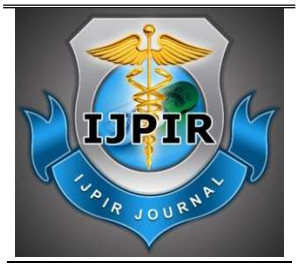
*Research Article*

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# Development and evaluation of thiolated chitosan based mucoadhesive pellets using extrusion and spheronization technology

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## ABSTRACT

Mucoadhesive pellets of diclofenac were prepared using Thiolated Chitosan polymers by Extrusion and Spheronization method. The characterization of pellets such as Particle size, SEM, Friability, Sphericity, and Angle of repose was carried out. Thiolated chitosan pellets possessed good mucoadhesive action. Thiolated Chitosan pellets showed higher mucoadhesion with increasing the quantity of polymer. This was due to the high value of the thiol moieties on chitosan leading to a much higher concentration of thiolate anions, which are responsible for the reaction with thiol groups within the mucus gel layer that improves mucoadhesion based on the covalent attachment.

**Keywords:** Thiolated chitosan, Diclofenac, Extrusion and Spheronization

## INTRODUCTION

Chitosan is a biodegradable polymer obtained from natural sources by alkaline deacetylation of chitin. Chitosan have generated much interest pharmaceutical applications either in drug transport or in controlled drug delivery system. It is also have many valuable properties like biocompatible, mucoadhesive, gel forming capacity at lower pH, permeation enhancement and all these properties makes chitosan a good candidate for the development of conventional and novel drug delivery system such as Colon drug delivery, pH sensitive Targeted drug delivery, Transdermal drug delivery, Gene drug delivery and Gastroretensive drug delivery [1-5]. However, practical use of chitosan has been mainly confined to the unmodified forms. For a breakthrough in utilization, especially in the field of controlled drug delivery, Chemical modification of chitosan is useful for the association of bioactive molecules to polymer and controlling the drug release profile, which will introduce desired properties and enlarge the field of the potential applications of chitosan.[6,7] Researchers have focused on modification of chitosan and their potential application towards drug delivery system.[8,9] Thiloated chitosan have been recognized as good candidates for mucoadhesive drug delivery system.[10]

In recent years, pellets produced by Extrusion-spheronization techniques have received much attention in pharma industry/academia research. Multiparticulate systems (such as pellets, beads or minitablets normally filled into hard capsules)

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offer several advantages over conventional single-unit matrix formulations. These include less risk of dose dumping, less inter and intra-subject variability and a higher degree of dispersion in the gastro-intestinal tract, thus minimizing irritation associated with high local drug concentrations.

The aim of the present study is to investigate the effect of new chitosan derivatives namely thiolated chitosan on the mucoadhesive strength of the pellets and dissolution release of Diclofenac sodium to improve its release properties at GIT pH value and potentially its bioavailability.

## EXPERIMENTAL METHODS

### Drug and excipients interaction studies

The compatibility of drug and excipient is important prerequisite before formulation. It is therefore necessary to confirm that the drug does not react with the polymers and excipient under experimental conditions and affect the shelf life of product or any other unwanted effects on the formulation. Drug and excipients compatibility was studied using FTIR spectral analysis.

### Preparation of pellets by extrusion and spheronization

Seven different formulations of pellets containing diclofenac sodium were prepared by extrusion and spheronization method. The component of the formulations was shown in Table 01. The solid powders of Diclofenac sodium, MCC, were accurately weighed and mixed by hand in a polyethylene bag for 10 min to obtain a homogeneous physical mixture and add Lactose, Chitosan or Thiolated chitosan as a different percentage mixed with thoroughly. The powder mixture was mixed with water as a binder for 20 minutes to achieve a consistency of a damp mass. The wet mass was then passed through a single screw extruder (EXT 30, RikonPharma, Thane, Mumbai, India) with a 1.0mm screen at 150 rpm. The extrudates were processed in a spheronizer (SPH 150, RikanPharma, Thane, Mumbai, India) fitted with a cross-hatched plate rotated at 300rpm for about 5 min. The obtained pellets were dried at 40ºC for 12 h.

**Table 01: Composition of the Formulation**

| Ingredients | Formulations | | | | | | |
|---|---|---|---|---|---|---|---|
| | FC | F1 | F2 | F3 | F4 | F5 | F6 |
| Diclofenac sodium | 33.3% | 33.3% | 33.3% | 33.3% | 33.3% | 33.3% | 33.3% |
| Thiolated chitosan | - | 20% | 30% | 40% | 50% | 60% | 66.7% |
| Chitosan | 50% | - | - | - | - | - | - |
| MCC | 08.35% | 23.35% | 18.35% | 13.35% | 08.35% | 3.35% | - |
| Lactose | 08.35% | 23.35% | 18.35% | 13.35% | 08.35% | 03.35% | - |
| Water | - | q.s | q.s | q.s | q.s | q.s | q.s |
| 1% acetic acid solution | q.s | - | - | - | - | - | - |

## EVALUATION OF PELLETS

### Surface Morphology

The Surface morphology of the pellets were studied by scanning electron microscopy (HITACHI, S-3400N, Japan). The samples were mounted on the SEM sample stab, using a double-sided sticking tape and coated with gold (200A) under reduced pressure (0.001 torr) for 5 min using an Ion sputtering device. The pellets were observed for morphology at acceleration voltage of 15 KV.

### Pellet shape

The pellets were characterized by geometric parameters determined by analyzing digital photographs of pellets scattered on a black surface, obtained with a digital camera SONY Cyber-shot 12 megapixel camera. The photographs were processed by UTHSCSA Image Tool 3.0 program. The geometric parameters Aspect Ratio, Sphericity were determined by applying the following equations.

$$AspectRatio = \frac{D_{max}}{D_{min}}$$

$$Sphericity = \frac{4\pi Ar}{Pm^2}$$

**Pellets size analysis**

The prepared samples of pellets (20g) were passed through a set of sieves number 10, 12, 14, 16, 18, 20 and 25 with aperture sizes of 2, 1.7, 1.4, 1.18, 1, 0.85 and 0.71 mm respectively. The samples were shaken for 10 min on a mechanical shaker (Scientific Engg Corporation, New Delhi, India). The samples retained on sieve number 18 (size ≈1mm) were used for further studies.

**Friability**

Friability of the pellets were determined by subjecting 10g of the pellets (Wi) were placed in the plastic chamber of a Roche Friabilator and subjected to impact testing at 25 rpm for 4min. The pellets were then screened using a sieve number 18 and weight of the pellets (Wf) retained on the sieve was measured. The weight loss (%) after friability testing was calculated.

$$Loss\ of\ Percentage = \frac{Initial\ Weight\,(Wi) - Final\ Weight\,(Wf)}{Initial\ Weight\,(Wi)} \times 100$$

**Angle of Repose**

Angle of repose is used to determine the flow properties of pellets. The method to find angle of repose is to pour the powder on a conical heap on a level, flat surface and measure the included angle with the horizontal.

$$\tan\theta = h/r$$

Where,
h = height of the heap,
r = Radius of the heap.

**Bulk Density**

Bulk density of the pellets was determined by pouring pellets into a graduated cylinder via a large funnel and measuring the volume and weight.

$$Bulk\ density = \frac{Weight\ of\ pellets}{Bulk\ volume\ of\ pellets}$$

**Tapped Density**

Tapped density was determined by placing a graduated cylinder containing a known mass of granules and mechanical tapper apparatus, which was operated for a fixed number of taps until the pellets bed volume has reached a minimum volume. Using the weight of the drug in the cylinder and this minimum volume, the tapped density may be computed.

$$Tapped\ density = \frac{Weight\ of\ pellets}{Tapped\ volume\ of\ granules}$$

**Carr's Index**

Carr's index is measured using the values of bulk density and tapped density. The following equation is used to find the Carr's index.

$$CI = \frac{(TD - BD)}{TD} X100$$

Where,
TD = Tapped density
BD = Bulk density

### Hausner's ratio

It is a measurement of frictional resistance of the drug. It was determined by the ratio of tapped density and bulk density.

**Hausner Ratio = $V_i$ / Vo**

Where,
$V_0$ = Bulk density
$V_i$= Tapped density

### Drug Content

300 mg of pellets equivalent to 100 mg of drug were accurately weighed and dissolved in 70 ml of Methanol for 15 min, make upto 100 ml with the same solvent, and filtered. 01 ml of the filtrate was diluted to 100 ml of Methanol. The content of Diclofenac was determined spectrophotometrically by measuring the absorbance at 276 nm. The results were expressed as mean values of three determinations.

### Swelling index

Swelling property of Chitosan pellets and Thiolated chitosan pellets were studied at pH 6.8. A weighed amount of pellets (10 g) were placed in a 100 ml measuring cylinder containing pH 6.8 media. Initial volume (Vo) was noted and change in physical volume was observed (Vt) at 6 h. The percentage of swelling was calculated using following formula:

**Percentage of Swelling = {Vt –Vo}/Vo * 100**

### Dissolution test

Dissolution studies of the pellets were performed in triplicate employing USP XIII dissolution rate test apparatus-1 (Electrolab, TDL-08L, India) simulating the GIT conditions. Weighed quantities of the pellets were loaded into the basket of the dissolution apparatus and 900 ml of dissolution medium of phosphate buffer pH 6.8. The temperature of the dissolution fluid was maintained at $37\pm5^0$ C with a stirring speed of 100 rpm. The samples withdrawn predetermined intervals 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hrs and were filtered (0.22 µm, Millipore). The drug quantity was determined using spectrophotometrically at 276 nm.

### Mucoadhesive studies

The mucoadhesive properties of the pellets were evaluated by in vitro wash-off test. A 2 cm wide and 2 cm long (2×2) piece of rat intestinal mucosa was tied onto a glass slide (3 in. long and 1 in. wide) using thread. About fifty pellets were spread onto the wet, rinsed, tissue specimen, and allowed to hydrate for 30 s. The prepared slide was hung onto one of the grooves of a USP 24 tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the test fluid at 37 °C contained in one liter vessel of the machine. At the end of 1 h, and at hourly intervals up to 6 h, the machine was stopped and the number of pellets still adhering to the tissue was counted. The test was performed at phosphate buffer, pH 6.8.

**% mucoadhesive = P1/ P2 * 100**

Where,
P1 = number of adhered pellets.
P2 = number of applied pellets.

## RESULTS AND DISCUSSION

Thiolated chitosan was prepared and the yield was found 90% . The conjugation reactions was carried out reacting chitosan and cysteine as the carriers of the thio group with 1-ethyl-3-(3-dimethylamino proyl) carbodiimide ( EDAC ) as catalyst. The characteristic absorption of the chitosan was the band at 1579.1 $cm^{-1}$, which is assigned to the stretching vibration of amino group of chitosan and 1438.6 $cm^{-1}$assigned to vibration of C-H. Another band at 3448.2 is due to amine NH symmetric vibration. The peak of 2927.8 $cm^{-1}$ is typical C-H vibration. The peaks around 896.0 and 1152.8 $cm^{-1}$ correspond to saccharide structure of chitosan. The broad peak at 1093.4 indicates C-O stretching vibration. IR spectrum of the Thiolated chitosan also shows three characteristics peak at

1152 , 1653.8 and 2304 $cm^{-1}$, corresponding to the vibration of the S-C bond, C=O bond of the amide bond and the H-S bond respectively, while the peak at 2928 $cm^{-1}$ is attributed to stretching of C-H bond.

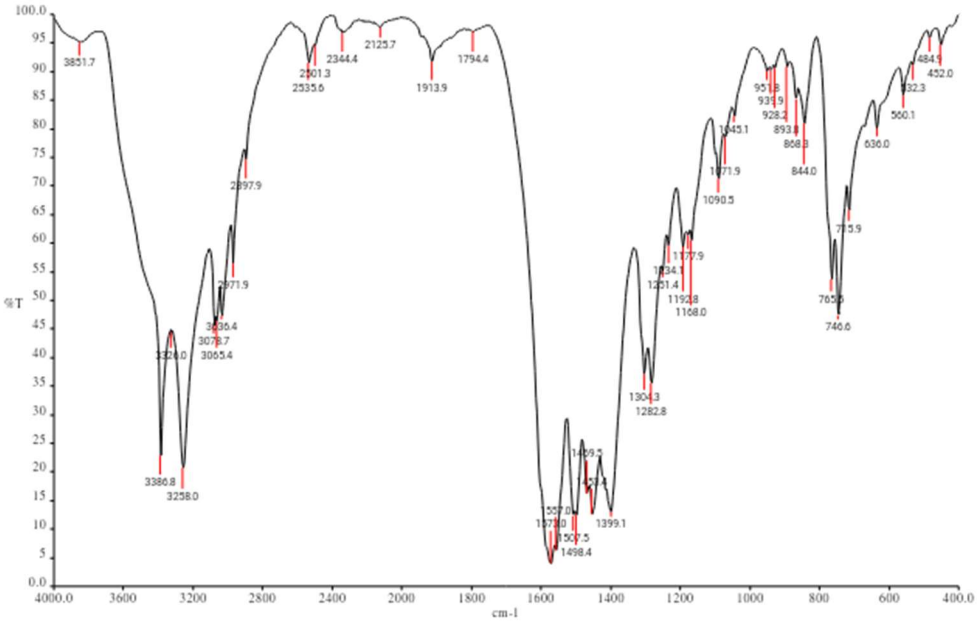


**Figure 1: FT-IR spectrum of Diclofenac sodium**

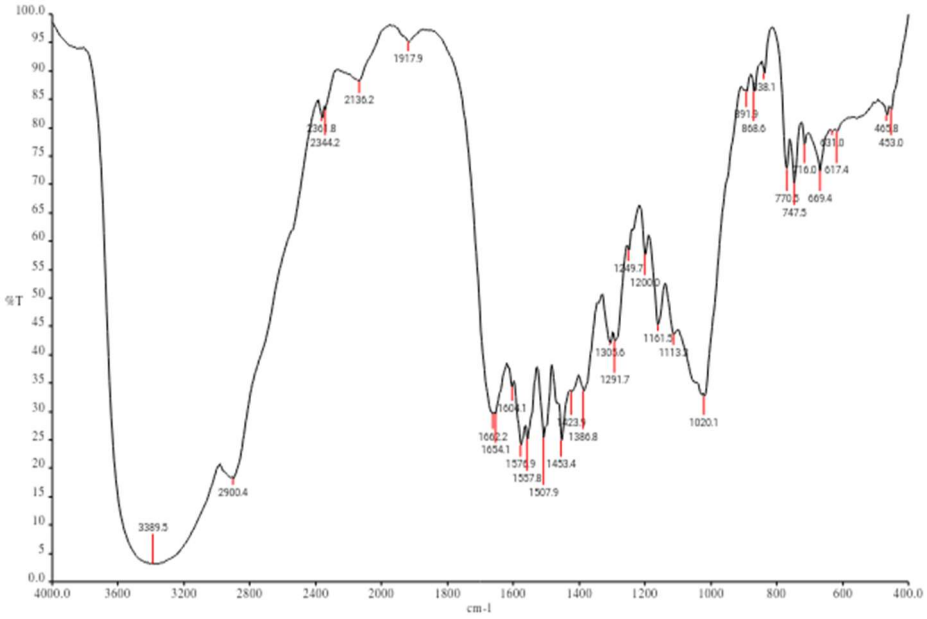


**Figure 2: FT-IR spectrum of Diclofenac sodium incorporated with Thiolated Chitosan pellets**

The IR spectrum of diclofenac sodium **(Figure 1)** showed characteristic peaks at 1167 and 682 $cm^{-1}$ for aromatic -C-Cl. The stretches between 800 and 600 $cm^{-1}$ also support the presence of -C-Cl group. The strong peaks in the region of 1600–1700–1800 $cm^{-1}$ indicate the presence of -C = O. Moreover, the presence of peaks in the region of 1250, 1283, and 1044 $cm^{-1}$ confirms the -C-O- group. The peaks at 1603, 1507, and 869– 716 $cm^{-1}$ confirm the presence of an aromatic ring.

The peaks of diclofenac sodium loaded Thiolated chitosan pellets **(**Figure 2**)** were similar (but with lesser intensity) to the spectrum of diclofenac sodium. The peaks of various functional groups as described in the IR spectrum of diclofenac sodium were also present in the pellets loaded with diclofenac sodium without any shift or change. These observations revealed the intact nature of the diclofenac sodium present in the pellets. From these results, the absence of drug–polymer interaction and the stability of the drug in the pellets were confirmed.

## Evaluation of pellets

Characterization of Thiolated chitosan pellets was performed. Particle of the pellets formulations was found in the range of 0.7 to 2 mm. Scanning electron microscopy (SEM) photomicrographs of Thiolated chitosan pellets are shown in Figure 3. The pellets are found discrete, spherical with a slightly rough surface. Aspect ratio and Sphericity of selected pellets were calculated and the result was shown in Table 02. The value of aspect ratio for the pellets (1.13 to 1.16) was found satisfactory. The Sphericity value of 1.00 corresponds to a perfect sphere. The Sphericity of all the pellets was found in the range 0.8968 to 0.9964.

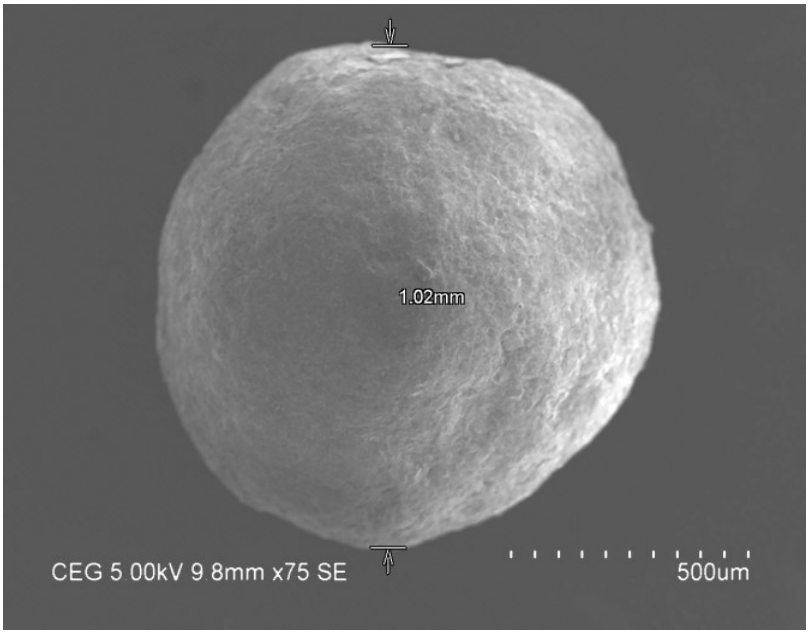


**Figure 3: Scanning Electron Microphotographs of Thiolated chitosan Pellets**

**Table 02: Characterization of Pellets**

| Formulations | Aspect Ratio | Sphericity |
|---|---|---|
| F1 | 1.21 | 0.9964 |
| F2 | 1.21 | 0.9554 |
| F3 | 1.20 | 0.8991 |
| F4 | 1.17 | 0.9948 |
| F5 | 1.17 | 0.9490 |
| F6 | 1.16 | 0.8968 |

The pellets were evaluated for bulk density (BD), tapped density (TD), compressibility (Carr's) index and angle of repose and the results were shown in Table 3. The bulk density of different formulations F1, F2, F3, F4, F5 and F6 were found in the range of 0.25 to 0.30 and tapped density from 0.27 to 0.32 respectively. The compressibility index was found to be in the range

6.25% to 10.34%. Hausner's ratio was found to be 1.06 to 1.11. Angle repose of all the pellets showed less than 23˚ and the results indicated that good flow property. The improved flow ability of pellets may be due to good sphericity and small size of granules. The drug content (%w/w) of different pellets was found in the range of 95.34 to 98.56. The result indicates that drug was uniformly distributed in the pellets.

**Table 3: Evaluation parameters of the pellets**

| Parameters | Formulation | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| Friability (%w/w) | 0.30 | 0.30 | 0.20 | 0.30 | 0.30 | 0.20 |
| Angle of repose | 22º20'±0.48 | 21º09'±0.84 | 21º0'±0.54 | 22º19'±0.58 | 21º86'±0.75 | 21º26'±0.53 |
| Bulk Density (gm/ml) | 0.28±0.01 | 0.29±0.01 | 0.30±0.01 | 0.27±0.01 | 0.26±0.01 | 0.25±0.01 |
| Tapped Density (gm/ml) | 0.31±0.01 | 0.32±0.02 | 0.32±0.01 | 0.30±0.01 | 0.29±0.01 | 0.27±0.01 |
| Carr's Index (%) | 9.677 | 9.37 | 6.25 | 10.00 | 10.34 | 7.40 |
| Hausner's ratio | 1.10±0.04 | 1.10±0.02 | 1.06±0.04 | 1.11±0.04 | 1.11±0.05 | 1.07±0.01 |
| Drug content | 95.34 | 96.72 | 97.86 | 96.10 | 97.32 | 98.56 |

## Swelling Index

Swelling index of Chitosan and Thiolated chitosan pellets in pH 6.8 are shown in **Table 04.** The higher degree of swelling index was observed in Thiolated chitosan as compared to Chitosan. This swelling was observed due to the presence of the hydrophilic thiolated group instead of the amino groupand so breaking of the intramolecular hydrogen bonding and creating more space for water within the polymer matrix.

**Table 04: Swelling Index**

| S. No | Formulations | Swelling Index (%) |
|---|---|---|
| 1 | FC | 49.3 |
| 2 | F1 | 98.3 |
| 3 | F2 | 113.7 |
| 4 | F3 | 139.1 |
| 5 | F4 | 167.5 |
| 6 | F5 | 190.3 |
| 7 | F6 | 212.1 |

## Mucoadhesive studies data

The mucoadhesion test for Chitosan and Thiolated chitosan pellets was performed at pH 6.8continuously for 6 h and report are shown in Table 05. The percentage of Chitosanpellets adhering to tissue at pH 6.8 after 6 h varied from 40 to 3, whereas the percentage of Thiolated chitosan pellets pellets adhering to tissue at pH 6.8 after 6 hours varied from 81 to 61. Higher mucoadhesion was seen in Thiolated chitosan pellets as compared to the Chitosan pellets. Thiolated Chitosan pellets showed higher mucoadhesion with increasing the quantity of polymer. This was due to the high value of the thiol moieties on chitosan leading to a much higher concentration of thiolate anions, which are responsible for the reaction with thiol groups within the mucus gel layer that improves mucoadhesion based on the covalent attachment.

**Table 05: Percentage of pellets adhering to tissue in Phosphate buffer pH 6.8**

| Formulations | Time in hours | | | | | |
|---|---|---|---|---|---|---|
| | 1 hr | 2hr | 3hr | 4hr | 5hr | 6 hr |
| **FC** | 40 | 31 | 25 | 16 | 9 | 3 |
| **F1** | 68 | 59 | 53 | 49 | 40 | 39 |
| **F2** | 69 | 61 | 56 | 53 | 50 | 44 |
| **F3** | 71 | 63 | 60 | 55 | 50 | 46 |
| **F4** | 73 | 65 | 61 | 59 | 55 | 53 |
| **F5** | 76 | 72 | 69 | 65 | 63 | 60 |
| **F6** | 81 | 78 | 73 | 70 | 65 | 61 |

### *In-vitro* Drug Release

The in vitro drug release profile of diclofenac sodium from Thiolated chitosan pellets and Chitosan pellets was shown in Figure 4. The release of diclofenac sodium from Thiolated chitosan pellets showed prolonged drug release in Phosphate buffer pH 6.8. Whereas chitosan pellets release the drug within short period of time. The sustained release of Thiolated chitosan is due to presence of hydrophilic thiolated groups inside of amino groups and so breaking the intermolecular hydrogen bonding and creating more space for water within the polymeric matrix. The degree of swelling of thiolated chitosan polymer was multifold increase when compared to chitosan. The result revealed that the concentration of thiolated anion increases which forms an electrostatic repulsive force between the negative charges.[64]The slow release of hydrophobic drug from the thiolated pellets could also be attributed to complex formulations between drug and thio group present on the polymer chain. There are different factors involved in the complex formulations such as wander walls interactions and hydrogen bonding between drug and thiolated chitosan.

In vitro release of Diclofenac sodium from pellets clearly indicate that the drug release rates are inversely proportional to the amount of the polymer in each pellets. All the Thiolated chitosan pellets was found to retard the drug release up to 12 hrs whereas Chitosan pellets was observed fast release within 6 hours. This might be due to increased swelling ability of thio polymer could have increased diffusion path length (gelling rate) for the drug which could have retarded the drug release from the formulation.

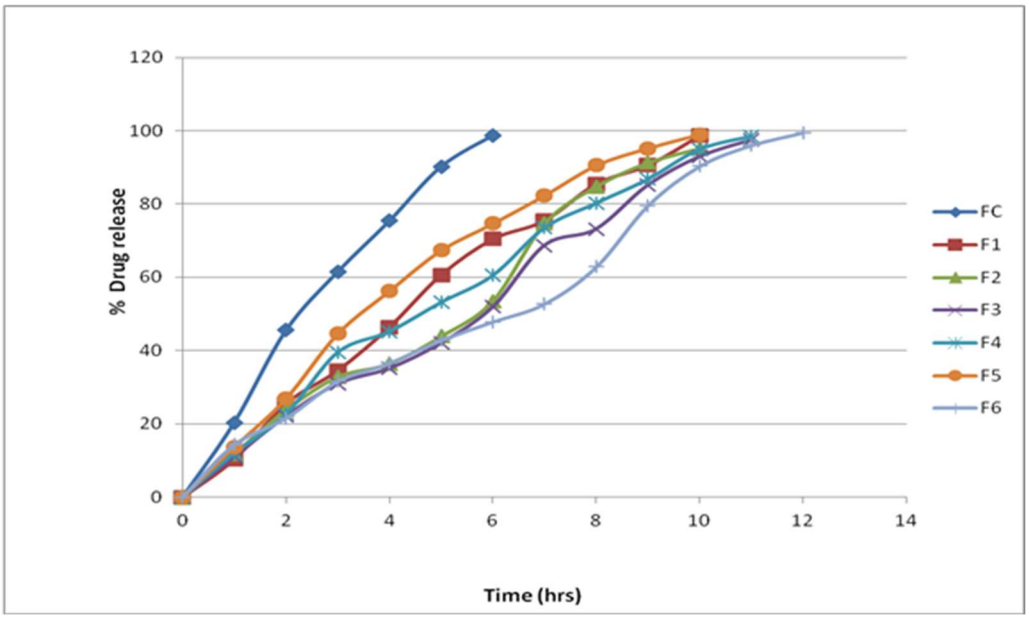


**Figure 04: *In-vitro* drug release of pellets in Phosphate buffer pH 6.8**

## CONCLUSION

Mucoadhesive delivery system of Diclofenac sodium pellets were successfully prepared using Thiolated Chitosan polymers by Extrusion and Spheronization method. The result of characterization of pellets such as Particle size, SEM, Friability, Sphericity, and Angle of repose was found satisfactory. This formulation have many advantages over the commercial tablet which include higher bioavailability, rapid onsite of action, less risk of dose-dumping, less inter and intra-subject variability and a higher degree of dispersion in the gastro-intestinal tract, thus minimizing irritation associated with high local drug concentrations. In addition to, better control of pain and inflammation enhanced absorption of drug achieved by the chitosan derivative polymers.

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