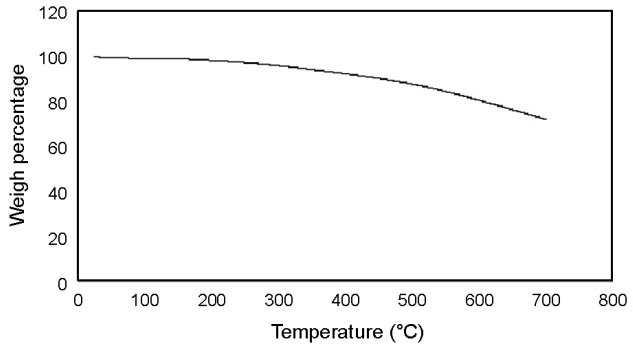

Fig. 5. Results obtained from TGA; the carboxylated multi-walled carbon nanotube (MWCNT-COOH)

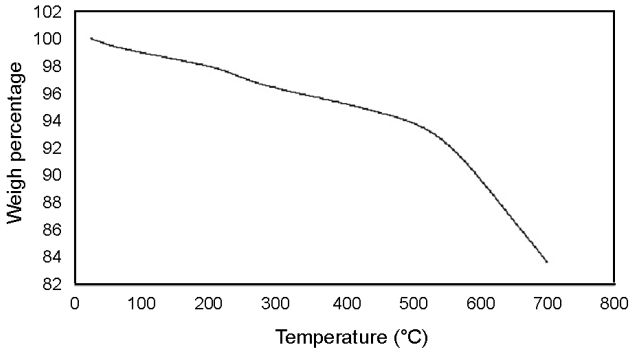

Fig. 6. Results obtained from TGA; the final functionalized multi-walled carbon nanotube

are analyzed. On the other hand, Fig. 6 shows a structure with a modified surface, indicating a reduction in the structure's resistance at high temperatures.

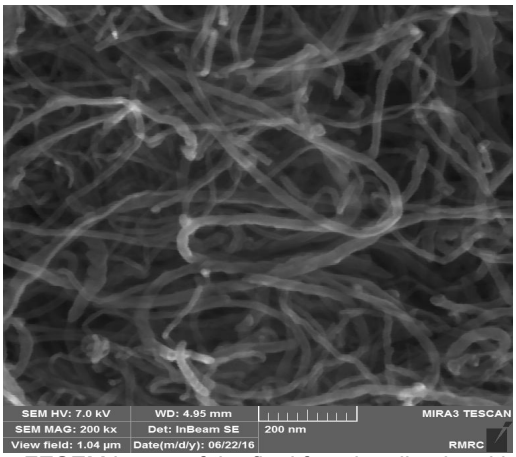

Fig. 7. FESEM image of the final functionalized multi-walled carbon nanotube (MWCNT- P-amino acetanilide).

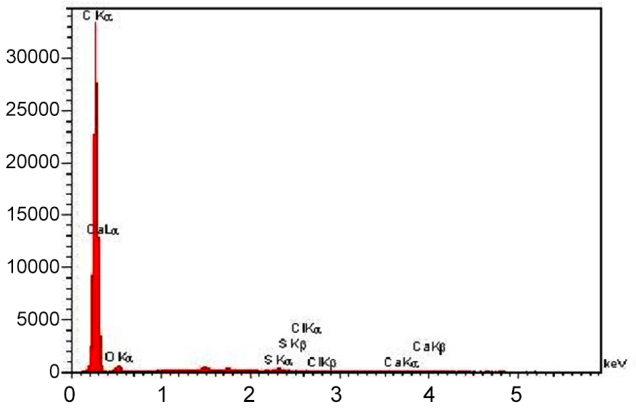

Fig. 8. Result EDAX of MWCNTs-COOH

SEM microscopy

Fig. 7 is the SEM image in a 200 nm scale of the initial carboxylated carbon nanotube .The bumps surface and increase in the diameter is due to the several chemical reactions and the covalent bond (bonding) of the functional groups on the wall of nanotubes. It verifies the modification of the nanostructure's surface.

Assessment of functionalization by EDAX

Further evidence for the multifunctionalization of pristine MWCNTs is provided by energy dispersion spectroscopic (EDAX) analysis. The EDAX spectrum of MWCNTs-COOH (Fig. 8) and MWCNTs- P-amino

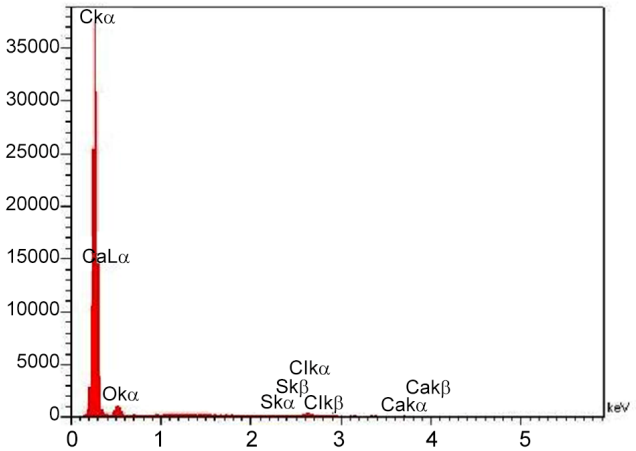

Fig. 9. Result EDAX of MWCNTs-COOH and MWCNTs- P-amino acetanilide

Table 1. Result EDAX of MWCNTs-COOH and MWCNTs- P-amino acetanilide

MWCNTs-COOH	Element	C	O	Cl	S	Ca
	Wt%	92.42	6.87	0.14	0.31	0.26
MWCNTs-P-amino acetanilide	Element	C	O	Cl	S	Ca
	Wt%	90.62	8.71	0.41	0.16	0.09

Table 2. Validation parameters for standard solutions of buspirone drug

Line equation	Correlation coefficient (R2)	Linear range	RSD% (experimental)	LOD (mg mL^{-1})	LOQ (mg mL^{-1})
Y=0.0534x+0.0361	0.995	1-30	0.97	13	43

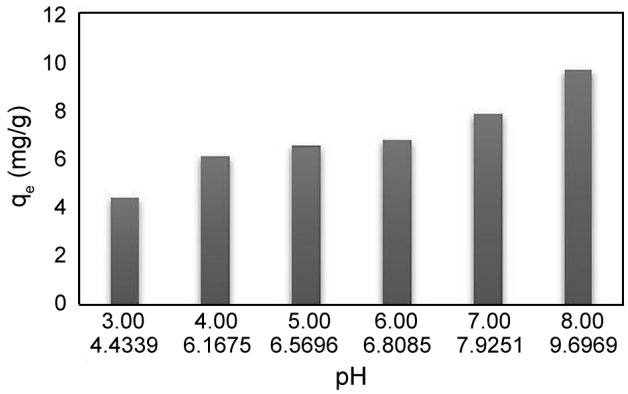

Fig. 10. The effect of pH on the sorption of buspirone drug by the nanoadsorbent

acetanilide (Fig. 9) is shown in table 1.

Determination of Optimum pH & mixing time

First, the maximum wavelength of buspirone drug was determined (238 nm). Then, in the maximum wavelength, the drug's linear range is drawn between 1 and 30 µg mL^{-1} and the computation of drug's concentration in reviewing parameters is studied in this range. The method's validation parameters are listed in Table 2. The percentage of the drug adsorbed with the concentration of 20 µg mL^{-1} in different pH (3-8) was studied by HPLC which the best result of sorption was obtained in pH=8. Its experimental results are shown in Fig. 10. Then, by adjusting pH= 8 in the

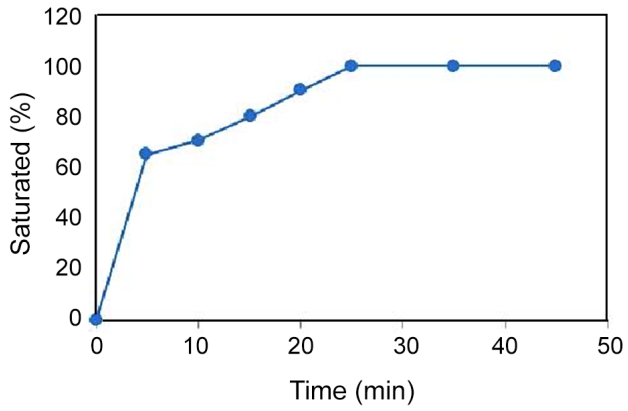

Fig. 11. The effect of time on the sorption of buspirone drug by the nanoadsorbent

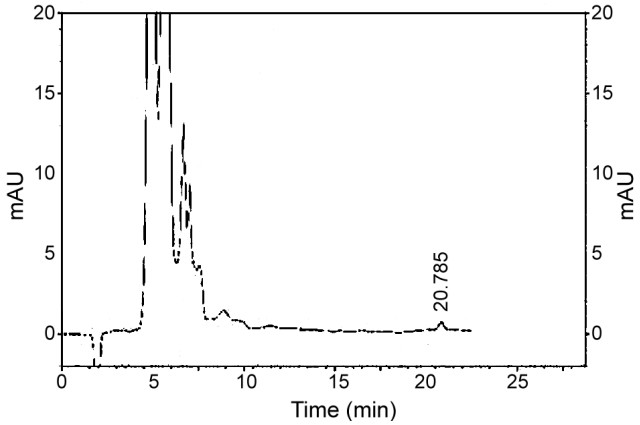

Fig. 12. HPLC results of sorption of drug in the blood plasma

solution as the optimum pH, the optimum time was determined in the same concentrations of the drug (20 µg mL^{-1}). Given Fig. 11, the maximum sorption was obtained in 20 min.

HPLC Results

After the determination of optimum conditions of sorption and recovery of buspirone drug by the functionalized carbon nanotube, a solution containing the drug was prepared in the blood plasma and placed near the nanoadsorbent. Then, the drug adsorbed by the acetonitrile solvent on the nanoadsorbent was recovered and injected to the device, based on the results of spectrums provided in Figs. 12 and 13. The

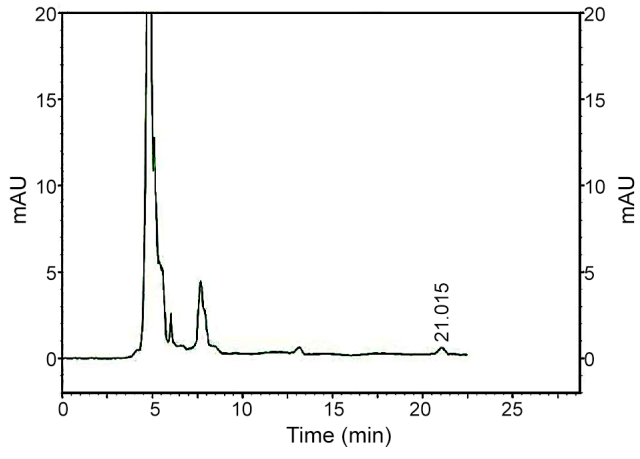

Fig. 13. HPLC results of sorption of drug in the urine

Table 3. The sorption percentages of drug in plasma by HPLC

Name	Sorption of Drug
Sample retesion time	21.015
Sample area	1021
Standard retesion time	20.971
Area standard	9411
C (mg/L)	0.108
Adsorbed %	89.200

Table 4. The sorption percentages of drug in urine by HPLC

Name	Sorption of Drug
Sample retesion time	20.785
Sample area	1123
Standard retesion time	20.971
Area standard	9411
C (mg/L)	0.119
Adsorbed %	88.100

sorption percentages of plasma and urine result shoes in Tables 3 and 4.

CONCLUSIONS

Chemical reformation and solubility of Carbon nanotubes made a vast criterion for research in this field. Many functionalization reactions by covalent and non-covalent mechanisms have been reported for Carbon nanotubes by many researchers. In this research adding functional group by covalent mechanism in multi membrane nanotubes in order to make nanostructures capable of reacting with solute drug in Plasma was done. This would be an approach to extract, preconcentrate and measure drugs from Plasma. In general, by functionalizing the multi-walled carbon nanotube and studying the effect of different factors on the sorption of buspirone drug on the adsorbent, the best absorption was obtained in pH= 8 and 20 min. Finally, by applying optimum conditions, the efficiency of adsorbent in the adsorption was evaluated in the plasma and urine. The maximum adsorption of drug was (89%) of drug were determined through HPLC with a low concentration. The maximum adsorption of drug was (88%) of drug were determined through HPLC with a low concentration.

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AUTHOR (S) BIOSKETCHES

Shirin Reshad, M.Sc., Department of Chemistry, Karaj Branch, Islamic Azad University, Karaj, Iran

Zahra Azizi, Assistant Professor, Department of Chemistry, Karaj Branch, Islamic Azad University, Karaj, Iran, *E-mail: zahra.azizi@kiau.ac.ir , zahraazizi@yahoo.com*

Elham Moniri, Associate Professor, Department of Chemistry, Varamin Branch, Islamic Azad University, Varamin, Iran