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FABRICATION AND CHARACTERIZATION OF COLON TARGETED IBUPROFEN NANOPARTICLES

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ABSTRACT

Aim: The objective of our study was to formulate a nanoparticulate drug delivery system that provides controlled delivery of ibuprofen directly targeting colon leading to optimized dosage, reduced side effects ultimately increasing bioavailability and to explore formulation optimization parameters. **Methodology:** Colon targeted ibuprofen nanoparticles were prepared by solvent evaporation method. Analysis of formulation was performed for surface morphology, percentage entrapment efficiency, particle size, zeta potential, content uniformity, in vitro drug release and Stability assessment. **Result:** The ultimate optimized conditions found were 1500rpm, 0.1% W/V Tween 80 stabilizer and 1:10 organic to aqueous ratio. Among 6 formulations of ibuprofen nanoparticles analyzed, F1 (with 0.1% Tween 80 stabilizer) was found to be optimized with highest percentage release 99.64%, mean particle diameter 586 nm, smooth surfaces, 88.36% encapsulation efficiency, zeta potential-49mV and controlled release of drug over 8 hours. **Conclusion:** The optimized stable formulation F1 results showcased that 1500 rpm, 0.1% W/V Tween 80 stabilizer and 1:10 organic to aqueous ratio are optimized parameters of colon targeted ibuprofen nanoparticles preparation with formulation being cost effective with its remarkable enhancement of bioavailability.

Keywords: Ibuprofen, Nanoparticles, Solvent Evaporation, Colon, Controlled release

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world and the fourth most common cause causing cancer-related death. The third most common cancer in men (10%) and second most in women (9.2%), by gender **[1]**.It is one of the most prevalent disease, mainly in women, in those having age range from 65-74.As ibuprofen, an anti-inflammatory medicine, if given orally, can cause GIT related problems, for this, we need such Drug Delivery System(DDS) that provide a targeted delivery to colon in controlled manner with fewer side effects. For this, Colon Targeted DDS developed ,in order to, treat with CRC(IBD or IBS) that is specific to colon **[2]**. CTDDS is an ideal route for those drugs that degrade in stomach and small intestine. It also prevent the drugs to undergo first-pass metabolism, thus, decreases systemic side effects with a targeted delivery to colon **[3]**.This study mainly aims to prepare colon targeted ibuprofen nanoparticles in order to mitigate the colon related diseases.

Recent years have seen considerable interest in colon-targeted drug delivery systems because of their potential to maximize pharmacological efficacy while reducing systemic side effects **[4].** Ibuprofen is popular among non-steroidal anti-inflammatory medications (NSAIDs) due to its strong analgesic and anti-inflammatory qualities. One interesting approach to address the disadvantages of traditional NSAID therapy, such as gastrointestinal discomfort and systemic absorption, is the incorporation of ibuprofen into nanoparticulate formulations **[5].** Exciting results have been observed in studies investigating the creation of ibuprofen-loaded nanoparticles for targeting the colon, including improved treatment outcomes in colon diseases, sustained drug release, and improved bioavailability at the target **[6]**. These developments highlight the potential of nanotechnology to transform local therapies for gastrointestinal diseases by improving drug delivery.
Ibuprofen being a non-steroidal anti-inflammatory medicine (NSAID), reaches its pharmacological

effectiveness by inhibiting non-selectively the cyclooxygenase (COX), which is necessary for the synthesis of prostaglandins production, hence lessening the production of prostaglandins, which serve as the inflammatory mediators causing colonic inflammation. Ibuprofen turns down the inflammation cascade responses in colon by inhibiting both cyclooxygenase isoforms, COX-1 and COX-2. As a result, this all pharmacological activity leads to the mitigation of symptoms associated with colonic diseases that may include IBS,IBD, Colorectal cancer etc. **[7]**.

Efficacy of ibuprofen in colon related problems have been investigated by various clinical trials. A meta-analysis was done to question the efficacy of non-steroidal anti-inflammatory medications (NSAIDs), including ibuprofen, in producing remission in ulcerative colitis patients. The trial revealed that NSAIDs, particularly ibuprofen, were useful in promoting clinical remission in people with mild to moderate ulcerative colitis **[8]**. Additionally, a randomized controlled experiment was conducted by Higgins and he found that ibuprofen induced therapy lessened inflammation and improved clinical presentation in Crohn's disease patients. These results highlight the pharmacological potential of ibuprofen in addressing the symptoms of colonic disorders, therefore stressing its value as a pharmacotherapeutic.

Although use of ibuprofen and other NSAIDs in colonic inflammatory conditions has it's associated side effects with it but still they are not as much as compared to corticosteroids. Moreover, these systemic side effects can be reduced by changing the formulation design and switching to nanoparticle formulation.**[9]**.

When it comes to treating illnesses like colon cancer and irritable bowel syndrome (IBS), nanoparticles provide exceptional benefits [10]. Their small size makes it possible for them to travel through the digestive system quickly and precisely, arriving at the colon. Because of their irregular motility, nanoparticles provide controlled release mechanisms that ensure maintained therapeutic levels in the colon, effectively relieving symptoms in cases of irritable bowel syndrome (IBS). Furthermore, nanoparticles can be engineered to selectively accumulate in tumor tissues during the treatment of colon cancer, improving medication efficacy while reducing systemic side effects. Their versatility in carrying different payloads, like as imaging agents or chemotherapeutic medications, increases their usefulness for monitoring and diagnosing medical conditions **[11]**. Nanoparticles stand out as a viable approach for tackling particular issues in colon-related illnesses because of their focused delivery and customizable qualities. This gives hope for more effective and personalized therapeutic treatments and their utility in diagnostic and therapy monitoring. The objective of our study was to introduce a nanoparticulate drug delivery system that provides controlled delivery of ibuprofen directly targeting colon leading to optimized dosage, reduced side effects and ultimately increasing bioavailability. This approach was implemented to boost effectiveness of treatment of diseases related to colon. Furthermore, the leverage of biocompatible and biodegradable nanoparticles was to enhance targeting and safety ultimately leading to patient compliance.

MATERIALS AND METHOD

Materials

Ibuprofen (≥98%), Tween 80 (72.4 %), Ethyl cellulose (>99%), Dichloromethane (>99.8%) and Methanol (99.90%) were procured from Merck Pakistan. Freshly prepared distilled water was utilized in whole process.

Pre-formulation Studies

Crystallinity

The instrument was calibrated beforehand to ensure accurate measurements. After that, a drop of water was added to the drug sample to create a paste that could be spread. This combination was then equally spread out over an ocular micrometer slide. Finally, the crystallinity of the sample was assessed by looking at its crystal habit under a microscope.

Size Distribution

At first, every sieve was precisely weighted. Then, the finest sieve was placed at the bottom of the stack, followed by the coarsest sieve, in ascending order of mesh size. On top of the stack's top sieve was a sample of the drug. After that, the entire stack was shaken for a certain time to let the particles go through the sieves according to size. The amount of drug that was retained on each sieve and the amount that went through each sieve were measured after shaking. The percentage of drug particles that made it through each sieve was determined using this data, which gave information about the size distribution of the ibuprofen particles.

Melting Point

The melting point instrument was carefully cleaned and calibrated to provide accurate readings. Next, the sample was put into the melting point device. The sample's first melt was noted, revealing the exact temperature at which the drug began to melt. Next, the sample was given time to melt completely, and this was recorded. This demonstrated the temperature range at which the medication completely melted.

Hygroscopicity

Initially, the object's exact weight was determined by weighing an empty, dried china dish. The dry drug sample was also weighed individually to

determine its starting weight. It was also measured how much the dish and the dried medication weighed together. Next, the porcelain dish and the dried drug sample were placed in an absorbent environment: a room with controlled humidity or a dryer with known humidity. The sample was placed in this arrangement and left to absorb moisture from the surrounding air for a predetermined period of time. The cup and the wet drug sample were weighed again after the exposure interval to determine the final drug weight after moisture absorption. The weight differences between ibuprofen before and after exposure to the absorbing media revealed information about its hygroscopicity, or capacity to absorb moisture from the surrounding environment.

Flow Properties

Using the appropriate equipment, the bulk volume of the ibuprofen sample was first determined. After a predetermined number of taps, the sample's tapped volume was determined. Furthermore, the cone method was utilized to ascertain the ibuprofen's angle of repose. The material was spread out freely via a funnel onto a level surface, forming a cone-shaped mound. The slope and surface of the cone-shaped pile were measured to find the angle of repose.

Solubility Analysis

Ibuprofen's solubility was ascertained using the equilibrium solubility method, which involved agitating an excess of the drug in a constant amount of solvent until equilibrium or saturation was reached in a vortex mixer. Following that, Whatman filter paper (No. 1) after which the concentration was measured at 222 nm using a UV spectrophotometer. Ibuprofen's solubility in distilled water and pH throughout the gastric tract were assessed. I. e. pH 1.2, 4.5 and pH 6.8

Partition Coefficient

Ibuprofen was dissolved in 100 mL of phosphate buffer (PH) to create a stock solution. The dosage of the medication was 6 mg. At 0.3 M, the ibuprofen solution's ultimate concentration has been reached. By diluting the stock solution in 0.1 M phosphate buffer, different standard dilutions were made from it to achieve an ibuprofen concentration range of 0.01 - 0.10 M. Next, using a UV spectrophotometer set to 222 nm, the absorbance of these solutions was measured on the same preparation day. A thermometer, a water bath, a magnetic stirrer, and an 800 ml beaker were utilized in the process to determine the octanol/water partition coefficients (P o/w) for Ibuprofen. A 200 ml beaker was filled with about 50 ml of 0.1 M phosphate buffer (pH = 8) and 50 ml of 1-octanol. The beaker was then submerged in water until the solution's temperature reached 37 c^{o}. Ibuprofen, precisely weighed at 6 mg, was added to the solution and allowed to stand for two hours. The two phases that had formed were separated, and the aqueous phases were added to a UV-visible spectrophotometer. Ultimately, the partition coefficients of Ibuprofen between octanol and water were determined.

Method of Formulation Preparation

The method employed for the preparation of colon targeted ibuprofen nanoparticles was solvent evaporation method. Ethyl cellulose was dissolved in mixture of dichloromethane and methanol (1:1), each solvent of 5ml. Then ibuprofen was dissolved in it. This solution of drug and polymer was added drop-wise into freshly prepared 0.1% aqueous Tween 80 solution at 1500 rpm. Stirring was continued until the organic solvents mixture had evaporated. Resulting solution containing Gel like fibers was heated and homogenized (at 1500 rpm) for breaking fibers. Nanoparticles were recovered by centrifuging at 3000 rpm, filtering, washing and drying

Post Formulation Evaluation

Surface Morphology Studies

The nanoparticles were added in deionized water and were subjected to sonication for a period of thirty minutes. A circular metal plate was used with carbon double tape. A drop of the nano dispersion was applied to the double tape (1mm×1mm) and left to dry. The morphology was scanned using a Hitachi S-3700N scanner from Japan.

Determination of Particle Size

Nanoparticle Tracking Analysis (NTA), is a popular technique for determining, visualizing and analyzing the nanoparticles in detail. It measures the Brownian motion of the particles, which is related to their size. The nanoparticle suspension was diluted in a suitable solvent to ensure individual particles and minimize interactions. The Zetasizer was adjusted to measure the above prepared sample by setting temperature at 25°C and refractive index was also set at 1.550. This is important for accuracy of the process. After we inserted the prepared sample in the Zetasizer, it automatically tracked the Brownian motion of particles for te size distribution analysis. Collected data was used to calculate the autocorrelation function and by putting all above into consideration the hydrodynamic diameter and size distribution of the nanoparticles were determined.

Determination of Zeta Potential

Zeta potential is a crucial parameter that reflects the surface charge of nanoparticles suspended in a liquid. The nanoparticle suspension was diluted in an appropriate electrolyte solution. The electrolyte helps conduct electricity and screen charges, improving measurement accuracy. The suspension was placed in a special cell with an electric field applied. Charged nanoparticles migrate towards the electrode with opposite polarity. The velocity of the moving

nanoparticles was measured using the electrophoretic mobility (velocity per unit electric field) was used to calculate the zeta potential using the Smoluchowski equation

Percentage Yield / Contents Uniformity

For the estimation of percentage yield, weighed dried nanoparticles in g (actual yield) and then divide it with the theoretical yield (wt. of drug+polymer), multiplied it with 100, as given below in formulae:

$$percentage\ yield = \frac{wt.of\ nanoparticles}{wt.of\ drug + polymer} \times 100\%$$

By applying the given formula, as the actual yield 0.121g and theoretical yield 0.13g, so the calculated percentage yield obtained was 93%. From this, it can be concluded that this method was very useful for the formulation of ibuprofen nanoparticles.

Content Uniformity

For the determination of content uniformity, first step was to prepare a sample, for this,100mg or 0.1g of nanoparticles weighed, then, dissolved the nanoparticles in 10ml of phosphate buffer (PH 7.4) by vortex mixing and then, filtered it to remove the impurities. The next step was to measure the absorbance at 222nm by using UV-Vi's spectrophotometer. As 100% percentage yield contains 50mg of drug, so for 16% contains 8mg of drug content/amount. From the data, it was shown that 121 mg of nanoparticles contain 8mg of ibuprofen. For 100mg or 0.1g of nanoparticles, obtain its ibuprofen content (i.e. 6.6mg). Then the concentration per ml calculated based on the above calculation. Calibration curve made for the known concentrations of ibuprofen solution, then the absorbance measured of each standard solution and plot a calibration curve using the equation:

$$y = mx + b$$

Then dilutions made at a ratios of 1:10, 1:100, and 1:1000 in phosphate buffer solution in order to obtain it concentrations within the range of the calibrated curve. The absorbance of each diluted sample measured using UV-vis spectrophotometer. At last, the ibuprofen content measured by using the formulae:

$$Ibuprofen\ content = conc.\left(\frac{mg}{ml}\right) \times dilution\ factor$$

% yield also calculated for each of the dilutions by measuring ibuprofen amount in sample with the amount in diluted samples. Then compare the %yields of each of the diluted sample solutions. The higher the % yield means the effectiveness of this method for the ibuprofen nanoparticles formulation.

In-Vitro Drug Release

In vitro dissolution studies were conducted for ibuprofen nanoparticles to analyze percentage of drug release in specified time intervals. For that USP dissolution test apparatus II (paddle type) was used. 300ml of phosphate buffer (pH 7.4) was prepared using distilled water. A dialysis sack containing fixed quantity of formulation was tied to paddle of dissolution apparatus. Constant temperature (37 ± 0.5°C) was provided. Samples of 5ml were taken after intervals of 0.5, 1,2,4,6 and 8 hours and 5ml buffer was poured back in basket every time after withdrawing sample. Dilutions were made by adding 9ml of Phosphate buffer of pH 7.4 to each sample. Absorbance was observed of these dilutions using UV spectrophotometer at 400nm.

Kinetic Modeling of Drug Release

Calibration curve was obtained by using UV absorbance. Then theoretical yield was calculated and percentage release of six formulations was calculated. The curve was obtained against time in hours and percentage release of formulations. Different kinetic models from dd solver were applied to check release kinetics of our formulations. The models applied were Zero-order, First-order, Higuchi model, Korsmeyer Peppas and Hixson-Crowell.

Encapsulation Efficiency

An easy and effective technique was used to encapsulate the pre-made ibuprofen nanoparticles to improve their stability and bioavailability. The encapsulation efficiency of ibuprofen in ethyl cellulose nanoparticles was evaluated using UV-Vi's spectroscopy. The nanocarriers were purified through centrifugal filtering to remove un-encapsulated medication. After two further filtration washings, around 100 µL of concentrated product was left over. The product was then placed in a glass vial, dried overnight in a vacuum chamber, and resuscitated in an organic solvent to dissolve the nanocarriers and release the medicine. Standard curves for each component in the organic solvent were created before usage, and solvents were chosen to avoid interference with the medicinal molecule's spectral peak.

$$encapsulation\ efficiency(EE) = \frac{drug(encapsulated)(mg)}{drug(added)(mg)} \times 100$$

Infrared Spectroscopy (IR) and Differential Scanning Calorimetry (DSC)

With the use of an FTIR spectrometer (WQF 520, Beijing Rayleigh Analytical Instrument Corporation, BRAIC China), the spectra of pure ibuprofen, ethyl cellulose, and ibuprofen loaded ethyl cellulose nanoparticles were captured. Using a potassium bromide (KBr) press, the pellets were made, and spectra covering the wave number range of 4000 to 400 cm−1 were recorded. The Perkin-Elmer DSC-7 model was utilized to conduct DSC scans on ethyl cellulose nanoparticles that were loaded with ibuprofen. To calibrate the device, indium was used. Drier nitrogen was used as the exhaust gas while all five milligram samples were heated in aluminum

pans. 10°C/min heating over a 30-230°C range was used for the analysis.

RESULTS AND DISCUSSION

Pre-Formulation Studies

Ibuprofen's crystalline structure, which displays needle-like or prismatic forms, suggests a clearly defined crystal lattice arrangement **[12]**. The medication does not readily absorb moisture, which helps to keep it stable and prolongs its efficacy and shelf life. Ibuprofen's low bulk density of 0.378 g/cc and tap density of 0.609 g/cc indicate that its flow qualities are noticeably poor **[13]**. These poor flow characteristics are further confirmed by the 38% Carr's index and the 1.61 Hausner's ratio. Furthermore, the angle of repose, which is 49.57 degrees, indicates that handling and processing issues may arise in creating a stable cone of ibuprofen powder **[14]**. Ibuprofen has a melting point that ranges from 75 to 78°C, which is consistent with its recognized characteristics and a crucial factor to take into account while formulating the medication and its thermal stability **[15]**. To ensure constant drug content and acceptable dissolution profiles, it is imperative to do additional study on the size distribution in the 200-450 micron range. This will help to understand particle size homogeneity and suitability for formulation into solid dosage forms **[16]**. Ibuprofen's Log P was measured using the shake-flask method as 1.56, suggesting a preference for the organic octanol phase over water. This is consistent with its lipophilic nature because of the carboxylic acid group, which facilitates absorption in living organisms. The value, however, is less than the literature's estimates (2.48-3.6). The disparity may be explained by a short shaking period and slow equilibration kinetics. Log P values for ibuprofen could be more accurate if the procedure was optimized by adding more mixing time, changing the solvents, or employing different methods **[17]**. Interesting details about ibuprofen's behavior in various pH environments, including 1.2, 6.8, and 7.4, are revealed by the solubility analysis of the drug. The significant increase in solubility seen at higher pH values emphasizes how pH affects ibuprofen's solubility; the drug's ionization is probably responsible for the increased dissolution rates. The solubility's dependence on pH has noteworthy consequences for pharmaceutical formulations, as preserving sufficient solubility is essential for medication effectiveness and bioavailability. The solubility of ibuprofen at acidic pH and acetate buffer at pH 1.2 is notably low, which poses a challenge to drug delivery and may result in irregular absorption kinetics and reduced therapeutic efficacy. Optimizing the delivery of ibuprofen and improving its clinical utility may be possible by addressing these issues with novel formulation techniques like pH adjustment or salt formation **[18]**.

Table 1: Bulk characterization.

Solubility solvent	Concentration (µg/ml)
Water	21
Acetate buffer pH 1.2	70
Phosphate buffer pH 6.8	200
Phosphate buffer pH 7.4	310

Table 2: Solubility analysis through UV spectrophotometer.

Parameters	Outcomes
Crystallinity	Needle or prismatic shape
Hygroscopicity	Non hygroscopic
Flow properties	Very poor flow properties Bulk density ….0.378 g/cc Tap density….0.609 g/cc Carr’s index (%) ….38% Hausner’s ratio….1.61 Angle of repose….49.57
Melting point	75-78 °C
Size distribution	200-450 microns

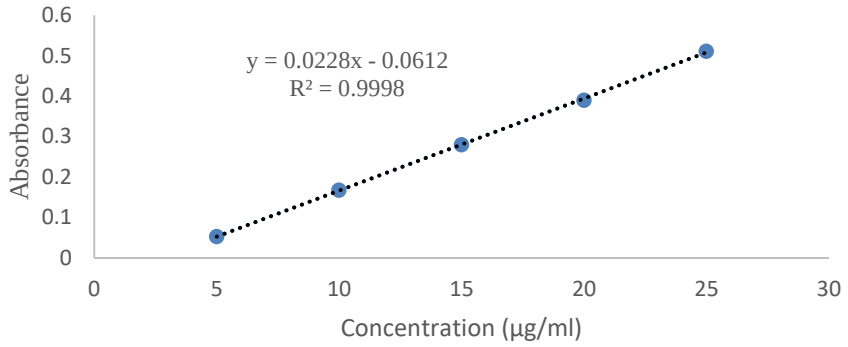

Figure 1: Standard calibration curve.

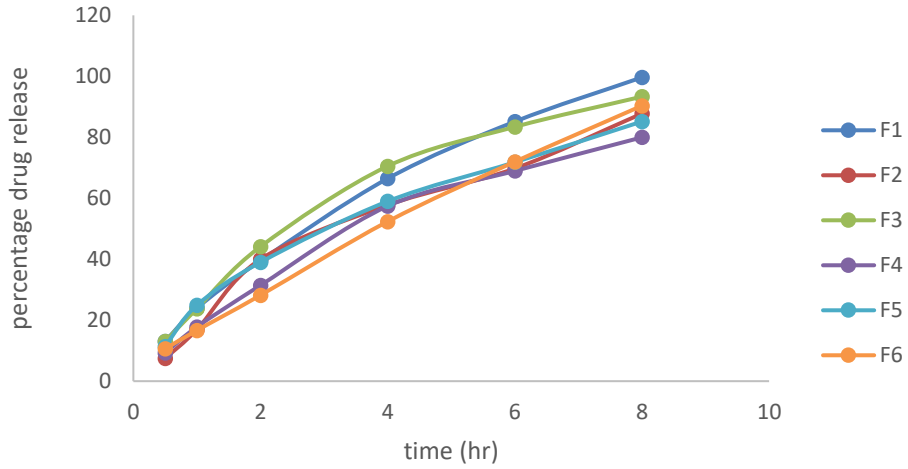

Figure 2: Drug release of formulations of ibuprofen nanoparticles.

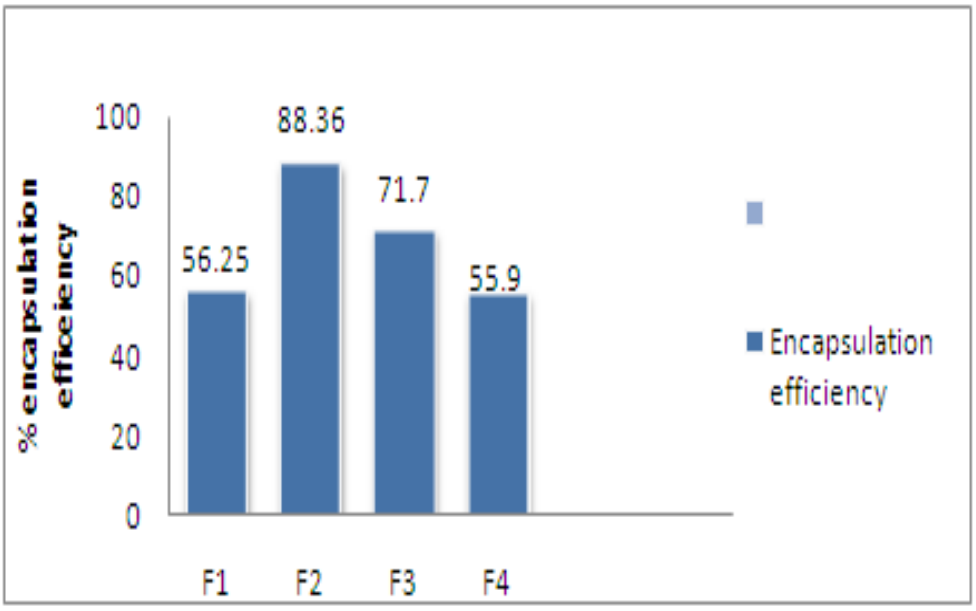

Figure 3: Encapsulation efficiency.

Table 3: Kinetic analysis.

Formulation	Zero-order F=k0*t			First-order F=100*[1-Exp(-k1*t)]			Higuchi F=kH*t^0.5			Korsmeyer-Peppas F=kKP*t^n				Hixson-Crowell F=100*[1-(1-kHC*t)^3]		
	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	N	R^2	AIC	MSC
F1	0.91	39.65	2.09	0.98	30.88	3.55	0.95	36.14	2.67	1.00	22.40	4.96	0.68	0.99	22.57	4.94
F2	0.91	38.66	2.03	0.98	27.76	3.85	0.93	37.08	2.30	0.98	31.68	3.20	0.69	0.98	28.66	3.70
F3	0.83	43.03	1.42	1.00	17.07	5.74	0.96	34.45	2.85	0.98	32.02	3.25	0.61	0.99	22.19	4.89
F4	0.91	37.85	2.02	1.00	15.11	5.81	0.94	35.33	2.44	0.99	28.40	3.60	0.69	0.99	23.76	4.37
F5	0.84	40.95	1.47	0.99	23.37	4.40	0.97	30.46	3.22	0.99	24.78	4.17	0.61	0.97	30.40	3.23
F6	0.98	30.24	3.50	0.97	31.76	3.24	0.90	39.18	2.01	1.00	11.28	6.66	0.81	0.99	25.48	4.29

Post Formulation Parameters

Surface Morphology Studies

All formulations exhibited spherical shape and were in the nano range. SEM scans of F1 formulation showed smooth, spherical particles with little porosity **[19].**

Percentage Yield

The successful and efficient synthesis process used for synthesizing ibuprofen nanoparticles is indicated by the high percentage yield of 93%. A high yield percentage means that a significant amount of the desired product—in this case, nanoparticles—was achieved throughout the production or chemical process. This is a good outcome since it shows that the procedure followed was successful in transforming the polymer and medication starting components into the required nanoparticles. For the manufacturing of nanoparticles to be both cost-effective and versatile, the synthesis method's efficiency is essential. A lesser amount of material is wasted when there is a high percentage yield, which lowers production costs and increases the process's viability for large-scale manufacturing. A high yield also suggests that fewer resources are needed to produce the necessary amount **[20]**.

Content Uniformity

A crucial factor in pharmaceutical formulations that guarantees consistency and dependability in medication dose is content homogeneity. In our study, UV spectroscopy was used to evaluate the content homogeneity of ibuprofen nanoparticles. The procedure comprised dissolving a certain quantity of nanoparticles in phosphate buffer, assessing UV absorbance, and connecting it to the concentration of ibuprofen. Ibuprofen content in various dilutions could be measured using the calibration curve produced by the yield calculation, which was based on UV absorbance at 222 nm. We assessed content homogeneity by computing the concentration of ibuprofen in several dilutions. A content uniformity of 96% shows that ibuprofen is distributed consistently throughout samples in the nanoparticles. This is essential to reduce the possibility of under- or overdose, guarantee that each dosage contains the prescribed amount of the active substance **[20]**.

Encapsulation Efficiency

A high drug loading capacity and successful ibuprofen loading were shown by the amazing 88.36% encapsulation efficiency of ibuprofen nanoparticles in this investigation, which was much higher than previously reported values. These nanoparticles are a promising drug delivery system because of their high encapsulation efficiency, which might allow them to deliver enough ibuprofen to the intended location for better therapeutic efficacy. They might also lower dosage frequency, increasing patient compliance and minimizing side effects.

Determination of Particle Size

The average hydrodynamic diameter of 586.9 nm aligns with what we anticipated, and the narrow size distribution (PDI = 0.15) suggests a high degree of control over particle size (**Fig 4)**. The low PDI (0.15) signifies a monodisperse population, a significant advantage for many applications. Consistent particle size is crucial for predictable behavior in areas like drug delivery, catalysis, or electronics. For instance, monodisperse nanoparticles in drug delivery ensure uniform drug release rates and improve targeting efficiency.

Zeta Potential Measurement

While a zeta potential of exactly 0 mV indicates no net surface charge, particles in this state tend to aggregate readily due to weak repulsive forces. For stable suspensions, we generally aim for an absolute value greater than ±30 mV. Our result of -49.8mV signifies a strong repulsive force between the nanoparticles due to their like charges, preventing them from clumping together (**Fig 5).**

In-Vitro Drug Release

In vitro drug release investigations are critical for determining the consistency of the rate and duration of drug release. Determining the role of polymer breakdown in drug release from nanoparticles is critical for ensuring controlled release performance. In vitro diffusion experiments help in estimating the rate of drug release and extent of drug release from nanoparticles. From the dissolution profiles in **Fig 2**, it can be seen that the percent release of ibuprofen increases with time, indicating a gradual release of drug from nanoparticles.

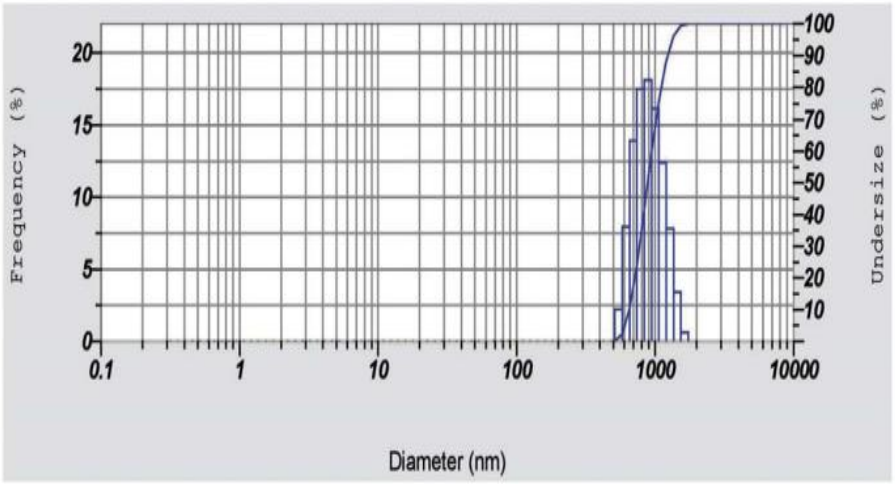

Figure 4: Mean particle diameter report of F1 formulation.

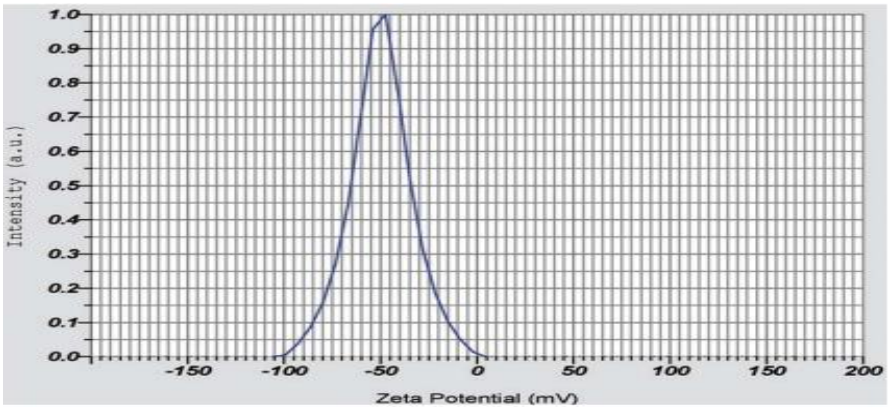

Figure 5: Zeta potential report of F1 formulation.

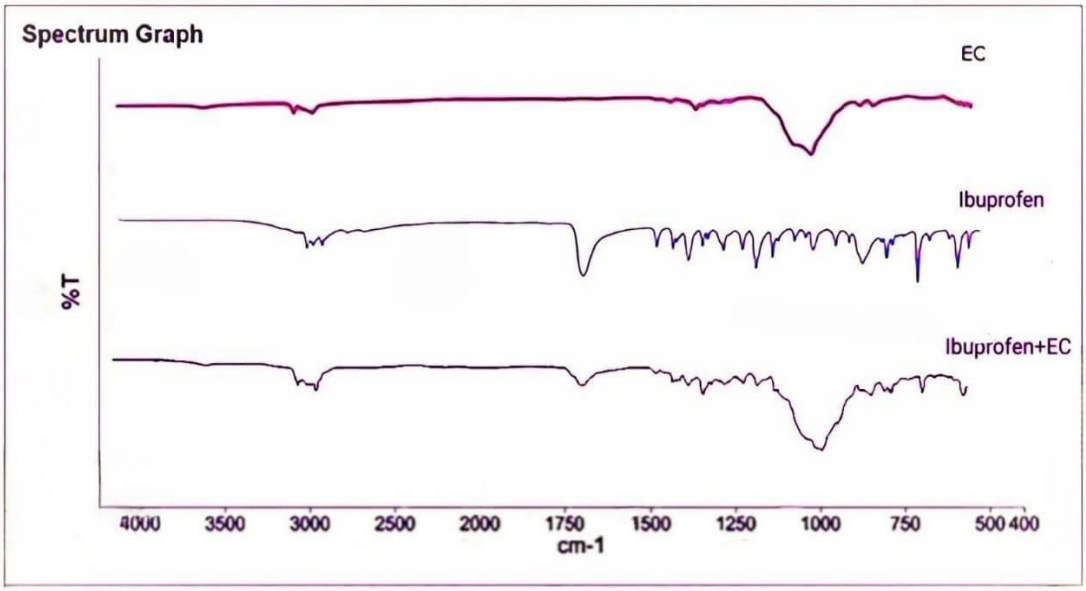

Figure 6: FTIR of ibuprofen, ethylcellulose, ibuprofen plus ethylcellulose.

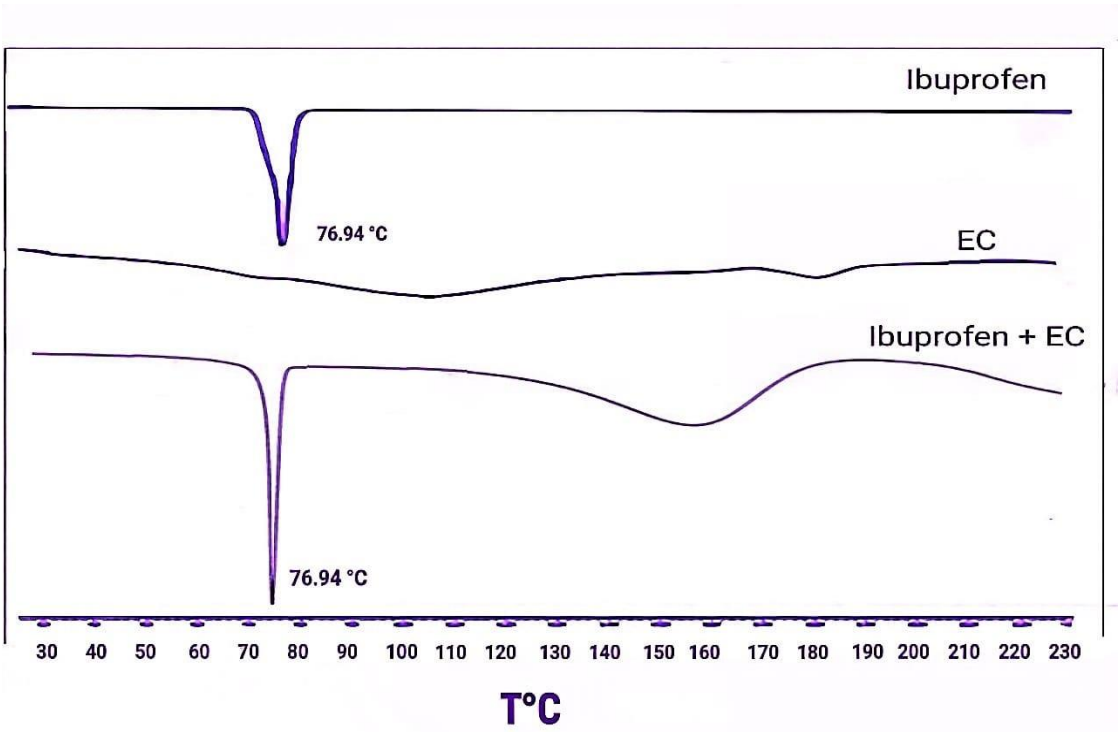

Figure 7: DSC analysis of Ibuprofen, ethylcellulose, Ibuprofen plus ethylcelullose.

Drug Release Kinetics

Our investigation of drug release kinetics from colon-targeted ibuprofen nanoparticles included the use of the DD solver program, which allowed us to analyze a variety of models such as zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell (**Table 4**) **[21]**. Zero-order kinetics demonstrate a steady drug release rate throughout time, regardless of concentration, indicating a controlled release pattern that is critical for therapeutic optimization. In contrast, first-order kinetics reveals concentration-dependent fraction of drug release per unit time. The observed increase in drug elimination with increasing concentrations emphasizes the importance of dose-response interactions in drug delivery systems. The Higuchi Model characterizes drug release as a diffusion process based on Fick's Law, with release being dependent on square root of time and closely resembling zero order kinetics being independent of concentration **[22, 23]**. The Korsmeyer-Peppas Model is specifically tailored to describe drug release from polymeric systems **[24]**.

Formulation 1's Zero-order model had R^2 of 0.9114, an AIC of 39.6482, and an MSC of 2.0901, which did not match the required requirements ($R^2 \geq 0.95$ and MSC > 3.5), suggesting inadequate alignment with this model. The First-order kinetics model, on the other hand, demonstrated high adherence, with a R^2 of 0.9794, an AIC of 30.8803, and an MSC of 3.5514, indicating a release mechanism influenced by drug concentration as well as driven by dissolution kinetics **[25]**. The Higuchi model demonstrated insufficient alignment, with R^2 (0.9506), AIC (36.1401), and MSC (2.6748) values with MSC falling below the specified requirements, indicating a deviation from this model. Formulation 1 has a good fit to the Korsmeyer-Peppas model, with a R^2 of 0.9964, an AIC of 22.3998, and an MSC of 4.9648, meeting the criteria. The n value : 0.679, derived from Korsmeyer-Peppas model, implies a non-Fickian diffusion mechanism, which means that release is influenced by both diffusion and dissolution mechanisms. Similarly, conformance to the Hixson-Crowell model, with R^2 = 0.9949, AIC = 22.5746, and MSC = 4.9357, indicates a diffusion-controlled mechanism, possibly depending on change in surface area over time. The overall trend suggests that formulation follows controlled release pattern, which is most likely attributed to nanoparticles dissolution and subsequently diffusion of ibuprofen. The high adherence to first-order kinetics, Korsmeyer-Peppas model, and Hixson-Crowell indicates that the drug release involves combination of diffusion and dissolution mechanisms. Additionally, the non-Fickian diffusion process derived from the Korsmeyer-Peppas kinetic model shows that release of ibuprofen is modulated by both diffusion through matrix and drug particle disintegration **[26]**.

The drug's release kinetics in Formulation 2 reflect a complicated interaction of components. While the zero-order model did not fit well, the formulation

with R^2, AIC, and MSC values of 0.9064, 38.6618, and 2.0349, respectively, with AIC lying outside the normal acceptable range. Formulation did follow first-order kinetics model, indicating a decrease in ibuprofen's release rate over the course of time owing to drug concentration and dissolution kinetics **[27].** Furthermore, it revealed compatibility with the Korsmeyer-Peppas model as evidenced by R^2 value of 0.9791 and AIC value of 31.6762, implying a release mechanism controlled by diffusion and dissolution processes. The Hixson-Crowell model also postulated a diffusion-controlled process.

Formulation 3 also showed good adherence to the first-order model, with parameters like R^2, AIC, and MSC 0.9977, 17.0694, and 5.7437, respectively, all coming within the specified required range. Also it differed from the Zero-order and Higuchi models. This formulation closely followed the Korsmeyer Peppas model, with R^2, AIC, and MSC values: 0.9801, 32.0227, 3.2515 respectively, meeting the required criteria of specifications, indicating anomalous diffusion, as evidenced by the 'n' value (0.611).

Formulation 4 deviation from the zero-order with R^2: 0.9052, AIC: 37.8453 and MSC: 2.0223 and Higuchi model with R^2: 0.9376, AIC:35.3325 and MSC: 2.4411 highlighting the complexities of drug release. Given this, alignment with the first-order model implies a controlled release in which the rate of drug release declines exponentially over time, most likely governed by parameters such as drug concentration and dissolution kinetics. The 'n' value (0.686), which is characteristic of a non-Fickian diffusion process, demonstrates the complexities of ibuprofen transport inside the formulation matrix.

Furthermore, compliance to the Hixson-Crowell model as evidenced by R^2, AIC, and MSC values; 0.9909, 23.7567, 4.3704 respectively, confirms the diffusion-controlled drug release, where over time, change in surface area influences drug release kinetics.

As formulation 5 is showing good adherence to first order model, Korsmeyer- Peppas model but not all parameters of zero order, Higuchi model and Hixon Crowell are in required specifications which indicate the drug release in this situation is likely a complicated interaction of diffusion, polymer swelling, and erosion, resulting in non-linear release kinetics of formulation over time[19]. in case of formulation 6, it is inconsistent with the Higuchi model, but Given the simultaneous following of zero-order, first-order, Hixson-Crowell, and Korsmeyer-Peppas models with n value 0.810, indicates non-Fickian diffusion mechanism or anomalous , the drug release mechanism is likely to be complex, involving factors such as dissolution, diffusion through the matrix, erosion, as well as swelling of the polymer matrix. However, among all these formulations following similar release kinetics, Formulation 1's 99% drug release highlights its value as an optimal formulation for colon targeting of nanoparticulate ibuprofen.

Stability Analysis

DSC Analysis

Using Differential Scanning Calorimetry (DSC), the relationship between ibuprofen and ethyl cellulose was investigated. DSC examines thermal characteristics to find alterations in a mixture's physical state or interactions. At 76.94°C, pure ibuprofen showed a sharp endothermic peak that indicated its melting point. There was no discernible shift in the peak when ethyl cellulose was added, indicating a lack of meaningful interaction. This is consistent with FT-IR analysis, which shows that there were no significant chemical interactions during encapsulation. The fact that the endothermic peak of ibuprofen has not been affected by encapsulation indicates that its thermal behavior remains intact **[28]**.

FTIR Analysis

Key structural features and compatibility were revealed by Fourier Transform Infrared (FT-IR) analysis of ibuprofen nanoparticles targeted towards the colon and coated with ethylcellulose. Ibuprofen's presence and molecular integrity following nanoparticle formation were confirmed by characteristic peaks, including a strong band at 1720 cm^-1 representing carbonyl stretching of the isopropionic acid group and a peak at 3000 cm^-1 indicating hydroxyl stretching vibration. Notably, ethylcellulose coating did not significantly alter the distinctive peaks of ibuprofen, suggesting that the drug's chemical structure was preserved. The absence of discernible changes indicates that ibuprofen and ethylcellulose interact chemically very little. This positive result implies that ibuprofen nanoparticle coating can be effective without sacrificing its integrity. This compatibility is encouraging for drug delivery targeted at the colon because sustained-release formulations often contain ethylcellulose, which provides protection against stomach breakdown. Ibuprofen nanoparticles are guaranteed to retain their essential characteristics upon colon delivery, thanks to FT-IR analysis **[28]**.

CONCLUSION

The formulation F1 concluded, at 1500 rpm, 0.1% W/V Tween 80 stabilizer and 1:10 organic to aqueous ratio, as an optimized and stable preparation for colon targeted ibuprofen nanoparticles. It could be, being cost effective with its remarkable enhancement of bioavailability, more appropriate choice for subsequent clinical trials and marketing.

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