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ORIGINAL RESEARCH ARTICLE

Fabrication and characterization of CNF/PLGA nanocomposite system for encapsulation of bacoside A_3

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ABSTRACT

The inception of nanocellulose-based biodegradable polymeric composites has accomplished astounding application in constructing superior biomaterials. The featuring characteristics of nanocellulose like bio-compatibility, low-cost production, abundance, and toxic-free nature, have paved the way for application in drug delivery. The stability and sustainability of medicinally important constituents of the herbs are also a major concern for the phytopharmaceuticals and nutraceutical industries. Hence, the goal of the present study was to fabricate a composite system of cellulose nanofiber/poly-lactic-co-glycolide (CNF/PLGA) to encapsulate Bacoside A_3 (BA_3) of Bacopa monnieri plant extract. The stability and sustained release of BA_3 at three different pH conditions from the fabricated system were evaluated for its application in the nutraceutical industry. The CNF/PLGA composite system had more storage stability (64%) of BA_3 than pure Bacopa extract (47%) for 45 days. The fabricated composite system maintained the antioxidant properties of Bacopa extract. The release of BA_3 was sustained in the CNF/PLGA matrix for up to 24 hours (pH = 9) compared to the control. The release kinetics implies that the BA_3 was effectively restrained in the CNF/PLGA nanocomposite matrix and follows the Korsmeyer-Peppas model and anomalous diffusion mechanism. The hydrolysis of PLGA and mechanical strength of CNF would be responsible for the sustained release of BA_3 from the composite system. In summary, the Bacopa extract CNF/PLGA composite system could be an option for the nutraceutical/pharmaceutical product with improved stability and sustained release of its active constituents.

INTRODUCTION

The eminence of nanocellulose-based polymeric structures has gained momentum due to their wellspring of extraordinary vital properties. Because of their characteristics, they are predominantly entitled to several applications like reinforcement for composite, filler, support, or coatings. Therefore, selecting surface-tailored cellulose nanofibers (CNF) would be a viable route for designing the novel composite system. Furthermore, the profound characteristics of CNF, such as particle size, surface charge, modification, and biocompatibility, have an important effect on the drug release and delivery process. The TEMPO (2, 2, 6, 6-tetramethylpiperidine-1-oxyl radical) oxidized cellulose nanofibers (CNF-TO) could be an option to

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overcome the issue related to encapsulation efficacy due to its narrow particle size, high surface area, an abundance of surface carboxyl and hydroxyl groups (Ono *et al.,* 2022; Isogai *et al.,* 2011; Saito *et al.,* 2007). The abundant hydroxyl and carboxyl groups present at the surface of CNF-TO can serve as anchors for the binding of PLGA and phytochemicals through hydrogen bonding or van-der-Wall attractions. A combination of both matrices for the fabrication of composite systems with higher reproducibility and high aspect ratio and can be easily formulated with improved drug efficacy and specificity was earlier reported by Zheng *et al.,* (2018) and Jun *et al.,* (2020). Nanostructured biodegradable polymeric composites are an important and exciting option for encapsulating the herbal extract (Ilomuanya *et al.,* 2022; Tang *et al.,* 2021; Soltanzadeh *et al.,* 2021; Torres *et al.,* 2021). In this regard, poly (lactic-co-glycolic acid) (PLGA) is already reported as the most thriving biodegradable biopolymer. It is used as a delivery vehicle for various pharmaceutical compounds like antibiotics, anticancer drugs, vitamins, DNA, siRNAs, proteins, and peptides (Zhi *et al.,* 2021; Alkahtani *et al.,* 2021; Jaworska *et al.,* 2021; Ramalho *et al.,* 2021; Sun *et al.,* 2021; Hwang *et al.,* 2022; Butreddy *et al.,* 2021; Dalila *et al.,* 2021). Despite numerous prospective conveniences, PLGA suffers demerits such as poor hydrophilicity, weak cell adhesion, acidic degradation products, and mechanical stability. To overcome these shortcomings, fabricating a composite system would be an excellent option for applying PLGA.

Bacoside A, a potential agent for the treatment of cognitive disease, is primarily a mixture of four triglycosidicsaponins: Bacoside A_3 (1), Bacopaside II (2), jujubogenin isomer of Bacopasaponin C (3) and Bacopasaponin C (4) (Shekhar *et al.,* 2021). Bacoside A_3 (BA_3) has been documented for its low therapeutic effectiveness due to poor solubility and low stability against pH and temperature, further limiting its applications as a pharmaceutical product. The cognitive-enhancing activities of BA_3 were already reported by several researchers (Sekhar *et al.,* 2021; Bhanadari *et al.,* 2020). Previous researchers have approached improving the cognitive and neuroprotective actions through different bioavailable formulations of bacosides (Amalraj *et al.,* 2021; Luo *et al.,* 2020; Kumar *et al.,* 2021). Various scientific investigations have followed numerous methods like phospholipid base carrier solid lipid nanoparticles (Vitthal *et al.,* 2013) PLGA nanoparticles (Jose *et al.,* 2014), block polymers (Ravedekar *et al.,* 2021), zinc oxide nanoparticles (Bhardwaj *et al.,* 2018) for the improvement of the therapeutic index of bacosides. However, issues like release under varying pH and storage stability are still unexplored.

To address this problem, a bioderived nanocarrier-based delivery system could be a promising approach including their versatility, biodegradability, stability, toxicity, and biocompatibility for the construction of carrier matrix for herbal constituents.

So far as current study is the first to develop a nanocomposite matrix of CNF/PLGA for extended-release of BA_3 at different pH along with improving its stability and shelf life. Hence, to facilitate this aim, firstly, the CNF was modified through TEMPO oxidation, and then its composite system with PLGA was developed and loaded with *Bacopa* extract (BAE). After that, the effect of different parameters like pH, and temperature were investigated on the release and stability of BA_3.

MATERIALS AND METHODS

Bacopa monnieri (L.) plant was collected from the experimental farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, U.P., India. For the CNF isolation, the distilled waste of citronella (*Cymbopogon winterianus*) (DWC) was taken from an oil extraction unit of CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plant), Lucknow. The chemical used (TEMPO (Sigma Aldrich) of analytical grade with a purity of 98-99%. Poly (D, L Lactide-co-Glycolide) having lactide:glycolide ratio of 50:50 with a molecular weight of 24,000-38,000, poly sorbate-80, and Polyvinyl alcohol (PVA), were procured from Sigma Chemicals, Mumbai (India). Dialysis membrane (molecular weight cut-off: 10000-12000 Da with flat width 23.21 mm, average diameter 14.3 mm, and capacity approximately 1.61 ml cm^{-1}) was procured from Hi-media Labs, India. All other chemicals and solvents used in the study were of high purity analytical grade and purchased from Merck (used as received).

Isolation of CNF from DWC

First, the cellulose was isolated from DWC according to Mishra *et al.,* (2018); and Sun *et al.,* (2004), thereafter, TEMPO-mediated oxidation

combined with ultrasonication was performed with some modification, which is briefly provided inside the supplementary data (SA-I) (Saito *et al.,* 2007).

Extraction of bacoside and quantification of Bacoside A_3

The methanolic extract of *Bacopa monnieri* was prepared according to Mathur *et al.,* (2002). The chromatogram and infra-red (IR) spectra of *Bacopa* extract are provided in SA-II.

Bacoside A_3 loaded CNF/PLGA nanocomposite preparation.

The composite system was prepared according to the method described by Rescignano *et al.,* (2014), with slight modifications. Briefly, PVA aqueous solution (1% w/v) was freshly prepared by dissolving a specific amount of PVA at 80° C for 2 hours (Rescignano *et al.,* 2014). The aqueous dispersion of CNF (2% w/v) was separately prepared after sonication of presoaked CNF suspension for 2 minutes. Accurately weighed amount of PLGA and *Bacopa* extract (480 mg) (BAE) was dissolved in dichloromethane and distilled water separately under magnetic stirring for 2 hours to create o/w emulsion. For this, the supernatant of *Bacopa* extract was slowly added to the organic phase of PLGA under sonication (10 minutes) in the ice bath. The mixture was again gently mixed under stirring to ensure proper mixing. The aqueous dispersion of CNF containing 1% PVA was then mixed into the resultant organic phase of PLGA and BAE using a probe sonicator at an output of 40 W in an ice bath for 20 minutes again. This resulting w/o/w emulsion was gently stirred at room temperature for 12 hours after adding 20 mL of 0.1% PVA to ensure proper evaporation of the organic phase. The resulting suspension was centrifuged at 15,000 rpm for 20 minutes at 4 °C to separate the nanocomposites and washed thrice with distilled water to remove the emulsifier. The washed composite system was then freeze-dried for 24 hours using a lyophilizer (Labconco freeze dryer-free zone 2.5 plus-7522800-USA). A control of PLGA composite without CNF was also prepared.

Coating of matrix with polysorbate-80 (P-80)

The coating of Polysorbate-80 (P-80) favored the brain uptake of the drug by mimicking low-density lipoproteins (LDL) and allowed the interaction with LDL receptors. So the composite matrix was coated with P-80, according to the method described by Jose *et al.,* 2014 (Jose *et al.,* 2014; Kreuter *et al.,* 2002).

Characterization of nanocomposite of CNF/PLGA

Loading of BA_3 was confirmed by Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM), and X-Ray diffraction (XRD) analysis, respectively. FT-IR (NICOLET-380) was used to determine the functional group and interaction between both matrices. While the changes in morphological features and crystallinity due to BA_3 loading on the matrices were performed using SEM (SEM-JEOL, JSM-6100) and XRD (ULTIMA-IV, Rigaku). The FT-IR without loaded CNF and PLGA used in the study is provided in supplementary data (SA-VI). The particle size and zeta potential of materials were analysed by using Zetasizer Nano ZS (Malveran, instruments U.K.) after 1:10 dilution of the composite matrix in deionized water at 25 °C using a disposable quartz cell. The reported values correspond to mean ± S.D. of thrice repeated measurements of each set.

Evaluation of encapsulation efficiency

Encapsulation efficiency (EE%) of BA_3 was calculated by taking the ratio of the BA_3 content in the freeze-dried composite system to the initial BA_3 concentration in loaded extract. The 10 mg of the composite matrix was vortex with 5 mL of methanol for 1 hour, centrifuged, and then filtered through a 0.22 μm membrane filter. The HPLC conditions used for the evaluation of BA_3 concentration are provided in SA-III.

Determination of antioxidant and total phenolic content of BAE-loaded matrices

Since the release of BA_3 was checked in P-80 coated matrices. Therefore the bioactivity like total antioxidant capacity (TAC), entire phenolic content (TPC), and DPPH (1,1-diphenyl-2 picrylhydrazyl) scavenging activities were determined only those samples and compared with pure BAE. TAC of the extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.,* (1999). The antioxidant activity was expressed as the number of ascorbic acid equivalent. TPC was determined using the method of McDonald *et al.,* (2001) with slight modifications (McDonald *et al.,* 2001). The determination of the radical scavenging activity was carried out using the DPPH scavenging assay as described by Lee *et al.,* (2016) with modifications.

In-vitro release profile of BA_3

To estimate the sustained release behavior of BA_3 from an optimized composite system and assessment of the stability of BA_3 at three different pH viz. 2.0 (distilled water), 7.5 (phosphate buffer) and 9.0 (phosphate buffer). The releasing was performed using the dialysis bag technique at a constant temperature of 37 °C under continuous stirring for 24 hours. 50 mg of BA_3-loaded matrix was placed inside a dialysis bag sealed at both ends, and immersed into the 15 mL of each medium. 500 µL of aliquots were withdrawn at a predetermined time interval and the medium was replenished again by a respective medium solution. PLGA nanocomposite system without CNF and the pure extract were also performed. The content of BA_3 was determined by the HPLC-PDA system according to pre-described conditions. The analysis was performed in a triplicate manner.

Formulation of release kinetics

The data obtained from *in vitro* release studies of *Bacopa* extract loaded surface-modified CNF/PLGA(CPLGA), PLGA nanocomposite were fitted to various models such as zero order, first order, Higuchi and Korsmeyer-Peppas to ascertain the kinetic modeling of BA_3 release (Dash *et al.,* 2010).

Determination of stability of BA_3 in loaded nanocomposite matrix

Since PLGA is a biodegradable polymer, the stability of BA_3 was determined at 4 °C in ambient conditions for up to three months, and the BA_3 present in the pure extract was taken as a control. The matrix was placed in a tightly packed glass vial and stored at 4 °C. At a definite time interval, the matrix was taken out, and re-suspended into methanol and vortex for 1 hour. The content of BA_3 was determined in the supernatant by using HPLC-PDA system.

RESULTS AND DISCUSSION

Analysis of BA_3

In our results, the extract showed around 3.2% of BA_3 in the methanolic extract of *B. monnieri*. The study conducted by Anand *et al.,* (2014) found the BA_3 content upto 3.5% in *Bacopa* extract. Shahid *et al.,* (2016) also estimated the bacoside A3 and it was 37.5 $\mu g mg^{-1}$ in the methanolic extract of *B. monnieri*. *Bacopa* extract is considered as a very rich source of antioxidants and phenols, hence TPC, TAC, and DPPH of *Bacopa* extract (BAE) was also determined. The value of TPC was 25 mg of $GAEg^{-1}$ of dried extract, while TAC was 164.5 mg of $AAEg^{-1}$. The value of DPPH was 80% for the concentration of 100 $mgml^{-1}$ of *Bacopa* extract. Our results were corroborating with the findings of Alam *et al.,* (2012), who evaluated the TPC content and TAC content of methanolic extract of *B. monnieri* and found 21.4 mgg^{-1} of GAE and 261.5 mgg^{-1} of AAE respectively (Alam *et al.,* 2012).

The spectral analysis was carried out by FT-IR for assuring the abundance of chemical groups present in BAE. IR spectrum obtained for bacosides presented typical molecule bands and peaks: 3300–3000 cm^{-1} (O–H), 1609 cm^{-1} (C = O), 1513 cm^{-1} (C = C), 1377 cm^{-1} (C–OH), and 1260 cm^{-1} (C–O–C). Similar FT-IR spectra of groups at 3300 cm^{-1}, 2844 cm^{-1}, 1514 cm^{-1}, 1717 cm^{-1} were obtained by many authors for *Bacopa* extract (Saoji *et al.,* 2017; Thakkar *et al.,* 2017).

Characterization of the composite system

The isolated fibers (CNF-TO) had a length: 84 nm, crystallinity index: 77%, zeta potential: -37 mV and carboxyl content: 9.34 mmol g^{-1}. Besides the composite system, *Bacopa* extract loaded on neat PLGA was also characterized to get information about the interaction between BAE and PLGA and the influence of the CNF on it. The visual appearance of both composites demonstrated the remarkable variability in their morphology (Fig. 1). The surface of CPLGA was smooth with ridges, suggesting the matrix's homogenous nature. At the same time, PLGA did not show a homogenous surface, suggesting that extract is not evenly distributed throughout the matrices. The SEM monographs of CPLGA and PLGA (Fig. 1) implied that CPLGA had interwoven thread-like structure. The probable reason for this was the adsorption of BAE on the interconnected and swelled surface of PLGA-CNF surface. At the same time, the PLGA matrix (Fig.1) resulted in nonspherical particle shape with sufficient aggregation after extract loading. The amorphous nature of BAE provokes the homogenous distribution of extract inside designed matrices. The encapsulation efficiency of the composite matrix was around 43%.

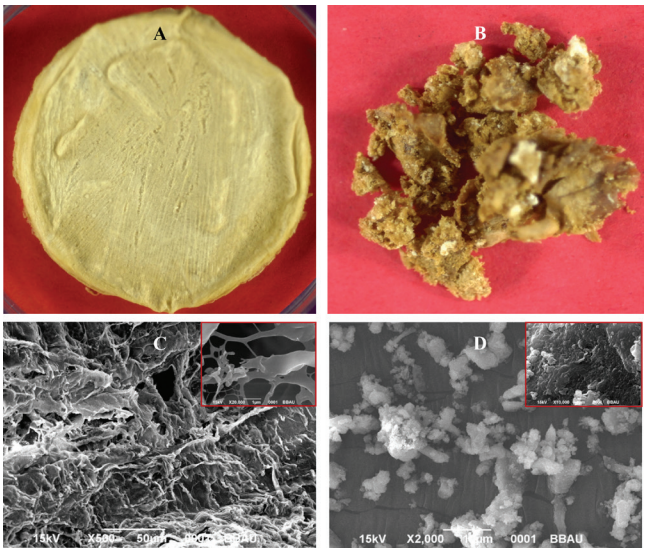

Figure 1. Fabricated composite of *Bacopa* extract loaded. CPLGA (A), neat PLGA (B); SEM of CPLGA(C), PLGA (D)

The FT-IR spectra (Fig. 2) showed the similarity between the uncoated matrices and coated matrices and implied that the P-80 coating did not alter the functionality of the composite system. But the broad peak with decreased intensity between 3300–3600 cm^{-1} indicated the presence of free hydroxyl groups of P80. Jose *et al.,* (2016) also found an increased-O-H band after encapsulation of carboplatin on PLGA nanoparticles (Jose *et al.*, 2016). A significant shift of the band (from 1728 cm^{-1} to 1740 cm^{-1}) in both matrices suggests a direct interaction of ester group (–C O) of polysorbate 80 groups with the composite surface (Joshi *et al.*, 2016).

X-ray diffraction analysis was used to check the interaction between PLGA and CNF and to assess the changes that occurred by incorporating BAE into the matrix (Fig. 3a). The characteristic peak of cellulose II at 2θ = 19.8° (Miller indices of 110) was detected in CNF. The amorphous nature of PLGA did not impart any characteristic peaks in the matrix (Ignjatović *et al.*, 2016). But after the incorporation of BAE emergence of two new peaks at 2θ = 28.5° and 40.8° was detected. It was reported previously that *Bacopa monnieri* extract has a wide range of XRD peaks, which vary between 2θ = 20-75° (Mohana and Padma, 2016).

Particle size distribution is an important physicochemical property of composite that determines the uptake and biological fate of the particular system (Ghasemian *et al.*, 2013). The size of the obtained composite system was around 172 nm. The polydispersity index (PDI) of matrices was found to be in a range of 0.220-0.650. The composites' low PDI values indicated a uniform composite system size. The zeta potential of the fabricated composite system was found to be -34.90 mV to -32.20 mV. The carboxylic groups present on PLGA's free surface are responsible for the generation of negative zeta potentials (Alshetaili and Abdullah, 2021).

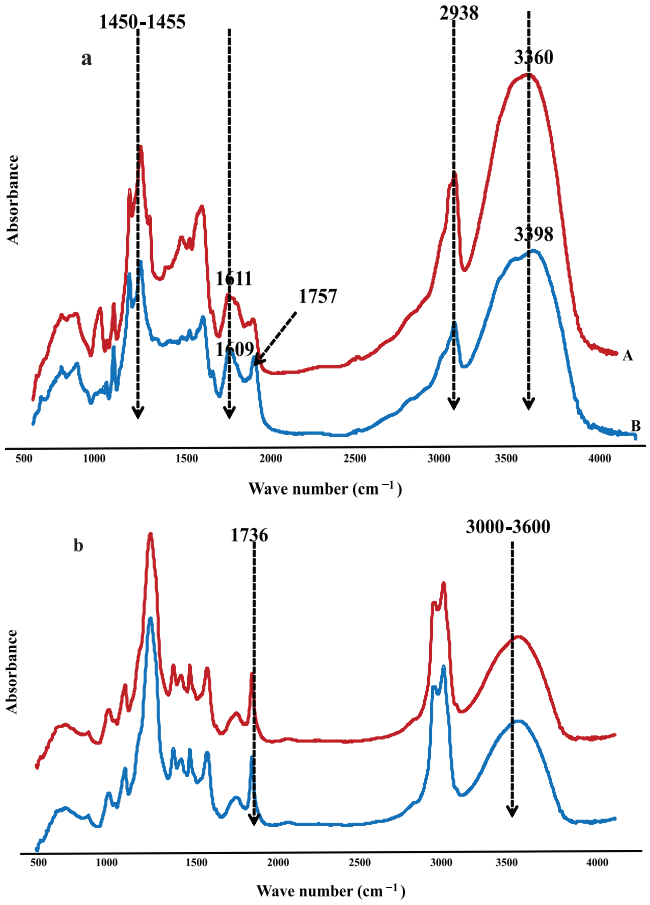

Figure 2. FT-IR spectra of (A): CPLGA, (B): PLGA, a. without P-80 coating b; with P-80 coating

Antioxidant properties of matrices

B. monnieri is well known for its potent antioxidant properties (Kishore *et al.*, 2017). Hence, the antioxidant properties of BAE loaded composite system were assessed and compared with that of pure extract. Total antioxidant capacity (TAC), DPPH (for the free radical scavenging potential of the matrix), and total phenolic content (TPC) of the composite and extract were also determined (Table 1). The total antioxidant capacity of the composite system was significantly lower than that of pure

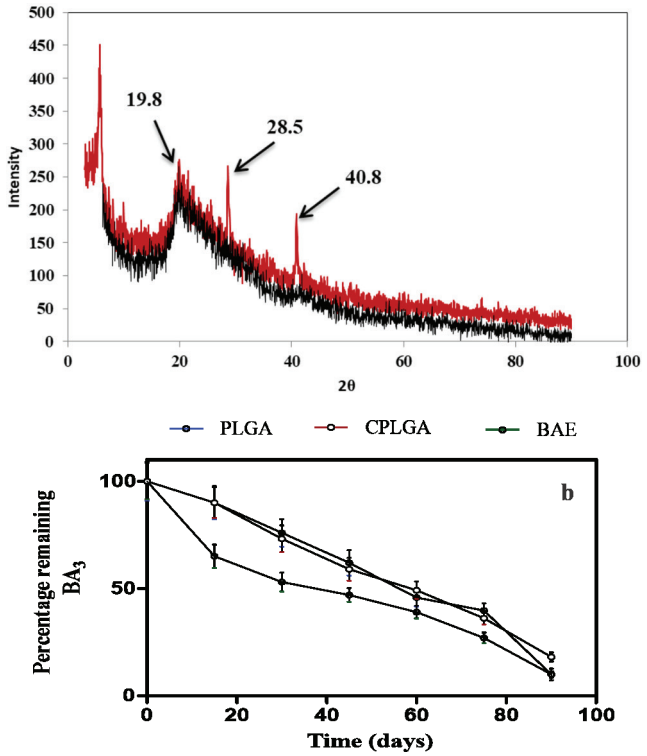

Figure 3 a. XRD of CPLGA matrix: black lines (neat matrix) and red lines (loaded with BAE) b. Stability of BA_3 from *Bacopa monnieri* extract

extract. The decrease in TAC of the composite system was probably due to less availability of the bioactive compound for the reaction with radicals or the time lag between both reactions (Pereira *et al.,* 2015). Conversely, the TPC values were higher composite system as compared to pure extract. The increased value of TPC might be due to the interaction between extract and composite matrix. Polymer characteristics like hydrophilicity, porosity, interaction with other polymers, and binding with extract components affect the loading efficiency (Trifković *et al.,* 2014). The strong interaction between bioactive molecules of the extract with the matrix, as shown in the FT-IR results, might be responsible for the increased TPC as it prevents the loss of phenols through diffusion from the matrix during the preparation of matrices (Ćujić *et al.,* 2016). However, no significant changes were observed (among the composite matrix and control) in the DPPH reduction percentage after loading of BAE ($p<0.05$).

Release kinetics of Bacoside A_3 (BA_3)

The release of BA_3 was performed by using a dialysis bag technique at 37 °C, at three different pH (Fig. 4 A, B, and C). BA_3 in *Bacopa* extract without loading was taken as control. The overall releasing pattern exhibited a biphasic pattern in which a burst release event was followed by sustained release of BA_3 by CPLGA at all pH. The biphasic releasing pattern of drug-loaded PLGA nanoparticles was reported previously (Encinas-Basurto *et al.,* 2017; Gülsel *et al.,* 2022; Mohammad *et al.,* 2021). Part I of the Fig. 4 A, B, and C shows burst release, while part II demonstrates the sustained release of BA_3. The burst effect was due to the immediate release of surface-bound bioactive compounds. The concentration gradient established after an hour was responsible for prolonging the sustained release of BA_3. The CPLGA showed more sustained release (24 hours) of BA_3 at pH (9) (Fig. 4 C). However, in pure BAE, 100 % of BA_3 was released within 6 hours.

The *in-vitro* release data of BA_3 was fitted into four different kinetics models, i.e., zero-order, first-order, Higuchi, and Peppas models. The values of the regression coefficient for different models are presented in Table 2. Out of four models, only the Peppas model showed the best model with $r^2 = 0.999$ (Table 2).

The Peppas equation (Korsmeyer-Peppas release model) provides additional information about the release behavior on the basis of n values (Yang and Washington, 2006). The present study showed that in all cases, the value of n ranged between $<0.45< n<0.89$ (Table 2), indicating the anomalous diffusion mechanism, which is a combination of diffusion and polymer degradation/

Table 1: Particle characteristics and biological properties of composite matrix

Composite	PS (nm)	ZP (mv)	PDI	TAC (µg AAeq mg^{-1})	TPC (µg GAeq mg^{-1})	DPPH (%)
CPLGA	172±1.67	-34.9±1.68	0.22±0.22	$25.8±0.79^b$	$11.2±0.75^b$	$76.0±1.9^a$
PLGA	200±1.40	-32.2±1.13	0.25±0.23	ND	ND	ND
BAE	ND	ND	ND	$64.5±1.50^a$	$5.65±0.21^a$	$79.5±2.2^a$

PS: Particle size, ZP: Zeta potential, PDI: Polydispersity index, TAC: Total antioxidant capacity, TPC : Total phenolic content, values are in Mean ± SD and different letters in the superscripts of the same column indicate the significantly different values ($p<0.05$), ND: Not determined.

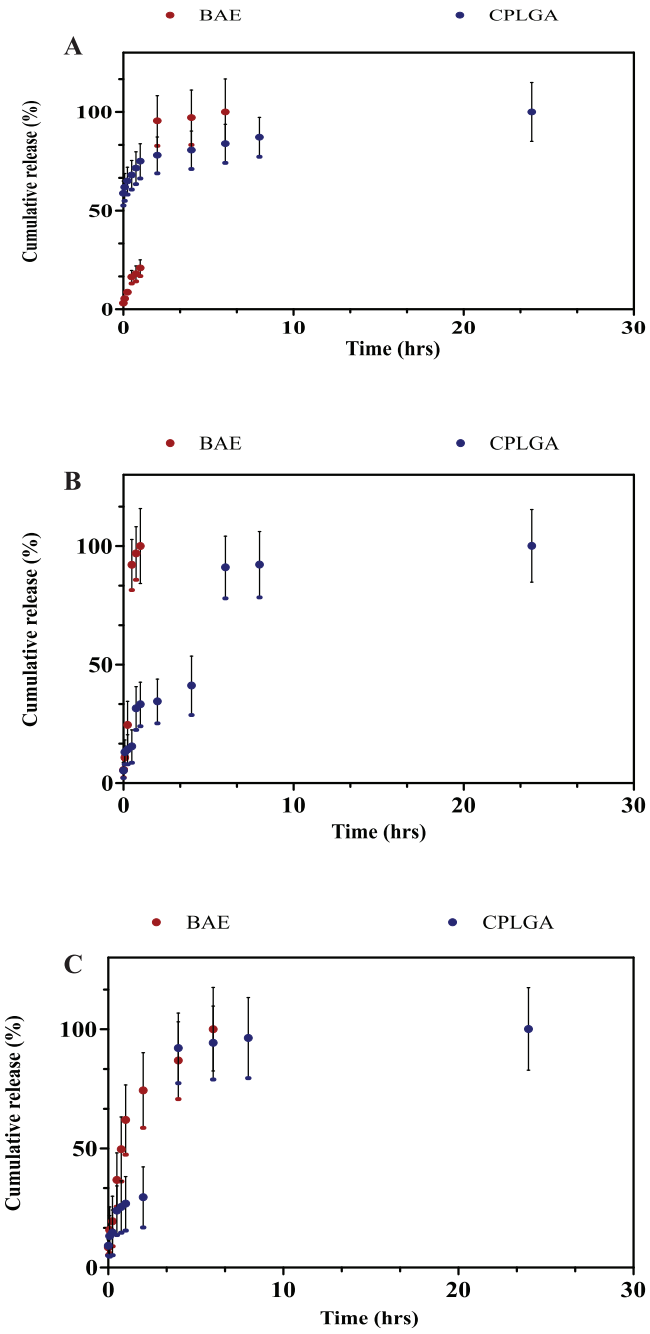

Figure 4. Cumulative release pattern of BA_3 (A): pH= 2.1, (B): pH= 7.4, (C): pH= 9.

erosion dominated release phenomena. This process occurred as follows surface-bound BA_3 release after the burst phenomenon (showed by part I). After that derived concentration gradient diverted to the release toward the plateau (showed by part II), which might be due to the slow diffusion of BA_3 from the matrix. The degradation of polymer chains and bonding between BA_3 and matrix was the possible reason for BA_3 release at elevated pH. The diffusion control release of BA_3 is consistent with the other reports where PLGA nanoparticle is used for sustained drug delivery. Ciprofloxacin-loaded PLGA nanoparticles also showed the erosion-controlled diffusion of the drug from the matrix (Türeli *et al.*, 2017). The release of carboplatin from PLGA matrix was also reported as diffusion-controlled (Jose *et al.*, 2016). *Brassica napus* extract, and curcumin-loaded PLGA nanoparticles and composites also showed the degradation of the polymeric chain for the diffusion of bioactive compounds (Shabestarian *et al.*, 2021; Chen *et al.*, 2021)

This hydrolysis-induced autocatalysis of PLGA may be the possible mechanism of BA_3 release (Ramazani *et al.*, 2016). The addition of CNF to PLGA may provide mechanical strength to the composite system (Josi *et al.*, 2021), leading to forbidding the erosion and chemical degradation of the PLGA. This may cause the sustained release of the BA_3. The possible reason for slightly more release of BA_3 from CPLGA at acidic pH was that acidic pH catalyzes the cleavage of susceptible ester bonds of PLGA as well as hydrogen bonding between BA_3 and matrix.

Stability study

Since the PLGA is a biodegradable polymer, lyophilization is necessary to maintain the drug's and carrier matrix's stability. The stability study of pure BAE-loaded PLGA and CPLGA was performed at 4°C (Fig. 3b). After analyzing the content of

Table 2: Kinetic coefficient of different kinetic models

	pH (2.1)		pH (7.4)		pH (9.0)	
Kinetic model	BAE	CPLGA	BAE	CPLGA	BAE	CPLGA
Zero order	0.398	0.751	0.154	0.649	0.432	0.539
First Order	0.340	0.677	0.133	0.385	0.297	0.422
Higuchi	0.639	0.948	0.361	0.850	0.732	0.780
Korsmeyer-Peppas	0.871	0.974	0.821	0.900	0.880	0.917
Diffusion exponent	0.620	0.080	0.300	0.560	0.360	0.540

BA_3upto 90 days, the results showed that both the matrices were able to retain the approx. 50% of BA_3 concentration up to 60 days, while neat *Bacopa* extract (BAE) showed 50% of BA_3 concentration at 30th days. By the end of 90th day, the content was decline up to 82% in CPLGA while 90% degradation was detected in PLGA. Hence, CPLGA had more suitability for improvement of BA_3 stability under controlled conditions (4°C).

The material characteristics and stability study suggested that the incorporation of CNF in PLGA improved the particle size, zeta potential, and stability of the composite system. Hence, it is reasonable that only CPLGA may be used for further study of antioxidant properties and control release of *Bacopa* extract.

CONCLUSION

The present study has successfully formulated a composite system for encapsulation of $BA_3$with a particle size of 172.6 nm, a zeta potential of -35.3 mv, and encapsulation efficiency of 42.7%. The *in-vitro* experiment of BA_3 release from the dialysis bag technique at three different pHs demonstrated the sustained release of BA_3 in the composite system as compared to pure extract. These findings could be of great significance for encapsulation which could improve its propensity for sustained release, stability during transportation, the shelf life of phyto-products and thereby suggesting its application in pharmaceuticals or nutraceutical purposes. This study focused only on the release of BA_3. However, the *in-vitro* and *in-vivo* release kinetics of other constituents of the *Bacopa* extract is required for other applications, such as the delivery of Bacoside A in the central nervous system.

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HIGHLIGHTS

- Bacoside A_3 (BA_3) was encapsulated in the composite system of cellulose nanofiber/poly-lactic-co-glycolide (CNF/PLGA).
- The composite showed sustained release of BA_3 for 24 hours at pH=9.
- The composite was able to improve the stability of encapsulated extract for upto 45 days as compared to pure extract.

List of Abbreviations

Cellulose nanofibers – CNF

TEMPO-oxidized cellulose nanofibers- CNF TO

Poly (lactic-co-glycolic acid)- PLGA

Bacoside A_3 - BA_3

Bacopa extract- BAE

Total phenolic content - TPC

Total antioxidant capacity -TAC