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Preparation, characterisation, and docking studies of biocompatible nanocarrier for the sustained drug release of an anticancer drug- Etoposide

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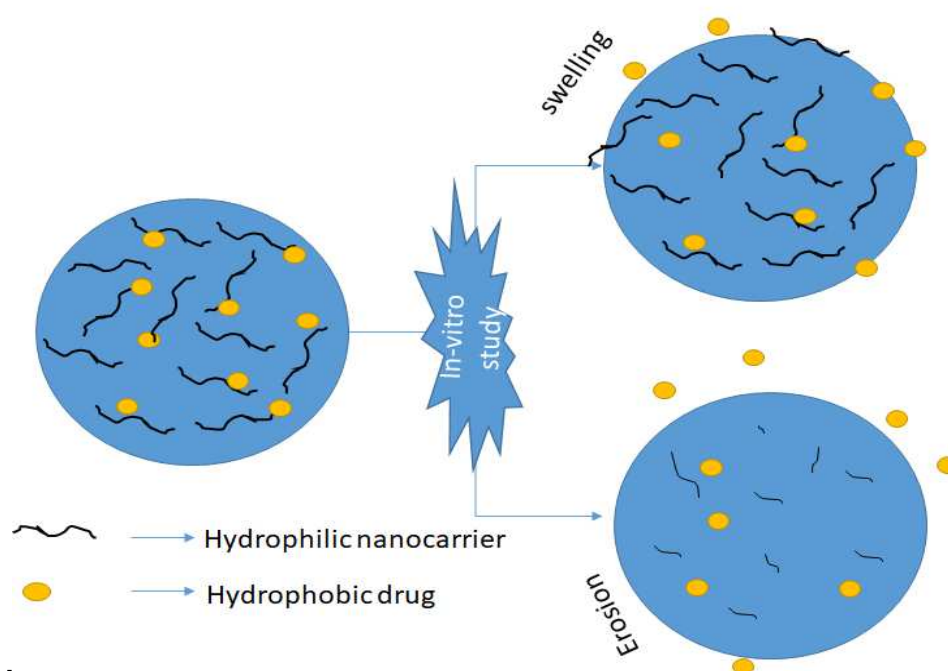
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Abstract

Etoposide is used in the treatment of testicular, prostate, bladder, gastric, and lung cancers, but its therapeutic application is restricted by poor aqueous solubility, limited bioavailability, and dose-dependent toxicity. The objective of this study was to develop a novel biodegradable nano-carrier system for the sustained delivery of Etoposide. The etoposide-loaded caseinate-sodium alginate PEC nanocarriers were prepared by polymer complexation. Molecular docking with AutoDock Vina v1.2.3 and protein-ligand interaction profiling were used to examine etoposide interactions with α - and β -casein. The nanocarriers were characterised using SEM, FTIR, XRD and DLS. In vitro drug-release data were evaluated using zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models. The caseinate-sodium alginate complex formed micelles with hydrophilic regions on their surfaces, allowing them to interact with the aqueous environment. Molecular docking studies performed using AutoDock Vina predicted favourable binding scores for etoposide against α -casein ($-7.0 \text{ kcal mol}^{-1}$) and β -casein ($-6.1 \text{ kcal mol}^{-1}$), with protein-ligand interaction profiling (PLIP) confirming four hydrogen bonds and hydrophobic contacts as the primary stabilising interactions. Physicochemical characterisation confirmed the formation of the nanocomplex, its structural integrity, and stability. The formulation provided controlled and sustained etoposide release throughout the study period. Kinetic modelling indicated non-Fickian transport, suggesting that both swelling and matrix erosion contributed to drug release. The etoposide-loaded caseinate-sodium alginate PEC nanocarrier demonstrated sustained drug delivery over the 48-hour study period, indicating potential for prolonged therapeutic action.

Keywords: biomaterials, controlled release, encapsulation, in vitro model(s), nanoparticle, pharmacokinetics

Graphical abstract



Introduction

One of the primary causes of morbidity and death globally, cancer is a significant public health concern. In accordance with World Health Organization (WHO) estimates, there will be over 35 million cancer cases globally by 2050, a 77% rise from 20 million in 2022. The recent revelation that India, in particular, has one of the highest cancer burdens in the world emphasises the urgent need for better treatment approaches. [1].

Chemotherapy remains a cornerstone of cancer treatment; however, a significant number of anticancer drugs have restricted bioavailability, inadequate solubility, low biocompatibility, and numerous adverse reactions. Unfortunately, the majority of chemotherapy drugs are unable to distinguish between rapidly proliferating malignant cells and their healthy counterparts, which can have detrimental effects. Furthermore, cancer cells may develop multidrug resistance (MDR), a self-defence mechanism, in response to chemotherapy, thereby reducing treatment efficacy. Together, these limitations significantly hinder the therapeutic benefits of conventional chemotherapy. To overcome such drawbacks, a wide range of nanocarrier-based delivery systems have been investigated. For example, employing molecular dynamics simulations to encapsulate 5-fluorouracil (5-FU) into carbon nanotubes (CNT) and boron nitride nanotubes (BNNT), creating dual-sensitive nanogels for the extended local administration of doxorubicin in triple-negative breast cancer cells, and green synthesis of hyaluronic acid-coated, thiolated chitosan nanoparticles targeting CD44 for cervical cancer [2–5]. All of these studies demonstrated how sophisticated nanocarriers can minimise systemic negative effects while enhancing drug stability, bioavailability, and therapeutic efficacy. A wide range of nanocarriers, including silica nanoparticles, Lipid nanoparticles, poly(ethylene glycol)-poly(ϵ -caprolactone)

nanoparticles, Nanogels, Glyceride Lipids, Liposomes, PEGylated nanoliposomes, Strontium Carbonate Nanoparticles, Tripalmitin nanoparticles, PLGA-MPEG, and PLGA-Pluronic Block Copolymers, Poly (L-lactic Acid), have been successfully employed to encapsulate drugs, thereby improving their half-lives in circulation, making the drugs more effective and safer for use, and also reducing their toxicity [6–14].

The well-known topoisomerase II inhibitor etoposide induces cytotoxicity by stabilising the cleavage complex, a transient intermediate in topoisomerase II's catalytic cycle. - thereby preventing DNA re-ligation and ultimately leading to cell death. Etoposide is a Biopharmaceutical Classification System (BCS) Class IV drug with solubility and permeability limitations, which shows enhanced drug bioavailability with mesoporous silica nanoparticles[15]. Similar results were obtained from a protein-based nanocarrier encapsulating etoposide for cancer therapy, which investigates the development of a novel biosynthetic and unfolded protein nanocarrier for loading and delivery of etoposide[16].

Drug delivery systems play a critical role in providing controlled release of drugs, which is essential for optimising therapeutic outcomes while minimising potential side effects[17]. Among them, researchers have placed a lot of emphasis on nanocarriers because of their capacity to improve drug release over time, offering a regulated and prolonged release profile. Both lipid nanoparticles and polymer nanoparticles have demonstrated sustained-release properties for drug delivery[1]. For instance, lipid nano capsules have been developed as drug delivery systems with small sizes that facilitate intracellular uptake and exhibit P-gp inhibitory properties, resulting in sustained drug release. Similarly, polymeric nanoparticles such as PLGA-based nanoparticles have been used to deliver drugs with long circulation times, thereby sustaining drug release over several days[18].

Among natural polymers, caseinate has emerged as a promising carrier owing to its biocompatibility and ability to form stable complexes with different types of molecules. The caseinate-chitosan complexes were formed by maintaining the proper pH. The sedimentation and stability studies for the caseinate-chitosan nanocarrier were carried out to ensure that the state of the nanocarrier is favourable for drug delivery[11]. Sodium caseinate- gum Arabic nanoparticles were prepared by slow acidification of the system with glucono-d-lactone (GDL) or titration with HCl. The formation of electrostatic complexes was studied as a function of pH. The colloidal behaviour of the complexes was observed to ensure the stability of the nanoparticles[19]. Sodium caseinate nanoparticles were injected with thymol using the high shear homogenization technique. The encapsulation reduced the dimension of sodium caseinate and increased the zeta potential, making it a suitable nanocarrier for drug delivery[20]. Curcumin was encapsulated in zein nanoparticles and coated with sodium compounds to stabilise the nanocarrier complex, which in turn helps to improve their water stability. This formulation also helped in the controlled release of the drug, as well as improved encapsulation efficiency and antioxidant activity of curcumin[21].

Alginate is a polysaccharide-based anionic biopolymer commonly employed in the fabrication of anticancer drug delivery systems because of its non-toxic, biocompatible, and biodegradable properties. Most of the components come from brown algae and are connected by a single bond. Alginate develops crosslinked networks when exposed to divalent and trivalent cations such as calcium and barium ions, and other minerals. For oral drug delivery applications, alginate-based systems are useful because they release a significant amount of medication in colonic pH

conditions and very little in stomach pH settings. Because of their size, hydrophilicity, sensitivity to certain microenvironments, and/or time-controlled administration, three-dimensional alginates-which combine the greatest features of hydrogels and nanoparticles-have demonstrated significant promise in drug delivery [22]. Recently, several polymers and several synthetic polymer chains have been covalently attached to the alginate backbone for use across diverse biomedical applications, among them poly (N-isopropylacrylamide-co-N-hydroxymethyl acrylamide), poly (crylonitrile/polyethylene glycol), poly (ethylene glycol methacrylate), and poly (N-isopropylacrylamide-co-N, N-diethylacrylamide)[3,4,23–26].

The use of polyelectrolytes (PECs) to encapsulate active ingredients such as drugs and DNA has gained popularity in recent years due to their straightforward, inexpensive preparation techniques, such as coacervation and layered self-assembly, compared with more conventional techniques like solvent evaporation and emulsification. Previous studies have been conducted on drug delivery systems using caseinate[19], zein [27], and chitosan with different counter-polymers [27-30], demonstrating improved stability and effective sustained release of various hydrophobic drugs [8].

Polyelectrolyte complexation with oppositely charged polymers has enhanced the drug transport capabilities of chitosan; however, the application of these complexes has been constrained by the intrinsic characteristics of chitosan. The effective synthesis of chitosan/chitosan/sodium polyacrylate (PAAS) polyelectrolyte complex hydrogels (CPG) was synthesised by crosslinking the two polymers using epichlorohydrin (ECH) as the crosslinking agent, a step that suppressed chitosan protonation within the alkali/urea solvent system[28–30]. Furthermore, sustained drug delivery of rhodamine B and Nile red was demonstrated using chitosan-dextran sulphate nanoparticles[31]. Alginate-caseinate nanocarrier for encapsulating the chemotherapy drug 5-Fluorouracil has not been used to date for sustained drug release. The potential of polyelectrolyte-based nanomaterial creation, characterisation, and in vitro investigation for the long-term delivery of hydrophobic medications is emphasised in this study.

Materials and methods

Ethanol, methanol, citric acid powder, caseinate, sodium alginate, and active pharmaceutical ingredient (API) etoposide were purchased from Chemscene, India. Distilled water served as the solvent for all preparative steps, and analytical-grade chemicals and reagents were used throughout.

A citric acid solution was prepared by dissolving citric acid powder in distilled water at ambient temperature, with the pH adjusted and held within the 1.5-2.5 range. Caseinate was then dissolved in this citric acid medium to form the caseinate solution, whereas the sodium alginate solution was independently formulated by dispersing sodium alginate powder in distilled water.

Both these solutions were combined and subjected to a magnetic stirrer at 800 rpm at room temperature for 30 minutes, causing a polyelectrolyte complex (PEC) to develop via the coacervation method. The formed PEC was centrifuged at 4000rpm for 8 minutes in six intervals to separate the particles. The settled particles were then filtered, separated, vacuum-dried, ground, and pulverized[27,32]. Both the powdered PEC and the supernatant obtained by centrifugation were used for subsequent studies[32].

PEC samples were prepared using both sodium alginate and caseinate in distilled water, respectively, with different weight/volume fractions ranging from 0.5% to 2.5% w/v. Specifically,

samples were prepared at concentrations of 0.75%, 1.0%, and 2.5% w/v in the ratio of 1:1. Following the optimisation of the weight-per-volume ratio for the polyelectrolyte complex(PEC), etoposide was added to a caseinate-citric acid solution, which was then combined with the sodium alginate solution and stirred to encapsulate it in the caseinate-sodium alginate PEC system[27]. The 3 sample solutions were prepared with etoposide loadings of 0.5mg, 0.7 mg, and 1.0 mg [32]. The preparation method is depicted schematically in **Figure 1**. All the samples were used for different analyses. The supernatant and residue collected after centrifugation were stored in the refrigerator and were further used for in vitro studies.

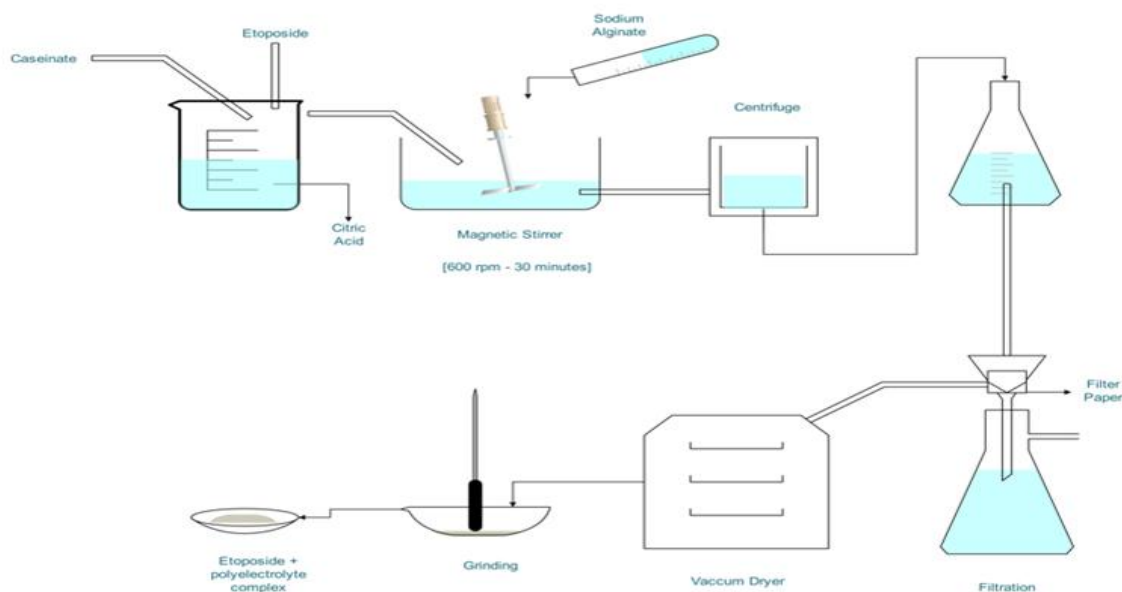


Figure 1 Schematic representation of the preparation and synthesis of Etoposide-encapsulated caseinate-sodium alginate polyelectrolyte complex.

Encapsulation efficiency (EE)

The ratio of drug weight encapsulated in a nano-carrier system to total drug added is known as the encapsulation efficiency (EE). In this study, to determine EE, etoposide-loaded caseinate-sodium alginate PEC was centrifuged at 4000 rpm for 8 minutes at room temperature for 6 intervals. The resulting supernatant was further diluted with methanol and analysed using Ultraviolet spectroscopy to quantify the concentration of non-encapsulated drug [1].

The encapsulation efficiency has been calculated using the following formula:

$$\text{Encapsulation Efficiency (\%)} = \left(\frac{\text{Quantity of drug that is encapsulated}}{\text{Total quantity of drug added}} \right) \times 100\%.$$

Characterisation Techniques

After the synthesis, the nanocarriers with and without drug should be characterised with the help of FTIR, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Dynamic Light Scattering. FTIR spectra were recorded using an Avatar model 330, with a wavenumber range of 4000-400 cm^{-1} and a resolution of 0.01 to 0.001 degrees. Samples were prepared using both attenuated total reflectance (ATR) for liquid samples and the potassium bromide (KBr) pellet technique for powdered nanocarriers to ensure accurate characterisation. A Rigaku Ultima IV diffractometer with a Cu-K α radiation source was used to record the diffraction patterns, with a scan rate of 2°/min and a step interval of 0.02. Using a Bruker Quantax 200-Z10 EDS detection system and field emission scanning electron microscopy (FESEM) on a SIGMA HV (Carl Zeiss) scanning electron microscope, the surface morphology of the nanocarrier was investigated. Images were captured on an instrument with a resolution of 0.5-1.0 nm[25,32].

The optical properties of the samples were analysed using an integrating sphere attachment. The JASCO V-750 UV-visible spectrophotometer can perform diffuse reflectance spectroscopy with a wavelength range of 170-1100 nm and a resolution of 0.1 nm[16]. The particle distribution (z-average diameter) and surface charge (zeta potential) of the nanocarrier using a Zetasizer nano-zs ZEN3600; Malvern Instruments, Worcestershire, UK. Each sample was dissolved in an appropriate solvent for analysis, and measurements were performed at room temperature. [7,33].

In-vitro drug release studies

In vitro studies for supernatant

Using a dialysis bag, etoposide's sustained-release behaviour of the caseinate-sodium alginate PEC was evaluated using the dialysis bag method. Both the supernatant and powdered formulations were analysed. Three separate dialysis bags (knotted at both ends) were each loaded with 10 mL of three distinct etoposide-loaded nanocarrier formulations (both supernatant and diluted powder with buffer) at concentrations of 1 mg, 0.7 mg, and 0.5 mg, respectively. The dialysis bags were then submerged in a beaker filled with 150 mL of phosphate buffer (pH 7.4) and maintained under magnetic stirring at 25°C for 48 hours, running at 150 rpm.

Sampling time points were set at 0,1,2,4,6,8,10,12,24,32, and 48 hours; an equal volume of freshly made phosphate buffer solution was used in place of 10 mL of release medium. Finally, to determine the quantification of etoposide release in the phosphate buffer, the collected samples were analysed using ultraviolet-visible spectroscopy (UV-Vis); the etoposide release rate was determined by graphing the absorbance readings obtained at a wavelength of 284nm[32]. Similarly, drug release rate for the previously mentioned compositions was carried out at 37°C to simulate physiological temperature. The absorbance values obtained at 284nm were used to calculate and compare release rates across different formulations.

In vitro studies for the settled powder

The dialysis bag approach was used for the release of etoposide from caseinate-sodium alginate nanocarrier powder. First, 10mg of the etoposide nanocarrier complex of different compositions (1.0 mg, 0.7 mg, 0.5 mg drug-nanocarrier formulations) was dissolved in 10 mL buffer. Three separate dialysis bags were loaded with 10 mL of three distinct drug-nanocarrier formulations of etoposide-encapsulated complex, and were transferred to each bag. After that, the dialysis bags were placed into a vessel containing 150mL of phosphate buffer adjusted to pH 7.4. Each beaker was kept under magnetic stirring at 25°C for 48 hours, running at 150 rpm. 10 mL of phosphate buffer was collected at determined times (for 0th hr, 1st hr, 2nd hr, 4th hr, 6th hr, 8th hr, 10th hr, 12th hr, 24th hr, 32nd hr, and 48th hr) and replaced with freshly prepared phosphate buffer solution.

A UV spectrophotometer was used to analyse the structural changes in the etoposide-loaded PEC by measuring the absorbance of the samples over a specific wavelength range. Ultraviolet-visible spectroscopy (UV-Vis) was used to analyse the collected samples in order to measure the etoposide release in the phosphate buffer. The drug released from the absorbance recorded at a wavelength of 284 nm was used to estimate the release rate of etoposide, which was computed by graphing the values.[32]. The Schematic representation of the in vitro studies is shown in **Figure 2**. Similarly, UV-Visible spectroscopy was used to determine the drug release rate for the aforementioned formulations, and the findings were obtained at 37°C.

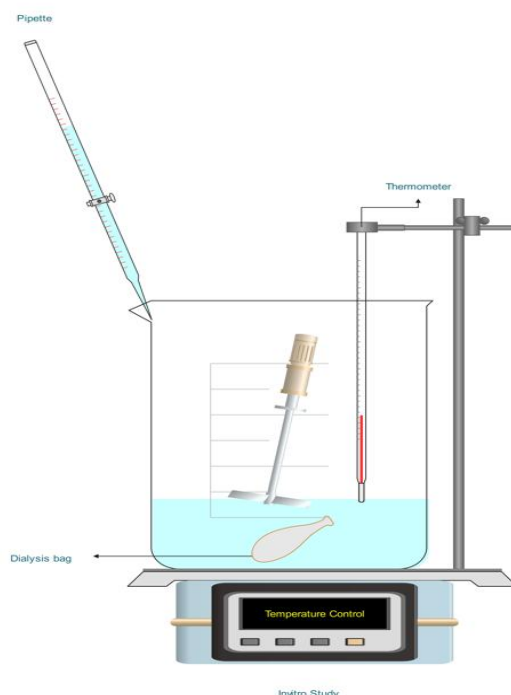


Figure 2 Schematic representation of the in vitro study setup.

Kinetic study

Kinetic modelling was applied to the experimental data derived from in vitro observations to clarify the fundamental process controlling drug release[7,33,34].

The results were compared to several kinetic models in order to characterise the release profile. The models used were the zero-order release model, Korsmeyer-Peppas, Higuchi, and first-order release models.

Zero-order release model

The drug release kinetics were first evaluated using the zero-order model, expressed by the equation:

$$Q_t = Q_0 + K_0 t$$

Where the initial amount of the drug is Q_0 the total amount of drug released at time 't' in hours is Q_t , the zero-order release constant is K_0 . Zero-order release behaviour was assessed by plotting the cumulative fraction of drug released, obtained from the in vitro experimental data, as a function of elapsed time.

First-order release model

Equation:

$$\log Q_t = \log Q_0 + K \frac{t}{2.303}$$

Where the initial amount of drug is Q_0 , the cumulative amount of drug released at time 't' in hours is Q_t , and the first-order release constant is K . The data from in vitro investigations were displayed as log cumulative percentage drug release vs. time to examine the drug release kinetics for first-order release.

Higuchi release model

Diffusion-governed release behaviour was examined by applying the Higuchi model, expressed as:

$$Q = K_H t^{\frac{1}{2}}$$

Or

$$\frac{M_t}{M_0} = K t^{\frac{1}{2}}$$

Where the overall amount of drug released at time 't' in hours is Q , and the Higuchi constant is K_H . Analysis under this model involved graphing the cumulative drug release percentage as a function of the square root of elapsed time.

Korsmeyer-Peppas release model

To describe the drug release mechanism, the Korsmeyer-Peppas model was applied. The model can be expressed as:

$$F = \left(\frac{M_t}{M} \right) = K_m t^n$$

Where F is the fraction of drug released at time ' t ', M_t is the amount of drug released at time ' t ', M is the total amount of drug in the dosage form, K_m is the kinetic constant, n is the diffusion or release exponent, and ' t ' is time in hours.

The value of the release exponent ' n ' provides insight into the release mechanism:

- $n < 0.45$ suggests quasi-Fickian diffusion
- $n = 0.45$ indicates Fickian diffusion.
- $0.45 < n < 0.89$ anomalous diffusion or non-Fickian diffusion.
- $0.89 \leq n \leq 1$ denotes case-2 relaxation or non-Fickian case 2 diffusion.
- $n > 1$ signifies non-Fickian super case 2 [34].

Application of the Korsmeyer-Peppas model to characterise release kinetics involved plotting the logarithm of cumulative percentage drug release against the log of time, using data generated from the in vitro release experiments.

Computational studies

Molecular docking studies were performed to explain the binding mechanism of etoposide within the caseinate-alginate polyelectrolyte complex (PEC) and to rationalise the experimentally observed drug encapsulation and sustained-release behaviour. Protein-ligand interactions were characterised using the Protein-Ligand Interaction Profiler (PLIP v3.0.0; Schake et al., 2025)

Molecular Docking

Receptor and Ligand Preparation

Since sodium caseinate is composed predominantly of α -casein and β -casein, both proteins were selected as representative receptor models. Three-dimensional structures of α -casein (UniProt ID: P02662) and β -casein (UniProt ID: P02666) were retrieved from the AlphaFold Protein Structure Database. The predicted local distance difference test (pLDDT) confidence scores for residues constituting the predicted binding regions were (state mean $\pm$ range, e.g. 82 ± 6) for α -casein and (mean $\pm$ range) for β -casein, indicating acceptable local structural confidence for exploratory blind docking despite the low global confidence expected for an intrinsically disordered protein.[36]. Structure preparation was performed in AutoDock Tools; water molecules were stripped from the structures, polar hydrogens were added, Gasteiger partial charges were assigned, and files were converted to PDBQT format.

The three-dimensional structure of etoposide (PubChem CID: 36462) was retrieved from the PubChem database, energy-minimised using the MMFF94 force field, and converted to PDBQT format. A sodium alginate tetrameric oligomer (alternating β -D-mannuronate/ α -L-guluronate) was constructed to represent the alginate backbone. Because AutoDock Vina applies a generalised

empirical scoring function, its application to the polysaccharide receptor is an approximation; accordingly, the alginate binding affinity is interpreted as a qualitative comparative indicator rather than a quantitative prediction.

Docking Protocol

Blind molecular docking was performed using AutoDock Vina v1.2.3 to avoid bias towards any predefined binding site. Docking grid boxes encompassing the entire receptor surface were constructed using the Axis-Aligned Bounding Box (AABB) method: minimum and maximum atomic coordinates were extracted from each PDBQT file, the geometric centre was calculated as the midpoint of each axis, and a 10 Å margin was added in every direction to ensure unrestricted ligand sampling. For α -casein, the coordinate ranges spanned X: -47.304 to 35.056 Å, Y: -35.515 to 59.298 Å, Z: -68.983 to 60.783 Å; for β -casein, X: -63.108 to 28.620 Å, Y: -41.718 to 62.341 Å, Z: -60.659 to 52.947 Å. Twenty binding poses were generated for each receptor-ligand pair, ranked by predicted binding affinity. The top-ranked pose was selected for interaction analysis. Pose reproducibility was assessed by computing the RMSD among the top five poses for each complex; poses within 2.0 Å RMSD of the top-ranked conformation were considered to occupy the same binding mode. All five top-ranked poses for both casein complexes satisfied this criterion, confirming a well-defined and reproducible predicted binding mode.

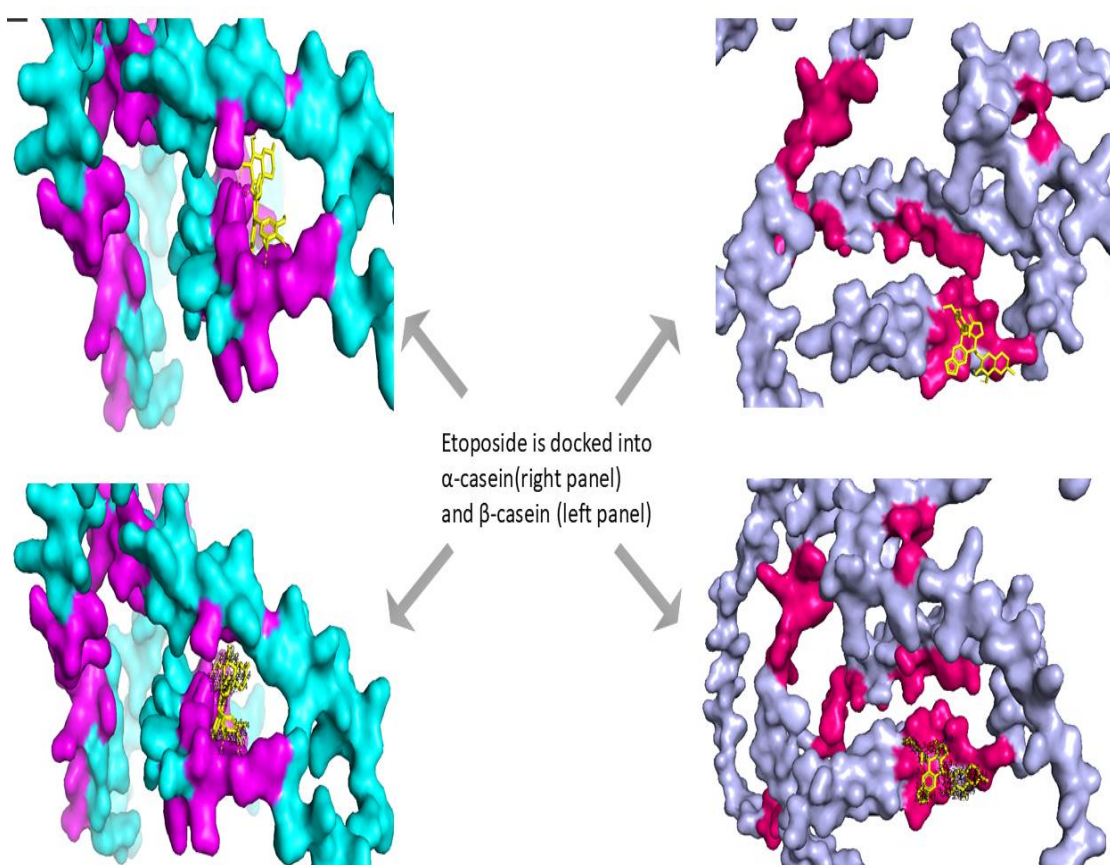


Figure 3 Docking poses of etoposide in α -casein (left) and β -casein (right). Ligand shown in stick representation; PLIP-identified H-bond interactions shown as dashed lines; hydrophobic contacts shown as dotted surfaces.

Results and discussion

Characterisation

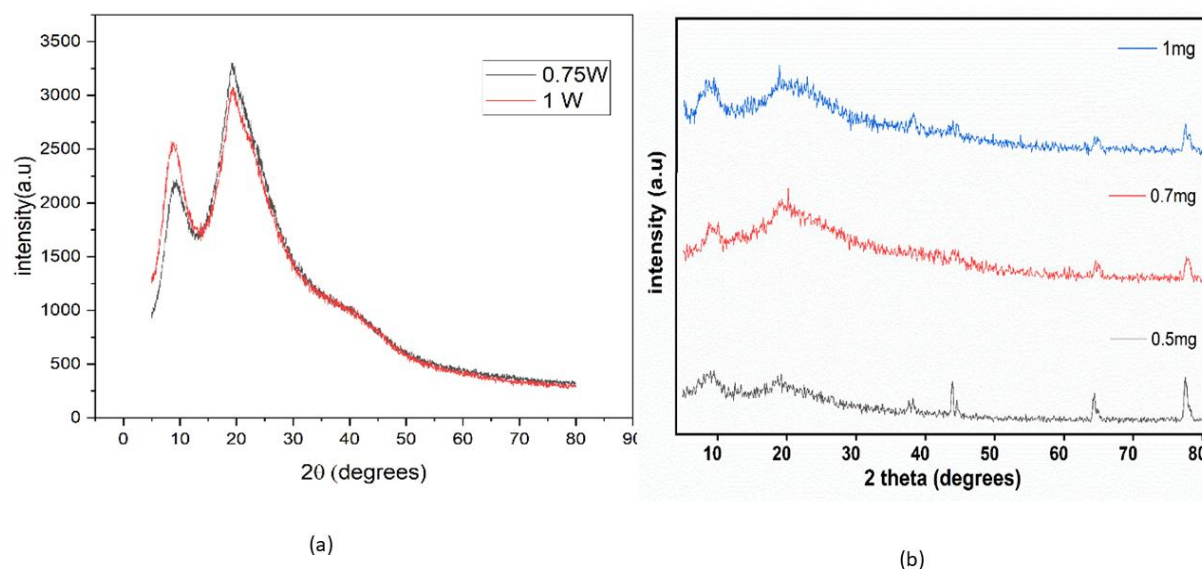


Figure 4 (a) Two different ratios of nanocarrier without drug, (b) three different ratios of drug encapsulated in nanocarrier.

The PEC is naturally hydrophilic. Both the inherent properties of the individual components as well as the interactions between them during complex formation are responsible for the hydrophilic aspect of the surface of the caseinate-sodium alginate complex. This consists of caseinate, which is amphiphilic in nature; the phosphoryl groups in caseinate contribute to its hydrophilic character. Sodium alginate is hydrophilic. The negatively charged carboxylate ions of sodium alginate interact with the positively charged regions of the caseinate molecules. A hydrophilic nanocomplex can encapsulate a hydrophobic drug molecule in its matrix. The active ingredient dispersed or dissolved in the hydrophilic matrix undergoes controlled release through diffusion. The nanocomplex exhibits pH- and redox-dependent swelling and degradation, thereby sustainably releasing the encapsulated drug in the tumour environment[37,38].

For Caseinate-Sodium Alginate PEC, peaks were observed at around 22.54° and 42.3°, as shown in **Figure 4(a)**. Similar results were observed for the PEC formation between sodium alginate and casein for the controlled release of Vanillin and amoxicillin[24,32]. For the sample 1 W/V, the peak at 22.54° represents the semicrystalline nature of the material, suggesting the presence of an electrostatic interaction between rich positively charged regions of caseinate (CA) and negatively charged carboxylate groups of sodium alginate (NaAlg) **Figure 4(a)**. Another peak observed at 9.02° arises from pronounced hydrogen-bond formation occurring between the two interacting species. A small peak at 42.3° denotes the strong intermolecular hydrogen bonding within the complex. These XRD peak positions reflect interplanar spacings within the crystalline domains of the complex and are not a measure of particle size; the nanoscale dimensions of the PEC carrier are established independently by the DLS measurements reported in Section 3.1[39,40].

For the sample with 0.75 W/V concentration, the peak around 22.54° indicates that CA/NaAlg PEC has a semi-crystalline structure and an electrostatic interaction between rich positively

charged regions of CA and negatively charged carboxylate groups of NaAlg (**Figure 4(a)**). Another peak observed at 9.02° and at 42.3° can be attributed to robust hydrogen-bonding interactions established between the two components. As above, these XRD-derived spacings reflect crystal structure rather than particle size; the nanocarrier dimensions of the PEC are confirmed by the DLS measurements in Section 3.1 [41].

For the 0.5mg sample, peaks were observed around 8.8° , 44° , and 77.5° . For the 0.7mg sample, the peaks around 9.35° , 20° , and 77.8° were observed; and for the 1mg sample, the peaks were observed around 8.7° , 20.8° , and 24° . Comparable findings were reported for the 1 mg composition, which showed similarities to the vanillin-loaded CA/NaAlg PEC (peak at 19°) and the etoposide-loaded PCL implant (characteristic peaks at 21.21° and 23.61°), both suggesting a semi-crystalline structure. The decrease in peak intensity has been observed when the drug is encapsulated in a nanocarrier, as shown in **Figure 4(b)**[41].

To understand the chemical structure of the caseinate-sodium alginate PEC, FTIR was performed, confirming the presence of functional groups. Characteristic peaks corresponding to the amino group ($-NH_2$) in caseinate, the carboxylic group ($-COO$) in sodium alginate, and the hydroxyl group ($-OH$) in sodium alginate were observed (**Figure 5**).

From the FTIR results of these two prepared PEC samples, it was observed that, for 0.75W/V (**Figure 5 (a)**), the bands for corresponding functional groups were identified at 941.36 cm^{-1} (C-O-C), 1083 cm^{-1} (C-O), 1526 and 1693 cm^{-1} (C=O), 3120 cm^{-1} (NH), 3236 cm^{-1} ($-OH$), 3419 and 3499 cm^{-1} (NH_2). For the concentration of 1W/V (**Figure 5(a)**), the bands for corresponding functional groups were identified at 937.24 cm^{-1} (C-O-C), 1165.88 cm^{-1} (C-O), 1542.8 cm^{-1} (C=O), 3114 and 3190 cm^{-1} (NH), 3367 cm^{-1} ($-OH$), 3419 and 3429 cm^{-1} (NH_2).

Stretching vibrations attributable to NH_2 , NH, C=O, $-OH$, C-O-C, and C-O groups were observed respectively near 3466 cm^{-1} , 3137 cm^{-1} , 1646 cm^{-1} , 3466 cm^{-1} , 937 cm^{-1} , and 1096 cm^{-1} , providing supporting evidence for polyelectrolyte complexation (non-covalent electrostatic association) between sodium alginate and caseinate. A band consistently appearing between 1500 and 2000 cm^{-1} across all spectra reflects the stretching mode of the $-CO-NH$ linkage, formed via electrostatic association between the protonated amine groups of caseinate and the carboxylate moieties of sodium alginate. Collectively, these spectral features verify that the expected functional groups and their characteristic vibrational signatures are consistently present across all the samples examined, concluding that the prepared samples are of correct compositions and their molecular interactions have taken place. Based on these results, the compositions can be used for drug loading. For 0.75W/V (**Table 5(b)**), the peaks for corresponding functional groups were identified as 931 cm^{-1} (C-O-C), 1080 cm^{-1} (C-O), 1622 cm^{-1} (C=O), 3051 cm^{-1} (NH), 3240 cm^{-1} ($-OH$), 3406 and 3475 cm^{-1} (NH_2)[2,41].

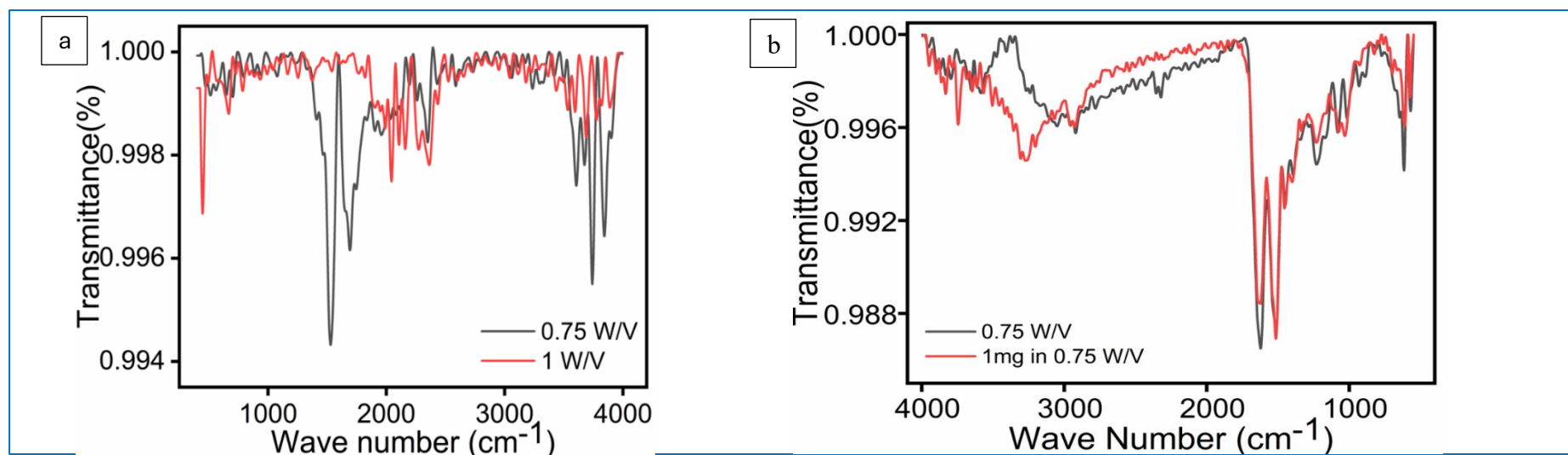


Figure 5 FTIR results for samples with (a) 0.75W/V and 1W/V of nanocarrier, (b) ratios 0.75W/V nano-carrier and 1 mg of drug in 0.75W/V.

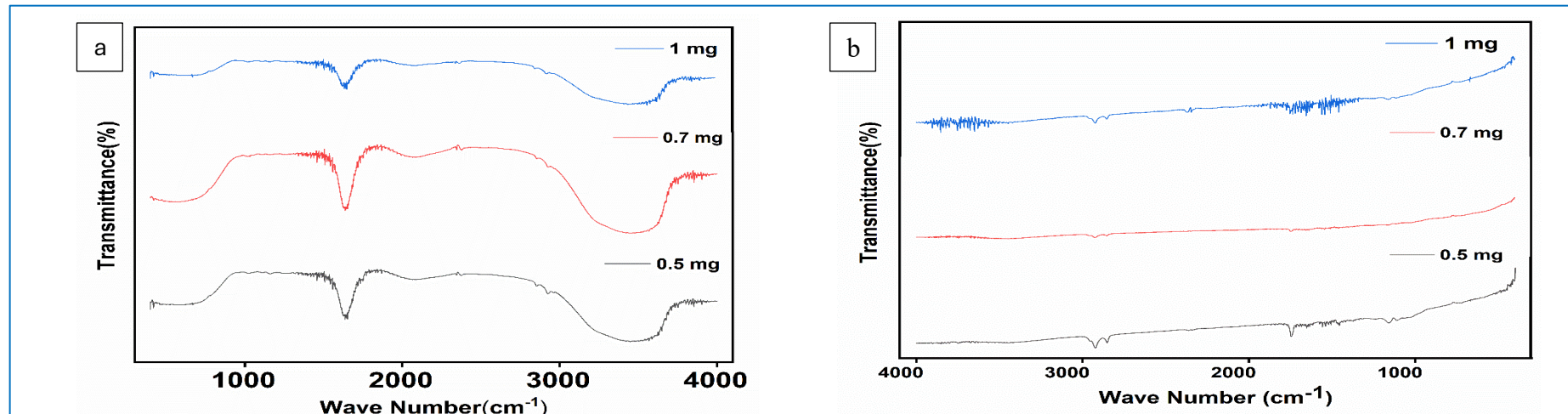


Figure 6 Combined FTIR results for (a) different ratios of supernatant, (b) different ratios of powder.

The primary specialised bands at 590.22 cm⁻¹, 3512.37 cm⁻¹, 2927.94 cm⁻¹, 1639.49 cm⁻¹, and 1467.83 cm⁻¹ in the FTIR of the supernatant of the etoposide-loaded nanocarrier (0.5 mg) correspond to the stretching vibrations of -OH, C-H stretching vibrations of aliphatic C-H bonds, stretching vibrations of the carbonyl group (C=O), and CH₂ bending vibrations, respectively (**Figure 6(a)**). As seen in the **Figure 6(b)**, the FTIR of the powder for the etoposide-loaded nanocarrier (0.5 mg) revealed the main specialised bands at 2927.94 cm⁻¹, 1159.22 cm⁻¹, 1462.04 cm⁻¹, and 1745.58 cm⁻¹, which correspond to the C-H stretching vibrations of aliphatic C-H bonds, C-O stretching vibrations, and -C=O stretching vibrations, respectively.

In a 0.7 mg etoposide-loaded nanocarrier, the FTIR of the supernatant indicated the primary specialised bands at 576.72 cm^{-1} , 1467.83 cm^{-1} , 1633.71 cm^{-1} , and 3448.72 cm^{-1} , which correspond to aromatic C-H bending vibrations, CH₂ bending vibrations, -C=C stretching vibrations, and -OH stretching vibrations, respectively. The FTIR spectrum of the powder for the 0.7 mg etoposide-loaded nanocarrier revealed the main specialised bands at 2927.94 cm^{-1} , 1745.58 cm^{-1} , and 3448.72 cm^{-1} , which correspond to the stretching vibrations of C-H, -C=O, and -OH, respectively. FTIR of the supernatant in a 1.0 mg etoposide-loaded nanocarrier revealed the primary specialised bands at 667.37 cm^{-1} , 1467.83 cm^{-1} , 1641.42 cm^{-1} , 2927.94 cm^{-1} , and 3500.8 cm^{-1} , which are linked to C-O-C stretching vibrations, CH₂ bending vibrations, -C=C, C-H, and -OH stretching vibrations, respectively [2,41].

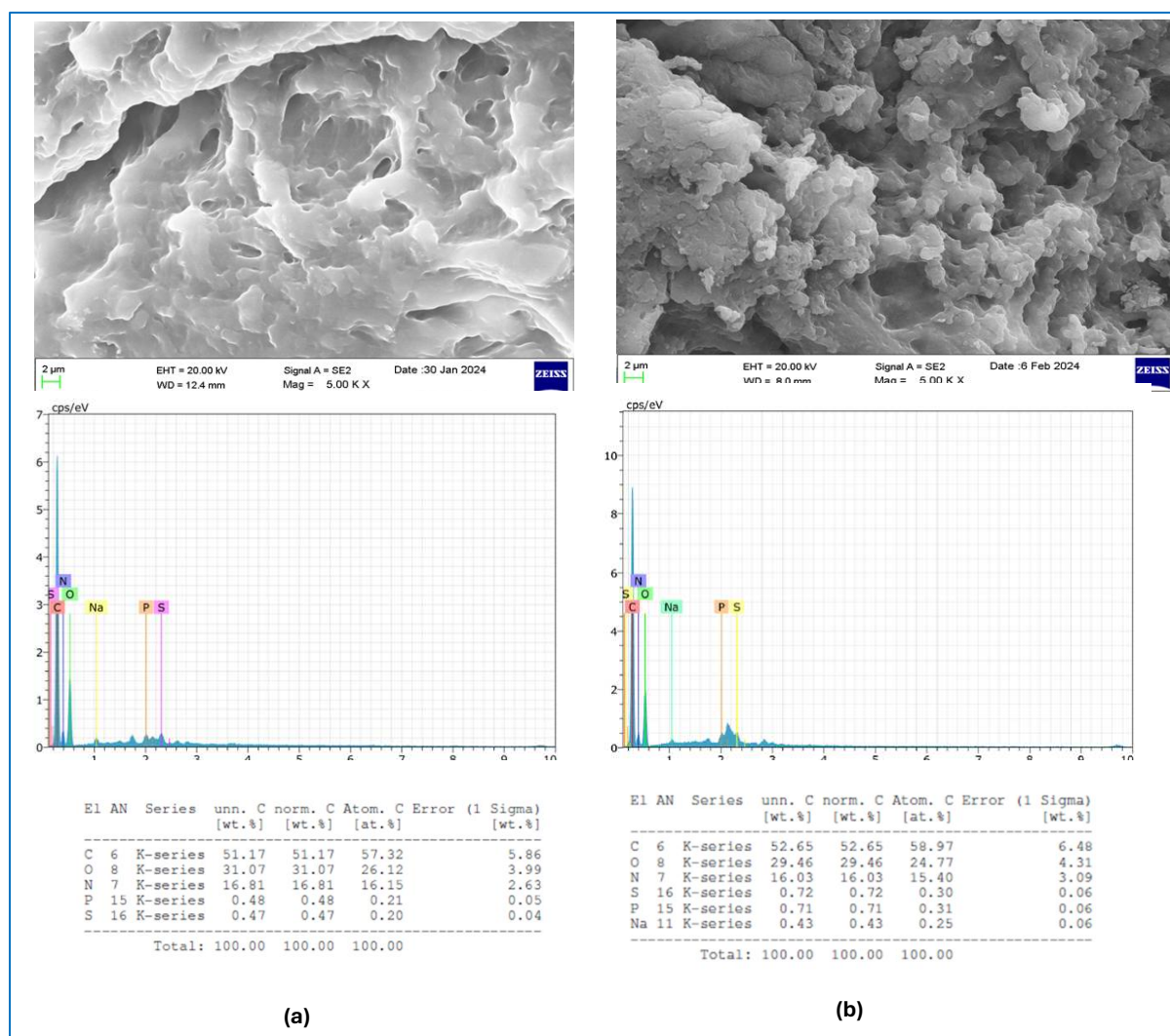


Figure 7 (a) SEM image of nanocarrier (composition 0.75w/v), (b) SEM image of drug-encapsulated nanocarrier (1 mg drug in 0.75w/v), (a) EDS image of nanocarrier (composition 0.75w/v), along with elemental mapping, (b) EDS image of drug-encapsulated nanocarrier.

By comparing the SEM images of the nanocarrier and drug-encapsulated nanocarrier (**Figure 7(a,b)**), open pores were observed in the PEC without drug, whereas in the case of the drug-encapsulated PEC, the pores are closed, which indicates that the drug has occupied the pores of

the PEC. Similar results were obtained from novel carboxymethyl chitin nanoparticles for anti-cancer drug delivery[26,42,43].

From the EDS analysis, as shown in **Figure 7(a,b)**, it was observed that the etoposide-encapsulated nanocarrier contained characteristic elemental peaks corresponding to the components of the polymer matrix. The sodium peak was identified, originating from sodium alginate, while sulfur and phosphorus signals likely arose from caseinate. These additional elements confirm the contribution of the carrier materials rather than etoposide alone, since etoposide does not contain sulfur and phosphorus in its structure.

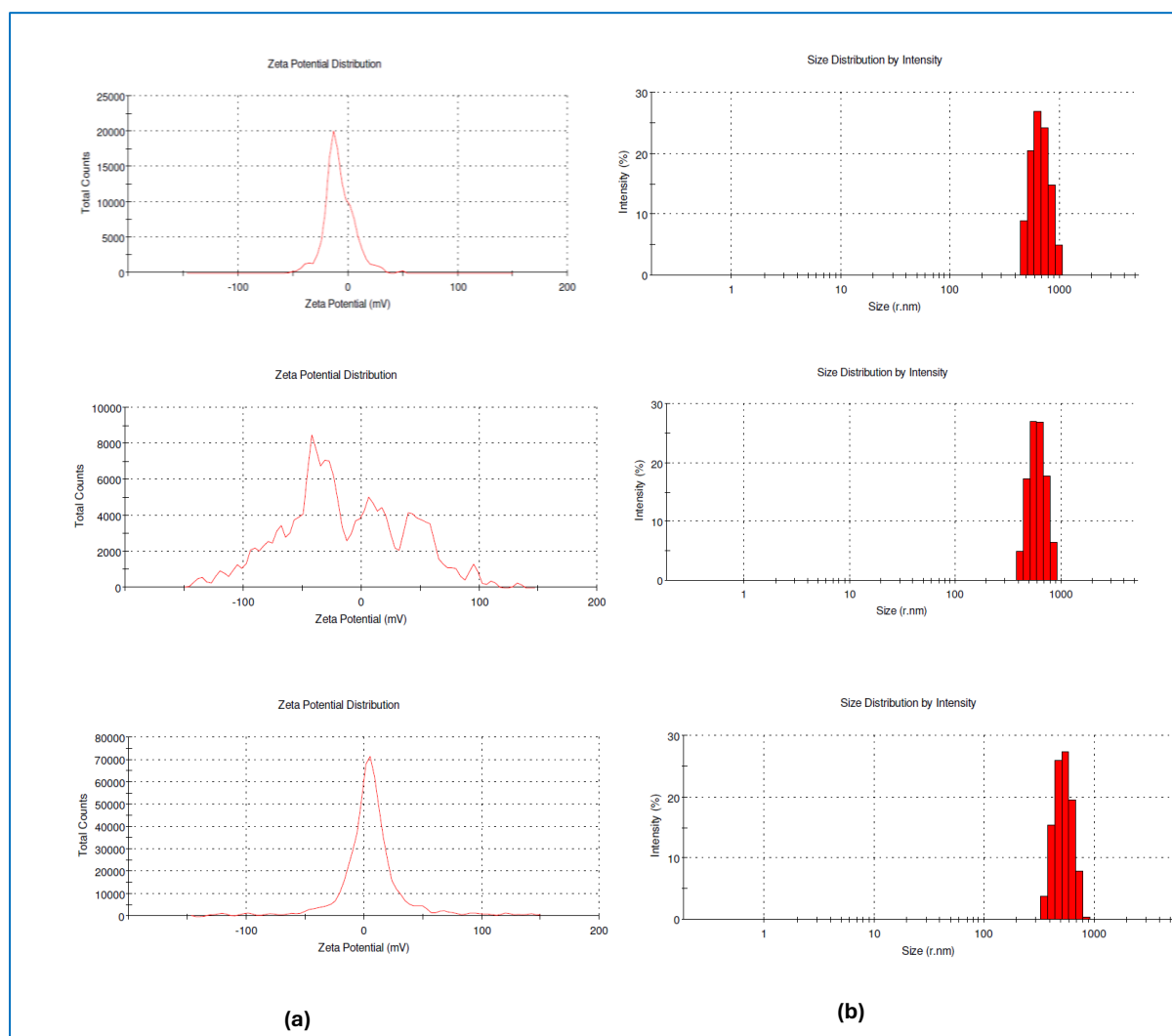


Figure 8 (a) Zeta potential for 0.5mg sample, 0.7 mg sample, 1 mg sample; (b) Particle size analyser for 0.5 mg, 0.7 mg, 1 mg.

While comparing the zeta potential results of all three compositions, as shown in **Figure 8(a)**, it is evident that the surface residual charge for each composition ranges from -20 mV to +40 mV. The results obtained for the 0.5mg sample: the mean surface charge, or zeta potential, is -8.05 mV; similarly, for the 0.7sample the average zeta potential was recorded as -8.05 mV; the 0.7mg formulation likewise exhibited an average surface charge of -19.3 mV, while the 1mg formulation showed a mean zeta potential value of 2.90mV. These relatively low zeta-potential magnitudes

indicate limited electrostatic stabilisation, suggesting that steric and/or polymer-mediated stabilisation may also contribute to dispersion stability, with no visible agglomeration observed. The negative zeta potentials observed for 0.5 mg and 0.7 mg samples are expected due to the presence of ionised carboxylate groups from sodium alginate, which shows a net negative surface charge. Similar results were obtained from functionalised mesoporous silica nanocarriers loaded with etoposide, as well as a niosome-based etoposide delivery platform developed for treating various cancer types[15,33]. From the results of the particle size analyser, as shown in **Figure 8 (b)**, it was found that for all the compositions, the particle sizes are in the range 600nm-800nm; hence, we can say that after the encapsulation of the drug, the size of the complex does not change. The results obtained for the 0.5mg sample were that the average diameter is 626.5nm; similarly, for the 0.7mg sample, the average diameter is 777.5nm, and for the 1mg sample, the average diameter is 690.9nm. Similar results were obtained from etoposide-encapsulated functionalized mesoporous silica nanoparticles and a novel niosomal drug delivery system of etoposide for targeting different types of cancer[15,33].

Encapsulation Efficiency

The calibration curve was plotted for the known concentration of the drug sample using the absorbance value at 284 nm. The absorbance at 284 nm was found for the different compositions of drug-encapsulated nano-carrier of 0.7 w/v, and the encapsulation efficiency was calculated[2,44].

Table 1 Encapsulation efficiency for different ratios of drug-encapsulated nano-carrier

Sample (composition of drug-encapsulated nano-carrier of 0.7 w/v)	Encapsulation Efficiency (%)
0.7 mg drug	46
0.5 mg drug	56.8
1 mg drug	26.48

The drug-to-polymer ratio plays a major role in encapsulation efficiency. As shown in **Table 1**, we can observe that as the drug concentration increases, the encapsulation efficiency decreases, which could mean limited availability of polymer binding sites; when the drug concentration exceeds the polymer's encapsulation capacity, the excess drug remains unbound and is lost to the surrounding medium. Despite the constant ratio of acrylamide and ethylene glycol dimethacrylate, the encapsulation efficiency decreases with the increase in the drug concentration, suggesting that the polymer network reached saturation at lower concentrations [45]. A comparable trend has been documented for 5-fluorouracil formulations, in which encapsulation efficiency declined as drug concentration rose in preparations involving PEL, R-20, and R-5 carriers[46]. Likewise, in Alginate/poly-L-arginine/chitosan ternary complex microcapsules, increasing the drug loading within the gel beads correspondingly increased the fraction of drug lost to the surrounding liquid medium during the encapsulation process[47]

Kinetic Study

The drug release patterns of etoposide-loaded nanoparticles were analysed using zero-order, first-order, Higuchi model, and Korsmeyer-Peppas model, demonstrating sustained release qualities [2]. Furthermore, the release rate of etoposide from its carrier is crucial for the biological effects of the drug[48]. Taken together, these findings indicate that etoposide's sustained-release kinetics are complex and influenced by carrier formulation and environmental conditions.

By integrating and synthesising the research findings discussed in this literature review, this review underscores the importance of advanced analytical techniques and the dissemination of kinetic models. Ultimately, a deeper understanding of sustained release kinetics will contribute to the design and optimisation of drug-loaded PECs for improved therapeutic efficacy and patient compliance. Zero-order kinetics of several samples of the in vitro release profile of caseinate-sodium alginate nanocarriers loaded with etoposide, as shown in Figure 1S.

Table 2 In vitro drug release for zero-order kinetics of caseinate-sodium alginate nanocarriers loaded with etoposide

Composition	R^2	k (rate constant)
0.5mg @25°C - supernatant	0.8358	0.1341
0.5mg @25°C - powder	0.9811	0.2402
0.5mg @37°C - supernatant	0.9795	0.5688
0.5mg @37°C - powder	0.9714	0.2069
0.7mg @25°C - supernatant	0.8708	0.1258
0.7mg @25°C - powder	0.9922	0.1644
0.7mg @37°C - supernatant	0.9765	0.2959
0.7mg @37°C - powder	0.9134	0.6707
1 mg @25°C - supernatant	0.85	0.1091
1 mg @25°C - powder	0.9847	0.1939
1mg @37°C - supernatant	0.9765	0.3437
1mg @37°C - powder	0.8537	0.4356

From the zero-order plots for both supernatant and powder, as shown in **Table 2**, it can be observed that the R^2 values are in the range 0.80-0.99; it can be concluded that the plots are fit, and the rate constant is independent of concentration. And from the R^2 values, it is deduced that 1 mg @25°C of powder showed the best fit with the value of 0.9847. A maximum release of 33% of the drug was seen for 0.5mg of the drug encapsulated in a nanocarrier.

First-order kinetics of several samples of the in vitro release profile of caseinate-sodium alginate nanocarriers loaded with etoposide are shown in Figure 2S.

Table 3 In vitro drug release for first-order kinetics of caseinate-sodium alginate nanocarriers loaded with etoposide.

Composition	R^2	k (rate constant)
0.5mg @25°C - supernatant	0.4857	0.0185
0.5mg @25°C - powder	0.8257	0.0243
0.5mg @37°C - supernatant	0.7746	0.0244
0.5mg @37°C - powder	0.5281	0.0161
0.7mg @25°C - supernatant	0.5518	0.0204
0.7mg @25°C - powder	0.5686	0.028
0.7mg @37°C - supernatant	0.69	0.0251
0.7mg @37°C - powder	0.6276	0.0213
1 mg @25°C - supernatant	0.5278	0.019
1 mg @25°C - powder	0.8769	0.0213
1mg @37°C - supernatant	0.666	0.0255
1mg @37°C - powder	0.5642	0.0188

While comparing the values of R^2 for first-order models, as shown in **Table 3**, the values are in the range 0.5-0.7, which is comparatively very low, from which it can be inferred that the drug release does not adhere to the reaction's initial order.

Higuchi kinetic release model of several samples of the in vitro release profile of caseinate-sodium alginate nanocarriers loaded with etoposide is shown in Figure 3S.

Table 4 In vitro drug release for Higuchi release model kinetics of Caseinate-sodium alginate nanocarriers loaded with etoposide.

Composition	R^2	k (rate constant)
0.5mg @25°C - supernatant	0.9794	1.0707
0.5mg @25°C - powder	0.915	1.7141
0.5mg @37°C - supernatant	0.9528	1.4957
0.5mg @37°C - powder	0.985	4.4593
0.7mg @25°C - supernatant	0.9789	0.984
0.7mg @25°C - powder	0.9757	0.0165
0.7mg @37°C - supernatant	0.9475	2.1365
0.7mg @37°C - powder	0.8689	0.1869
1 mg @25°C - supernatant	0.977	0.9772
1 mg @25°C - powder	0.9329	1.3818
1mg @37°C - supernatant	0.957	2.4873
1mg @37°C - powder	0.9666	3.4145

Korsmeyer-Peppas kinetic release model of several samples of the in vitro release profile of caseinate-sodium alginate nanocarriers loaded with etoposide, as shown in Figure 4S.

Table 5 In vitro drug release kinetics for the Korsmeyer-Peppas model of caseinate-sodium alginate nanocarriers loaded with etoposide.

Composition	R^2	k	n
0.5mg @25°C - supernatant	0.8412	0.0868	0.6504
0.5mg @25°C - powder	0.9501	0.2209	0.7486
0.5mg @37°C - supernatant	0.8738	0.2853	0.738
0.5mg @37°C - powder	0.8433	0.6946	0.5374
0.7mg @25°C - supernatant	0.9122	0.2287	0.7007
0.7mg @25°C - powder	0.9382	0.5987	0.8895

0.7mg @37°C - supernatant	0.946	0.1086	0.7778
0.7mg @37°C - powder	0.9613	0.3491	0.7679
1 mg @25°C - supernatant	0.8938	0.2236	0.6691
1 mg @25°C - powder	0.9191	0.1495	0.6185
1mg @37°C - supernatant	0.9281	0.065	0.7971
1mg @37°C - powder	0.9067	0.3931	0.6497

From the plots for the Korsmeyer-Pappas model, as shown in **Table 5** it was observed that the n values are in the range $0.45 < n < 0.89$, from which the drug release follows non-Fickian diffusion. So, it can be concluded that pure diffusion is not occurring; as the buffer gets in contact with the drug-nanocarrier complex, the hydrophilic nanocarrier absorbs the solution and swells, and after that, the drug is released [37,41,49,50].

From all the above-mentioned plots, from the R^2 values, it can be concluded that zero-order is the best fit for the drug release studies, which indicates that for a maximum of 48 hours, the overall drug release amount is in the range 5 -16 % for supernatant and the range of 5- 35% for powder. So, with reference to the literature survey, it can be concluded that 99% of the drug will be released in 2 weeks. Hence, concluded that the drug is released in a sustained manner [33,50].

Rate of Drug Release

Rate of the reaction:

$$r_A = -k \frac{dC_A}{dt}$$

Where r_A is the rate of the reaction, dC_A is the difference in concentration, and dt is the difference in time.

For the rate of drug release as shown in Figure 5S to 7S (Supplementary), all the compositions for both temperatures, 25°C and 37°C, follow sustained release from the 0th hour to the 48th hour; mostly between the 0th and 10th hour. Hence, sustained release is being achieved.

Binding Affinity Analysis

The docking results demonstrated energetically favourable and spontaneous interactions between etoposide and all three receptor models (**Table 6**). Among the receptors, α -casein displayed the highest binding affinity ($-7.0 \text{ kcal mol}^{-1}$), followed by β -casein ($-6.1 \text{ kcal mol}^{-1}$). The sodium alginate oligomer showed a substantially lower affinity ($-3.661 \text{ kcal mol}^{-1}$). The considerably stronger affinity of the casein proteins is consistent with their amphiphilic character and hydrophobic domain architecture, which can accommodate poorly water-soluble molecules such as etoposide through hydrophobic contacts and directed hydrogen bonding. The comparatively weak interaction predicted for alginate supports its functional role as a structural scaffold and diffusion regulator within the PEC rather than as a primary drug-binding component.

Table 6 AutoDock Vina binding affinities and PLIP-confirmed interactions for etoposide docked into α -casein, β -casein, and sodium alginate oligomer.

Complex	Binding Affinity (kcal mol ⁻¹)	Confirmed Hydrogen-Bond Residues (PLIP)	Key Hydrophobic Residues (PLIP)
α -CN-Etoposide	-7.0	Glu45 (sidechain OH), Val46 (backbone NH), Phe47 (backbone NH/C=O) - 4 H-bonds confirmed by PLIP	Phe43 (3.67 Å)
β -CN-Etoposide	-6.1	Gln55 (side chain C=O) - 1 H-bond confirmed by PLIP	Phe48 (3.54-3.99 Å; 4 contacts)
NaAlg-Etoposide	-3.661	Not determined (polysaccharide; qualitative only)	Not determined

Protein-Ligand Interaction Analysis

Non-covalent interactions in the docked complexes were characterised using PLIP v3.0.0 (Schake, Bolz et al., 2025). The results are summarised in **Table 7**. In the α -casein-etoposide complex, PLIP identified one hydrophobic contact and four hydrogen bonds. The hydrophobic contact involved Phe43, whose aromatic ring engaged a carbon centre of etoposide at a distance of 3.67 Å, consistent with a C-H/ π or hydrophobic van der Waals interaction. Among the four hydrogen bonds, two involved backbone atoms of Val46 and Phe47 (protein as donor; D $\cdots$ A distances of 3.19 and 3.75 Å, donor-hydrogen $\cdots$ acceptor angles of 133.9° and 148.6°, respectively), one involved the Glu45 sidechain carboxylate acting as donor (D $\cdots$ A = 4.03 Å, angle = 112.6°), and one represented the etoposide hydroxyl group (O3) donating to the backbone carbonyl of Phe47 (D $\cdots$ A = 2.80 Å, angle = 110.3°). In all cases, the hydroxyl group at O3 of etoposide constituted the hydrogen-bond acceptor or donor, consistent with the known capacity of etoposide's phenolic hydroxyl to engage in directional electrostatic interactions.

In the β -casein-etoposide complex, PLIP identified four hydrophobic contacts, all involving Phe48 (distances 3.54-3.99 Å), and a single strong hydrogen bond between the etoposide O3 hydroxyl (donor) and the side-chain carbonyl oxygen of Gln55 (acceptor; D $\cdots$ A = 2.76 Å, angle = 157.5°). The near-linear geometry of this interaction (157.5°) is indicative of a particularly strong and directional hydrogen bond. The cluster of four hydrophobic contacts with Phe48 suggests that the phenylalanine side chain adopts a geometry that wraps around the methylenedioxy or aromatic ring system of etoposide, providing a hydrophobic cradle that stabilises the binding pose.

Collectively, these findings demonstrate that etoposide binding within the casein matrix is governed by a complementary array of hydrophobic interactions and directed hydrogen bonds, with the phenolic hydroxyl group at O3 serving as the primary pharmacophoric element mediating protein-drug recognition. The greater number of PLIP-identified contacts in the α -

casein complex, together with its lower predicted binding energy, supports the conclusion that α -casein is the dominant drug-binding subunit within the caseinate component of the PEC.

Table 7 PLIP-validated hydrogen-bond interactions between etoposide and casein residues ($D\cdots A \leq 4.1$ Å; $D-H\cdots A$ angle $\geq 90^\circ$; PLIP v3.0.0).

Complex	Residue	Donor Atom	Acceptor Atom	$D\cdots A$ (Å)	$D-H\cdots A$ ($^\circ$)
α -CN	Glu45	O ϵ (sidechain)	O3-etoposide (hydroxyl)	4.03	112.6 $^\circ$
α -CN	Val46	N (backbone NH)	O3-etoposide (hydroxyl)	3.19	133.9 $^\circ$
α -CN	Phe47 (i)	O3-etoposide (hydroxyl)	O2-Phe47 (backbone C=O)	2.80	110.3 $^\circ$
α -CN	Phe47 (ii)	N (backbone NH)	O3-etoposide (hydroxyl)	3.75	148.6 $^\circ$
β -CN	Gln55	O3-etoposide (hydroxyl)	O ϵ -Gln55 (sidechain C=O)	2.76	157.5 $^\circ$

Correlation between Computational and Experimental Findings

The computational investigations provided molecular-level mechanistic insight that directly complements and contextualises the experimental characterisation of the etoposide-loaded caseinate-alginate PEC nanocarrier. The convergence of results across four independent analytical approaches - molecular docking, PLIP interaction profiling, FTIR/XRD/SEM characterisation, and in vitro drug-release kinetics - provides a coherent and mutually reinforcing mechanistic picture.

Molecular docking and PLIP analysis demonstrated that etoposide forms multiple favourable non-covalent interactions with both α -casein (-7.0 kcal mol $^{-1}$; four PLIP-confirmed hydrogen bonds and one hydrophobic contact) and β -casein (-6.1 kcal mol $^{-1}$; one strong hydrogen bond and four hydrophobic contacts), with the phenolic hydroxyl at O3 of etoposide identified as the primary pharmacophoric atom in both complexes. These molecular-level interactions are directly reflected in the FTIR spectra, where shifts in the hydroxyl stretching frequency (~ 3400 cm $^{-1}$) and the emergence of an amide I carbonyl band in the drug-loaded PEC are consistent with hydrogen-bond formation between the etoposide hydroxyl and the casein backbone amide and side-chain carbonyl groups identified by PLIP (Val46 N-H, Phe47 N-H/C=O, Gln55 C=O).

The SEM images of the drug-loaded PEC showed closure of the open pore architecture observed in the blank nanocarrier, consistent with etoposide occupying the hydrophobic cavities predicted by docking within the α -casein and β -casein binding regions. The reduction in crystallinity observed by XRD upon drug encapsulation further supports molecular-level dispersion of etoposide within the protein matrix, as expected from non-covalent, non-specific blind docking rather than co-crystallisation. The DLS-measured particle diameters (626.5-777.5 nm) and negative zeta potentials (-8.05 to -19.3 mV for 0.5 and 0.7 mg formulations) indicate limited

electrostatic stabilisation of the drug-loaded PEC (Section 3.1), with the anionic alginate shell likely contributing a partial, non-dominant stabilising charge alongside steric/polymer-mediated effects.

The sustained-release kinetics observed *in vitro* - best described by the zero-order model (R^2 up to 0.9922) and characterised by non-Fickian (anomalous) diffusion in the Korsmeyer-Peppas analysis ($n = 0.54-0.89$) are mechanistically coherent with the docking and PLIP findings. The multiple hydrogen bonds and hydrophobic contacts between etoposide and the casein proteins reduce the drug's chemical potential gradient, retarding its diffusion from the polymer matrix. The swelling and progressive erosion of the hydrophilic alginate network, reflected in the Korsmeyer-Peppas n values exceeding 0.45, provide the additional diffusional resistance responsible for the observed anomalous transport. Together, these findings demonstrate that the sustained-release behaviour of the developed nanocarrier results from the synergistic contributions of molecular drug-protein stabilisation by caseins and macroscopic diffusion control by the biodegradable alginate-caseinate polymeric framework, a conclusion that is experimentally validated and computationally rationalised.

Conclusion

By varying the composition of caseinate-sodium alginate, the optimum polymer concentration for the formation of the polyelectrolyte complex (PEC) was identified as 0.75% (w/v). Three etoposide-loaded nanocarrier formulations containing 0.5 mg, 0.7 mg, and 1.0 mg of drug were successfully prepared using the optimised PEC composition. The encapsulation efficiencies were 56.8%, 46.0%, and 26.48%, respectively, indicating that the 0.5 mg formulation exhibited the highest drug-loading performance. The release kinetics of the drug were assessed against four established mathematical models- zero-order, first-order, Higuchi, and Korsmeyer-Peppas among which the zero-order model exhibited the closest agreement with the experimental data, thereby supporting a sustained and controlled pattern of drug release. The Korsmeyer-Peppas release exponent ($n = 0.60-0.89$) indicated a non-Fickian diffusion mechanism, demonstrating that both diffusion and polymer swelling/erosion contributed to drug release. The release profile showed sustained drug release over the first 48 h; based on comparable literature systems, near-complete release would be anticipated on a multi-week timescale, though this was not directly measured here. Furthermore, molecular docking and protein-ligand interaction analyses complemented the experimental findings by revealing favourable hydrogen-bonding and hydrophobic interactions between etoposide and the casein proteins, providing a molecular basis for the observed encapsulation efficiency and sustained-release behaviour. The consistency between the computational predictions and the experimental characterisation supports the hypothesis that the prolonged release results from a combination of drug-protein interactions and the diffusion-regulating properties of the alginate-caseinate polymeric network, a hypothesis that independent computational validation and molecular dynamics simulation could test further. Overall, the developed etoposide-loaded caseinate-sodium alginate nanocarrier demonstrated promising potential as a sustained drug delivery system, and future *in vivo* studies will be undertaken to evaluate its therapeutic efficacy and safety

Ethical Approval and consent to participate

Not relevant

Consent to publish

Not relevant

Availability of Data and Materials

All data supporting the findings of this study are available within the paper and its Supplementary Information.

Competing Interests

The authors of this study affirm that they have no known conflicting financial interests or personal ties that might have influenced the work presented.

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Declaration of Interests Statement

Regarding the content of this work, the authors have no conflicts of interest to disclose.

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