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Research Article

FORMULATION AND IN VITRO EVALUATION OF pH-SENSITIVE CHITOSAN BEADS OF FLURBIPROFEN

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ABSTRACT

The object of present study was to develop controlled release pH-sensitive chitosan beads of flurbiprofen. Thiolactic acid was used for making the thiolated chitosan. Thiol containing chitosan (TKCS), soluble in, water was synthesized by graft copolymerization technique. The TKCS beads were prepared using tripolyphosphate (TPP), at pH 4.0. The morphology of TKCS beads were examined by scanning electron microscopy (SEM). TKCS beads were prepared using flurbiprofen as model drug at three different concentrations (2%, 3%, 6% w/w). The in-vitro release behaviour was studied in phosphate buffer solution at various pH. The release rate of flurbiprofen from TKCS beads was significantly more at higher pH. The release rate of flurbiprofen at pH 7.4 was higher than that of at pH 1.2 (simulated gastric fluid) due to ionization of thiol groups and high solubility of flurbiprofen in an alkaline medium. These results indicated that the TKCS beads may be useful for controlled drug delivery through oral administration by avoiding the drug release in the highly acidic gastric fluid region of the stomach.

KEY WORDS: pH-Sensitive, Thiol, chitosan beads, Chitosan, Flurbiprofen, Tripolyphosphte.

INTRODUCTION

Recently, several technical advancements have led to the development of various novel drug delivery systems (NDDS) that could revolutionize the method of drug delivery and hence could provide definite therapeutic benefits[1]. Here, the interest is in the chemical modification of CS to improve its solubility and widen its application. Among various methods graft copolymerization is most attractive because it is a useful technique for modifying the chemical & physical properties of natural polymer. Chitosan bears two types of reactive group that can be grafted first the free amino groups on deacetylation and second the hydroxyl group on the C3 and C6 carbon deacetylated units[2]. The grafting of chitosan, modifies its properties that is possible to maintain some interesting characteristic such mucoadhesivity, biocompatibility and biodegradability[3, 4]. In this

work thiolactic acid was grafted in chitosan using glutaraldehyde by means of coupling reaction. The grafted chitosan was used for the preparation of beads using tripolyphosphate to improve the release in gastric fluid[5]. Flurbiprofen, a non-steroidal anti-inflammatory drug (NSAID) of the propionic acid class is used for the relief of pain and inflammation associated with rheumatoid arthritis and osteo arthritis and for the inhibition of entraoperative miosa[6]. The physicochemical properties of flurbiprofen and its short half-life make it suitable for administration by oral route.

MATERIALS AND METHODS

Flurbiprofen was obtained as gift sample from FDC Mumbai, Polymer - Chitosan was purchased from Sigma - Aldrich (Athens Greece), Thiolactic acid was also purchased from Sigma - Aldrich (Germany), Gluteraldehyde was purchased from merck co. (Germany). All other chemicals and reagents were of analytical grade and used without further purification.

Synthesis of Thiol-Containing Chitosan (TKCS)

Chitosan (0.05gm) was dissolve in 1% acetic acid solution. Then thiolactic acid (0.3ml) was added drop wise to the chitosan solution. Then gluteraldehyde (0.4ml) was added to the above reaction mixture. The pH of the solution was maintained at 5.0. The reaction was conducted at 50^0C for 4 hours. Then the reaction mixture was poured into acetone solution (200ml) for precipitation. The precipitate was washed repeatedly with ethanol and acetone. The product was dried in vacuum at room temperature [7].

Preparation of thiol containing Beads

Thiol containing chitosan beads were prepared by ionotropic gelation process with counter polynion tripolyphosphate (TPP). Thiol containing chitosan dissolved in 2% w/w acetic acid solution and stirred for 1 day to obtain a transparent and homogeneous solution. The thiol containing solution with or without drug was dropped through a syringe needle (0.45 mm gauge) into the 15% TPP in aqueous phase. Solidified white beads were formed immediately and allowed to stand for 1 hr in the solution with occasional agitation at room temperature. The pH of the TPP solution was adjusted to pH 4.0 with a 1.0 M HCl solution. The gel beads were filtered, washed with distilled water repeatedly, and dried under vacuum at room temperature for 2 days. Drug loaded thiol containing chitosan beads were prepared by taking three different percentage of Flurbiprofen (2%, 3%, 6%) with respect to polymer. Drug was dispersed in thiol containing solution for 1 day. Chitosan beads were prepared from same procedure[8].

PHYSICOCHEMICAL CHARACTERIZATION

Micromeritic properties

The beads were characterized for their micromeritic properties such as mean particle size, tapped density, % compressibility, etc. The mean particle size of beads were determined using optical microscope (Magnus MLX-DX, Olympus,India) fitted with an ocular and stage micrometer. Average 200-300 particles were measured and particle size was calculated.

Tapped density was determined by placing beads in a graduated cylinder on a mechanical tapper apparatus, which was operated for a fixed number of taps until the granular volume has reached to minimum. This tapping method was used to calculate tapped densities and compressibility index, as follows[9]. (Equation 1 and 2): ***Tapped density=Mass of beads/Vol. of beads after standard tapping (1)***

$$\%\ \text{compressibility index} = (1 - V/Vo)$$

Here, V and Vo are the volumes of the sample after and before the standard tapping respectively.

Morphology

Scanning electron microscopy (SEM) was done to study the morphology of beads .The sample for SEM were prepared by sprinkling the powder on a both side adhesive tape stuck to a stub. Gold palladium coating on the prepared stub was carried out by using sputter coater

(POLARON model SC-76430). The thickness of coating was about 200Å. The coated stubs were randomly scanned under electron microscope (LEO-430, UK).

Percentage yield of beads

The prepared beads were collected and weighed. The actual weight of obtained beads divided by the total amount of all non-volatile material that was used for the preparation of the beads multiplied by 100 gives, the % yield of beads[10]. (Equation-3): ***% yield=Actual weight of product obtained/Total weight of excipients and drug x 100 (3)***

Drug loading efficiency

To determine the loading efficiency 10mg beads were taken and dissolved in 10ml phosphate buffer (pH 7.4), thus the solution was filtered to separates shell fragments. The estimation of drug was carried out by using U.V. double beam spectrophotometer (shimadzu U.V. 1700 series) at the λmax of 247 nm. The loading efficiency was calculated as follows[10]. (Equation 4): ***Loading efficiency =Calculate drug content/Theoretical drug content x 100 (4)***

Swelling test

Dried beads were carefully weighed and immersed in Phosphate buffer (pH 7.4) and simulated gastric fluid (pH 1.2) at 37^0 C. At predetermined time intervals, swellen beads were taken out and the excess water was blotted with filter paper from the surface, and then weighed on a sensitive balance. The following equation was used to determine the swelling degree[11]. (Equation 5):

Swelling degree %=[(X_w–X_d)/X_d]X100 (5)

Here, X_d and X_w represent the mass of drug and mass of swollen beads respectively. The swelling behavior of the chitosan and thiol containing chitosan beads in solution of pH 1.2 and 7.4 at 37ºC are shown in figure 2 and 3.

***In –vitro* drug release at different pH**

The different drug release rate from beads was determined by using USP XXIII basket type dissolution apparatus. A weighted amount of beads placed in a non-reacting muslin cloth that had a smaller mesh size than that of beads. The mesh was tied with a nylon thread to avoid the escape of any beads. The dissolution test was performed in 900ml of phosphate buffer at various pH (5.8, 7.4, 8.0) and simulated gastric fluid (pH 1.2) at 37^0C and placed at 120 rpm as per USP XXIII drug release test prescribed for flurbiprofen extended release beads[12,13]. At specified time intervals, 5ml aliquots were withdrawn, filtered, diluted with the same medium and assayed at 247nm for flurbiprofen using a U.V. double beam spectrophotometer (Shimadzu U.V.- 1700 series). Samples withdrawn were replaced with equal volume of the same dissolution medium. All the experiments as specified above were conducted in triplicate.

STATISTICAL ANALYSIS

In this study, the result are given as mean ± standard deviation (SD) student T-test and one way analysis of variance (ANOVA) were applied to find out the signification difference in drug release from different batches by using graph pad instant software, programmed at statistically significant difference,i.e. $P < 0.05$.

RESULTS AND DISCUSSION

The pH sensitive thiol containing chitosan beads were prepared by ionotropic gelation process with counter polyanion TPP. By this method the formulation of fluribiprofen loaded beads were developed using three different percentage of drug 6%, 3%, 2% respectively for chitosan beads and thiol containing chitosan beads. The chitosan was used in different ratio and three formulation were developed (CS_1, CS_2, CS_3) and for thiol containing chitosan was also used in different ratio ($TKCS_1$, $TKCS_2$, $TKCS_3$).

The thiol containing chitosan was found to be soluble in both water and acetic acid. (Table 1)

Table 1: Batch specification of prepared beads:

S.No.	Batch Code	% of drug	% TPP solution	Polymer amount(mg)	pH of TPP
1	CS1	6	15	500	4
2	CS2	3	15	1000	4
3	CS 3	2	15	1500	4
4	TKCS 1	6	15	500	4
5	TKCS 2	3	15	1000	4
6	TKCS 3	2	15	1500	4

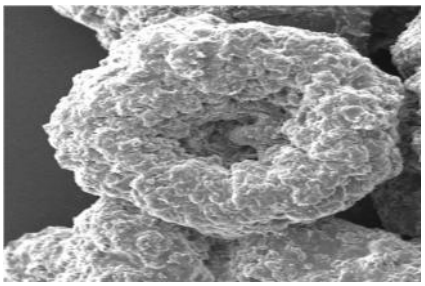
The mean particle size of the beads were found to be ranging from 1120.00 ± 30.00 µm to 1280.00 ± 20.00 µm for chitosan beads and 1451.00 ± 18.00 µm to 1572.00 ± 20.00 µm for thiol containing chitosan beads. The tapped density was found to be ranging from 0.326 ± 0.003 to 0.698 ± 0.006 for chitosan beads and it was from 0.356 ± 0.004 to 0.610 ± 0.005 for TKCS beads. Compressibility index was in range from 6.66 ± 1.02 to 9.28 ± 1.06 for chitosan beads and 6.66 ± 1.02 to 12.00 ± 10.0 for thiol containing chitosan beads. (Table 2)

Table 2: Micromeritic properties of beads:

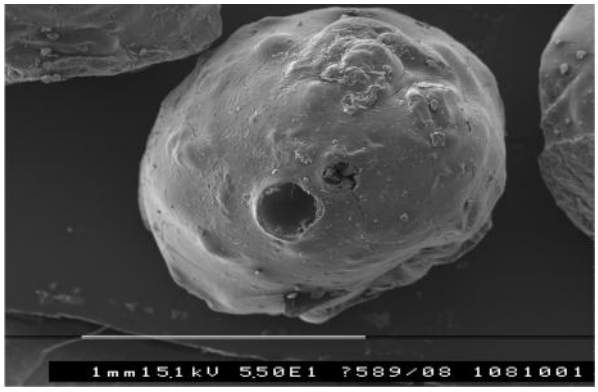
S.No.	Batch Code	Drug %	Loading efficiency	Yield %
1	CS 1	6	72.16 ± 1.52	90.21± 1.12
2	CS 2	3	83.61 ± 1.67	92.30 ± 1.31
3	CS 3	2	90.12 ± 2.88	91.40 ± 3.02
4	TKCS 1	6	70.51 ± 2.70	85.10 ± 1.01
5	TKCS 2	3	80.31 ± 3.56	84.25 ± 1.02
6	TKCS 3	2	89.50 ± 1.61	33.19 ± 2.19

SEM micrograph confirmed that prepared chitosan beads and thiol containing chitosan beads were spherical in shape having perforated surface. (Fig-1)

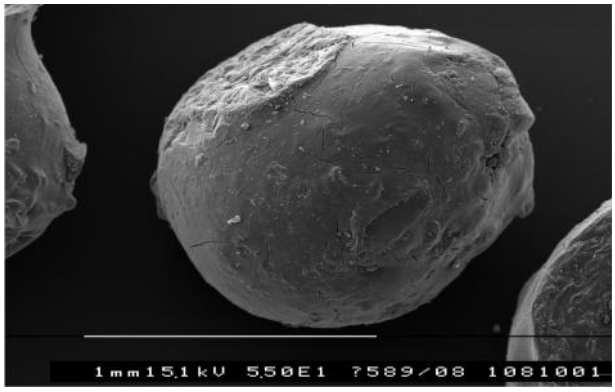
Figure 1: Scanning electron micrograph of beads



(a). Thiol containing Chitosan beads with drug Flurbiprofen



(b) Chitosan beads with drug Flurbiprofen



(c). Chitosan beads without drug

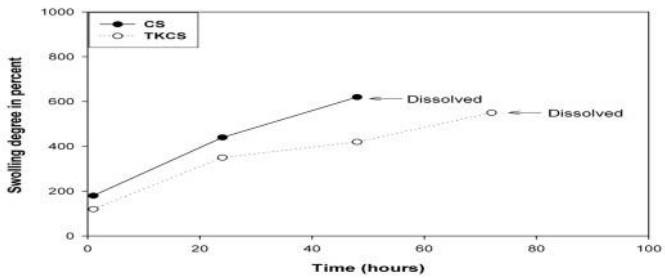
The maximum and minimum percentage yield of chitosan beads was found to be 91.04 ± 3.02 to 92.30 ± l.2l and thiol containing chitosan beads was found to be 83.19 ±l2.19 to 85.10 ± 1.09. The entrapment efficiency of chitosan beads and thiol containing chitosan beads was found to be good. The maximum loading efficiency was found in batch CS_3 (90.12±2.88%) and batch $TKCS_3$ (89.50±1.61) beads. (Table 3)

Table 3: Formulation Parameters for beads

S. No.	Batch Code	Mean particle size (µm)	Tapped density[(b)] (g/cm^3)	Compressibility Index[(b)]
l	CSl	1120 + 30	0326 + 0.003	928 + 106
2	CS 2	1260 + 10	0.562 + 0.002	6.66 +2.01
3	CS 3	1280 ± 20	0.698 ±0.006	7.69 ± 1.61
4	TKCSl	1451±18	0.356 ± 0.004	8.12 ± 1.02
5	TKCS2	1561 ±2l	0.530 ± 0.003	6.66 ± 1.02
6	TKCS3	1572 ± 20	0.610 ± 0.005	11.20 ± 2.13

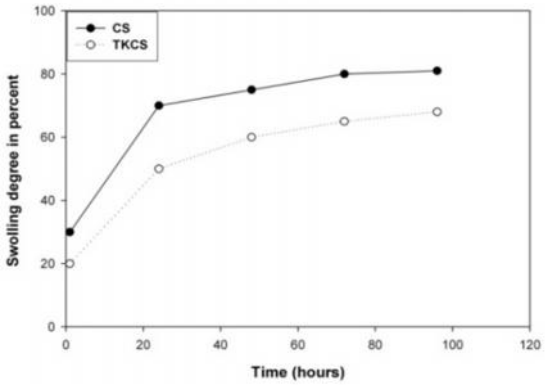
The swelling property in chitosan (CS) beads and thiol containing chitosan (TKCS) was found in pH 1.2 at 37^0C. TKCS beads were found to be dissolved within 3 days while the chitosan beads slowly swelled and were found to be dissolved after 4 days. (Fig-2)

Figure 2: Swelling behavior of CS & TKCS beads at pH 1.2



No disintegration and dissolution was found in CS and TKCS beads at pH 7.4. This may be due to lack of protonation of amino groups at pH 7.4, and swelling reached up to stable equilibrium much more rapidly in pH 7.4 than in pH 1.2. This expected behavior, attributed to high amount of interchain linkages in the well cross linked chitosan, tripoly phosphate (TPP) network, the swelling percentage was much higher at pH 1.2 than that of pH 7.4 (Fig-3)

Figure 3: Swelling behavior of CS & TKCS beads at pH 7.4



The in vitro drug release was performed at different pH, the percentage. The release rate of flurbiprofen was found to be higher at pH 7.4 and 8.0 than that of at pH 1.2 and 5.8. All formulations of beads released small amount of drug at pH 1.2. The release of drug was slightly more in thiol containing chitosan beads (TKCS1, TKCS2, TKCS3) than that of simple chitosan beads (CS1, CS2,CS3) due to more swelling of thiol containing chitosan beads than chitosan beads. (Figure 4, 5, 6 & 7)

Figure 4: Release profile of Flurbiprofen in simulated gastric fluid at pH 1.2

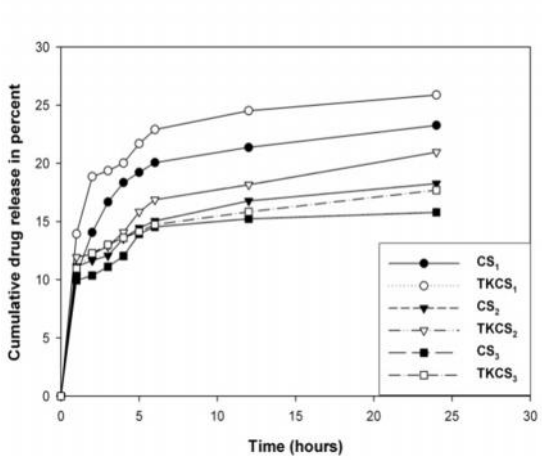


Figure 5: Release profile of Flurbiprofen in phosphate buffer at pH 7.4

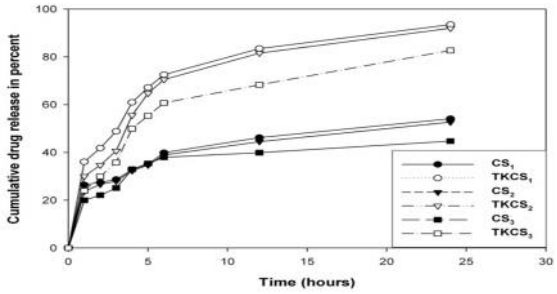


Figure 6: Release profile of Flurbiprofen in phosphate buffer at pH 5.8

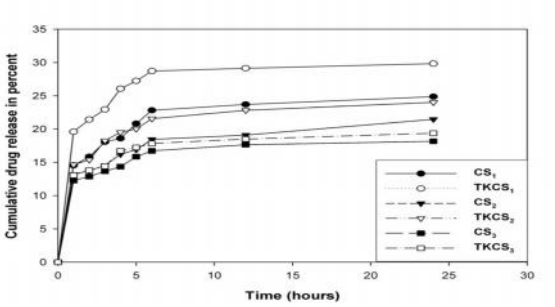
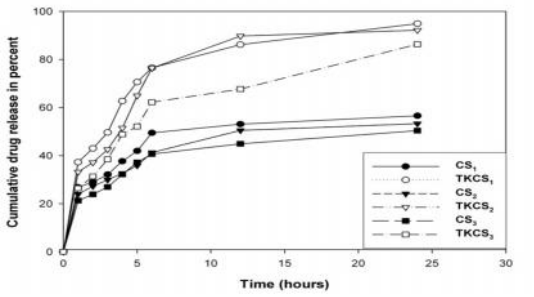


Figure 7: Release profile of Flurbiprofen in phosphate buffer at pH 8.0



The release of drug in thiol containing chitosan beads (TKCS1,TKCS2,TKCS3) was very much higher than the chitosan beads (CS1, CS2, CS3). Higher release rate of flurbiprofen at pH 7.4 & pH 8.0 than that at pH 1.2 and pH 5.0 may be due to electrostatic and hydrophobic repulsion between carboxyl group of flurbiprofen and the methyl group of modified TKCS in the pH 7.4 and pH 8.0. This study showed that the percentage of drug release increased with an increasing pH of dissolution medium. The reason may be formation of complexes between carboxyl group of flurbiprofen and amino group of chitosan at pH 1.2 and poor solubility of drug at pH 1.2.

CONCLUSION

Novel thiol containing chitosan beads to be used as controlled drug delivery system. The beads were prepared under mild condition at room temperature at pH 4.0 and the loading efficiency of a model drug, flurbiprofen, was about 85%. The TKCS beads were found to be pH sensitive, the swelling degree of beads and the ionization of thiol groups influenced the release profile of the drug at various pH. The release rate in simulated intestinal fluid (pH 7.4) was higher than that in simulated gastric fluid (pH 1.2), enabling the drug delivery or release to take place preferentially in the intestine, avoiding drug leakage in stomach. According to this result TKCS beads represents a promising tool as successful drug carriers for controlled drug delivery systems.

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