FORMULATION AND CHARACTERIZATION OF CHRONO MODULATED COLON TARGETED DRUG DELIVERY OF VEDOLIZUMAB (ENTYVIO) DRUG

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Abstract
This study focuses on the development and characterization of a novel chrono-modulated colon-targeted drug delivery system for Vedolizumab (Entyvio), an important drug used for inflammatory bowel disease. The formulation aims to optimize drug release timing to synchronize with the circadian rhythm of the gastrointestinal tract, enhancing therapeutic efficacy and reducing potential side effects. The formulation process involves the encapsulation of Vedolizumab in specialized carriers designed to delay drug release until reaching the colon. Various physicochemical parameters, such as bulk density, tapped density, compressibility index, and angle of repose, are evaluated to ensure optimal formulation characteristics. In vitro dissolution studies are conducted to assess drug release profiles at different time intervals, mimicking the circadian rhythm. Results demonstrate the successful development of the chrono-modulated formulation, with promising delayed drug release in line with the desired timing for colonic drug delivery. Variations in drug release kinetics among batches highlight the need for rigorous quality control measures. Additionally, accelerated stability studies suggest the formulation's stability under defined conditions, although further investigations are warranted to assess long-term stability. This research contributes to the advancement of targeted drug delivery systems and offers potential benefits for patients requiring Vedolizumab therapy. The findings underscore the importance of optimizing drug release timing for enhanced therapeutic outcomes and serve as a foundation for future studies in the field of chrono-modulated drug delivery systems.

Keywords: Vedolizumab, inflammatory, chrono-modulated, drug delivery systems.

Introduction to Colon-Targeted Drug Delivery

Colon-targeted drug delivery is a specialized approach in the field of pharmaceuticals that aims to deliver therapeutic agents directly to the colon for the treatment of various local and systemic diseases. The colon, being the terminal part of the gastrointestinal tract, presents unique challenges for drug delivery due to its selective permeability, acidic pH, and the presence of gut microbiota. Conventional drug delivery systems often face limitations in achieving effective drug concentrations in the colon, leading to reduced therapeutic outcomes and increased side effects.[1,2]

The need for colon-targeted drug delivery arises from the prevalence of diseases that specifically affect the colon, such as inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, as well as colon cancer. Additionally, many drugs intended for systemic conditions may have adverse effects when released in the upper gastrointestinal tract, necessitating site-specific targeting to the colon.[3]

Chrono-modulated drug delivery is a promising technique used to regulate drug release at specific times, ensuring optimal therapeutic levels during the desired period of action.[4] This approach takes advantage of the circadian rhythm and biological variations in the colon, which exhibit different physiological conditions at various times of the day. By delivering drugs in a time-controlled manner, chrono-modulated drug delivery systems can maximize drug efficacy while minimizing side effects.[5]

Targeted drug delivery systems have emerged as a crucial area of research and development in the field of pharmaceutical sciences, driven by the need for enhanced therapeutic efficacy, reduced side effects, and improved patient compliance. Conventional drug delivery methods often lack the ability to selectively deliver drugs to the desired site of action, leading to suboptimal treatment outcomes and systemic toxicity. This limitation is particularly evident in the treatment of complex diseases, such as cancer and chronic conditions affecting specific organs or tissues.[6]

One of the primary reasons for the demand for targeted drug delivery systems is the ability to achieve site-specific drug release.[7] By directing the therapeutic agents to the exact location of the disease or the intended target within the body, these systems can significantly increase drug concentration at the site of action while minimizing

exposure to healthy tissues. This localization of drug delivery not only enhances the drug's effectiveness but also reduces the risk of adverse effects, improving patient safety and tolerability.[8]

Tiwari et al.,(2012) reported that "Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. For the treatment of human diseases, nasal and pulmonary routes of drug delivery are gaining increasing importance. [9] Tewabe et al.,(2021) reported that "Nanomedicine is an advanced version of Paul Ehrlich's "magic bullet" concept. Targeted drug delivery is a system of specifying the drug moiety directly into its targeted body area (organ, cellular, and subcellular level of specific tissue) to overcome the aspecific toxic effect of conventional drug delivery, thereby reducing the amount of drug required for therapeutic efficacy.[10] Kwon et al.,(2012) reported that "Targeted drug delivery to tumor sites is one of the ultimate goals in drug delivery. Recent progress in nanoparticle engineering has certainly improved drug targeting, but the results are not as good as expected. This is largely due to the fact that nanoparticles, regardless of how advanced they are, find the target as a result of blood circulation, like the conventional drug delivery systems do.[11] Ali et al.,(2019) reported that "Targeted drug delivery system improves the efficiency and safety of the therapeutic agents by managing the pharmacokinetics and pharmacodynamics of drugs. Currently, numerous drug carrier systems have been developed with different sizes, architectures and characteristics surface properties.[12]

Erkoc et al.,(2015) reported that "In this overview, recent developments on stimuli sensitive systems for targeted drug delivery and various delivery routes have been described. Novel technologies used to address challenges associated with the delivery of large drug molecules along with clinical applications have been presented."[13]

The aim of this study is to formulate and characterize a chrono-modulated colon-targeted drug delivery system for Vedolizumab (Entyvio) to optimize its release profile, improve therapeutic efficacy, and minimize systemic side effects.

Drug Profile

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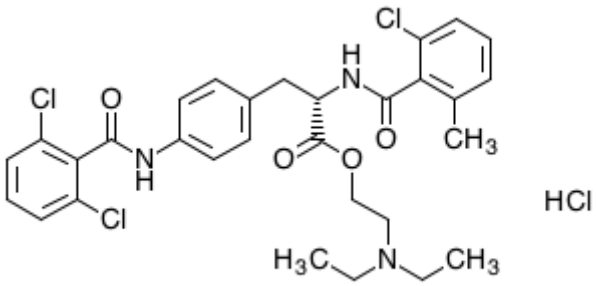
DRUG	VEDOLIZUMAB
Chemical structure	 D10028
Chemical Formula	**C6528H10072N1732O2042S42**
Generic Name	**Vedolizumab**
Half-life	**Vedolizumab has a long terminal elimination half life of 336 to 362 hr.**
Clearance	**Vedolizumab has a low clearance of 0.180 to 0.266 ml/hr/kg.**

Molecular Weight	**Approximately 147 kDa**
Route Of Administration	**Intravenous (IV) route.**

Material & Method

Evaluation of Blend Flow Properties

Bulk density

- Weigh an empty and dry graduated cylinder or measuring cup (M1) accurately and record the weight.
- Carefully pour the powder into the graduated cylinder using a funnel until it reaches the desired volume.
- Gently tap the cylinder to settle the powder and remove any air gaps. You can use your hand or a tapping apparatus.
- Weigh the cylinder with the settled powder (M2) and record the weight.
- Calculate the bulk density using the formula:

Bulk Density (g/ml) = (M2 - M1) / Volume of the cylinder (ml)

Tapped density

Tapped Density Measurement:

- If the bulk density measurement was performed, transfer the powder from the graduated cylinder to the tapping apparatus.
- Set the tapping apparatus to tap the container for a specific number of taps (e.g., 1000 taps).
- After tapping, weigh the container with the tapped powder (M3) and record the weight.
- Calculate the tapped density using the formula:

Tapped Density (g/ml) = (M3 - M1) / Volume of the cylinder (ml)

Compressibility Index:

The Compressibility Index is calculated using the tapped density and bulk density of the powder. It provides valuable information about the powder's cohesion and how it will behave during processes like tabletting, capsule filling, and powder handling. The formula to calculate the Compressibility Index is:

Compressibility Index (%) = [(Tapped Density - Bulk Density) / Tapped Density] × 100

Where:

- Tapped Density is the density of the powder after tapping or vibration (g/ml).
- Bulk Density is the density of the powder in its loose, untapped state (g/ml).

Hausner's Ratio

Hausner's Ratio is a measure of the powder's flowability and compressibility. It is calculated by dividing the tapped density by the bulk density of the powder. The formula for Hausner's Ratio is as follows:

Hausner's Ratio = Tapped Density / Bulk Density

Where:

- Tapped Density is the density of the powder after tapping or vibration (g/ml).
- Bulk Density is the density of the powder in its loose, untapped state (g/ml).

Angle of repose

The procedure for measuring the Angle of Repose involves allowing the powder to flow through a funnel onto a flat surface until a conical pile is formed. The height and radius of the pile are measured, and then the Angle of Repose is calculated using the formula:

Angle of Repose (θ) = arctan (h / r)
Where:
h is the height of the conical pile (cm)
r is the radius of the conical pile base (cm)

Preparation of core Tablet Blends

Accurately weighed quantities of drugs and excipients were passed through sieve no. 20 and 40, respectively. Drug and excipients were added in geometric proportions in a polybag and mixed thoroughly. The lubricants were finally added to the blends to get the lubricated blend of flurbiprofen. The flow properties of the blends were evaluated. Formulation of Vedolizumab core Tablets Vedolizumab core tablets were formulated using direct compression method.

Formulation Table For Core Tablets

Ingredients (mg)	Batch -1	Batch -2	Batch -3	Batch -4
Vedolizumab	75	100	75	100
Microcrystalline Cellulose	75	75	75	75
Crospovidone	5	4	5	4
Magnesium stearate	1.5	1.25	1.25	1.5
Talc	2	2.25	2.5	2.25

Characterization of core Tablets

Weight Variation: The weight variation was carried out by weighing 20 randomly selected core-tablets from each batch. The average weight was calculated and compared with the individual core-tablet weights.

Thickness: Ten tablets were randomly selected from each batch and the thickness of each individual tablet was measured using a Digital Vernier Calliper.

Hardness: Six tablets were randomly selected from each formulation and the hardness of each tablet was determined using Pfizer hardness tester. It was expressed in kg/cm 2.

Drug Content: The mini tablets were powdered. Powder equivalent to the dose of one capsule was weighed accurately. Powder equivalent to the dose of Vedolizumab was weighed and transferred to a 100 ml volumetric flask and volume was made up using methanol. Further, 1 ml of this solution was withdrawn and transferred to a 10 ml flask and volume was made up using methanol. Further dilutions were done suitably and UV absorbance was taken at 247 nm.

Colon Targeted Drug Delivery Systems

Final colon targeted drug delivery system were formulated using C3 coating formula. Formulation C3 from coating system was selected. The selected formulations of SMEDDS (liquids SMEDDS, slurry SMEDDS) and mini tablets were filled in ethyl cellulose coated capsules(C3) to prevent the release of drug in stomach and small intestine and finally release the drug in colon. The prepared systems were then subjected to evaluation for drug content and in vitro release. Colon targeted drug delivery formulations are as shown in

Colon Targeted Drug Delivery Formulations

Formulations	Core:coat ratio	Coat Weight	MCC
Batch -1	1:1	100	20
Batch -2	1:1.25	125	25
Batch -3	1:1.5	150	30
Batch -4	1:1.75	175	35
Batch -5	1:2.0	200	40

Drug Content:
Coated capsule containing drug equivalent to 100 mg formulation was placed into a 100 ml volumetric flask and volume was made up using methanol to give 1000 µg/ml solution. This solution was shaken for 15 min until the drug dissolved in the solvent.

In vitro Dissolution Studies
The prepared capsules were subjected to in-vitro dissolution studies using an 8 station USP (type 1) basket dissolution apparatus. The dissolution studies were carried out in 0.1 N HCl pH 1.2 for 2 hrs, in phosphate buffer pH 7.4 for 3 hrs and in phosphate buffer pH 6.8 for next 6 hours at 37±0.5°C & rotation speed was maintained at 50 rpm. At regular time interval of 1 hour, 5 ml of sample was withdrawn from the dissolution medium & replaced with equal volume of fresh medium. The sample withdrawn was subjected to UV-Visible spectrometry in shimadzu UV-1800 spectrophotometer for determination of drug release. Absorbance was measured at 247 nm for 0.1 N HCl pH 1.2, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 respectively.

Release Kinetics: The rate and mechanism of release of enteric coat based capsule and ethyl cellulose coated capsule of flurbiprofen were analyzed by fitting the dissolution data into various kinetic models.

Zero-order Equation: Zero-order release kinetics, cumulative amount of drug released vs time and the release rate data are fitted to the following equation:

$C = K_0 .t$

First Order Equation: First-order release kinetics, log cumulative percentage of drug remaining vs time and the release rate data are fitted to the following equation:

$C = 100 \times (1 - e^{-Kt})$

Higuchi's Equation: The Higuchi release, cumulative percentage of drug released vs square root of time and the release rate data are fitted to the following equation:

$Q = Kt1/2$

Where, K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Stability Studies: Stability of dosage form has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specification.

Procedure
From the batches of Vedolizumab colon targeted formulations, optimized formulation F2 and F3 were evaluated for stability studies as per ICH guidelines. Optimized formulations were stored at 40°C ± 2°C/75 ± 5% RH (accelerated stability conditions) for 3 months. The capsules were evaluated for weight variation, appearance and in vitro drug release after storage for 3 months. The values for in vitro drug release were calculated and were compared for change in dissolution profile.

Result & Discussion

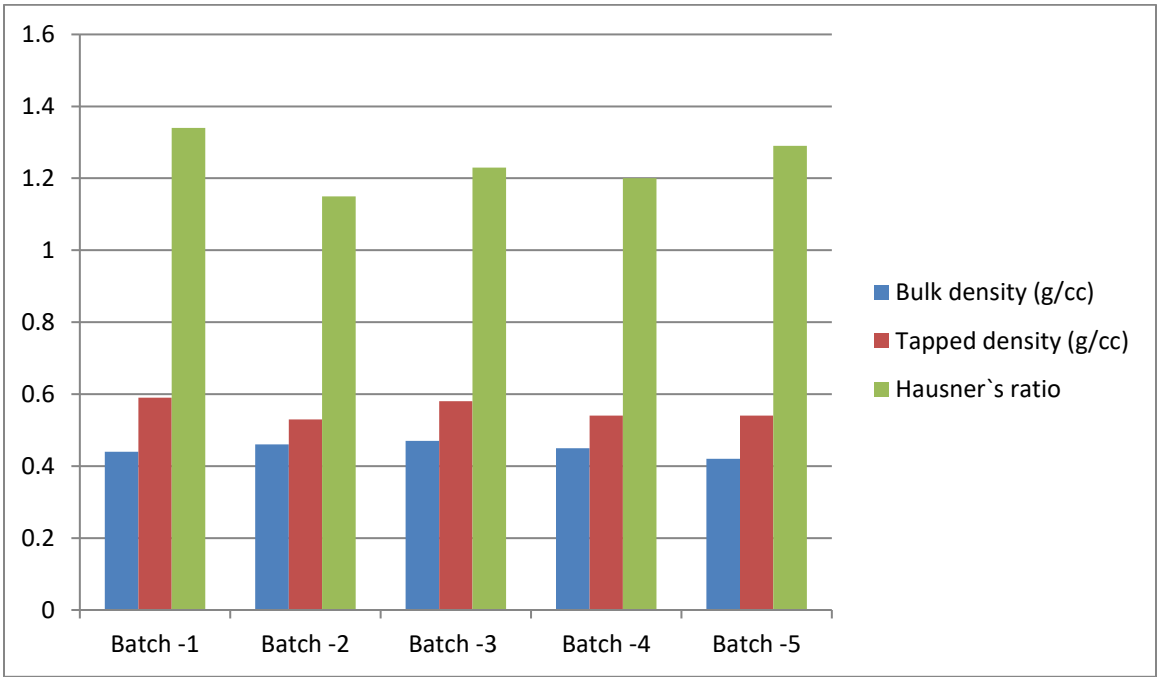
Evaluation of Bulk and Tapped Density for Different Batches:

The obtained results show slight variations in the bulk density and tapped density among the different batches. Batch-3 exhibited the highest bulk density (0.47 g/cc) and tapped density (0.58 g/cc) values, indicating a higher degree of compaction and packing efficiency compared to the other batches. On the other hand, Batch-5 showed the lowest bulk density (0.42 g/cc) while maintaining a similar tapped density to Batch-4 (0.54 g/cc).

Hausner's ratio, which is calculated as the ratio of tapped density to bulk density, provides insight into the flowability of the material. In this study, the Hausner's ratio ranged from 1.15 to 1.34 across the different batches. Batch-2 exhibited the lowest Hausner's ratio, indicating better flow properties compared to the other batches.

Evaluation of micrometric properties:

Evaluation Parameters	Bulk density (g/cc)	Tapped density (g/cc)	Hausner`s ratio
Batch -1	0.44	0.59	1.34
Batch -2	0.46	0.53	1.15
Batch -3	0.47	0.58	1.23
Batch -4	0.45	0.54	1.20
Batch -5	0.42	0.54	1.29



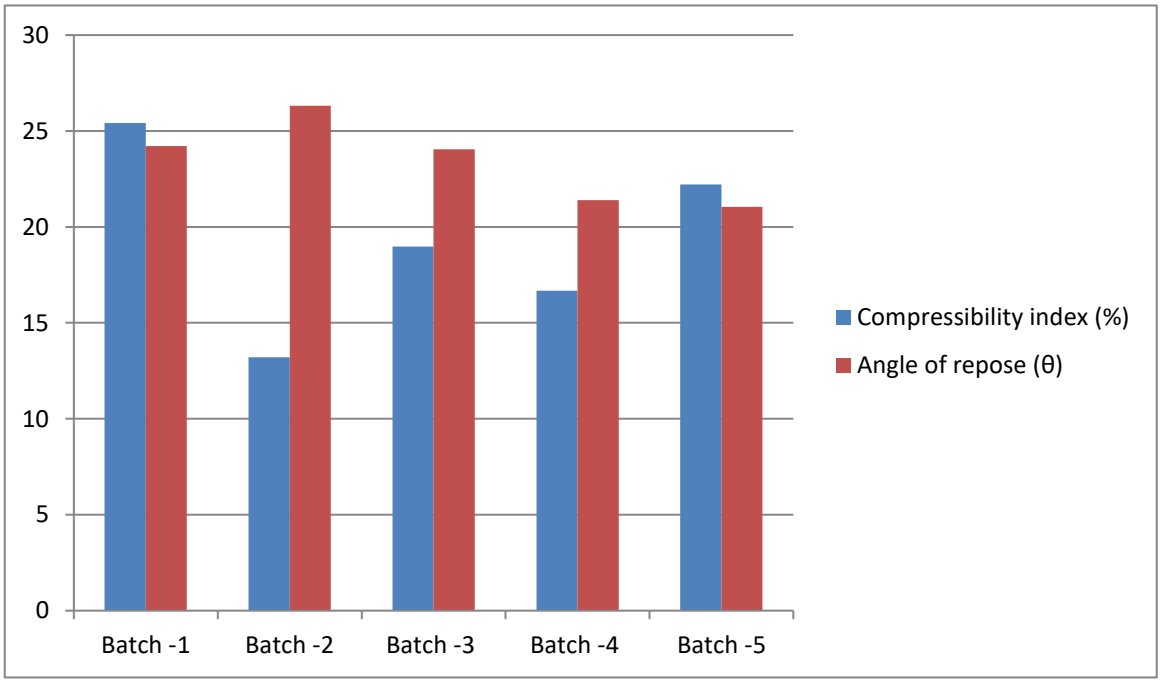
Evaluation of Micromeritic studies:

The obtained results indicate variations in the compressibility index and angle of repose among the different batches. Batch-2 exhibited the lowest compressibility index (13.21%), suggesting that it is the most compressible among the batches. Conversely, Batch-1 showed the highest compressibility index (25.42%), indicating a lower ability to undergo compression.

Regarding the angle of repose, Batch-2 exhibited the highest value (26.31°), indicating poorer flowability and particle-packing behavior compared to the other batches. On the other hand, Batch-4 demonstrated the lowest angle of repose (21.40°), suggesting better flow properties and improved particle-packing characteristics.

Evaluation of Micromeritic studies

Evaluation Parameters	Compressibility index (%)	Angle of repose (θ)
Batch -1	25.42	24.22
Batch -2	13.21	26.31
Batch -3	18.97	24.05
Batch -4	16.67	21.40
Batch -5	22.22	21.05



Characterization of core Tablets

The obtained results show variations in weight variation, average thickness, and drug content among the different batches. Batch-1 and Batch-5 displayed the highest weight variation, with values of 19.20% and 19.52%, respectively, suggesting potential inconsistencies in the dosage unit weights within these batches. Batch-2 showed the lowest weight variation (14.67%), indicating better uniformity in weight.

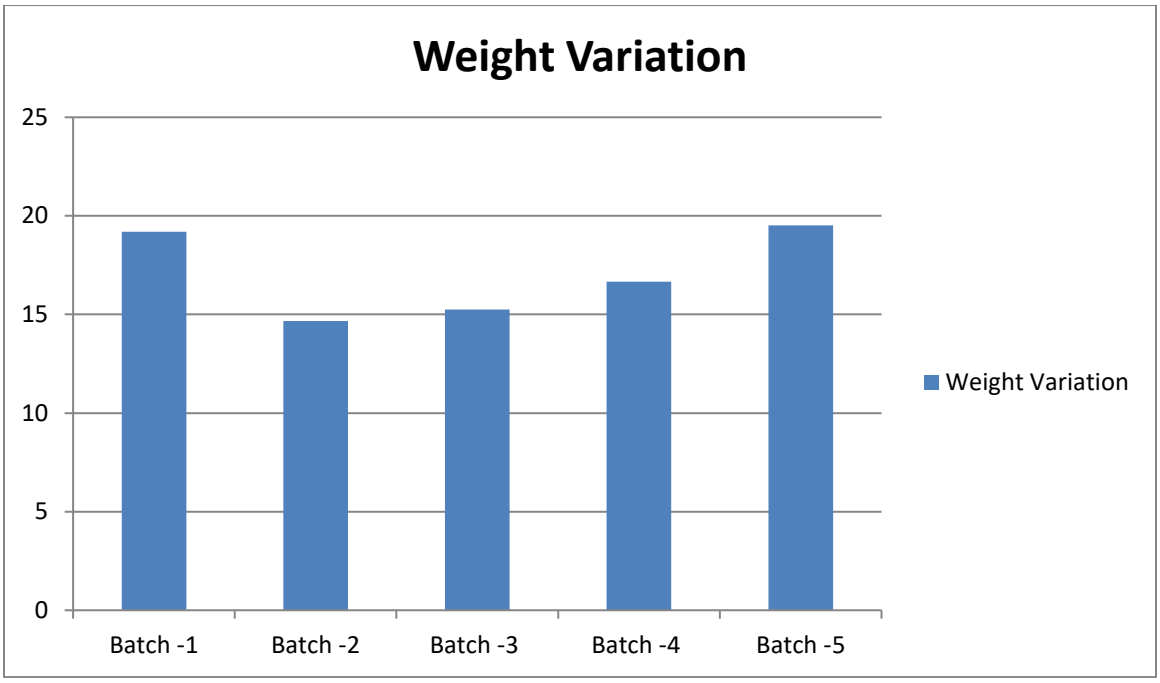
Regarding average thickness, Batch-1 and Batch-5 had the highest values (2.65 mm and 2.67 mm), while Batch-2 had the lowest value (2.34 mm). These differences may have implications for the disintegration and dissolution properties of the dosage units.

In terms of drug content, Batch-3 exhibited the lowest percentage (90.56%), which may raise concerns about the potency and efficacy of the medication in this particular batch. On the other hand, Batch-1 and Batch-5 showed the highest drug content (97.56% and 97.46%), suggesting good uniformity in drug distribution within these batches.

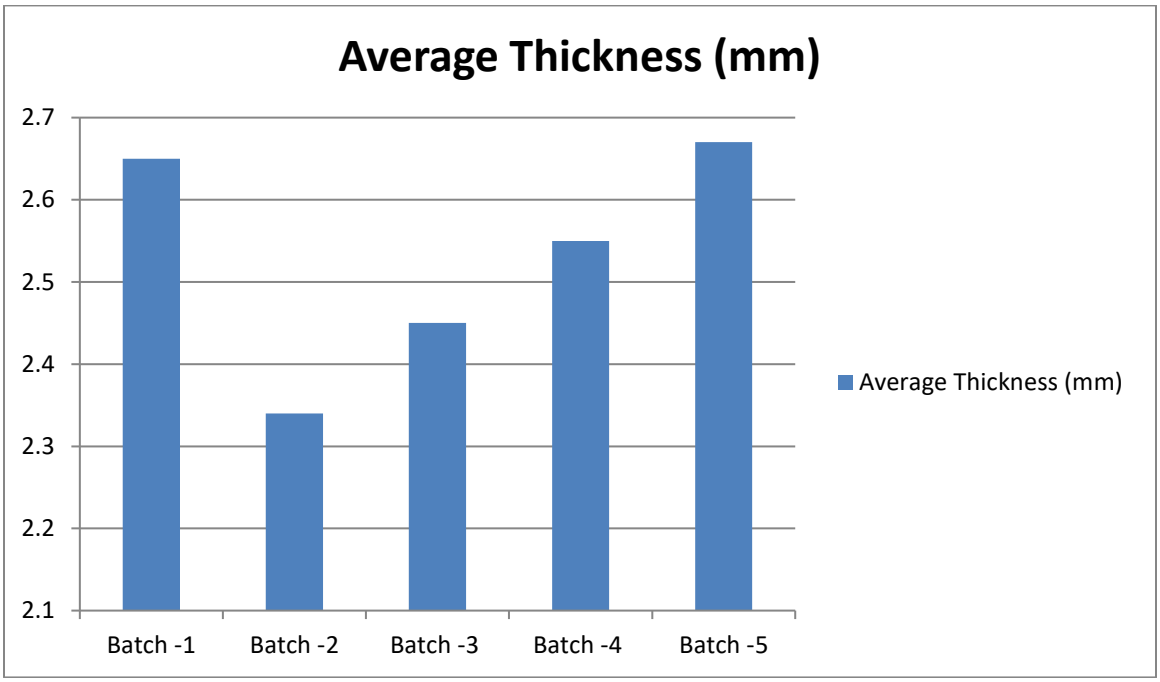
Characterization of core Tablets

Evaluation Parameters	Weight Variation	Average Thickness (mm)	% Drug Content
Batch -1	19.20	2.65	97.56
Batch -2	14.67	2.34	96.24
Batch -3	15.25	2.45	90.56
Batch -4	16.67	2.55	95.46
Batch -5	19.52	2.67	97.46

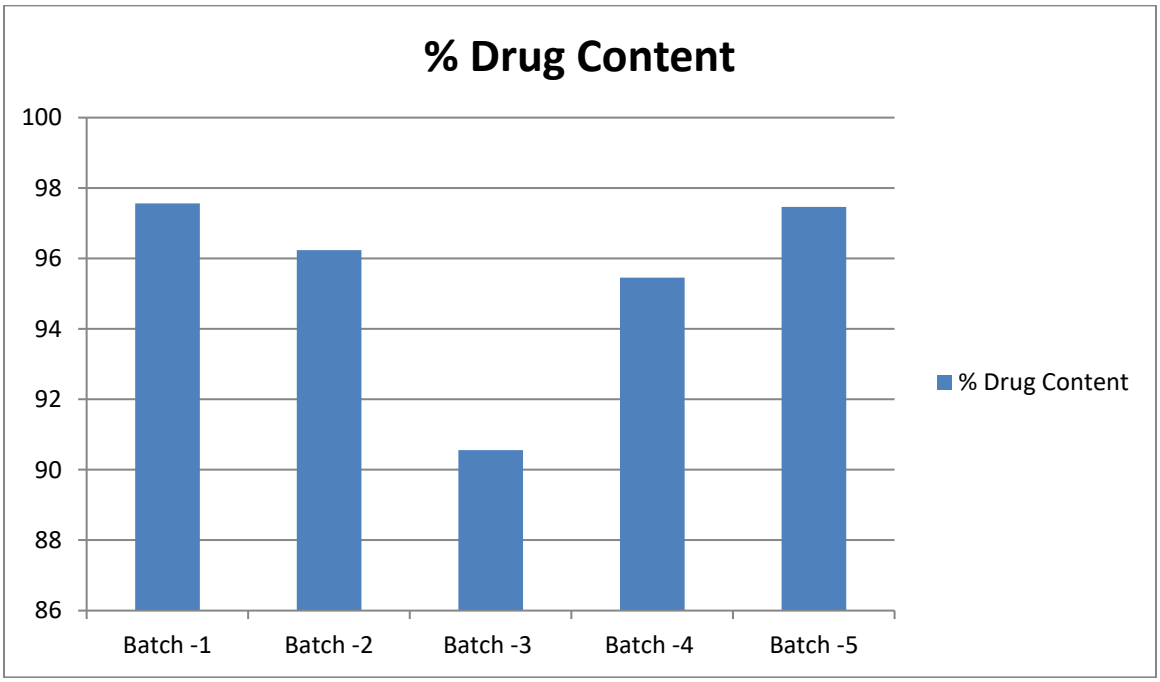
Characterization of core Tablets (Weight Variation)



Characterization of core Tablets (Average Thickness (mm))



Characterization of core Tablets (% Drug Content)

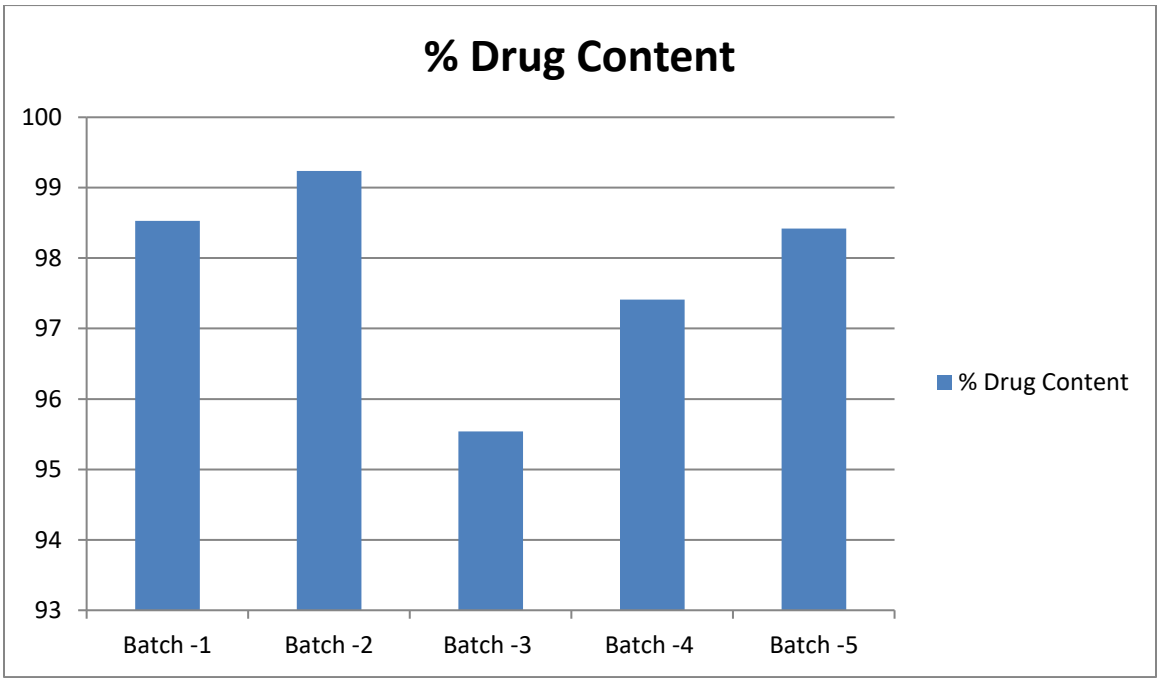


Colon Targeted Drug Delivery System:
Colon targeted drug delivery system were prepared according to the above procedure, in which the selected coating formula. These systems were evaluated for drug content, in vitro drug release and the results are as discussed below

Drug Content: The drug content of all the formulations of Colon Targeted Drug Delivery System is as discussed below

Evaluation Parameters	% Drug Content
Batch -1	98.53
Batch -2	99.24
Batch -3	95.54
Batch -4	97.41
Batch -5	98.42

The drug content of all the formulations of Colon Targeted Drug Delivery System is as discussed below:

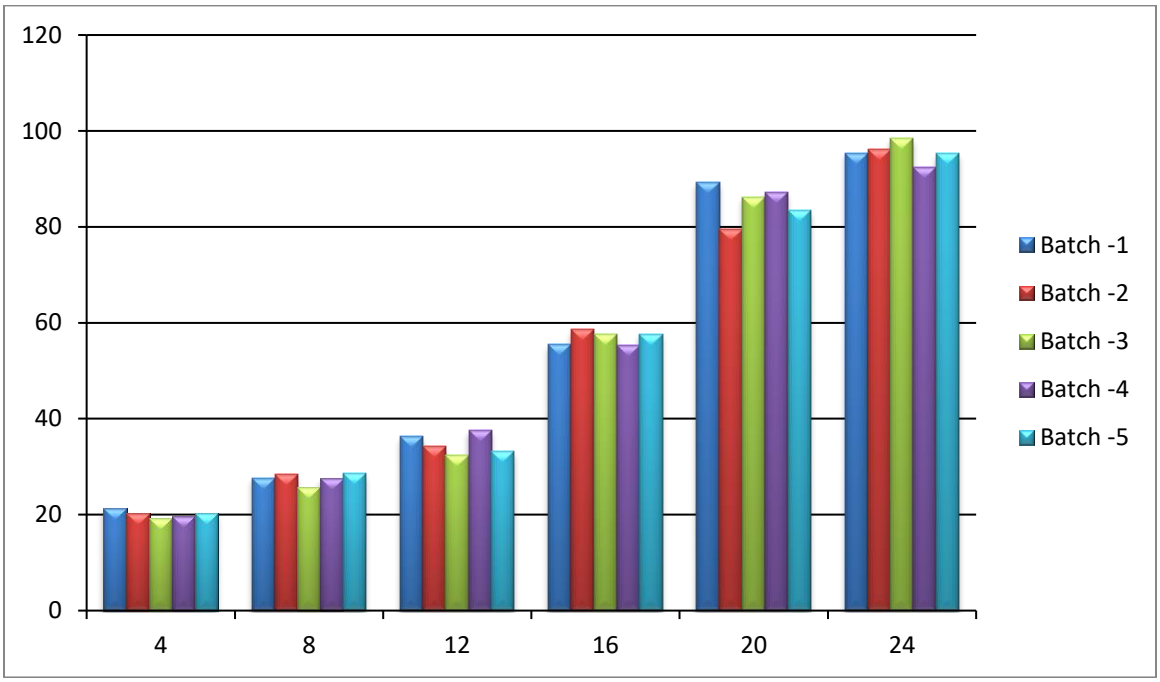


In vitro dissolution studies

The dissolution profiles show variations in drug release among the five batches. At 4 hours, Batch-1 and Batch-5 exhibited the highest drug release (21.22% and 20.24%, respectively), while Batch-3 showed the lowest release (19.25%).

In vitro dissolution studies of optimised formulation:

Time (h)	Batch -1	Batch -2	Batch -3	Batch -4	Batch -5
4	21.22	20.25	19.25	19.65	20.24
8	27.65	28.47	25.65	27.55	28.65
12	36.25	34.24	32.36	37.55	33.21
16	55.45	58.64	57.68	55.27	57.65
20	89.25	79.55	86.21	87.24	83.46
24	95.45	96.15	98.46	92.45	95.21



Stability Studies:

The accelerated stability studies were carried out according to ICH guidelines. After a period of one month, the samples were observed for any change on appearance. It was observed that capsules were devoid of any change in color or appearance of any kind. It was also noted that capsules were free of any kind of microbial or fungal growth or bad color. Hence there was no change in appearance of capsules. Similarly, no significant change was seen in drug content of both the formulations.

Table : Stability Study Formulation

Evaluation Parameters	Physical Appearance	% Drug Content
Batch -1	No change	99.54
Batch -2	No change	97.26
Batch -3	No change	96.55
Batch -4	No change	95.41
Batch -5	No change	97.40

Summary

The evaluation of various physical and performance parameters for five different batches of materials or pharmaceutical formulations revealed variations in their properties. These differences could impact industrial applications and product performance. Further investigations are needed to understand the underlying factors behind these variations and their implications. The findings emphasize the need for quality control, optimization of processing, and potential adjustments for consistent and effective outcomes. Stability studies indicate short-term stability, but extended assessments are recommended for longer-term and diverse storage conditions.

In conclusion, the comprehensive evaluation of multiple batches of materials and pharmaceutical formulations highlighted significant variations in their physical properties, flow characteristics, dosage parameters, and drug release profiles. These variations underscore the importance of in-depth investigations to identify the root causes behind these differences and their potential impact on industrial applications and product performance. The findings emphasize the necessity of stringent quality control measures, process optimization, and formulation

adjustments to ensure consistent and efficient outcomes. Additionally, the positive results from accelerated stability studies provide initial assurance of short-term stability, paving the way for further assessments to ascertain longer-term stability under varied storage conditions.

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