DOXORUBICIN LOADED SILICA NANOTUBES: AN INVESTIGATION OF THE RELEASE BEHAVIOR

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This study aimed to investigate the release behavior of doxorubicin(DOX) from nanostructured silica tubes as carrier. The nanostructured silica nanotubes were synthesized according to the sol-gel method using tetraethoxysilane (TEOS) as silica precursor in the presence of cetyl trimethylammonium bromide (CTAB) and D-sorbitol derived organogelator as structure directing agents.

The silica nanotubes were successfully prepared and their morphology has been examined with scanning electron microscopy (SEM). The release of doxorubicin was carried out by using two different release media, of pH values 7.4 and 5.5 respectively. The release was carried out by using HPLC system and the results have been reported indicating the difference in the release behavior between these two different pH-media.

Keywords: Doxorubicin, silica nanotubes, drug delivery

1. Introduction

Considering the therapeutic efficiency, the use of carrier materials for drugs, protects the biomedicine from the body conditions until the carrier delivers the drug to the targeted site, a process known as drug delivery system [1][2]. This system can reduce the side effects and improve the pharmacological profiles for different drug molecules such as anticancer agents. Many materials such as polymers, carbonates, metals and their oxides, [3] liposomes and dendrimers were investigated as drug delivery systems to avoid the side effects and reduce toxicity of these drugs towards the non-infected organs [4].

Using hollow materials such as mesoporous silica nanocomposite as delivery system has been widely investigated [5] in nanobiomedical field due to their biocompatibility, large pore volume and tunable pore size. Tuning the properties of the silica mesoporous materials leads to *in-vivo* control drug release

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[6]. In addition to their biodegradability and biocompatibility silica nanocomposites are considered as bioactive materials which can be prepared via a sol-gel method. This method starts with the hydrolysis and condensation of the silica precursor such as TEOS at low temperature [7] In the presence of CTAB as template the precursor will surround this template which can be removed by heat decomposition or centrifugation [2]. The sol-gel method receives careful attention due to its low temperature manufacturing conditions, and to the homogeneity, compatibility with other substances and stability towards thermal/ temperature changes that the obtained products exhibit. The derivatives of silica nanocomposites which were prepared by this method have been utilized as carrier for drug molecules and for biological molecules as well [8]. The possibility of adding the drug during the formation of the silica nanocomposite or after the preparation of nanocomposites opens a wide range of drug delivery applications [7].

The use of silica nanotubes as a resulted material of sol-gel method is an advanced route for drug delivery systems according to their inner and outer surfaces for loading a higher amount of drug molecules. Moreover, the open ends of these nanotubes can act as gates to control the release of the drug molecules [9]. In addition, the variety of functionalization of the silica nanotubes can provide selectivity toward the attachments or the moieties inside the tubes such as the drug molecules, as well as functionalizing the outside surface to specify the targeted site. Another advantage of the silica nanotubes is the mechanical strength which makes them less affected by the physiological conditions. Because of the easiness to achieve the template synthesis in addition to the above explained properties, nanostructured silica nanotubes are considered as novel inorganic drug carriers [10]. The release of the drug from the matrix of silica nanotubes can be stimulated by different external factors such as chemical stimulus or ultrasound [11]. Therefore, a pH-responsive delivery system based on silica nanotubes can be prepared, and thus the drug release depends on the pH changes [12].

High toxicity of the anticancer agents negatively affects the entire human body. Therefore, targeting the cancer cells with chemotherapy via an appropriate drug delivery system aims to reduce their toxicity even in the case of high doses. Thus, such delivery system can increase the therapeutic efficiency on the tumor site, simultaneously increasing safety. Doxorubicin (DOX) is a chemotherapy agent for various kinds of cancers including liver cancer [13], bone cancer [14], breast cancer, ovarian carcinoma, and Kaposi's sarcoma [15]. This drug is classified as anthracycline antibiotic, but it is cytotoxic [14] with serious side effects. For example, doxorubicin causes cardiac toxicity, acute nausea, vomiting, immune system disorder, heart tissues damage and hear loss with dermatological irritations [13].

Chemically, doxorubicin is sensitive to several chemical, physical, and physiological factors [16]. Based on all the foregoing mentioned above the need

arises to find a suitable drug delivery system for this drug. For this purpose sol-gel derived silica materials have been widely investigated by many researchers [14]. The release of the drug from such derivatives can be controlled by simple diffusion followed by degradation of the matrix of the drug carrier. For advanced release control the researches have investigated the stimuli-responsive materials and the dependence on external factors such as light, ultrasound and magnetic field, and furthermore, on the internal factors such as in-situ metabolic substances and pH [17].

In this work ~~a~~ nanostructured silica tubes have been prepared based on sol-gel method by using TEOS as silica precursor in the presence of CTAB and D-sorbitol derived organogelator as structure directing agents, followed by loading the doxorubicin into the nanotubes matrix, while the release behavior of the drug has been studied for two different pH values The cumulative drug release was demonstrated by using a HPLC system. Whereas, the release period rationalized according to the interactions between the silica material and the doxorubicin molecules was briefly detailed.

2. Experimental

2.1. Materials and Method

The chemicals for preparing silica nanotubes were tetraethoxysilane (TEOS) as silica precursor, cetyl trimethylammonium bromide (CTAB), as templating agent, α-methyl benzyl amine (MBA), ethanol and 25% ammonium aqueous solution. All of them were from Sigma-Aldrich co. and used as obtained from the source. 1,3 : 2,4-bis-O-(p-Nitrobenzylidene)-D-sorbitol (NO2-DBS) has been prepared as previously reported [18]. Demineralized water with type 2~10MΩ) was prepared by a Millipore Elix5 device which has been employed in all experiments. A commercial solution of Doxorubicin: (7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione (of 2mg/mL concentration) was used for all experiments.

2.2. Equipment

The HPLC system that was used was Agilent 1100, UV-Vis spectra applied in Thermo scientific evolution 220. The morphology of prepared material was investigated by scanning electron microscopy (SEM), using a HITACHI S2600N equipment. Powder samples for SEM analyses were well dispersed in acetone and then covered with a thin silver layer deposited by dc-sputtering.

2.3. Synthesis of nanostructured silica nanotubes

Silica nanotubes were prepared following the method previously reported [19]. In a typical procedure for a solution of 1% organogelator in 3mL absolute ethanol, 30 mg (1 equiv.) of organogelator and 5-7 mg (~0.25 equiv.) of CTAB have been dissolved with heating and intermittent stirring followed by the addition of 40 mg (2 equiv.) of MBA and then the mixture was homogenized by 5 min. sonication. To the viscous solution 0.27 mL (16 equiv.) of TEOS partially hydrolyzed with pre-stirring for 15 min with 0.1 mL water have been added with continuous stirring for other 5 min. The prepared sample was sealed inside a glass tube and left for maturation for around 170 hrs. (7 days). Afterwards the sample has been heated for 6 hrs. at 100^{o}C, then the temperature was raised to 200°C for extra 2 h., under (~10 mm Hg.) vacuum. The resulted solid has been calcined at 650^{o}C for 6 hrs. (heating rate 5^{o}C/ min) under atmospheric pressure.

2.4. Drug loading

The loading of the doxorubicin was done according to the physical adsorption of the drug on the silica nanotubes method. This loading occurs by stirring for 24 hours, in the dark, of silica nanotubes (5mg) with aqueous solutions of the drug with three different concentrations: 600 μg doxorubicin /mg of silica (sample a), 300 μg doxorubicin /mg of silica (sample b) and 150 μg doxorubicin/mg of silica (sample c), respectively. The resulted materials (a, b, c) were centrifuged and each washed with 20 mL of PBS. Encapsulation efficiency was estimated by determination the amount of unloaded drug in combined supernatant and washings by 480 nm UV-Vis spectroscopy using a calibration curve.

2.5. Calibration curve

The calibration curve has been established by preparing different concentrations of the doxorubicin then submitted to 480 nm UV-Vis spectroscopy The results are presented in (Table 1).

Table 1
UV-Vis Calibration curve data at 480 nm wave length.

Conc. (mg/mL)	Abs
0.0020	0.0381
0.0040	0.0659
0.0080	0.1506
0.0120	0.2348
0.0200	0.3862
0.0400	0.7659

2.6. Drug release

The drug release experiments of doxorubicin were carried out at room temperature in two PBS solutions at pH 7.4 and at pH 5.5, respectively: a sample of 0.5mg drug loaded material was placed in filtration paper with dimensions 5*5 cm shaped as envelope and immersed in 150 mL water as release medium continuously stirred at 400 rpm. Aliquots of the release medium were continuously collected using a HPLC pump and analyzed though UV-detector of the system.

3. Results and discussion

3.1. Silica nanotubes synthesis

Silica nanotubes have been synthesised by using a templating sol-gel method with TEOS as silica precursor and a combination of structure directing agents, NO_2-DBS as organogelator and CTAB as surfactant respectively, catalysed by an organic amine (MBA) according to the previously reported procedure [15]. Scanning electron microscopy (SEM) was applied (Fig. 1) to investigate the morphology of the resulted material. Around 100-300 nm diameter silica tubes were obtained with lengths of several micrometres, with inner channels of 80-100 nm in diameter.

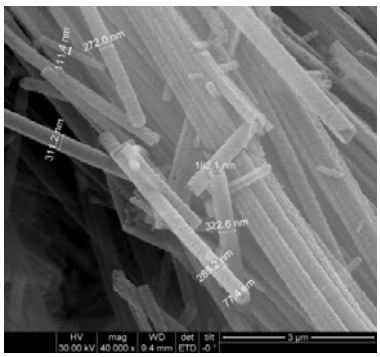

Fig. 1. SEM analysis illustrates the morphology of the silica nanotubes and their diameters.

3.2. Encapsulation of doxorubicin

The results of the encapsulation efficiency obtained by UV-Vis are presented in (Table 2).

Table 2

The result of encapsulation efficiency of silica nanotubes.

	Mixed DOX. in μg/ mg of Silica nanotubes.	Encapsulated DOX. in μg/ mg of Silica nanotubes.	Encapsulation efficiency %
1	600	100	16.6
2	300	50	16.6
3	150	50	33.3

From the results of encapsulation efficiency it can be recognized that the highest efficiency (33.3 %), was obtained in the experiment involving the lowest DOX amount (150 μg/ mg silica) while the efficiency of the other samples was the same (16.6%). An explanation might be the saturation of the silica nanotubes' surface with the voluminous drug molecule due to numerous possibilities of H-bonding with residual silanol moieties and self-assembly of the drug by π- stacking. Thus the adsorption of DOX at the surface hinders further penetration of the drug into the pores of the material and leads to modest values for the encapsulation efficiency. In designing this encapsulation experiment we made the hypothesis of loading the drug into the inner channel of the silica nanotubes with 80-100 nm diameters as revealed by SEM micrograph.

The DOX molecule geometry has been optimized for the energy minimum utilizing Hyperchem at the semi-empirical AM1 level of theory and we estimated the area (7.38 nm^2) and the volume (1.366 nm^3) of the molecule, being comparable with literature data [23]. So, taking into account the calculated dimensions of the drug and the characteristics of the silica material we may conclude that saturation with self-assembled doxorubicin molecules has also occurred at the entrance of the inner channels of the nanotubes, thus preventing the exploitation of the entire encapsulation space provided by this particularity of the silica material. The use of more concentrated drug solutions in encapsulation experiments favors only the saturation of the surface and self association of the drug, respectively, and a decrease in efficiency of 50%.

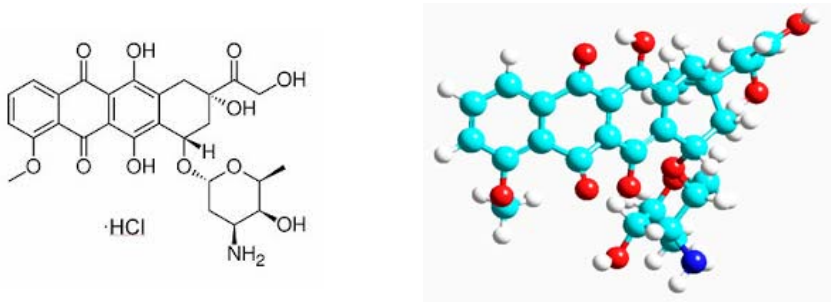

Fig. 2. Minimized geometry for doxorubicin using Hyperchem.

3.3. Drug release

The release experiments were conducted with the drug loaded nanotubes that have the highest encapsulation efficiency, i.e. 33.3%. Two media have been used for doxorubicin release, each of 50 mL of PBS solution, with pH 7.4 respectively 5.5 for a release period in dark conditions of 28 h and 2.3 h respectively.

Fig. 3 presents the release experiment at pH 7.4 exhibiting a constant increase in the first 4 hours followed by a prolonged but also constant release for the next 16 hours. After that period the drug starts to decompose due to the high sensitivity of the doxorubicin according to several conditions including pH changes and the analytical ones [22].

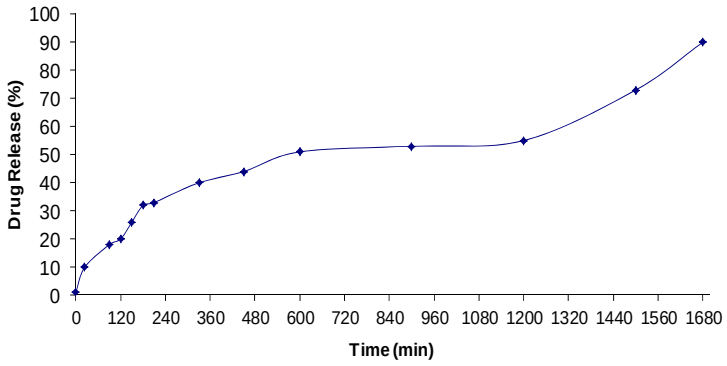

Fig. 3. Cumulative doxorubicin release from the nanostructured silica tubes at pH 7.4.

Fig. 4 presents the release experiment at pH 5.5. The reason of using such acidic medium for the release study is related to the cancer cells, characterized by this acidic medium. The drug loaded nanotubes in this medium shows completely different release behavior since the release of the doxorubicin began after 70 minutes (Fig. 4).

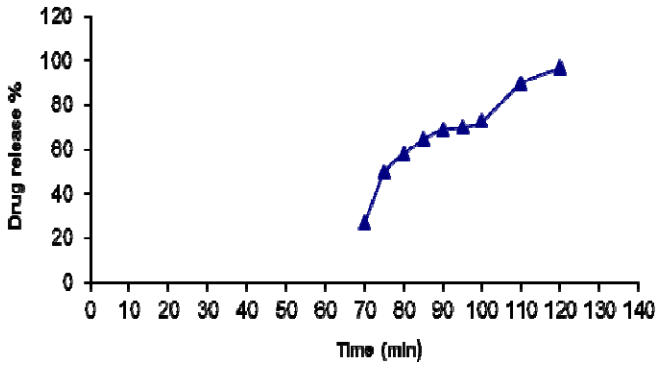

Fig. 4. Cumulative doxorubicin release from the nanostructured silica tubes at pH 5.5.

An induction period can be noticed in the drug release in acidic medium which maybe refers to the protonation of the doxorubicin NH_2 groups in this medium. We may assume that in this period the amount of the released drug was not properly detected by the device. Meanwhile, the release of doxorubicin in this acidic medium was faster than that in the pH 7.4 medium. The reason of the fast release (120 min.) is the electrostatic repulsion between the positively charged doxorubicin molecules and the surface of silica nanotubes. The release behavior is consistent with the previously reported work [20] which confirmed the advantage of using such a delivery system. Since the cancer cells have an acidic pH, while the normal tissues have a neutral one, that tends to become basic. Therefore, a faster release in the acidic medium will be demanded. as confirmed by a recent review in the same context [21].

It is worth mentioning that there are many factors affecting the release behavior of doxorubicin from the silica matrix: for example, hydrogen bonding, electrostatic interactions and hydrophobic interactions between the drug and the silica material [23]. The geometry of the doxorubicin molecule plays also an important role in the release kinetics: the voluminous molecules with the tendency of self-association, aglomerated toward the ends of the inner chanels of the silica nanotubes require an induction time in order to be transformed in the protonated form when release is performed in acidic conditions.

4. Conclusions.

We have investigated the encapsulation and the release of an anticancer drug, doxorubicin using nanostructured silica nanotubes prepared via a sol-gel

method. The best encapsulation efficiency was obtained for a lower drug: nanostructured material ratio of 3:100 due to a significant volume of the encapsulated drug and steric hindrance in the inner channels of the material which influenced the diffusion process. Release experiments in neutral and acidic media exhibit different behaviors, obtaining a constant release of the drug for about 16 hours in neutral conditions and the existence of a delay in release in acid medium due to slow pre - protonation of the drug inside the channels, followed by a fast release.

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