Nanosized Titania Reservoirs for Epilepsy Treatment

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ABSTRACT

We carried out elaborate studies on phenytoin encapsulation within the titania matrix. We changed the parameters of the sol-gel synthesis to play with the drug-matrix interactions and titania morphology to design the release kinetics. First, we showed that hydroxyl groups of titania matrix are the main interaction sites with hydantoin ring of phenytoin. We confirmed that there may be two possible complexes formed between the drug and the matrix, where one or two hydroxyl groups participate. The probability of each complex depends on the hydroxyl group coverage of the titania surface, which, in its turn, depends on the water/alkoxide ratio used in the sol-gel synthesis. Second, we varied the water/alkoxide ratio to investigate how this would affect the morphology of the matrix and, as a result, the drug release kinetics. Finally, we applied characterization techniques to study the morphology of the matrix to explain the release 'in vitro'.

Keywords: titania, phenytoin, epilepsy, drug release, drug-matrix interactions

1 INTRODUCTION

Phenytoin sodium is one of antiepileptic drugs widely used in the neurological practice. This drug reduces the excess of cerebral activity by the inhibition of the electrical conductivity between the neurons due to the blockage of sodium channels sensible to the voltage. The principal problem of all the neurological drugs is the penetration through the hematoencephalic barrier, which results in very small amounts of the drugs reaching the epileptic center. For this reason, a large concentration of the drug remains in the blood system, which produces very aggressive adverse effects, among which are renal and hepatic dysfunctions. One way to reduce the side effects is to administrate the drug directly into the epileptic focus. Nanotechnology opens up this possibility by development of nanostructured implantable devices able to release the drug *in situ* in a controlled way. The time of the release may vary from a few weeks up to one year depending on the reservoir design and the necessity of a patient. After the drug has been released, the reservoir remains intact, which allows to remove the device once the service life has finished. The introduction and remove of the device is done by the stereotactic surgery, which is the medical technique of a minimum invasion. Thus, this would be a very convenient way for a patient to receive the treatment.

2 MATERIALS AND METHODS

Titania reservoirs were synthesized by sol-gel method. Titanium (IV) tetrabutoxide ($Ti(OC_4H_9)_4$, 98%, *Sigma Aldrich*) was contineously added (0.1 ml/min) to the mixture of deionized millipore filtered water, filtered ethanol (96 %, *Metrochem*) and sodium phenytoin (99%, *Sigma Aldrich*) at 25°C under constant stirring. The molar ethanol/alkoxide ratio was kept constant and equal to 8. The sodium phenytoin/alkoxide ratio was fixed to 7.5 mg per 1 g of alkoxide (1.175 mol %). The molar ratio water/alkoxide r_w was taken as 4, 8 and 16. The resulting homogeneous sol was then left to gelate for 24 h under constant stirring and after that was dried at room temperature. The white powder was then dried at 40°C in a vacuum for 24 h.

NMR studies were performed with the *Bruker Instrument* model *Advance II-300* using a 4 mm CP-MAS probe (31P-15N) at 5 KHz. High resolution TEM microstructural characterization was carried out in a field emission electron microscope *FEI F-30* of 300 kV by *Phillips*. Drug release test was performed 'in vitro' at 37°C in a phosphate buffer solution with pH=7.2. A pellet of 50 mg was introduced into 300 ml of the buffer at constant stirring of 100 rpm. The samples of 3 ml were taken from the solution and measured with a UV-Vis spectrophotometer (*Perkin-Elmer Lambda 40*) and then returned to the solution to avoid concentration change. The concentration of the drug was defined at the wavelength of 212 nm using the calibration curve. The percentage of released phenytoin was calculated by normalization the released amount to the total amount of the drug in the pellet.

3 RESULTS AND DISCUSSION

In the particular case of the drug incorporated into the sol-gel titania, there are two principal questions that one should address: (i) does the synthesis process affect the structure-activity relation and the stability of the

drug and (ii) what functional groups of the matrix and the drug participate in the interaction? There are different types of interactions that can be found in the modern drug delivery systems: electrostatic (Coulombic), hydrophobic, or hydrogen-type. Sol-gel titania, if it is not calcinated, has a surface covered with hydroxyl groups with the average density of 5 OH/nm^2. These terminal hydroxyls can interact with a heteroatom of the drug molecule serving as adsorption sites favoring the drug distribution inside the matrix. Naturally, the number of OH groups capable of binding the drug would define the amount of the drug that can be carried by the matrix, whereas the strength of the interaction would influence the drug diffusion out of the reservoir. The two parameters together will influence the release profile. Thus, the surface coverage by OH groups determines the adsorption behavior and the surface reactivity.

3.1 Phenytoin-Titania Interactions

The solid state ^{13}C NMR study allowed us to determine that phenytoin is attached to the matrix without any changes in the structure and to establish what part of the molecule couples to the titania hydroxyl groups [2]. The comparison of the two spectra for pure phenytoin and the one encapsulated into the titania matrix (Fig. 1) revealed that the same signals are present in both cases with the only difference of the peaks in the aliphatic region of the spectrum for phenytoin titania. These peaks correspond to the nonhydrolyzed butyl radicals attached to titania. The slight shift of the signals for encapsulated phenytoin as compared to pure phenytoin implies that the structure of the phenytoin molecule in the matrix is more rigid than 'free' phenytoin. Due to the largest shifts for the two carbons of the hydantoin ring it becomes clear that the hydantoin ring in the phenytoin molecule is the system that interacts with OH groups of the titania matrix. To answer the question how exactly the interaction takes place, we suggested the possible complexes between the hydantoin ring and titania hydroxyl groups, calculated using the *Gaussian 03* [1] package of programs within the Density Functional Theory (DFT) formalism, and shown in Fig. 2.

The last complex proposed (tridentate C-III) has three simultaneous weak hydrogen-type interactions: two hydroxyl groups of titania interact with two oxygen atoms (of carbonyl groups) of phenytoin and there is an oxygen bridge from titania to a proton of the amine group of phenytoin. The calculated Gibbs energies show that C-III is more favorable in comparison to C-I and C-II. Since hydroxyl groups of titania participate in the complex formation, phenytoin adsorption on titania should significantly depend on the hydroxylation degree of titania. The experimental evidence of the presence of C-III complex was obtained by comparison of carbonyl region of IR-spectra calculated for different complexes

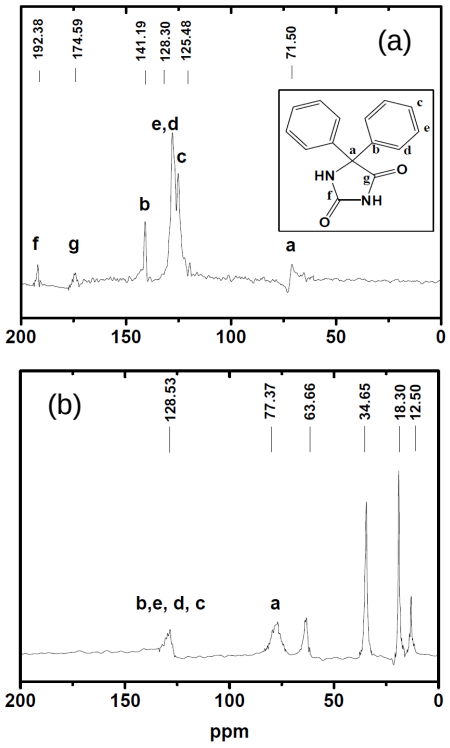

Figure 1: Solid state ^{13}C NMR spectrum of (a) pure phenytoin and (b) phenytoin-titania complex. The peak letters indicate corresponding phenytoin atoms in the phenytoin structure given on the right.

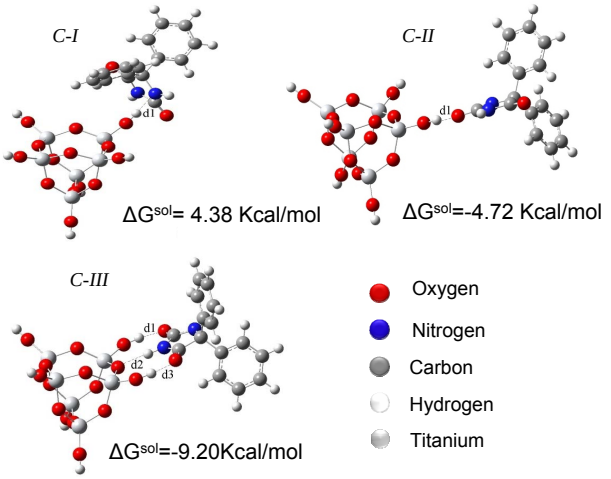

Figure 2: Optimized geometries of phenytoin-titania complexes: $C-I$ and $C-II$ are monodentate complexes, and $C-III$ is the tridentate complex. The corresponding corrected free Gibbs energies on formation of each complex are given below.

with the experimental IR-spectrum. Even though the carbonyl group signals do not disappear completely, as suggested in an 'ideal' theoretical system, a significant reduction of the signals suggests the presence of rather large amounts of C-III, though it is hard to conclude in what proportion to C-II and unbound phenytoin it is formed. Since the amount of hydroxyl groups on the titania surface is crucial for the phenytoin load in titania reservoirs, the hydroxylation degree was analyzed by IR and TGA/DSC analyses. It was found that with increase of water/alkoxide ratio r_w, the hydroxylation degree increases up to $r_w = 16$ and then decreases for $r_w = 24$. Water/alkoxide ratio $r_w = 16$ was concluded to be the most favorable to bind the largest amount of the drug because of the highest hydroxyl group coverage. The next step in the research was to study how different r_w would affect the phenytoin release 'in vitro'.

3.2 Water-Alkoxide Ratio

As it was mentioned above, titania reservoirs were synthesized by the sol-gel method. The surface properties were characterized by the Brunauer-Emmett-Teller (BET) method, crystallinity - by High Resolution Transmission Electron Microscopy (HRTEM), hydroxyl group coverage - by IR spectroscopy combined with a homemade vacuum heating cell under nitrogen atmosphere [3]. These parameters were considered in the connection with the drug release 'in vitro'.

To give an idea about the structure and morphology of the prepared materials, it is important to notice that the structure of the reservoirs is rather complex. The primary particles formed during the polycondensation are of the size of about 3 - 5 nm (Fig. 3a). The primary particles almost immediately aggregate, forming the primary aggregates of about 50 nm size [4]. Slitlike micropores of 2.5 nm are formed as a result of aggregation of the primary aggregates with the formation of the secondary aggregates. The secondary aggregates are much larger but they also can aggregate between them during the sample drying, forming the structure shown in Fig. 3b with macropores comparable to the aggregate sizes. The agglomerates have different sizes ranging from 0.1 up to 0.8 μm, building up a porous structure with large distribution of pore sizes.

Interestingly, it was found that the specific surface area increases with the addition of phenytoin to the reaction due to the difference in the particle growth at larger pH (pH=10 for the solution of phenytoin sodium in water). In the case of different r_w, it was observed that the surface area first increases and then decreases, while crystallization degree decreases with the increase of water content in the reaction. Titania synthesized in this way is mainly amorphous, however, when the samples were observed under a high resolution electron microscope (HRTEM), the regions with the crystalline

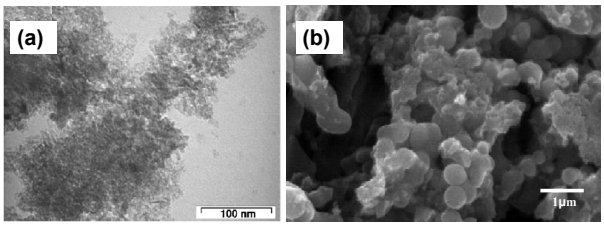

Figure 3: (a) TEM image showing nanoparticle agglomeration and (b) SEM image showing the spherical morphology.

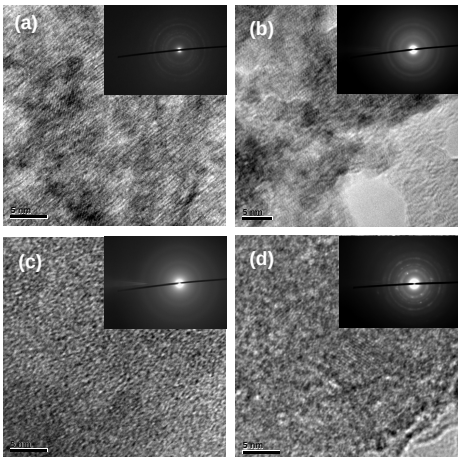

Figure 4: HRTEM micrographs with corresponding diffraction patterns of phenytoin-titania reservoirs synthesized with different water/alkoxide ratios r_w: (a) $r_w = 4$, (b) $r_w = 8$, (c) $r_w = 16$, and (d) $r_w = 16$ titania reference (without phenytoin).

structures corresponding to anatase titania were found (Fig. 4). Thus, there is an indication of a small degree of crystallinity on the nano scale in the material. Moreover, the degree of crystallinity depends on the water/alkoxide ratio r_w and decreases with the increase of r_w [3].

It was possible to characterize the OH group coverage in an accurate way, excluding the contribution of the sample humidity and physically adsorbed water. The results showed that the hydroxyl group coverage increases with increase of r_w from 4 to 16. Fig. 5 shows the drug release kinetics of phenytoin from the reservoirs with different water/alkoxide ratios r_w.

For all three samples the release profiles are similar in shape and characterized by the two regimes: the initial fast release described by the short-time (ST) release rate followed by the long-time sustained release with lower release rate (LT). The initial release rate increases with the increase of water content in the reaction. It is correlated with the size of macropores formed between the secondary aggregates of titania nanoparticles. The size of the secondary aggregates grows with increase of r_w,

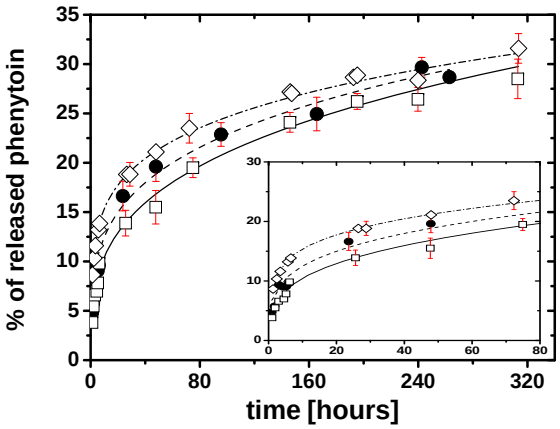

Figure 5: Release kinetics of phenytoin to buffer from 50 mg of titania reservoirs synthesized with different water/alkoxide ratios r_w: squares $r_w = 4$, circles $r_w = 8$ and diamonds $r_w = 16$. The lines indicate the Fick's second law fits: solid for $r_w = 4$, dashed for $r_w = 8$ and dash-dotted for $r_w = 16$. The inset shows closer look to the initial release stage.

thus, during the initial release period, there is a drug discharge with the highest release rate and drug amount for $r_w = 16$. Then, the initial discharge slows down with the decrease of r_w.

The constant long-time release rate is affected mainly by the following factors: morphology of the surface (surface area, porosity and pore size) and in bulk (crystalline or amorphous), interactions between the matrix and the drug, and the diffusion of the molecules within the matrix. These parameters interplay in such a way that LT release rate first slightly increases with increase of water content from 4 to 8 and then decreases for $r_w = 16$. The combination of morphology, degree of hydroxylation, and crystallinity allows sample $r_w = 8$ to liberate faster than other samples during the long-term stage.

There are different empirical and semiempirical approaches that have been developed to interpret the release mechanisms. One of the simplest empirical equation is the so-called power law equation based on Fick's second law of diffusion:

$$M_t/M_\infty = kt^n, \qquad (1)$$

where M is the amount of drug released after an instant t and infinite times, k is the constant that correlates with the diffusion coefficient and n is the exponent characterizing the release mechanism. If the Fickian diffusion takes place, n is equal to 0.5, 0.45 and 0.43 for a thin film, a cylinder and a sphere, respectively. For porous matrix n is expected to take lower values [5], [6]. However, given the simplifications introduced for this model, the analysis based on the power law should be taken with precaution. The values of parameter n are very low ($n < 0.45$ for all the samples) and vary from 0.2 to 0.3. This suggests that the release process is controlled by non-Fickian diffusion. The titania matrix has the pores quite heterogeneous in length, surface roughness and fractality, which may be the reason for the complex transport behavior.

4 CONCLUSIONS

Elaborate studies on phenytoin encapsulation within the titania matrix were performed. The NMR studies confirmed that the drug does not undergo substantial modifications during the sol-gel process. The tridental complex $C - III$ is the most favorable to form as confirmed by theoretical calculations and IR-spectroscopy. The amount of hydroxyl groups on the titania surface is crucial for the phenytoin load in titania reservoirs. The drug release profile corresponds to a non-Fickian diffusion with the most efficient process for sample with $r_w = 16$. Drug release kinetics studies revealed that there are two steps in the release process: the initial or short time stage and the long time stage. The initial release rate was found to increase with increase of water/alkoxide ratio in the reaction, whereas the long time release rate increased first with increase of r_w from 4 to 8 and then decreased for $r_w = 16$.

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REFERENCES

[1] *Gaussian 03*, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman *et al*, *Gaussian, Inc.*, Wallingford CT, (2004), Revision D.01.

[2] T. Lopez, K. Espinoza, A. Kozina, A. Galano, and R. Alexander-Katz, J. Phys. Chem. C, 114 20022-20027 (2010).

[3] T. Lopez, K. A. Espinoza, A. Kozina, P. Castillo, A. Silvestre-Albero, F. Rodriguez-Reinoso, and R. Alexander-Katz, Langmuir, 27, 4004-4009 (2011).

[4] B. E. Heredia-Cervera, A. A. Gonzalez-Azcorra, G. Rodriguez-Gattorno, T. Lopez, E. Ortiz-Islas, and G. Oskam, Sci. Adv. Mater. 1, 63 (2009).

[5] N. A. Peppas, Pharm. Acta Helv. 60, 110-111 (1985).

[6] N. A. Peppas and R. W. Korsmeyer, in: *Hydrogels in Medicine and Pharmacy*, Peppas, N. A., (Ed.) in *Medicine and Pharmacy* 3, 109-136, CRC Press, Boca Raton (1986).