Valproate-Loaded Hydrogel Nanoparticles: Preparation and Characterization

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ABSTRACT: Developing a simple and efficient approach to formulate biodegradable nanoparticles for intravenous delivery of sodium valproate (a hydrophilic small molecule drug chronically used in epileptic patients), is the principal objective of the current study. To fabricate particles via ionotropic gelation approach, a polycation polymer (chitosan) along with a polyanion (tripolyphosphate) was utilized in the presence of sodium valproate, and the Taguchi experimental design method was drawn upon so as to determine the optimum conditions of nanoparticle generation. In the following step, the researchers investigated sodium valproate-loaded nanoparticles to explore various features of the nanoparticles including drug loading parameters, particle size distribution, zeta-potential, morphology, stability, yield, and *in vitro* drug release profile. Nanoparticles with sizes of 63 ± 1 nm (number-based) and 79 ± 3.21 (volume-based) were obtained with slightly negative zeta–potential, which was more positive in drug-loaded nanoparticles than the unloaded ones. The TEM imaging of the hydrogel nanoparticles manifested spherical shapes and corroborated the size achieved via particle size analyzer. The loading efficiency, loading amount, and loading ratio were determined to be 21.81 ± 3.90%, 10.31 ± 1.82 (mg sodium valproate/g nanoparticle) and 23.70 ± 4.54%, respectively, in optimum conditions. Moreover, there was observed a gradual drug release for nearly a week consisting, in average, about 94.64 ± 2.71% of the nanoparticles' drug content. In a nutshell, the present study introduces a practical, simple, and effective ionotropic gelation approach to generate sodium valproate-loaded nanoparticles, leaving open a window of promising prospects in the field of intravenous long-term delivery of this chronically used drug. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 4686–4693, 2012

Key words: sodium valproate; hydrogel nanoparticles; chitosan; tripolyphosphate; *in vitro* characterization

INTRODUCTION

Nanotechnology has recently been delved into as a highly efficient approach for localized (i.e., spatially controlled) and/or timed (temporally controlled) delivery of both small and large molecular weight drugs and even genes for different therapeutic purposes.[1] Several types of nanoparticulate systems have been attempted as potential drug delivery systems.[2]

Hydrogel nanoparticles (popularly referred to as nanogels, a registered trademark of Superateck Pharma, Montreal, Canada) are a subcategory of nanoscale particulate materials that have recently undergone substantial experimentation. These nanogels are of considerable potential and capability due to the fact that they contain the characteristic features of the beneficial properties of hydrogels (due to their high degree of hydrophilicity) and nanoparticles (because of their small size) in drug delivery.[3] Therefore, it seems that the world of pharmacy will benefit from features of hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility of these particles and all the advantages of nanoparticles, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired biophase, e.g., tumor sites. Different methods have been adopted to prepare nanoparticles of hydrogel consistency. Besides the commonly used synthetic polymers, active research is focused on the preparation of nanoparticles using naturally occurring hydrophilic polymers. Among the natural polymers, chitosan has been reported most frequently in preparation of nanogels.[4] Chitosan is a biodegradable polysaccharide prepared via partial deacetylation of chitin, a copolymer of glucosamine and *N*-acetyl-*d*-glucosamine linked together by $\beta^{1,4}$ glycosidic bonds. Chitosan has been widely used in pharmaceutical and medical applications because of its favorable biological properties such as biodegradability, biocompatibility, low-toxicity, bacteriostatic, fungistatic, anticancerogen, and anticholesterolemic properties.[5] As we have pointed out elsewhere,[3] chitosan nanoparticles can nowadays be prepared via a

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number of processes. Chitosan, in its cationic solution has the ability to spontaneously gel forming and contact with multivalent polyanions because of the formation of inter- and intramolecular crosslinkages. Of the various polyanions investigated, tripolyphosphate (TPP) is the most widely used because of its nontoxic properties and fast gel-forming ability as well.[6,7] Many researchers have explored the capacity of chitosan-TPP nanosystem for loading by peptides, proteins, oligonucleotides, and plasmids DNA for potential pharmaceutical uses.[8–10] In comparison to other nanoparticle systems, the chitosan-TPP nanogels forms under mild conditions, are homogeneous with adjustable sizes, and possess positive surface charge that can be easily modulated by varying the processing conditions.[7]

Valproic acid is a widely used antiepileptic drug available for use since the 1960s. Sodium valproate is the sodium salt of valproic acid designated chemically as 2-propylpentanoate. Several clinical trials as well as decades of experience have documented the efficacy and safety of sodium valproate in adults and children for the treatment of generalized seizures (tonicclonic, myoclonic, and absences), partial seizures (simple and complex), and compound/combination seizures (including those refractory to treatment with other antiepileptic drugs).[11] The chronic clinical use of sodium valproate, the linear correlation between plasma concentration and the effect of the drug, and the well known pharmacokinetic features of valproate make as a candidate for nano-formulations. It is hypothesized in the current study that the association of sodium valproate with nanocarriers will provide relatively steady plasma levels of valproic acid with lesser fluctuations which, in turn, promote more reliable control of seizures. It decreases the number of drug doses, thus facilitating convenience and ensuring compliance on the part of the patient. The finding of this experiment can also lead to minimize the requirement for therapeutic drug monitoring and help to reduce the toxic side effects of valproate therapy, not only by reducing the therapeutically necessary dosage but also by inhibition of the formation of toxic metabolites, and, particularly, may result in increased brain-to-plasma concentration ratio of the drug to reduce dose-related side effects in the periphery while increasing the dose efficacy in the central nervous system. In this study, accordingly, hydrogel nanoparticles loaded by valproate are prepared and characterized *in vitro* in terms of the drug delivery performance-related properties.

MATERIALS AND METHODS

Materials

Chitosan (minimum deacetylation degree (DD) of 85%, Sigma-Aldrich, St. Louis, USA, Lot. no. 212F498-89) and Sodium tripolyphosphate (Sigma-Aldrich) were purchased locally. Sodium valproate was kindly donated by Rouz Darou Pharmaceuticals (Tehran, Iran). Other chemicals and reagents were of proper degrees of purity and all purchased locally.

Preparation of nanoparticles

The hydrogel nanoparticles were prepared using the ionotropic gelation method, i.e., via ionic crosslinking of chitosan (CS) using TPP. The method has already been optimized with respect to all parameters in terms of their single as well as combination effects on the finally resulted particle sizes, using a Taguchi orthogonal array design. Specifically, in optimum condition the polyanion TPP solution [5% (w/v)] was added to polysaccharide chitosan solution [0.3% (w/v) in acetate buffer of 0.67*M*, pH = 4] in a drop-wise manner, with the volume ratio of 1: 8 (TPP: CS) over a 2-min time period with constant magnetic stirring (1500 rpm) at 25°C and the stirring was continued for a 20 min. Finally, nanogels dispersion was separated from out-of-range undesired associations via centrifuging the samples at 3000 rpm for 5 min. The most effective factors on the size of nanogels were, by rank, TPP/chitosan volume ratio, chitosan concentration, temperature, addition time of TPP solution to chitosan solution, and TPP concentration.

Preparation of sodium valproate-loaded nanoparticles

Incorporation of sodium valproate into the nanoparticles was performed by dissolving sodium valproate in the polyanion TPP solution to obtain sodium valproate final concentrations of 0.5, 1, 2.5, 5, and 10 mg/mL. After mixing with oppositely charged polymer solution during the preparation procedure, to find the optimum drug concentration in terms of the loading parameters. The rest of preparation method was carried out according to the previous section. All the experiments were triplicated.

In vitro characterizations of valproate-loaded nanoparticles

The statistical central and dispersion indices of the particle sizes of freshly prepared chitosan nanoparticles were determined using a laser-diffraction based instrument called Particle Size Analyzer (Shimadzu, model SALD-2101, Japan). The particle size measurements were performed using a quartz cell in the manual mode. Samples were diluted to appropriate concentrations with distilled/filtered water. Triplicated samples were analyzed in each case.

Since, the surface zeta-potential of the drug-loaded nanoparticles is one of the major determinants of the

biofate of the carriers, the zeta-potential of the optimized valproate-loaded nanoparticles was measured using a zetameter, (Zetasizer® 3000-HS, Malvern Instruments, UK), working based on photon correlation spectroscopy technique.

Particle morphology and possible aggregation was examined by transmission electron microscopy (TEM) (Philips, model CM10). Samples were immobilized on copper grids. They were dried at room temperature and then, examined using a TEM without being stained.

For chemical characterization, Fourier transform infrared spectroscopy (FTIR) was used. Valproate-loaded hydrogel Nanoparticles were freeze-dried, and then, their FTIR spectra were obtained with KBr pellets using a FTIR spectroscope (Shimadzu, model 8000 series, Japan).

Drug assay

A reversed-phase high performance liquid chromatography (HPLC) method with UV detection was developed and used throughout the study to quantify valproate concentrations in samples of loaded nanoparticles. The chromatographic system consisted of a C_{18} column (Chromolith® Performance, 100×4.6 mm^2, Merk, Germany) connected by a pre-column guard with the same packing and a binary mixture of phosphate buffer (0.025*M*, pH 6) and acetonitrile (60: 40) as stationary and mobile phases, respectively. A pump-controller unit (Knauer, Smartline®, model 1000, Berlin, Germany) and a Rheodyne injection device (Rheodyne, Model 71251, CA) equipped by a 50 μL loop were used for solvent delivery (flow rate 1 mL/min) and sample injection, respectively. The analyte detection was made by a UV-detector (Knauer, model 2500, Berlin, Germany) at wavelength of 210 nm. The chromatograms were processed using compatible software (Knauer, Eurochrom®, Berlin, Germany). A series of validation tests were carried out on the method.

Loading parameters of sodium valproate-loaded nanoparticles

The total drug concentrations in nanodispersions were determined following the destruction of the nanoparticles via addition of 0.015 mL/mL $HClO_4$ (70%) to the dispersions, and then, measurement of the drug concentration in resulting solution using the developed HPLC method. The unloaded drug concentrations were determined following the separation of nanoparticles from the aqueous medium containing unloaded drug via filtration through a 50 nm membrane filter (Millipore®, Bedford, MA), and then, measurement of the amount of free (unloaded) drug in the filtrates using the developed HPLC method. The loaded amount (LA) of sodium valproate in nanoparticles was determined using the Equation:

$$\begin{aligned}\text{LA} = {} & (\text{total valproate in (mg)} \\ & - \text{unloaded valproate in (mg)})/ \\ & (\text{nanoparticles weight in (g) obtained by freeze} \\ & - \text{drying method})\end{aligned}$$

The loading ratio (LR) was determined using the equation:

$$\begin{aligned}\text{LR} = {} & [(\text{total valproate concentration} \\ & - \text{unloaded valproate concentration})/ \\ & \text{total Na valproate concentration}] \times 100\end{aligned}$$

Finally, the loading efficiency (LE) of sodium valproate by the prepared nanoparticles was calculated as:

$$\begin{aligned}\text{LE} = {} & (\text{LA}/\text{total Na valproate added during} \\ & \text{the loading procedure}) \times 100\end{aligned}$$

In vitro release of valproate from the hydrogel nanoparticles

In vitro release profile of valproate from the prepared hydrogel nanoparticles was determined as follows; the valproate-loaded nanoparticles were prepared using the optimal methodological setup and the final nanodispersion was then, divided into fifteen 1-mL portions in 1.5 mL polypropylene micro tubes. The samples were shaken gently (15 rpm) while being incubated in 37°C using a vertically shaking incubator designed and assembled in-house. At the beginning of the test and at 1, 2, 4, 8, 12, 24, 48, 72 h, and 1, 2, and 3 weeks and also at 1, 2, and 3 months intervals, one of the aliquots was harvested and after a 1: 10 dilution with distilled/filtered water the total drug concentration in nanodispersions was determined by destruction of the nanoparticles by adding 0.015 mL $HClO_4$ (70%) to 1 mL of dispersion followed by HPLC analysis for drug content. At the same time the free drug content in each sample was determined after passing the nanodispersion through a 50 nm membrane filter (Millipore®, Bedford, MA) and HPLC analysis of the drug content in filtrate. The percent ratio of free-to-total valproate concentrations in each sample was the ordinate of release profiles. The release experiment was repeated three times.

Stability of sodium valproate-loaded nanoparticles in suspension form

To evaluate the stability of drug-loaded hydrogel nanoparticles *in vitro*, a drug-loaded nanoparticle

sample was prepared and divided into two portions: one portion was refrigerated at 2–8°C and the other was kept at room temperature. Particle size distributions of the two samples were monitored at the beginning of the test and also after 1, 2, 4, 8, 12, 24, 48, 72 h, as well as 1, 2, and 3 weeks. These experiments were carried out three times.

Preparation of nanopowder form of drug carriers

To prepare a suitable nanopowder form from nanoparticles, cryoprotective excipients such as glucose, mannitol, sucrose, glycerin, and tween 80, which are necessary part of the freeze-drying procedure, were added to the nanoparticle dispersions, each at concentrations of 1, 3, and 5%. The nanoparticle dispersions were frozen at −32°C for a minimum of 12 h, and then, dried at −55°C and 0.5 kPa for 24 h. Finally, all dried samples were reconstituted in distilled/filtered water to appropriate concentrations and their sizes were evaluated by particle size analyzer, as mentioned earlier in section "*In vitro* characterizations of valproate-loaded nanoparticles".

Determination of nanoparticles yield

The yield of nanoparticle preparation was determined after the separation of nanoparticles from out-of-range waste materials. Aqueous suspensions resulting from nanogelation procedure, as described earlier in section "Preparation of nanoparticles" were centrifuged at 3000 × *g* for 5 min, and then, nanodispersion (i.e., supernatant) as well as the waste material (i.e., centrifuge pellet) were freeze-dried separately until reaching a constant weight of each portion. Nanoparticles yield (NPY), by convention, was defined as:

$$\begin{aligned}\text{NPY} = [&(\text{Dried weight of nanodispersion}\\ &-\text{nominal weight of acetate buffer components added}\\ &-\text{nominal weight of sodium valproate added}\\ &-\text{nominal weight of cryoprotectant added})/\\ &\text{sum of nominal weights of chitosan and}\\ &\text{TPP added initially}] \times 100.\end{aligned}$$

RESULTS AND DISCUSSION

Nanoparticle preparation

Nanoparticles of chitosan were prepared via the ionotropic gelation technique. This method utilizes the ionic interaction between the positively charged chitosan and the negatively charged TPP, based on the ability of chitosan to form a gel after contacting with polyanions by forming inter- and intramolecular linkages. The optimum combination of independent variables and their corresponding estimates between real- and orthogonal-values were altered by Taguchi experimental design. In this design the optimum condition is calculated based on the dominancy of signal/noise ratio of each factor level. After the initial estimation, the optimal setting was validated by nanoparticles preparation using the specified conditions followed by the particle size analysis in each case. As a result, the optimum setting indicated earlier in nanoparticle preparation method was reliable and highly robust with the outputs being highly consistent with the theoretical data.

In vitro characterization of sodium valproate-loaded chitosan nanoparticles

The final particle sizes corresponded to the optimal setting and were 62 ± 2.01 nm (number-based diameter) and 78 ± 2.09 nm (volume-based diameter) for unloaded nanoparticles, and 63 ± 1 nm (number-based diameter) and 79 ± 3.21 nm (volume-based diameter) for valproate-loaded ones. The number-based diameter is the diameter of a sphere having the same length as the particle in question; whereas the diameter of a sphere having the same volume as the particle is the volume-based diameter. In other words, the modal diameter of the semilogarithmic particle size distribution graph consists of the relative percentage of particles in each size class based on the number of particles on the *Y*-axis is the number-based diameter; while the modal diameter of this graph with the relative percentage of particles based on the volume they occupy on the *Y*-axis is the volume-based diameter.[12]

The particles obtained in the present study were unidisperse (unimodal curves) with very suitable sizes (below 100 nm), in terms of both central tendency indices and dispersity indices with respect to the intended intravenous drug delivery. Most importantly, all the mean, median, and modal diameters remain without any significant changes after the loading procedure ($P > 0.05$). Furthermore, the size dispersity of the nanoparticles population was about the same in valproate-loaded and unloaded nanoparticles. This means that in optimum condition [CS 0.3% (w/v), TPP 5% (w/v), TPP/CS 1/8, temperature 25°C, Addition time of TPP solution to CS solution 2 min, sodium valproate 2.5 mg/mL], the drug loading procedure has no significant effect on particle size and size distribution of the nanoparticles. Our diameters of about 60 nm obtained in current study for valproate-loaded hydrogel nanoparticles are remarkably lower compared to 300–400 nm for insulin-loaded hydrogel nanoparticles[13,14] and >120 nm for ammonium glycyrrhizinate-loaded hydrogel nanoparticles,[15] both prepared by ionotropic gelation process.

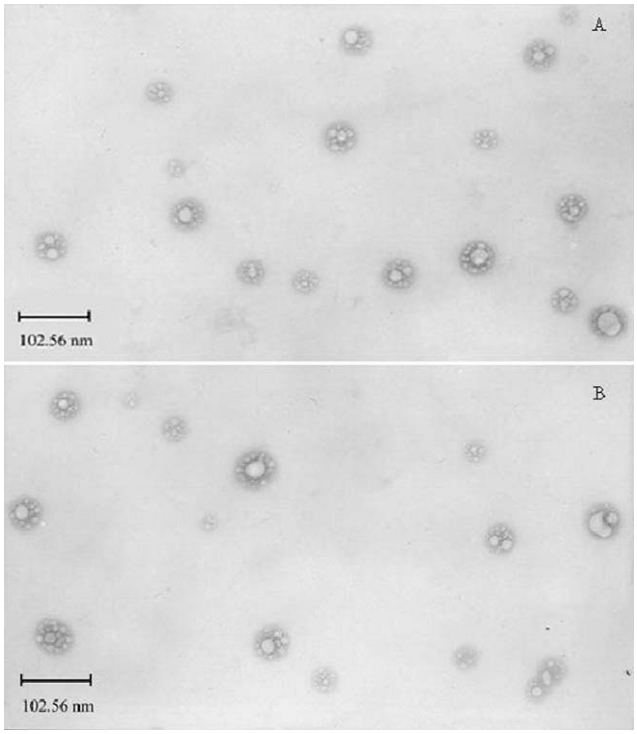

Figure 1 (A) TEM images of hydrogel nanoparticles prepares without sodium valproate (unloaded sample) and (B) the particles loaded with sodium valproate (2.5 mg/mL). (CS 0.3% (W/V), TPP 5% (W/V), TPP/CS 1/8, Temperature 25°C, Addition time of TPP solution to CS solution 2 min.

In all these instances, the size of nanoparticles increased when the agent was loaded, while the size of our nanoparticles did not significantly change after sodium valproate loading. Interestingly, the small size of obtained sodium valproate-loaded nanoparticles in this study is a favorable prerequisite for a long circulating drug delivery system.

The zeta-potential distribution diagrams of the unloaded as well as valproate-loaded nanoparticles, being both monodispersed, show an overall mean zeta-potential of slightly negative, i.e., −6.80 and −9.01 mV for valproate-loaded and unloaded nanoparticles, respectively. These values, while deserving further improvements, again emphasize the applicability of the prepared nanoparticles for the long circulating intravenous delivery. The drug loading has clearly shifted the zeta-potential of nanoparticles toward positive, which was expected considering the protonated, i.e., neutral, nature of sodium valproate in acidic pH of the loading condition, which being incorporated in particle surface, causes the net surface density of negative charges to be relatively decreased. There are studies on nanoparticles obtained via the same preparation method that have reported positive final zeta-potential.[13,14]

Figure 1 shows the morphological characteristic of nanoparticles obtained by TEM. The size analysis of sample particles under TEM (while confirming the size profiles obtained from our particle size analysis) indicates that the size ranges of valproate-loaded and unloaded chitosan-TPP nanoparticles are apparently identical, both having spherical shapes. In addition, the presence of some 'satellite' particles around the main particles as a typical behavior of the samples is noteworthy. The shapes of particles are approximately spherical and smooth in texture with almost a homogenous structure, which can be attributed to the relatively gentle preparation condition of nanoparticles. Various studies have also reported spherical shapes[16,17] but the evidence from the formation of polyhydrons, instead of spheres is published.[18]

Figure 2 shows FTIR spectra of unloaded as well as valproate-loaded hydrogel nanoparticles. In characterizing the FTIR spectra, originally, there are three determinant peaks of chitosan at around 3400 cm^{-1} of OH, 1100 cm^{-1} of C—O—C, and 1600 cm^{-1} of NH_2. As expected, the peak at 3400 becomes wider as TPP becomes conjugated to chitosan. This event is evident in our spectra and is attributed to the resulting enhanced hydrogen bindings. In addition, in chitosan-TPP nanoparticles peak of 1600 cm^{-1} of NH_2 bending vibrations shifts to around

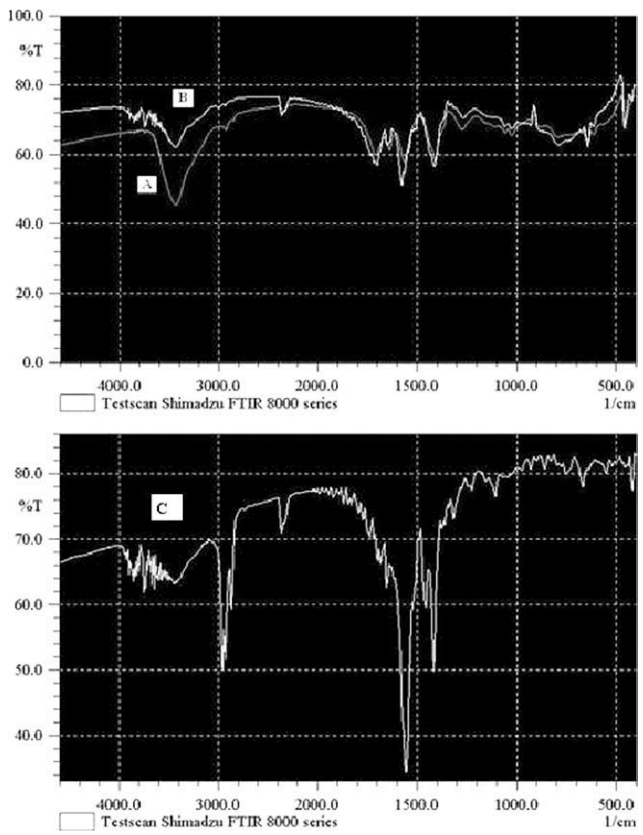

Figure 2 (A) FTIR spectra of unloaded nanoparticles, (B) sodium valproate-loaded nanoparticles, and (C) sodium valproate.

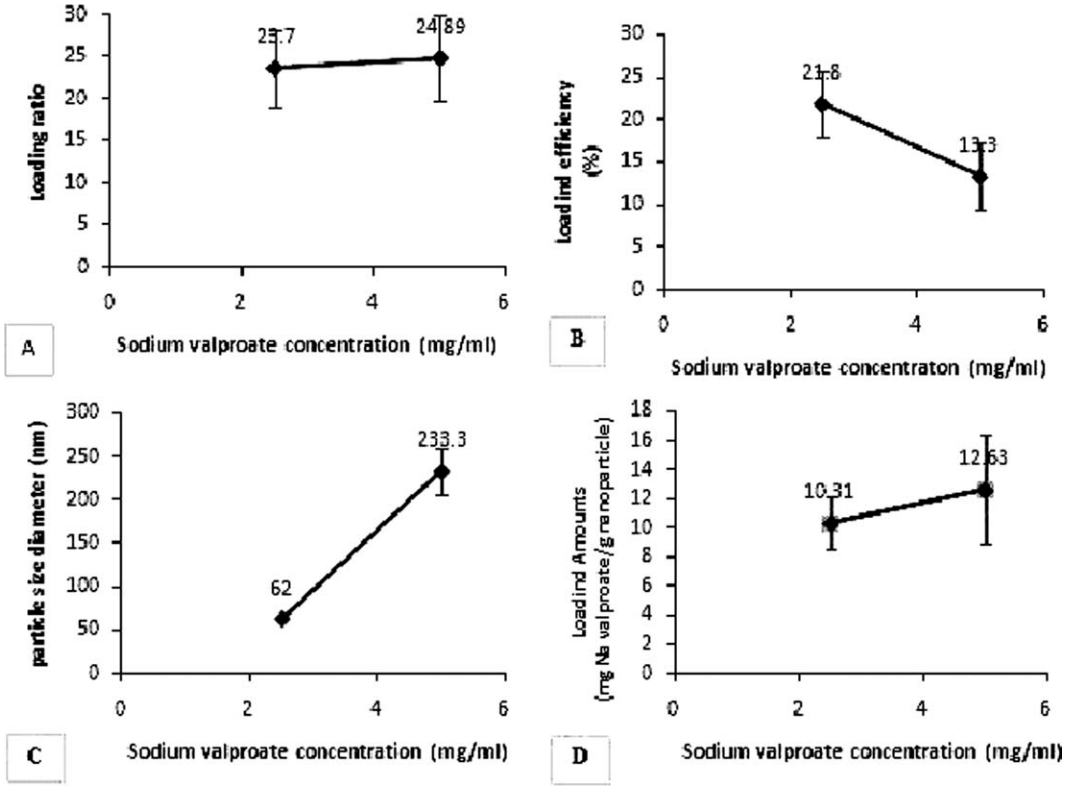

Figure 3 The effects of sodium valproate concentration on loading ratio (A), Loading efficiency (B), Particle size diameter (C), and Loading amounts (D) of drug-loaded nanoparticles.

1500 cm^{-1} and a new sharp peak at 1630 cm^{-1} appears. The FTIR spectrum is consistent with the result of chitosan film modified by phosphate, and it could be attributed to the linkage between phosphate and ammonium ions. So we suppose that the phosphate groups of TPP were linked with ammonium groups of chitosan in nanoparticles. Compared with the spectrum of sodium valproate, in the spectrum of sodium valproate-loaded nanoparticles, the absorption peak of about 1550 cm^{-1} (carboxyl group absorption peak) disappears and a new shoulder peak at 1453 cm^{-1} (salt of carboxyl) appears. The results indicate the presence of the electrostatic interactions between carboxyl groups of sodium valproate and amino groups of chitosan.

Sodium valproate encapsulation and release

The loading parameters of sodium valproate in prepared nanogels at two different drug concentrations are displayed in Figure 3. It is notable that for the concentrations 0.5 and 1 mg/mL no quantifiable valproate concentrations were obtained. Also for the drug concentration of 10 mg/mL the drug was precipitated during the nano-gelation procedure. Therefore, only the data for two concentrations of 2.5 and 5 mg/mL are shown in Figure 3.

A promisingly high drug loading ratio obtained with the valproate concentration of 2.5 mg/mL while with no harmful effects on the most important parameter of particle size. However, the higher drug concentration of 5 mg/mL suffered this limitation in a way that it made an about fourfold increase in particle mean diameter. Considering the loading ratio, a ratio of about 25% was achievable even in the latter higher concentration. It means that the drug loading capacity is not the limiting factor in this case, and even if we did not have the solubility problem of the drug in nanoparticles preparation condition, it seems probable that with the concentration of 10 mg/mL we could attain higher drug loading amounts. In other words, if the limiting factors of particle size growth or drug solubility can somehow overcome the achievement of higher drug loading seems quite possible with this procedure. Considering this behavior along with the chemical structure of valproate, it seems that the drug is loaded in nanoparticles via some kind of electrostatic interactions strong enough to keep the drug in association with the hydrophilic nanogels. The possibility of the physical 'caging' effect can be ruled out considering the drug's small structure. Also the involvement of any significant chemical bound can also be excluded, considering the FTIR data. The presence of multiple hydrogen bonds with chitosan

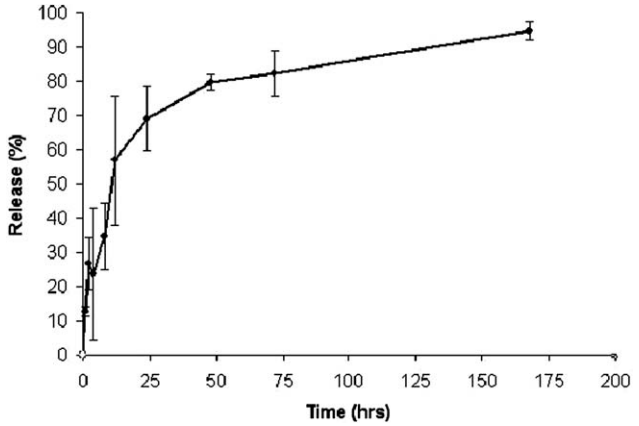

Figure 4 The release profile of sodium valproate from hydrogel nanoparticles. CS 0.3% (W/V), TPP 5% (W/V), TPP/CS 1/8, Temperature 25°C, Addition time of TPP solution to CS solution 2 min, sodium valproate 2.5 mg.

molecules, without crowded sodium valproate molecules competing for amine groups and experiencing spatial exclusion from each other may also be considered to play at least a partial role in this scenario.

Sodium valproate concentration of 2.5 mg/mL was selected as optimum, in which the sizes of nanoparticles were similar to the sizes obtained in optimum condition of particle fabrication without the drug while the loading ratio was not significantly different from higher concentrations.

The experiment on the possibility of drug decomposition by acidic condition of nanoparticles destruction and/or drug adsorption to filter showed no significant changes resulting from none of the factors in drug concentrations, thereby insuring the validity of the data obtained in drug loading estimation method.

There have been several other similar studies applying similar nanogels and method, which resulted in higher loading efficiencies of 55%,[13] 80%,[14] and more than 90%,[19] but this was possibly because of the larger sizes of the obtained nanoparticles (300–400 nm, 250–400 nm, 500–710 μm, respectively).

The release profiles of sodium valproate from hydrogel nanoparticles while being shaken at 37°C are shown in Figure 4. It should be noted that because the particles were completely deformed and aggregated after 1 week, the data beyond 1 week were practically useless and hence are not presented. Drug released from nanoparticles and subsequent biodegradation are important for developing successful formulations. The release rates of drug from nanoparticles depend upon: (1) desorption of the surface-bound/adsorbed drug; (2) diffusion through the nanoparticles matrix; (3) diffusion through the polymer wall (in case of nanocapsules); (4) nanoparticle matrix erosion; and (5) a combined erosion/diffusion process. In the case of matrix devices such as the one we prepared in this study, drug is uniformly distributed/dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than the matrix degradation, then the mechanism of drug release becomes mainly governed by Fickian diffusion, otherwise it depends upon degradation rate. Considering that we have performed the drug release experiments directly on the nanosuspension, as prepared, without the replacement of the medium, to avoid any shock because of the external conditions on the nanoparticles, and also considering the drug loading ratio in nanodispersion, it can be concluded from the release data that there is an initial burst release in the first 24 h of sampling, including about 60% of the loaded drug. This release rate becomes gradually lower followed by a linear phase between the second and seventh days, including about 20% of the drug. The burst release is hypothetically caused by a portion of the drug associated weakly to nanoparticles, e.g., adsorbed onto the surface of the microsphere.[20] The terminal apparently zero-order release, however, seems to be related to a portion of the drug either bound to nanoparticles more strongly, e.g., via electrostatic associations or entrapped deeply inside the nanogel structures.

Stability of sodium valproate-loaded nanoparticles in suspension form

According to the results obtained from particle stability studies, nanoparticle sizes remained unchanged for two weeks with no apparent difference in temperature sensitivity (Fig. 5) and increased about fivefold on three weeks in both temperature conditions. However, the volume-based particle size

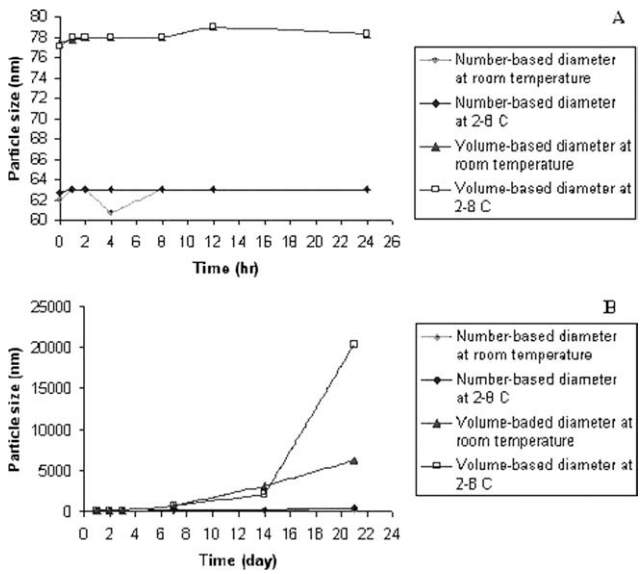

Figure 5 Particle size of sodium valproate-loaded hydrogel nanoparticles during stability test. (A) 0–24 h (First day) and (B) 1–21 days.

profiles showed dramatic increase after one week, indicating the occurrence of some rare giant associations. The whole body of these data indicates excellent particle stability, at least for one week, in addition to no observable swellability of the particles in aqueous media, both features being very important with respect to our long-circulating therapeutic targets.

Preparation of freeze-dried nanopowder form of drug-loaded nanoparticles

From a series of tests using simple sugars or nonionic surfactants as cryoprotectants, the best result, i.e., regaining particle sizes of the nanoparticles after reconstitution was obtained with 5% (w/v) concentration of glucose. The average particle size after reconstitution was 90 nm. Using 5% (w/v) of glucose, the appearance of the cake (the dried material) became as a regular white powder, i.e., neither hardened nor fluffy, which is the best for a freeze-dried formulation. When other cryoprotectants were used, the appearance of the dried material was translucent and sticky. Redispersion of the nanoparticles was possible with other agents, but as a form of visible aggregates. The good cryoprotective results with glucose probably arise from its ability to bind with water molecules in the amorphous phase during the freezing step. A part of the water in the frozen glucose remained nonfrozen [even 32% (w/w)], which, in turn, acts as a plasticizer and spacing matrix, thus reducing the pressure of ice crystals against the nanoparticles and preventing harmful aggregation caused by the frozen materials.

Nanoparticles weight-based yield

Our approximate yield was calculated to be 82.9 ± 3.98 on the basis of the polymer and TPP used and the waste material seems to be unconjugated chitosan and aggregated nanoparticles. The yield of the nanoparticles formation is very promising in terms of the capability of the system to be economically scaled up.

CONCLUSIONS

The collective body of the data in this study indicates that the very simple and available ionotropic gelation method used for fabrication of the valproate-loaded nanoparticles results in practically acceptable drug loading, particularly considering the small molecule drug used, with particles having:

- Very high hydrophilicity, above 90% in water;
- Ideal particle size of about 60 nm with suitable dispersion and no rare large particles/associations
- Reasonable zeta-potential of about −6.5 mv;
- Spherical uniform shapes;
- Release-control of the drug up to 95% release during the first week, but deserving improvements;
- FTIR spectrum evidencing the formation of chemical associations resulting in nanoparticles formation
- Capability of being reversibly freeze-dried with minimum deviations from initial particle sizes upon redispersion.

All the aforementioned features, being favorable with respect to a long-time delivery of the drug upon intravenous administration, represent a successful drug-loaded nanoparticulate system, which hold high promises in terms of intravenous long-term delivery of this chronically used drug, mainly in epilepsy cases. In addition, taking the chemical structure of the loaded drug into account, small molecules which are highly problematic in the way to prepare nanoparticles for drug delivery, the loading as well as *in vitro* characterization results propose a delivery system with high potentials and capacities for a vast number of other drugs. A series of further improvements beyond the scope of the current study are underway in our lab with the main goal of optimizing the system for entering the *in vivo* studies and ultimately, hopefully clinical trials.

References

1. Moghimi, S. M.; Hunter, A. C.; Murray, J. C. Pharmacol Rev 2001, 53, 283.
2. Sahoo, S. K.; Labhasetwar, V. Drug Discov Today 2003, 8, 1112.
3. Hamidi, M.; Azadi, A.; Rafiei, P. Adv Drug Deliv Rev 2008, 60, 1638.
4. Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi, T. M. J Control Release 2004, 100, 5.
5. Hejazi, R.; Amiji, M. J Control Release 2003, 89, 1.
6. Kawashima, Y.; Handa, Y. T.; Takenaka, H.; Lin, S. Y.; Ando, Y. J Pharm Sci 1985, 74, 264.
7. Gan, Q.; Wang, T.; McCarron, P. Colloids Surf B 2005, 44, 65.
8. Shu, X. Z.; Zhu, K. J. Int J Pharm 2000, 201, 51.
9. Sezer, A. D.; Akbuga, J. Int J Pharm 1995, 121, 113.
10. Aydin, Z.; Akbuga, J. Int J Pharm 1996, 131, 101.
11. Davis, R.; Peters, D. H.; McTavish, D. Drugs 1994, 47, 332.
12. Sinko, P. J.; Singh, Y. Martin's Physical Pharmacy and Pharmaceutical Sciences, 6th ed.; Lippincoll Williams & Wilkins; a Woltcrs Kluwcr business: Philadelphia, 2011, Chapter 18.
13. Fernandez-Urrusuno, R.; Cavlo, P.; Remunan-Lopez, C.; Vila-Jato, J. L.; Alonso, M. J. Pharm Res 1999, 16, 1576.
14. Pan, Y.; Li, Y.; Zhao, H.; Zheng, J.; Xu, H.; Wei, G.; Hao, J.; Cui, F. Int J Pharm 2002, 249, 139.
15. Wu, Y.; Yang, W.; Wang, C.; Hu, J.; Fu, S. Int J Pharm 2005, 295, 235.
16. Sannan, T.; Kurita, K.; Iwakura, Y. Makromol Chem 1976, 177, 3589.
17. Genta, I.; Pavanetto, F.; Conti, B.; Giunchedi, P.; Conte, U. Proc Int Symp Control Release Bioact Mater 1994, 21, 616.
18. Gan, Q.; Wang, T. Colloids Surf B: Biointerfaces 2007, 59, 24.
19. Ko, J. A.; Park, H. J.; Hwang, S. J.; Park, J. B.; Lee, J. S. Int J Pharm 2002, 249, 165.
20. Zhou, S. B.; Deng, X. M.; Li, X. H. J Control Release 2001, 75, 27.