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Original Research Article

PREPARATION AND EVALUATION OF POROUS PELLETS LOADED WITH INDOMETHACIN FOR CONTROLLED RELEASE

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Abstract: The aim of the present study was to prepare and evaluate microporous pellets loaded with Indomethacin is an oral Non-steroidal anti-inflammatory drug, indicated for the treatment of rheumatoid arthritis. Indomethacin is indicated for the treatment of rheumatoid arthritis, alone or in combination with other NSAID'S drugs. Indomethacin is also indicated for the treatment of ankylosing, spondylitis, osteoarthritis.

The frequent adverse effects of NSAIDs like Indomethacin are gastro-intestinal disturbances. Enteric coated formulations minimize these symptoms. Multi-particulate system such as porous pellets disperse freely in the gastro-intestinal tract, contributes to maximum drug absorption, reduced peak plasma fluctuations and have less side effects. Porous pellets were prepared by extrusion /spheronization method.

Keywords: Indomethacin Pellets, Controlled release, Extrusion/Spheronisation.

Introduction: Controlled release delivery systems provide a uniform concentration or amount of drug at absorption site and thus after absorption, allow maintenance of plasma concentration within a therapeutic range, which minimizes side effects and also reduces frequency of administration. These products typically provide benefits over immediate release formulations, including greater effectiveness, in the treatment of chronic conditions, reduced side effects, and greater patient convenience due to simplified dosing schedule.

A number of design options are available to control or modulate drug release from a dosage form. In present research work, porous pellets system has been designed for Indomethacin.

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The most frequent adverse effects of Indomethacin like other NSAIDs are gastro-intestinal disturbances. Controlled release or enteric coated release formulations minimize these symptoms. Multi-particulate system such as pellets are often used as oral solid dosage form since they offer therapeutic as well as technological advantages over single unit dosage forms. They disperse freely in the gastro-intestinal tract, contributes to maximum drug absorption, reduced peak plasma fluctuations and less side effects. Furthermore, pellets also allow the formulator to modify the drug release by coating the pellets and a mixture of pellets with different release characteristics can be used to obtain the desired release profile

Method: Preparation of Pellets: The powdered MCC and Sodium Chloride were passed through a 40 mesh sieve. The powders were granulated with water to get a good dough mass of extrudable consistency. The volume of the binder required was noted and the quantity of the binder used was calculated. The wet mass was extruded in to short cylinders using a cylinder roll type gravity feed extruder with a roller speed setting of 100 rpm. A granulating cylinder with 1.0 mm pore size was used and extrudates were obtained. Spheronization of the extrudates was carried out in the spheronizer using a serrated plate. The spheronization speed was varied from 300rpm to 1500rpm and spheronization time was varied from 2 min to 20 min to get pellets of good sphericity. Drying of pellets was carried out in a tray drier.

Drug loading[19]: Dried pellets were collected and the NaCl fraction was removed from the pellets by aqueous extraction: 30g of pellets were placed on to a 500 mL bottle top filter (membrane filter); the filter was placed on a 2-L flask and connected to a vacuum pump. An aliquot of 2 L of water was poured on to the filter in steps of 250 mL to extract the NaCl fraction. Later the pellets were oven dried at 40°C.

The drug was loaded by immersing the pellets into the drug solution. It was out carried by immersing 1g of pellets into the 2.5% indomethacin in methanol solution for 24 hrs. After 24 hrs the pellets were collected and oven dried at 40°C.

Formulation of pellets: The optimal formula and processing conditions arrived at in some earlier experiments on the extrusion / spheronization process standardization was used as guidelines for processing. MCC and Sodium Chloride (NaCl) in different ratios were used to make pellets. Water is used as a binder. The quantity of the binder used was sufficient to maintain loss on drying.

Table 1: Formulation chart for the preparation of pellets

Formulation code	Drug %	MCC %	NaCl %
F1	2.5	90	7.5
F2	2.5	80	17.5
F3	2.5	70	27.5
F4	2.5	60	37.5
F5	2.5	50	47.5
F6	2.5	40	57.5
F7	2.5	30	67.5

Characterization of Pellets: a. Angle of repose: Angle of repose was assessed to know the flowability of pellets, by a fixed funnel method. A funnel with the end of the stem cut perpendicular to its axis of symmetry was securely arranged above the graph paper of

height which was placed on a flat horizontal surface. Indomethacin pellets were carefully poured through the funnel until the apex of the conical pile just reaches the tip of the funnel. The radius (r) and height of the pile (h) were then determined. The angle of repose (θ) for samples were calculated using the formula[35],

Angle of repose (θ) = $\tan^{-1}$ (h / r) **(1)**

Angle of repose represents whether the given sample was free flowing or not. The relationship between angle of repose and flowability is shown in Table 2. The mean of three determinations was used to calculate the angle of repose from each of the formulation.

Table 2: Relationship between angle of repose and Sample flow

Angle of repose (θ)	Flow ability
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

b.Compressibility

Carr's index is a dimensionless quantity, which proved to be useful to the same degree as the angle of repose values for predicting the flow behavior. Apparent bulk density was determined by pouring the bulk samples into a graduated cylinder. Tapped density was determined by placing a graduated cylinder containing a known mass of powder on a mechanical tapper apparatus (Electro lab tap density tester). Samples were tapped until no further reduction in volume of the sample was observed. Carr's index is calculated using the formula given below and the relationship between compressibility and flow property is shown in Table 3. The mean of three determinations was used to calculate the compressibility index from each of the formulation. [36]

$$\text{Carr's index} = \frac{\text{(Tapped density – Bulk density)}}{\text{Tapped density}} \quad (2)$$

Table 3: Relationship between powder flowability& % compressibility

% Compressibility range (Carr's index)	*Flow description*
5-15	*Excellent (free flowing granules)*
12-16	*Good (free flowing powder granules)*
18-21	*Fair (powdered granules)*
23-28	*Poor (very fluid powders)*
28-35	*Poor (fluid cohesive forces)*
35-38	*Very Poor*
> 40	*Extremely poor*

Pellet Sphericity: Pellet size and shape were determined using an image analysis system. Photomicrographs were taken with a digital camera. The obtained images were processed by image analysis software to characterize each individual pellet by mean Feret diameter (FD) (average of 180 calliper measurements with an angle of rotation of 1°), aspect ratio (AR) (ratio of longest Feret diameter and its longest perpendicular diameter) and two-dimensional shape factor (*e*R)

$$e\text{--}R = \frac{2\pi r \pm \sqrt{1-b \div l}}{P_m} \quad (3)$$

Where *r* is the radius, *Pm* the perimeter, *l* the length (longest Feret diameter) and *b* the width (longest perpendicular diameter to the longest Feret diameter) of the pellet[19].

Compatibility Studies: Drug is in intimate contact with one or more excipients, which could affect the stability of the drug. The knowledge of the drug excipient interactions is essential for selecting appropriate excipients. This was studied using FT IR spectrophotometer and Differential Scanning Calorimetry (DSC).

Differential Scanning Calorimetry (DSC): DSC is a technique in which the difference in heat flow between the sample and a reference is

recorded versus temperature All dynamic DSC studies were carried out on Du Pont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with empty cell as the reference. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10º c/min. The runs were made in triplicate. The scanning temperature for reference pure drug and formulation are the same when dynamic measurements are performed, and hence the required heat energy for chemical transformation is directly recorded on a heat flow versus temperature graph. The energy is measured as Joules per kilocalorie[38]

Fourier Transform Infrared Spectroscopic (FT IR) studies[39]: FTIR analysis was carried out for pure drug and for pellets with and without drug using KBr pellet method on FTIR spectrophotometer .Drug was mixed with KBr and spectra was taken. FT-IR spectrum of pure drug Indomethacin was compared with FT-IR spectra of Indomethacin formulations. Disappearance of peaks or shifting of peaks in any of the spectra was studied using the apparatus FTIR- 8400-S, Shimadzu, Japan.

Evaluation of Pellets:

Percentage yield: Determining whether the preparation procedure chosen for incorporating a drug into the polymers is efficient and is of prime importance. The raw materials, amount of active compound, MCC, and other process parameters are deciding factors for the yield of the product during the preparation of pellets. [40] The yield was determined by weighing the Indomethacin pellets and then finding out the percentage yield with respect to the weight of the input materials, i.e., weight of drug and polymers used. The formula for calculation of % yield is as follows;

$$\% \text{ yield} = \frac{\text{Wt.of pellets} \times 100}{\text{Wt.of drug} + \text{Wt of polymers}} \quad (4)$$

Drug loading and encapsulation efficiency: Drug loading is important with regard to release characteristics. Generally, increased drug loading leads to an acceleration of the drug release. Drug entrapment efficiency represents the proportion of the initial amount of drug, which has been incorporated into the pellets.

100 mg of Indomethacin pellets were weighed and transferred to 100 ml volumetric flask containing pH 7.4 phosphate buffers. From this, 1 ml of solution was transferred to 10 ml volumetric flask and diluted up to the mark. Further 1 ml of this solution is diluted to 10 ml and absorbance was measured at 319 nm. The drug content was calculated by using the formula

$$\text{Amount of drug} = \frac{\text{Concentration from standard graph} \times \text{dilution factor}}{1000} \quad (5)$$

Percentage encapsulation efficiency is found out by calculating the amount of drug present in 100 mg of pellets. It is further calculated by using formula

% Encapsulation Efficiency = (b)/a X 100 (6)

Where, a is the theoretical drug content and b is the drug entrapped

In vitro drug release studies: The *in vitro* release of drug from the pellets was carried out in basket type dissolution tester USP XXIII, TDT-08L, with auto sampler containing 900 ml of pH 1.2 buffer for the first 2 hrs and in 7.4 pH phosphate buffer for the next 22 hrs. The volume of the dissolution media was maintained at 900 ml with constant stirring (100 rpm) and temperature of bath was maintained at 37 ± 0.5°C. Aliquots (10 ml) of dissolution media were sampled at specified time points and replaced with fresh media immediately after sampling. Samples were analyzed for drug content by UV Visible spectroscopy .The release data obtained were fitted into various mathematical models to know which mathematical model is best fitting for the obtained release profile. [41]

Dissolution studies were carried out for all the batches of the prepared formulations (09 batches) and commercial formulation, the details of which are given in Table 4.

Table 4: Dissolution media used for prepared and commercial formulation

SlNo.	Formulations	Quantity used	Dissolution media	
			For first 2 hrs	For next 22 hrs
1	F1-F7	Equivalent to 75 mg of Indomethacin	pH 1.2 buffer	pH7.4 phosphate buffer
2	Indocin®SR	Equivalent to 75 mg of Indomethacin	pH 1.2 buffer	pH 7.4 phosphate buffer

Stability studies: It is necessary to perform stability testing to find out the extent of deterioration and to ensure the degradation has not exceeded an acceptable level assuring the safety of the patient and the activity of the product[45].

Degradation of active ingredients in pharmaceutical formulations may occur by hydrolysis, oxidation, reduction, racemization, ring cleavage, photolysis, decarboxylation and isomerization. Physical degradation of pharmaceutical products may occur due to loss of water, loss of volatile constituents, absorption of water, crystal growth, polymorphic changes and colour changes.

If pharmaceutical preparations or new formulations are stored under normal conditions, their instabilities are detectable only after long storage periods. Such a method is time consuming and uneconomical. In an attempt to reduce the time required to obtain information about instabilities, various stress tests are undertaken. The most common stress conditions used are temperature, humidity and light.

Pharmaceutical preparations may often exhibit physical or chemical reactions that may end in instability due to which the product gets deteriorated. This deterioration may lead to:

- Reduction in the activity of the product
- Formation of toxic products
- An inelegant product

Short-term stability study: Stability is defined as the ability of a particular drug or a dosage form in a specific container to remain with its physical, chemical, therapeutic and toxicological specifications.

A drug formulation is said to be stable if it fulfills the following requirements:

- It contains at least 90% of the stated active ingredient
- It contains effective concentration of the added preservatives, if any
- It does not exhibit discoloration or precipitation, nor develops foul odour
- It does not develop irritation or toxicity

Optimized formulation of the pellets was selected for stability studies Formulations were packed in a screw capped bottle and studies were carried out for 90 days by keeping at

- $25 \pm 2^{o}C$ and $60 \pm 5\%$ RH
- $40 \pm 2^{o}C$ and $75 \pm 5\%$ RH

Samples were withdrawn on 15^{th}, 45^{th} & 90^{th} day and were analyzed for drug content spectrophotometrically at 319 nm.

Result:

Optimization of process parameters for pelletization: Evidence has been proved in recent years MCC possess physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable porous pellets to release the entrapped drug in the intestinal lumen. In the present study, extrusion/spheronization method was optimized in order to prepare porous pellets by using MCC, NaCl to entrap the drug. The present method is quite different from other methods. Indomethacin is water in-soluble drug and could be entrapped into water in-soluble polymer by extrusion/spheronization method and porous pellets were prepared.

The pellets were prepared by using Avicel PH 101, as polymer and sodium chloride (NaCl), as a pore forming agent by extruder/spheronization technique. The technique was optimized using

the parameters for pelletizations are shown in table 7. When the ratio of MCC was 70 % w/w produces spherical and hard pellets, suitable for pharmaceutical uses. But, when the ratio of MCC was 90 %, 80 %, 50 %, 40 %, 30 % w/w produces rod shaped, egg shaped, and semi spherical and brittle pellets respectively. These pellets are not suitable for pharmaceutical purpose. In the present study it was found that the ratio of MCC was 70 % w/w, resultant pellets did not have any surface irregularities and non aggregated.

An attempt was made to prepare porous pellets by using 10, 20, 40, 50, 60, 70 % w/w of NaCl as a pore forming agent fail to produces the required pores in the porous pellets. The increased or decreased of NaCl responsible for shrinking of pore in the pellets. Maximum drug load was obtained, when the optimum ratio of 30 % w/w NaCl was used as pore forming agent. Produces suitable pore to entrap more amount of the drug.

In the present study, it was found that optimum spheronization speed was found to be 1250 rpm to produce spherical pellets. It was observed that with increase in stirring speed from 1250 to 1500 there was a decrease in average size of the pellets and produces semi spherical pellets. When the stirring speed was 1000 rpm, 700 rpm and 300 rpm produces semi spherical, egg shaped and rod shaped pellets respectively. It was also found that optimum stirring time was found to be 15 min to produce spherical pellets. When the stirring time was 20 min, there was a decrease in yield and produces semi spherical pellets. When the stirring time was 10 min, 5 min and 2 min, it was observed that some amount of wetted mass adhere to the spheronizer resulting in lower recovery of yield and produces semi spherical, egg shaped and rod shaped pellets respectively. Repeat batches treated at an optimized rate mentioned above proved to produce reproducible sizes, showing that spheronization speed and stirring time were well controlled. In the present study, to produce the spherical porous pellets, an optimum drug concentration 2.5 % w/v was used. It was found that higher the amount of drug will show presence of crystals on surface of pellets which is determined by SEM study which were unsuitable for pharmaceutical uses.

Table: Optimization of process parameters for pelletization.

Parameters	Formulation	Ratio	Description of pellets
MCC:NaCl (w/w)	F1	90:10	Rod shape and brittle
	F2	80:20	Egg shape and brittle
	F3	70:30	Spherical and hard
	F4	60:40	Spherical and brittle
	F5	50:50	Semi spherical and brittle
	F6	40:60	Semi Spherical and hard
	F7	30:70	Spherical and brittle
Spheronization Speed (rpm)	F3	300	Rod shape
		700	Egg shape
		1000	Semi spherical
		1250	Spherical
		1500	Semi spherical
Spheronization speed (time)	F3	2	Rod shape
		5	Egg shape
		10	Semi spherical
		15	Spherical
		20	Semi spherical

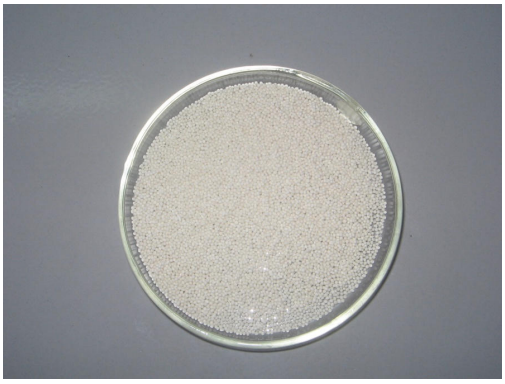


Figure 1: Pellets obtained by process of extrusion spheronizer

Figure 2: Indomethacin loaded pellets filled in size 0 capsule

Characterization of Pellets

Micromeritic properties: Generally the multiparticulate drug delivery systems are formulated as single unit dosage forms in the form of capsule or tablet. Such multiparticulate systems should possess the required and better Micromeritic properties. The obtained data average size, angle of repose (θ), tapped density, granular density, % compressibility index (CI) along with related parameters are presented in Table 8. The values of θ^0 and CI ranged from 23.45to 26.30and 8.76 to 9.39respectively indicating that the obtained values were well within the limits. This result clearly shows that the prepared pellets have reasonably good flow potential.

The values of tapped density ranged between 0.834 to 0.902gandcm3. Density difference between the formulations is negligible and the density values of formulations were well within the limits, indicating that the prepared pellets were non-aggregated and spherical in nature.

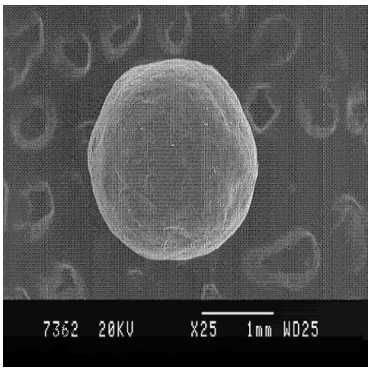
Table 2: Micromeritic properties and Particle size analysis

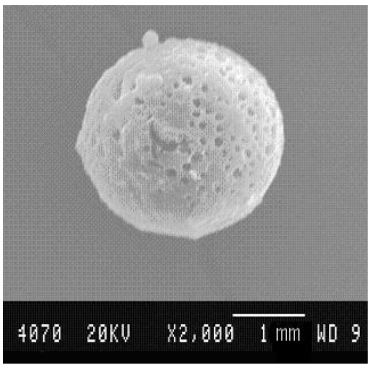
Formulation code	Average size(μm)	Angle of repose θ^0	Tapped density (g/cm^3)	Granule density (g/cm^3)	Carr's index (%)	Friability (%)
F1	1125±0.56	26.54±0.92	0.84±0.64	1.06±0.88	9.12±0.32	0.53±0.78
F2	1179±0.45	25.42±0.25	0.86±0.92	1.07±1.78	8.79±0.99	0.52±0.45
F3	1345±0.23	23.45±0.88	0.90±1.43	1.05±1.45	9.39±0.53	0.43±0.82
F4	1239±0.55	26.30±0.65	0.89±1.01	1.05±0.96	8.93±0.98	0.47±0.36
F5	1238±1.02	25.25±0.46	0.83±0.55	1.04±0.72	8.76±1.76	0.45±0.78
F6	1279±0.92	25.98±0.74	0.83±0.82	1.07±0.81	8.69±2.01	0.49±0.22
F7	1253±0.36	24.66±0.45	0.84±0.62	1.08±0.18	8.85±1.11	0.55±0.91

***Standard deviation, n = 3**

Scanning electron microscopy: Scanning electron microscopy (SEM) is one of the most commonly used method for characterizing drug delivery systems, owing in large part of simplicity of samples preparation and ease of operation. Scanning electron microscopy was carried out in order to characterize surface morphology, texture and porosity of the coating films. In this study the sample was prepared by placing the formulation F3 samples in pH 7.4 buffer solutions for 24 hours followed by drying the samples at 30° C for 24 hours. The samples

were mounted on aluminium mount and sputtered with gold. Sample was scanned at an accelerating voltage of 20 kV. Scanning electron micrographs obtained are given in figures 15, 16 and 17. Figure 15 shows the surface topography of the pellets, where a smooth surface can be observed with its optimal, spherical shape.SEM photographs reveal the absence of drug particles on the surface of pellets showing uniform distribution of the drug in the pellet. It also shows the approximate diameter of pellets ranging from 1.2 – 1.4 mm. A small degree of etching on the surface can be observed due to effect of the pH 7.4 phosphate buffer as a dissolution media. Figures 15 and 16 shows the SEM micrograph of porous pellets formed of formulation F3 at the resolution of 2000x. The fine pore formation of the dimensions in microns can be clearly observed.





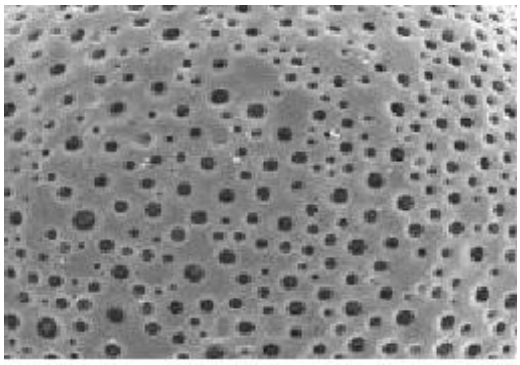


Figure 3: SEM of non porous pellet, Figure 4: SEM of porous pellet (F3) Fig 5: SEM of porous pellet (F3)

Internal pore structure: CT-scanning of the porous structure (Figure 18) detected one pore system with a branched structure. (Equivalent diameter > maximum inscribed diameter) Table 10.

These pore system represented 99% of the total porosity volume, indicating that nearly all pores are incorporated in an interconnected network.

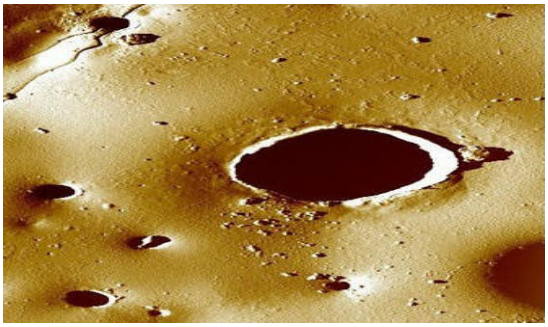


Figure 6: CT- scan of porous structure

Table 10: CT- scan analysis of the internal structure of a porous pellet obtained by Nacl extraction.

Maximum inscribed diameter (µm)	Equivalent diameter (µm)	Porosity represented by pore system (%)
62	325	98

Pellet Sphericity: Image analys (Fig 19) is revealed an AR (n=303, ±SD) of 1.15±0.08, an e_R of 0.94 ± 0.03 and FD of 946±142 µm, indicating that spherical pellets were obtained, and 92 % of the pellets were found in the 1135-1245µm.

AR = Aspect Ratio

FD= Feret diameter

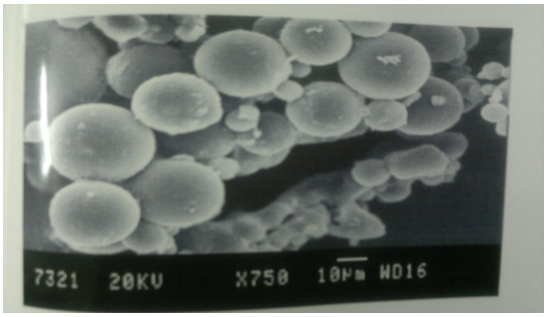


Fig 7: Image analysis of pellets

Table 11: Analysis of pellet Sphericity:

AR	e_R	FD (µm)
1.15±0.08	0.94±0.03	946±142

Fourier Transform Infra Red spectrum (FT-IR): FT IR spectra were obtained for of Indomethacin pure drug and Indomethacin loaded pellets and are presented in Figure. 20. The following characteristic bands were observed. The characteristic peaks of the pure drug were compared with the peaks obtained for formulation F3 and are given in Table 12. From the data it is observed that a similar characteristic peak of Indomethacin and Formulation F3 was appears with minor differences. The characteristics peaks found both in pure drug of Indomethacin and formulation F3, hence it appears there is no chemical interaction between drug and polymer and it can be concluded that the characteristics bands of pure drugs were not affected after successful loading.

FTIR studies:

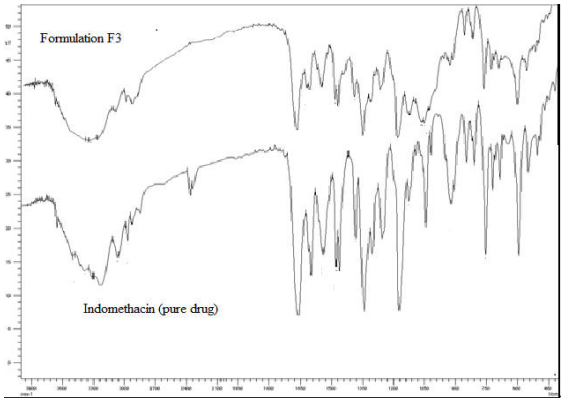


Figure 20: FT-IR of pure drug and Formulation (F3)

Table 12: Peak positions of pure Indomethacin and formulations with intensity range:

Groups	Peak positions in pure drug(cm-1)	Peak positions in formulation (cm-1)	Intensity range (cm-1)
Aromatic C-H stretching C-C stretching	3400- 2500	3400.6 2500.4	3030-3200 1620
Aliphatic---C-H stretching C-H bending	2968.55 1467.88	2972.1 1473.66	2962-2853 1485-1445
N-H stretching	3431.8	3446.91	3400
C=C Stretching aromatic	1539.25	1587.8	1450-1600
O-CH_3 deformation	1450	1455	1450-1480

Differential scanning calorimetric (DSC) studies: To understand the compatible state of the drug, DSC studies were carried out on pure drug, drug loaded pellets and empty pellets. The thermo grams obtained are shown in Figure21. The data obtained from the DSC scans for the Indomethacin and Indomethacin loaded pellets are given in Table 13 in terms of onset of melt (To), melting points (Tm) and completion of melt (Tc). Indomethacin exhibits a sharp endothermic peak at to 161.25^0C. It was observed that presence of the endothermic peak at to 162.02^0C in the drug loaded pellets indicated, that the drug retains its identity in the prepared pellets. The melting points of the drug and Polymers were estimated by open capillaries and found agrees well with the DSC data.

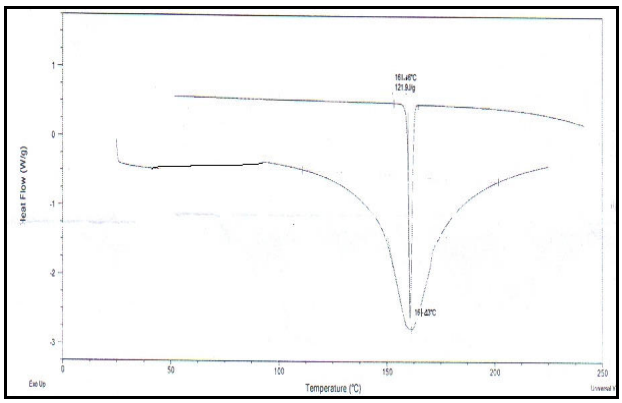


Figure 21: Comparison of DSC thermogram of pure drug and formulation F3

Table13: *DSC data obtained for Indomethacin and formulation* F3

SL. No.	Drug and Formulation	T_o	T_m	T_c	Melting range
1	Indomethacin	156.64	161.25	162.88	6.24
2	Formulation F3	158.35	162.02	165.12	6.77

Where, T_o – Onset of melt, T_m - Melting point and T_c – Completion of melt

- Indomethacin exhibits a sharp endothermic peak at 161.25°C.
- Presence of the endothermic peak at 162.02°C in the drug loaded pellets indicated that there is no interaction between drug and polymer.

Evaluation of the pellets formulation:

Determination of Drug Content: The prepared formulations were analyzed for drug content and the data is reported in Table 14.The drug content was found to be within the limits which show that the drug was uniformly distributed in all the formulations.

Table 14: Result of % Yield of pellets formulations $F_{1\ to}$ F_7

Sl. No.	Formulation Code	% Yield			
		Trial I	Trial II	Trial III	Mean ± S.D*
1	F_1	89.2	89.5	90.8	90.33±0.1527
2	F_2	93.1	92.4	93.6	93.03±0.6027
3	F_3	95.7	97.9	96.1	96.5±1.1718
4	F_4	95.8	92.9	93	93.9±1.6462
5	F_5	93.6	95	94.1	94.21±0.7094
6	F_6	96.9	93.8	94	94.9±1.734
7	F_7	96.1	94.7	95	95.23±0.737

***Standard deviation, n = 3**

Drug loading and Encapsulation efficiency: The percent of drug loading in the formulations was found to be in the range of 16.56 to 19.32 %. The percentage encapsulation efficiency was found to be 94.50 to 96.19 %. The results obtained are given in Table 15. The test for drug content was carried out to ascertain whether the drug is uniformly distributed in the formulation. The obtained results are reported in Table 15. Drug loading and entrapment efficiency increase with increase in the polymer concentration. From the results it can be inferred that there is a proper distribution of Indomethacin in the pellets and the deviation is within the acceptable limits. The decrease in the drug content in the product probably can be due to the decrease in pore size and concentration. The bar graphs are shown in figure 22 and 23.

Table15: Drug loading and encapsulation efficiency of pellets

Formulation	Drug loading(%)	Encapsulation efficiency(%)
F1	16.56±0.23	94.50±0.75
F2	16.97±0.65	95.69±0.83
F3	19.32±0.44	96.19±0.33
F4	18.61±0.76	95.98±0.46
F5	18.21±0.53	95.32±0.87
F6	17.12±0.89	94.89±0.64
F7	18.11±0.43	95.10±0.11

***Standard deviation, n = 3**

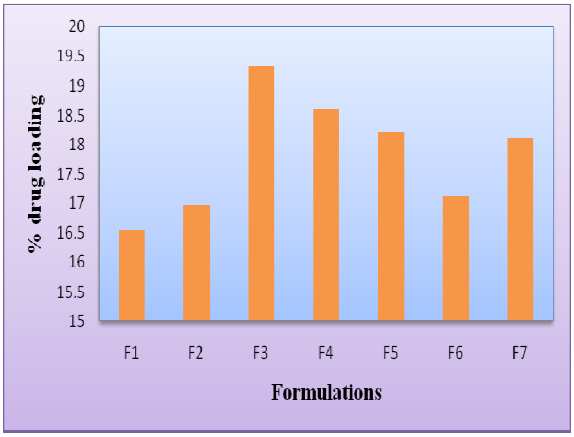


Figure 22: graph of % drug loading

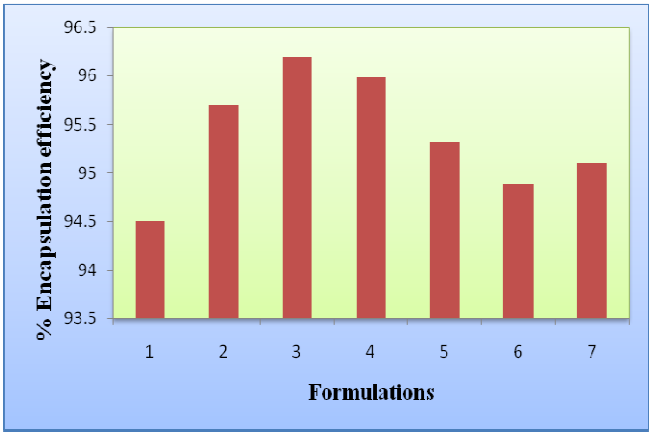


Figure 23: graph of % Encapsulation efficiency

***In vitro* drug release:** The *in vitro* release studies were carried out for all formulations in both pH 1.2 and pH 7.2. The *in vitro* release data for the Indomethacin formulations and marketed sample Indocin ®SR-75mg.are given in the Table 16 & 17 and the corresponding graphs are represented in Figure 25. The release profile of pellets in both media clearly indicates that the concentration of polymers and pores formed decreases Indomethacin release from pellets .The increase in the concentration of the polymers and decrease in pore concentration decreases the drug release from the matrices, as the pore concentration and pore size increases results in immediate release of drug. It was observed that there is no significant release of drug at gastric pH from pellets. At the end of 24th, *in-vitro* drug release from formulation F1 to F7 was found to be 72.22to 91.41in the intestinal environment as shown in the Fig24. The decrease in the drug release from the pellets was due to hydrophobicity of polymer. The release kinetics is mainly depends on the concentration of the polymers used, increase in the polymer concentration results in the controlled release of the drug from the pellets. The *in-vitro* drug release was considerably retarded from pellets as compared to Indocin ®SR-75 mg

Table 16: % Cumulative drug release of formulations

Time (hr)	% Cumulative drug release						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
1	1.46±0.25	2.21±0.76	2.72±0.64	3.56±0.26	3.76±0.46	3.84±0.78	3.95±0.84
2	3.92±0.13	5.52±0.64	9.13±0.75	7.18±0.11	7.84±0.67	8.36±0.45	8.08±0.11
3	5.67±0.27	10.58±0.53	11.25±0.45	13.04±0.54	14.2±0.84	12.83±0.36	10.6±0.92
4	8.38±0.42	19.78±0.43	23.41±0.21	25.39±0.41	28.34±0.54	32.78±0.74	17.5±0.42
6	19.01±0.11	33.91±0.32	38.65±0.17	30.49±0.78	41.25±0.66	42.69±0.84	35.2±0.53
8	26.22±0.45	38.53±0.59	49.34±0.89	41.72±0.51	46.49±0.78	53.64±0.92	41.25±0.63
10	36.76±0.65	45.69±0.87	57.92±0.55	46.49±0.42	53.11±0.35	60.28±0.82	52.56±0.74
12	41.68±0.67	52.69±0.78	68.22±0.65	53.11±0.66	64.96±0.22	65.39±0.64	63.36±0.56
16	51.84±0.76	58.24±0.44	77.25±0.53	64.96±0.55	72.47±0.78	74.69±0.54	70.05±0.94
20	64.78±0.55	70.59±0.36	85.36±0.74	72.47±0.53	82.48±0.83	78.36±0.66	77.18±0.43
24	72.22±0.41	83.94±0.65	91.41±0.33	82.48±0.18	85.36±0.65	82.65±0.56	81.84±0.21

***Standard deviation, n = 3**

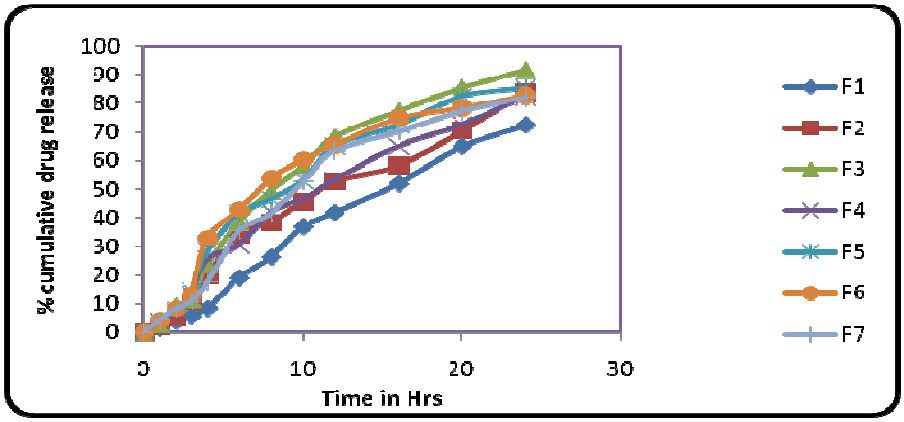


Figure 24: % cumulative drug release (F1-F7)

Release behaviour of Formulation (F3): Formulation F3 shows 91.43 % release in 24 h. There is significant increase in release of drug at pH 7.2

Comparison with a marketed product: Formulation F3 was compared with a marketed product Indocin SR, to be given once in 24 hours. In the figure. 25. The percentage of drug released from Indocin SR and F3 is plotted as a function of time. The dissolution data is tabulated in table 17. It is evident that prepared Indomethacin pellets show better release profile than the marketed product.

Table 17: Comparison of dissolution profiles

Time (hrs)	CUMULATIVE % DRUG RELEASE	
	F3	Indocin®SR
0	0	0
1	2.72±0.64	7.79±0.43
2	9.13±0.75	13.58±0.55
3	11.25±0.45	18.36±0.23
4	23.41±0.21	18.66±0.31
6	38.65±0.17	38.3±0.22
8	49.34±0.89	41.5±0.76
10	57.92±0.55	51.36±0.82
12	68.22±0.65	67.7±0.74
16	77.25±0.53	76.5±0.65
20	85.36±0.74	87.6±0.45
24	91.41±0.33	93.36±0.34

***Standard deviation, n = 3**

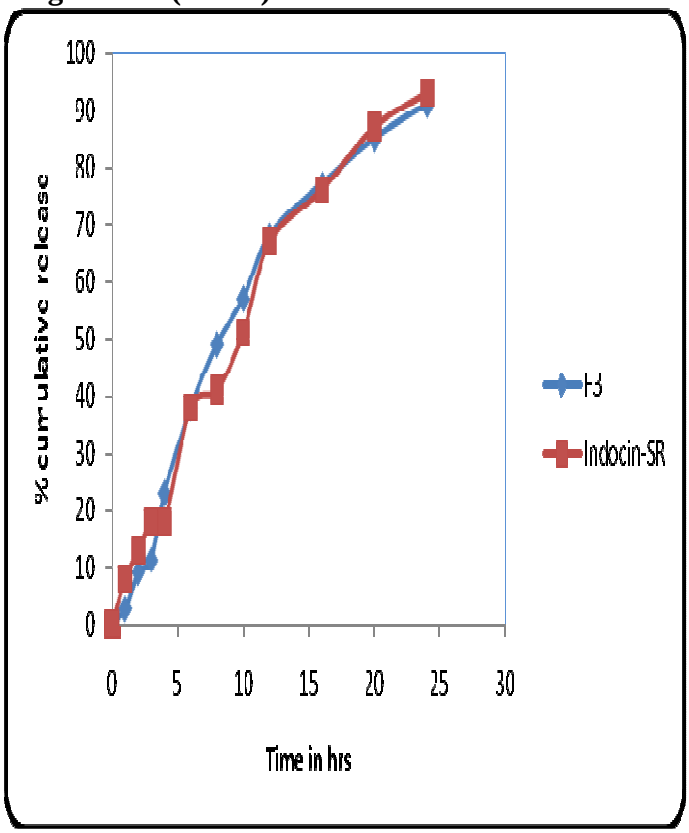


Figure 25: % Cumulative drug release of F3 and Indocin®SR

Release kinetics: The release pattern of the drug from the formulations was obtained by plotting log % cumulative drug retained versus time in h and log % cumulative drug release versus log time in h. The data obtained is given in Table 18. The kinetic plots are shown graphically in Figures 26.

Table 18: Release data of Indomethacin pellets

Time (Hr)	Log % cumulative drug retained						
	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
1	1.99	1.99	1.98	1.98	1.98	1.98	1.98
2	1.98	1.97	1.95	1.96	1.96	1.96	1.96
3	1.97	1.95	1.94	1.93	1.93	1.94	1.93
4	1.96	1.9	1.88	1.87	1.85	1.82	1.87
6	1.9	1.82	1.78	1.84	1.76	1.75	1.84
8	1.86	1.78	1.7	1.76	1.72	1.66	1.73
10	1.8	1.73	1.62	1.72	1.67	1.59	1.72
12	1.76	1.67	1.57	1.67	1.54	1.53	1.67
16	1.68	1.62	1.35	1.54	1.43	1.4	1.54
20	1.54	1.46	1.16	1.43	1.24	1.33	1.43
24	1.44	1.2	0.93	1.24	1.16	1.23	1.21

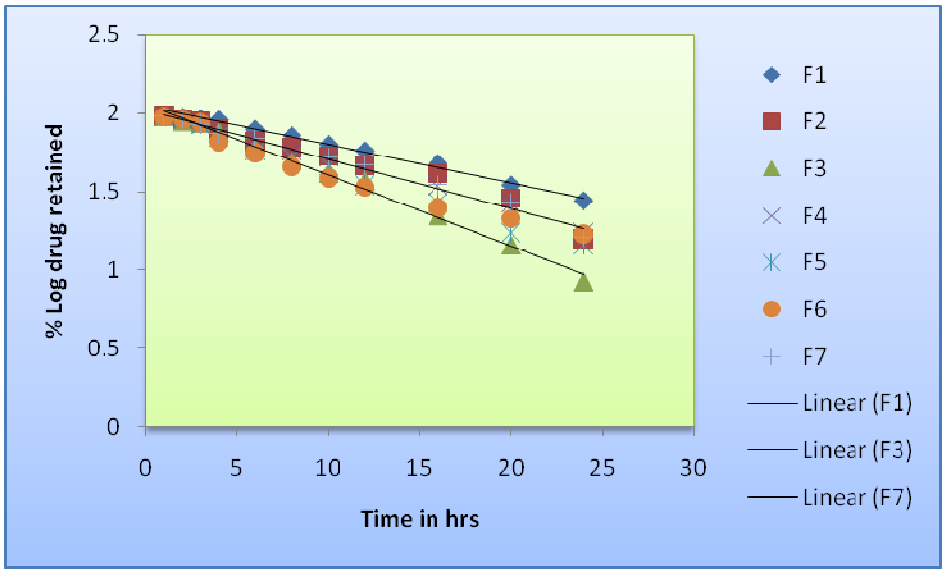


Figure 26: Release kinetics of Indomethacin pellets

Curve fitting: The *in vitro* release studies data was fitted into various mathematical models to determine which the best-fit model. The various parameters n-time exponent, k-release constant and R-regression coefficient, were also calculated.

Koresmeyer-Peppas equation:

$M_t/M_\infty = 1 - A (\exp -Kt)$

$\log (1 - M_t/M_\infty) = \log A - kt/2.303$

- **R = regression co-efficient**
- **n = time exponent**
- **k = release rate constant.**

If the value of 'n' determined from Korsmeyer-Peppas equation was below 0.45, which indicates that the drug release from the formulation follows Fickian diffusion, if 'n'value is between 0.5-0.85, indicates Non-Fickian diffusion or anomalous mechanism (relaxation controlled) and if 'n'value is above 0.89, indicates Super case II transport.

The release data obtained were fitted into various mathematical models. The data obtained from in-vitro release studies was fit into Koresmeyer Peppas Model. The various

parameters, the intercept (A), Release constant (K) and Regression co-efficient (R^2) obtained are given in table 19.

Table 19: Data of various parameters of model fitting for *in vitro* drug release

Formulations	A	R^2
F1	1.2406	0.9915
F2	1.0371	0.9727
F3	1.3188	0.9910
F4	1.0625	0.9761
F5	1.1503	0.9842
F6	1.2905	0.9732
F7	1.0215	0.9623

In all the cases the value of intercept A were found to be more than 0.89. This indicates that the releases of drug from all the formulations were found to be super case-II transport that is release by more than one mechanism.

Higuchi Plot: The amount of drug released versus square root of time was plotted. The plot should be linear if the release of drug from the delivery system is diffusion controlled. The plots were linear and the results inferred that drug release from the pellets formulation was by non- Fickian diffusion. The data are reported in Table 20 and the graph shown in Figure 27.

Table 20: Higuchi plot data of Indomethacin pellets formulations

Square root Of Time	% cumulative drug release						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
1	1.46	2.21	2.72	3.56	3.76	3.84	3.95
1.4	3.92	5.52	9.13	7.18	7.84	8.36	8.08
1.7	5.67	10.58	11.25	13.04	14.2	12.83	10.6
2	8.38	19.78	23.41	25.39	28.34	32.78	17.5
2.4	19.01	33.91	38.65	30.49	41.25	42.69	35.2
2.8	26.22	38.53	49.34	41.72	46.49	53.64	41.25
3.1	36.76	45.69	57.92	46.49	53.11	60.28	52.56
3.4	41.68	52.69	68.22	53.11	64.96	65.39	63.36
4	51.84	58.24	77.25	64.96	72.47	74.69	70.05
4.4	64.78	70.59	85.36	72.47	82.48	78.36	77.18
4.8	72.22	83.94	91.41	82.48	85.36	82.65	81.84

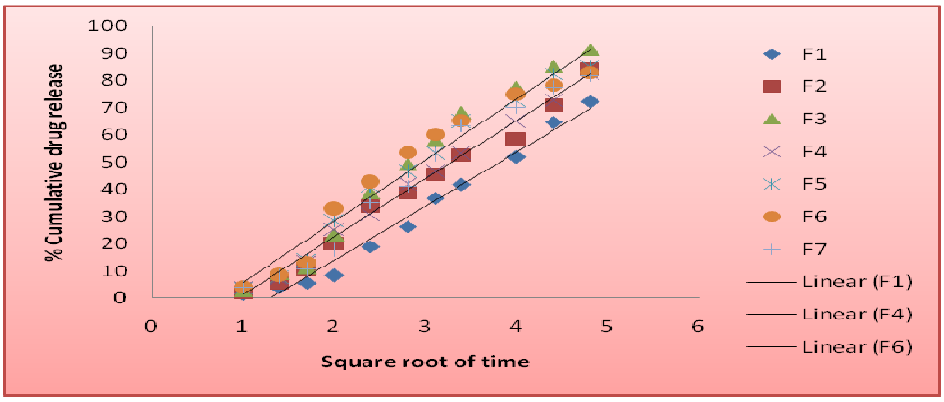


Figure 27: Higuchi plot graph of Indomethacin pellets formulations

Stability studies: The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized formulation (F3) was subjected to stability studies according to ICH guidelines by storing at 25ºC/60% RH and 40ºC/75% RH for 90days. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The obtained data is presented in Table 21. From the Table, it is clear that the formulation did not undergo any chemical changes/interaction during the study period.

Table 21: Stability studies of formulation F3

Stability condition	Sampling (in days)	Drug content (in %) Mean ± SD*
25ºC/60% RH	0	100.00 ± 0.20
	15	99.40 ± 0.48
	45	99.10 ± 0.85
	90	98.70 ± 1.17
40ºC/75% RH	0	100.00 ± 0.52
	15	99.10 ± 1.12
	45	98.20 ± 0.72
	90	97.50 ± 0.79

** Standard Deviation, n=3*

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