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RESEARCH ARTICLE

Potential use of Nanostructured Lipid Carriers mediated neuronal delivery of Carbamazepine

Salar Masoomzadeh[1, 2, 3], Somayeh Sadighian [2], Tooba Gholikhani[3,4], Kobra Rostamizadeh [1*]

[1] Department of Medical chemistry, School of pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.

[2] Department of Pharmaceutical Biomaterials, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.

[3] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

[4] Nanora Pharmaceuticals, Tabriz, Iran.

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ABSTRACT

Carbamazepine (CZB) as a medication for epilepsy and anxiety therapy, presenting a complex pharmacokinetic profile and severe side effects. Therefore, the development of strategies that significantly decrease the side effects and frequency of administration, together with a simple administration, is still a great need. In this respect the use of lipid-based nano systems, such as nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN) is highly efficient. In this process, the lipids were used are Cetyl palmitate and Coconut oil to made NLCs, 75°C water bath was prepared; which was included surfactants, Tween80 and Sodium Lauryl Sulfate (SLS), the particles produced by adding the above-mentioned lipids, dissolved in Dichloromethane in this environment. Physiochemical tests were carried out, In vitro drug release studies were performed by HPLC system. Optimized drug-NLCs showed spherical morphology and their average particle size were 170 ± 2.6 nm, PDI of 0.273 ± 0.017. These particles zeta potential and entrapment efficiency were − 23.2 ± 1.2 mV and of 97.1 ± 2.1% respectively. Taken together, the therapeutic effectiveness of CZB was markedly augmented in CZB-NLCs; thereby, NLCs could be appropriate carriers for the delivery of epilepsy medications to the brain with higher drug release potential.

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INTRODUCTION

Seizures are a group of diseases that are observed in many different forms and for many reasons [1]; Seizures can be caused by a CNS infections[2] to genetic damage , head trauma and hits but in all kinds of the seizures with each of the reasons the main cause of occurrence is shifting the brain networks from balanced forms to hyperactive and hypersynchronous state[3]. Many drugs are used in this field that work by inhibiting the sudden brain neuranal actions [4] and the neurons in the specific focal points of the brain (at the for example complex partial seizure) but one of the most useful of them is Carbamazepine, a drug that is used in many kinds of diseases other than seizures[5] like the bipolarity[6], neuropathic pains[7], and mental illnesses[8].Carbamazepine inhibits sodium channel firing [9] and prevents the cascade and it applies its effects by reducing polysynaptic nerve response and in addition inhibiting post-tetanic potentiation [10]. The high frequency of its administration is served as a major disadvantage for this drug. Lipid based drug delivery systems like liposomes, can be beneficial in this regard. This research was carried out to solve this issue by making use of nanostructured lipid carriers (NLCs). NLCs are the group of lipid-based nanoparticles that were introduced to the pharmaceutical industry for almost a century;

* Corresponding Author Email: *Rostamizadeh@gmail.com*

nanostructured lipid carriers are spherical structures that have three layers that from outside to inside are composed of thin hydrophilic layer, solid lipophilic layer and the liquid oil core[11]; these carriers are so similar to Solid Lipid Nanoparticles but they have some advantages over them like their capacity for drug loading[12], stability in drug-releasing and physical stability [13]. Using NLCs can help the drug to have a controlled and sustained releasing[14]. These carriers may protect the drug from unwanted metabolic damages[15] in the body as well as delivery to unwanted targets[16]. In this study, a sustain released NLC formulation of Carbamazepine with improved pharmacokinetic parameters was developed.

MATERIALS AND METHODS

Materials

Cetyl palmitate, Tween 80, Sodium Lauryl Sulfate (SLS) and Span 60 were produced by Iranian factory Pars-shimi and the Coconut oil was provided by the same company but produced by Mancolimited factory of India; Hydrochloric acid (Merck, Germany); Potassium chloride and Sodium chloride (Merck, Germany); Monopotassium phosphate and Disodium phosphate ($Na_2HPO_4.2H_2O$) were prepared from Merck company of Germany; Methanol, Acetonitrile and Formic acid were produced by German company Merck and they were bought from Pasargad Iranian company as an importer; Dichloromethane (Emertat, Iran); and Carbamazepine as active compound of this research was supplied by Arastoo Pharmed Iranian company.

Preparation of NLCs

For the preparation of NLCs, an aqueous phase containing surfactants at a concentration of 25 mg/mL was stirred until complete dissolving. This phase was heated at 75 °C; then was added to the lipid phase (containing 60 mg of coconut oil and 140 mg of acetyl palmitate in 11 mL of dichloromethane) under continuous stirring. The composed emulsion was homogenized by using a homogenizer (Heidolph, Germany) at 12,500 rpm for 30 min and consequently. Then to obtain NLC, the emulsion is brought to room temperature. At the end, the NLCs was checked by using SEM method.

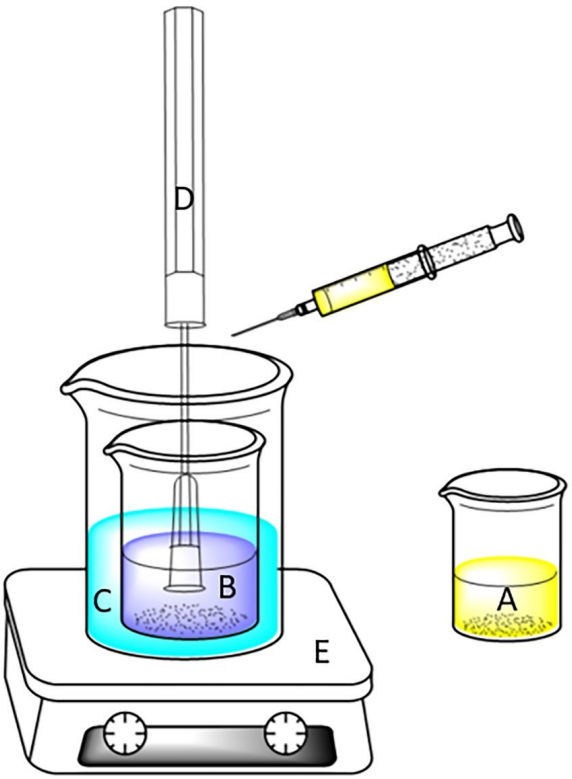


Fig.1. Schematic picture of making nanostructured lipid carriers (Pure and Drug-loaded).
A: Lipophilic solution (was dripped into the B); B: Aqueous Solution; C: Water bath; D: Homogenizer; E: Heater-Stirrer

Determination of NLCs Particle Size and Ζ-potential

The average size of the synthesized NLCs and their surface charge was measured by using a Nano Zetasizer.

Stability of nanoparticles

The pH of the NLCs solution was decreased to 0.5-1 with dropwise addition of Hydrochloric acid, then the solution was centrifuged at 20000 rpm for 20 minutes and the precipitate was then dried for 12 hours at 40 ° C; the dried sediment was dispersed at 50 mL of distilled water by sonicating and the size of the particles was investigated by Dynamic Light Scattering (DLS).

Characterization of NLCs

Differential scanning calorimetry (DSC) patterns of NLC, Carbamazepine, Carbamazepine loaded NLC were collected on DSC-60 (Shimadzu, Japan). In this study the heating rate was considered 10 ◦C/ min and the temperature range was 20–260 ◦C.

The FT-IR spectra of CZB, CBZ-NLC was obtained by Fourier trans form infrared spectroscopy. ATR method was considered for pure CZB while the KBr disk method considered for the rest samples. The scanning wavenumber was between 500–4000 cm−1 and resolution have been 1 cm−1. Encapsulation efficiency (EE) and drug loading (DL) of Carbamaz with 255 nm for λex were calculated by using the data obtained from an Shimadzu HPLC spectrophotometer. EE (%) = loaded drug / added drug × 100. DL (%) = loaded drug/weight of NPs × 100 equations[17].

invitro Drug release

Dialysis techniques can be use to investigate *in-vitro* release[18, 19]. In short, 30 mg of drug-loaded NLCs in an immersed dialysis bag in 25 ml of PBS solution and stored at pH 7.4 and 37 °C temperature. At a predetermined time, interval, 2 ml of sample was taken simultaneously and was replaced with the same volume of fresh buffers to continue the status of the sink.

$$Actual\ drug\ concentration\ at\ time\ Z\left(\frac{\mu g}{ml}\right)=Cz+\left(\frac{v}{V}*\Sigma Co\right)$$

Cz= Calculated drug concentration at time z v= 1 ml V= 25 ml Co = All concentrations from time 0 to z-1

For calculating releasing percent this formulation was used (25 ml is the volume of each sample vial):

$$Drug\ releasing\ percent=\left(\frac{Actual\ drug\ concentration\ at\ time\ Z\times 25}{Total\ dissolved\ nano\ drug\times CBZ\ loading\ percent}\right)\times 100$$

RESULTS AND DISCUSSION

Physical stability of NLCs

Size of nanoparticles was investigated with and without drugs to check the effect of drug loading on the particles size and the method efficacy; the data obtained shows that the drug loading can't affect the particle's size so much and doesn't make them larger than the expected limit.

Checking the size stability by using the process of part 2.4 was shown that the size of the particles after the process has not changed significantly and only their PDI of them was increased from 0.33 to 0.42.

PDI reports the Polydispersity Index of the particles size[20]; all of the PDIs which were have been measured in this article are between 0.08-0.7, which in this range, distribution algorithms work best.

Investigation of NLCs Ζ-potential

The Ζ-potential of the pure NLC particles was about -24 and this data for drug-loaded NLCs was estimated at -28 (Fig.2); this proves that the drug loading cannot cause the reduction of the surface potential of particles and follow by doesn't cause a reduction of stability of nano solution and particles attraction to each other.

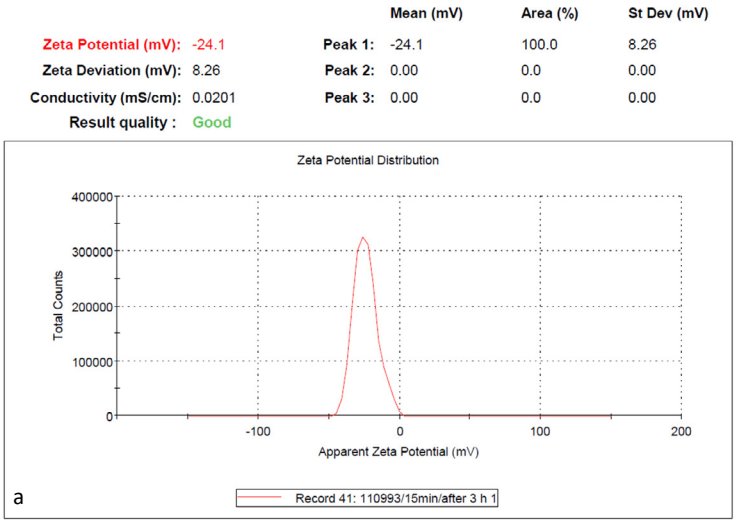
SEM, DSC, and FT-IR results

SEM

The result of SEM is reported in Fig.3. in this image the structure and structural system of the particles can be observed.

DSC

The results show that the melting point of the drug was decreased in drug-loaded NLCs in comparison with pure carbamazepine (the melting point of the drug was decreased by about 20°C in drug-loaded NLCs.); the recorded charts exactly report that the melting point of nanoparticles weren't changed so much and it should say that the recorded melting point is exactly for Cetylpalmitate (because the coconut oil appears in liquid form even at room temperature.); at Fig.4 section C the recorded peaks, in order from left to right, reports



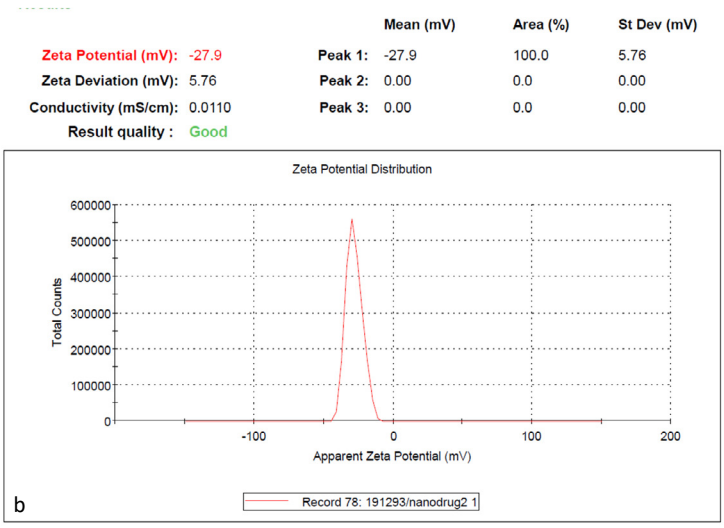


Fig.2. Ζ-potential diagrams. (a: pure NLCs, b: drug-loaded NLC

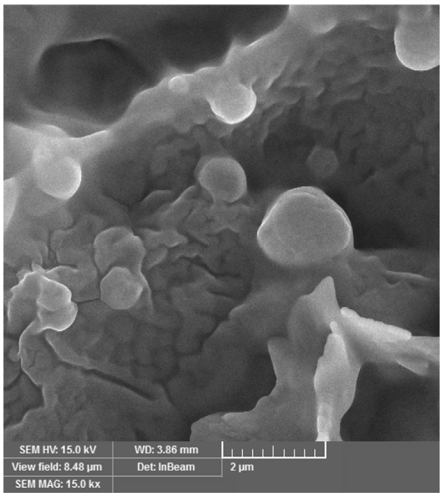


Fig.3. SEM image of Carbamazepine loaded NLCs.

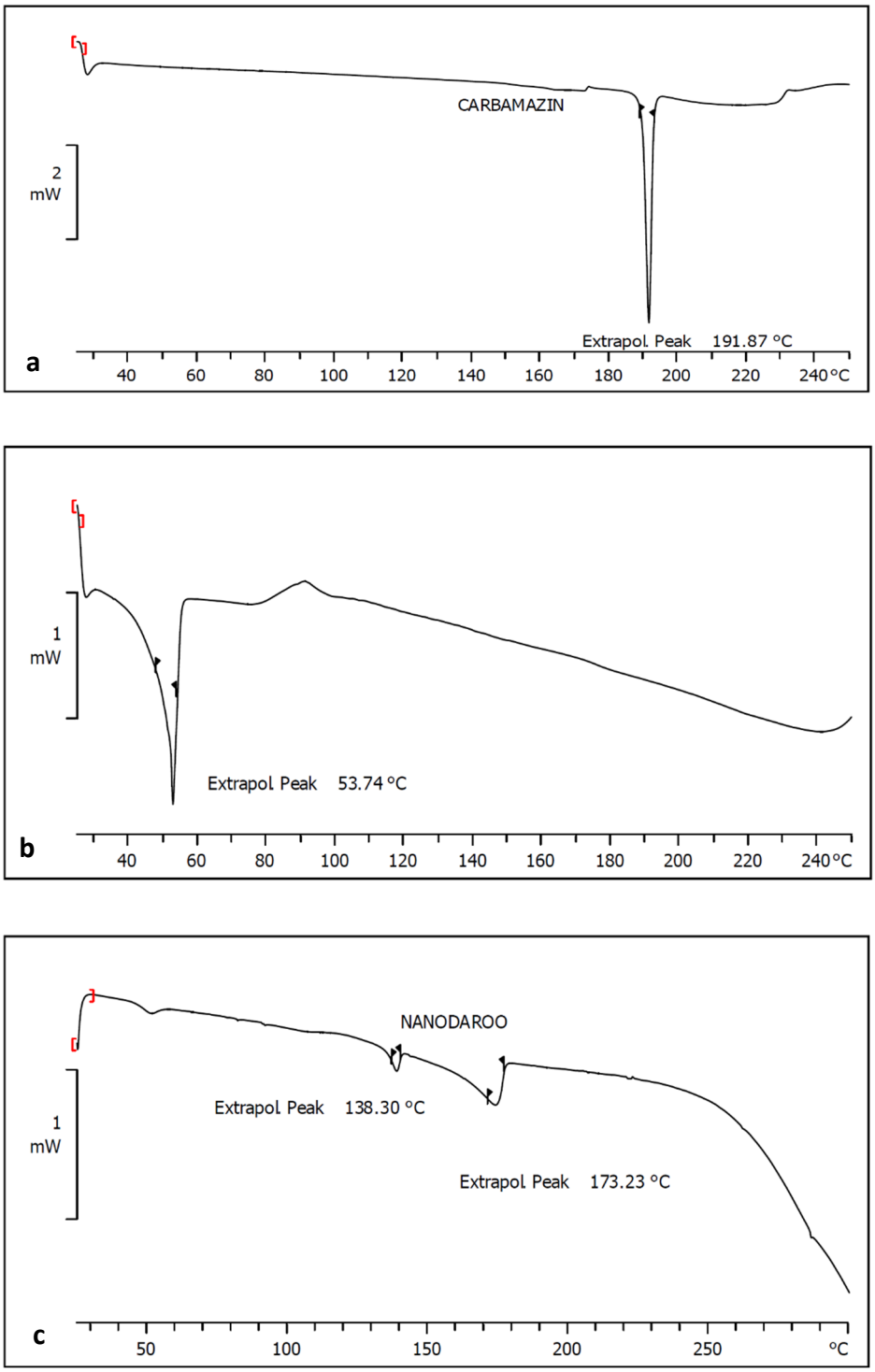


Fig.4. DSC results (a= pure drug; b= Pure NLCs; c= drug-loaded NLCs).

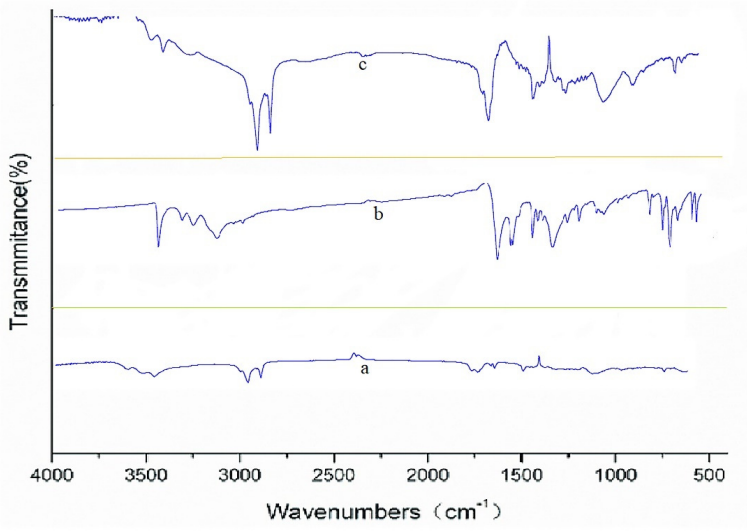


Fig.5. FT-IR results (a= pure NLCs; b= Pure drug; c= dug-loaded NLCs).

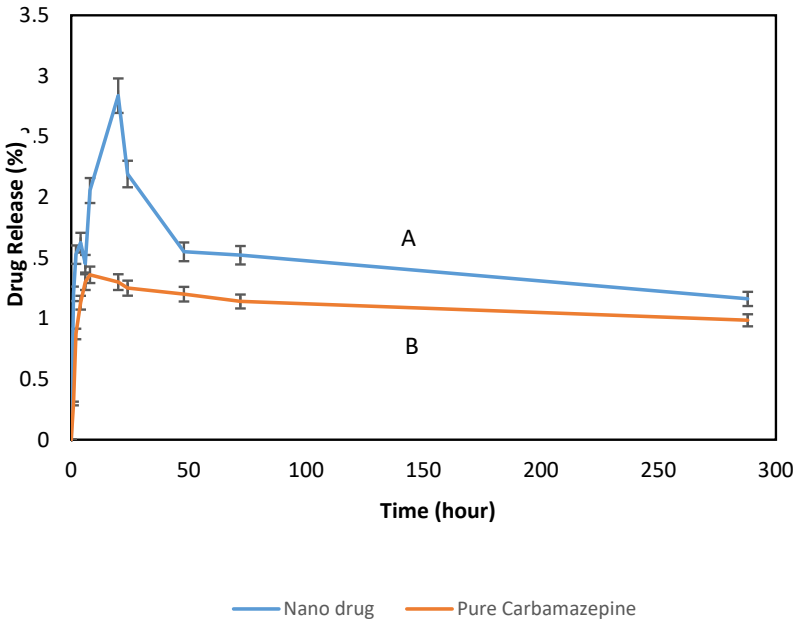


Fig.6. Invitro drug release diagram (a total drug for nano-drug due to calculated loading is 534 µg, and for the pure drug is 5100 µg).

the melting point of pure Cetylpalmitate, pure drug, and drug loaded NLCs.

FT-IR

Examining FT-IR charts shows the results that are given in the above diagram; the results shows that the possible reactions in the nanoparticle manufacturing process cause some changes in IR peaks recording, for example, the peaks of free and hydrogenated amines at the diagram of pure carbamazepine were recorded at 3100 to 3460 (cm^{-1}) [21] but the peaks of same structures have migrated to the range of 2849-2918 cm^{-1} the drug-loaded NLCs diagram.

Calculating the drug loading in NLCs

By using the data which are obtained from studies the loading percent of carbamazepine in NLCs was calculated about 1.78% (the process was exactly described at section 2.7.).

Invitro Drug release

To analyze the drug invitro releasing the data of the process which was described at section 2.8 was used[22]; by using these data and calibration diagram[23] the data table was obtained using which the release diagram from loaded-NLCs was drawn; the data of the samples which were taken from vials of pure NLCs, reports nothing because of no HPLC peaks in the λ which was set for the detection of carbamazepine(287nm)[24, 25]; the data of pure drug-releasing was reports the needed time for reach the maximum drug concentration in PBS environment (these data used to draw the diagram of pure drug-releasing in Fig.5). According to these results, it seems that this carrier can be used for carbamazepine delivery to the patients who need to use this drug because after 288 hours the concentration of a drug is still good.

CONCLUSION

Nowadays, various cases are considered about a drug; The targeted effect of the drug on the disease, the half-life of the drug and its side effects are among the most important characters, which can greatly affect the amount of drug consumption. This research proves that NLCs can be a suitable carrier for carbamazepine and reduce the frequency of drug administration as it has increased the CBZ blood concentration. These carriers can affect the drug usage frequency by continuous drug releasing. Reducing daily and maybe weekly drug dosage using can increase the patients companionship in the course of treatment and this is one of the most important achievements of a research. Also loading the drug in NLCs causes the more drug releasing in compare with pure drug (even up to double). The loading of carbamazepine in NLCs can also reduce the side effects of carbamazepine due to the gradual increase in concentration (instead of a sudden increase).

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AUTHOR CONTRIBUTIONS

All of the authors were involved in all stages of the article writing.

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