Bilayered Beeswax Microspheres: A Novel Approach for Colon-Specific Delivery of Etoricoxib

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ABSTRACT

Background: Colon-specific drug delivery systems are critical for drugs requiring targeted release at the site of action, such as Etoricoxib, a nonsteroidal anti-inflammatory drug (NSAID). This study explores the development of a novel colon-specific system using beeswax as a natural core material, optimizing drug release for enhanced therapeutic outcomes and patient compliance.

Objective: To develop a colon-specific drug delivery system for Etoricoxib utilizing beeswax as a natural core material, optimize key formulation parameters, and evaluate encapsulation efficiency, drug content, and release profile.

Methods: Core microspheres were fabricated using a hot-melt microencapsulation process, with the drug-to-beeswax ratio, emulsifier concentration, and pH of the external phase optimized. Bi-layered microspheres were created by coating the core with cellulose acetate butyrate or Eudragit S 100 using an emulsion-solvent evaporation technique. The physicochemical properties were characterized using solubility studies, melting point determination, UV spectroscopy, FTIR, DSC, and SEM. The microsphere formulations were assessed for encapsulation efficiency, drug content, and particle size. In vitro dissolution studies were conducted to evaluate the drug release profile.

Results: Among the core microsphere formulations, F7 showed the highest encapsulation efficiency (86.87%) and drug content (9.69%). For double-walled microspheres, the C2 formulation, coated with Eudragit S 100, exhibited superior performance, with encapsulation efficiency of 78.87% and drug content of 8.89%. SEM images revealed smooth, spherical microspheres. In vitro dissolution tests indicated a controlled and delayed release mechanism for the C2 formulation, exhibiting zero-order release or Case II transport. The use of beeswax as the core material and Eudragit S 100 as the enteric coating resulted in an effective colon-targeted drug delivery system.

Conclusion: The study successfully developed a novel colon-specific drug delivery system for Etoricoxib using beeswax as the core material and Eudragit S 100 as an enteric coating. The formulation demonstrated optimized encapsulation efficiency, drug content, and a controlled release profile, offering potential advantages in therapeutic efficacy and patient compliance.

Keywords: Etoricoxib, colon-specific drug delivery, beeswax, microencapsulation, Eudragit S 100.

1. INTRODUCTION

A novel drug delivery system (DDS) aims to efficiently transport the active pharmaceutical ingredient (API) to its target site while ensuring controlled release to meet the body's therapeutic needs. Over the years, DDS technologies have revolutionized healthcare by enabling precise monitoring of drug release rates and facilitating targeted drug delivery to specific anatomical locations [1]. An optimal DDS ensures that the active ingredient reaches its intended site of action and is administered at a predetermined rate that aligns with the body's therapeutic requirements, thereby improving patient outcomes. Drug carrier technologies involve the attachment of therapeutic agents to various carrier particles such as lipids, nanoparticles, or microspheres, each offering distinct advantages for drug delivery [2]. Among these, microparticles, microspheres, and microcapsules are commonly used in multiparticulate systems due to their favorable structural and functional properties [3]. These carriers enable drug administration through various routes, including liquids (solutions, suspensions, and parenteral formulations), semisolids (gels, creams, and pastes), and solids (capsules, tablets, and sachets), making them versatile and convenient delivery platforms.

Microcarriers, with sizes typically greater than 100 nm, are advantageous over nanoparticles in terms of local action and reduced systemic distribution. Their larger size prevents them from being carried through the lymphatic system, allowing for localized drug delivery [4]. Microspheres, which consist of drug particles distributed in a continuous phase formed by one or more compatible polymers, have been increasingly studied for their potential in controlled release applications [4]. Etoricoxib, a non-steroidal anti-inflammatory drug (NSAID), acts by inhibiting the cyclooxygenase-2 (COX-2) enzyme, which plays a key role in the synthesis of prostaglandins involved in pain and inflammation [5]. The growing global prevalence of colonic diseases, including inflammatory bowel disease (IBD), underscores the urgent need for effective local therapies that improve drug safety and efficacy [6]. Colonic drug delivery systems (CDDS) are specifically designed to address these challenges, offering solutions for controlled and localized drug release. However, successful colon-targeted drug delivery is influenced by multiple factors such as the interaction between the drug and the gastrointestinal (GI) tract, the type of DDS used, and the physicochemical properties that affect GI transit time.

One of the primary goals of oral CDDS is to prevent premature drug release in the stomach and small intestine, delaying the release until the drug reaches the colon. Various strategies, such as mucoadhesive systems, pH-dependent formulations, and gastro-retentive dosage forms, have been developed to improve the performance of colonic drug delivery and therapies for conditions like IBD [7, 8]. These systems typically involve the encapsulation of the active pharmaceutical agent in a protective shell that regulates drug release through three key mechanisms: pH sensitivity in the GI tract, modulation of GI transit time, and enzymatic activation by colonic flora. Polysaccharide-based microcarriers, for instance, are designed to resist digestion in the small intestine but can be degraded by colonic enzymes, enabling targeted drug release in the colon [9, 10].

This study seeks to develop a novel colon-specific drug delivery system by utilizing beeswax as a natural coating material for microspheres containing Etoricoxib as the active ingredient. Beeswax, a complex mixture of diesters, hydrocarbons, free fatty acids, and fatty alcohol esters, is chosen for its natural, edible properties and its ability to withstand environmental factors such as humidity, oxygen, and digestive conditions over extended periods [11, 12]. Beeswax has been extensively used in pharmaceutical applications as a gastro-resistant coating, particularly for anti-inflammatory drugs, and has shown promise in formulating delayed-release formulations for anticancer agents [13, 14]. The use of beeswax as a coating material offers multiple benefits, including enhanced protection of the drug from premature release and improved release characteristics. The hot-melt method will be employed to fabricate beeswax microspheres, with critical process parameters such as the drug-to-beeswax ratio, emulsifier concentration, and pH of the external phase adjusted to optimize the production of the core microspheres. Following this, double-walled microspheres will be created using the emulsion-solvent evaporation technique, with cellulose acetate butyrate and Eudragit S 100 chosen as the enteric coating materials for the outer layer. The final aim is to develop an advanced DDS that optimizes drug release in the colon, enhancing therapeutic efficacy while minimizing systemic side effects [18].

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Etoricoxib, beeswax, and Eudragit S 100 were obtained as gift samples from Sai Mirra Inno Pharm Pvt. Ltd. (Chennai, India). Tween 80 was procured from Kamlesh Enterprise (Mumbai, India), while Span 80 was sourced from Jeevika Specialities Pvt. Ltd. (Nagpur, India). Ethanol was purchased from Gayatri Industries (Mumbai, India), and acetone was procured from Griver Enterprises (Jaipur, India). Light Liquid Paraffin was obtained from Excell Impex (Gujarat, India).

2.2. Characterisation of Pure Drug

2.2.1. Solubility studies

The solubility of Etoricoxib was evaluated in various solvents, including THF, ethanol, water, acetone, and methanol, to identify the most suitable for formulation. A 10 mg sample of Etoricoxib was added to 10 mL of each solvent and stirred for 24 hours at room temperature. Solvents with lower solubility were heated at 37°C to aid dissolution. The solutions were filtered, and drug concentrations were determined using a UV-Visible spectrophotometer at 225 nm [19].

2.2.2. Determination of Melting point

The melting point of Etoricoxib was determined using the open capillary method. A clean capillary tube sealed at one end was filled with Etoricoxib by repeatedly tapping the tube until

the drug was compactly packed. The capillary tube was then affixed to the melting point apparatus, which was programmed for an automatic temperature increase. The temperature at which the drug began to melt was carefully observed and recorded as the melting point of Etoricoxib [20].

2.2.3. Determination of λ_{max} of Etoricoxib

The UV spectrum of Etoricoxib was scanned using a Shimadzu UV-visible spectrophotometer (Model UV-1800). A solution with a concentration of 6 µg/ml was prepared and scanned over the wavelength range of 200–400 nm. The wavelength at which maximum absorbance occurred (λmax) was identified as 236 nm [21].

2.2.4. Preparation of standard stock solution and various concentrations

2.2.4.1. Preparation of Standard Stock Solution:

30 mg of Etoricoxib was accurately weighed and dissolved in methanol. The solution was diluted to 100 ml in a volumetric flask, resulting in a stock solution with a concentration of 300 µg/ml (0.3 mg/ml) [22].

2.2.4.2. Preparation of Calibration Standards:

Aliquots of the standard stock solution were taken to prepare a series of concentrations ranging from 2 µg/ml to 10 µg/ml. These aliquots were diluted with phosphate buffer solution (pH 6.8) to achieve the desired concentrations [23].

2.2.4.3. Measurement of Absorbance

Wavelength: The absorbance of each diluted standard solution was measured at 236 nm using a spectrophotometer. Blank: Phosphate buffer solution pH 6.8 was used as the blank for zeroing the spectrophotometer [24].

2.2.4.4. Construction of Calibration Curve:

Plotting: Absorbance values obtained from the spectrophotometer were plotted against the corresponding concentrations of Etoricoxib (2 µg/ml to 10 µg/ml).

Curve: The calibration curve was constructed by plotting absorbance (y-axis) against concentration (x-axis). Linear regression analysis was performed to determine the equation of the line (y = mx + b), where 'm' is the slope (absorptivity coefficient) and 'b' is the y-interce 【25】.

The calibration curve was employed to determine the unknown concentrations of Etoricoxib in samples by correlating their absorbance readings at 236 nm. This approach ensures both accuracy and reproducibility in analytical measurements【26】. To prepare specific concentrations of Etoricoxib solutions, aliquots of the standard stock solution were diluted with phosphate buffer (pH 6.8) in 100 ml standard flasks. A 3 µg/ml solution was prepared by diluting 1 ml of the stock solution, while 1.6 ml, 2 ml, 2.4 ml, and 3 ml of the stock solution were used to prepare 4.8 µg/ml, 6 µg/ml, 7.2 µg/ml, and 9 µg/ml solutions, respectively. These dilutions ensured precise and consistent preparation of calibration standards for spectrophotometric analysis.

2.2.5. Differential Scanning Calorimetry of Pure drug

The thermal behavior of Etoricoxib was analyzed using a Differential Scanning Calorimeter (DSC-60 Plus). For the analysis, Etoricoxib was placed in hermetically sealed, pierced aluminum pans to ensure sample integrity during the experiment. The sample was subjected to a controlled heating rate of 10°C/min, with the temperature range spanning from 25°C to 170°C. Throughout the experiment, an inert atmosphere was maintained by purging the system with nitrogen gas at a constant flow rate of 50 mL/min【27】. This setup provided accurate thermal characterization of the pure drug.

2.2.6. Fourier transform infrared spectroscopy studies

Fourier transform infrared spectroscopy (FT-IR) studies were carried out to study the compatibility of drug and excipients. To check interaction of pure Etoricoxib, Etoricoxib and bees wax, and also for core microsphere, IR spectra were recorded on (FT-IR Bruker STS 1900FT) by scanning in the range between 4000 and 650 cm^{-1} [28].

2.3. Formulation of Core microsphere

Etoricoxib microspheres were prepared using beeswax through a hot-melt microencapsulation technique. Beeswax was melted in a china dish using a water bath, ensuring the bath temperature exceeded the wax's melting point. Etoricoxib, previously sieved through a No. 40 sieve, was uniformly dispersed in the melted wax and stirred until a homogeneous mixture was achieved using a magnetic stirrer. This mixture was then emulsified in 100 ml of a hot aqueous solution of acetate buffer at pH 4.1, which included an emulsifier, and stirred mechanically at 800 rpm using a mechanical stirrer. After 3 minutes of emulsification, the system was cooled in an ice bath to 10°C while continuously stirring. After 20 minutes, the solidified microspheres were collected by filtration, washed with distilled water, and finally air-dried at room temperature for 48 hours [29].

Table 1: Formulation of Etoricoxib core microspheres using Beeswax

Formulation code	Etoricoxib: Beeswax ratio	RPM	Emulsifier	pH	Shape
F1	1:4	800	PVA 1 %	6.1±0.1	Aggregate
F2	1:6	800	PVA 1 %	6.1±0.1	Spherical
F3	1:6	800	PVA 1.5 %	6.1±0.1	Spherical
F4	1:6	800	PVA 1 %	4.1±0.1	Spherical
F5	1:6	800	PVA 1.5 %	4.1±0.1	Spherical
F6	1:6	800	Tween 80	4.1±0.1	Spherical
F7	1:6	800	Tween 80 + Span 80	4.1±0.1	Spherical

2.4. Characterization of Core microspheres

2.4.1. Percentage yield of Etoricoxib Core microspheres

After preparation the core microspheres were dried overnight at room temperature. Recovery is the ratio of the weight of microspheres obtained to that total weight of solid contents charged at the beginning of the microencapsulation process [30].

Percent yield = weight of microspheres (g) / weight of all species charged (g) × 100

2.4.2. Percentage Entrapment efficiency of Etoricoxib Core microspheres

Percentage entrapment efficiency is the percentage of drug encapsulated in the microspheres relating to the initial quantity used. The appropriate number of core Microspheres in 100 ml of phosphate buffer pH 6.8 was placed in an ultrasonic bath at 70°C to remove Etoricoxib from beeswax completely. After cooling to room temperature, it was filtered through 0.45 μm filter paper. 1 ml supernatant was taken and diluted to 10 ml with phosphate buffer solution of pH 6.8. After filtration, the absorbance of Etoricoxib at 236 nm was measured using a UV-visible spectrophotometer (Model UV-1800). The measured absorbance was then converted to the amount of Etoricoxib by using standard calibration curve [31]. Percentage encapsulation efficiency was calculated as follows,

Encapsulation efficiency % = entrapped amount of drug per g microsphere / theoretical amount of drug per g microsphere × 100

2.4.3. Drug content of Etoricoxib core microspheres

Etoricoxib-incorporated wax microspheres from each batch were chosen and powdered in a mortar. 100 mg of drug-loaded wax microspheres were carefully weighed and put to a 100mL volumetric flask. To this, 100mL DCM was added and swirled for 60 minutes, until the entire

drug had leached out. After filtering the solution, 1mL was removed and put to a 10mL volumetric flask with 10mL (10µg/mL) of phosphate buffer at pH 6.8. The drug content was calculated using a UV-visible spectrophotometer (Model UV-1800) at 236 nm with pH 6.8 phosphate buffer as a blank [31].

2.5. Formulation of Etoricoxib double walled microspheres

Solvent evaporation was used to prepare double-walled microspheres. The core microspheres were dispersed in 10 ml of coating solution containing coating polymer in Ethanol: Acetone (2:1), resulting in a coat:core ratio of 5:1. The aforementioned organic phase was then put into 70 cc of light liquid paraffin containing 1%w/v Span 80. The system was then stirred continuously at room temperature with a stirrer for 3 hours to allow the solvent to evaporate. Finally, the double-walled microspheres were filtered, washed in n-hexane, and freeze dried overnight [32].

Table 2: Formulation of Etoricoxib Double walled microspheres

Coating	Polymer	Coat: Core ratio	Shape
C1	Cellulose Acetate Butyrate (CAB)	5: 1	Spherical
C2	Eudragit S 100 (ES 100)	5: 1	Spherical

2.6. Characterization of Etoricoxib Double walled microspheres

2.6.1. Differential Scanning Calorimetry of Etoricoxib Double walled microspheres

DSC was conducted using a Differential Scanning Calorimeter (Model DSC-60 plus). The C2 formulation was hermetically sealed into pierced aluminium pans and heated at a constant rate of 10°C/min over a temperature range of 25 to 170°C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 5mL/min [33].

2.6.2. Percentage yield of Etoricoxib Double walled microspheres

After preparation the double walled microspheres were dried overnight at room temperature. Recovery is the ratio of the weight of microspheres obtained to that total weight of solid contents charged at the beginning of the microencapsulation process [34].

Percent yield = weight of microspheres (g) / weight of all species charge

2.6.3. Percentage entrapment efficiency of Etoricoxib Double walled microspheres

The percentage entrapment efficiency is the ratio of medicine encapsulated in microspheres to the initial quantity used. An appropriate number of double-walled microspheres in 100 ml of phosphate buffer pH 7.4 were immersed in an ultrasonic bath at 70°C to completely remove Etoricoxib from beeswax. After cooling to room temperature, the sample was filtered using 0.45 µm filter paper. 1 ml of supernatant was diluted to 10 ml with a pH 7.4 phosphate buffer solution. Following filtering, the absorbance of Etoricoxib at 236 nm was measured with a UV-Visible Spectrophotometer (Model UV-1800) [51]. The absorbance was measured and translated to the quantity of Etoricoxib using a standard calibration curve. The percentage encapsulation efficiency was calculated as follows [35].

% of Encapsulation efficiency = entrapped amount of drug per g microsphere / theoretical amount of drug per g microsphere × 100

2.6.4. Drug content of Etoricoxib Double walled microsphere

Each batch's etoricoxib-double walled microspheres were selected and powdered in a mortar. 100 mg of drug double walled microspheres were carefully weighed and put to a 100mL volumetric flask. To this, 100mL DCM was added and swirled for 60 minutes, until the entire drug had leached out. After filtering, 1mL of the solution was removed and added to a 10mL volumetric flask with 10mL (10µg/mL) of phosphate buffer at pH 7.4. The drug content was determined UV spectrophotometrically at 236 nm with pH 7.4 phosphate buffer as a blank [36].

2.6.5. Scanning Electron Microscopy of Etoricoxib Double walled microsphere

The morphology and surface appearance of double walled microspheres was found by using Scanning Electron Microscopy (SEM, Model - ZEISS EVO 18). The particles were freeze dried, coated with gold palladium to achieve a film of 20nm thickness [37].

2.7. *In-vitro* dissolution study of Core microspheres and Double walled microspheres

Core and double-walled microspheres were tested for drug release in vitro using the USP Dissolution Apparatus II at 37±0.5°C and 100 rpm. The investigation was conducted in (pH 1.2) solution for the first two hours, then dissolution in (pH 6.8) (PBS) for the next four hours, and finally in (pH 7.4) (PBS) until the completion of the study . A 5 ml sample was taken at predefined intervals and replaced with fresh dissolving medium. The removed samples were pipetted into a succession of 10 ml volumetric flasks, and the volumes were filled to the mark with a dissolving medium. After proper dilutions, the samples' absorbance at 236 nm was measured using a Shimadzu UV-Visible spectrophotometer (Model UV-1800) [38]. The cumulative % drug release was determined, and a graph of the percentage cumulative drug release vs time was created.

2.8. *In-vitro* Release kinetics of Double walled microspheres

The *in-vitro* release data were fitted to mathematical models to ascertain the mechanism and kinetics of drug release from the microspheres. The zero-order, first-order, Higuchi, and Korsmeyer-Peppas models were among the kinetic models [39].

2.9. Stability studies of Double walled microspheres

A pharmaceutical preparation is considered stable if, during its shelf life, it meets its physical, chemical, microbiological, therapeutic, and toxicological requirements in a certain container or closure system. Stability testing shows how a drug's or product's quality changes over time in reaction to environmental factors such as temperature, humidity, and light. This data is used to establish retest intervals, optimal storage conditions, and shelf life [40].

The stability protocol for this study was developed utilizing the ICH "Q1AR2" criteria. The stability of microspheres during storage was tested in a Thermo Lab Scientific chamber at 40°C ±2°C/75% RH ± 5% for 3 months, following ICH recommendations [63]. The formulation's storage stability was then determined by examining the microspheres' physical stability (color change), entrapment efficiency, and cumulative drug release characteristics (dissolution study) after 0, 30, 60, and 90 days [41].

3. RESULTS AND DISCUSSION

3.1. Solubility studies of Etoricoxib Pure Drug

The solubility of Etoricoxib, the pure drug, was evaluated across various solvents, including tetrahydrofuran, water, ethanol, acetone, methanol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF). The observations revealed that the drug was insoluble in water, slightly soluble in ethanol, soluble in acetone and methanol, and freely soluble in DMSO, DMF, and tetrahydrofuran. These findings are summarized in Table 3.

Table 3: Solubility Studies of Etoricoxib pure drug

S. No.	Solvent	Insoluble	Sparingly Soluble	Soluble	Freely soluble
1.	Water	✓	-	-	-
2.	Ethanol	-	✓	-	-
3.	Acetone	-	-	✓	-
4.	Methanol	-	-	✓	-
5.	DMSO	-	-	-	✓
6.	DMF	-	-	-	✓
7.	THF	-	-	-	✓

3.2. Determination of melting point of Etoricoxib Pure Drug

The melting point of the pure drug, Etoricoxib, was determined using the open capillary method. The drug exhibited a melting point of 136.63°C, which falls within the standard range of 134–138°C for Etoricoxib. This consistency with the typical melting point range indicates the purity of the drug.

3.3. Determination of λ_{max} of Etoricoxib Pure Drug

The λmax of Etoricoxib was determined using a UV-visible spectrophotometer (Model UV-1800). The identification of the pure drug was conducted through its UV spectrum obtained within the range of 200–400 nm. The λmax of Etoricoxib was found to be 236 nm, as illustrated in Figure 1

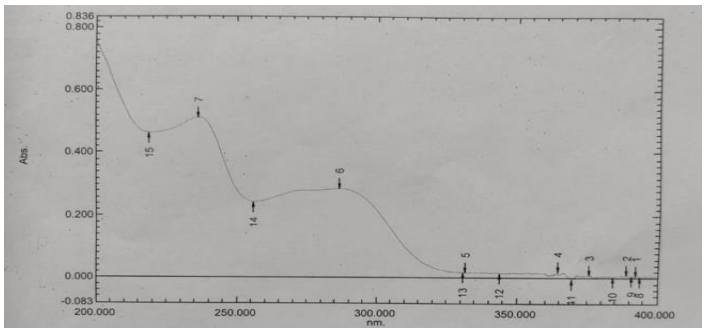

Figure 1: UV Spectrum of Etoricoxib Pure Drug

3.4. Standard calibration curve of Etoricoxib Pure Drug

The results and the graph of the standard calibration curve for Etoricoxib are presented in Table 4 and Figure 2, respectively. The λmax was determined to be 236 nm. The linearity equation was found to be y=0.081x with a coefficient of determination R^2=0.998. These findings indicate that Etoricoxib adheres to Beer-Lambert's law.

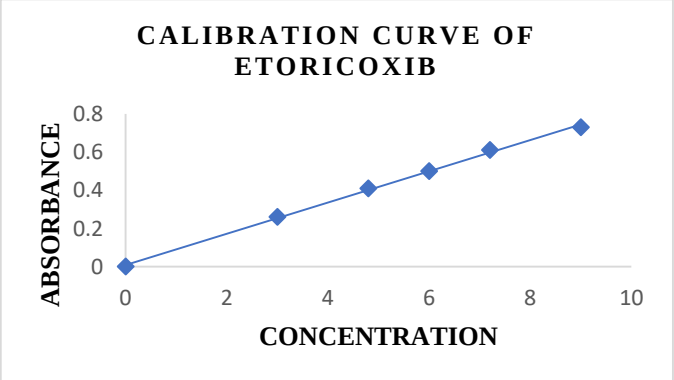

Figure 2: Calibration Curve of Etoricoxib pure drug

Table 4: Statistical Data for Calibration Curve of Etoricoxib pure drug

S. No.	PARAMETERS	VALUES
1.	λ_{max} (nm)	236
2.	Slope	0.081
3.	R^2	0.998

3.5. Differential Scanning Calorimetry of Etoricoxib Pure drug

The differential scanning calorimetry (DSC) analysis of Etoricoxib was conducted using a DSC-60 plus instrument. The thermogram exhibited a distinct endothermic peak at 136.63°C, corresponding to the melting point of Etoricoxib. This observed melting point is consistent with the reported range of 134–138°C, confirming the purity of the drug. The sharpness of the endothermic peak further indicates the crystalline nature of the drug. The DSC thermogram of Etoricoxib is presented in Figure 3, demonstrating the thermal behavior and confirming the stability of the drug under the specified experimental conditions. These findings validate the identity and quality of Etoricoxib for its intended applications.

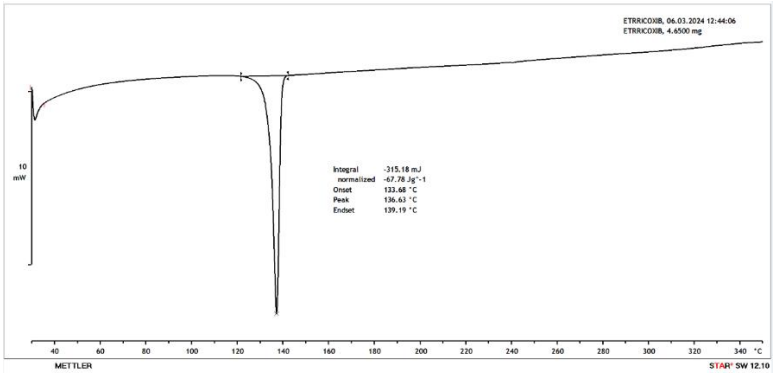

Figure 3: Differential Scanning Calorimetry of Etoricoxib Pure Drug

3.6. Fourier transform infrared spectroscopy studies

The FTIR spectra of Etoricoxib, its combination with Beeswax, and Beeswax-based microspheres are shown in Figures 4, 5, and 6. The FTIR spectrum of Etoricoxib (Figure 4) displays characteristic peaks at around 3400 cm^{-1}, indicating the presence of the hydroxyl group (-OH), as well as peaks at approximately 1600 cm^{-1} and 1730 cm^{-1} corresponding to the aromatic rings and carbonyl groups, respectively. These functional groups are crucial for the pharmacological activity of Etoricoxib.

When Etoricoxib was combined with Beeswax (Figure 5), the spectrum showed that the main functional groups responsible for the drug's pharmacological effects remained largely unchanged. The characteristic peaks of Etoricoxib were still evident, suggesting that there were

no significant chemical interactions between the drug and Beeswax. This implies that the drug is compatible with Beeswax, maintaining its pharmacological activity. In the FTIR spectrum of Beeswax-based microspheres (Figure 6), the characteristic peaks of both Beeswax and Etoricoxib were present, confirming the stability of the drug within the Beeswax matrix. The absence of any new peaks or significant shifts indicates that the drug is well incorporated into the microsphere formulation without undergoing any major chemical changes.

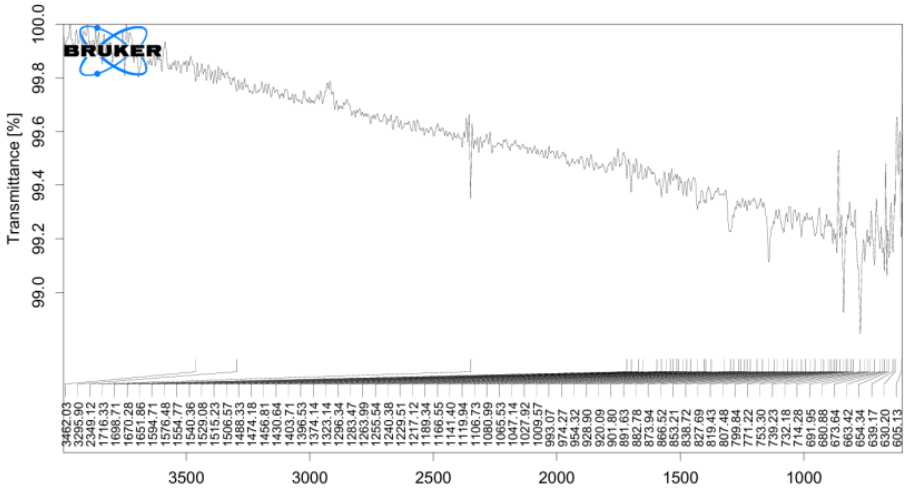

Figure 4: FTIR Spectrum of Etoricoxib Pure Drug

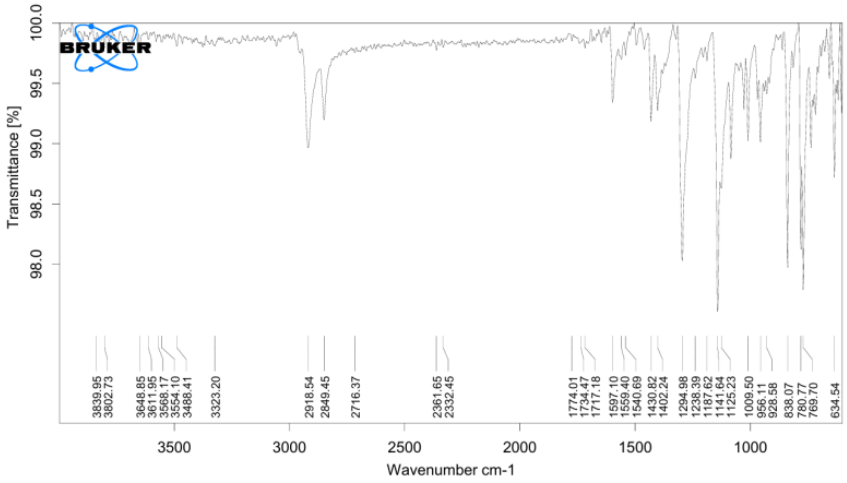

Figure 5: FTIR spectrum of Etoricoxib and Bees wax

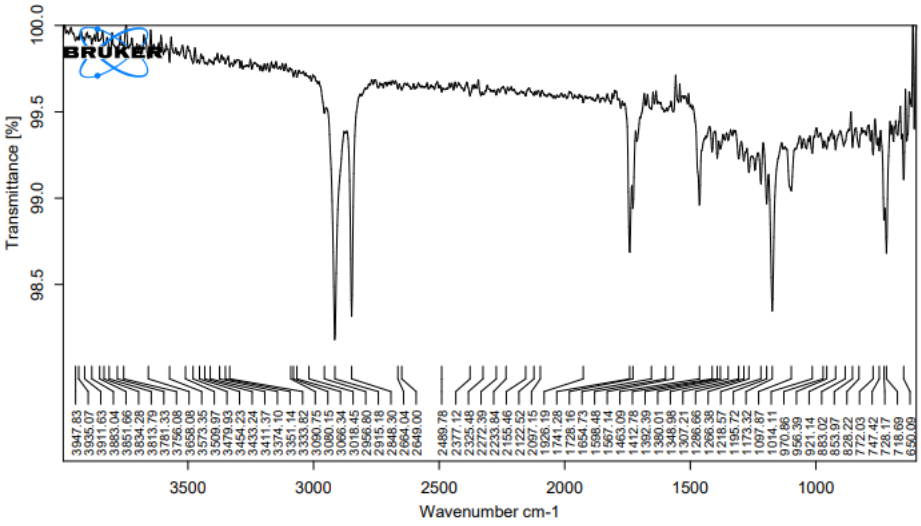

Figure 6: FTIR spectrum of Etoricoxib Bees-wax-based Microspheres

3.7. Physical characteristics of Etoricoxib Core microspheres

The physical characteristics of the core microspheres, including percentage yield, entrapment efficiency, and drug content, are presented in Table 5.

3.7.1 Percentage yield of Etoricoxib core microspheres

The Etoricoxib core microsphere yield ranged from 72.38% (F2) to 88.19% (F7), with F7 achieving the highest due to optimized formulation parameters, as shown in Table 5. Recovery was influenced by the pH of the external phase, with higher recovery at pH 4.1 ± 0.1 compared to pH 6.1 ± 0.1, indicating better emulsion stability in acidic conditions. Increased emulsifier concentration and altered emulsifier types also improved recovery, particularly in F5 and F7, by enhancing emulsion droplet stability and preventing coalescence. These findings emphasize the role of pH, emulsifiers, and stabilization techniques in optimizing microsphere production.

3.7.2 Percentage Entrapment efficiency of Etoricoxib core microspheres

Encapsulation efficiency varied significantly, influenced by the Drug:Beeswax ratio, pH, and surfactant properties (Table 5). A 1:6 ratio improved formulation customization, avoiding droplet aggregation seen at 1:4. Efficiency was below 42% at pH 6.1 but increased at pH 4 due to reduced Etoricoxib solubility, with F5 achieving 48.58%. Surfactants with HLB >15 showed better solubilizing efficiency. The optimal HLB value of 9, achieved with a Tween 80 and Span 80 mixture, stabilized the lipid phase and minimized drug loss, leading to F7's highest efficiency (86.87%). Smaller microspheres formed at HLB values between 13.5 and 14.5, while lower HLB values increased sizes. These findings underscore the importance of optimizing emulsifier concentration, HLB values, and pH to enhance drug entrapment and microsphere stability, as demonstrated by F7.

3.7.3 Drug content of Etoricoxib core microspheres

The drug content of beeswax microspheres ranged from 3.74% (F2) to 9.69% (F7) (Table 5). F7 showed the highest drug content due to its superior entrapment efficiency, achieved through optimized formulation parameters, including a balanced HLB value and appropriate emulsifier concentration, minimizing drug loss. Lower drug content in F2 reflects reduced entrapment efficiency, underscoring the importance of fine-tuning formulation parameters. F7's 9.69% drug content highlights its effectiveness in maximizing drug loading in microsphere-based delivery systems.

Table 5: Physical characteristics of Etoricoxib Core microspheres

Formulations	Percentage yield	Entrapment efficiency %	Drug content
F1	84.66%	33.15%	5.97
F2	72.38%	37.72%	3.74
F3	74.28%	41.15%	4.44
F4	73.57%	48.01%	5.34
F5	78.23%	48.58%	5.82
F6	82.09%	42.29%	4.57
F7	88.19%	86.87%	9.69

3.8. Differential scanning calorimetry of Etoricoxib Double walled microsphere

The DSC study of the Double-walled microsphere formulation C2 (Figure 5) revealed an endothermic peak at 135.84°C**,** indicating that the drug does not interact with the polymers (Eudragit S 100 and beeswax). The absence of any significant shifts suggests that the drug remains in its native form, confirming its stability and integrity within the formulation. This indicates no chemical interaction between the drug and the polymers used.

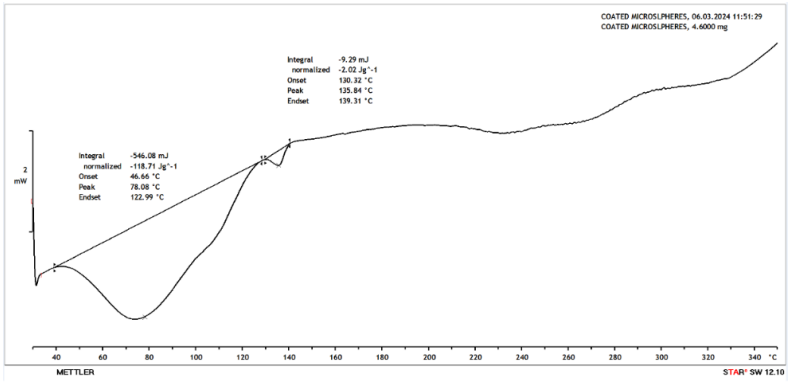

Figure 5: Differential scanning calorimetry of Etoricoxib Double walled microsphere C2 Formulation

3.9. Physical characteristics of Etoricoxib Double walled microspheres

3.9.1. Percentage yield of Etoricoxib Double walled microspheres

The percentage recovery of the coated microspheres was found to be 83.23% for C1 and 88.12% for C2, as shown in Table 6. The increase in the coat ratio led to a higher yield of the coated microspheres, with C2 achieving the greatest yield of 88.12%. This suggests that a higher coat ratio improves the encapsulation process, leading to better recovery and yield of the coated microspheres. The results indicate that optimizing the coat ratio can enhance the overall efficiency of the microsphere formulation.

3.9.2. Percentage entrapment efficiency of Etoricoxib Double walled microspheres

The percentage entrapment efficiency of the double-walled microspheres was assessed using formulation F7, which demonstrated the highest encapsulation efficiency of 86.37% and appropriate spherical particle formation. For the second coating process, cellulose acetate butyrate (CAB) and Eudragit S 100 were selected to coat the F7 beeswax microspheres. The results, as shown in Table 6, revealed that the C2 formulation had an increased encapsulation efficiency of 78.87%, compared to C1, which had 70.87%**.**

The study indicated that while the two polymeric coatings differed, the presence of the second wall led to an increase in particle size. Microspheres coated with Eudragit S 100 were found to be larger and more spherical than those coated with CAB**.** The twofold coating process resulted in a 1.8% loss of drug entrapment for Eudragit S 100 and a 1.7% loss for CAB**.** The higher viscosity of the Eudragit S 100 organic phase, which inhibited drug migration to the outer phase, was attributed to the lower drug loss in this formulation.

3.9.3. Drug content of Etoricoxib Double walled microspheres

The percentage drug content for the double-walled microspheres was found to be 7.87% for C1 and 8.89% for C2**,** as shown in Table 6. The C2 formulation exhibited the highest drug content, making it the **s**uperlative formulation**.** The increased drug content in C2 compared to C1 indicates better drug incorporation and entrapment in the formulation. This suggests that the second coating with Eudragit S 100 led to a more efficient encapsulation process, resulting in a higher concentration of the active pharmaceutical ingredient in the microspheres. Thus, C2 is the most effective formulation in terms of drug content, highlighting its potential for optimized drug delivery.

Table 6: Physical characteristics of Etoricoxib Double walled microspheres

Formulation code	**Percentage Yield (%)**	**Entrapment Efficiency (%)**	**Drug Content**
C1	83.23%	70.87%	7.87
C2	88.12%	78.87%	8.89

3.10. Scanning electron microscopy of Etoricoxib Double walled microspheres

The surface morphology of the Double walled microspheres C2 formulation was analyzed using Scanning Electron Microscopy (SEM, Model: ZEISS EVO 18) to assess its shape and surface characteristics. As shown in Figure 6, the SEM images revealed that the microspheres had a smooth-textured, spherical polymer surface. The SEM analysis confirms the high quality and consistency of the formulation.

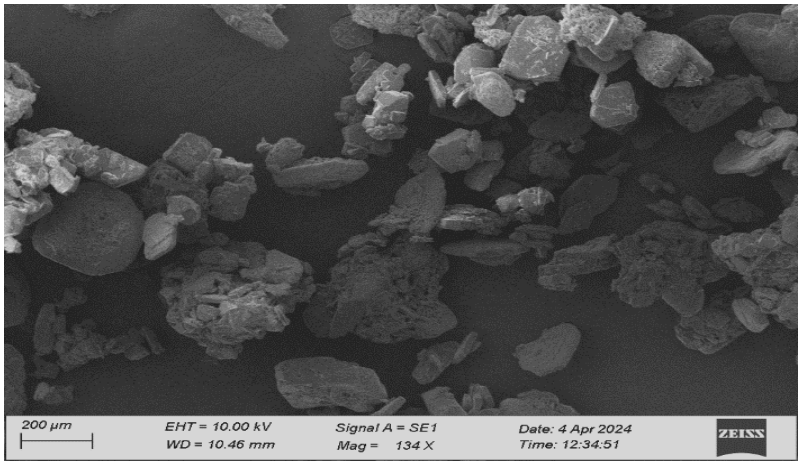

Figure 6: SEM of Etoricoxib Double walled microspheres C2 Formulation

3.11. *In-vitro* dissolution of Etoricoxib Core microspheres and Double walled microspheres

The In-Vitro Drug Release of both beeswax microspheres and double-walled microspheres was studied using a USP Dissolution Apparatus II at 37±0.5°C with a rotation speed of 100 rpm. The release profiles of Etoricoxib for F5, F6, F7 beeswax microspheres and C1 and C2 double-walled beeswax microspheres are illustrated in Figures 7,8 & 9. It was observed that in the acidic medium (pH 1.2), the drug was only marginally released from the basic beeswax formulations (F5, F6, F7), with the release being minimal from C1 and negligible from C2 double-walled microspheres. In contrast, at pH 6.8, the drug release from C1 and C2 formulations started slowly but increased significantly at pH 7.4.

As summarized in Tables 7, 8, and 9, after two hours, the percentage drug release from formulations F5, F6, and F7 in the acidic medium (pH 1.2) ranged from 6.8% to 14.98%, while the release for C1 was 4.88%, and C2 showed no release. At the end of six hours, the drug release for C1 and C2 formulations ranged from 3.3% to 6.64%, while for F5, F6, and F7, it ranged from 15.53% to 29.46% in the simulated small intestinal liquid (pH 6.8). At the end of the 24-hour release period, the drug release for F5 was 67% in the colon-simulated liquid (pH 7.4), while F6 and F7 formulations showed a high drug release of 90% to 95%. The C2 microspheres, on the other hand, showed a release of 75.62%, indicating a slower, more controlled release profile.

These results demonstrate that the double-walled microspheres (C1 and C2) exhibited a controlled release, with a slower and more sustained release in neutral and basic media, while the plain beeswax microspheres (F5, F6, and F7) released a higher amount of the drug over time, particularly in the simulated intestinal and colon media. The findings suggest that the C2 formulation has the potential for extended and controlled drug release, making it suitable for targeted drug delivery applications.

Table 7: Percentage Cumulative drug release of Etoricoxib Core microspheres F5, F6, F7 and Double walled microspheres C1, C2 at pH 1.2

Time (hrs)	F5	F6	F7	C1	C2
1	6.4%	8.79%	7.46%	0.75%	0
2	8.75%	13.75%	14.98%	4.88%	0

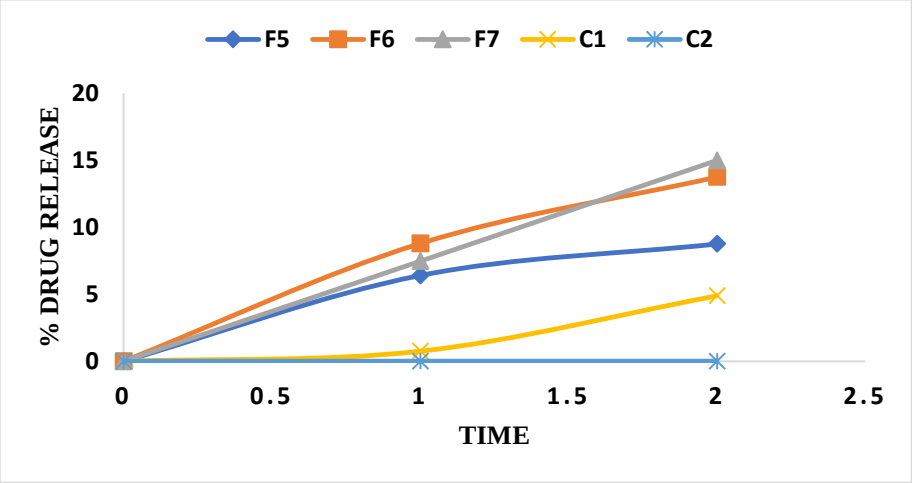

Figure 7: Percentage Cumulative drug release of Etoricoxib Core microspheres F5, F6, F7 and Double walled microspheres C1, C2 at pH 1.2

Table 8: Percentage Cumulative drug release of Etoricoxib Core microspheres F5, F6, F7 and Double walled microspheres C1, C2 at pH 6.8

Time (hrs)	F5	F6	F7	C1	C2
3	10.4%	15.38%	18.88%	5.46%	0%
4	12.38%	16.53%	20.29%	5.75%	0%
5	13.89%	18.48%	23.51%	6.13%	0.37%
6	15.53%	22.62%	29.46%	6.64%	3.3%

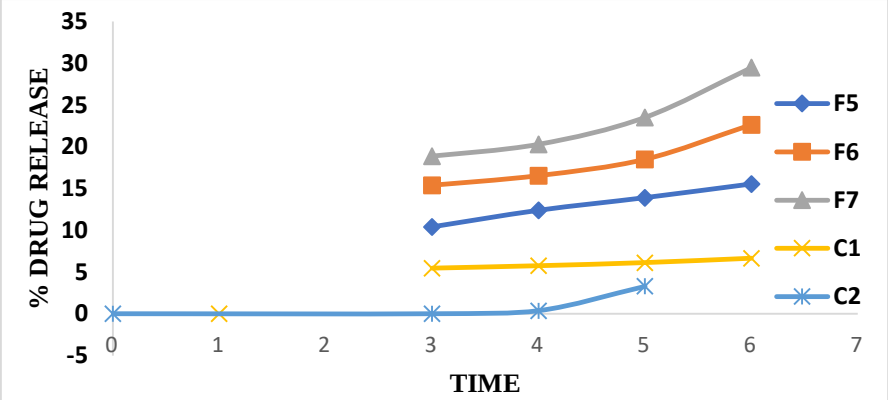

Figure 8: Percentage Cumulative Drug Release of Etoricoxib Core microspheres and Double walled microspheres at pH 6.8

Table 9: Percentage Cumulative Drug Release of Etoricoxib Core microspheres F5, F6, F7 and Double walled microspheres C1, C2 at pH 7.4

Time (hrs)	F5	F6	F7	C1	C2
12	31.17%	45.01%	52.85%	37.94%	28%
18	49.96%	67.25%	73.48%	62.5%	54.88%
24	66.33%	90.64%	94.14%	82.35%	75.62%

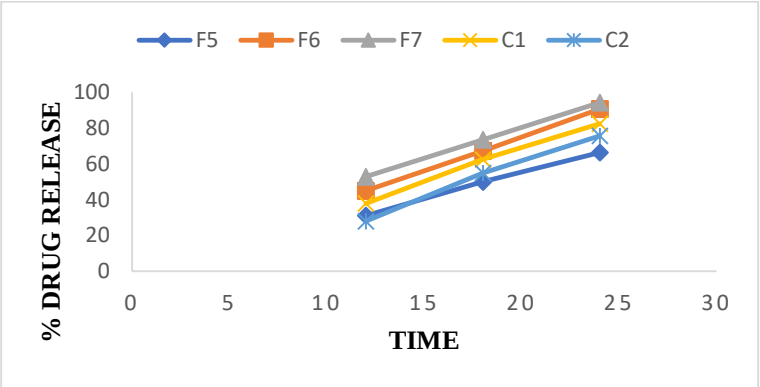

Figure 9: Percentage Cumulative Drug Release oof Etoricoxib Core microspheres F5, F6, F7 and Double walled microspheres C1, C2 at pH 7.4

3.12. *In-vitro* Release kinetics of Etoricoxib double-walled microsphere formulation (C2)

The kinetic analysis of drug release from the C2 double-walled microspheres indicates a controlled and sustained release profile. The release follows a zero-order kinetic model with a high correlation coefficient ($R^2 = 0.9929$), suggesting a consistent drug release rate over time. While the first-order kinetic model shows a moderate correlation ($R^2 = 0.8994$), it is not the dominant mechanism. The Higuchi model, with a correlation coefficient of $R^2 = 0.9071$, indicates that drug release is primarily driven by diffusion through the polymer matrix. The Korsmeyer-Peppas model provides the most accurate description, with a high correlation coefficient ($R^2 = 0.9996$) and an exponent ($n = 1.3741$) revealing a non-Fickian release

mechanism, which combines diffusion and polymer relaxation. These findings, summarized in Table 10**,** underscore that the C2 highlight that the C2 formulation is best characterized by the Korsmeyer-Peppas and zero-order models, demonstrating its effectiveness in delivering a controlled and prolonged therapeutic action.

Table 10: *In-Vitro* kinetic data of Etoricoxib Double Walled Microsphere (C2)

Formulation code	**Zero-Order Kinetics**		**First-Order Kinetics**		**Higuchi Model**		**Korsmeyer-Peppas Model**	
Double-walled Microsphere (C2)	K **(mg/h)**	R^2	K **(h^{-1})**	R^2	K **($mg/h^{1/2}$)**	R^2	n	R^2
	3.1523	0.9929	0.1849	0.8994	14.155	0.9071	1.3741	0.9996

3.13. Stability studies of Etoricoxib Double walled microsphere of C2 formulation

The C2 formulation was evaluated under accelerated conditions (40°C ± 2°C/75% ± 5% RH) for three months. Negligible changes were observed in entrapment efficiency and cumulative drug release as shown in **Table 11**, indicating excellent stability. This stability is attributed to the optimized formulation and compatibility of Eudragit S 100 and beeswax, confirming the suitability of C2 microspheres for long-term storage and practical application.

Table 11: Stability Studies of Etoricoxib Double walled microsphere of C2 formulation

Sampling day	**Colour**	**% Entrapment efficiency**	**% Cumulative drug release**
0 days	NC	78.87±0.52%	75.62±0.64%
30 days	NC	78.79±0.78%	75.60±0.66%
60 days	NC	77.96±0.84%	75.59±0.81%
90 days	NC	77.81±1.01%	74.98±1.07%

4. CONCLUSION

In conclusion, this study successfully developed a colon-specific drug delivery system for Etoricoxib, utilizing beeswax as a core material and Eudragit S 100 as an enteric coating. The optimized C2 formulation demonstrated a high encapsulation efficiency of 78.87% and drug content of 8.89%, with a controlled and delayed drug release profile, following a zero-order and Case II transport mechanism. SEM analysis confirmed the formation of smooth, spherical microspheres, further validating the suitability of the formulation for colon-targeted drug delivery.

The minimal drug release at acidic pH (1.2) and gradual release at pH 6.8, followed by a significant release at pH 7.4, emphasized the potential of this system for sustained therapeutic efficacy. Additionally, the bi-layer coating approach, particularly with Eudragit S 100, enhanced drug encapsulation and controlled release, making it a promising strategy for colon-specific delivery systems. Stability studies confirmed the long-term stability of the formulation, with minimal variations in encapsulation efficiency and drug release.

This research highlights the effectiveness of double-walled microspheres in improving the encapsulation and release properties of Etoricoxib, offering a promising solution for targeted, controlled, and sustained drug delivery. These findings have significant implications for improving the therapeutic outcomes of colon-specific drug delivery systems, with potential applications in the treatment of inflammatory bowel diseases and other related conditions.

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6. CONFLICT OF INTEREST

The authors have declared no conflicts of interest

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