An in vitro controlled release study of valproic acid encapsulated in a titania ceramic matrix


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Abstract
Despite the therapeutic efficacy of valproic acid towards numerous diseases, its poor bioavailability and systemic side effects pose significant barriers to long term treatment. In order to take advantage of controlled release implants of valproic acid, the drug was encapsulated into titania ceramic matrices via a sol–gel process. The integrity and structure of valproic acid-containing matrices were characterized through the use of FESEM, TEM, and BET analyses. In vitro controlled release studies and kinetic analyses were performed under ambient conditions (25 °C, atmospheric pressure) and controlled release behaviors were studied using a GC–MS method. Results showed first order dependence in the rate of valproic acid release as a function of drug concentrations in the titania ceramic device. A marked dependence on the surface area and pore size distribution with drug loading was also observed. This research opens new possibilities for the design of novel time-delayed controlled release systems for valproic acid encapsulates.

1. Introduction
An ideal drug distribution system is one which delivers the drug when and where it is required. In addition to this, the drug dosage required to elicit the desired therapeutic response should be as small as necessary. In practice, such a system should provide a programmable concentration–time profile that produces an optimum therapeutic response. This goal can only be achieved to a limited extent using conventional delivery systems. During the past two decades, significant advances have been made in the area of controlled release as evidenced by the increasing number of patents, publications, as well as commercially controlled-release products for the delivery of a variety of bioactive agents ranging from pharmaceuticals to agricultural and veterinary compounds [1–5]. This proliferation of interest is a reflection of the growing awareness that by achieving predictable and reproducible release rates of bioactive agents, particularly pharmaceuticals, to the target environment for a desired duration, optimum biological responses, prolonged efficacy, decreased toxicity as well as reduction of required dose level as compared to the conventional mode of delivery can be effectively achieved [6]. Bioactive agents can be incorporated into silica xerogel either by adsorbing the drug onto the surface of the heat-treated silica xerogel [7–9] or by adding the drug during the sol–gel manufacturing process [10–12]. The self-assembly of organized nanoscopic structures is of interest in both colloidal and material science [13]. These advantages permit high delivery efficiency, precise control of the dose for prolonged time periods (i.e., days, weeks, or months) and a reduction in toxicity. Temporal controlled drug delivery systems often use either synthetic or natural materials as for example, hydrophilic homopolymers or amphiphilic copolymers, etc., as drug carriers in the form of micro/nanospheres, micro/nanocapsules, dendrimers, micelles, liposomes, and hydrogels [3,4]. Typically, these devices are designed to operate under static conditions, which require a constant rate of drug transport from the carrier to the live environment [14].

The engineering of the porosity in nanostructured materials, such as TiO2, SiO2, etc., is an area of technological and scientific interest [15–18]. Sol–gel derived titania, an inexpensive, non-toxic and biocompatible material [19–21], has been studied as a carrier material for various drugs, such as sodium phenytoin [22], valproic acid [23], and temozolomide [24]. Controlled release over an extended duration, which can be achieved by controlling the dissolution, the diffusion, and the relaxation processes [24,25], is...
highly beneficial for drugs that are rapidly metabolized and eliminated from the body following administration. Controlled release systems aim to improve the effectiveness of drug therapy.

The purpose of this study is (i) to develop a controlled release system for valproic acid release (VPA), which can be achieved by encapsulating the molecules in a sol–gel derived titania network, and (ii) to investigate the influence of synthetic control parameters on drug release kinetics and porosity of the sol–gel materials.

2. Experimental

2.1. Synthesis

Nanostructured TiO$_2$ was prepared using sol–gel chemistry methods. The synthesis reactor consists of a three-necked round bottom reactor equipped with a vertical condenser fitted to the middle neck. A separatory funnel was connected to one of the two side necks. 24.13 ml of deionized water and varying amounts of valproic acid were added slowly to 102.34 ml of 2-propanol (Alfa Aesar, A Johnson Matthey Company, USA) under vigorous stirring to the remaining neck on the reactor. Following the addition of water and valproic acid (VPA), this neck was closed using a rubber stopper. 50 ml of the alcoxide precursor (titanium (IV) isopropoxide) (Alfa Aesar) was added dropwise to the solution over a 4 h period using the separatory funnel fitted to the flask. This mixture was stirred continuously over a period of 24 h at room temperature. Using this procedure, a stable colloidal suspension of approximately 175 ml was obtained. The excess water and alcohol were removed at room temperature and 10 kPa pressure over a seven-day period using a vacuum rotavapor (Buchi, R-2015). Approximately 20 g of amorphous titania were obtained using this procedure. Four different titania samples were obtained through the addition of 624.1, 249.64, 124.82, and 0 mg of VPA respectively. The concentrations of valproic acid obtained using these amounts of VPA added to 20 mg of titania resulted in a desired range of release rates previously shown to be effective in the control of epileptic seizures in animal models [19].

2.2. Characterization techniques

Scanning electron microscopy (SEM) was performed using a Hitachi-4800 Field Emission Scanning Microscope operated at 3 kV to investigate porous morphology and nanostructure.

The high resolution transmission electron microscopy (TEM) images were obtained using a TEM microscope, JEOLJ 010, operated at 200 kV and equipped with an energy dispersive spectroscopic (EDS) microanalysis system (Oxford). The images were obtained using a CCD Mega Vision (III) camera.

Nitrogen adsorption–desorption isotherms were obtained using a Micromeritics ASAP 2020 instrument. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas ($S_{BET}$). By using the Barrett–Joyner–Halenda (BJH) model, the pore volumes and pore size distributions were obtained using the adsorption and desorption branches of the isotherms.

Several pellets of each sample having an approximate weight of 1 g were synthesized. Preparation of these samples was performed at a pressure of 1.5 metric tons over a 3 min period using a pellet maker (Carver Laboratory Press).

2.3. Controlled drug release

2.3.1. Static release

With the exception of the sample which did not contain valproic acid, each sample was placed in a glass vial. 30 ml of the solvent, methanol (Aldrich), was then slowly added to each sample using a pipette. Because analysis was performed using GC–mass spectroscopy, methanol was chosen as the solvent of preference in order to avoid interference of water or viscous organic fluids. Each vial was covered by aluminum foil in order to protect the titania samples from light. Sampling was performed by removing a small sample of fluid from a precisely measured location in the vial. The positioning of the measurement site was located half way up the central axis of the vial. Sampling was performed after an initial period of 18 h, followed by subsequent measurements at 24 h intervals over a total time frame of 800 h. Analysis was performed using gas phase chromatographic/mass spectroscopy (GC–MS, Varian). The remaining liquid samples were returned to the vial following each measurement. It should be pointed out that when this approach is used, the concentration of VPA encapsulated within the titania and that in the solution varied over the time frame used in the study. Under these conditions, the concentration gradient will approach zero when equilibrium between adsorption and desorption is reached.

2.3.2. Dynamic drug release

Unlike the static drug release, in which the concentration of valproic acid in solution was allowed to vary, the solvent was replaced following each concentration measurement. This procedure results in the maximum possible concentration gradient between valproic acid in the solid and that in the solution, as the concentration of valproic acid in solution is always very close to zero.

2.3.3. GC–MS quantitative analysis

Quantitative analysis was performed on liquid samples using a Varian 450-GC gas chromatograph and a Varian 300-MS SQ mass spectrometer. Both instruments use electron ionization (EI). GC conditions were as follows: column pressure: 60 kPa, linear velocity: 36.7 cm/s, column flow: 1.0 ml/min, pulse pressure was: 70 kPa, pulse duration: 0.25 min and a total run time of 19 min. For analysis, 2 μl of the sample was placed in the injector and run at a 1:20 split ratio. The quantity of VPA released was calculated from the integrated peak, which corresponded to VPA and was confirmed by mass spectroscopy. The retention time for this peak was 6.1 min.

2.3.4. Reverse release (adsorption)

Two blank TiO$_2$ pellets, which did not contain VPA and were synthesized as described in Section 2.1, were placed in separate glass vials. The vials contained 6.05 mg and 12.11 mg of VPA in 30 ml of methanol, respectively. The VPA molecules in solution were adsorbed (reverse release) into the titania phase. The extent of the adsorption was monitored over an 800 h period using GC–MS as described above.

2.3.5. VPA release models

Three dissolution–diffusion kinetic models were used to fit the in vitro VPA–TiO$_2$ release profiles [26–29].

(1) The zero-order model describes the dissolution process and can be expressed as:

\[ N_t - N_0 = -kt \]

(2) The first-order model can be used to express the rate of release from systems in which the dissolution rate depends on the concentration of VPA in the titania as follows:

\[ \log \frac{N_t}{N_0} = -kt \]
(3) The modified Freundlich method can be used to explain the experimental data in terms of ion exchange and diffusion-controlled processes using the following equation:

\[
\frac{N_0 - N_t}{N_0} = -kt^a
\]

In these equations, \(N_0\) and \(N_t\) represent the amount of VPA in the TiO\(_2\) matrix present at release times 0 and \(t\), respectively. \(k\) is the corresponding release rate constant, and \(a\) is an arbitrary constant whose chemical significance is not clearly resolved [26].

3. Results

3.1. SEM, TEM and EDS analysis of TiO\(_2\) nanostructures

Field emission scanning electron microscopy (FESEM) images of the TiO\(_2\) particles prepared using sol–gel chemistry at low temperature (\(\sim 25^\circ C\)) are shown in Fig. 1. It shows the uniformity of the aggregates, which consist of individual particles which are in the nanoscale range. In order to investigate the structure of the TiO\(_2\) particles to which valproic acid had been added during synthesis, a portion of the material was sampled by scratching the tablet surface and then analyzed by HRTEM. The nanoparticles were dispersed in ethanol following ultra-sonication for 5 min prior to analysis. The resulting TEM image of a single aggregate is shown in Fig. 2. The particle structure of the TiO\(_2\) aggregates consists of individual nanoparticles, which are approximately in the 50–100 nm range.

Energy dispersive spectroscopy (EDS) analysis, which was carried out during TEM acquisition, of the TiO\(_2\) particles showed only titanium and oxygen peaks (figure not shown for the sake of brevity). This implies that, following synthesis at low temperature under vacuum conditions, the nanostructured support material is free of contaminants and unreacted precursors.

3.2. Surface analysis using nitrogen adsorption/desorption

The surface areas of the TiO\(_2\) nanostructures were obtained using nitrogen adsorption/desorption isotherms at 77 K and relative pressures (\(P/P_0\)) ranging from 0 to 1. Typical isotherms for the TiO\(_2\) nanostructures with different drug loadings are shown in Fig. 3. The corresponding BET isotherms were of type IV in accord with the IUPAC classification [30]. These isotherms are typical of mesoporous materials as they show a large increase in nitrogen uptake at high relative pressures and have noticeable adsorption/desorption hysteresis loops [31].

These observations are consistent with the corresponding pore diameter distributions determined from the adsorption isotherms, which show pore diameters to be in the 2–3 nm range. These results are also tabulated and are shown in Table 1.

These results show that the surface areas are very large and go through a maximum at a loading which corresponds to 6.05 mg of VPA/g of titania. A maximum of 2.9 nm in the pore diameter was also observed at this same VPA loading. One can visualize this as occurring due to the extra surface created by the further addition of valproic acid. However, the addition of larger concentrations of valproic acid, beyond a critical amount, results in some pore mouth

![Fig. 1. SEM images of nanoporous TiO\(_2\) aggregates, which contain encapsulated valproic acid, dried at room temperature under vacuum (10 kPa).](image1)

![Fig. 2. TEM image of valproic acid containing TiO\(_2\) aggregates.](image2)

![Fig. 3. Nitrogen adsorption–desorption isotherms at 77 K for the titania nanostructured particles at different concentrations of VPA as follows: (□) 0, (△) 6.05, (▲) 12.11 and (○) 30.3 mg VPA/g TiO\(_2\).](image3)

<table>
<thead>
<tr>
<th>VPA Encapsulated Samples</th>
<th>BET [m(^2)/g]</th>
<th>(V) [cm(^3)/g(^b)]</th>
<th>(d_{BET}) [nm](^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>545.2</td>
<td>302</td>
<td>2.40</td>
</tr>
<tr>
<td>6.05</td>
<td>681.7</td>
<td>451</td>
<td>2.90</td>
</tr>
<tr>
<td>12.11</td>
<td>656.3</td>
<td>357</td>
<td>2.40</td>
</tr>
<tr>
<td>30.30</td>
<td>587.2</td>
<td>322</td>
<td>2.10</td>
</tr>
</tbody>
</table>

\(^a\) Total pore volume, obtained from the volume of \(N_2\) adsorption at \(P/P_0 = 0.9957\).

\(^b\) Pore diameter, estimated using the adsorption–desorption branches of the isotherm.
blocking, which show lower surface area (Table 1), with a consequent decrease in the rate of drug release [32].

3.3. In vitro static VPA release

VPA static release profiles from the VPA–titania device (i.e., the amount of VPA released as a function of time) are shown in Fig. 4. The static release pattern (Fig. 4, curves 1, 2 and 3) shows two distinct release rate regions. The initial rate of release is characterized by a sharp increase below 100 h followed by a much slower release rate increase ending in equilibrium. The two samples with the highest VPA loading, 12.11 and 30.3 mg VPA/g TiO₂, result in a valproic acid release of 45 and 22% respectively, while the sample having a lower concentration, 6.05 mg VPA/g TiO₂, shows a release which is somewhat higher (data calculated from Fig. 4).

3.4. In vitro dynamic release

The results associated with the dynamic release are shown in Fig. 5a and b. In this study the entire solvent was changed following each measurement. Using this procedure, the maximum possible concentration gradient between the solid and the liquid phase is achieved. Due to desorption of the drug from the solid, this concentration gradient is decreased following each measurement. Fig. 5a shows the dependence of the valproic acid concentration remaining in solution prior to the removal of the liquid phase. The plots all converge to zero following a period of approximately 800 h, Fig. 5a. The total amount of valproic acid removed following each measurement was also calculated and is expressed numerically as function of time as shown on the right ordinate (Fig. 5a). These results can be integrated and expressed as the percentage of valproic acid released as a function of time (Fig. 5b).

From these observations it is clear that in the dynamic release mode, which could be compared to biological system where the drug molecules are continually consumed [19] a large concentration gradient is maintained with the result that release occurs over longer periods of time. This is shown in Fig. 6 in which the integrated dynamic release rate is plotted as a function of time. The time over which drug release occurs increases noticeably as the concentration of VPA in the titania matrix is increased. For the titania sample with a VPA loading of 30.3 mg VPA/g of titania, release occurs over a period of approximately 500 h. In fact, Fig. 6 shows that the entire drug that was loaded initially is quantitatively released during the reported period. This observation may
enable us to optimize the drug dose to the specific needs of the patient.

3.5. Reverse release study

Because adsorption–desorption equilibrium was obtained when a solid sample of titania in which valproic acid had been encapsulated was placed in methanol, we decided that it might be constructive to approach the position of equilibrium from the opposite direction. In order to do this, two samples of pure titania weighing 1 g each were placed in two separate glass vials with 30 ml of methanol to which 12.11 and 6.05 mg of valproic acid had been added respectively. The concentration of VPA dissolved in the methanol was monitored daily over a period of 800 h. The results of this study are shown in Fig. 7 in which we have also included the results shown in Fig. 4. The adsorption results are shown using solid lines while desorption data are shown with dotted lines. During the adsorption experiment, equilibrium was established following a period of approximately 350 h. A region of rapid adsorption followed by a region of slower adsorption was observed for both samples. The concentration of valproic acid in solution following equilibrium was found to be approximately 1.5 and 3 mg of VPA/g of titania respectively (curves 1 and 2). When these concentrations are compared to those obtained at equilibrium for the desorption process, i.e.; 4.5 and 6 mg of VPA/g of titania respectively, as shown in curves 1' and 2' it becomes clear that the desorption process becomes more challenging for valproic acid.

3.6. Kinetic analysis

In order to obtain additional insight regarding the release process we applied three dissolution–diffusion kinetic models (zero-order, first-order, and modified Freundlich model; Fig. 8a–c) to our data and calculated the corresponding kinetic parameters and linear correlation coefficients (R^2).

In general, the zero order and the modified Freundlich models are not suitable to explain the controlled drug release data obtained in this study. This is reflected by the relatively poor fit to the data (Fig. 8a and c). The plots do not fit linear relationships and also have low correlation coefficients (R^2 = 0.10–0.43). The first order model fits the release data much better (Fig. 8b), with linear correlation coefficients of R^2 = ~0.98 for VPA (Table 2) and a high degree of linearity. The rate-controlling step in the rate of drug release shows a first order dependence on the concentration of the VPA in the titania matrix and the diffusion process is governed by the concentration gradient of the drug in the titania matrix and that found in the bulk phase of the liquid. Additionally, the kinetic model predictions suggest that the release mechanism is independent of VPA loading (see Table 3).

4. Discussion

Our results suggest that the surface–adsorbate interactions for the lower loadings of valproic acid are stronger than those observed at higher concentrations of VPA and hence a lower rate of release is observed. As described in Fig. 3, the combination of high surface areas and narrow pore size distribution makes these materials ideal for use as implantable devices for use in controlled drug release applications. This maximum in surface area as a function of drug loading has been previously reported [32] and is most likely due to an increase in the surface roughness as a consequence of the incorporation of an additional amount of VPA. Controlled drug release from the pore surface may also depend on the length and shape of the diffusion path [33]. The particle size distribution for the individual titania nanoparticles is quite narrow (Fig. 2) which is indicative of a relatively short diffusion path. Bulky or branched organic molecules, such as valproic acid (2-propylpentanoic acid) (see Scheme 1), are more likely to adsorb or be encapsulated in the larger mesopores because of restricted transport and molecular size [34]. In addition, interpenetrated voids with a possible size of 1–2 nm inside the pore walls of the substrate are large enough to encapsulate VPA molecules. In fact, well before the titania solidification step, the VPA molecules are adsorbed or encapsulated in the nanopores during the nucleation step of the TiO2 formation in the liquid phase [35]. The voids facilitate mass transfer, leading to a large encapsulation within the inner surface sites.

The structure of valproic acid contains a carboxylic acid group attached to the central atom of a straight hydrocarbon atom chain which contains seven carbon atoms. Hence, one would expect strong interactions between titania and VPA through OH–TiO2 hydrogen bonds [23,24]. Subsequent multilayer adsorption forces are considerably weaker resulting in a faster rate of release (Figs. 4 and 5a). The extended period for VPA release, as shown in Figs. 4 and 6, from nanostructured titania is largely due to the strong electrostatic interactions between positively charged Ti4+ surface sites and polarized VPA molecules. VPA has a large dipole moment, with a COO− functional group in each molecule. Because of the strong interaction between titania and the VPA monolayer in contact with the titania, the desorption of this more strongly bound monolayer, will require larger concentration gradients than VPA molecules adsorbed on subsequent layers [36]. The slower release profile which is observed as a result of the drug surface interaction is of benefit in epileptic treatment, as the initial fast release enables the establishment of a therapeutic dose, and the subsequent sustained release allows maintenance of this dose over a long period of time [23,36,37].

The gradual approach to equilibrium is undoubtedly due to the presence of an adsorption–desorption process (Fig. 6). Initially there is a large concentration gradient between the surface and

<table>
<thead>
<tr>
<th>VPA in TiO2 matrix (mg/g TiO2)</th>
<th>R^2</th>
<th>k_1 (h^−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.05</td>
<td>0.969811</td>
<td>0.00594</td>
</tr>
<tr>
<td>12.11</td>
<td>0.983072</td>
<td>0.00556</td>
</tr>
<tr>
<td>30.30</td>
<td>0.984222</td>
<td>0.00247</td>
</tr>
</tbody>
</table>

k_1 is the equilibrium VPA release rate constant.

Table 2

<table>
<thead>
<tr>
<th>N0 (mg)</th>
<th>Nl (mg)</th>
<th>k1 (h^−1)</th>
<th>k2 (h^−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.05</td>
<td>4.5</td>
<td>0.00594</td>
<td>0.0222</td>
</tr>
<tr>
<td>12.11</td>
<td>6.3</td>
<td>0.00556</td>
<td>0.0061</td>
</tr>
<tr>
<td>30.3</td>
<td>8.0</td>
<td>0.00422</td>
<td>0.0155</td>
</tr>
</tbody>
</table>

Table 3

| Valproic acid (red ball: O, gray ball: C and white ball: H). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Scheme 1.
Fig. 7. VPA (a) adsorption (solid line)–desorption (short dotted line) kinetics and (b) parabolic diffusion reverse release model of 6.05 (blue solid line; curve 1) and 12.11 (green solid line; curve 2) mg of VPA/g TiO₂ in 30 ml solvent (methanol). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 8. Dissolution–diffusion kinetic models of (a) zero-order, (b) first-order and (c) modified Freundlich model for VPA release from VPA–titania matrix. The linear modeling is shown as the solid curve and the experimental, dynamic release which is similar as the released drug should be consumed by metabolic process, points are as (1 ○) 30.3, (2 ▲) 12.11, and (3 △) 6.05 mg VPA/g TiO₂ to methanol over 800 h.
the bulk liquid methanol phase. This large concentration gradient favors desorption. However, as the concentration of valproic acid in the liquid phase increases, the adsorption process becomes dependent on the rate at which the drug is consumed. If no consumption occurs, the results obtained using the static approach will apply (Fig. 4). On the other hand, when there is complete drug consumption, the results described using the dynamic model will apply (Fig. 5a and b). The expected behavior is likely somewhere in between the two models.

The adsorption–desorption state at equilibrium, which is shown in Fig. 7a, can be expressed as

$$N_{\text{ads}} + N_1 = N_0$$  \hspace{1cm} (4)

where $N_{\text{ads}}$ is the adsorbed VPA concentration at equilibrium, $N_1$ is released/desorbed VPA concentration at equilibrium and $N_0$ is total concentration of VPA respectively. Taking the derivative,

$$\frac{d}{dt}(N_{\text{ads}} + N_1) = 0$$  \hspace{1cm} (5)

or,

$$\frac{dN_{\text{ads}}}{dt} + \frac{dN_1}{dt} = 0$$  \hspace{1cm} (6)

where

$$\frac{dN_{\text{ads}}}{dt} = -k_1 N_0$$

and

$$\frac{dN_1}{dt} = k_2 N_1$$

where $k_1$ and $k_2$ are the constant for desorption and adsorption respectively.

$$-k_1 N_0 + k_2 N_1 = 0$$  \hspace{1cm} (7)

solving

$$k_2 = \frac{k_1 N_0}{N_1} = \frac{k_1 N_0}{N_{\text{ads}} - N_0}$$  \hspace{1cm} (8)

It should be noted that desorption follows first order kinetics, whereas the adsorption does not. This means that the adsorbed layers on the open or active sites of the matrix are different than that of desorption. It is possible that during desorption, there is a considerable amount of VPA in the bulk while during adsorption most of VPA in the liquid phase increases, the adsorption process becomes diffusion-controlled and VPA on the surface of TiO$_2$ particles diffuse into the medium solution via a concentration gradient. This may be related to the much smaller size of the TiO$_2$ particles. As smaller particles provide more side edges/surfaces and shorter diffusion path, the bulk matrix can more readily diffuse toward the edge/surface in a relatively continuous way even at the beginning of release.

5. Conclusion

A VPA-loaded, nanostructured TiO$_2$ matrix was synthesized using the sol–gel method, and characterized by elemental analysis, FESEM, TEM and GC–MS. TEM and SEM show aggregates of small particles (50–100 nm), leading to a material with high surface area (~650 m$^2$ g$^{-1}$), as confirmed with BET. VPA release into methanol was followed over 800 h under two conditions, ‘dynamical release’, where 100% VPA is assumed to be consumed, and ‘static conditions’, where the VPA concentration in the solution reaches a steady-state in an adsorption/desorption equilibrium. Dynamic release showed a first-order desorption kinetics, with the kinetic parameter ($k$) of 2.47 × 10$^{-3}–5.94 × 10^{-3}$ h$^{-1}$. From static release data, kinetic parameters ($k_1$) of 4.22 × 10$^{-3}–5.94 × 10^{-3}$ h$^{-1}$ ($k_2 = 2.20 × 10^{-3}–15.5 × 10^{-3}$ h$^{-1}$) were derived. These in vitro studies should be helpful to designing sol–gel derived nanocarriers for effective use in controlled release VPA for treatment.

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References


