

FORMULATION AND EVALUATION OF CONTROLLED RELEASE INDOMETHACIN MICROPARTICLES

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Abstract

Indomethacin loaded alginate microparticles were prepared by using the ionic cross linking technique using calcium chloride. The effect of sodium alginate concentration was evaluated with respect to particle shape, entrapment efficiency and in vitro release behaviors. The effect of various concentrations of hydroxyl propyl methylcellulose (HPMC 50cps), polyvinyl pyrrolidone (PVP K 25) and calcium chloride on the % drug release was also evaluated. By changing formulation parameters the shape and entrapment efficiency were found to be altered. The desired in vitro release profile was obtained by altering the sodium alginate concentration only. The kinetic modeling of the release data indicates that indomethacin release from alginate microparticles follows first order and the release mechanism was case II transport. The fourier transform infrared spectroscopy (FTIR) study confirmed the absence of any drug polymer interaction. Differential scanning calorimetry (DSC), X-ray diffraction (XRD) studies revealed that the crystallinity of the drug decreases when loaded in the alginate microparticles. Indomethacin associated adverse effects can be reduced by using controlled release formulations. The controlled release microparticles loaded with indomethacin were evaluated for various in vivo parameters such as ulcer index, lipid peroxidation (LPO), reduced glutathione (GSH) and catalase (CAT) levels. Ulcer index and other biochemical parameters (except GSH levels) were found to be significantly different when compared to pure drug which is desirable. Therefore, loading of indomethacin in controlled release microparticles found to have fewer side effects when compared to pure drug which is clearly evident from various in vivo tests conducted. Both in vitro and in vivo studies confirmed beyond doubt the advantage of control release of indomethacin in the effective therapeutic management of inflammation and proved to be better than conventional dosage form with substantial decrease in side effects.

Key words: Controlled release, Indomethacin, Microparticles

Konrollü Salım Gösteren İndometasin Mikrokürelerinin Formülasyonu ve Geliştirilmesi

İndometasin yüklü aljinat mikroküreleri, iyonik çapraz bağlanma tekniği ile kalsiyum klorür kullanılarak hazırlanmıştır. Mikroküreler sodyum arjinat konsantrasyonunun etkisi, partikül şekli, hapsedilme etkinliği ve in vitro salım özellikleri incelenerek değerlendirilmiştir. HPMC 50 cps, PVP K25 ve kalsiyum klorürün farklı konsantrasyonlarının ilaç salımı üzerine etkisi de incelenmiştir. Formülasyon parametrelerinden partikülün şekli ve hapsedilme etkinliğindeki değişimlerin önemli olduğu belirlenmiştir. İstenen in vitro salım profili sadece sodyum aljinat konsantrasyonunu değiştirerek elde edilmiştir. Salım bulgularının kinetik modellenmesi sonuçlarına göre indometasinin İrjinat mikrokürelerden salımının birinci derece kinetiğiyle uyumlu olduğu ve salım mekanizmasının "olgu II transport"ına göre gerçekleştiği bulunmuştur. FTIR çalışmalarıyla herhangi bir ilaç-polimer etkileşimi olmadığı kanıtlanmıştır. DSC ve XRD çalışmaları, ilacın kristal yapısının arjinat mikrokürelerine yüklenmesi sonucunda azaldığını doğrulamıştır. İndometasinle tedavide görülen yan etkiler, kontrollü salım formülasyonları kullanılarak azaltılabilir. İndometasin yüklü kontrollü salım mikroküreleri, ülser indeksi, LPO, GSH, CAT düzeyleri gibi çeşitli in vivo parametreler yönünden değerlendirilmiştir. Ülser indeksi ve diğer biyokimyasal parametreler (GSH düzeyleri dışında) beklendiği şekilde, ilacın tek başına alınmasına göre anlamlı farklılıklar göstermiştir. Buna göre, kontrollü salım mikrokürelerine indometasin yüklenmesi sonucu yan etkilerin, ilacın tek başına verilmesine kıyasla daha az olduğu çeşitli in vivo testlerle açıkça kanıtlanmıştır. İn vivo ve in vitro çalışmalar sonucunda, enflamasyonun etkin tedavisinde indometasinin kontrollü salımının avantajı doğrulanmış ve yan etkilerin önemli oranda azalması nedeni ile konvansiyonel dozaj şekline göre üstünlüğü kanıtlanmıştır.

Anahtar kelimeler: Kontrollü salım, İndometasin, Mikropartikül

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INTRODUCTION

The goal of designing an ideal drug delivery system is to achieve a constant release of drug from the delivery system, reduced dosing frequency and adverse effects due to plasma fluctuations and improved patient compliance. Microparticles will give controlled release of drug having the above mentioned advantages.

Indomethacin is 10 to 40 times potent when compared to other salicylates in relieving pain, reducing swelling and tenderness of the joints. But the incidence and severity of side effects with indomethacin can limit its therapeutic activity which is related to its initial high concentrations in the plasma exhibited by the conventional dosage forms (1). So an attempt was made to control the release of indomethacin from the dosage form, by incorporating it into alginate microparticles.

Alginates are naturally occurring substances, found in brown algae have received much attention as a vehicle for controlled drug delivery. Alginates can be considered as block polymers, which mainly consists of mannuronic acid (M), glucuronic acid (G) and mannuronic – gluconic (MG) blocks. Dropwise addition of aqueous alginate solution to the aqueous solution containing calcium ions causing spherical gel formation termed as alginate bead. Alginate is known to be nontoxic when taken orally and also have a protective effect on the mucous membranes of the upper gastrointestinal tract. The dried alginate beads have the property of reswelling and they can act as controlled release system. Their reswelling property is susceptible to pH, which protects the acid sensitive drug from gastric juice (2).

The present study was focused on optimization of the formulation of controlled release indomethacin microparticles and also evaluation of % entrapment efficiency, physicochemical interaction studies, *in vivo* studies such as evaluation of anti-inflammatory activity and assessment of ulcerogenic potential of the optimized formulation.

MATERIALS

Indomethacin was procured from Madras Pharmaceutical Ltd., Chennai, India. Sodium alginate (NaAlg), hydroxy propyl methyl cellulose (HPMC 50cps), sodium carboxy methyl cellulose (NaCMC) and polyvinyl pyrrolidone (PVP K25) were purchased from SD.Fine-Chem Ltd., Mumbai, India. Calcium chloride, sodium hydroxide flakes and potassium dihydrogen orthophosphate were obtained from Qualigens fine chemicals, Mumbai, India.

METHODS

1) Preparation of alginate microparticles (3,4)

A general method of preparation of microparticles is outlined as follows. Sodium alginate dispersion was prepared by dispersing required quantity of sodium alginate in specified quantity of distilled water with vigorous stirring (Mechanical stirrer, Remi motors Ltd., Mumbai, India). Indomethacin was weighed (Electronic balance, Shimadzu, Japan) and dispersed in sodium alginate dispersion with vigorous stirring. Above prepared sodium alginate and indomethacin dispersion was added slowly drop by drop with the help of syringe (10 ml) having 0.7 x 32 mm needle into gently agitated double the volume of calcium chloride solution (1% w/v), which results in the formation of microparticles. The microparticles were stirred for 30 min and left aside for specified time along with calcium chloride solution. Then they were filtered and rinsed with water. The microparticles obtained were air dried for 24 hrs, followed by drying in a hot air oven (Tempo instruments and equipments Pvt. Ltd. Mumbai, India) at 50°C if needed.

II) Evaluation of microparticles

1) *Percentage entrapment efficiency*: Known quantity of alginate beads was added to 50 ml of pH 7.2 phosphate buffer and kept aside for about 12 hr. Then the volume was made up to 100 ml and filtered through whatmann filter paper No.40. The absorbance of the filtrate was measured at 318.6 nm (UV/visible spectrophotometer, Systronics 117, India). Percentage entrapment efficiency was calculated as follows:

$$\text{Percentage entrapment efficiency} = \frac{\text{Actual loading}}{\text{Theoretical loading}} \times 100$$

2) *In vitro dissolution studies*: *In vitro* dissolution studies for the microparticles were carried out by using U.S.P. dissolution apparatus II (Paddle type, 08L, Electrolab, Mumbai, India). The test conditions are given in Table 1.

Test procedure: 900 ml of dissolution medium pH 7.2 phosphate buffer was transferred into vessels of dissolution apparatus and was allowed to reach the temperature of $37 \pm 0.2^\circ\text{C}$. Preweighed microparticles were rapidly placed into vessels and paddles were rotated at the speed of 50 rpm. 5 ml of samples were withdrawn at 15 min, 30 min, 45 min, 60 min, 90 min, 2 hr and for every hour up to 12 hrs from each vessel and immediately replaced with fresh buffer. The samples were then filtered through a whatmann filter paper No. 40 and the absorbances of above samples were measured at 318.6 nm using UV/Visible spectrophotometer.

Drug release kinetics: The percentage of drug release data was fitted in zero order and first order plots to know the order of release. Zero order plots (5) are derived from plotting cumulative percent drug release versus time. First order plot (6) is derived from plotting log percent drug remaining versus time. Further the data was fitted into Higuchi (7), Hixson-Crowell (8) and Korsmeyer- Peppas equations (9) to know the release mechanism.

3) Scanning Electron Microscopy (SEM)

SEM images of the microparticles were recorded using a Hitachi S520 scanning electron microscope (Japan) at the required magnification. A working distance of 33.5 mm was maintained and the acceleration voltage used was 10 KV with the secondary electron image (SEI) as a detector. Prior to examination samples were gold coated under vacuum to render them electrically conductive.

III) Physicochemical interaction studies

Physicochemical interaction studies were carried out by using different instruments as follows.

a) Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectral measurements were performed using Thermo Electron FTIR spectrometer to confirm the presence of any interaction between the polymer and drug. The microparticles were finely ground with KBr to prepare the pellets under a hydraulic pressure of 600 psi and spectra were scanned between 400 and 4000 cm^{-1} .

b) Differential Scanning Calorimetry (DSC)

DSC curves of the indomethacin, sodium alginate microparticles and indomethacin loaded sodium alginate microparticles were recorded using modulated differential scanning calorimeter (Model TAG 1000). The analysis was performed by heating the 2-3mg samples on a aluminum crimp pans at a rate of $10^\circ\text{C} / \text{min}$ under a nitrogen atmosphere (50 ml, min^{-1})

c) XRD Studies

The X-ray diffraction pattern of the samples were recorded using a Rigaku Geigerflex diffractometer equipped with Ni-filtered Cu K α radiation ($\lambda=1.5418\text{\AA}$), goniometer speed-2°/min. voltage -30 Kv and current – 20 mA.

IV) In vivo evaluation of the optimised formulation

The main objective of the present study is to assess whether the anti-inflammatory activity of indomethacin microparticles is prolonged for longer periods of time and also to study whether potency is retained or enhanced when compare to the pure indomethacin.

Further, the study was also planned to evaluate any change in ulcerogenic potential of indomethacin loaded microparticles when compared to pure drug. The protocol is approved by Institutional Ethical committee (Reg. no. 1016/a/2006, 05/01/2008, 105).

i) Evaluation of anti-inflammatory activity of indomethacin loaded alginate microparticles

Male albino rats weighing 150-200g were used. The animals were kept in a light controlled on a 12 hr cycle. Food and water were available ad libitum. The animals were fasted overnight before the experimentation and given free access to water.

The animals were divided into 3 groups each containing 6 animals i.e. control (Group I), standard (Group II) and test groups (Group III). Blank alginate microparticles, pure indomethacin, indomethacin loaded alginate microparticles were dispersed in 1% w/v NaCMC solution. All the animals were treated as per treatment schedule (Table 2). After 1 hr paw edema was induced by injecting 50 μ l of 1% w/v carragenan into the subplantar region of the left hind paw. Paw volume was determined upto 6 hr at every hour in all the groups using plethysmometer (10) and percent inhibition of edema was calculated up to 6 hr.

ii) Assessment of ulcerogenic potential of indomethacin loaded microparticles:

Healthy male albino rats weighing between 150-200 g were used for the study and housed individually in polypropylene cages maintained under standard conditions (12 : 12 hr light and dark cycles; $25 \pm 22^\circ\text{C}$. 35 – 60% humidity). The rats were fed with animal feed pellets (Gold mohur) and were given water ad libitum.

The rats were divided into 3 groups each containing 6 animals i.e. control (Group I), standard (Group II) and test groups (Group III). The animals were kept on fasting for 48 hr before test water was given ad libitum. Blank alginate microparticles, pure indomethacin, indomethacin loaded alginate microparticles were dispersed in 1% w/v NaCMC solution. All the animals were treated as per treatment schedule (Table 3). Then the animals were sacrificed after 6hr (11).

The stomachs were isolated and opened along the greater curvature, the mucosa was washed under slow running tap water and the number and size of ulceration was scored as per the method of Rao *et al.* (12), The parameters such as gastric pH and ulcer index were measured as per the procedure published (12,13).

The following parameters were measured.

a) Gastric pH (13)

Using broad range pH paper the pH of the stomach contents were noted.

b) Ulcer index

The washed stomachs were fixed on a cork plate and the number and severity of the ulcers were measured using the following scores.

Severity score:

- 0 = Normal colored stomach.
- 0.5 = Red coloration.
- 1 = Spot ulcer.
- 1.5 = Hemorrhagic streaks.
- 2 = Ulcers \geq but \leq 5.
- 3 = Ulcers $>$ 5.

Calculation:

Ulcer index (UI) was calculated as follows:

$$\text{Ulcer Index} = \text{UN} + \text{US} + \text{UP} \times 10^{-1}$$

Where,

- UN = Average of ulcers per animal.
- US = Average of severity score.
- UP = % of animals with ulcer.

iii) Evaluation of antioxidant activity

The isolated stomachs of all groups were homogenized in chilled phosphate buffer (pH 7.4) using a homogenizer. The homogenates were centrifuged at 800 rpm for 5 min at 4°C (REMI C-24) to separate the molecular debris. The supernatant so obtained was centrifuged at 10,000 rpm for 20 min at 4°C (REMI CM-12) to get the post mitochondrial supernatant (PMS) (14), which was used to assay the *in vivo* antioxidant parameters like lipid peroxidation (LPO) (15), catalase (CAT) (16) and reduced glutathione (GSH) (17).

0.5 ml of PMS was taken and 0.5 ml of Tris hydrogen chloride buffer was added and incubated at 37°C for 2 h and then 1 ml of ice cold trichloroacetic acid was added, centrifuged at 1000 rpm for 10 min. From the above, 1 ml of supernatant was taken and added 1 ml of thiobarbituric acid and the tubes were kept in boiling water bath for 10 min. The tubes were removed and brought up to room temperature and 1 ml of distilled water was added. Absorbance was measured at 532 nm by using a UV-VIS-spectrophotometer. Blank samples were prepared without tissue homogenate. Activity was calculated as follows:

$$\frac{3 \times \text{Absorbance of sample}}{50.156 \times (\text{mg of tissue taken})} = \mu\text{M} / \text{mg tissue}$$

Estimation of reduced glutathione (GSH) from PMS

0.75 ml of PMS was mixed with 0.75 ml of 4% sulfosalicylic acid and then centrifuged at 1200 rpm for 5 min at 4°C (REMI CM-12, India). From the above, 0.5 ml of supernatant was taken and added to 4.5 ml of 0.01M DTNB. Absorbance was measured at 412 nm by using a UV-VIS-spectrophotometer. Blank samples were prepared without PMS. Activity was calculated as follows:

$$\frac{3 \times \text{Absorbance of sample}}{13.6 \times (\text{mg of tissue taken})} = \mu\text{M of GSH} / \text{mg tissue}$$

Table 1. Dissolution test conditions.

Parameter	Specification
Dissolution medium	pH 7.2 phosphate buffer
Volume of medium	900 ml
Temperature of medium	37±0.2°C
Paddle rotation speed	50 rpm
Sampling time interval	15 min, 30 min, 45 min, 60 min, 90 min, 2 hr & for every hour up to 12 hr
Detecting wavelength	318.6 nm

Table 2. Treatment schedule for anti-inflammatory activity.

Group	Drug	Dose (mg/kg)	Purpose
I	Blank alginate microparticles	At a dose equivalent to indomethacin loaded alginate microparticles	To serve as control
II	Pure indomethacin	5	To serve as standard
III	Indomethacin loaded alginate microparticles	At a dose equivalent to 5 mg/kg pure indomethacin	To serve as test

Table 3. Treatment schedule for assessment of ulcerogenic potential.

Group	Drug	Dose (mg/kg)	Purpose
I	Blank microparticles	At a dose equivalent to indomethacin loaded microparticles	To serve as control
II	Pure indomethacin	30	To serve as standard
III	Indomethacin loaded microparticles	At a dose equivalent to 30 mg/kg pure indomethacin	To serve as test

c) *Estimation of catalase (CAT) from PMS*

0.4 ml of homogenate was diluted 20 times with phosphate buffer (pH 7.0). Test sample was prepared by taking 2 ml of diluted homogenate and 1 ml of H₂O₂ was mixed. The blank sample was prepared by adding 1 ml of phosphate buffer pH 7.0 to 4 ml of the diluted homogenate. The absorbances of test and blank were measured at 240 nm for 2 min with 60 sec intervals by using a UV–VIS-spectrophotometer. Statistical analysis was carried out to know the differences whether are significant or not.

RESULTS AND DISCUSSION

Optimization of the formulation

Optimization is to make the formulation as perfect, effective or functional as possible. Various trials were carried out by altering the concentration of excipient and by including, excluding some excipients so as to obtain the formulation that will show the best results as required.

The first trial was carried out to evaluate the cross linking time required for obtaining proper gelled droplets or microparticles prepared with various concentrations of sodium alginate (0.5, 1, 2, 3, 4% w/v) and 1% w/v calcium chloride without drug. In this trial it was found that above 2% w/v sodium alginate concentration gave spherical shape microparticles without agglomeration and crosslinking time of 2hr is sufficient to obtain properly gelled microparticles.

The second trial was carried out to prepare the microparticles having 1% w/v indomethacin with 0.5%, 1%, 2%, 3%, 4%, 5%, w/v sodium alginate dispersion and 1% w/v calcium chloride solution and to evaluate the optimum concentration of sodium alginate to obtain spherical microparticles without agglomeration. Above 2% sodium alginate concentration gave microparticles which are spherical and discrete in nature when compared to lower concentrations. However 5% w/v sodium alginate concentration is more viscous in nature, it was difficult to pass through the needle. The % entrapment efficiency, assay and *in vitro* dissolution studies were carried out (Table 4, Figure 1). It was found that as the concentration of polymer was increased the cumulative % drug release decreased. 3% w/v sodium alginate concentration was selected for further trials as it gave spherical, discrete spheres with sufficient entrapment efficiency eventhough the percentage drug release was lower. The drug concentration was fixed as 1% w/v for further trials.

The objective of trial 3 was to increase the % entrapment efficiency at least 70% and to get the 75% cumulative release of drug at the end of 12th hr. For this purpose stirring speed was increased to 1000 rpm. Further, the trials were carried out at drug dispersion time of 3hr and 4hr. The % entrapment efficiency, assay and *in vitro* dissolution studies were carried out (Table 5, Figure 2). The % entrapment efficiency was found to be increased but the cumulative % drug release was not changed when compared to trial 2. The drug dispersion time of 3hr was selected for further studies as the % entrapment efficiency, assay values and percentage drug release were not significantly different for 4hr dispersion time.

Trial 4 was carried out by incorporating different concentrations HPMC 50cps (0.1, 0.5, 1.0 and 1.5), an hydrophilic polymer (to increase the drug release) in the microparticles prepared as per the specifications of trial 3 and to monitor the effect of different concentrations HPMC on the % drug release. The % entrapment efficiency and *in vitro* dissolution data were shown in Figure 3. It was found that there was no effect of HPMC on the release of indomethacin from microparticles.

Trial 5 was carried out to find out the effect of different concentrations of PVP K 25, an hydrophilic polymer, on the drug release from the microparticles prepared with 3% w/v sodium alginate, 3hr drug dispersion time at 1000 rpm. The % entrapment efficiency, assay and *in vitro* dissolution studies were carried out (Table 6, Figure 4). It was concluded that PVP K 25 did not

have any effect on drug release as there was no change in drug release between the trial 5 and trial 3. Even though the microparticles with the lower concentrations of sodium alginate have higher drug release, the 3% w/v sodium alginate was selected because it gives spherical shape, discrete microparticles with a hope that drug release may be increased by using other excipients. But this was not achieved. Therefore 2% w/v sodium alginate concentration was selected for further studies.

Table 4. Formulation data of trial 2.

S.No.	Concentration of Indomethacin (%w/v)	Sodium alginate concentration (%w/v)	% Entrapment efficiency	Drug assay (mg/100mg)
1	1	0.5	56.83	0.57
2	1	1	49.32	0.49
3	1	2	43.60	0.44
4	1	3	48.72	0.49
5	1	4	61.40	0.61
6	1	5	56.50	0.57

Table 5. Formulation data of trial 3.

S.No.	Concentration of Indomethacin (%w/v)	Concentration of Sodium alginate (% w/v)	Concentration of Calcium chloride (%w/v)	Drug dispersion time (hr)	% Entrapment efficiency	Drug assay (mg/100mg)
1	1	3	1	3	96	0.96
2	1	3	1	4	97	0.97

Table 6. Formulation data of trial 5.

Concentration of Indomethacin (%w/v)	Concentration of Sodium alginate (% w/v)	Concentration of PVP K25 (%w/v)	Concentration of Calcium chloride (%w/v)	% Entrapment efficiency	Drug assay (mg/100mg)
1	3	0.5	1	90.9	0.91
1	3	1.0	1	90.0	0.90
1	3	1.5	1	93.0	0.93
1	3	2.0	1	96.0	0.96

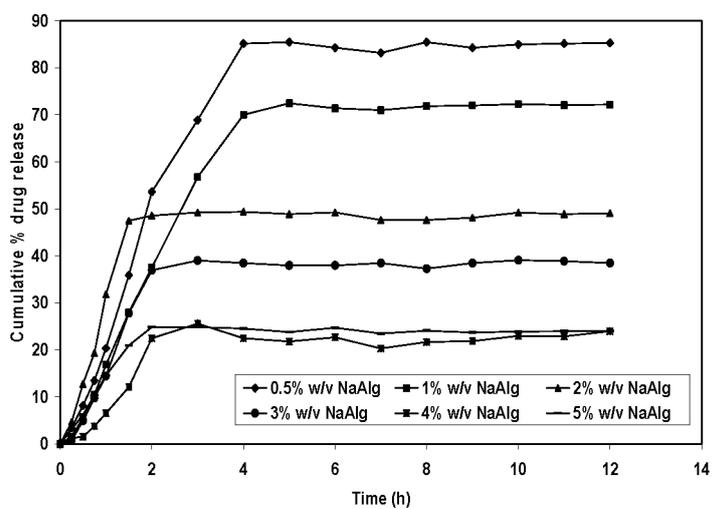


Figure 1. Dissolution profiles of trial 2.

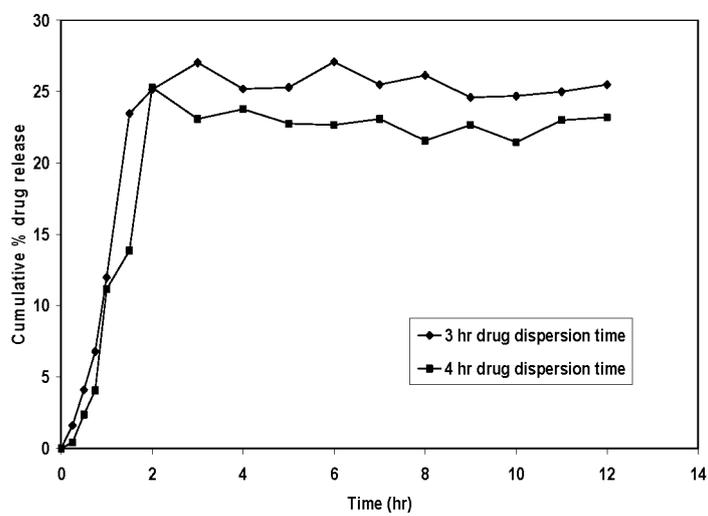


Figure 2. Dissolution profile of trial 3.

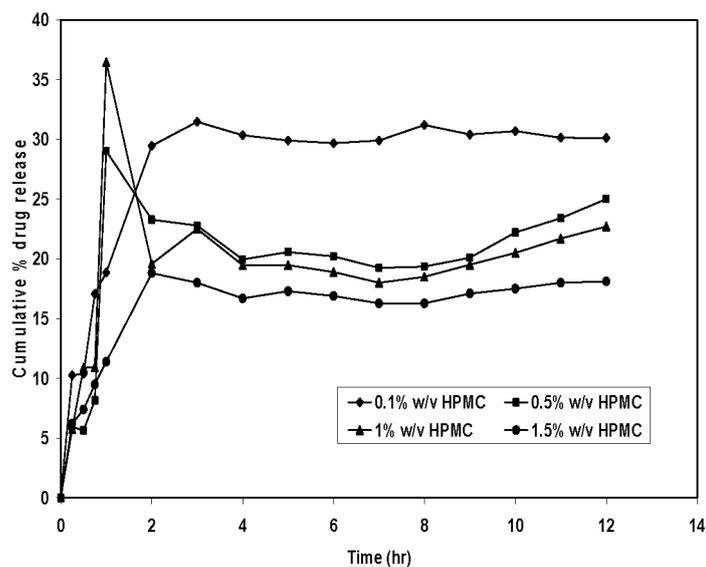


Figure 3. Dissolution profile of trial 4.

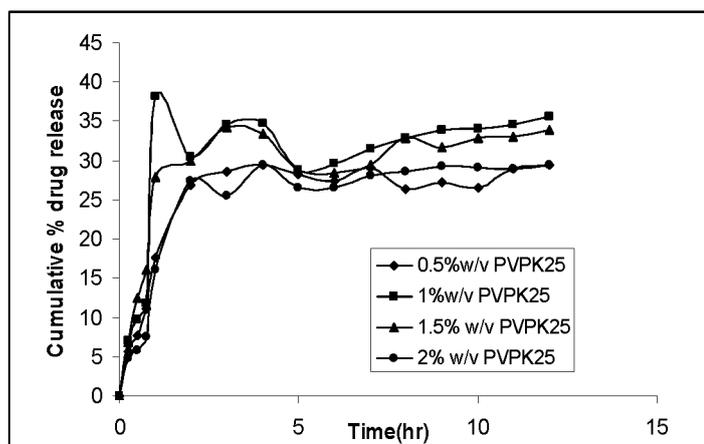


Figure 4. Dissolution profile of trial 5.

In trial 6, 2% w/v sodium alginate microparticles prepared with 0.5%, 0.75%, 1% w/v calcium chloride solution to see if there is any effect of the concentration of calcium chloride on % drug release. The percentage entrapment efficiency, assay and *in vitro* dissolution studies were performed (Table 7, Figure 5). It was found that there was no effect of calcium chloride on drug release, but higher the calcium chloride concentration, the sphericity of the microparticles was found to be increased. In this trial, 2% w/v sodium alginate concentration selected as the microparticles were more spherical and discrete in nature when compared to the microparticles prepared with 1% w/v sodium alginate. However the percentage drug release was found to be unchanged. So 1% w/v sodium alginate concentration was taken for further studies,

even though the microparticles were not that spherical when compared to 2% w/v sodium alginate microparticles.

Trial 7 was carried out with the aim to improve the percentage entrapment efficiency of microparticles prepared with 1% w/v sodium alginate, as in trial 2 by increasing the stirring speed to 1000 rpm for 1hr. The percentage entrapment efficiency was found to be significantly increased with the change in stirring speed. There was no change in percentage drug release with these microparticles (prepared in trial 7) (Table 8, Figure 6) when compared to the microparticles prepared in trial 2. The % drug release met the USP specifications.

Trial 8 was carried out with an aim that different concentrations of calcium chloride have any effect on percentage drug release of microparticles prepared in trial 7. The percentage entrapment efficiency and *in vitro* dissolution studies were carried out (Table 9, Figure 7). It was found that there was no effect of calcium chloride on percentage entrapment efficiency and percentage drug release. But with the increase in the concentration of calcium chloride the spherical nature and discrete properties of microparticles also increased, so 1% calcium chloride concentration was selected. Finally in this trial the formula of controlled release indomethacin alginate microparticles were optimized i.e. microparticles prepared with 1% w/v sodium alginate, 1% w/v indomethacin and 1% w/v calcium chloride.

The fluctuations in dissolution profiles for the formulations prepared (Trial 4, 5 & 6 and Figure 3-5) may be due to the usage of HPMC 50 Cps, PVP K25 and also due to the differences in the entrapment efficiency.

Table 7. Formulation data of trial 6.

Concentration of Indomethacin (%w/v)	Concentration of Sodium alginate (% w/v)	Concentration of Calcium chloride (% w/v)	% Entrapment efficiency	Drug assay (mg/10mg)
1	2	0.5	43.5	0.44
1	2	0.75	42.9	0.43
1	2	1.0	43.6	0.44

Table 8. Formulation data of trial 7.

Concentration of Indomethacin (%w/v)	Concentration of Sodium alginate (% w/v)	Concentration of Calcium chloride (% w/v)	% Entrapment efficiency	Drug assay (mg/100mg)
1	1	1	72.6	0.73

Table 9. Formulation data of trial 8.

Concentration of Indomethacin (%w/v)	Concentration of Sodium alginate (% w/v)	Concentration of Calcium chloride (% w/v)	% Entrapment efficiency	Drug assay (mg/100mg)
1	1	0.5	71	0.71
1	1	0.75	73	0.73
1	1	1	72.6	0.73

Drug release kinetics

Drug release data from the optimized indomethacin alginate microparticles, was fitted in various equations such as zero order, first order, Higuchi, Hixson-Crowell cube root and Korsmeyer-Peppas. The order of release of indomethacin from alginate microparticles follows first order as R^2 value of first order plot is superior than zero order plot. The mechanism of release of indomethacin from microparticles is controlled by the diffusion but not by dissolution as the R^2 value of Higuchi model is higher than Hixson Crowell cube root plot.(Table 10 (a) and Table 10 (b)).

Table 10 (a). Kinetic equations.

Plot	Kinetic equations
Zero order plot	$Q = Q_0 - K_0 t$
First order plot	$\text{Log } C = \text{Log } C_0 - K_1 t / 2.303$
Higuchi plot	$Q = Kt^{1/2}$
Hixson – Crowell cube root law plot	$M_0^{1/3} - Mt^{1/3} = K_t$
Korsmeyer – Peppas plot	$M_t/M_\infty = Kt^n$

Table 10 (b). Kinetic equation and corresponding regression values.

Plot	Regression R ²	RMS values	K±S.E
Zero order plot	0.7639	10663.86	0.4868±14.8605
First order plot	0.8393	36305.29	0.01±0.0985
Higuchi plot	0.9017	12557.99	0.3135±9.5515
Hixson – Crowell			
cube root law plot	0.8154	28208.9	0.0490±2.1398
Korsmeyer – Peppas			
plot (n = 0.9893)	0.7236	36596.52	0.0066±0.3280

In order to know the diffusion mechanism, the data was fitted into Karsmeyer Peppas equation. The n and the coefficient of correlation (R²) obtained for the respective model were 0.9893 and 0.7236. Generally n value < 0.45 indicates Fickian release, > 0.89 indicates case II transport and between 0.45 – 0.89 indicates anomalous (or) Fickian release of drug from the polymer matrix. In the present study the value of n for release of indomethacin from alginate microspheres indicated that the release from microspheres followed super case – II transport mechanism controlled by swelling and relaxation of the polymer chains. This was further confirmed by RMS and k values (Table 10 (a) and Table 10 (b)).

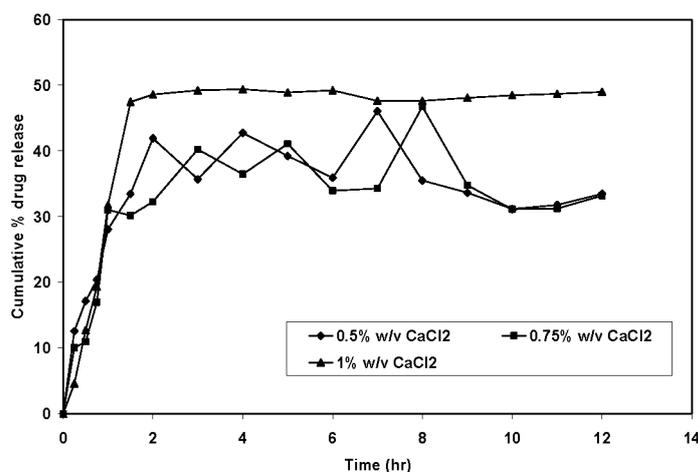


Figure 5. Dissolution profile of trial 6.

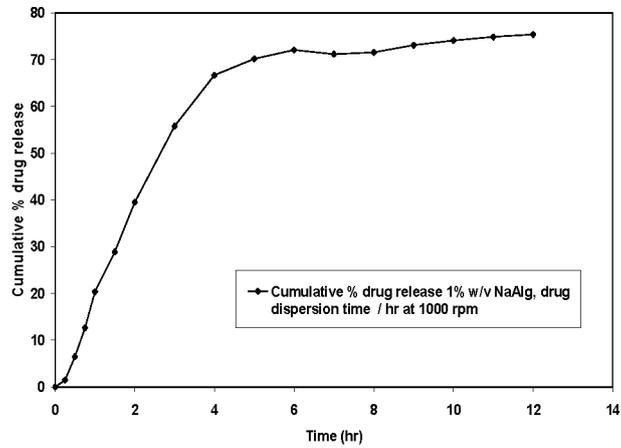


Figure 6. Dissolution profile of the trial 7.

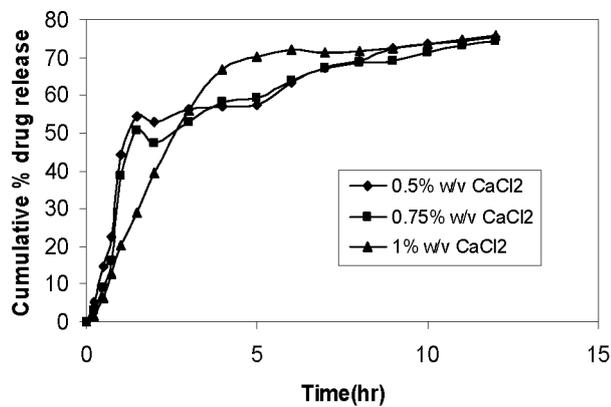


Figure 7. Dissolution profile of trial 8.

SEM Studies

SEM images of single indomethacin loaded alginate microparticles taken at 60x magnifications are shown in Figure 8. Microparticles were not that spherical in nature and their surfaces were slightly rough. However polymer debris had seen around some particles. Some extent of agglomeration was also there. This may be due to lower concentration of 1% w/v sodium alginate which was ought to be selected for getting better % drug release as per USP specifications.

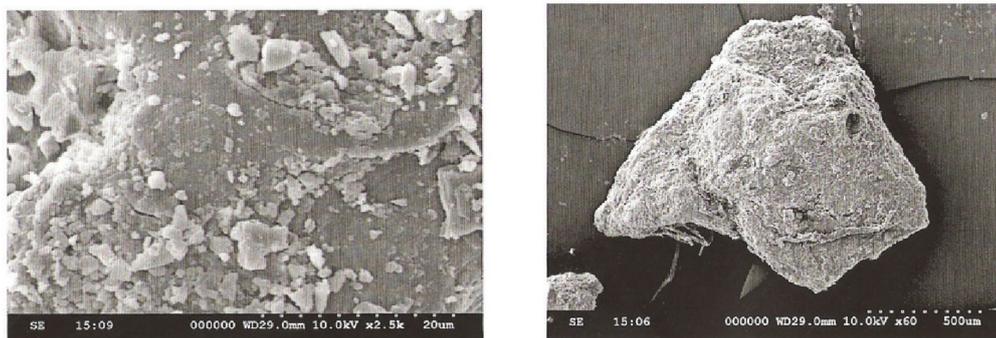


Figure 8. Scanning electron micrographs of indomethacin loaded alginate microparticles.

Physicochemical interaction studies

To know the chemical interaction, stability of indomethacin in alginate microparticles the FTIR spectral analysis was carried out (Figure 9). It was found that there was no any change in the peaks of pure indomethacin in indomethacin loaded microparticles. They appeared along with peaks of polymer sodium alginate. So indomethacin was chemically stable when incorporated in alginate microparticles. Similar results were reported by some research workers also (18,19).

To know the physical state of indomethacin in alginate microparticles, DSC analysis was carried out (Figure 10). The sharpness, intensity of endothermic peak in the indomethacin loaded alginate microparticles decreased when compared to endothermic peak of pure indomethacin. Finally it was concluded that a slight decrease in crystallinity of indomethacin was observed when loaded in the alginate microparticles. Similar results were obtained by Sunit Kumar Sahoo (18).

The XRD spectral analysis was carried out for blank sodium alginate microparticles, indomethacin loaded alginate microparticles and pure indomethacin (Figure 11). In the XRD spectra of indomethacin loaded alginate microparticles many peaks of the pure indomethacin observed but with less intensity and decreased sharpness, indicating that decrease in crystallinity of indomethacin when incorporated into alginate microparticles. Similar results were obtained by Sunit Kumar Sahoo (18).

In vivo evaluation of the optimised formulation

In vivo studies were carried out for optimized indomethacin loaded alginate microparticles to assess the control release of indomethacin *in vivo* and to screen for any change in the ulcerogenic potential of indomethacin when loaded in alginate microparticles.

Evaluation of anti-inflammatory activity

In the animals treated with pure indomethacin (Group I), the mean paw edema volume was significantly decreased when compared to the animals treated with blank alginate microparticles (Group I), which was indicated by relevant percentage inhibition of paw edema. But after 3rd hour the percentage inhibition of the paw edema was decreased.

In the case of animals treated with indomethacin loaded alginate microparticles (Group III) percentage inhibition of the paw edema was observed to be less at the initial hours when

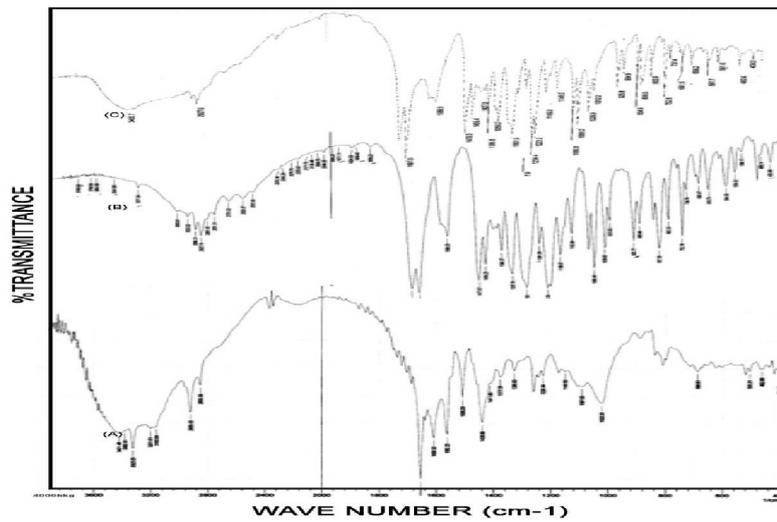


Figure 9. FTIR spectra for blank alginate microparticles, pure indomethacin and indomethacin loaded alginate microparticles (FTIR spectrum of blank alginate microparticles (A), FTIR spectrum of pure indomethacin (B), FTIR spectrum of indomethacin loaded alginate microparticles (C)).

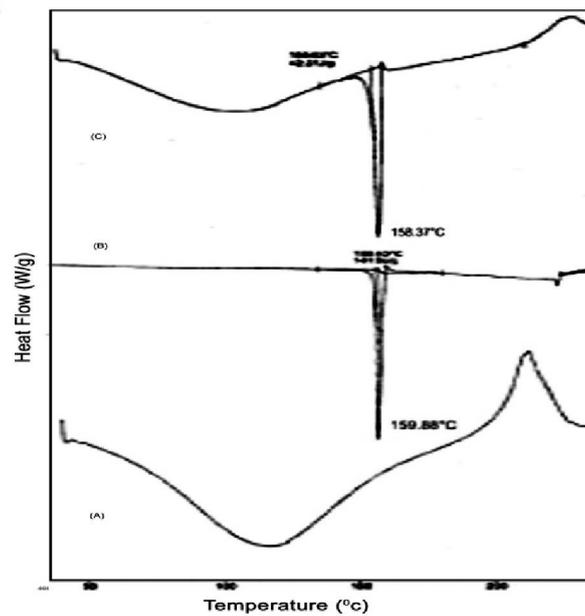


Figure 10. DSC thermogram for blank alginate microparticles, pure indomethacin and indomethacin loaded alginate microparticles (DSC thermogram of blank alginate microparticles (A), DSC thermogram of pure indomethacin (B), DSC thermogram of indomethacin loaded alginate microparticles (C)).

compared to animals treated with pure indomethacin (Group III). But it was significantly higher at the 6th hour. The percentage inhibition of the paw edema was gradually increased with time up to 6 hours in the group treated with indomethacin loaded alginate microparticles. It indicates controlled release of indomethacin was successfully achieved and this was confirmed by *in vitro* dissolution data (Fig 12).

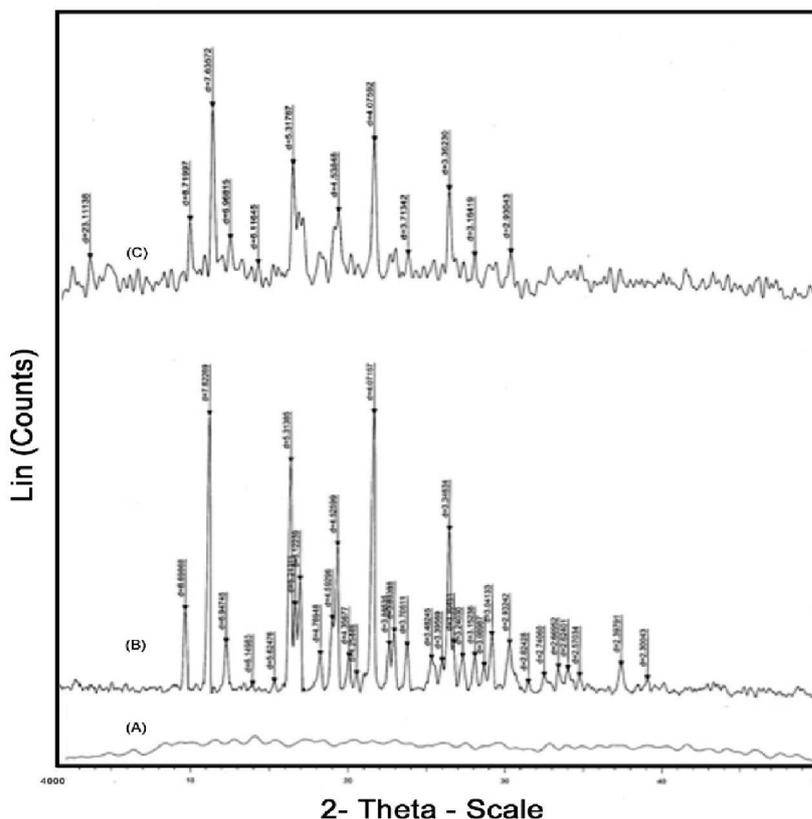


Figure 11. X-RD spectrum for blank alginate microparticles, pure indomethacin and indomethacin loaded alginate microparticles
 (A) - X-RD spectrum of blank alginate microparticles
 (B) - X-RD spectrum of pure indomethacin
 (C) - X-RD spectrum of indomethacin loaded alginate microparticles

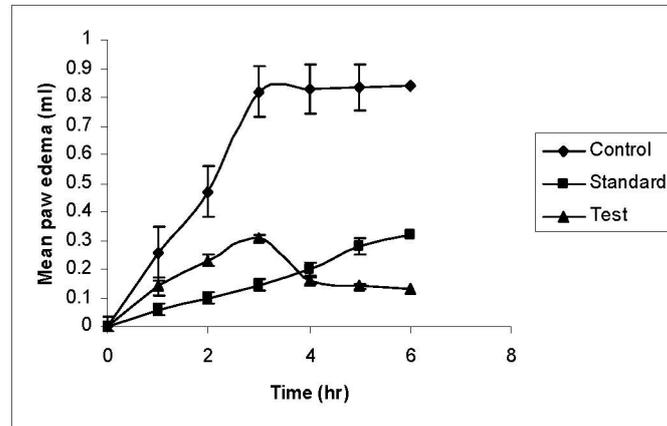


Figure 12. Anti-inflammatory activity of pure indomethacin and indomethacin loaded alginate microparticles

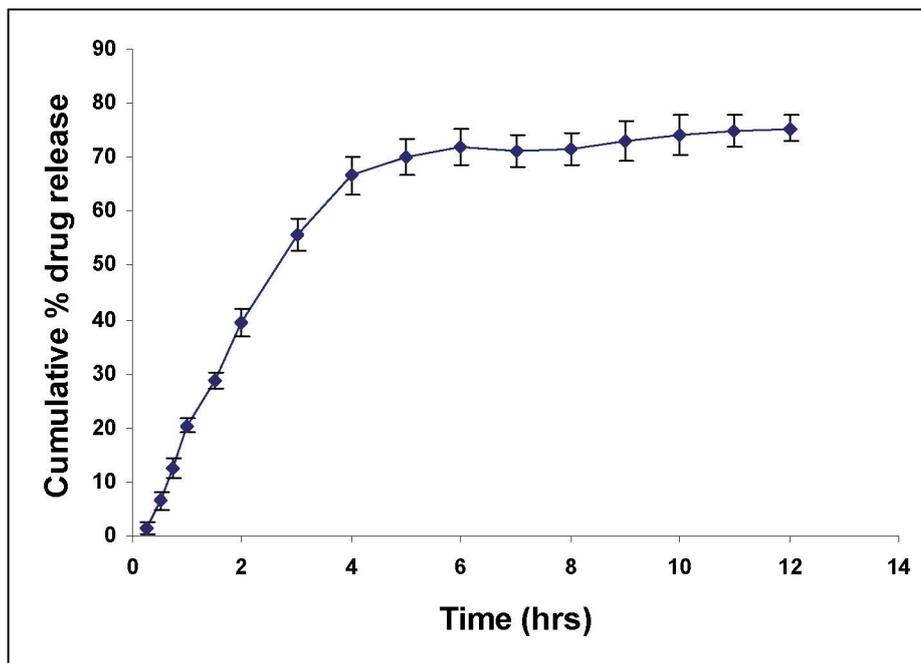


Figure 13. Dissolution Profile of Trial 7

Evaluation of ulcerogenic potential of indomethacin loaded microparticles

The isolated stomachs of indomethacin treated group animals (II) showed the streaks, spot ulcers and redness. Whereas redness was observed in the stomachs of animals treated with blank microparticles (I) and the indomethacin loaded microparticles (III).

The cause for gastric mucosal damage in animals treated with pure indomethacin (II) may be due to high plasma concentrations of indomethacin occurred initially (1). Gastric mucosal damage was due to the interference of indomethacin with cyclooxygenase pathway and oxidative stress developed by indomethacin (19).

In the stomachs of rats treated with indomethacin loaded microparticles (III) redness was observed and gastric mucosal damage was found to be decreased. This may be due to the controlled release of indomethacin from the microparticles and there may not be initial high plasma concentrations of indomethacin.

The ulcer index of the standard group (animals treated with pure indomethacin, II) was found to be 22.12 which was the highest amongst all the groups. It was observed that there was a considerable decrease in the value of ulcer index i.e. 14.37 and 13.87 for test group (animals treated with indomethacin loaded microparticles, II) and control (animals treated with blank microparticles I) respectively (Table 11).

Evaluation of antioxidant activity

The LPO values of the animals treated with indomethacin loaded microparticles (III) were to be less than animals treated with pure drug (II) which is desirable. Oxidative stress is associated with peroxidation of cellular lipids, the concentration of LPO products may reflect the degree of oxidative stress. Higher levels of LPO is responsible for tissue damage and subsequent disorders associated with indomethacin formulated as microparticles (III) showed a significant decrease in LPO as compared to pure drug (19). This was reflected in the UI values also. Hence, indomethacin microparticles were found to be better in reducing the tissue damage when compared to pure drug (Table 12).

Table 11. Comparison of pH, ulcer index between standard and test groups.

Group	Treatment	pH	Ulcer Index
I	Blank microparticles at a dose equivalent to indomethacin loaded microparticles	3 ± 1.154	13.87
II	Pure indomethacin 30 mg/kg	2.5 ± 1.732	22.12
III	Indomethacin loaded microparticles at a dose equivalent to 30 mg/kg pure indomethacin	4 ± 0.912	14.37

Table 12. Comparison of LPO, catalase & GSH between standard & test groups

Group	Treatment	LPO ($\mu\text{m/gm tissue}$)	Catalase (Kat units/ g protein/min)	GSH ($\mu\text{m/gm tissue}$)
I	Blank microparticles at a dose equivalent to indomethacin loaded microparticles	0.0625 ± 0.0025	0.0139 ± 0.00117	0.033 ± 0.00143
II	Pure indomethacin 30 mg/kg	0.128 ± 0.0123^a	0.0069 ± 0.00133^a	0.0377 ± 0.002
III	Indomethacin loaded microparticles at a dose equivalent to 30 mg/kg pure indomethacin	$0.087 \pm 0.00264^{b,c}$	0.0145 ± 0.001^d	0.0367 ± 0.0018

Comparison made between groups II, III versus I.

a = $P < 0.001$ when compared to control (G-II)

b = $P < 0.001$ when compared to control (G-III)

c = $p < 0.01$ when compared to standard (G-III)

d = $p < 0.001$ when compared to standard (G-III)

Catalase reduces hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals there by protecting the cellular constituents from oxidative damage in peroxisome. Catalase concentration in the animals treated with pure indomethacin (II) decreased significantly when compared to the animals treated with indomethacin loaded microparticles (III) (Table 12). Decreased catalase concentration is the indicative of oxidative stress caused by the pure indomethacin which causes gastric morphological alterations, such as mucosa hemorrhagic infarct, mucosa cell necrosis, leukocyte infiltration mucosa hemorrhagic erosion. Oxidative stress was decreased when indomethacin was given in the form of microparticles, which is desirable. So, controlled release of indomethacin decreases the side effects as mentioned.

There was no significant difference in GSH values for all the groups tested. This clearly indicates that there is no influence of indomethacin (pure as well as microparticles) on this parameter. Similar findings were reported by Valcheva *et al.*, in their work (Table 12) (20).

CONCLUSION

Indomethacin is an ideal drug for converting into the controlled release formulation and, sodium alginate was used as a vehicle for controlled drug delivery. In the present investigation indomethacin loaded microparticles were successfully developed with desirable release profiles as per USP specifications and optimized the variables. Both *in vitro* and *in vivo* studies

confirmed beyond doubt the advantage of control release of indomethacin in an effective therapeutic management of inflammation and proved to be better than conventional dosage form with substantial decrease in side effects (mainly the ulcerogenic potential). The method developed is simple, effective, reproducible and also a significant contribution in the field of pharmaceutical technology. Further studies are required to develop a suitable dosage form/s for human use.

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