



# Bromelain Loaded Lipid-Polymer Hybrid Nanoparticles for Oral Delivery: Formulation and Characterization

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## Abstract

Bromelain (Br), a mixture of proteolytic enzymes from pineapple (*Ananas comosus*), has various therapeutic potentials; however, its low bioavailability has limited the clinical applications specifically in oral delivery as the most common convenient used route of administration. In the present study, a lipopolymeric nanoparticle (NP) containing Br was developed to enhance its stability and oral delivery efficiency. Firstly, Br was loaded into poly (D, L-lactide-co-glycolide acid) (PLGA) and PLGA-phosphatidylcholine (PLGA-PC) NPs using double emulsion solvent evaporation technique. Then, Br integrity and activity were investigated using SDS-PAGE and gelatin test. The stability and release profile of Br from synthesized NPs were evaluated at different pH values of the digestive system. Furthermore, cytotoxicity, cellular uptake, and the amount of Br passage from Caco-2 cells were explored. The results showed PLGA-PC-Br NPs had higher encapsulation efficiency (83%) compared to PLGA-Br NPs (50%). In addition, this NP showed more Br released in neutral (20.36%) and acidic (34%) environments compared to PLGA-Br NPs after 5 days. The delay in the release of Br from PLGA-PC-Br NPs versus the faster release of Br from PLGA-Br formulation could assure that an appropriate concentration of Br has reached the intestine. Intestinal absorption study demonstrated that lipid polymer NPs were able to pass through Caco-2 cells about 1.5 times more (98.4%) than polymeric NPs (70%). In conclusion, PLGA-PC NPs would be considered as a promising lipid-polymer nanocarrier for effective intestinal absorption of Br.

**Keywords** Bromelain · Oral administration · Lipid-polymer nanoparticles · PLGA

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## Introduction

Bromelain (Br) is a complex of proteinase extracted from the fruit and stem of the pineapple plant [1, 2]. It has various therapeutic potentials, such as fibrinolytic, antithrombotic, antimicrobial, anticancer, wound and burn repair, and pain relief, as well as anti-inflammatory effects [3, 4]. It can also be used as a feasible candidate for the treatment of COVID-19 symptoms [5]. Despite its numerous pharmacological effects, Br has low bioavailability in oral delivery as the most common convenient and extensively used route of administration due to its early liver first-pass effect, poor penetration of the intestinal mucous membrane, and the restriction of gastric wall transmission [6].

Over the past decades, numerous nanocarriers, including liposomes, polymer particles, and dendrimers, have been developed to overcome the barriers in oral delivery of drugs [7, 8]. Nanocarrier properties, such as their size, high surface-to-volume ratio, and desirable drug release profiles, can provide better access to the target tissue and drug release in a controlled steady-state [9]. Encapsulation of molecules with low solubility into nanoformulations might facilitate their transfer into the bloodstream, thereby, avoiding rapid clearance and increasing bioavailability [10, 11].

Different studies have been conducted on nanoformulations of Br including PLGA, chitosan, Katira gum, noisome, and lipid core nanocapsules to enhance its therapeutic efficiency [12]. Br was also applied to synthesize NPs, overcome the mucus barrier against NPs, and enhance particle uptake. Br was used for the biosynthesis of gold NPs from chloroauric acid as a reducing and capping agent [13]. Also, Br was conjugated to poly (acrylic acid) (PAA) NPs to enhance the permeability and bioavailability of polymer in oral administration [14]. Br was also used in the surface functionalization of silica NPs to enhance particle uptake in endothelial, macrophage, and cancer cell lines, while imposing minimal impact on cellular viability [15]. By the encapsulation of Br into Katira gum NPs, its anti-inflammatory effects increased due to enhanced absorption and acid proteases protection of Br [16]. Furthermore, Br loaded onto multiple-wall lipid-core nanocapsules illustrated enhanced anti-proliferative effect against breast cancer cells (MCF-7) [17]. Hyaluronic acid-conjugated PLGA NPs containing Br had more cytotoxicity in *in vivo* model of Ehrlich's ascites carcinoma, compared to Br-PLGA NPs and free form [18]. Br-PLGA NPs were coated with Eudragit L30D polymer to introduce stability against the gastric acidic conditions [19].

Poly (D, L-lactide-co-glycolide acid) (PLGA), as a biodegradable and FDA-approved polymer, is a suitable carrier for the delivery of drugs and genes due to desirable biocompatibility features, no toxicity, no immune response, simple synthetic methods, and controlled degradation rate [20–22]. This polymeric nanoparticle (PNP) has some disadvantages, such as low half-life and tissue specificity, hydrophobic surface area, and low passage through the intestinal wall [23]. Therefore, structural modification is required to use PLGA-based NPs for oral intake. Lipid-polymer hybrid nanoparticles (LPNPs) specifically deliver drugs to the tissue and improve physicochemical properties. The polymer could control the drug release kinetics while the lipid component is responsible for the loading efficiency, permeation ability, and prevention of hepatic metabolism of the compound [23, 24]. The oral administration of LPNPs has been widely used for the promotion of mucus penetration, as well as the improvement of cell entry and cellular transport [25]. In their study, Fei et al. indicated that loading of berberine onto LPNPs improved its oral delivery efficiency [26]. In the present study, a novel lipid-polymeric formulation containing Br was developed to enhance the stability of Br in an acidic environment and facilitate

oral Br delivery uptake through the intestinal wall. In addition, the physicochemical properties of synthesized NPs, as well as *in vitro* Br release and activity, were evaluated. Furthermore, cellular uptake and apparent permeability of formulations were investigated in Caco-2 cells.

## Materials and Methods

### Materials

Br and PLGA (50:50 (MW 30,000–60,000 Da)) with acid terminated were acquired from Sigma-Aldrich (Darmstadt, Germany). Hanks Balanced Salt Solution (HBSS) was purchased from Bio idea (Iran). HTS Transwells were provided by Corning company (Schneidorf, Germany). L- $\alpha$ -phosphatidylcholine (95%) (Soy) was obtained from Avanti Company. Other chemical compounds were purchased from Merck (Darmstadt, Germany).

### Preparation of the PLGA NPs containing Br

The PLGA NPs loaded with Br were prepared using (W/O/W) double emulsion solvent evaporation method with some modifications [27]. In brief, PLGA polymer (20 mg) and Br (1 mg) were dissolved in DCM (1 ml) and distilled water (200  $\mu$ l), respectively. Then, Br solution (aqueous phase 1) was added drop by drop to PLGA solution (oil phase). Thereafter, the mixture was sonicated using a probe sonicator at 90% amplitude for 5 min to make the W/O emulsion. Subsequently, W/O emulsion was added to 2% cold polyvinyl alcohol (PVA) (5 ml) as the second aqueous phase to form a double emulsion (W/O/W).

After 10 min of sonication, the double emulsion was added to 0.1% PVA and stirred overnight to evaporate the organic solvent. Thereafter, the sample was centrifuged at 14,000 rpm for 20 min at 4 °C and washed three times with distilled water to remove residual PVA. Finally, the NPs were suspended in distilled water (1 ml) and kept at 4 °C for subsequent experiments. Furthermore, Br-free NPs were also synthesized as described here.

### Br-Loaded Lipid-Polymer NPs

Br-loaded lipid-polymer NPs (LPNPs) were also prepared using same as the above mentioned method except that phosphatidylcholine (PC) (10 mg/ml in ethanol, 500  $\mu$ l) was added to 4.5 ml of cold 2% PVA in the second emulsion preparation step. The ratio of polymer to PC was considered 4:1 [28].

## Physicochemical Characterization of Synthesized NPs

### Particle Size and Zeta Potential

Zetasizer Nano ZS (Malvern Instrument, UK) was used for the determination of the size and zeta potential of NPs. To this purpose, 100  $\mu$ l of the synthesized NPs was suspended in 1 ml of filtered deionized water and sonicated to form a homogeneous suspension. Three independent measurements were performed.

## Atomic Force Microscopy

The surface morphology of NPs containing Br was investigated by atomic force microscopy (AFM). For this object, the desired amount of NPs suspension was placed on a layer of mica, dried at room temperature, and used for imaging [29].

## Determination of Encapsulation Efficiency (EE %) and Loading Capacity (LC %)

The percentages of encapsulation efficiency (EE %) and loading capacity (LC %) of Br in NPs were indirectly determined. During the synthesis of NPs, the supernatants were collected and used to measure the unloaded Br using the BCA protein assay kit (Parsstous, Iran) at 562 nm. The supernatant of empty PLGA was used as control. Equations (1) and (2) were used to measure the encapsulation efficiency and loading capacity, respectively [30].

For LC% calculation, we used lyophilized form of NPs to obtain the total weight of NPs (mg).

$$EE(\%) = \frac{\text{Initial amount of Brin formulation} - \text{Amount of Brin supernatant}}{\text{Initial amount of Brin formulation}} \times 100 \quad (1)$$

$$LC(\%) = \frac{\text{Initial amount of Brin formulation} - \text{Amount of Brin supernatant}}{\text{Total weight of NPs}} \times 100 \quad (2)$$

## Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The integrity of Br before and after encapsulation in PLGA NPs was investigated using the SDS-PAGE method (Bio-Rad Miniprotean II system). Electrophoresis was conducted with 15% acrylamide gel. The samples, including PLGA-PC-Br, PLGA-Br, empty PLGA and PLGA-PC, and free Br, were mixed with loading buffer (ratio of 1:1) and boiled at 95 °C in different time points for Br (5 min), PLGA-Br, and empty PLGA (30 min)[31]. SDS-PAGE was run for 45 min, 20 mA, 120 V, and 160 V for stacking gel and separating gel, respectively. Finally, the gel was stained with Coomassie blue 0.1% (w/v) and analyzed.

## In vitro Br Release Profile

The release of Br from NPs was investigated in different pH values (6.8 and 1.2). Neutral environment (PBS 0.1 M, pH=6.8) and acidic environment (hydrochloride buffer (HCL) (0.7% v/v) containing NaCl (0.2% w/v), pH=1.2) are regarded as simulated intestinal fluid (SIF) and simulated gastric fluid (SGF), respectively. Br-free NPs and Br-loaded NPs (containing 200 µg of Br) are suspended in both neutral and acidic buffer (800 µl) in sink condition and incubated in shaker incubator (37 °C, 100 rpm) (ISH2040, Germany) for 3 days. After 0, 1, 2, 3, 4, 24, 48, 72, 96, and 120 h, the samples were centrifuged at 14,000 rpm for 20 min. Subsequently, the amount of released Br in supernatant was assayed using

BCA assay kit (Parstous, Iran). The percentage of released Br was determined according to Eq. (3) [32].

$$\text{Released Br \%} = \frac{\text{Amount of released Br}}{\text{Total amount of Br}} \times 100 \quad (3)$$

### Br Activity Assay

Br encapsulated into PLGA and PLGA-PC (containing 200 µg Br) was suspended in 1 ml phosphate-buffered saline (PBS) and incubated in a shaker incubator (37 °C, 100 rpm). After 48 h, samples were centrifuged at 14,000 rpm for 20 min. The supernatant (100 µL) was mixed with 5% gelatine solution (2.5 ml) at 45 °C and remained at room temperature for 20 min. Br as a proteolytic enzyme degrade gelatine into amino acids and oligo peptides. Subsequently, 3% hydrogen peroxide solution (10 µL) was added to them to stop the reaction.

The initial pH of the samples was measured using a pH meter (Mettler Toldedo, Switzerland). To increase the pH value first to 6.9 which degrade all amino acids, and then to 7.8, sodium hydroxide (NaOH 0.05 N) was added in two steps. After one step in which the pH value increases to 6.9, 37% formaldehyde (1 ml) was added to samples to break the amino acid groups and release the acidic hydrogens (H<sup>+</sup> ions) from amino acids, and the pH value was measured again. Consequently, in order to neutralize acidic hydrogens, NaOH was added again to increase the pH value to 7.8. The total amount of NaOH added to the samples was calculated. It is related to the amount of acidic hydrogen that is dependent on the amino acid groups and enzymatic activity. For the standard curve, the different concentrations of Br (0.5, 0.25, 0.125, and 0.006 mg in 100 µL of deionized water) were prepared, and their Br activities were determined.

### Stability in the Different pH of Digestive System

Different pH values of 1.2 (HCL buffer), 3.5 (acetate buffer), and 6.8 (PBS) were used to investigate the effect of pH on the stability of PLGA-Br and PLGA-PC-Br formulations [33]. For this purpose, 100 µl of the synthesized NPs suspended in 1 ml double-distilled water was added to solutions with different pHs. After 3 h, their size changes were assessed using Zetasizer Nano ZS (Malvern Instrument, UK).

### In Vitro Studies

#### Cytotoxicity Assessment Based on MTT Assay

Colon epithelial carcinoma cell line (Caco-2) as an extracellular model of enterocytes (small intestine cells) was cultured in DMEM (Gibco) high-glucose medium (4.5-g glucose) containing 10% fetal bovine serum and 1% penicillin–streptomycin antibiotic. The MTT assay was performed to evaluate the cytotoxicity of free Br and NPs containing Br on Caco-2 cells. Caco-2 cells ( $1 \times 10^4$ ) were seeded in each well of the 96-well plates (Greiner, UK). After 24 h of incubation, the cells were treated with different concentrations of free Br, PLGA-Br, and PLGA-PC-Br (containing 12.5–100 µg Br/ml). The MTT reagent (20 µL) was poured in each well after 48 h to evaluate the formation of formazan crystals by

living cells. To dissolve these crystals, DMSO (100  $\mu\text{L}$ ) was added to each well and was shaken (Behdad, Iran) at 300 rpm for 10 min. The absorbance was measured by ELISA plate reader at 570-nm wavelength (Infinite NanoQuant M200, Tecan, Switzerland) [34].

## Cellular Uptake

Caco-2 cells were seeded at a density of  $2 \times 10^5$  per well in 24-well plates (HTS Transwell, Corning, Schnell Dorf, Germany) with 0.4- $\mu\text{m}$  pore size. Caco-2 cells were cultured on the upper side of the Transwell membrane to simulate the formation of a cellular monolayer resembling the intestinal cells. During 5 days, different volumes of 400  $\mu\text{L}$  and 1000  $\mu\text{L}$  of fresh DMEM medium were added to the upper and lower chambers of the Transwell plate, respectively. Culture medium was changed to HBSS (Hanks' Balanced Salt Solution pH (roughly 7.0–7.4)) as transport media after the formation of cellular monolayer 1 h before cell treatment. Thereafter, PLGA-Br or PLGA-PC-Br NPs containing 100  $\mu\text{g}/\text{ml}$  of Br were added to the top chamber. At different time points (1, 2, and 4 h), the lower chamber medium which contained the NPs that transfer from top to bottom of the plate was collected (100  $\mu\text{l}$ ) and replaced with the same amount of fresh Hanks buffer. For the release of Br from NPs, the collected medium was shaken in a shaker incubator for 24 h (Behdad, Iran). Following that, the medium was centrifuged at 15,000 rpm, and the Br concentration was quantified using a BCA assay kit. Absorbance was measured using a microplate reader at the wavelength of 562 nm (Infinite NanoQuant M200, Tecan, Switzerland) [35]. Furthermore, Blank NPs were used as a negative control. To evaluate the interaction between NPs and Transwell, the NPs were added to the chamber under the same condition but without the cells. Another measured parameter was the apparent permeability coefficient ( $P_{app}$ ) which is indicative of drug (Br) passage over a unit of area per 1 s (Eq. (4)).

$$P_{app} \left( \frac{\text{cm}}{\text{s}} \right) = \left( \frac{dQ}{dT} \right) \times \frac{1}{(A \times C_0)} \quad (4)$$

In the above equation,  $dQ/dT$  shows the drug permeation rate ( $\mu\text{g}/\text{ml}/\text{s}$ ),  $A$  is the area of transwell ( $\text{cm}^2$ ),  $C_0$  denotes the initial concentration of Br ( $\mu\text{g}/\text{cm}^3$ ), and  $t$  refers to the time elapsed for the test (s).

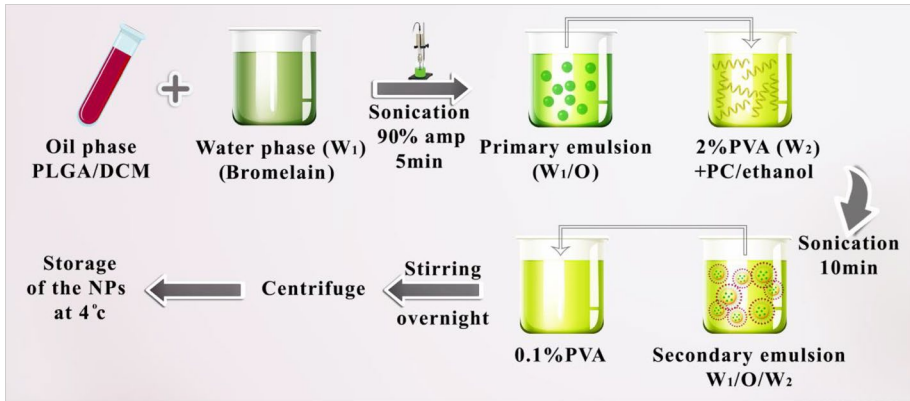
## Data Analysis and Statistical Computing

The results were expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD) with at least 3 replications. Statistical analysis was performed in Prism 8 software (Graphpad, La Jolla, CA, USA) using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. A  $p$ -value  $\leq 0.05$  was considered statistically significant ( $*P \leq 0.01$ ,  $**P \leq 0.001$ , and  $***P \leq 0.0001$ ).

## Results

### Synthesis and Characterization of Br Encapsulated into LPNPs

In the present study, Br was loaded into PLGA-lipid NPs using the double emulsion solvent evaporation (W/O/W) method as displayed in Scheme 1.



**Scheme 1** Schematic illustration of Br loaded LPNPs

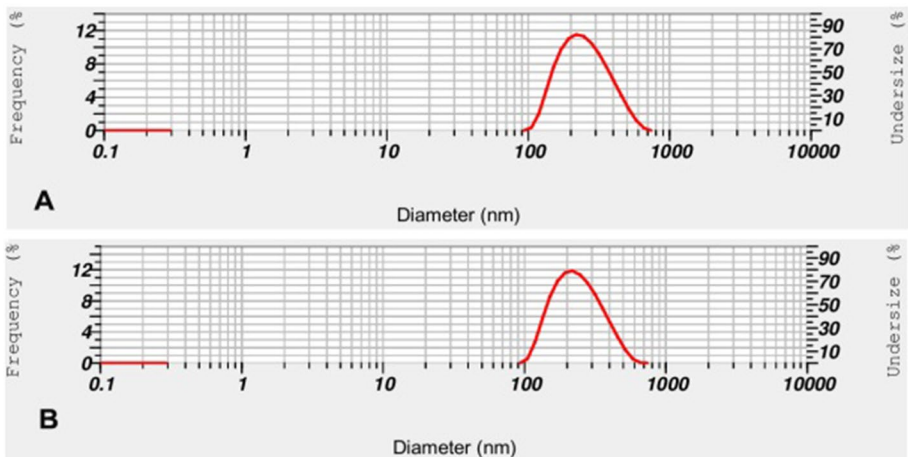
The mean size of negatively charged PLGA-PC-Br and PLGA-Br was obtained about  $209.4 \pm 7.30$  nm and  $229 \pm 9.31$  nm, respectively. Polydispersity index of less than 0.3 revealed the uniform size distribution of NPs (Table 1 and Fig. 1).

AFM results demonstrated that PLGA-Br and PLGA-PC-Br had a spherical structure with the NPs with smooth surface properties (Fig. 2).

As illustrated in Table 2, both the loading capacity and encapsulation efficiency of PLGA-PC-Br (LPNPs) were higher, compared to the PLGA-Br (PNPs).

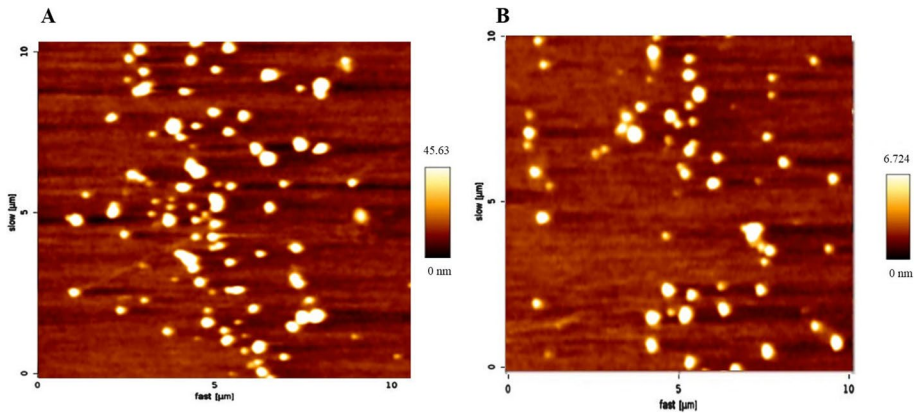
**Table 1** Particle size and zeta potential of synthesized NPs

Formulation	Z-average (nm)	PDI	Zeta potential (mV)
PLGA-Br	$229 \pm 9.31$	0.21	$-43.1 \pm 0.6$
PLGA-PC-Br	$209.4 \pm 7.30$	0.134	$-42.1 \pm 0.55$



**Fig. 1** The size profile of the PLGA-Br (A) and PLGA-PC-Br (B) NPs obtained by DLS analysis. The intensity of scattered light is plotted as a function of the hydrodynamic diameter





**Fig. 2** **A** Morphology of the NPs by AFM at the magnification of 10  $\mu\text{m}$  for PLGA-Br NPs, and **B** PLGA-PC-Br NPs

**Table 2** Encapsulation efficiency (EE%) and loading capacity (LC%)

Formulation	EE %	LC %
PLGA-Br	50%	4.5%
PLGA-PC-Br	83%	7.6%

SDS-PAGE was performed to confirm the presence of Br in the synthesized NPs. As shown in Fig. 3, the band of Br after encapsulation was the same as that of free Br.

The size of NPs was measured at different pH values which represented different parts of the digestive system (Table 3). The results suggested that the average particle size of NPs was not significantly affected by different pH values after 3 h ( $P < 0.005$ ).

## Br Activity

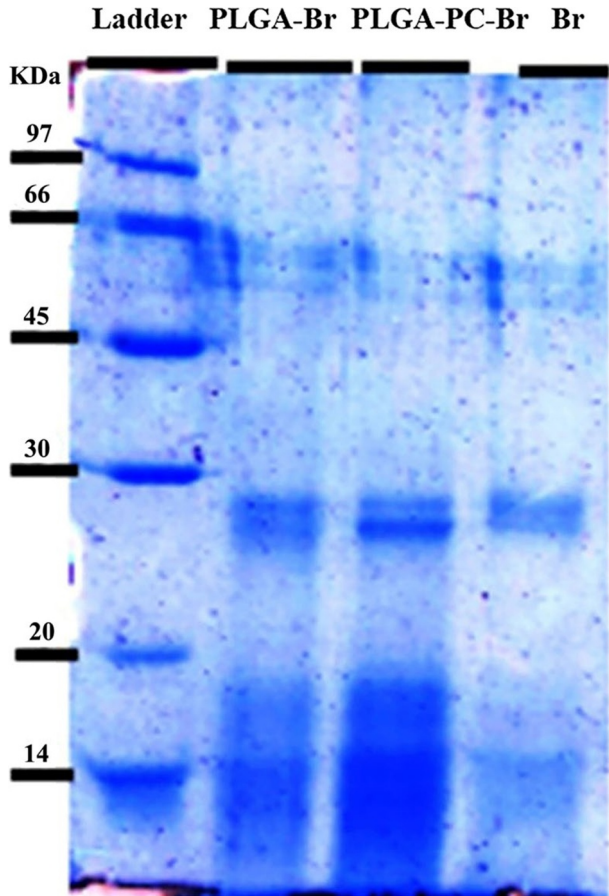
The enzymatic activity of Br loaded into PLGA formulations is related to the volume of NaOH 0.05 M that is needed to neutralize H ions of amino acid groups of gelatine produced in the presence of Br. The results indicated that 63% and 97% of Br activity were preserved in the PLGA-Br and PLGA-PC-Br formulations, respectively.

## Br Release from NPs in Neutral and Acidic Medium

The in vitro release profile of Br-loaded PLGA and PLGA-PC NPs was evaluated at two different pH values of 1.2 and 6.8. According to Fig. 4A, after 5 days, the release of Br from PLGA-PC NPs was 34% and 20.36% in acidic and neutral medium, respectively. At this time, Br was released from non-lipidic formulation (PLGA) of 30.1% and 18.8% in acidic and neutral media, respectively (Fig. 4B).



**Fig. 3** SDS PAGE of free Br and NPs containing Br

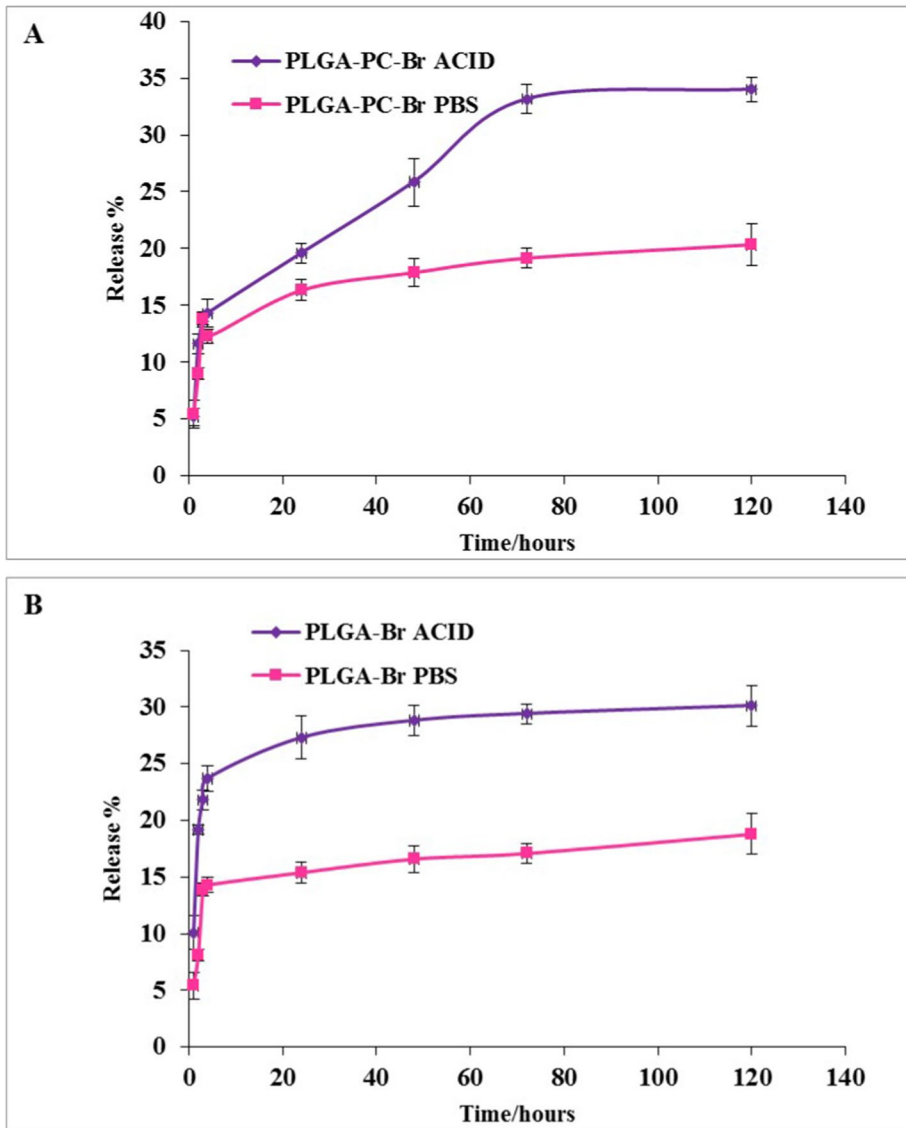


**Table 3** NP sizes at different pH values after 3 h

Formulation	Initial size (nm)	Final size (nm) at pH=1.2	Final size (nm) at pH=3.5	Final size (nm) at pH=6.8
PLGA-Br	229 ± 9.31	213.9 ± 2.47	227.4 ± 0.53	189.9 ± 2.17
PLGA-PC-Br	209.4 ± 7.3	211.7 ± 7.6	199.8 ± 0.32	174.2 ± 9.46

**MTT Assay**

MTT assay was performed on Caco-2 cells to determine the toxicity of free Br and different forms of NPs on the intestinal cells. The results indicated that free Br had more cellular toxicity, compared to synthesized NPs, which showed cell viability more than 80% in all studied concentration (Fig. 5).



**Fig. 4** Release profile of Br from NPs in two simulated environments of stomach and intestine **A** PLGA-PC-Br and **B** PLGA-Br

### In Vitro Study of Cellular Uptake

The evaluation of passage of different formulations across the Caco-2 monolayer provides information about the passive or active permeability of oral delivery platforms in vitro. Our results demonstrated that the cumulative transport for PLGA-PC-Br and PLGA-Br NPs across the intestinal barrier after 4 h of incubation was approximately 98.4% and 70%, respectively (Fig. 6). On the other hand, the results also indicated that in the absence of

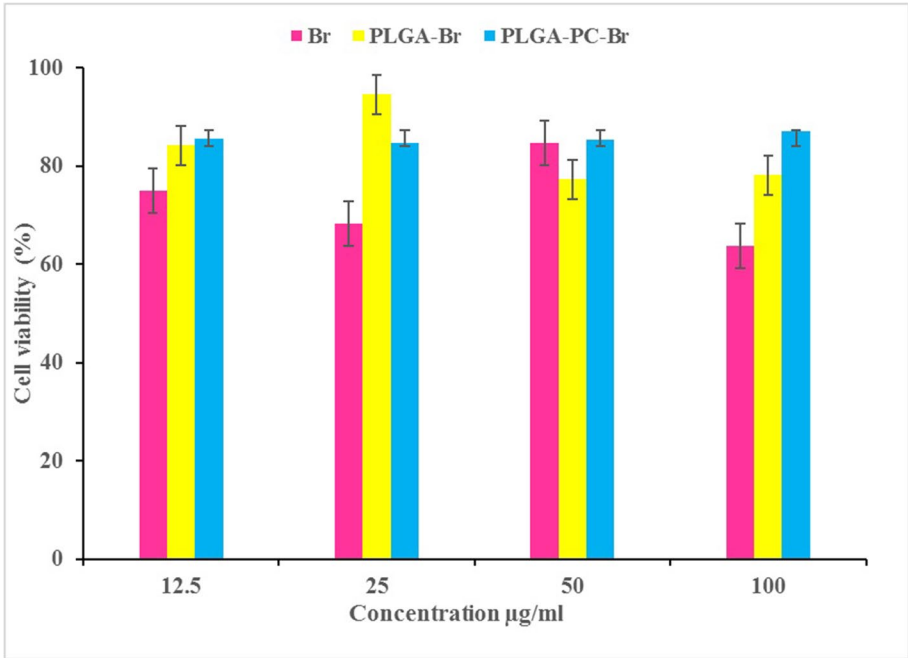


Fig. 5 MTT assay of Br NPs on Caco-2 cells to determine the toxicity of NPs, ( $P \leq 0.05$ )

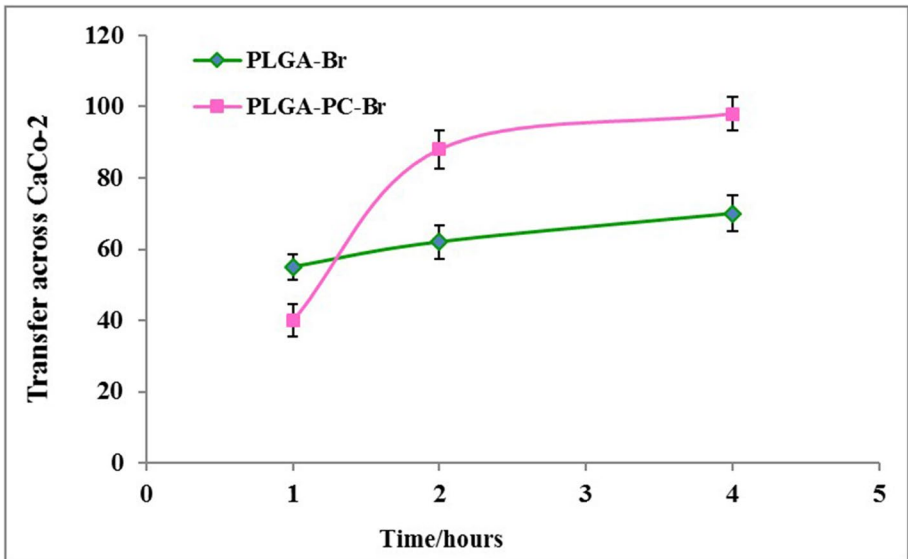


Fig. 6 Percentage of Br passage from Caco-2 cell monolayer in Transwell plate at different time points

the cells, the transport of PLGA-PC-Br and PLGA-Br NPs through the pore was approximately 99.4% and 98.6%, respectively, after 1 h.

### Apparent Permeability Coefficient ( $P_{app}$ ) for Two Formulations of Br

The apparent permeability coefficient  $P_{app}$  indicates the permeation speed of Br and allows comparison between the two formulations. The  $P_{app}$  for the formulation containing PC was significantly higher, compared to the formulation without PC ( $P \leq 0.0001$ ) (Fig. 7).

## Discussion

Br is a combination of proteolytic enzymes extracted from the fruit or stem of pineapple. Increased stability of Br with various therapeutic potentials is an important consideration for its clinical application; therefore, different strategies have been developed for this purpose.

It was shown that nanocarrier systems could deliver therapeutic agents from the intestinal mucous membrane more efficient than free drugs through the endosomal route and flowing drug release through the lysosomes to the blood [36].

In a study by Sharma et al., Br was loaded into nanostructured lipid carriers (Br-NCs) with high drug loading and stability. Results indicated the efficiency of orally administered Br-NCs in rheumatoid arthritis [37].

In the present research, Br was encapsulated into PLGA and PLGA-PC NPs using the double emulsion solvent evaporation technique to enhance therapeutic efficiency, especially in oral administration. In this strategy, the organic phase consisted of Br and PLGA, while the aqueous phase included PVA as an emulsifier and stabilizing agent, which is

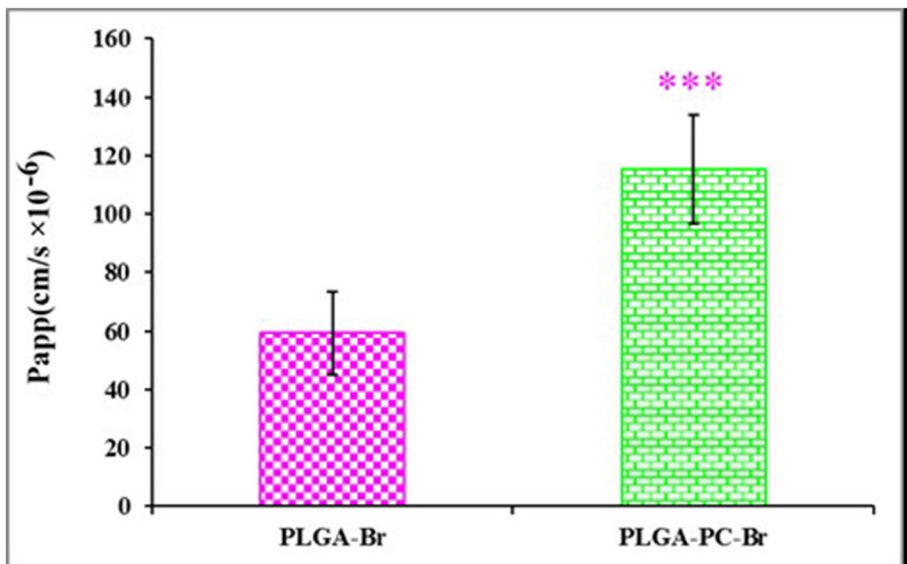


Fig. 7 Comparison of  $P_{app}$  for PLGA-Br and PLGA-PC-Br NPs

found to be at the PLGA NP surface. It was demonstrated that PVA could prevent self-aggregation of NPs in aqueous medium and control shape and colloidal stabilization of NPs [38]. The ratios of Br/polymer (1:20) and polymer/lipid (4:1) were selected according to the our previous study [28].

It was shown that the smaller size of NPs could definitely improve the efficiency of cellular uptake and be highly feasible for oral chemotherapy [39]. In the current study, the size of all synthesized particles was in the nano range (about 200 nm). Lower particle size with higher encapsulation efficiency was observed in PLGA-PC NPs, compared to polymeric NPs, which may be due to increases in the miscibility of formulation. This result was also similar to other reports [33, 40]. In general, over the course of formation process of LPNPs, lipids act as an agent that reduces leakage of incorporated drug and protect inner core from degradation by blocking the water infiltration [41]. Zhang et al. showed the reduction of surface tension induced by PCs resulted in efficient kinetic stability of nanoemulsion [42]. Moreover, PC could stabilize oil-in-water (o/w) mixtures and develop bioavailability and solubility of hydrophobic drugs [43]. High encapsulation efficiency could reduce the Br therapeutic dose, thereby decreasing the side effects. The synthesized NPs also showed a negative charge due to the presence of carboxyl terminal of the PLGA and the polar head group of the phospholipid. However, there is no significant difference in surface charge of PLGA-PC and PLGA may be due to the orientation of the hydrophobic tails of PC to the PLGA core while its negative groups oriented to the surface of NPs.

The surface charge of NPs has an important effect on Br delivery in the small intestinal epithelium. The interactions between the positive charge NPs and the negative charge mucus layer are unavoidable and might increase Br release or mucosal adhesion. On the contrary, negatively charged NPs directly propagate into the mucosal layer and, subsequently, into the intestinal epithelium [44, 45]. The stability of the synthesized NPs was evaluated in different simulated GIT fluids (pH 1.2, pH 3.5, pH 6.8) while passing through the gastrointestinal tract. No significant changes ( $p < 0.05$ ) were observed in particle sizes of NPs. In addition, the stability of Br-loaded NP suspension was also assayed after 1-month storage at 4 °C. There were no significant changes in the particle size, PDI, and zeta potential of NPs (data not shown). This result was similar to the study by Nabeel Ibrahim et al. [46].

In the release study, Br had a burst release at first four hours, followed by a slower and continuous release. The maximum release of Br from nanocarriers was obtained 30–35% after 5 days. This is an ideal NP formulation in oral administration which makes sure that the drug is not completely released in the gastrointestinal tract. Therefore, PLGA NPs could be uptaken from the intestinal cells via endocytosis pathway followed by passing from lysosomes or transcytosis at the basal side of intestine.

After 5 days, more released Br was observed in an acidic medium, compared to the neutral medium in both nanoformulations. These results could be attributed to the penetration of excess hydrogen ions into NPs at an acidic medium, resulting to break down. Delay in the release of Br in acidic medium from PLGA-PC-Br formulation versus the faster release of Br from PLGA-Br formulation could assure that an appropriate concentration of Br has reached the intestine. Comparable results were reported in another study, which encapsulated berberine in PEG-lipid-PLGA and demonstrated a delayed release in the acidic medium [26].

The oral administration of biodegradable nanocarriers is limited due to stomach degradation and the sink effect played by the duodenum [47]. It was demonstrated that LPNPs have more potential for in vivo cellular delivery, as compared to PNPs and liposomes. The results of the present study indicated that the cumulative transport for PLGA-PC-Br NPs across the intestinal barrier was more than PLGA-Br NPs. LPNPs have the potential to enhance the physical stability of NPs and prevent the first-pass metabolism [25].

## Conclusion

In the current study, polymeric NPs (PLGA) and lipid-polymeric NPs (PLGA-PC) were synthesized for oral delivery of Br. Br release profile in PLGA-PC NPs showed a delay in the rate of more Br release in the acidic medium versus the faster release of Br from PLGA NPs. It points to the appropriate concentration of Br in the intestine. It was also indicated that Br passed through the Caco-2 cells 98.4% and 70% in PLGA-PC-Br and PLGA NPs, respectively. The apparent permeability coefficient for the lipid-based formulation was higher than the polymeric formulation. In conclusion, PLGA-PC-Br NPs are advantageous over PLGA NPs for oral administration due to their higher cellular uptake and better physical properties. Nevertheless, further studies are needed to assess the potential of these novel synthesized NPs in vivo.

**Author Contribution** Mahboubeh Ebrahimian: Performed the experiments, analyzed data, assisted in writing, and revised and edited the manuscript. Fatemeh Mahvelati: Performed the experiments. Bizhan Malaekeh-Nikouei: Edited the manuscript. Ezzat Hashemi: Edited the manuscript. Fatemeh Oroojalian: Assisted in writing. Maryam Hashemi: Conceived the idea, designed experiments, provided reagents, analyzed data, and wrote, edited, and revised the entire manuscript.

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**Availability of Data and Material** All authors take full responsibility for the data, analyses and interpretation, and the conduct of the research. We have full access to all the data and have the right to publish any and all data.

**Code Availability** Not applicable.

## Declarations

**Ethics Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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