# FORMULATION AND EVALUATION OF MESOPOROUS CARRIERS FOR ORAL DRUG DELIVERY

[1] Mrs. Smita Akshay Chavan, [2]Mrs. Aparna Ajinkya Swami
[1] M.Pharm, [2]M.Pharm
[1] Pharmaceutics
[1] Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India.

**ABSTRACT**

The present research work focuses on the use of mesoporous substance like Syloid 244 FP (amphorous silicon dioxide) and Montmorillonite in the drug delivery system as a drug carrier. Solid dosage form (pellet) was formulated by using extrusion-spheronisation technique. The syloid 244 FP have good entrapment capacity of drug as compare to the montmorillonite. The Syloid 244 FP doesn't shows any change in drug content even after change in its proportion with drug. In comparison to that MMT changes in drug content with change in its proportion with drug. For MMT pellets with increase in concentration of MMT increase the hardness of pellets. SEM confirms the smooth and regular surface of MMT pellets as compare to rough and irregular surface of Syloid 244 FP. In comparison to GR product the optimized pellets containing (drug-mesoporous material-polymer composite) showed same acid protection for initial 2 hours. Following that the release was found to gradual upto 5 hours. The marketed Sustained release tablet also shows sufficient acid protectionof drug in initial 2hrs, followed by sustained release up to 12-24 hours. While optimized pellets of mesoporous material (MMT and Syloid 244 FP) the initial acid protection upto 2 hours and then sustained release upto 6 hours. The Stability studies of optimized formulations did not show any significant change in drug content when kept at accelerated stability condition and there was no significant difference in values of % drug content as well as % drug release after 6 hours observed during the stability studies conducted upto 90 days. The mean paw edema volume shows the inflammation was sufficiently decreased in test group as compare to control group and standard group. Optimized formulation shows significant ($P< 0.001$) reduction in inflammation upto 4 hours. The optimized test formulation of Syloid 244 FP shows the higher percent reduction of edema in rats paw upto 4hours as compared to the mmt formulation which shows maximum reduction upto 3 hours.

**Keywords:** Dicolfenac Sodium (DS), Montmorillonite, Syloid 244fp, Gastro retentive (GR).

## Introduction

Mesoporous carriers is apparent as a promising and novel drug vehicle due to its unique its structure that preserving a level of chemical stable, surface functionality and biocompatibility ensure the control and target release of a variety of drug molecules. There are different types of commercially available mesoporous carrier that can be incorporated into the polymer matrix to form polymer nanocomposites[1]. The correct selection of particle absolutely necessary to ensure effective penetration of polymer or its precursor into the interlayer spacing of the reinforcement and result in the desired exfoliated or intercalated product. Most commonly used mesoporous carriers include:Montmorillonite organoclays (MMT), Carbon fibers,Polyhedral Oligomericsilsesquioxane (POSS),Carbon nanotubes, Silica (N-silica), Aluminum oxide (Al2O3), Titanium oxide (TiO2). In this Paper selection of mesoporous carries were selected are montmorillonite and syloid 244fp, the selection of polymer was sodium alginate and drug was used diclofenac sodium[2,6].

## Material and Method

Diclofenac Sodium was obtained as gift sample from Bhalaji Drug, Ahmedabad, Gujarat, sodium Alginate (food grade) were provided from LobaChemie Pvt. Ltd. Mumbai, Montmorillonite was obtained from Sigma-Aldrich Chemicals Pvt. Ltd. Bangalore, Syloid 244 FP were provided from Grace Davison Chemicals India Pvt. Ltd, Hydroxy propyl methyl cellulose (LR) were obtained from Colorcon Asia Pvt. Ltd. Goa, Micro Crystalline Cellulose PH 101( LR) was provided by Centaur Pharmaceuticals Pvt. Ltd. Pune, Disodium Hydrogen Phosphate (AR) was provided by Loba Chemie Pvt. Ltd. Mumbai, Potassium Dihydrogen Phosphate (AR) was provided by Loba Chemie Pvt. Ltd. Mumbai., Sodium Chloride (AR) was obtained from Loba Chemie Pvt. Ltd. Mumbai, Hydrochloric acid (AR) were provided by Loba Chemie Pvt. Ltd. Mumbai.

## METHOD[5,6]

To formulate pellets with desired characteristics like physical appearance and sphericity, the parameters like speed of spheronization and time of spheronization were optimized.The trial batches of pellets were prepared by using extrusion spheronization process. The ingredients including polymers and MCC were weighed accurately, sieved through sieve no.80 and mixed geometrically in a mortar and pestle. The mixture was granulated with binder solution (2.5 % HPMC 50 CPs) and distilled water to form wet mass. This wet mass was then extruded through extruder having 1 mm extruder screen. Extrudate was then spheronized using a Spheronizer; equipped with 3 mm friction plate at the range of 2000 to 2500 rpm for 15 to 20 min as optimized earlier. Spheronized pellets were dried an overnight. These dried pellets were screened through sieve no.40 and evaluated.

## Performulation[4,19,20]

### 1) Compatibility study of drug with excipients in pellets formulation

FTIR spectroscopy can be used to investigates and predict any physicochemical interaction between different components, in formulation and therefore it can be applied to the selection of suitable chemically compatible excipients.The pure drug and formulation ( Montmorillonite and Syloid 244FP) sample and KBr were taken as 1:300 ratio and scanned from 4000$cm^{-1}$ to 400$cm^{-1}$ in FTIR spectrophotometer (Jasco MV 4100).

**2) Differential Scanning Calorimetry (DSC) analysis:**

DSC measurements were performed on a Differential Scanning Calorimeter equipped with an intra-cooler (DSC Mettler STAR SW 9.20, Switzerland). pan, and the samples were heated under nitrogen gas flow (20 ml/min) at a scanning rate of 10 0C per min from 250 to 1200$^0$C. An empty aluminium pan was used as reference.

**Performulation Result and Discussion**

**1) Drug-Excipient compatibility study using FTIR spectroscopy**

IR spectra of drug and pellets formulation are shown fig.1 and 2 for A2 and B2

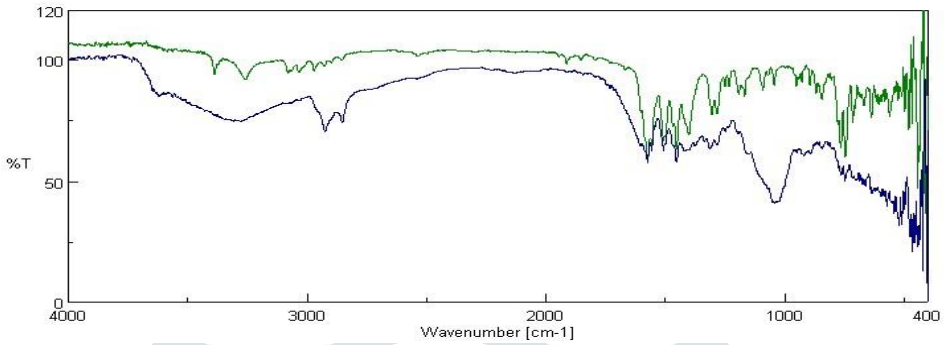



**Figure 1 FT-IR spectra of DS (Green) and A2 pellet formulation (Blue)**

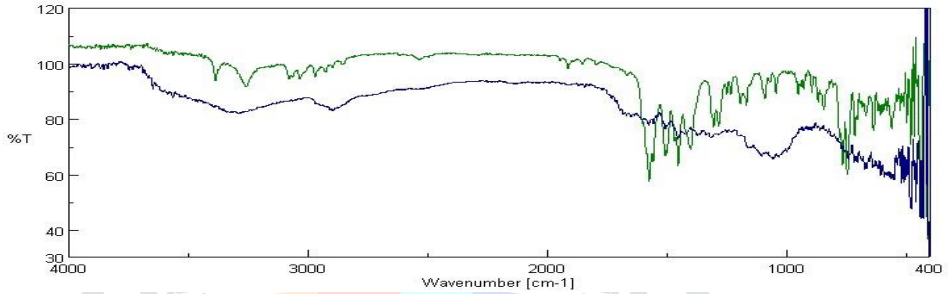



**Figure 2 FT-IR spectra of DS (Green) and Pellets formulation (Blue)**

From FT-IR spectra, all characteristic peaks of drug were retained in pellets formulation with very less or negligible shifting (figure 1,2). These indicate that no any structural or chemical interaction between drug and pellets formulation.

**2) Differential Scanning Calorimetry (DSC) Analysis**

DSC thermograph of the DS (Fig.3) showed sharp endothermic peak at temperature of 290.30$^o$C. Endothermic peak at this region is due to the melting of DS. From DSC it was confirmed that drug has melting point at about 290.30$^o$C.

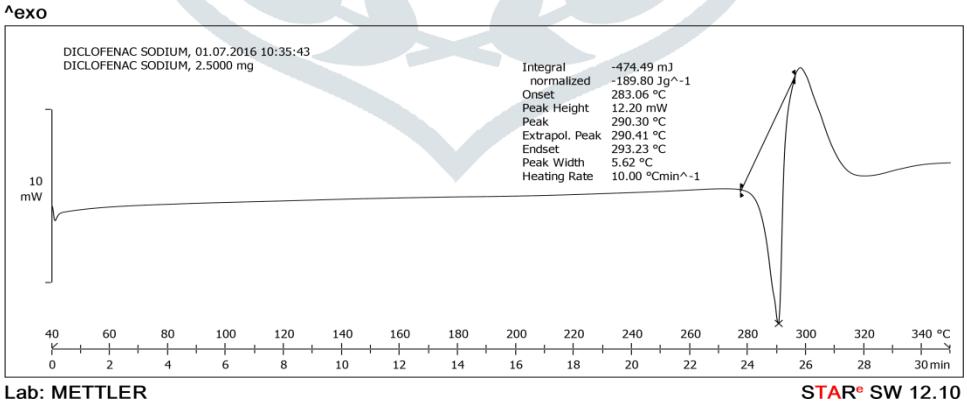



**Figure 3 dsc curve of diclofenac sodium**

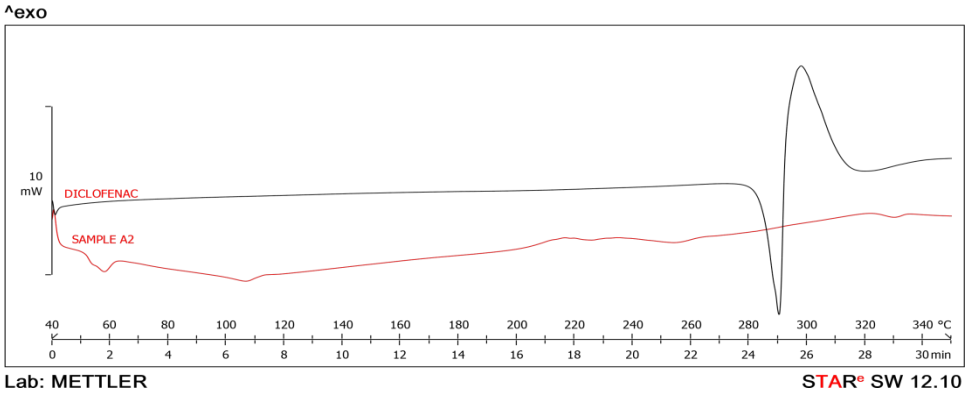



**figure 4 DSC curve of A2Formulation and DS**

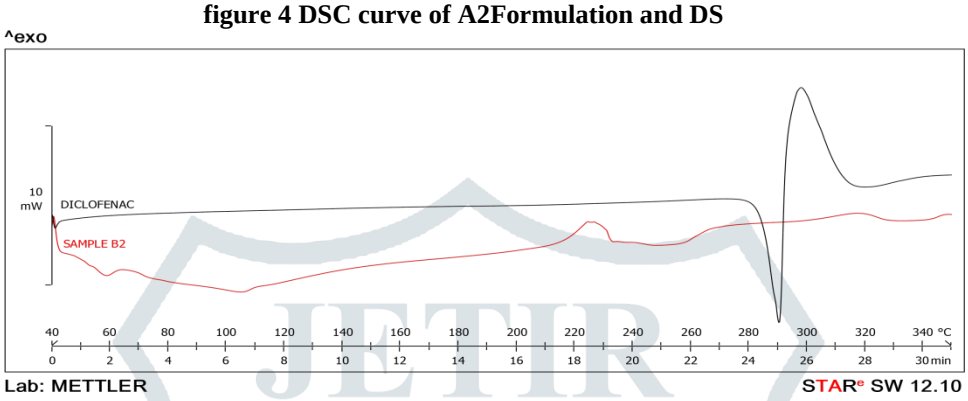



**Figure 5 DSC curve of formulation B2 and DS**

DSC is considered as a tool to investigate the melting behavior of crystalline materials. A2 and B2 of mesoporous formulation was optimized. DSC thermograms of DS showed endothermic peak onset at $283.06^0$C and $290^0$C respectively. Endothermic peak at this region is due to the melting of drugs.

DS loaded pellets prepared by the spheronisation-extrusion method showed a large endothermic peak. Absence of drug that conforms DS in mesoporous carriers is completely entrapped.

**Experimental**[2,9,10,16]

**Preparation of pellets by using drug (DS)**

The pellets were prepared by using two mesoporous materials (Syloid 244 FP, Montmorillonite by following the procedure already explained in 11.5. The composition of pellets with drug is shown in table 1, 2.

**Table 1. preliminary batches of montmorillonite**

| **Formulation** | **$A_1$** | **$A_2$** | **$A_3$** | **$A_4$** | **$A_5$** |
|---|---|---|---|---|---|
| DS (mg) | 1000 | 1000 | 1000 | 1000 | 1000 |
| Sodium Alginate (mg) | 500 | 500 | 500 | 500 | - |
| Montmorillonite (mg) | 2000 | 1000 | 500 | - | 1000 |
| HPMC 50 CPs (2.5% w/v) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| MCC PH 101 (mg) | 500 | 1500 | 2000 | 2000 | 2500 |
| Distilled water | q.s | q.s | q.s | q.s | q.s |

**Table 2. Preliminary batches of Syloid 244 FP**

| **Formulation** | **$B_1$** | **$B_2$** | **$B_3$** | **$B_4$** |
|---|---|---|---|---|
| DS (mg) | 1000 | 1000 | 1000 | 1000 |
| Sodium Alginate (mg) | 900 | 500 | 1000 | - |
| Syloid 244 FP (mg) | 1000 | 500 | - | 500 |
| HPMC 50 CPs (2.5% w/v) | 2.5 | 2.5 | 2 | 2.5 |
| MCC PH 101 (mg) | 2500 | 2000 | 2500 | 2500 |

| | | | | |
|---|---|---|---|---|
| Distilled water | q.s. | q.s. | q.s. | q.s |

**Evaluation of prepared pellets**

**1) Process efficiency**

The percent efficiency of method was determined by using the following equation-

$$\text{Process efficiency} = \frac{\text{wt.of solid dispersion prepared}}{\text{wt.of drug and polymer taken}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

**2) Morphological Characteristics and Flow Properties of Pellets**

All the batches were studied for the morphological features (pellet size, and shape) using digital motic microscope. The prepared pellets were evaluated for the bulk and tapped density,carr's compressibility index, angle of repose, and Hausner's ratio. (Equation 2 to 6)

a) **Bulk Density:** Apparent bulk density was determined by placing pellets into graduated cylinder and measuring the volume and weight "as it is". Bulk density was calculated by equation 2

$$\text{Bulk density} = \frac{\text{Weight of the powder}}{\text{Bulk volume of powder}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

b) **Tapped Density:** It was determined by placing graduated cylinder, containing a known mass of pellets 20 g sample was poured gently through a glass funnel in to a 100 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was not obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated by equation 3.

$$\text{Tapped density} = \frac{\text{Weight of the powder}}{\text{Tapped volume of powder}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

c) **Carr's Index:** A volume of pellets were filled into a graduated glass cylinder and repeatedly tapped for a known duration. The volume of pellets after tapping was measured. Carr's index calculated by equation 4

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

**Table 3. Relationship between % Compressibility and Flowability.**

| Carr's index | Type of Flow |
|---|---|
| 5-15 | Excellent |
| 12-16 | Good |
| 18-21 | Fair to passable |
| 23-35 | Poor |
| 33-38 | Very poor |
| >40 | Extremely poor |

**d) Angle of repose:**

Flow ability of pellets was determined by calculating angle of repose by funnel method. The funnel should have 10 mm inner diameter of stem should be fixed at a height of 2 cm over a platform. About 20 g of sample should slowly pass along the wall of the funnel till the tip of the pile formed and touches the stem of the funnel. A rough circle was drawn around the pile base and the radius of conc was measured. Angle of repose was calculated from the average radius using equation 5.

$$\text{Angle of repose} = \theta = \tan^{-1}\frac{h}{r} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5$$

Where,
Θ= Angle of repose
h = Height of the pile
r = average radius of the powder conc.

**e) Hausner ratio:** It provides an indication of degree of densification which could result from vibration of the feed hopper. Hausner ratio closer to 1 indicates good flow ability of materials. It was calculated by equation 6

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk Density}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 6$$

A Hausner ratio ≤ 1.25 and ≥1 is considered as good and poor flow respectively.

**3) Friability,Drug Content and Hardness**

**a) Friability**

Accurately weighed quantity of pellets (3±0.025 gm) was placed in a friabilator and tumbled for 100 revolutions at 25 rpm. 25 glass beads (3mm in diameter,) were used for attrition effect to occur. Subsequently, the pellets were sieved through sieve no. 60. The weight loss (%) is calculated as:

$$\% F = \frac{Wi - Wr}{Wi} X\ 100 \quad \ldots\ldots 7$$

Where, Wi- initial weight of pellets before friability testing, and

Wr - the weight of pellets retained above the sieve after friability testing.

The friability was carried out in triplicate (n=3), and data was expressed as mean ± Std.D.

**b) Drug content**

Accurately weighed 100 mg crushed pellets and dissolved in 10 ml of methanol. The solution was stirred for 15 min and filtered through whatman filter paper no.41. After suitable dilution, the filtrates were analyzed by spectrophotometrically at 275 nm. The drug content was carried out in triplicate (n=3), and data was expressed as mean ± Std.D.

$$\% \text{ Drug Content} = \frac{\text{Drug amount present}}{\text{Initial amount of drug}} \times 100 \quad \ldots\ldots 8$$

**c) Hardness**

The hardness of all formulated batches wastested by using Brookfield Texture Analyser. The hardness was carried out in ten times (n=10), and data was expressed as mean ± Std.D.

**4) Scanning Electron Microscopy**

The outer macroscopic structure of clay was investigated using Scanning Electron Microscope (JSM 6360, JOEL). Samples were mounted on metal stubs by means of double faced adhesive tape and were sputtered with a thin layer of platinum under vaccum. This coated cuvette placed in the microscope's chamber. The SEM images was taken at acceleration voltage of 20kV, chamber pressure 0.6 mm of Hg, with original magnification of 2000.

**5) *In-Vitro* Drug Release Studies**

In-vitro release of the drug from pellets of all formulation batches was determined using USP Type Iapparatus (Basket). The in-vitrorelease test was performed in 900 ml of 0.1N HCl for initial 2 hrs and further the medium was replaced by 7.4 pH buffer both maintained at 37 ± 0.5°C. Basket speed was kept 50 rpm. At predetermined time interval aliquots were collected and replaced with respective fresh dissolution medium (37 ± 0.5°C). The filtered samples was analyzed spectrophotometrically at 275nm (0.1N HCL) and 275nm (PBS pH6.8). The dissolution was carried out in triplicate (n=3) and data was expressed as mean ± Std.D.

**6) Selection of optimized batch**

The selection of optimized batch is done on bases of drug content, hardness and drug release of the mesoporous pellets.

**7) Stability study of optimized pellets formulation**

In any rationale design and development of dosage forms for drugs, the stability of active component must be major criteria in determining their acceptance or rejection. In the stability studies the product is exposed to accelerated conditions of temperature and humidity. However the studies will take longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature for short period of time.

The optimized formulation filled and sealed in aluminium packaging coated inside with polyethylene, and various replicates were kept in humidity control stability chamber (Oswal's Scientific) maintained at $40^0 \pm 2^0$C for three month. At end of studies, samples were analysed for every month including zero day analysis for the drug content and in vitro release profile.

**8) Evaluation of In-Vivo anti-inflammatory Activity of Optimized pellet formulation**

The pellets was evaluated for anti-inflammatory activity in animals by using Female, Wistar rats (weighing 100-200 g) of local strain and using carrageenan induced rat paw edema inflammatory method. Animals were divided in 3 groups as control, Standard and test (6animals each). The formulation used as test. The dose equivalent amount (10 mg/ kg) of pellets was crushed and dispersed in DW and dispersed in distilled water and administered orally 30 min prior injection of carrageenan solution. Standard dose of marketed formulation (Voveran tablet) in DW was administered orally to standard group. Control group received vehicle. Increases in paw edema volume after 30 min, 1 to 4 hours of carrageenan injection were measured by using Plethysmometer. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Institute (Approval No.MCP/IAEC/170/2015). The experiments were carried in accordance with guidelines of CPCSEA.

- **Statistical analysis**

Results are expressed as mean ±SD. The statistical analysis was performed by Two way Analyses of Variance (ANOVA) followed by Dunnet's t- test. The $P<0.05$ was considered as statistically significant.

% inhibition of inflammation was calculated using equation .9

$$\% \text{ Reduction of edema} = \frac{C - T}{C} \times 100 \quad \ldots\ldots 9$$

Where,

C= % swelling of control group (untreated)

T= % swelling of treated group

**Result and Discussion**

**1) Evaluation process efficiency**

All the Formulations of pellets were evaluated by process efficiency. The result as a percent yield are shown in table 4 and 5

**Table 4. Process efficiency of extrusion spheronization process of MMT**

| Batch | % Yeild |
|---|---|
| $A_1$ | 63.76 |
| $A_2$ | 69.07 |
| $A_3$ | 34.32 |
| $A_4$ | 84.15 |
| $A_5$ | 75.7 |

**Table 5. Process efficiency of extrusion spheronization process of Syloid 244 FP**

| Batch | % Yeild |
|---|---|
| $B_1$ | 50.46 |
| $B_2$ | 69.2 |
| $B_3$ | 84.15 |
| $B_4$ | 66.5 |

All the Formulations shown the satisfactory yield in the range of 34.32% to 84.15% (Table 4, 5).It can be noted that the change in concentration of clay in formulation, there was change in process efficiency of the extrusion spheronization process.

From table 5 change in concentration of the Syloid 244 FPcauses change in process efficiency. With increase in the concentration of Syloid 244 FP, percent yield decreased. From table 5 appropriate concentration (1g) of MMT gave the good yield/ increase in process efficiency. If the concentration is high or low than 1 gm,percentage yield of MMTpellets is decrease.

From the above results shows that MMT shows good process efficacy as compare to Syloid 244 FP 244fp.

**2) Evaluation of Morphological Characteristics and Flow Properties of Prepared Pellets**

The flow properties of all Formulations are shown in Table 12.6 and 12.7

**Table 6. morphological and flow properties of MMT**

| Formulations | Bulk density* ($gm/cm^3$) | Tapped density* ($gm/cm^3$) | Carr's index* (%) | Hausners ratio* (H) | Angle of repose*(θ) |
|---|---|---|---|---|---|
| $A_1$ | 0.7584±0.05 | 0.7946±0.02 | 4.55±1.13 | 1.0477±0.22 | 28.22±1.43 |
| $A_2$ | 0.6766±0.04 | 0.7152±0.07 | 5.39±0.99 | 1.057±0.16 | 31.28±2.19 |
| $A_3$ | 0.6529±0.034 | 0.6529±0.034 | 5.008±0.25 | 1.052±0.002 | 28.06±2.60 |
| $A_4$ | 0.7464±0.03 | 0.7938±0.04 | 5.97±0.61 | 1.0635±0.19 | 27.22±2.51 |
| $A_5$ | 0.5752±0.01 | 0.6103±0.03 | 5.75±0.75 | 1.061±0.06 | 28.65±2.64 |

* All reading taken in triplicate, n ± SD

**Table 7. morphological and flow properties of Syloid 244 FP**

| Formulations | Bulk density* ($gm/cm^3$) | Tapped density* ($gm/cm^3$) | Carr's index* (%) | Hausners ratio* (H) | Angle of repose*(θ) |
|---|---|---|---|---|---|
| $B_1$ | 0.6337±0.01 | 0.6676±0.02 | 5.07±1.09 | 1.0534±0.23 | 28.45±3.01 |
| $B_2$ | 0.53±0.06 | 0.56±0.06 | 5.35±0.46 | 1.056±0.16 | 31.11±1.06 |
| $B_3$ | 0.7464±0.03 | 0.7938±0.04 | 5.97±0.61 | 1.0635±0.19 | 27.22±2.51 |
| $B_4$ | 0.7249±0.03 | 0.7465±0.01 | 5.07±1.09 | 1.0297±0.03 | 33.21±1.95 |

* All reading taken in triplicate, n ± SD

The observed values of angle of repose, bulk density and tapped density are in the range of 27.22 to 33.21, 0.53 to 0.7584 ($gm/cm^3$) and 0.56 to 0.7946 ($gm/cm^3$) respectively. The value of Carr's index below 15% indicates excellent flow characteristics whereas that above 25% indicates poor flow ability. Hausner's Ratio (H) is an indirect index for ease of powder flow. The flow properties of the all formulations were found to be excellent.

The Syloid 244 FP and MMT shows the good morphological and flow properties.

**3) Friability, Drug content and Hardness**

All the Formulations of pellets were evaluated by friability testing, precent drug content and hardness. The result as a percent friability, percent drug content and hardeness are shown in table 8 and 9.

**Table 8 percent friability, drug content, hardness of MMT**

| Batch | % Friability* | % drug content* | Hardness* (gm) |
|---|---|---|---|
| $A_1$ | 0.03±0.090 | 107.8±0.062 | 1305.8±0.051 |
| $A_2$ | 0.026±0.088 | 84.34±0.038 | 1106.6±0.865 |
| $A_3$ | 0.023±0.083 | 54.45±0.024 | 909.3±0.060 |
| $A_4$ | 0.7±0.113 | 104±0.012 | 419.5±0.980 |
| $A_5$ | 0.33±0.085 | 100±0.011 | 1183.7±0.943 |

* All reading taken in triplicate, n ± SD

**Table 9 percent friability, drug content, hardness of Syloid 244 FP**

| Batch | % Friability* | % drug content | Hardness *(gm) |
|---|---|---|---|
| $B_1$ | 1±0.079 | 93.78±0.036 | 839.9±0.933 |
| $B_2$ | 0.633±0.086 | 94.73±0.024 | 714.9±0.860 |
| $B_3$ | 0.66±0.113 | 104±0.012 | 419.5±0.980 |
| $B_4$ | 0.7±0.098 | 100±0.036 | 546.8±1.101 |

* All reading taken in triplicate, n ± SD

All the Formulations, the percent friability was less than 0.5-1 % indicates all Formulations are in acceptable range.As result shown of Syloid 244 FP (table12.9) the Syloid 244 FP is less friable as compare to the MMT (table 12.8). Although the both batch shows less than 1%. Hence the MMT is having high percent friability.

All the Formulations have shown percentage drug content nearly 100% . Drug content of all the Formulations are found in the range of 54.45 to 104%. Therefore extrusion spheronization technique can be used effectively for preparation of pellets. The Syloid 244 FP shows good percent drug content. The Syloid 244 FP have good entrapment capacity of drug as compare to the montmorillonite. The Syloid 244 FP doesn't shows any change in drug content even concentration of Syloid 244 FP changes. With increase in the concentration of MMT, the drug content increases. So the Syloid 244 FP shows better drug content as compared to MMT.

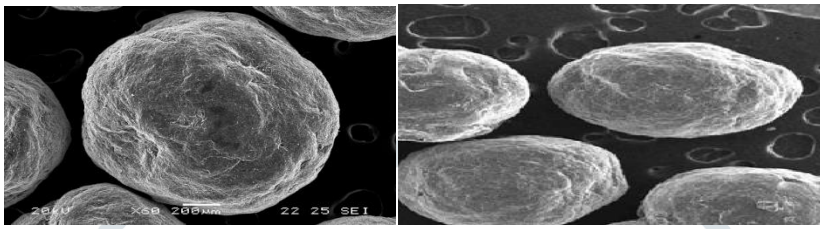
In present work, the hardness of all Formulations were tested by using Texture Analyser and result are shown in table 8and 9. The Syloid 244 FP shows low hardness as compare to the MMT. The Syloid 244 FP texture is smooth and fuffy in the nature. So the hardness decrease as the concentration of Syloid 244 FP is reduced. In MMT the concentration of MMT increase, there is increase in the hardness of pellets.

**4) Evaluation of pellets for Scanning Electron Microscopy (SEM)**

The scanning electron microscopic (SEM) evaluation is important for determining the surface morphology, size, shape.

(a) (b)

**figure 6 SEM images of MMT [a, b] pellets**



(a) (b)

**figure 7 SEM images of Syloid 244 FP [a,b] pellets**

Surface of pellets as shown in SEM images also good and size of pellets was found to be 200µm and ratio of length to width (Aspect ratio) is 1.03 which indicates pellets are spherical in shape.

The Syloid 244 FP shows irregular surface, whereas the MMT shows smooth surface.

**5) Evaluation of pellets for In-Vitro Drug Release**

All formulations of pellets were evaluated for in-vitro drug release upto 7 hours. The results are shown in table 10 and 11.

**Table 10 cumulative drug release (%) of MMT pellets**

| Time | $A_1$ | $A_2$ | $A_3$ | $A_4$ | $A_5$ |
|---|---|---|---|---|---|
| **0.1N HCl** | | | | | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5 | 1.20±0.05 | 1.874±1.49 | 1.32±0.06 | 2.78±0.04 | 2.34±0.57 |
| 10 | 1.35±0.12 | 2.38±0.08 | 1.45±0.09 | 3.89±0.10 | 2.56±0.06 |
| 15 | 2.45±0.07 | 2.56±0.14 | 2.09±0.23 | 9.23±0.13 | 3.13±0.08 |
| 30 | 2.89±0.11 | 2.74±0.11 | 2.45±0.12 | 15.45±0.17 | 3.29±1.34 |
| 60 | 2.93±0.23 | 2.77±0.07 | 2.56±0.17 | 29.98±0.06 | 3.45±0.10 |
| 90 | 3.03±0.17 | 2.84±0.19 | 2.63 ±0.03 | 41.45±0.08 | 3.56±1.03 |
| 120 | 3.12±0.19 | 2.92±0.35 | 2.72±0.18 | 56.19±0.07 | 6.10±0.06 |
| **PBS pH7.4** | | | | | |
| 150 | 24.22±0.14 | 26.1±0.05 | 31.84±0.12 | 77.02±0.09 | 28.29±0.87 |
| 180 | 38.32±0.04 | 40.5±0.05 | 42.05±0.21 | 74.14±0.06 | 29.63±0.56 |
| 240 | 37.68±0.18 | 58.5±0.01 | 47.04±0.24 | 61.61±0.05 | 37.89±0.78 |
| 300 | 47.8±0.05 | 69.3±0.02 | 56.6±0.03 | | 48.91±0.15 |
| 360 | 61.3±0.04 | 82.8±0.01 | 59.7±0.19 | | 67.5±0.75 |
| 420 | 73.2±0.18 | 78.3±0.27 | 48.8±0.08 | | |

*All values are expressed as mean± SD, n=3.

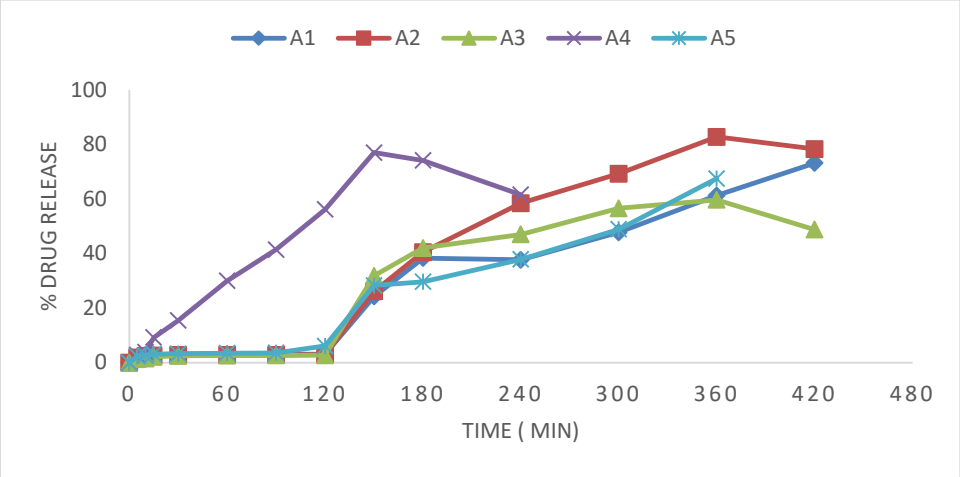



**Figure 8 cumulative drug release (%) of MMT pellets**

**Table 11 cumulative drug release (%) of Syloid 244 FP pellets**

| Time | $B_1$ | $B_2$ | $B_3$ | $B_4$ |
|---|---|---|---|---|
| **0.1 N HCl** | | | | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5 | 1.26±0.10 | 0.23±0.04 | 2.78±0.04 | 1.89±0.93 |
| 10 | 1.67±0.87 | 1.45±0.06 | 3.89±0.10 | 2.65±0.87 |
| 15 | 2.41±0.05 | 2.07±0.78 | 9.23±0.13 | 7.08±0.06 |
| 30 | 2.76±0.08 | 2.64±0.08 | 15.45±0.17 | 10.65±0.73 |
| 60 | 2.97±0.06 | 2.76±0.73 | 29.98±0.06 | 13.02±0.08 |
| 90 | 3.28±0.78 | 2.87±0.10 | 41.45±0.08 | 16.78±0.78 |
| 120 | 3.56±0.23 | 3.07±0.56 | 56.19±0.07 | 19.38±0.67 |
| **PBS pH 7.4** | | | | |
| 150 | 32.24±0.10 | 45.3±0.06 | 77.02±0.09 | 23.90±0.87 |
| 180 | 41.66±0.34 | 51.5±0.78 | 74.14±0.06 | 28.85±0.65 |
| 240 | 43.57±0.06 | 66.3±0.87 | 61.61±0.05 | 34.78±0.10 |
| 300 | 52.25±0.73 | 72.9±0.32 | | 49.89±0.37 |
| 360 | 53.25±0.13 | 81±0.67 | | 53.67±0.23 |
| 420 | 58.94±0.45 | 78.3±0.08 | | |

*All values are expressed as mean± SD, n=3.

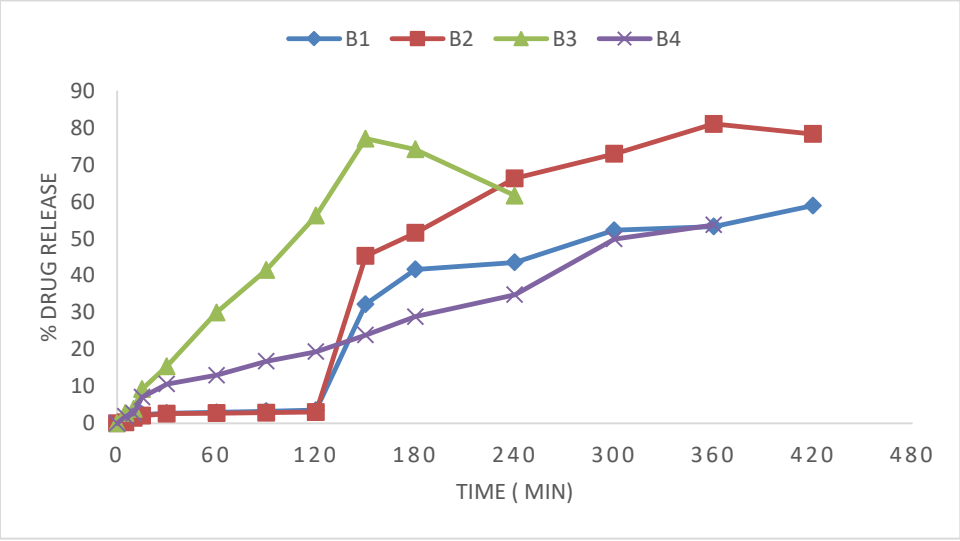



**figure 9 cumulative drug release (%) of Syloid 244 FP pellets**

The in-vitro drug release is depend on the concentration of mesoporous material (MMT and Syloid 244 FP).

The formulation A1 DS: MMT (1:2) ratio gives less release of DS which 61.3% upto 6 hours. The formulation A2 DS: MMT (1:1) ratio gives the desired drug release of DS which is 82% upto 6 hours.The formulation A3 DS-MMT (1:0.5) ratio protect drug in acidic pH and in PBS 7.4 shows slow drug release (59% within 6 hours). The formulation A4 MMT is not added, shows immediate drug release and also drug gets release acidic medium (upto 60%). The formulation A5 Sodium alginate is not added, shows drug release in acidic medium and PBS pH7.4 medium upto 67.5 within 6 hours.

The formulation B1DS–Syloid 244 FP (1:1) ratio shows 53% drug release in 6 hr. The formulation B2 reduces the DS–Syloid 244 FP (1:0.5) ratio gives desired drug release of DS (81% within 6 hrs). The formulation B3 Syloid 244 FP is not added, drug release in acidic medium and

in PBS shows immediate release (within 3 hours). The formulation B4 sodium alginate is not added, shows drug release in acidic medium and PBS pH7.4 medium upto 53% within 6 hours.
From drug release the A2 and B2 shows the optium release of DS.

**6) Selection of optimized batch**

The desired properties drug content, hardness and *In-vitro* drug release of trial Formulations is shown in table 12. The concentration of polymer and there combination affect the drug release of Syloid 244 FP and montmorillonite pellets. The combination of sodium alginate can successfully modify the drug release. (Figure 10)

**Table 12. Drug release and effect of polymer on drug release.**

| Trial Formulations | Drug content (%) | Hardness (gm) | Observation |
|---|---|---|---|
| $A_1$ | 107.8±0.062 | 1305.8±0.051 | DS-MMT(1:2) ratio gives less drug release of DS (61.3% within 6 hours) |
| $A_2$ | 84.34±0.038 | 1106.6±0.865 | DS-MMT (1:1) ratio gives the desired drug release of DS (82% within 6 hours) |
| $A_3$ | 54.45±0.024 | 909.3±0.060 | DS-MMT (1:0.5) ratio protect drug in acidic pH and in PBS 7.4 shows slow drug release(59% within 6 hours) |
| $A_4$ | 100±0.016 | 419.5±0.98 | MMT is not added, shows immediate drug release and also drug gets release acidic medium (upto 60%) |
| $A_5$ | 100±0.011 | 1183.7±0.943 | Sodium alginate is not added, shows drug release in acidic medium and PBS pH7.4 medium upto 67.5 within 6 hours |
| $B_1$ | 93.78±0.036 | 469.3±0.933 | DS–Syloid 244 FP (1:1) ratio shows 53% drug release in 6 hr. |
| $B_2$ | 94.73±0.024 | 714.9±0.860 | Reduce the DS–Syloid 244 FP (1:0.5) ratio gives desired drug release of DS (81% within 6 hrs) |
| $B_3$ | 100±0.012 | 419.5±0.98 | Syloid 244 FP is not added, drug release in acidic medium and in PBS shows immediate release (within 3 hours) |
| $B_4$ | 100±0.036 | 546.8±1.101 | Sodium alginate is not added, shows drug release in acidic medium and PBS pH7.4 medium upto 53% within 6 hours |

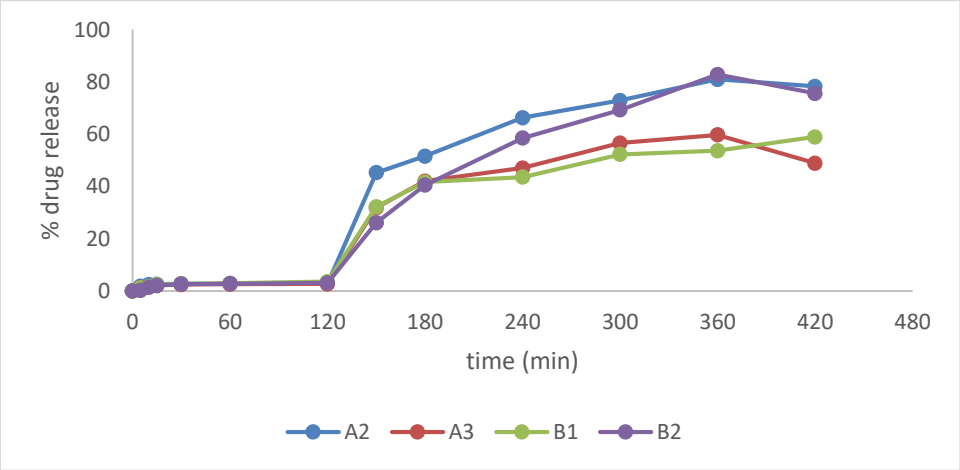



**Figure 10 Drug release profile of trial batch.**

Result shows the batch $B_2$ and $A_2$give desirable drug release and properties as compare to other Formulations. Hence, batch $B_2$ and $A_2$composition selected for sustain release phase
The drug content of the Syloid 244 FP is higher than the montmorillonite. But the hardness of the montmorillonite is greater than the Syloid 244 FP. The drug release of the montmorillonite and Syloid 244 FP is the same.

**7) Comparisonof optimized formulations with marketed formulation**

The formulations of both mesoporous carrier which shows better results of drug release are chosen for comparison with the marketed formulation (Rectin 100 SR tablet, Voveran GR tablet 50mg). Among all above batch B2 and A2 were selected for in-vitro drug release comparison and release pattern shown in Fig 11.

**Table 13 comparison optimized formulation with marketed tablet**

| Time | B2 | A2 | GR | SR |
|---|---|---|---|---|
| **0.1N HCl** | | | | |
| 5 | 0.23±0.04 | 1.874±1.49 | 1.89±0.08 | 0.511±0.98 |
| 10 | 1.45±0.06 | 2.38±0.08 | 1.94±0.07 | 0.794±0.35 |
| 15 | 2.07±0.78 | 2.56±0.14 | 2.25±0.56 | 1.00±0.23 |
| 30 | 2.64±0.08 | 2.74±0.11 | 2.25±0.78 | 1.257±0.09 |
| 60 | 2.76±0.73 | 2.77±0.07 | 2.25±0.08 | 1.488±0.54 |
| 90 | 2.87±0.10 | 2.84±0.19 | 2.309±0.78 | 2.131±0.12 |
| 120 | 3.07±0.56 | 2.92±0.35 | 3.42±0.08 | 2.363±0.67 |
| **PBS pH 7.4** | | | | |
| 150 | 45.3±0.06 | 26.1±0.05 | 93.6±0.56 | 9.09±0.34 |
| 180 | 51.5±0.78 | 40.5±0.05 | 94.14±0.45 | 19.58±0.56 |
| 240 | 66.3±0.87 | 58.5±0.01 | 94.14±0.45 | 23.19±0.28 |
| 300 | 72.9±0.32 | 69.3±0.02 | 94.68±0.23 | 33.9±0.45 |
| 360 | 81±0.67 | 82.8±0.01 | 91.8±0.56 | 56.7±0.89 |
| 420 | 78.3±0.08 | 78.3±0.27 | 86.4±0.92 | 65.1±0.78 |
| 24 hrs | 73.8±0.09 | 73.2±0.12 | 92.34±0.11 | 96±0.23 |

*All values are expressed as mean± SD, n=3.

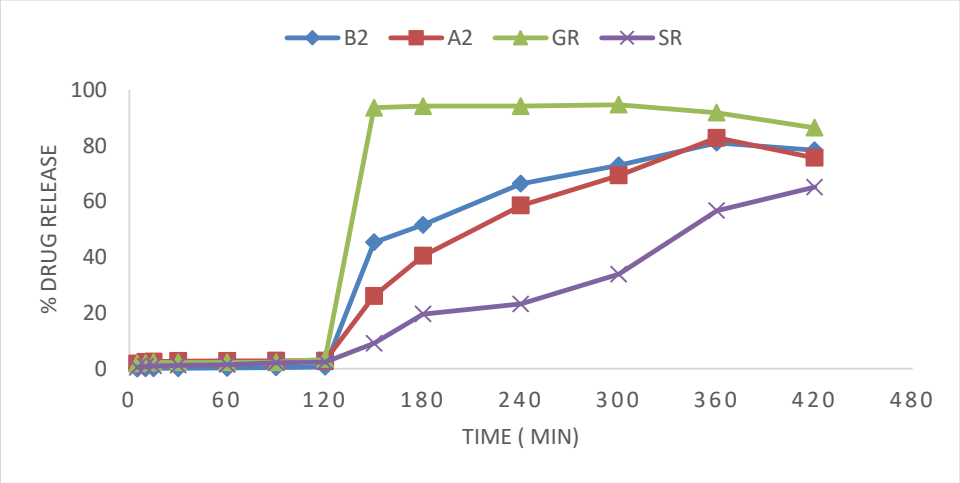



**Figure 11 comparison of optimized formulation with marketed tablet**

The marketed gastro-retentive tablet shows sufficient acid protection for initial 2 hrs.But it shows complete release of DS within 1 hr in PBS pH 7.4.The marketed Sustained release tablet shows slow release of DS in initial 2hrs, and sustained release up to 24 hrs in PBS pH7.4.While the diclofenac sodium loaded pellets shows the an extended release pattern with optimal acid protection for initial 2 hrs.A2 pellets formulation shows 82% drug release up to 6 hrs. And B2 pellets formulation 81% drug release up to6 hrs.

**8) Stability studies of optimized formulation**

The stability studies were carried out only for optimized formulations (A2 and B2). The sample were filled in capsules and wrapped in aluminum foil and kept at $40^0$± $2^0$C and $75^0$ ± 5% RH for three months to access their stability. After 90 days samples were withdrawn and retested for drug content and drug release studies.

For pellets of A2 (Syloid 244 FP) drug content and drug release was observed to be 84.34±0.038 and 82.8±0.01 at 6 hours for the initial period (0 day). After storage for 90 days drug content and drug release was observed 81.22±0.73 and 80.9±0.63 after 6 hours.

For pellets of B2 (MMT) drug content and drug release was observed to be 94.73±0.024 and 81±0.67at 6 hours for the initial period (0 day). After storage for 90 days drug content and drug release was observed 93.54±0.033and 79.56±0.37after 6 hours

The Optimized formulation did not show any significant change in drug content when kept at different accelerated stability chamber condition and there was no significant difference in values of % drug content as well as % drug release after 6 hours observed during the stability studies after 90 days.

**9) Evaluation of In-vivo Anti-inflammatory activity of optimized formulations**

Optimized formulation was evaluated for anti-inflammatory activity. Female Wistar rats of local strain were used for the anti-inflammatory study by carrageenan induced rat paw edema model.

**Table 14 animal grouping with treatment**

| Group | Number of animals | Treatment |
|---|---|---|
| Control | 06 | Normal Saline |
| Standard | 06 | Voveran(10 mg/ kg) |
| Test | 06 | A2 & B2 ( 10 mg/ kg) |

Protocol of the in-vivo Animal study was approved by Institutional Animal Ethical Committee (IAEC) (Approval No MCP/IAEC/170/2015.). The animals were kept for one week in the animal house before the experiment to be conducted, and they were maintained on proper supplies of food and water. Animals were grouped as Control, Reference and Test (each contains 6 animals). The test formulation was administered orally as dispersion in DW, and Voveran solution in DW is used as reference. Control group receives normal saline solution; dose equivalent to 10 mg/kg was administered orally to respective groups. 30 min after the dose, 0.1 ml of 1% carrageenan

solution in normal saline was injected sub plantarly into the right paw. After dosing paw volume was measured using plethymometer (VJ Instruments), at various time intervals for all groups.

**10) In-Vivo anti-inflammatory Activity**

**a) In-Vivo anti-inflammatory activity of A2**

The result of mean paw volume and percentage inhibition of rats is shown in table 15 and 17

**Table 15 results of in-vivo anti-inflammatory activity of A2**

| Group | Mean paw volume (ml) ±SEM | | | | |
|---|---|---|---|---|---|
| | Time (hrs.) | | | | |
| | 30 min | 1 | 2 | 3 | 4 |
| Control | 1.42±0.09 | 1.39±0.13 | 1.12±0.78 | 1.57±0.65 | 1.42±0.04 |
| A2 | 1.36±0.26* | 1.24±0.172* | 0.89±0.103** | 1.218±0.175** | 1.36±0.150* |
| Ref | 1.38±0.189 | 1.30±0.202 | 1.2±0.300 | 1.38±0.291* | 1.38±0.212* |

Values are expressed in mean ± SEM (n=6), * P<0.05, **P< 0.01 as compare to control, data analyze by ANOVA followed by Dunnets test. (SEM- Standard Error Mean)

The result of mean paw edema volume shows sufficient increase in control group, while in treated groups the inflammation was shown to be reduced as compared to control group. Optimized formulation shows significant (P<0.01) reduced in 30 min and upto 4 hrs. Thus the optimized test formulation (A2) shows optimal anti-inflammatory effect as compare with the reference.

The results of anti-inflammatory activity of optimized formulation and reference are shown in table 16

**Table 16 percent inhibition of edema in rat paw for marketed formulation and A2**

| Time | % Inhibition | |
|---|---|---|
| | Standard* | Test* |
| 30 min | 4.41±0.260 | 2.89±0.189 |
| 1 hr | 6.92±0.172 | 12.09±0.202 |
| 2 hrs | 7.69±0.103 | 25.84±0.300 |
| 3 hrs | 13.76±0.175 | 29.75±0.291 |
| 4 hrs | 19.55±0.150 | 5.55±0.212 |

*All values are expressed as mean± SD, n=3.

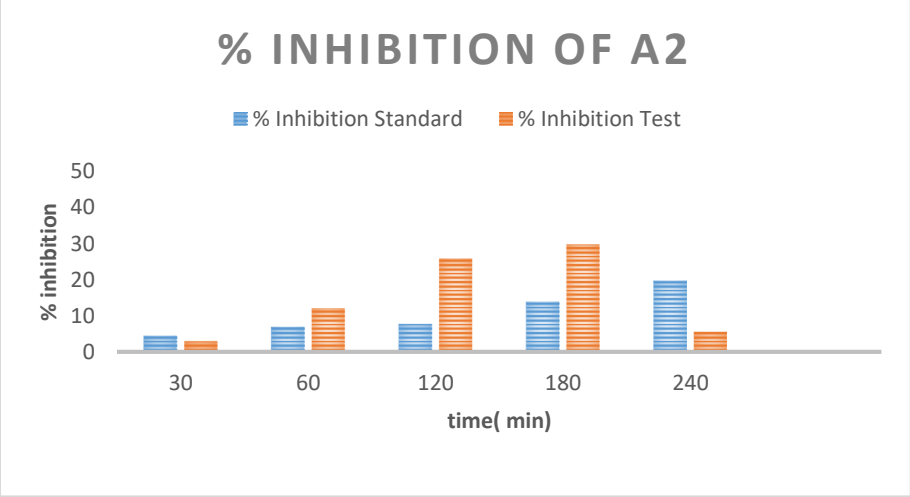



**Figure 12 percent reduction of edema in rat paw for marketed formulation and A2**

As compare to the marketed formulation MMT showed higher percentage inhibition till the 3 hours. Then the % percentage inhibition at 4 hour of MMT formulation is lower than the marketed formulation. As compare to in- vitro release the % inhibition is good and shows the optium.

**b) In-Vivo anti-inflammatory activity of A2**

The result of mean paw volume and percentage inhibition of rats is shown in table17 and 18

**Table 17 results of in-vivo anti-inflammatory activity of B2**

| Group | Mean paw volume (ml) ±SEM | | | | |
|---|---|---|---|---|---|
| | time (hrs) | | | | |
| | 30 min | 1 | 2 | 3 | 4 |
| Control | 0.9±0.03 | 0.912±0.05 | 1.05±0.03 | 0.902±0.09 | 1.03±0.02 |
| Ref. | 0.791±0.37* | 0.792±0.138* | 0.78±0.34** | 0.636±0.269* | 0.306±0.092** |
| B2 | 0.702±0.20* | 0.664±0.43** | 0.58±0.15** | 0.559±0.265** | 0.508±0.21** |

Values are expressed in mean ± SEM (n=6), * P<0.05, **P< 0.01 as compare to control, data analyze by ANOVA followed by Dunnets test. (SEM- Standard Error Mean)

The result of mean paw edema volume shows sufficient increase in control group, while in treated groups the inflammation was shown to be reduced as compared to control group. Optimized formulation shows significant (P<0.01) reduced in 30 min and upto 4 hrs. Thus the optimized test formulation (B2) shows optimal anti-inflammatory effect as compare with the reference.

The results of anti-inflammatory activity of optimized formulation and reference are shown in table 18

**Table 18 percent reduction of edema in rat paw for marketed formulation and B2**

| Time | % Inhibition | |
|---|---|---|
| | Standard | Test |
| 30 | 13.78±0.377 | 28.20±0.200 |
| 60 | 15.15±0.138 | 37.34±0.431 |
| 120 | 34.61±0..346 | 79.25±0.152 |
| 180 | 41.82±0.269 | 61.35±0.265 |
| 240 | 238.5±0.092 | 103.93±0.214 |

*All values are expressed as mean± SD, n=3.

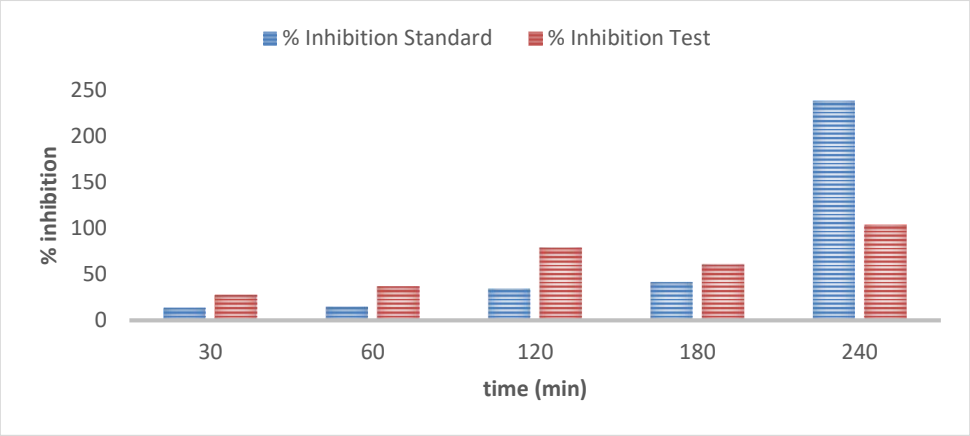



**Figure 13 percent reduction of edema in rat paw for marketed formulation and B2**

As compare to the marketed formulation Syloid 244 FP showed higher percentage inhibition till the 3 hours. Then the % percentage inhibition at 4 hour of Syloid 244 FP formulation is lower than the marketed formulation. As compare to in- vitro release the % inhibition is good and shows the optium.

The Syloid 244 FP showed the higher percent reduction (103%) of edema in rats paw within 4hrs. As the montmorillonite with 3 hrs shows the maximum inhibition. The shows the best results.

**Conclusion**

Orally administered modified release formulations are better over conventional multi dose delivery systems, particularly for long term therapeutic effect in case of chronic inflammatory conditions. Mesoporous carriers like MMT and Syloid 244 FP when intercalated with drug can used for the controlled release formulation. In the present work mesoporous carriers were evaluated by using DS as a model drug for their suitability as a carrier to produce a stable and effective oral dosage form. The present study shows that both the carriers showed best compatibility with drug and excipients used in the study. At the same time both the carriers showed proper flow properties moderate drug release (in-vitro) and stability in acidic environment in GI tract. The present work shows MMT has better process efficacy and showed good hardness to the pellets, whereas Syloid 244 FP has shown sufficient drug content and slightly better in-vivo efficiency of the pellets. The present study confirms the potential of both the mesoporous carriers in oral drug delivery system.

**References**

1. Altman R, Bosch B, Brune K, Patrignani P, Young C (2015), Advances in NSAID development: evolution of Diclofenac Products using pharmaceutical technology, Drugs 75: 859-77.
2. Diclofenac Epolamine, the American Society of Health-System Pharmacists. Retrieved 3 April 2011.
3. M. Sugunalakshmi, H. BavaBakrudeen, B. Abilash,P. N. Krishnan, A. B. Mandal, (2014), Inorganic clay mineral, Montmorrillonite for the Adsorption of Isoniazid drug, formulations and release studies, International Journal of Recent Advances in Engineering & Technology(2): 15-22.
4. J.P. Zheng, L. Luan, H.Y. Wang, L.F. Xi, K.D. Yao, (2007) Study on ibuprofen/montmorillonite intercalation composites as drug release system, Applied Clay Science 36: 297–301
5. Dutta N. K, Mazumdar K, Dastidar S.G, Park J. H, (2007), Activity of diclofenac used alone and in combination with streptomycin against Mycobacterium tuberculosis in mice, International Journal of Antimicrobial agents,30 (4):336-40.
6. Yueqi Wang, Hao Huang, Chungang Zhang , Yilin Tang, Jinzhuo Li , Xing Tang, CuifangCai (2013), Preparation of highly stable diclofenac potassium pellet with microcrystalline cellulose by extrusion-spheronization, Asian Journal of pharmaceutical sciences 8:356-361.
7. Nattawut Charoenthai, Peter Kleinebudde, SatitPuttipipatkhachorn, (2007), Influence of Chitosan Type on the Properties of Extruded Pellets With Low Amount of Microcrystalline Cellulose, AAPS PharmSciTech; 8 (3) Article 64.
8. R.S. Satoskar, S. D. Bhandarkar, (2007), Pharmacology and Pharmcotherapeutics, Popular Prakashan: 171.
9. Rouquerol, J., Avnir, D., Fairbridge, C.W., Everett, D.H., Haynes, J.M., Pernicone, N., Ramsay, J.D.F., Sing, K.S.W., Unger, K.K. (1994). Recommendations for the characterization of porous solids (technical report). Pure Appl. Chem. 66:1739-1758
10. Shegokar, R., Müller, R.H, (2010). Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives. Int. J. Pharm. 399:129-139
11. Euliss, L.E., DuPont, J.A., Gratton, S., DeSimone, J., (2006). Imparting size, shape, and composition control of materials for nanomedicine. Chem. Soc. Rev. 35:1095-1104.
12. Lehmann, V., Stengl, R., Luigart, A., (2000) On the morphology and the electrochemical formation mechanism of mesoporous silicon. Mater. Sci. Eng., B 69-70: 11-22.
13. Salonen, J., Lehto, V.-P., (2008) Fabrication and chemical surface modification of mesoporous silicon for biomedical applications. Chem. Eng. J. 137: 162-172.
14. Lehmann, V., Grüning, U., (1997). The limits of macropore array fabrication. Thin Solid Films 297: 13-17.

15. Salonen, J., Kaukonen, A.M., Hirvonen, J., Lehto, V.-P., (2008). Mesoporous silicon in drug delivery applications. J. Pharm. Sci. 97: 632-653
16. Salonen, J., Laitinen, L., Kaukonen, A.M., Tuura, J., Björkqvist, M., Heikkilä, T., Vähä-Heikkilä, K., Hirvonen, J., Lehto, V.P., (2005). Mesoporous silicon microparticles for oral drug delivery: Loading and release of five model drugs. J. Controlled Release 108: 362–374.
17. Zhao, D., Huo, Q., Feng, J., Chmelka, B.F., Stucky, G.D., (1998). Nonionic triblock and star diblock copolymer and oligomeric surfactant syntheses of highly ordered, hydrothermally stable, mesoporous silica structures. J. Am. Chem. Soc. 120: 6024-6036.
18. FDA, 1979. Database of Select Committee on GRAS Substances (SCOGS) Reviews: Silicon dioxides. Retrieved April 6th, 2011 from http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogsListing&id=276.
19. FDA, 2011. Inactive ingredient search for approved drug products: Silicon dioxide. Retrieved April 6th, 2011 from http://www.accessdata.fda.gov/scripts/cder/iig.
20. Paul DR., Robeson LM.,( 2008), Polymer nanotechnology: Nanocomposites. Polymer., (49): 3187-3204
21. Zhou NL., Zhang SQ., Shen J., Meng N.,( 2009) Controlled release and antibacterial activity chlorhexidine acetate (CA) intercalated in montmorillonite. Int. J Pharm., (382): 45-49.