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PREPARATION AND IN VITRO CHARACTERIZATION OF CARBAMAZEPINE-LOADED CHITOSAN-COATED/UNCOATED PLGA AND ZEIN NANOPARTICLES

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Abstract: The aim of this study is to prepare CBZ-loaded chitosan (Ch)- coated/uncoated poly(lactic-co-glycolic acid) (PLGA) and Zein (using 20 mg or 40 mg Zein) nanoparticles (CBZ-PLGA-Zein-NPs or CBZ-PLGA-Zein-Ch-NPs) and to characterize (Particle size, PDI, zeta potential, percent encapsulation efficiency (EE%), FT-IR, DSC and XRD analyzes, and *in vitro* release study) them *in vitro*. These nanoparticles were prepared using a modified emulsification-solvent evaporation method. The particle sizes of CBZ-PLGA-Zein(20)-NPs, CBZ-PLGA-Zein(40)-NPs and CBZ-PLGA-Zein(20)-Ch-NPs were found to be about 222 nm, 245 nm and 221 nm, respectively. The PDI value of all NP formulations was below 0.3. This indicates a narrow particle size distribution. The EE% values of CBZ-PLGA-Zein(20)-NPs, CBZ-PLGA-Zein(40)-NPs and CBZ-PLGA-Zein(20)-Ch-NPs were determined as about 64%, 56% and 62%, respectively. The coating of the optimum formulation (containing 20 mg Zein) with chitosan did not lead to a significant difference in the particle size and EE% value of this formulation ($P>0.05$). A sustained release of CBZ from all prepared NPs formulations was achieved until 48th h. In conclusion, CBZ-PLGA-Zein(20 mg or 40 mg)-NPs and CBZ-PLGA-Zein(20 mg)-Ch-NPs were successfully prepared and characterized *in vitro*.

Keywords: Epilepsy, Chitosan, Nanoparticles, PLGA, Zein

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1. Introduction

Epilepsy, which is a heterogeneous disorder characterized by epileptic syndromes, variable prognosis and diverse etiologies, affects around 65 million people worldwide and has many causes, each reflecting underlying brain dysfunction. Epileptic seizures, defined as the temporary occurrence of signs and/or symptoms caused by excessive and abnormal or synchronous neuronal brain activity, are quite common and often result in bodily injuries such as burns, fractures, and concussions (Stafstrom and Carmant, 2015; Kanner and Bicchi, 2022; Manole et al, 2023). Types of epilepsy are classified as generalized, focal (these are the most common), combined focal and generalized and unknown (Devinsky et al., 2018; Sarmast et al., 2020; Kanner and Bicchi, 2022). Surgery, vagus nerve stimulation, a special diet (ketogenic diet) and the use of antiseizure drugs are treatment approaches for epilepsy (Green et al., 2020). Epileptic seizures require long-term use of anti-seizure drugs due to their recurring nature. However, long-term exposure of the central nervous system to these drugs leads to adverse effects such as impairment of liver and kidney functions, psychiatric problems and cognitive impairment. In addition, pharmacotherapy has limited efficacy due to blood-brain barrier, untimely medication and drug-resistant epilepsy (Wu et al., 2022). In drug-resistant epilepsy, seizures persist despite the use (at effective daily doses) of at least two syndrome-adapted antiseizure medications, and its prevalence is approximately 30% (Guery and Rheims, 2021). With appropriate use of antiseizure drugs, seizure control is achieved in approximately 70% of cases (Ulamek-Koziol et al., 2019; WHO, 2024).

Carbamazepine (CBZ), used for epilepsy management and treatment, is a sodium channel blocker that inhibits action potential generation and causes a decrease in synaptic transmission. It is also approved for use in the treatment of trigeminal neuralgia and acute manic and mixed episodes (associated with bipolar *I* disorder*) (*Lo, 2014; Maan et al., 2024). CBZ is a Class II (Biopharmaceutics Classification System) drug with high permeability and low solubility. Therefore, its low solubility in water and dissolution rate are the determining factors for its oral bioavailability (Uzunović et al., 2010). Moreover, it is metabolized in the liver and cytochrome P450 3A4 is the main isoform responsible for CBZ-10,11-epoxide metabolite formation (Pearce et al., 2008; Maan et al., 2024). The most common side effects associated with CBZ use are dizziness, vomiting, nausea, ataxia and drowsiness. Rarely, it causes a few

serious skin reactions (Maan et al., 2024). In the literature, nano-sized drug delivery systems have been prepared to increase the solubility of CBZ, enhance its therapeutic efficacy, and reduce their potential adverse effects (Scioli Montoto et al., 2018; Zybina et al., 2018; Qushawy et al., 2019; Kandilli et al., 2020; Ugur Kaplan et. al., 2023).

Our aim was to prepare CBZ-loaded chitosan-uncoated/coated poly(lactic-*co*-glycolic) acid (PLGA) and Zein (Z) nanoparticles (CBZ-PLGA-Zein-NPs or CBZ-PLGA-Zein-Ch-NPs) and to characterize them *in vitro*. To our knowledge, this is the first study on CBZ-loaded PLGA+Zein NPs or PLGA+Zein+Ch NPs. PLGA is an FDA-approved, biocompatible and biodegradable copolymer widely used in the preparation of polymeric nanoparticles due to its low immunogenicity, minimal toxicity, and safety profile (Swider et al., 2018; Kandilli et al., 2020). Zein, the main protein found in corn, belongs to the prolamin protein class and is commercially available. It is soluble in water-glycols or water-alcohol mixture. Due to its biocompatibility, biodegradability and high coating capacity, this biopolymer has been used in the preparation of drug delivery systems (Pascoli et al., 2018; André de Almeida Campos et al., 2023).

2. Materials and Methods

2.1. Materials

CBZ was a gift from Biofarma İlaç San. Tic. A.Ş. (Türkiye). PLGA (50:50, Resomer RG 502 H, Ave. Mw 7000-17000 Da) and chitosan (low molecular weight; 50000-190000 Da) were bought from Sigma-Aldrich (Germany) and Sigma-Aldrich (Iceland), respectively. Zein and poly (vinyl alcohol) (PVA; Mw 30000-70000 Da) were obtained from Sigma-Aldrich (USA). All other materials used were analytical grade.

2.2. Preparation of Chitosan-Uncoated/Coated PLGA+Zein NPs

The uncoated PLGA-Zein NPs [CBZ-PLGA-Z(20)-NPs or CBZ-PLGA-Z(40)-NPs] were prepared by a modified emulsification-solvent evaporation method. Briefly, PLGA (80 mg) and CBZ (40 mg) were dissolved in dichloromethane (DCM). Also, Zein (20 mg or 40 mg) [PLGA:Zein ratio (4:1 and 2:1 w/w); Zein:CBZ ratio (1:1 and 1:2 w/w)] was dissolved in DCM:ethanol (1:1, v/v). The solution of PLGA and CBZ was added to the Zein solution under a magnetic stirrer (500 rpm, 15 min) to obtain an organic phase. The organic phase was added dropwise into the aqueous solution of PVA (3%, w/v) when stirring using an Ultraturrax T10 at 25000 rpm for 5 min (IKA, Germany). Then, it was sonicated using an ultrasonic probe for 5 min (using 60% power; Sonoplus HD 2070, Bandelin Electronics, Germany). The NPs dispersion was centrifuged (12500 rpm, 40 min, 15 °C) after removing the organic solvent under reduced pressure. The NPs were freeze-dried for 24 h (Martin Christ, Alpha 1-2 LD Plus, Germany).

Blank PLGA-Zein NPs [B-PLGA-Z(20)-NPs or B-PLGA-Z(40)-NPs] were also prepared using the same procedure without CBZ.

In addition, chitosan-coated blank or CBZ-loaded PLGA-Zein NPs [B-PLGA-Z(20)-Ch-NPs or CBZ-PLGA-Z(20)-Ch-NPs] were prepared by the above-mentioned procedure until the centrifugation step. Before centrifugation step, 0.1% (w/v) chitosan solution in 1% (v/v) aqueous acetic acid was added to the aqueous dispersion of the blank or CBZ-loaded PLGA-Zein NPs prepared using 20 mg of Zein (chitosan solution:PLGA-Zein NPs suspension, 1:2 v/v) and shaken in a water bath shaker (horizontal, 50 rpm) for 30 min at room temperature. Centrifugation and lyophilization steps were then performed as described above.

2.3. Characterization of Chitosan-Uncoated/Coated PLGA+Zein NPs

The morphological analysis of NPs was carried out using TEM (Hitachi HighTech HT7700, Japan). The images of the NPs were obtained at an accelerating voltage at 120 kV after being placed on a copper grid and dried at room temperature for 24 h. "Zetasizer Nano ZSP (Malvern Ins. Ltd, UK)" was used to measure the particle size (PS) of NPs (at a scattering angle of 173°) as well as the zeta potential (ZP) values of these formulations. The formulations were diluted (20-fold) before the measurements.

The CBZ content in the prepared NPs was determined as follows: 20 mL DCM:ethanol (1:1, v/v) mixture was added to the lyophilized NPs (20 mg) and kept in an ultrasonic bath for 15 min. Then, the mixture was stirred on a magnetic stirrer for 3 h and filtered (PTFE membrane filter; 0.45 μm). The CBZ content in the samples was determined using a validated HPLC method (Kandilli et al. 2018). The percentage of encapsulation efficiency (EE%) and percent drug loading (DL%) were calculated (Kandilli et al., 2020).

Also, the DCS, FT-IR and XRD analyzes were performed for NPs formulations. The conditions of these analyzes are given in Table 1.

Table 1. The conditions of DSC, FT-IR and XRD analyzes

Analysis	Device	Conditions
DSC	Differential Scanning Calorimetry (Hitachi STA 7300, Japan)	25–250 °C; 10 °C/min; a constant flow of nitrogen gas (200 mL/min)
FT-IR	Fourier Transform Infrared Spectroscopy (Shimadzu IRSpirit-T, Japan)	4000-400 cm^{-1}
XRD	PANalytical Empyrean diffractometer (Netherlands)	CuKα radiation (λ 1.5406 Å); 0.5° divergence slit; 2θ range of 10-90°

2.3.1. *In vitro* release studies

The release studies were performed in phosphate buffer (PB, pH 6.8) or HCl (pH 1.2) using the dialysis bag method. NPs were dispersed in 1 mL of release medium, and the dispersion was placed in a dialysis bag (MWCO 14000 Da; Sigma-Aldrich). The dialysis bags were immersed in the release media (50 mL) in the vials in a water bath shaker (horizontal, 50 rpm, 37±0.5 °C). Samples (1 mL) were withdrawn at selected time points, and 1 mL of release medium was added to maintain the "Sink Condition". The samples were filtered (PVDF membrane filter; 0.45 µm), and CBZ contents of the samples were analyzed by the HPLC method validated in our study (Kandilli et al. 2018).

3. Results and Discussion

The TEM images of NPs showed that almost spherical NPs were prepared (Figure 1). While the particle size values of NPs with 20 mg of Zein were smaller than 229 nm, the particle size values of NPs with 40 mg of Zein were smaller than 252 nm. The PDI value of all NP formulations was below 0.3, indicating a narrow particle size distribution. The EE% values of PLGA-NPs with Zein (20 mg or 40 mg) were about 64% and 56%, respectively (Table 2). The DL% values calculated for these formulations are given in Table 2. Considering the particle size, EE% and DL% values, PLGA NPs with 20 mg Zein were selected for coating with chitosan. Then, PLGA-Zein-Ch NPs were prepared by coating PLGA-Z(20)-NP with chitosan (mentioned in methods). There was no significant difference between the particle sizes of the CBZ-containing PLGA-Zein-NP formulations and their respective blank-NP formulations ($P>0.05$). However, increasing the amount of zein in the formulation (from 20 mg to 40 mg) caused a significant increase in NPs particle sizes ($P<0.05$) (Table 2). Moreover, the addition of chitosan to the formulation containing 20 mg Zein did not cause a significant change in particle sizes of NPs ($P>0.05$, Table 2).

In this study, ZP measurement was also carried out. ZP specifies the NP'surface charge and is important for predicting the physical stability of NPs dispersions. In our study, negative zeta potential values were obtained for PLGA- Zein (20 mg or 40 mg) NPs (absolute value less than or equal to approximately 12 mV; Table 2) due to the presence of terminal carboxylic groups in PLGA, the PVA adsorption on the surface of particles, and the rearrangement of Zein (Mura et al., 2011; Gagliardi et al., 2021).

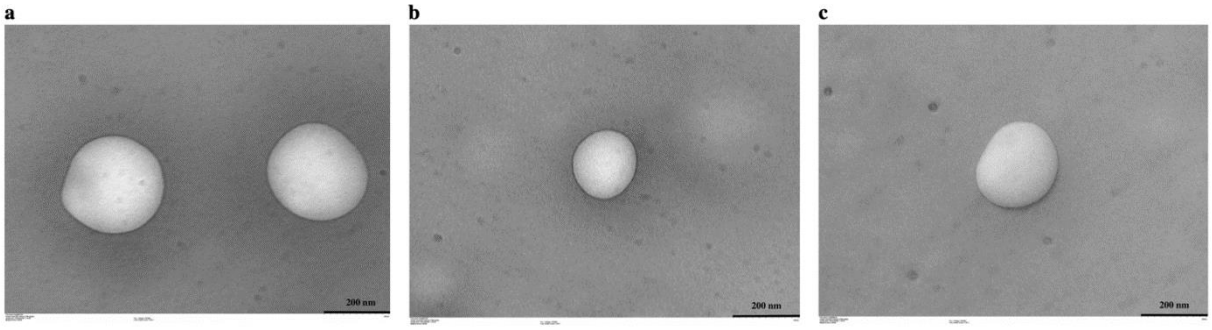

Figure 1. The TEM images of CBZ-PLGA-Z(20)-NP (a), CBZ-PLGA-Z(40)-NP (b) and CBZ-PLGA-Z(20)-Ch-NP (c). [CBZ: Carbamazepine; Ch: Chitosan; NP: nanoparticle, PLGA: poly(lactic-*co*-glycolic) acid; Z: Zein; CBZ-PLGA-Z(20)-NP: CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(40)-NP: CBZ-loaded PLGA-Zein nanoparticles with 40 mg Zein; CBZ-PLGA-Z(20)-Ch-NP: Chitosan-coated CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein].

Table 2. The particle size, PDI, zeta potential, EE (%) and DL (%) values for Blank- NPs and CBZ-loaded NPs

Formulation	Particle Size (nm) (n=9; X±SD)	PDI (n=9; X±SD)	Zeta Potential (mV) (n=9; X±SD)	EE% (n=6; X±SD)	DL% (n=6; X±SD)
B-PLGA-Z(20)-NP	227.91±9.87[a]	0.19±0.05	-12.11±1.45		
CBZ-PLGA-Z(20)-NP	222.06±14.20[b]	0.14±0.03	-8.51±1.36	63.96±2.08[c]	21.79±0.69
B-PLGA-Z(40)-NP	250.82±7.50[a]	0.21±0.01	-12.10±2.11		
CBZ-PLGA-Z(40)-NP	244.62±5.57[b]	0.18±0.03	-6.86±1.41	56.35±1.93[c]	16.32±0.50
B-PLGA-Z(20)-Ch-NP	226.20±11.40	0.20±0.02	39.98±2.63		
CBZ-PLGA-Z(20)-Ch-NP	221.46±6.12	0.19±0.02	37.69±5.89	62.28±3.09	21.33±0.63

[a]= $P<0.05$ between B-PLGA-Z(20)-NPs and B-PLGA-Z(40)-NPs; [b and c]= $P<0.05$ between CBZ-PLGA-Z(20)-NPs and CBZ-PLGA-Z(40)-NPs. [B= blank; CBZ= carbamazepine; Ch= chitosan; NP= nanoparticle, PLGA= poly(lactic-*co*-glycolic) acid; SD= standard deviation; X= mean; Z= zein; B-PLGA-Z(20)-NP= blank PLGA-Zein nanoparticles with 20 mg Zein; B-PLGA-Z(40)-NP= blank PLGA-Zein nanoparticles with 40 mg Zein; CBZ-PLGA-Z(20)-NP= CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(40)-NP= CBZ-loaded PLGA-Zein nanoparticles with 40 mg Zein; B-PLGA-Z(20)-Ch-NP= chitosan-coated blank-PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(20)-Ch-NP= chitosan-coated CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein].

Colloidal drug carriers with ZP values between -10 mV and +10 mV are considered neutral (Clogston and Patri, 2011). In addition, we found that PLGA-Zein (20 mg)-Ch NP formulations had positive zeta potential values (greater than 30 mV; Table 2) due to the adsorption of chitosan, a cationic polymer, on the surface of PLGA-Zein NPs (Pauluk et al., 2019; Dandamudi et al., 2021). The dispersions of NPs with ZP values of ≤−30 mV or ≥+30 mV are considered to have sufficient physical stability (Clogston and Patri, 2011; Ugur Kaplan et al., 2023). We carried out DSC and FT-IR analysis to determine whether there was an interaction between CBZ and other components in the NP formulation. Figure 2 shows the FT-IR spectra recorded for CBZ and blank- and CBZ-loaded NPs. CBZ's FT-IR spectrum showed the characteristic peaks that we detailed in our previous study (Kandilli et al., 2020). Additionally, it can be seen in Figure 2 that the FT-IR spectra of blank-NPs and the corresponding CBZ-loaded NPs are similar. Furthermore, Figure 3 shows DSC thermograms obtained for CBZ and blank- and CBZ-loaded NPs. DSC analysis is also used to evaluate of active substances in the formulations. The endothermic peaks seen at 171.5 °C and 191.3 °C (as distinct) in the DSC thermogram obtained for CBZ were not observed in the DSC thermograms of CBZ-loaded NPs formulations. This supports the result obtained by FT-IR analysis. Ma et al. (2020) reported that the DSC thermogram of CBZ-form III showed two endothermic peaks at 175 °C (related to its melting point) and 192 °C. It also stated that the endothermic peak related to its melting point was observed at 192 °C in the thermogram of CBZ-form I (Ma et al. 2020).

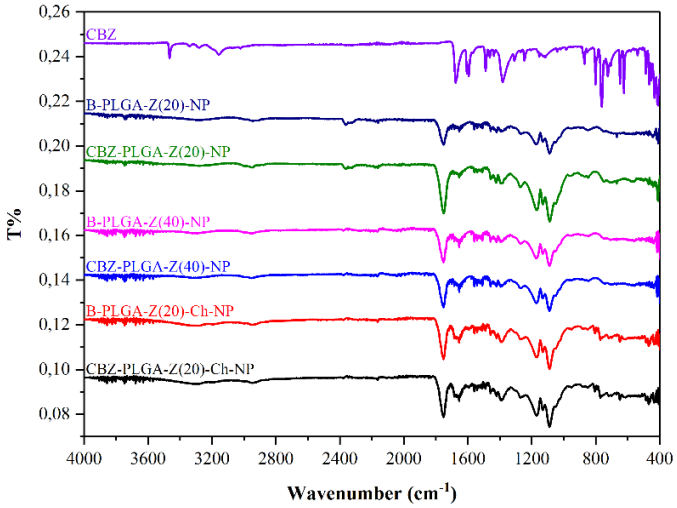

Figure 2. FT-IR spectra of free CBZ and all NP formulations [B= blank; CBZ= carbamazepine; Ch= chitosan; NP= nanoparticle, PLGA= poly(lactic-*co*-glycolic) acid; Z= zein; B-PLGA-Z(20)-NP= blank PLGA-Zein nanoparticles with 20 mg Zein; B-PLGA-Z(40)-NP= blank PLGA-Zein nanoparticles with 40 mg Zein; CBZ-PLGA-Z(20)-NP= CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(40)-NP= CBZ-loaded PLGA-Zein nanoparticles with 40 mg Zein; B-PLGA-Z(20)-Ch-NP= chitosan-coated blank-PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(20)-Ch-NP= chitosan-coated CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein].

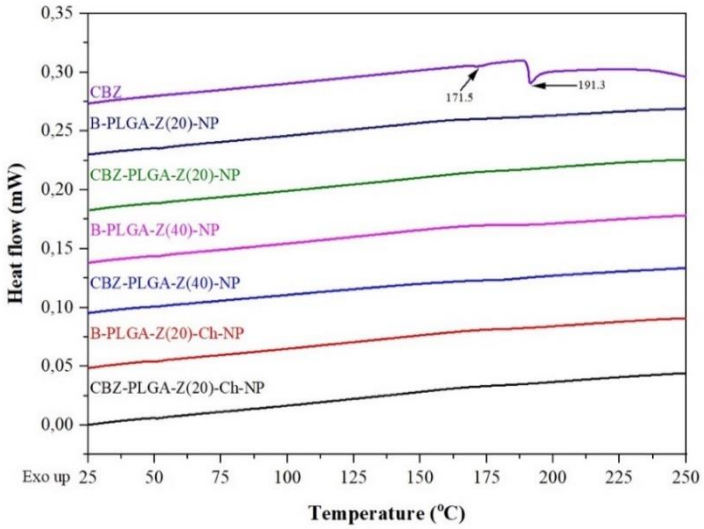

Figure 3. DSC thermograms of free CBZ and all NP formulations (Abbreviations used for the samples are as in Figure 2).

In addition, XRD patterns of free CBZ, blank- and CBZ-loaded nanoparticles were recorded (Figure 4). In the

XRD patterns of free CBZ, there are several distinct diffraction peaks at 13.2°, 15.4°, 16.0°, 19.6°, 25.1°, and 27.7°, 30.9°, 32.2° (related to CBZ form III), indicating its crystalline character. Similar XRD patterns for CBZ were obtained in previous studies (Wang et al., 2012; Caliandro et al., 2013; Pinto et al., 2014; Krstić et al., 2015). However, in the XRD patterns of CBZ-loaded NPs, several peaks related to CBZ appeared but in much less intensity. This supports the result obtained by DSC analysis and shows that CBZ is successfully entrapped within the prepared NPs.

EE% and DL% values calculated for the formulations are given in Table 2. A significant decrease ($P<0.05$) was observed in the EE% and DL% values by increasing the amount of zein in the formulation (from 20 mg to 40 mg). However, it was determined that the addition of chitosan to the formulation with Zein (20 mg) did not cause a significant change in the EE% and DL% values ($P>0.05$). Many factors (solubility of the active substance, the affinity of active substance to polymer, manufacturing process, etc.) have an impact on the EE (%). Therefore, the different physicochemical properties of the polymers with different chemical structures affect the entrapment/encapsulation process (Valo et al., 2009). In our study, increasing the amount of Zein in the formulation may have negatively affected the PLGA-CBZ interaction.

Liu et al. (2021) developed docosahexaenoic acid-loaded zein nanoparticles or Zein+PLGA nanoparticles. The EE (%) values obtained for docosahexaenoic acid-loaded nanoparticles prepared using PLGA+Zein were higher than the EE (%) values determined for nanoparticles prepared using Zein alone. Additionally, increasing the Zein:docosahexaenoic acid ratio from 10:1 to 50:1 in the formulation did not cause a significant change in the EE (%) values obtained for Zein+PLGA nanoparticles. It has been reported that PLGA can support the entrapment of docosahexaenoic acid, which is a hydrophobic polyunsaturated fatty and hardly soluble in water, into Zein + PLGA nanoparticles and allows to obtain increased EE% values (Liu et al., 2021).

In vitro release studies were carried out in the different release media (HCl pH 1.2 and PB pH 6.8). The release profiles of CBZ from the prepared NPs are given in Figure 5. While the CBZ release from PLGA NPs containing 20 mg or 40 mg Zein and PLGA-Zein-Ch NPs in the HCl (pH 1.2) release medium in the first hour was approximately 17%, 20% and 16%, respectively; in the PB (pH 6.8) release medium, it was approximately 15%, 20% and 14%, respectively. It indicates a burst drug release. Furthermore, at 48 h, CBZ release from PLGA NPs containing 20 mg or 40 mg Zein and PLGA-Zein-Ch NPs was determined to be approximately 100% for the three NP formulations (in HCl release medium) and 99%, 100% and 98% (in PB release medium), respectively (Figure 5). These results indicate that the pH of the release medium, the amount of Zein in the formulation, or the chitosan coating do not have a significant effect on the CBZ release from NPs. As a result, the release profiles of all NPs formulations showed that a pH-independent and sustained-CBZ release up to 48 h in both release media was achieved. In addition, PLGA nanoparticles exhibit a biphasic release pattern with an initial burst release of the active substance followed by sustained release (Kandilli et al. 2020; Sakhi et al., 2022).

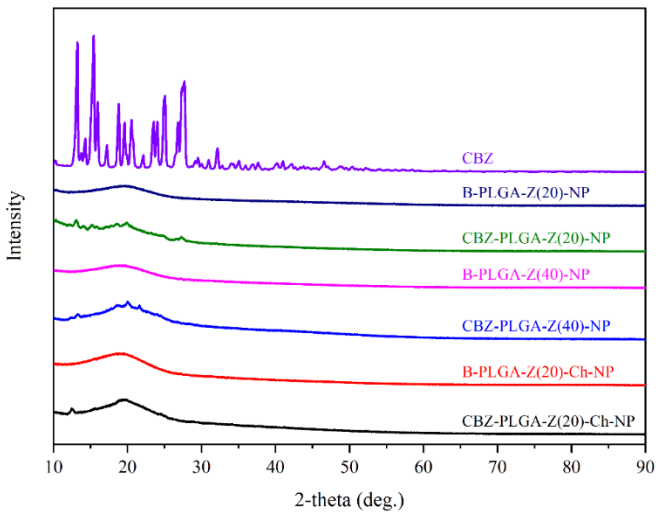

Figure 4. X-ray patterns of free CBZ and all NP formulations [B= blank; CBZ= carbamazepine; Ch= chitosan; NP= nanoparticle, PLGA= poly(lactic-*co*-glycolic) acid; Z= zein; B-PLGA-Z(20)-NP= blank PLGA-Zein nanoparticles with 20 mg Zein; B-PLGA-Z(40)-NP= blank PLGA-Zein nanoparticles with 40 mg Zein; CBZ-PLGA-Z(20)-NP= CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(40)-NP= CBZ-loaded PLGA-Zein nanoparticles with 40 mg Zein; B-PLGA-Z(20)-Ch-NP= chitosan-coated blank-PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(20)-Ch-NP= chitosan-coated CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein].

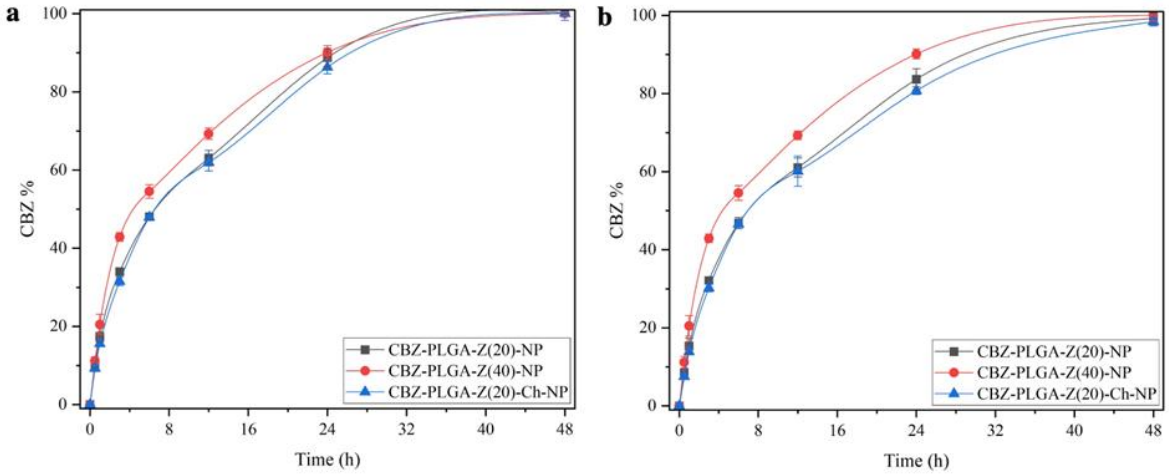

Figure 5. In vitro release profiles of CBZ-PLGA-Z(20)-NP, CBZ-PLGA-Z(40)-NP and CBZ-PLGA-Z(20)-Ch-NP at HCl pH 1.2 (a) and PB pH 6.8 (b) (X±SD; n=3) [CBZ= carbamazepine; Ch= chitosan; NP= nanoparticle, PLGA= poly(lactic-*co*-glycolic) acid; Z= Zein; CBZ-PLGA-Z(20)-NP= CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein; CBZ-PLGA-Z(40)-NP= CBZ-loaded PLGA-Zein nanoparticles with 40 mg Zein; CBZ-PLGA-Z(20)-Ch-NP= Chitosan-coated CBZ-loaded PLGA-Zein nanoparticles with 20 mg Zein].

Kandilli et al. (2020) developed CBZ and levetiracetam-loaded NPs. They performed an *in vitro* release study in pH 7.4-PB medium using a dialysis bag (cutoff MW 14,000 Da) method. It was observed that approximately 40% and 90% of CBZ were released from NPs within 3 h and 48 h, respectively. The release profile showed that an initial burst CBZ release was followed by sustained CBZ release (Kandilli et al., 2020).

Besides, Zybina et al. (2018) prepared the CBZ-loaded PLGA nanoparticles via high pressure homogenization followed by solvent evaporation. An in vitro release study was carried out by resuspending the lyophilized NPs in PBS (pH 7.4), diluting this suspension 25-fold with PBS, and then incubating at 37 °C under continuous shaking. They reported that about 90% of CBZ was released from NPs after 1 h of incubation (Zybina et al., 2018).

4. Conclusion

CBZ-loaded PLGA NPs with Zein (20 mg or 40 mg) and CBZ-loaded PLGA-Zein (20 mg)-Ch-NPs were successfully prepared and characterized. Compared to the NP formulation with 40 mg Zein (PLGA:Zein, 2:1 w/w and Zein:CBZ, 1:1 w/w), the NP formulation with 20 mg Zein (PLGA:Zein 4:1 w/w and Zein :CBZ 1:2 w/w) had a smaller average particle size and also a higher encapsulation efficiency value. Furthermore, the coating of the optimum formulation (containing 20 mg Zein) with chitosan did not lead to a significant difference in the particle size and EE (%) value of this formulation. However, chitosan coating allowed obtaining a zeta potential value above +30 mV, which is a suitable value for the physical stability of a colloidal dispersion. The positive zeta potential values indicate that the NPs were successfully coated with chitosan. A sustained release of CBZ from all prepared NPs formulations was achieved until 48th h.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.B.U.K.	M.Ç.
C		100
D	50	50
S		100
DCP	100	
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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