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Hybrid Phosphotungstic acid -Dopamine (PTA-DA) Like-flower Nanostructure Synthesis as a Furosemide Drug Delivery System and Kinetic Study of Drug Releasing

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Abstract

The work aimed to focus on the synthesis of the ratio 1:1 from phosphotungstic acid -dopamine nano-structure in tris(hydroxymethyl)-aminomethane (Tris)- HCl solution at pH ranged 8.5-9.5. That leads to prepared a light yellow colored nanostructure powder. The SEM analysis gave reasonable shapes for this prepared nanostructure such as flower-like nanostructure. These nano compounds were used as Furosemide drug delivery, which loaded on its surface at 12h at pH about 9.5. This pH value is suitable for loading drug inside the prepared nanostructure, that attitude to the phosphotungstic acid (POM) compound is semistable in basic media, hence it opens it to bond and perminented of loading drug in basic medium (pH 9.5). The kinetic study for relasing of this drug found to be pseudo first order.

Keywords: Polyoxometalates (POMs); polyoxotungestic acid –dopamine nanostructure; dopamine; Furosemide drug; drug delivery system.

1.Introduction

Polyoxometalates (POMs) regards as a large group of anionic polynuclear metal-oxo nanoclusters, which are recognized as addenda atoms with general formula [MxOy]n. Generally, this formula consists of two or more high oxidation states (either a d^0 or d^1 electronic configuration) transition metals (M) such as preferably tungsten (W) or molybdenum (Mo), and less frequently niobium (Nb), tantalum (Ta) and vanadium (V), or mixture from these mention metals, linked together through oxo-ligands (O) in coordination (y) ranged from 4 to 7[1-3]. The coordination number of addenda ions in monomeric MO_4 fragments can be elevated from four to be six in polyanions when acidified, and the terminal O_2 ligands can form pπ–dπ interactions with the metals as double bonds [2]. In 1826, Poly-oxometalates discovered the first POM species by Berzelius[4]. Poly-oxometalates are classified into three families based on their structure and composition. As seen in **table 1.** The Scheele has first reported this cluster in 1783[4]. Since POMs are soluble and accumulate in the water, their stable biological systems are depressed. This behavior is attributable to the thermodynamic and kinetic instability of many POMs structures at physiological pH, making them simple targets for reactions that produced various structures [7]. Phosphotungstic acid (PTA) is one of the active POMs compounds that beyond two class of POMs as isopolyanions with the chemical formula $H_3PW_{12}O_{40}$. This compound is having a Keggin structure $[X^{n+}M_{12}O_{40}]^{(8-n)-}$, where (X) is the central atom P and (M) is W, these polybasic acids include a complex anion called a heteropolyanion, in this structure the heteropolyatom is surrounded by an oxometallate polyhedrons cage. In general, three modified structures of heteropolyanions using a center atom (X) is mostly P or Si with M is commonly W or Mo with less common transition metals such as V, Nb, Ta to produce a complexing heteroatom: a) Keggin structure $[X^{n+}M_{12}O_{40}]^{(8-n)-}$, b) Dawson structure $[(X^{n+})_2M_{18}O_{62}]^{(16-2n)-}$ and c) Anderson structure $[X^{n+}M_6O_{24}]^{(8-n)-}$ [8,9]. Because of the reasonable structures of phosphotungstic acid as Keggin structure, it combined as self-assembly with other materials and producing a versatile application such

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as dopamine to synthesize WO_3 as photocatalyst[10], synthesis of nanostructure with dopamine, and loaded oral drug as drug delivery[11,12]. The purpose of this research was to prepare the hierarchical nanostructure self-assembled of phosphotungstic acid (PTA) as Keggin type of polyoxometalate with bio-molecule (dopamine) and then loaded Furosemide drug into it. The characterizations of these compounds were investigated, and it was estimated the ability of drug-releasing in neutral and acidic mediums.

Table 1. The classification of the Poly-oxometalates

Class	Info.	Referen -ces
first-class	-It includes the heteropolyanionic compounds, which building from a metal-oxide matrix with one or more p-, d- or f-block hetero atoms. They are made up of heteroanions like PO_4^{3-}, SO_4^{2-},and SiO_4^{2-} etc. - that are incorporated with vanadium-, tungsten-, or molybdenum-based metal oxide framework. - This category is widely used as a mono-, di-, and trilacunary clusters that attitude to the intrinsic stability of the resulting stable building block libraries, which is a construction of a larger aggregate by the incorporation of heteroanions	[4-6]
second class	-It includes the isopolyanions, which are made up of only one type of d^0 metal cations of producing a metal oxide matrix without any incorporation of heteroatoms or heteroanions. -These compounds in this class are less stable structural motifs than their heteropolyanion counterparts. Moreover, they have fascinating physical properties and can be used similarly as cluster-based building blocks. - The isopoly compounds are less used in medicine studied than the heteropolyoxometalates.	[4,6] [7]
third-class	-It includes gigantic size of molybdenum-based reduced POM nanosized clusters, which are called Mo-blue and Mo-brown species.	[4]

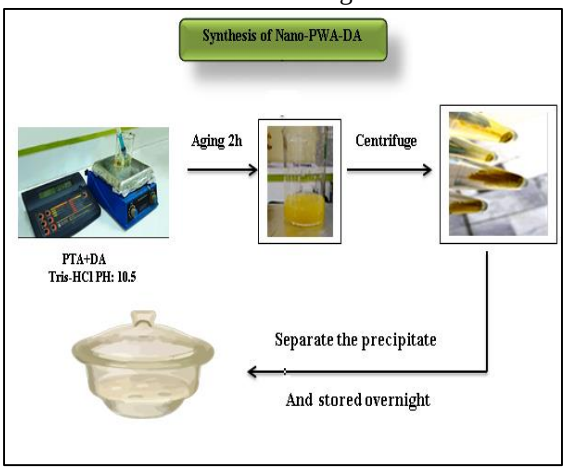
2. Experimental

A. Materials

Furosemide and Dopamine hydrochloride (DA) were purchased from TCl -America and Thermo Fisher (TMO) respectively. Phosphotungstic acid (PTA) and tris(hydroxymethyl)aminomethane (Tris) were supplied by HiMedia. The rest materials were used without any further purification methods.

B. Preparation of hierarchical nanostructures as a nanocarrier

For the synthesis of the hierarchical nanostructures in ratio 1:1, exact 1mg of dopamine was dissolved in 0.5 mL of Tris-HCl solution (10 mM, pH 10.5) as solution no. 1. Similarly, exact 1 mg of phosphotungstic acid was dissolved in a similar procedure as solution no. 2. As soon as possible, solution no. 2 was added and mixed with a solution no. 1. The produced solution was turned from colorless to visible yellowing for 0.5 min at 37 ºC. The resultant was aged for 2 h with shaking before being separated with centrifugation (5000 rpm, 5 min) and three deionization distal water washes[11,12]. As shown in **scheme 1.** The yellow hierarchical nanostructure was stored overnight in a desiccator that contains a silica gel.



Scheme 1. The schematic diagram for the synthesis process of the hierarchical nanostructures.

2.3 Loading of Furosemide drug on the hierarchical nanostructures

Furosemide (Lasix) was selected as the study's model drug. It's a diuretic and an anthranilic acid derivative that's used across the mouth. To get this drug to stick to the nanostructure[13], exact (1 mg/mL) of DA and (1 mg/mL) of PTA were dissolved in a certain volume of 10mM Tris-HCl solution until reaching its pH to 9.5. This solution was immersed in an aqueous solution of the drug (0.02 mg/mL, 5 mL) shaken for 12 h at 37 ºC. The yellow compound was collected and centrifuged at 5000 rpm for 5 min to remove the unbound drug, and then washed three times with ultrapure water, and then measured the concentration of supernatant at 270 nm using UV-Visible spectrometer.

The percentage of drug (Furosemide) loading on the prepared hierarchical nanostructures was calculated using the mention equation in refernce[14]:

$$Loading\ contant\ \% = \left[\frac{W_t - W_f}{W_{np}}\right] x100 \qquad \ldots(1)$$

Where: W_t is the total weight of the drug (Furosemide) fed, W_f is the weight of non-

encapsulated free (Furosemide) drug, and W_{np} is the weight of the hierarchical nanostructures.

2.4 In-vitro Releasing of Furosemide drug from the hierarchical nanostructure

The in-vitro releasing of this drug from the hierarchical nanostructure was performed in an acidic medium (pH 2.8 glycine-HCl buffer solution) and neutral medium (pH 7.4 Phosphate buffered saline (PBS) solutions) at 1-33 h.
The percentage of drug release was calculated with the following equation[15].

$$Release\ \% = \frac{Wt\ of\ drug\ released(mg)}{Wnp\ x\ \ \%\ loading\ drug} \times 100 \quad \ldots (2)$$

2.5 kinetic study for In-vitro Releasing of Furosemide drug from the hierarchical nanostructure

Kinetic studied of the drug (Furosemide)-releasing were applied to the experimental data to clarify the possible rate-controlling routes. The first-order kinetic equation and the second-order kinetic equation can be represented as equation 3 and equation 4 mention below[16], respectively.

$$Log\left(1 - \frac{C_t}{C_T}\right) = \frac{k_1}{2.303}\ x\ t \quad \ldots (3)$$

$$\frac{t}{C_t} = \frac{1}{CT} x\ t + \frac{1}{k_2 x\ CT^2} \quad \ldots (4)$$

Here:
t: time of release, C_t: releasing at time t, C_T: Total liberation in time ∞ , k_1: speed constant for 1st order, and k_2: speed constant for the 2nd order.

3.Results and Discussion

A. Structural Properties

The obtained results in **figure 1(a,b, and c)** demonstrated that the use of 1:1 ratio of phosphotungstic acid and dopamine could be well controlled by buffer Tris- HCl solution to reach for pH 9.5, which causes to incorporate both as-prepared PTA-DA hierarchical nanostructure. The prepared PTA-DA hierarchical nanostructure was successfully prepared in nanosize by applied the XRD data in Scherrer's equation (equation 5)[17-23].

$$L = \frac{k\,\lambda}{\beta\ \cos\theta} \quad \ldots (5)$$

where: k is the Scherrer's constant (0.94-0.85), λ is the wavelength of irradiation source(Cu kα) (used 0.15406 nm), 2θ a Bragg diffraction angle and β is (FWHM) the full half-maximum intensity width in degrees.
The mean crystal sizes for PTA, DA, and PTA-DA hierarchical nanostructure were found at 2θ (25.37°, 34.59°, 53.25°, 59.87°)[23], (25.45°, 34.18°, 52.36°, 25.93°), and (9.80°, 13.82°, 16.70°, 25.13°)[11], which equal to 87.944 nm, 68.560 nm, and 12.950 nm, respectively. The minimum mean crystal size occurs for PTA-DA hierarchical nanostructure, that due to strong bonding between PTA and DA in the produced PTA-DA hierarchical nanostructure. While the mean crystal size for PTA-DA hierarchical nanostructure raises with Furosemide loading to reach 16.060 nm. In the other words, in Figur 1 (d), the 2θ positions of PTA-DA hierarchical nanostructure after drug loading alters to 6.01°, 12.05°, 18.12°, and 24.78°), depended on Furosemide 2θ positions mention in reference [25].

A. Morphology of surfaces

The detailed morphology of prepared PTA- DA hierarchical nanostructures was measured using SEM image in **Figure 2 (c and d),** which clarified that the detailed morphology of prepared PTA- DA hierarchical nanostructures is a flower-like nanostructure, formed from a multitude of nano petals (thickness around 21 nm with central core to form 3D flower-like hierarchical structures)[26], with relatively smooth surfaces. But, the SEM images in Figure 2 (a and b) for DA and PTA demonstrated the morphology of the dopamine gives irregular smooth sheets shape with thickness size of about 30μm, while, the morphology of the PTA notices as a semi-spherical agglomerated nanoparticles (62.93 nm-266.50 nm) with high roughness.

B. Drug loading

The furosemide was loaded on the as-prepared flower-like hierarchical nanostructures in deionized water at a temperature of 37°C, and at 12h. The ratio of drug loading was found to be 58%.

C. Drug relasing in acidic and neutral mediums

After studied the loading of furosemide on the flower-like microspheres, the relasing of this drug was investigated in pH 2.8 using glycine-HCl buffer solution and pH 7.4 using Phosphate buffer saline (PBS) solution at a period time of relasing in ranged (1-33) hours. The data in **figure 3** indicates the rapid relasing of furosemide is happed under pH 7.4 and reach to 100 % at 12 h, that attitude to two influences the first is related with the drug stracture, hence the amine groups in Furosemide stracture are partially deprotonated at pH 7.4, but they are fully protonated at pH 2.8[27]. From the other side, the second reson is beyond to pH-responsive of nanostructure and the stability of polyoxymetalate (phosphotungsnic acid) that be less stable because the weak electrostatic

interactions between Furosemide and the flower-like nanostructure are obtained in neutral medium or basic medium[24]. However, the relasing of this drug is resisted and reached to about 50 % at 12 h that depended on elevated the coordination number of POM stracture from four to six in acidic medium by pπ–dπ interactions between the terminal O_2 ligands metals double bonds [2].

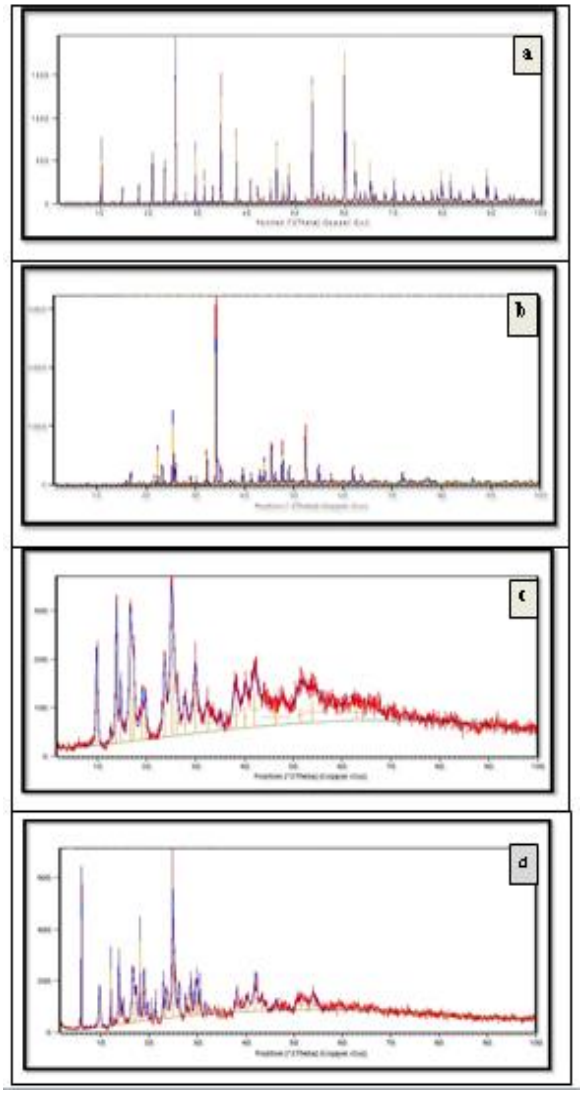


Fig. 1. XRD patterns of (a) PTA, (b) DA, (c) the as-prepared PTA- DA hierarchical nanostructures, and (d) Furosemide loading on PTA- DA hierarchical nanostructures.

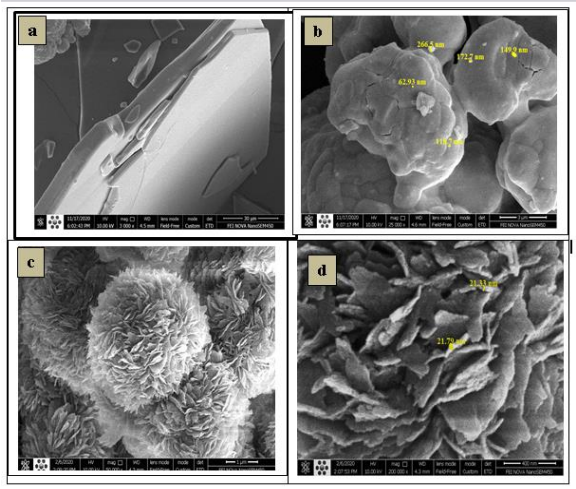


Fig. 2. SEM images of (a) DA. (b) PTA (c and d) PTA- DA flower-like hierarchical nanostructure and its nano petals

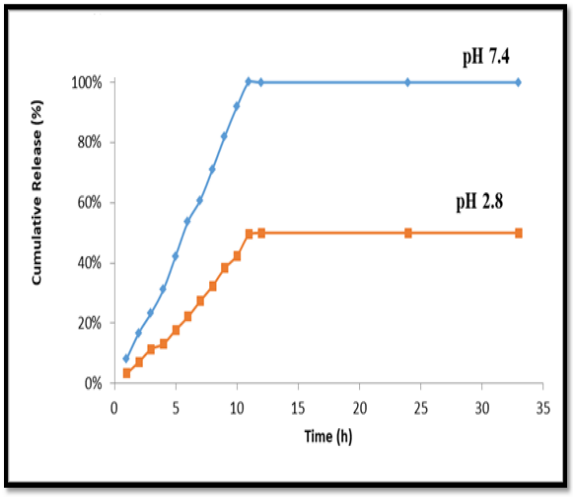


Fig.3.Furosemide release from flowerlike nanostracture in pH 2.8 and pH 7.4

D. Kinetics of Drug relasing in acidic and neutral mediums

Based on results in **figure 4(a and b), figure 5(a and b) and table 2**, that the kinetic study for Furosemide relasing in acidic medium and neutral medum show a good compliance with the pseudo first order equation, when the values of correlation coefficient R^2 in the pseudo first order equation plote is more value then its for the pseudo second order equation plote. Likewise, the calculated rate constant for the first order (k1) is having a maximum value in neutal medium.

Table 2. The correlation coefficient (R^2) and the rate constant (k_1,k_2) of the releasing kinetics reaction for Furosemid drugs.

The drugs	Type of pH solution	The correlation coefficient (R^2)		Rate constant(k)	
		Pseudo-first order	Pseudo-second order	k_1 h^{-1}	k_2 $L.h^{-1}\ mol^{-1}$
Furosemide	(pH 7.4)	0.9141	0.7614	0.2867	0.3212
	(pH 2.8)	0.9311	0.3398	0.2104	0.6238

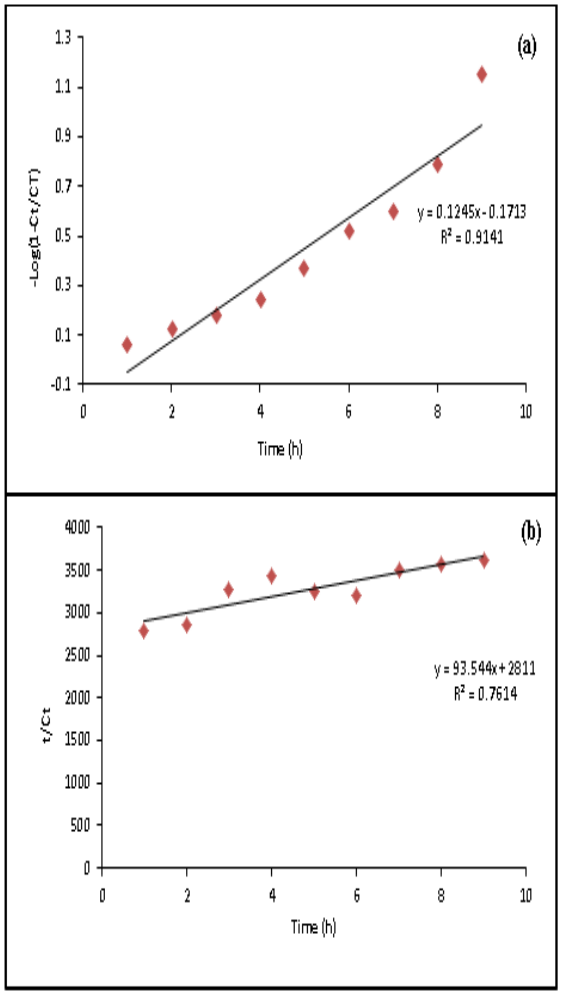


Fig. 4. (a)pseudo-first-order model of furosemide release, (b) pseudo-second-order model of furosemide release, in PBS solution pH 7.4

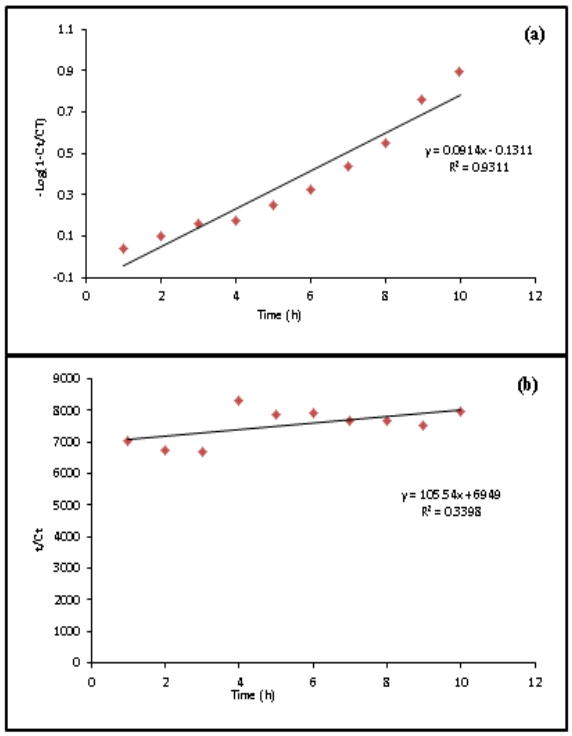


Fig. 5. (a) pseudo-first-order model of furosemide release, (b) pseudo-second-order model of furosemide release, in glycine-HCl buffer solution pH 2.8.

Conclussion

The PTA-DA hierarchical nanostructure exhabits a great potential as a Furosemide drug delivery, that due to give a relasing percentage equal to 100% at pH 7.4, 37 ^{o}C during 12 h.

This drug dielevery is successfully prepared in 1:1 ratio from PTA: DA as yellow powder. This stracture befor and after loaded this drug is conformed with XRD analysis and SEM image. The impact parameter on drug relasing is pH of solution. The maximum relasing of Furosemide is happened under neutral medium pH 7.4. The Furosemide loading on nanostructure is favour under basic medium pH 9.5. The Furosemide loading PTA- DA is having a like-flower nanostructure as 3 D nanostructure. The Furosemide relasing process in pH 2.8 and 7.4 obeys to pseudo first order kinetics.

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Conflicts of interest

There are no conflicts to declare.

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