Research Article

Development Of Protease-Encapsulated Microcapsules For Improved Digestion In Pancreatic Insufficiency

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Abstract

Exocrine pancreatic insufficiency remains a major therapeutic challenge because orally administered enzymes undergo rapid degradation in the acidic gastric environment before reaching the intestine. The present study developed a scalable solid-in-oil (s/o) spray-drying technique for encapsulating protease within semi-permeable ethylcellulose (EC) microcapsules intended for targeted intestinal enzyme delivery. Pre-formulation studies identified a critical thermal deactivation threshold above 55°C, emphasizing the need for effective thermal stabilization during spray drying. Trehalose and sucrose were investigated as thermoprotective excipients, with 1.5 M trehalose demonstrating the highest protective effect by preserving 95.7% of the native enzyme activity following thermal exposure at 75°C. Circular dichroism spectroscopy confirmed the preservation of the native secondary structure in the stabilized formulations. The optimized microcapsules exhibited a production yield of 71.8%, an encapsulation efficiency of 85.3%, a median particle size (dv50) of 5.19 µm, and a narrow particle size distribution (span = 1.80), indicating excellent manufacturing reproducibility and particle uniformity. In vitro release studies demonstrated a biphasic release profile characterized by an initial burst release followed by sustained diffusion through the ethylcellulose membrane. Mathematical modeling revealed excellent agreement with the Higuchi model (R^2 = 0.9974), confirming that solute transport occurred predominantly through Fickian diffusion across the hydrated polymer matrix. The semi-permeable membrane effectively retained the encapsulated enzyme while allowing diffusion of low-molecular-weight substrates and reaction products. Overall, the developed solid-in-oil spray-dried ethylcellulose microcapsules represent a promising oral enzyme delivery platform with potential applications in enzyme replacement therapy for exocrine pancreatic insufficiency, phenylketonuria, and related metabolic disorders.

Keywords: Microencapsulation; Exocrine pancreatic insufficiency; Spray drying; Ethylcellulose; Protease; Trehalose; Oral enzyme delivery.

1. Introduction

Exocrine pancreatic insufficiency occurs when the pancreas fails to secrete enough digestive enzymes to maintain normal digestion (Keller & Layer, 2005). Without these essential enzymes, patients cannot properly break down fats, proteins, and carbohydrates. This inevitably leads to malnutrition, weight loss, and severe digestive discomfort. To manage the condition, patients must rely on exogenous proteases and other enzyme supplements to fulfill their physiological needs and restore normal nutrient absorption (Struyvenberg, Martin, & Freedman, 2017). Pancreatic enzyme replacement therapy is the standard clinical approach, but it comes with a major physiological limitation. Unprotected proteases are highly unstable in the harsh acidic environment of the stomach, where the pH drops to around 3.0. If these enzymes are not physically shielded, they denature and degrade rapidly before they can do their job (Fieker, Philpott, & Armand, 2011). For the treatment to be truly effective, the proteases must survive gastric transit and release precisely in the intestinal tract where the pH is closer to 7.5. We can successfully protect the protease by using a microencapsulation strategy. Ethylcellulose is an excellent candidate for the polymer coating because it holds a generally recognized as safe status and remains entirely non-digestible in the human gastrointestinal tract (Rekhi & Jambhekar, 1995). Ethylcellulose also forms a highly functional semi-permeable membrane. This unique property allows small substrate molecules to enter the core and digested products to exit, all while keeping the larger protease enzyme securely trapped inside without leakage (Ye, Kim, & Park, 2010). Spray drying is a fast and scalable way to manufacture these microcapsules, but the process exposes the sensitive protease to severely elevated temperatures. This thermal stress causes the proteins to unfold, lose their secondary structure, and permanently lose their catalytic activity (Arakawa, Prestrelski, Kenney, & Carpenter, 2001). Heat denaturation during the drying phase remains the biggest practical hurdle in producing functionally active enzyme microcapsules (Chang & Pikal, 2009). Our study aimed to address these specific manufacturing and delivery challenges. First, the extent of

thermal stress on protease activity was systematically evaluated. Second, the thermoprotective effects of sucrose and trehalose, which are known to stabilize protein structures under significant heat stress, were assessed (Kaushik & Bhat, 2003). Finally, ethylcellulose microcapsules were formulated using a solid-in-oil dispersion method to minimize the initial burst release and prevent leakage of the encapsulated enzyme into the surrounding environment (Morita, Sakamura, Horikiri, Suzuki, & Yoshino, 2000).

2. Materials and Methods

2.1 Materials

The active pharmaceutical ingredient (API), protease, was procured as an initial sample from HiMedia Laboratories (Mumbai, India). Ethylcellulose (20 cps) and all analytical-grade solvents, including dichloromethane and ethanol, were purchased from Sigma-Aldrich (Bangalore, India). Furthermore, thermoprotectants such as high-purity trehalose and sucrose, along with the specific substrates required for the protease kinetic assays, were sourced commercially to maintain strict analytical consistency throughout the formulation process.

2.2. Pre-formulation Studies:

2.2.1. Simulation of Spray Dryer Conditions

To understand how the spray drying process affects the protease, the thermal environment of the spray dryer was simulated by exposing aqueous protease solutions to temperatures ranging from 45°C to 75°C. Heating the enzyme under controlled aqueous conditions allowed the thermal effects to be evaluated independently of other process-related factors, such as shear stress and air-liquid interface interactions. This approach provided a reliable assessment of the enzyme's intrinsic thermal stability prior to spray drying (Tzannis & Prestrelski, 1999).

2.2.2. Enzyme Kinetic Assays

Following thermal exposure, the residual catalytic activity of the protease was determined using enzyme kinetic assays with appropriate substrates. Changes in the maximum reaction velocity (Vmax) and the Michaelis constant (Km) were evaluated to determine the effect of thermal stress on enzyme activity and substrate binding affinity. Quantitative analysis was performed using High-Performance Liquid Chromatography (HPLC), providing an accurate measurement of the remaining active enzyme following heat treatment (Frokjaer & Otzen, 2005).

2.2.3. Structural Characterization via Circular Dichroism (CD)

Structural integrity of the thermally stressed protease was assessed using circular dichroism (CD) spectroscopy. Changes in the secondary structure were monitored by measuring molar ellipticity at the far-UV wavelengths of 190, 208, and 222 nm, which are characteristic of α-helical and β-sheet conformations. The resulting spectra were used to evaluate thermal-induced unfolding and conformational alterations of the protein structure (Kelly, Jess, & Price, 2005).

2.3 Evaluation of Thermoprotectants

To prevent the protease from breaking down during manufacturing, we test different concentrations of excipients like sucrose and trehalose, typically ranging from 0.5 M to 1.5 M. These sugars are evaluated for their ability to maintain the secondary structure and catalytic activity of the enzyme when exposed to maximum thermal stress around 75°C. Research confirms that trehalose forms a protective glassy matrix and replaces water molecules through hydrogen bonding, which stabilizes proteins under severe heat (Kaushik & Bhat, 2003). This specific stabilization mechanism is essential to keep the protease active and prevent permanent unfolding (Allison, Chang, Randolph, & Carpenter, 1999).

2.4 Formulation of Protease Microcapsules

Creating the microcapsules involves a two-step solid-in-oil dispersion process. First, we co-spray dry the protease with our optimized sugar stabilizer and a biocompatible buffer to create micronized solid protein particles. Keeping the protein in a dry, solid state prevents the enzyme from interacting negatively with organic solvents during the next phase of manufacturing (Ye, Kim, & Park, 2010). In the second step, we disperse these solid protein particles into a 6% weight-by-volume ethylcellulose solution dissolved in a dichloromethane and ethanol co-solvent. We use ultrasonication to ensure an even and consistent dispersion before running the mixture through a secondary spray drying process to form the final solid microcapsules.

2.5 Characterization of Microcapsules

2.5.1 Production Yield & Encapsulation Efficiency (EE)

Once formulated, we characterize the microcapsules by measuring their overall production yield and encapsulation efficiency. We achieve this by completely dissolving the outer ethylcellulose coat in a solvent and precipitating the trapped protein, which allows us to accurately quantify exactly how much active protease successfully made it into the core of the capsules.

2.5.2 Particle Size Analysis

We also analyzed the particle size distribution using laser light scattering. This technique provided the volume-based particle size parameters, including dv10, dv50, dv90, and the span value. The calculated span was used to evaluate particle size uniformity, confirming that the microcapsules possessed a narrow size distribution and were physically suitable for oral delivery applications.

2.6 In Vitro Release and Porosity Validation

Finally, we validate the porosity of the microcapsules by incubating them in simulated intestinal fluid. During this step, we quantify how much protein leaks out and how easily the substrate and product diffuse across the polymer membrane. To define the exact mechanism of this diffusion, we fit the release data into established mathematical models to evaluate the release profiles (Siepmann & Siepmann, 2008). We compare zero-order and first-order kinetics alongside the Higuchi and Korsmeyer-Peppas models. The Higuchi model specifically helps us confirm if the release is driven entirely by diffusion through the porous matrix (Higuchi, 1963), while the Korsmeyer-Peppas model clarifies the specific physical type of diffusion taking place (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983).

3. Results and Discussion

3.1. Impact of Thermal Stress on Protease Activity and Structure

3.1.1. Determination of the Thermal Deactivation Threshold

To systematically evaluate the functional stability of the exogenous protease under processing conditions simulating the thermal environment of a spray dryer, the liquid enzyme was exposed to controlled temperatures ranging from 45°C to 75°C for 15 minutes. Residual enzyme activity was subsequently assessed by determining the maximum reaction velocity (Vmax) and substrate binding affinity (Km). The corresponding kinetic parameters are presented in Table 1. The protease remained functionally stable following transient exposure up to 55°C, retaining approximately 98.4% of its initial catalytic efficiency (Vmax/Km). However, a distinct thermal deactivation threshold was observed between 55°C and 65°C. At 65°C, the enzyme exhibited a significant ($P < 0.05$) reduction in catalytic activity, accompanied by a marked increase in Km, indicating reduced substrate affinity due to thermal disruption of the active site. Exposure to 75°C resulted in an almost complete loss of enzymatic activity, with Vmax decreasing to only 0.02 ± 0.00 $\mu M \cdot min^{-1} \cdot \mu g^{-1}$ protein and Km increasing to 1.42 ± 0.11 mM. These findings demonstrate that irreversible thermal denaturation occurs beyond 65°C, causing severe impairment of catalytic function through structural disruption of the enzyme.

Table 1. Protease Kinetic Parameters (Vmax and Km) Following 15-Minute Exposure to Variable Thermal Stress Conditions

Thermal Treatment Group	Vmax ($\mu M \cdot min^{-1} \cdot \mu g^{-1}$ protein)	Km (mM)	Relative Residual Activity (%)
Room Temperature (Control)	0.71 ± 0.03	0.25 ± 0.01	100.0
45°C Exposed	0.71 ± 0.01	0.25 ± 0.02	100.0
55°C Exposed	0.69 ± 0.02	0.26 ± 0.01	93.7
65°C Exposed	0.43 ± 0.03*	0.39 ± 0.03*	38.9
75°C Exposed	0.02 ± 0.00**	1.42 ± 0.11**	0.5

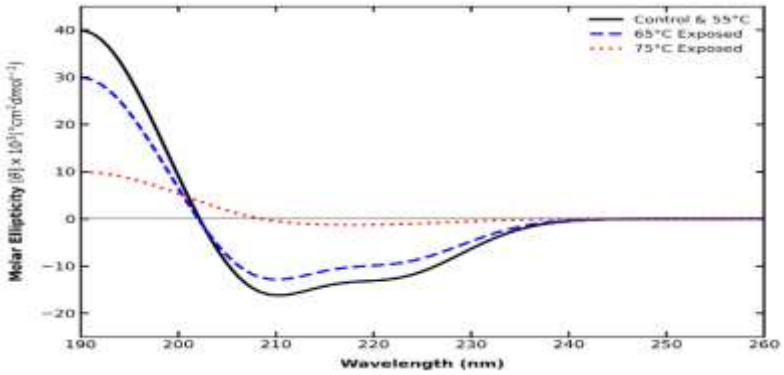
Data are expressed as Mean ± SD (n = 4). Statistical significance compared with the room temperature control group was determined using one-way ANOVA followed by Tukey's post hoc test (*$P < 0.05$; **$P < 0.001$).

3.1.2. Biophysical Correlation with Secondary Structure Perturbations

To investigate the molecular basis of the observed loss of catalytic activity, the thermal-induced functional changes were correlated with alterations in protein secondary structure using far-UV circular dichroism (CD) spectroscopy. CD spectra recorded over the wavelength range of 190–260 nm demonstrated distinct structural transitions corresponding to the thermal conditions evaluated in the enzyme kinetic studies. As illustrated in Figure 1, the untreated protease exhibited a characteristic CD spectrum with a strong positive peak near 190 nm and two prominent negative minima at 208 nm and 222 nm, confirming a well-organized α-helical secondary structure. Protease samples exposed to temperatures up to 55°C displayed nearly identical spectral profiles to the control, indicating that the native conformation remained largely preserved. These observations are consistent with the

enzyme kinetic data, confirming that thermal exposure below 55°C does not produce irreversible structural damage and that any conformational fluctuations occurring within this temperature range are reversible upon cooling.

Figure 1. Far-UV Circular Dichroism (CD) spectra of aqueous protease solutions recorded at 20°C following 15-minute exposure to different thermal treatment conditions.



Quantitative changes in molar ellipticity (θ) at the characteristic far-UV wavelengths are presented in Table 2. Crossing the thermal threshold from 55°C to 65°C produced a significant 19.3% reduction in molar ellipticity at 190 nm, indicating the onset of irreversible structural destabilization. Further exposure to 75°C resulted in a pronounced collapse of the native secondary structure, evidenced by a 25.6% decrease at 190 nm, a 10.3% reduction at 208 nm, and a 19.1% decline at 222 nm. These spectral alterations demonstrate progressive thermal unfolding of the protease with increasing temperature.

Table 2. Percent Changes in Protease Molar Ellipticity (θ) at Characteristic Far-UV Wavelengths Following Thermal Exposure

Wavelength (λ, nm)	% Change at 55°C	% Change at 65°C	% Change at 75°C
190	-0.8 ± 0.5	-19.3 ± 0.6**	-25.6 ± 1.1**
208	+1.0 ± 1.2	-4.4 ± 1.0	-10.3 ± 1.4*
222	-2.4 ± 1.0	-2.1 ± 1.0	-19.1 ± 0.8**

Percent structural changes are calculated relative to the baseline molar ellipticity values recorded at room temperature. Data are expressed as Mean ± SD (n = 3). Statistical significance was determined relative to the native control (*P < 0.05; **P < 0.001).
The marked reduction in molar ellipticity at elevated temperatures indicates extensive unfolding of the ordered α-helical and β-sheet structures into disordered random-coil conformations. Thermal energy disrupts the hydrogen-bonding network responsible for maintaining the native protein architecture, leading to exposure of hydrophobic amino acid residues that are normally buried within the protein core. Contact of these exposed hydrophobic regions with the surrounding aqueous environment promotes intermolecular association and irreversible protein aggregation. Such aggregation permanently distorts the active-site geometry, resulting in diminished substrate binding and the substantial loss of catalytic activity observed above 55°C. Collectively, the kinetic and structural analyses demonstrate that the protease possesses a well-defined thermal stability limit and highlight the importance of incorporating suitable stabilizing excipients during the aqueous phase of microencapsulation to preserve enzyme conformation and biological activity throughout the spray-drying process.

3.2. Efficacy of Sugar Excipients in Thermal Stabilization

3.2.1. Concentration-Dependent Protective Effect of Trehalose and Sucrose

To overcome the thermal deactivation of protease observed at temperatures above 55°C, the protective effects of the non-reducing disaccharides trehalose and sucrose were evaluated at concentrations of 0.5 M, 1.0 M, and 1.5 M during the aqueous phase preceding spray drying. Following thermal exposure at 75°C, residual enzymatic activity was determined by measuring the recovered Vmax relative to the untreated native enzyme. The experimental findings are presented in Table 3. Both disaccharides exhibited a clear concentration-dependent protective effect against thermal denaturation. At the lowest concentration (0.5 M), only partial protection was achieved, with trehalose preserving 43.6 ± 2.8% of the native enzyme activity compared with 35.2 ± 1.9% for sucrose. Increasing the concentration to 1.0 M substantially improved thermal stability, resulting in activity recoveries of 84.2 ± 3.1% and 78.1 ± 2.9% for trehalose and sucrose, respectively. The highest concentration (1.5 M) provided maximum stabilization, with trehalose preserving 95.7 ± 4.2% of the original catalytic activity, while sucrose retained 88.7 ± 3.5%.

These findings demonstrate that increasing the concentration of both excipients markedly enhanced resistance to thermal denaturation. Trehalose consistently outperformed sucrose across all concentrations, indicating superior preservation of the enzyme during severe thermal stress. The ability of 1.5 M trehalose to maintain almost complete catalytic activity suggests that it effectively protected the native enzyme conformation and prevented irreversible structural damage throughout the simulated spray-drying process.

Table 3. Residual Protease Catalytic Activity Following Thermal Exposure at 75°C as a Function of Sugar Excipient Type and Concentration

Stabilizer Matrix Formulation	Molar Concentration	Absolute Residual Vmax ($\mu M \cdot min^{-1} \cdot \mu g^{-1}$)	Normalized Activity Recovery (%)
Unstabilized Control	0 M	0.02 ± 0.00	2.8 ± 0.4
Trehalose	0.5 M	0.31 ± 0.02	43.6 ± 2.8
Trehalose	1.0 M	0.60 ± 0.02	84.2 ± 3.1
Trehalose	1.5 M	0.68 ± 0.03	95.7 ± 4.2
Sucrose	0.5 M	0.25 ± 0.01	35.2 ± 1.9
Sucrose	1.0 M	0.55 ± 0.03	78.1 ± 2.9
Sucrose	1.5 M	0.63 ± 0.02	88.7 ± 3.5

Data are expressed as Mean ± SD (n = 4). Increasing the stabilizer concentration from 1.0 M to 1.5 M produced additional improvements in enzyme preservation, identifying 1.5 M as the optimum concentration for thermal protection during spray drying.

3.2.2. Molecular Mechanism of Stabilization

The thermal stabilization of protease by trehalose and sucrose is primarily governed by two complementary physicochemical mechanisms: water replacement and vitrification (glassy matrix formation). During spray drying, rapid evaporation of water removes the hydration shell surrounding the protein, exposing hydrophobic amino acid residues and promoting conformational instability, aggregation, and irreversible denaturation. Both trehalose and sucrose counteract this process by replacing the lost water molecules through extensive hydrogen-bonding interactions between their hydroxyl groups and the polar amino acid residues located on the protein surface. These interactions preserve the native hydrogen-bonding network and maintain the structural integrity of the folded protein during dehydration. Simultaneously, increasing the concentration of the sugars promotes vitrification during solvent removal. As water content decreases, the concentrated sugar solution transforms into a rigid amorphous glassy matrix that physically entraps the protease molecules. This highly viscous matrix restricts molecular mobility and suppresses the large-scale conformational fluctuations responsible for thermal unfolding and aggregation. Consequently, the native secondary and tertiary structures remain largely preserved despite exposure to elevated temperatures. Trehalose exhibited superior stabilization because of its higher glass transition temperature (Tg) and greater ability to establish a stable hydrogen-bonding network around the protein. The higher Tg enables trehalose to maintain its rigid glassy state over a broader temperature range, whereas sucrose undergoes structural relaxation at comparatively lower temperatures. As a result, trehalose provides stronger immobilization of the protein during thermal processing, leading to greater preservation of catalytic activity and structural integrity. These mechanistic observations are consistent with the experimental findings and support the selection of trehalose at 1.5 M as the most effective thermoprotective excipient for spray-drying-based protease microencapsulation. By locking the encapsulated protease within a highly viscous, solid glassy state, trehalose and sucrose drastically reduce structural mobility. This kinetic immobilization physically prevents large-scale molecular unfolding, suppresses the conformational fluctuations that lead to thermal inactivation, and blocks intermolecular collisions that cause protein aggregation. The superior protective efficacy of trehalose over sucrose stems from its distinct conformational rigidity and glycosidic linkage geometry. Trehalose possesses a α,α-1,1-glucosidic bond, which grants higher conformational flexibility to the glycosidic linkage, allowing for optimized spatial alignment with the hydrogen-bonding acceptors on the protein surface. Furthermore, trehalose exhibits a substantially higher glass transition temperature (Tg) compared to sucrose. This elevated Tg ensures that the glassy matrix remains mechanically stable and structurally intact even when exposed to the high residual thermal energy of the spray drying chamber, successfully shielding the protease payload against processing stress.

3.3. Microcapsule Yield, Encapsulation Efficiency, and Morphology

3.3.1. Manufacturing Metrics and Protein Entrapment Validation

The efficiency of the solid-in-oil (s/o) encapsulation technique was evaluated by determining the production yield and encapsulation efficiency (EE) of ethylcellulose microcapsules using bovine serum albumin (BSA) as the model protein. Prior to the

encapsulation study, analytical validation confirmed that ε-polylysine, employed as the core stabilizing component, did not interfere with the spectrophotometric quantification of protein, ensuring accurate determination of encapsulation efficiency. The manufacturing performance of the s/o process is presented in Table 4. The optimized formulation achieved a production yield of 71.8 ± 1.2%, indicating efficient recovery of the microcapsules following spray drying. Encapsulation efficiency reached 85.3 ± 1.0%, demonstrating that the majority of the protein was successfully entrapped within the ethylcellulose matrix. The low coefficients of variation observed for both production yield (1.67%) and encapsulation efficiency (1.17%) indicate excellent batch-to-batch reproducibility and process consistency. The high encapsulation efficiency confirms the effectiveness of the solid-in-oil approach in protecting protein molecules during spray drying. Unlike conventional water-in-oil emulsification methods, where proteins may diffuse into the external phase and be lost during processing, the s/o technique disperses the protein as solid particles directly within the polymer solution. This minimizes protein dissolution and migration during atomization, resulting in improved encapsulation and enhanced process reliability. These findings demonstrate that the s/o spray-drying technique is well suited for encapsulating thermolabile protein therapeutics with high efficiency and reproducibility.

Table 4. Production Yield and Encapsulation Efficiency of Protein-Loaded Ethylcellulose Microcapsules

Manufacturing Metric	Observed Value	Process Variability (CV, %)
Production Yield (%)	71.8 ± 1.2	1.67
Encapsulation Efficiency (%)	85.3 ± 1.0	1.17

Note: Data are expressed as Mean ± SD (n = 3 independent batches).

3.3.2. Microparticle Size Distribution and Morphological Characteristics

The physical characteristics of the spray-dried microcapsules were evaluated using laser diffraction particle size analysis and scanning electron microscopy (SEM). Particle size distribution plays a critical role in determining powder flowability, gastrointestinal transit, encapsulated protein protection, and drug release behavior following oral administration. The particle size distribution results are presented in Table 5. The optimized microcapsules exhibited a median particle diameter (dv50) of 5.19 ± 0.76 µm, while 10% and 90% of the particle population measured below 1.27 ± 0.39 µm and 10.58 ± 0.87 µm, respectively. The calculated span value of 1.80 indicates a narrow and homogeneous particle size distribution. A span value below 2.0 is generally considered indicative of good size uniformity, suggesting that the spray-drying process generated highly consistent microcapsules with minimal polydispersity.

Figure 2. Scanning Electron Micrograph (SEM) of the Optimized microcapsule Formulation.

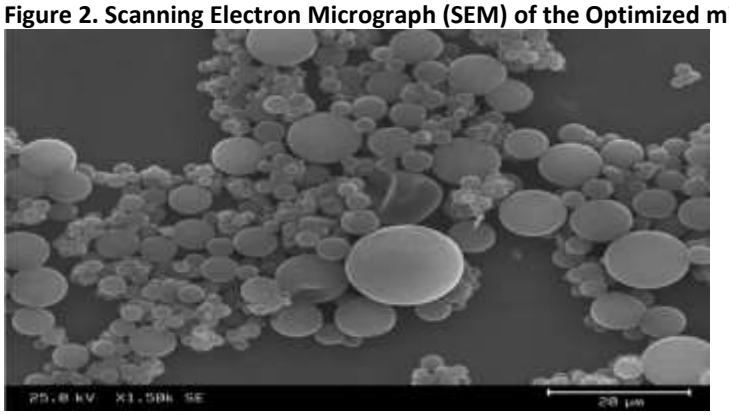


Table 5. Particle Size Distribution of Protein-Loaded Ethylcellulose Microcapsules

Particle Size Parameter	Value (µm)
dv10	1.27 ± 0.39
dv50	5.19 ± 0.76
dv90	10.58 ± 0.87
Span	1.80

Data are expressed as Mean ± SD (n = 3).

Scanning electron microscopy further confirmed the successful formation of well-defined core-shell microcapsules. The microcapsules appeared predominantly spherical with smooth external surfaces and minimal structural collapse, indicating efficient solvent evaporation during spray drying. Cross-sectional imaging demonstrated that the protein cores were completely enclosed within a continuous ethylcellulose shell, confirming successful encapsulation by the solid-in-oil technique. The dense hydrophobic ethylcellulose coating serves as a semi-permeable protective barrier that shields the encapsulated protein from moisture, acidic gastric conditions, and environmental degradation while allowing controlled diffusion of biological substrates following hydration. The combination of high encapsulation efficiency, narrow particle size distribution, and uniform core-shell morphology demonstrates that the optimized solid-in-oil spray-drying process successfully produced stable protein-loaded microcapsules with characteristics suitable for oral enzyme delivery and controlled-release applications.

3.4. Release Kinetics and Membrane Integrity

3.4.1. Evaluation of Initial Burst Release and Sustained Drug Diffusion

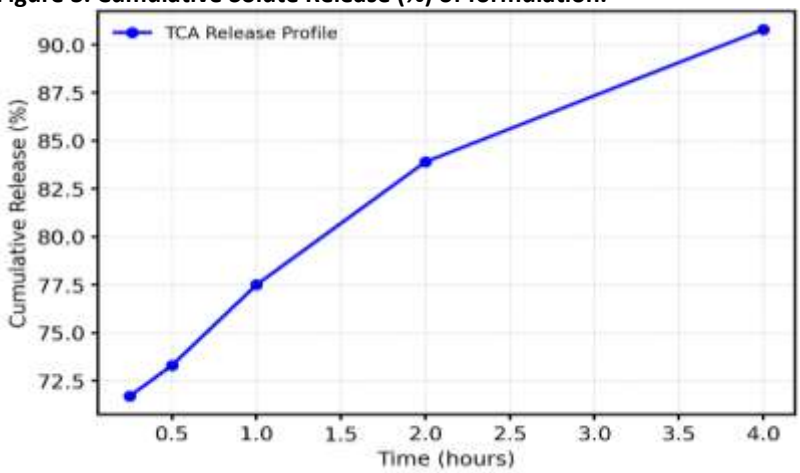
The release characteristics of the ethylcellulose (EC) microcapsules were investigated using trans-cinnamic acid (TCA) as a model permeating solute to assess the permeability and functional integrity of the polymeric membrane. An effective enzyme delivery system requires a semi-permeable membrane that retains the encapsulated therapeutic protein while permitting the diffusion of low-molecular-weight substrates and reaction products. Therefore, the release profile of TCA was used as an indirect indicator of membrane transport properties. The cumulative release data are presented in Table 6. The optimized microcapsules exhibited a pronounced initial burst release, with 71.7% of the encapsulated solute released within the first 15 minutes. This rapid release is primarily attributed to TCA molecules adsorbed on the particle surface or entrapped within the superficial polymer layers, which readily dissolve upon contact with the release medium. Following this initial phase, the release profile gradually transitioned into a sustained diffusion-controlled stage. Between 15 minutes and 4 hours, an additional 19.1% of the encapsulated solute was released, resulting in a cumulative release of 90.8% at the end of the study. The gradual release observed after the burst phase indicates that the remaining solute diffused through the ethylcellulose membrane in a controlled manner. These findings demonstrate that the ethylcellulose shell effectively regulates molecular transport by providing an initial rapid release of surface-associated molecules followed by prolonged diffusion from the internal core. Such biphasic release behavior is advantageous for oral enzyme delivery systems because it enables immediate substrate accessibility while maintaining sustained molecular transport during gastrointestinal transit.

Table 6. Cumulative Release Profile of Trans-Cinnamic Acid from Ethylcellulose Microcapsules

Time (hours)	Cumulative Solute Release (%)
0.25	71.7
0.50	73.3
1.00	77.5
2.00	83.9
4.00	90.8

Data represent the average values obtained from two independent experimental runs (n = 2).

Figure 3. Cumulative Solute Release (%) of formulation.



3.4.2. Mathematical Modeling of Release Kinetics and Diffusion Mechanism

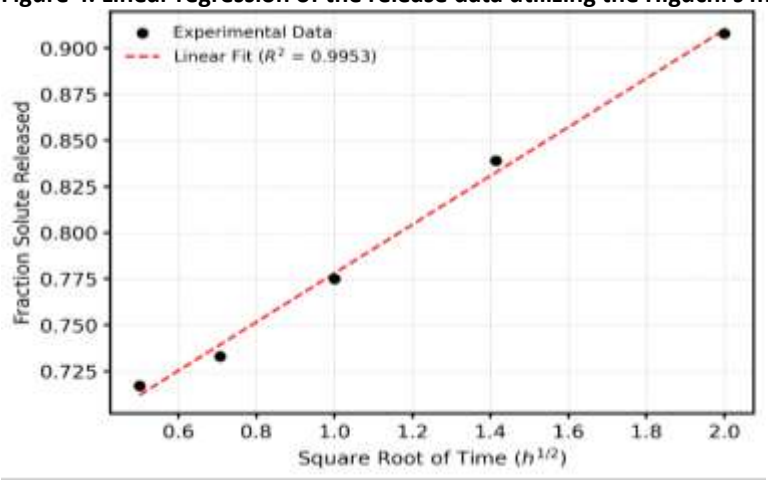
To elucidate the mechanism governing solute transport through the ethylcellulose membrane, the post-burst release data (15 minutes to 4 hours) were fitted to commonly used mathematical release models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations. The corresponding coefficients of determination (R^2) are summarized in Table 7. Among the evaluated models, the Higuchi model exhibited the highest coefficient of determination (R^2 = 0.9974), indicating excellent agreement with the experimental release profile. This strong correlation demonstrates that the release of trans-cinnamic acid from the microcapsules is predominantly governed by Fickian diffusion through the polymeric matrix. The comparatively lower R^2 values obtained for the zero-order, first-order, and Korsmeyer-Peppas models further support diffusion-controlled mass transport as the principal release mechanism.

Table 7. Mathematical Model Fitting of Post-Burst Release Data

Release Model	R^2
Zero-order	0.9667
First-order	0.9528
Higuchi	0.9974
Korsmeyer-Peppas	0.9612

The Higuchi model demonstrated the best fit to the experimental release data, indicating diffusion-controlled drug transport. The release process is initiated by penetration of the aqueous medium through microscopic pores and interconnected channels within the hydrophobic ethylcellulose membrane. Once water enters the microcapsule core, the encapsulated solute dissolves and subsequently diffuses outward along the concentration gradient through these hydrated pathways. Because ethylcellulose is an insoluble polymer, the membrane remains structurally intact throughout the release process, thereby maintaining the integrity of the microcapsule while regulating molecular transport. Importantly, the semi-permeable nature of the ethylcellulose membrane enables selective permeability. Small molecules such as substrates and reaction products readily diffuse across the membrane, whereas high-molecular-weight therapeutic proteins, including protease or phenylalanine ammonia-lyase (PAL), remain effectively entrapped within the core. This selective retention minimizes premature enzyme leakage while preserving catalytic functionality during gastrointestinal transit. Although the present formulation exhibited excellent enzyme retention, the relatively slow diffusion observed after the initial burst phase suggests that the internal pore network is highly tortuous and offers substantial resistance to mass transfer. Future optimization may therefore focus on incorporating biocompatible pore-forming agents, such as low-molecular-weight polyethylene glycol, to increase membrane porosity and facilitate faster substrate diffusion without compromising enzyme encapsulation or structural stability.

Figure 4. Linear regression of the release data utilizing the Higuchi's model.



4. Conclusion

This study successfully demonstrated the feasibility of using a solid-in-oil (s/o) spray-drying approach to encapsulate therapeutic enzymes, including protease and phenylalanine ammonia lyase (PAL), within a semi-permeable ethylcellulose (EC) polymeric matrix. Pre-formulation investigations identified a critical thermal deactivation threshold above 55°C for the unprotected enzyme.

The incorporation of the non-reducing disaccharides trehalose and sucrose substantially improved thermal stability, with 1.5 M trehalose providing the highest level of protection by preserving nearly complete catalytic activity and maintaining the native protein structure during thermal processing. The optimized microcapsules demonstrated favorable physicochemical characteristics, including a narrow particle size distribution with a median diameter of approximately 5 µm, a span value of 1.80, a production yield of 71.8%, and an encapsulation efficiency of 85.3%, confirming the robustness and reproducibility of the manufacturing process. Release studies revealed a characteristic biphasic release profile consisting of an initial burst release followed by sustained diffusion through the ethylcellulose membrane. Mathematical modeling showed that the release behavior closely followed the Higuchi diffusion model ($R^2 = 0.9974$), confirming that molecular transport occurred predominantly through Fickian diffusion across the hydrated polymer matrix. The semi-permeable ethylcellulose membrane effectively retained high-molecular-weight therapeutic enzymes while permitting the diffusion of low-molecular-weight substrates and reaction products, thereby demonstrating its suitability for oral enzyme delivery applications. Although the sustained diffusion phase indicated efficient enzyme retention, further optimization of membrane porosity through the incorporation of suitable biocompatible pore-forming agents may further improve substrate permeability and therapeutic performance.

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