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In-Situ Release of Antiepileptic Drugs from Nanostructured Reservoirs

Tessy Lopez, Anna Kozina, Emma Ortiz-Islas, Karla A. Espinoza and Richard Gonzalez

Laboratorio de Nanotecnología para Medicina, Instituto Nacional de Neurología y Neurocirugía 'MVS'; Departamento de Atención a la Salud, Universidad Autónoma Metropolitana Xochimilco; Departamento de Química, Instituto Nacional de Investigaciones Nucleares; Department of Chemical and Bimolecular Engineering, Tulane University
Mexico, USA

1. Introduction

The interest to 'in situ' drug delivery systems has been sparked by the advantages of these systems, such as ease of application, localized delivery for a site-specific action, prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort. There are different materials that can be potentially used as the implants, each of which has its advantages and disadvantages. Emulsion, liposomes, microcapsules and micells may be potentially used for certain applications Collins-Gold et al. (1990); Sharma (1997); Chen et al. (1997); Zhang et al. (1996), however, they still have some room for improvement. They are not the best systems for long-time delivery because of the stability, sterilization and low drug entrapment problems, as well as, in some cases, difficulty of manufacturing procedure or in control of the properties Hatfia & Amsdena (2002). As alternative, 'in situ' setting semi-solid drug depots are being developed. These implants are made of biodegradable polymers that solidify once injected into the site. Together with all the advantages, the main minus of these systems is the initial burst in the drug release connected with the release of the drug during the solidification time of the matrix. Also, some of the polymers require high temperature for injection or usage of organic solvents, which may result in necrosis or toxicity. Another polymer matrix (ethylene-vinyl acetate) was successfully used as an implant to deliver phenytoin into the brain Tamargo et al. (2002), however, the material manufacturing time is quite long, more than one month, which makes it difficult to apply routinely.

The recently developed sol-gel technology offers new possibilities for incorporating biologically active agents within inorganic titania or silica xerogels at room temperature, and for controlling their release kinetics from the gel matrix Chiriac et al. (2010); Quintar-Guerrero et al. (2010); Lopez et al. (2006); Lopez & Quintana et al. (2007); Lopez et al. (2007). This

sol-gel technique is inexpensive, versatile and simple and provides easily reproducible xerogel properties. Thus, such materials are good candidates to create 'in situ' delivery systems.

2. State of the art

Phenytoin (5,5-diphenyl hydantoin) is one of the major first-line antiepileptic drugs used in the treatment of generalized and partial (with or without secondary generalization) seizures. Also, it is used acutely in the management of life threatening status epilepticus and in the treatment of serial seizures. The mechanism of action is not definitely known but extensive research strongly suggests that its main mechanism is to block frequency-, use- and voltage-dependent neuronal sodium channels and, therefore, limit repetitive firing of action potentials. In chemical structure, phenytoin is related to the barbiturates. However, the use of phenytoin clinically is problematic for several reasons. Firstly, phenytoin has a low therapeutic index so that therapeutic and toxic doses are close to each other. Secondly, because of its saturable metabolism, the relationship between plasma concentration and dose is non-linear and difficult to predict Richens & Dunlop (1975). Thirdly, it has a long term toxicity profile, including adverse cosmetic effects Reynolds (1989), which is undesirable. Also, chronic drug administration can lead to many side effects, among which language and memory problems, intellectual decline and psychiatric illness. This occurs because only a certain amount of the drug overcomes the hematoencephalic barrier, which requires its higher dosage Lolin et al. (1994). Considering this, 'in situ' prolonged drug delivery represents an alternative that has excellent therapeutic benefits.

3. Sol-gel derived materials

The encapsulation of a drug inside an inorganic nanostructured matrix is a promising way to deliver the drug. The matrix is usually some metal oxide such as Titanium or Silicon dioxides or aluminosilicates of different structures. The structure may vary from highly organized (crystals, microtubes) to an amorphous one. Such a matrix has a high surface area and porosity allowing to accommodate rather large amounts of the drug. The drug may be incorporated either by adsorption into already existing structure or during the structure formation, namely the synthesis of the matrix. The latter method is more efficient because it allows encapsulation of larger amounts of the drug and its release during a longer period. The synthesis conditions of conventional chemical processes do not always allow addition of the drug during the synthesis, since many drugs are quite sensitive to the change of the synthesis parameters such as temperature, pH, etc. Also, the solubility of the drug influences the possibility of its encapsulation. However, in this case, the sol-gel method may overcome these difficulties and become a good option, since it allows the drug encapsulation under the mild conditions. In the typical sol-gel process the synthesis starts with a solution containing metal precursors, such as metal alkoxides, water as a hydrolysis agent, and alcohol as a solvent. The reacting mixture may also include acid or base as a catalyst. Metal alkoxides undergo hydrolysis and polycondensation at near room temperature forming a sol, in which polymers or fine particles are dispersed without precipitation. Further reaction connects the particles solidifying the sol into a wet gel, which still contains water and solvents. Vaporization of the solvent and water produces a dry gel, which is a porous material. Since the sol-gel process starts with a well mixed solution, the reaction may take place at lower temperatures as compared to

conventional mixtures. This enables incorporation of otherwise decomposing compounds such as many drugs. The drug is usually added to the initial mixture and during the process accommodates within the pores. Usually amorphous material has a distribution of the pore sizes. Thus, on release, firs, the drug situated inside the large pores comes out, then the one inside the mesopores, and, finally, the one inside the micropores. This permits reaching a desired level of the drug and then to have its prolonged liberation.
The release profile of the drug incorporated into titania matrix is expected to depend on the following factors: the reservoir surface properties affecting drug-matrix interactions, the morphology of the matrix, the degree of crystallinity, drug dissolution and diffusion, and the method of incorporation of the drug into the matrix. Since the majority of these factors may be controlled by the parameters of the sol-gel synthesis, the drug release kinetics, therefore, may be tuned by tailoring the processing parameters during the sol-gel reaction. Thus, one specific parameter of the synthesis can be varied in order to change the drug release profile. In the following sections we will discuss what are the main parameters influencing the release profile and how they affect the release kinetics 'in vitro'.

4. Phenytoin-titania reservoirs

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.

4.1 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 Lopez et al. (2010). The comparison of the two spectra for pure phenytoin and the one encapsulated into the titania matrix (Figs. 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 within 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

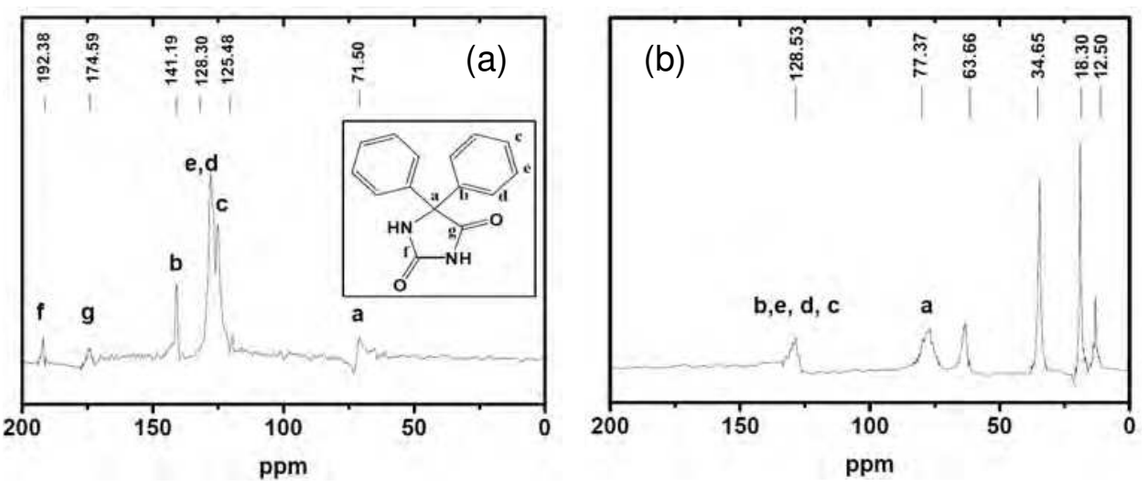

Fig. 1. Solid state ^{13}C NMR spectra of (a) pure and (b) titania encapsulated phenytoin. The peak letters indicate corresponding atoms in the phenytoin structure given on the right in (a).

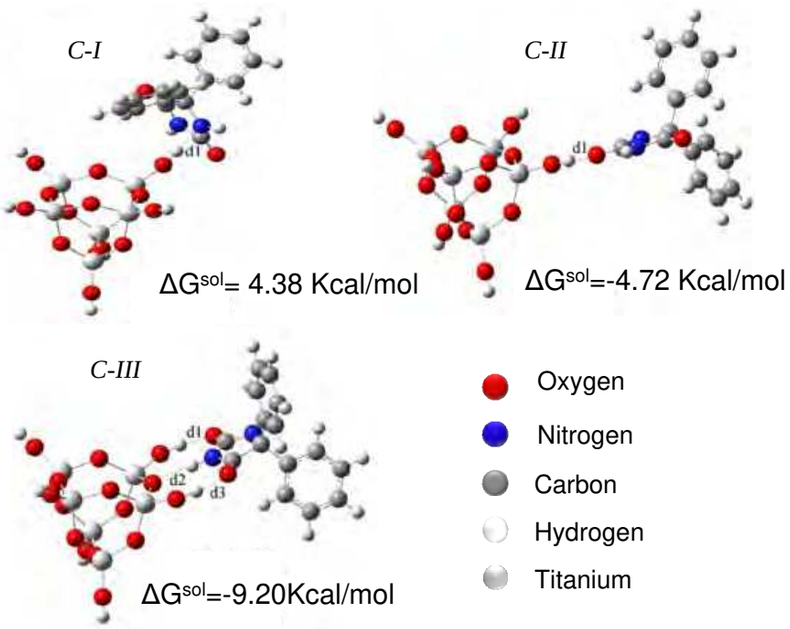

Fig. 2. Optimized geometries of phenytoin-titania complexes: $C-I$ and $C-II$ are monodentate complexes, and $C-III$ is the tridentate complex. The corresponding corrected free Gibbs energies on formation of each complex are given below.

and titania hydroxyl groups, calculated using the *Gaussian 03* Frisch et al. (2004) 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 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'.

4.2 Water-alkoxide ratio

As it was mentioned above, titania reservoirs were synthesized by the sol-gel method. Titanium(IV) tetrabutoxide was continuously added to the mixture of deionized Millipore filtered water, filtered ethanol and sodium phenytoin 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. The molar ratio of 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. 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 Lopez et al. (2011). 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 Heredia et al. (2009). 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 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 structures corresponding to anatase titania were observed (Fig. 4). Thus, there is an indication of a small degree of crystallinity on the nano

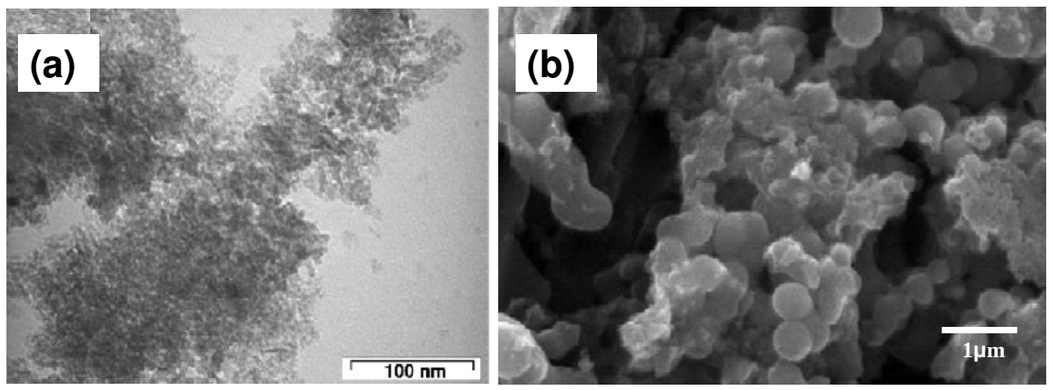

Fig. 3. (a) TEM image showing nanoparticle agglomeration and (b) SEM image showing the spherical morphology.

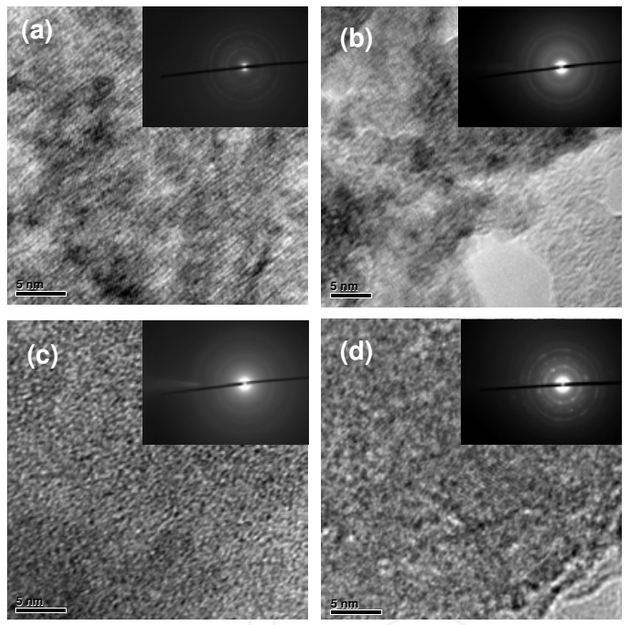

Fig. 4. HRTEM micrographs with corresponding diffraction patterns of phenytoin-titania reservoirs synthesized with different water/alkoxide ratios r_w: (a) $r_w = 4$, (b) $r_w = 8$, (c) $r_w = 16$, and (d) $r_w = 16$ titania reference (without phenytoin).

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 Lopez et al. (2011).

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 synthesized 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

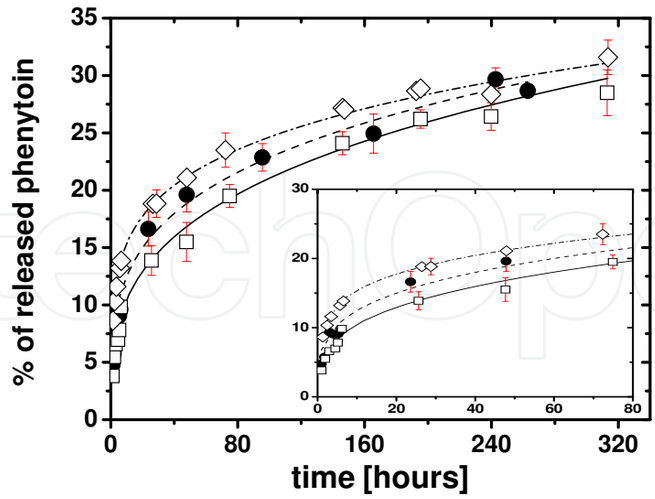

Fig. 5. Release kinetics of phenytoin to buffer from 50 mg of titania reservoirs synthesized with different water/alkoxide ratios r_w: squares $r_w = 4$, circles $r_w = 8$ and diamonds $r_w = 16$. The lines indicate the Fick's second law fits: solid for $r_w = 4$, dashed for $r_w = 8$ and dash-dotted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

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, 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: reservoir morphology on 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:

$$M_t / M_\infty = kt^n, \tag{1}$$

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 Peppas (1985); Peppas & Korsmeyer (1986). 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

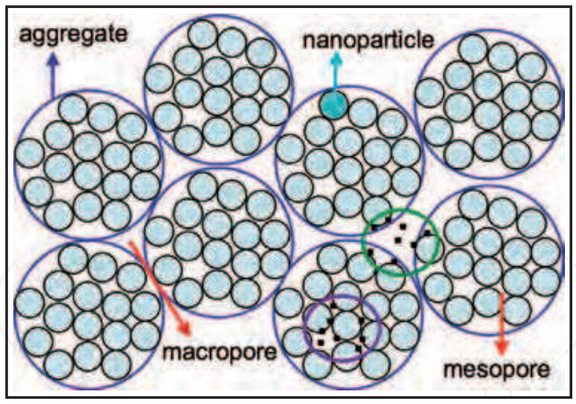

Fig. 6. (Schematic illustration of the morphology of the titania reservoirs, consisting of nanoparticles, aggregates, and macro- and mesopores. The green and purple encircled regions show phenytoin present in macropores and mesopores, respectively.

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.

4.3 Thermal treatment

With the purpose to determine the influence of the surface characteristics such as the effective surface area, porosity, and the average pore size of titania on the release kinetics of phenytoin, various titania-phenytoin reservoirs were prepared by the sol-gel method combined with the hydrothermal treatment of titanium (IV) isopropoxide in 1 M acetic acid. Control over the particle size was achieved by using the hydrothermal treatment at 220°C for different times: 1, 3, 8, 20 and 42 hours. The reservoirs were loaded with 5 wt% of phenytoin Heredia et al. (2009).

The obtained material consisted of pure anatase crystal phase independent of the heat treatment time. It was found that the average particle size defined from XRD measurements grows with the increasing treatment time. As it was previously mentioned, most nanoparticles are clustered in aggregates. The average aggregate size determined by dynamic light scattering was found to be in the range between 20 and 60 nm for the shortest and longest treatment time, respectively. The average number of nanoparticles per aggregate was found to be about 15-40 suggesting the development of porosity as shown in Fig.6.

The drug release kinetics were determined by measuring the UV-vis spectra of the buffer solution with the immersed reservoir as a function of time for a period of up to two months in a closed glass bottle. Fig. 7 shows the results of the release studies of five materials hydrothermally treated for different periods of time. It was found that the reservoirs are able to release phenytoin for more than 45 days, and the release kinetics are characterized by two regimes: an initial fast release and a subsequent slow release, similar to that observed in Fig.5. The duration of the initial fast release regime was found to depend on the hydrothermal treatment time, and decreases with nanoparticle and aggregate size. Unfortunately, the initial release rate was not quantified due to a generally non-linear behavior and insufficient data points. The slow release rate is independent of time and showed a weak dependence on the morphology of the nanomaterial. The phenytoin constant release rate was found to be

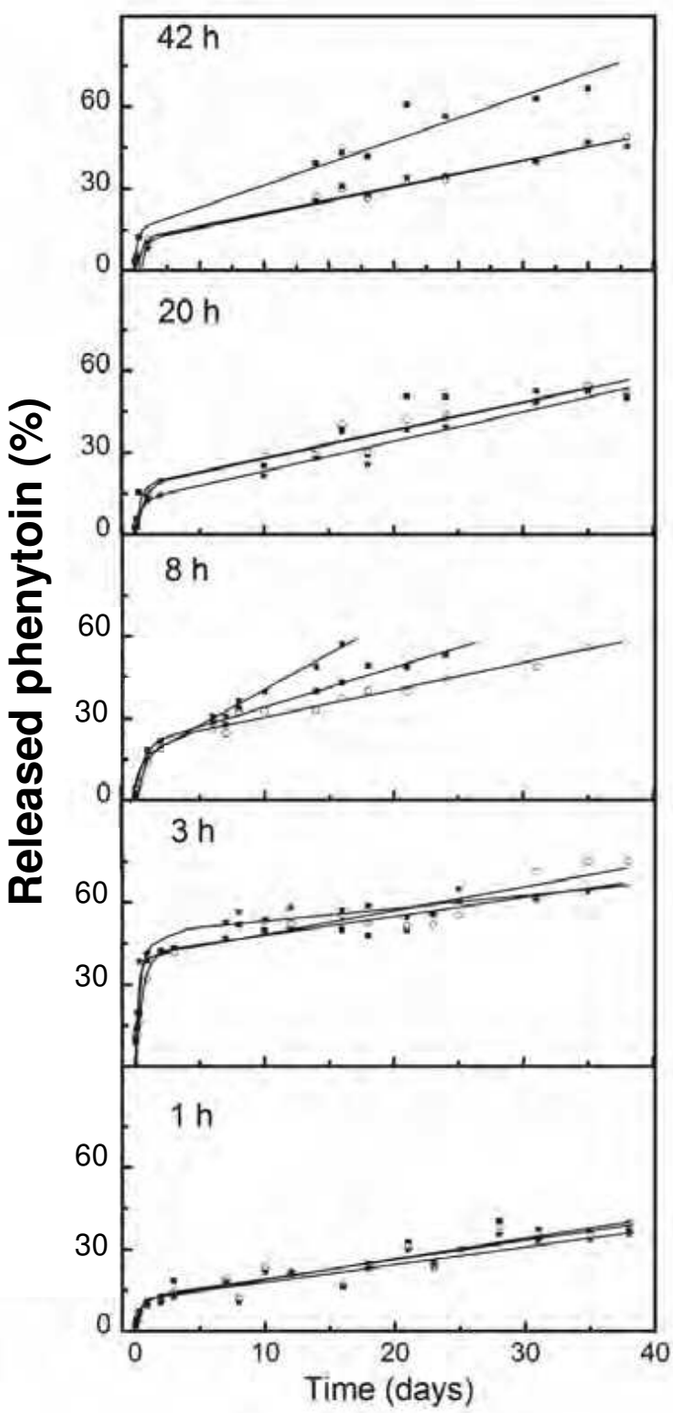

Fig. 7. Release kinetics for the titania reservoirs with different morphological properties. The released phenytoin is shown as a percentage of the initially incorporated amount of phenytoin. The kinetics were determined for three samples of each hydrothermal treatment duration. The straight lines correspond to linear fits after allowing for the initial fast release period to end.

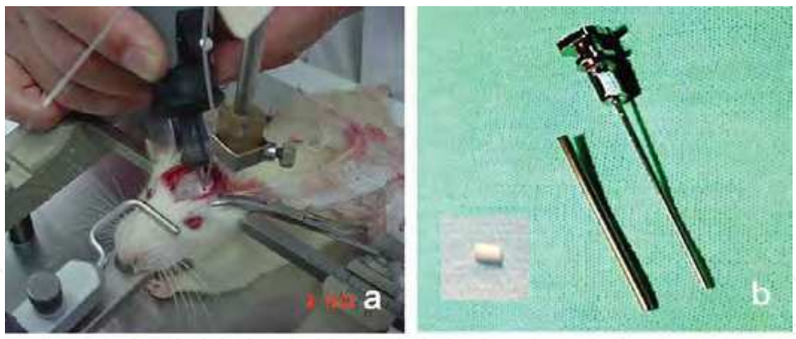

Fig. 8. (a) Stereotactic surgery is used to introduce the implant; (b) Cannula used for compressing the material to a cylinder and 1x1.5 mm titania cylinder implant.

between 0.017 mg/day and 0.030 mg/day, depending on the properties of reservoirs. One could distinguish between two main release rates: for the two smallest particles (1 and 3 h of thermal treatment) the rate is about 0.017 mg/day, while for the three largest particles (8, 20 and 42 h), the rate is about 0.030 mg/day. This trend follows the size of the mesopores, however, the dependence is remarkably weak. Taking into account that the pore size is at least a factor of two larger than the phenytoin molecules (varies from 4.3 to 12.6 nm for 1 and 42 h of treatment, respectively), the release rate is expected to be mainly related to the phenytoin-titania surface interaction. For strong interactions, the difference in specific surface area for the smaller mesopore and the larger mesopore nanomaterials would be expected to result in significantly different release rates. However, as it was shown, the release rate can be tuned to between 0.017 mg/day and 0.030 mg/day by control over the properties of the materials.

5. In vivo tests

Male Wistar rats (180-250 g) were used to study biocompatibility and effectiveness of the materials Lopez et al. (2006; 2007; 2009). All rats were induced epileptic convulsions following the Kindling model, where the rats were intraperitoneally injected with an aqueous solution of a subconvulsive dose of Pentilentetrazole (PTZ) (35 mg/kg). After each injection, observations were made for 20 min and the resulting seizures classified based on Racine's description for motor seizure activity in rats Racine (1972) as follows: 0 - normal activity; 1 - mouth and facial movements; 2 - head nodding; 3 - forelimb clonus; 4 - rearing; 5 - rearing and falling, loss of postural control, or full motor seizure activity. The animals were considered epileptics after exhibiting at least three consecutive phase 4 or 5 seizures. The material with or without phenytoin was then compressed to the form of a small cylinder with 1 mm diameter and 1.2 mm of height and stereotactically implanted into the temporal lobe of the rats (Fig. 8).
After the surgery, the animals were allowed to recover in their home cages with food and water. The effect of the implants were evaluated by further initiation of the seizure with PTZ on the rats with the reference and drug loaded reservoirs (six for each group). For the reference group of rats with a drugless TiO2 reservoir, all six rats kept on having epileptic events (Crisis-Tonic-Clonic-Generalized CTCG), thus, no curative effect was observed for these implants. The group of rats with implants showed a reduction of the intensity and frequency of the seizures, however, only about 45% of effectiveness of shielding was observed. One of the possible reasons for that may be a very small size of the implant, which results in

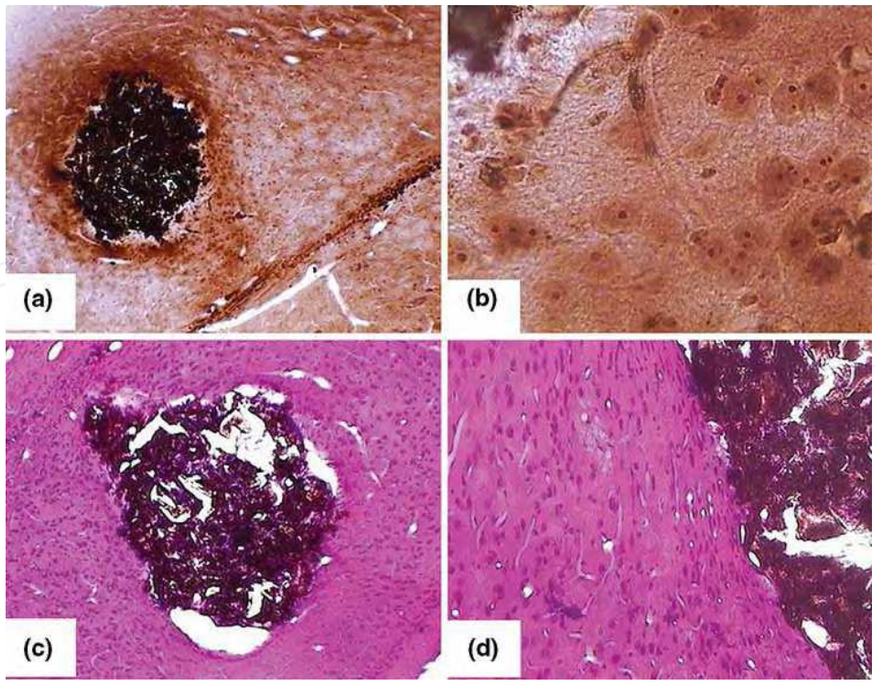

Fig. 9. Comparative histological study: (a) overall view of an histological section with the reservoir (20X); (b) The same as (a) at 100X; (c) Glial response to inflammation of the tissue in the neighborhood of the reservoir (20X); (d) Amplified limiting zone in (c) at 100X.

the release of phenytoin with the concentration lower than its terapeutic threshold. Another reason is that the release 'in vivo' may differ from 'in vitro', which requiers additional information to be able to really design the material. Our next steps would be to change the parameters of the matrix in order to find the best conditions for the reservoir to work 'in vivo'. Also, the size of the implant may be varied up to 4-5 mm in diameter Tamargo et al. (2002), which also may result in better protection.

A group of rats was sacrificed using an overdose of sodium phenobarbital administered by an intraperitoneal injection after 6 months following the implant. After that, they were perfused using a saline solution of 3.7% formaldehyde. The brains were extracted and conserved in 3.7% formaldehyde solution. The brain specimens were microtomed and conserved in a 4% formaldehyde solution for a period of 15 days. Sections (10 μm) were embedded in paraffin and viewed using an optical microscope. The sections were dyed using the Bielchowsky technique, which enables examination of the neuronal microfibrils and cell soma integrity. Implant position did not vary after 6 months in the basolateral amygdala, meaning that the reservoir was highly compatible with the nervous tissue. To confirm the lack of glial response to the implant, sections were taken of the implant zone for histological analysis and neuronal damage evaluation.

A comparative histological study Fig. 9 shows that the nerve cells are not adversely affected by the presence of the reservoir. The interfacial area between the implant and the surrounding tissue is devoid of inflammatory areas. This observation suggests that these ceramic implants can safely be used to deliver drugs to the damaged areas of the brain.

6. Outlook and prospects

An anticonvulsant drug phenytoin can be encapsulated into the sol-gel biocompatible titania and can be successfully implanted into the temporal lobe of the brain by low invasion stereotactic surgery. The implantation process is such that the damage of the surrounding tissue is minimal. The drug release from the implants is controlled by the parameters of the matrix such as its morphology, drug-matrix interaction strength, etc. Depending on the parameters of the synthesis, the release profile may be designed according to the necessities in terms of release rate and the amount of the released drug.

The first experiments 'in vivo' indicate that there is a certain degree of protection on the epileptic rats, even though no sharp fall of the seizure type was observed. Also, the biocompatibility tests revealed a good affinity between the material and the brain tissue. Thus, the first results are quite promising for the future application of the reservoirs.

One of the main prospects of the study is to achieve better protection 'in vivo' for longer time. Also, one needs to find a correlation between the drug release 'in vitro' and its effect and release profile 'in vivo'. This would allow generalization of design of the materials for different types of epilepsy patients and their needs. A very firm clinical stage is required before any commercialization of the materials.

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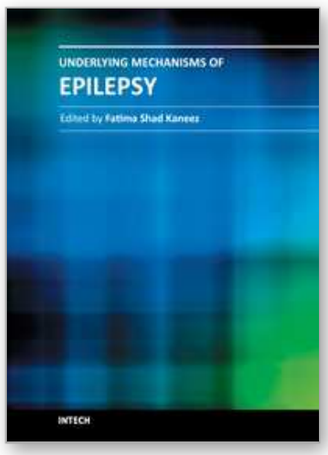

Underlying Mechanisms of Epilepsy

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This book is a very provocative and interesting addition to the literature on Epilepsy. It offers a lot of appealing and stimulating work to offer food of thought to the readers from different disciplines. Around 5% of the total world population have seizures but only 0.9% is diagnosed with epilepsy, so it is very important to understand the differences between seizures and epilepsy, and also to identify the factors responsible for its etiology so as to have more effective therapeutic regime. In this book we have twenty chapters ranging from causes and underlying mechanisms to the treatment and side effects of epilepsy. This book contains a variety of chapters which will stimulate the readers to think about the complex interplay of epigenetics and epilepsy.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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