

ORIGINAL ARTICLE

Preparation and evaluation of indomethacin loaded alginate microspheres

Příprava a hodnocení alginátových mikročastic s obsahem indometacinu

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Summary

Indomethacin-loaded alginate microspheres were prepared by the ionic cross linking technique using calcium chloride. The effect of calcium chloride concentration was evaluated with respect to the size, entrapment efficiency and shape (sphericity) of the particles. The entrapment efficiency and *in vitro* release profiles were found to be altered by changing various formulation parameters. The desired indomethacin *in vitro* release profile (as per USP specifications for extended release formulations) was obtained from microspheres prepared from gel containing 2% of sodium alginate and 2% of methyl cellulose hardened in 3% calcium chloride solution. The kinetic modeling of the release data indicated that indomethacin release from alginate microspheres followed Higuchi model and the release mechanism was diffusion. FTIR study confirmed the absence of any drug polymer interaction. DSC and XRD studies revealed that the crystallinity of the drug decreased when loaded in the alginate microspheres. The pharmacokinetic parameters were also evaluated in rabbits using HPLC technique and it was found that indomethacin loaded microspheres showed increased $t_{1/2}$ and AUC values. K_e value was less than that of pure drug.

This confirmed controlled release of the drug from microspheres leading to more residence time in the body within the therapeutic range providing longer duration of action which is preferable in chronic treatment of the diseases.

Key words: indomethacin • controlled release • Fickian diffusion • pharmacokinetics

Souhrn

Alginátové mikrosféry s indometacinem byly připraveny intovou gelací za použití chloridu vápenatého. Byl hodnocen vliv koncentrace chloridu vápenatého na velikost, enkapsulační účinnost a sféricitu připravených mikročastic. Bylo zjištěno, že změnou různých formulačních proměnných lze měnit enkapsulační účinnost i disoluční profil mikrosfér. Optimální *in vitro* disoluční profil indometacinu (podle USP specifikace s prodlouženým uvolňováním) byl dosažen ze vzorku obsahujícího 2 % natrium-alginátu a 2 % methylcelulosity tvrzeného v 3% roztoku chloridu vápenatého. Dle kinetického modelování disolučních dat byl Higuchi difúzní model vybrán jako vhodný. Dle FTIR studie nebyly potvrzeny žádné interakce mezi složkami mikročastic. DSC a XRD studie odhalily, že krystalinita léčiva inkorporovaného do mikročastic byla nižší ve srovnání s čistým léčivem. Farmakokinetické parametry byly získány na základě *in vivo* testů na králících s použitím HPLC techniky. Bylo zjištěno, že indomethacinové mikrosféry prokázaly vyšší $t_{1/2}$ a AUC ve srovnání s indomethacinovou suspenzí. Jednoznačně bylo tedy prokázáno řízené uvolňování léčiva z mikrosfér, poskytující delší působení léčiva v terapeutickém rozmezí, které je výhodné zejména v léčbě chronických onemocnění

Klíčová slova: indometacin • řízené uvolňování • Fickova difuze • farmakokinetika

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Introduction

Indomethacin is an important indole acetic acid non-steroidal anti-inflammatory drug which is used in the treatment of rheumatoid arthritis and other severe inflammatory diseases^{1, 2}. Indomethacin is an inhibitor of prostaglandin synthesis and is used for several inflammatory diseases, but in recent years indomethacin has also been recommended as the treatment of choice for low birth weight infants with *ductus arteriosus*. Patients with Bartter's syndrome have been treated successfully with indomethacin.

Indomethacin decreases the duration of morning stiffness. It relieves pain, reduces swelling and tenderness of the joints. In these actions it is 10 to 40 times more potent when compared to other salicylates. The main side effects of indomethacin are ulceration of the entire upper GIT, sometimes with perforations and hemorrhage and these associated adverse effects are due to initial high plasma concentration³ and are dose-related⁴. Occult blood loss may lead to anemia in the absence of ulceration⁴. In the case of rheumatoid arthritis, the frequent administration of the drug into the intra-articular cavity is very difficult. Oral conventional dosage forms are administered three or four times a day to maintain adequate and effective therapeutic concentration in blood, which is responsible for the occurrence of high initial peak plasma concentrations. However, it fails to protect the patients against morning stiffness⁵. The incidence and severity of side effects with indomethacin can limit its therapeutic activity. So controlled release is better to avoid the incidence and severity of side effects, moreover indomethacin fulfills many biopharmaceutic characteristics required for controlled release, as apparent partition coefficient of the drug is 1, dissociation constant of indomethacin is 4.5, which is ideal for acidic drugs, stable in the GI environment, it is not absorbed by carrier-mediated transport processes, it is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration⁶. The aim of this work is to formulate and characterize alginate microspheres for the controlled delivery of indomethacin and to carry out pharmacokinetic evaluation of the optimized formulation.

Materials and methods

Indomethacin (gift sample from Dr. Reddy's labs, Hyderabad, India), sodium alginate, calcium chloride and methylcellulose (Loba Chemie Pvt. Ltd., Mumbai, India). All the chemicals, solvents and reagents were of analytical grade.

Preparation of alginate microspheres⁷

Indomethacin alginate microspheres were prepared by ionotropic external gelation technique. Sodium alginate/methyl cellulose dispersion was prepared by dispersing specified quantity of sodium alginate and methyl cellulose in a specified quantity of distilled water with vigorous stirring and the specific quantity of indomethacin was dispersed with vigorous stirring (Table 1).

Table 1. Formulation chart of gels for indomethacin microspheres

Concentration (%)	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
Indomethacin	1	1	1	1	1	1	1	1
Sodium alginate	1	1	1	1	2	2	2	2
Methyl cellulose	–	–	–	–	–	1	1.5	2
Calcium chloride	1.5	2	2.5	3	3	3	3	3

The indomethacin polymer mixture was added slowly drop by drop with the help of a 20-gauge hypodermic needle fitted with a 10 ml syringe into calcium chloride solution, which resulted in the formation of microspheres. The microspheres were left aside for two hours in the calcium chloride solution, filtered and rinsed with distilled water. The microspheres were air-dried for 24 hrs at room temperature.

Entrapment efficiency⁸

Microspheres were powdered in a mortar and indomethacin was extracted from microspheres using ethanol, filtered and analyzed UV spectrophotometrically (1601 A, Shimadzu Corporation, Japan) after necessary dilution at 320 nm. Entrapment efficiency was calculated using the formula

$$\text{Entrapment efficiency} = \left[\frac{\text{Actual Loading}}{\text{Theoretical Loading}} \times 100 \right] \%$$

Particle size measurement⁹

Particle size and size distribution of different microsphere formulations were measured using an optical microscope (Olympus, Model HB, India), and the mean particle size was calculated by measuring 200 particles with the help of a calibrated ocular micrometer. The average particle size was expressed as the volume mean diameter in micrometers.

Fourier Transform Infrared Spectroscopy^{10, 11}

The indomethacin-loaded alginate microspheres were finely ground with KBr to prepare the pellets under a hydraulic pressure of 600 pounds for square inch and spectra were scanned between 400 and 4000 cm^{-1} (Shimadzu-8400 S, Japan) to confirm the presence of any interaction between the polymers and indomethacin.

Differential Scanning Calorimetry^{10, 11}

DSC thermograms of indomethacin and indomethacin-loaded sodium alginate microspheres were recorded using a modulated differential scanning calorimeter (DSC-60, Shimadzu Corporation, Japan). The analysis was performed by heating the 2–3 mg samples on an aluminium crimp pans at a rate of 10 $^{\circ}\text{C}/\text{min}$ in a nitrogen atmosphere (50 ml, min^{-1}) to know the thermal behaviour of the samples.

XRD Studies^{10, 11}

The X-ray diffraction pattern of the microspheres were recorded using a Rigaku Geigerflex diffractometer (Japan), equipped with Ni-filtered $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$), goniometer speed-2 $^{\circ}/\text{min}$ voltage – 30 Kv and current – 20 mA to know the crystallinity of the drug dispersed in the microspheres.

Scanning Electron Microscopy¹²⁾

SEM images of the indomethacin-loaded microspheres 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 to know the shape of the microspheres. Prior to examination, the samples were gold-coated under vacuum to render them electrically conductive.

Determination of sphericity¹³⁾

To determine sphericity, the tracings of microspheres (magnification 45 ×) were taken on a black paper using a Lucida camera, (Model-Prism type, Rolex, India) and the circulatory factor was calculated using the equation:

$$S = p^2 / (12.56 \times A), \quad [1]$$

where A is area (cm²) and p is perimeter (cm).

Dissolution studies¹⁴⁾

The dissolution studies of indomethacin and indomethacin microspheres were determined using a USP dissolution apparatus type II (Electrolab, Mumbai, India). The dissolution medium used was 900 ml of pH 7.2 phosphate buffer. 5 ml of sample solutions were withdrawn at predetermined time intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours) and then filtered through Whatman filter paper (No. 40). 5 ml of pH 7.2 phosphate buffer were replaced in the dissolution flask to maintain sink conditions. The amount of released indomethacin was analyzed spectrophotometrically at 320 nm.

Evaluation of release kinetics¹⁵⁾

To investigate the mechanism of indomethacin release from optimized formulation, the release data was analyzed for zero order, first order, Higuchi model, Hixon-Crowell and Korsmeyer-Peppas model. The data were presented in the following graphical representation and regression analysis was performed.

M_t versus t	(zero order)
Log cumulative % of drug remained versus t	(first order)
M_t versus square root of t	(Higuchi)
Cube root of drug percentage remaining	(Hixon-Crowell)
Log M_t versus log t	(Korsmeyer-Peppas)

M_t is the cumulative % of drug released at time t.

Korsmeyer et al.¹⁶⁾ derived a simple relationship which described drug release from a polymeric system:

$$M_t / M_\infty = k_t^n, \quad [2]$$

where M_t / M_∞ is the fraction of drug released at time t, k is the rate constant and n is the release exponent. Release curve where $M_t / M_\infty < 0.6$ was used to determine the exponent 'n' value. The n value was used to characterize different release mechanisms. For example, n = 0.45 for Case I or Fickian diffusion, 0.45 < n < 0.89 for anomalous behaviour or non-Fickian transport, n =

0.89 for Case II transport, and n > 0.89 for Super Case II transport. Fickian diffusional release occurs by the usual molecular diffusion of the drug due to a chemical potential gradient. Case II relaxational release is the drug transport mechanism associated with stresses and state-transition in hydrophilic glassy polymers, which swell in water or biological fluids. This term also includes polymer disentanglement and erosion. The rate constant 'k', coefficients of correlation (R²) and 'n' of each model were calculated by linear regression analysis.

HPLC analysis¹⁷⁾

Plasma samples analysis were performed using a Shimadzu (Japan) HPLC system equipped with a SPD-20A tunable absorbance detector and a Luna 5u C₁₈ (2) 100 A⁰ column (250 mm × 4.6 mm I.D) at ambient temperature (23–27 °C). The mobile phase was a mixture of 400 ml of sodium acetate buffer pH 3.6 and 600 ml of acetonitrile. The solution was filtered through 0.45 μm filter and degassed by sonication. The flow rate was 1 ml per minute. Detection was carried on at 320 nm wavelength. Prepared samples were injected to a HPLC column through a Rheodyne injector (HAMILTON, 702 NR) fitted with a 20 μl loop. A calibration curve was plotted for indomethacin in the range of 1–5 μg/ml. A good linear relationship was observed between the concentration of indomethacin and its peak area (R² = 0.9972).

Pharmacokinetic evaluation of the optimized formulation^{18–20)}

The optimized indomethacin microspheres were further evaluated for pharmacokinetic parameters. Pharmacokinetic study protocol was approved by the IAEC (Reg. No. IAEC/MNRCOP/CPCSEA/2013). Six male/female adult rabbits weighing between (1.5–2 kg) were used for the study. The animals were housed in individual cages under standard laboratory conditions of light, temperature and relative humidity. The study was conducted as open randomized design in which a single dose 10 mg/kg was administered to rabbits. The animals were divided into 2 groups containing 3 animals in each. For one group pure indomethacin was given as suspension with 1% sodium carboxy methyl cellulose and for another group indomethacin microspheres were given. After clipping off the hair on the ears of the rabbits, the blood sample of 1 ml was withdrawn at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8 and 12 hr intervals, using a 1 ml syringe with a 26 gauge needle (nominal outer diameter, 0.4636 mm, inner diameter, 0.260 mm and wall thickness, 0.101 mm). The withdrawn samples were collected in Eppendorf tubes containing 0.4 ml of 3% sodium citrate solution and centrifuged (Remi equipments, Mumbai, India) at 2,500 rpm for 15 min to separate plasma and analyzed for drug content by using HPLC (Schimadzu, Japan).

Results and discussion**Entrapment efficiency**

All formulations showed entrapment efficiency in the range from 64.17% to 86.33% (Table 2). Formulation F1 showed the lowest and F8 showed the highest entrapment

Table 2. Entrapment efficiency and particle size of formulations

Formulation	Entrapment efficiency (%)	Particle size (µm)
F1	64.17 ± 2.17	348.24 ± 1.87
F2	65.82 ± 1.61	366.25 ± 3.24
F3	81.76 ± 1.97	373.71 ± 2.34
F4	79.57 ± 1.26	379.28 ± 2.61
F5	71.58 ± 1.88	386.26 ± 2.09
F6	69.38 ± 2.33	401.27 ± 2.52
F7	71.82 ± 1.78	409.11 ± 2.55
F8	86.33 ± 1.55	423.65 ± 3.27

efficiency, respectively. The entrapment efficiency increased progressively with increasing alginate concentration in formulations F1 to F4. An increase in the alginate concentration resulted in the formation of larger microspheres entrapping greater amounts of the drug. This

may be attributed to the greater availability of the active calcium-binding sites in the polymeric chains and, consequently, the greater degree of cross-linking as the quantity of sodium alginate increased²¹. The decreased incorporation efficiency in F5 may be due to increased concentration of CaCl₂, which could be attributed to the formation of porous beads ensuring the diffusion of the drug out of the beads at the time of curing²². The entrapment efficiency increased progressively with increasing alginate and methyl cellulose concentration in formulations F6 to F7.

Particle size measurement

The mean particle size of the various formulations of alginate microspheres were between 348.24 ± 1.87 µm to 423.65 ± 3.27 µm. It was found that the particle size distribution of each formulation was within a narrow range but the mean particle size was different among the formulations (Table 2). The results indicated the proportional increase in the mean particle size of the microspheres with increasing amounts of sodium alginate

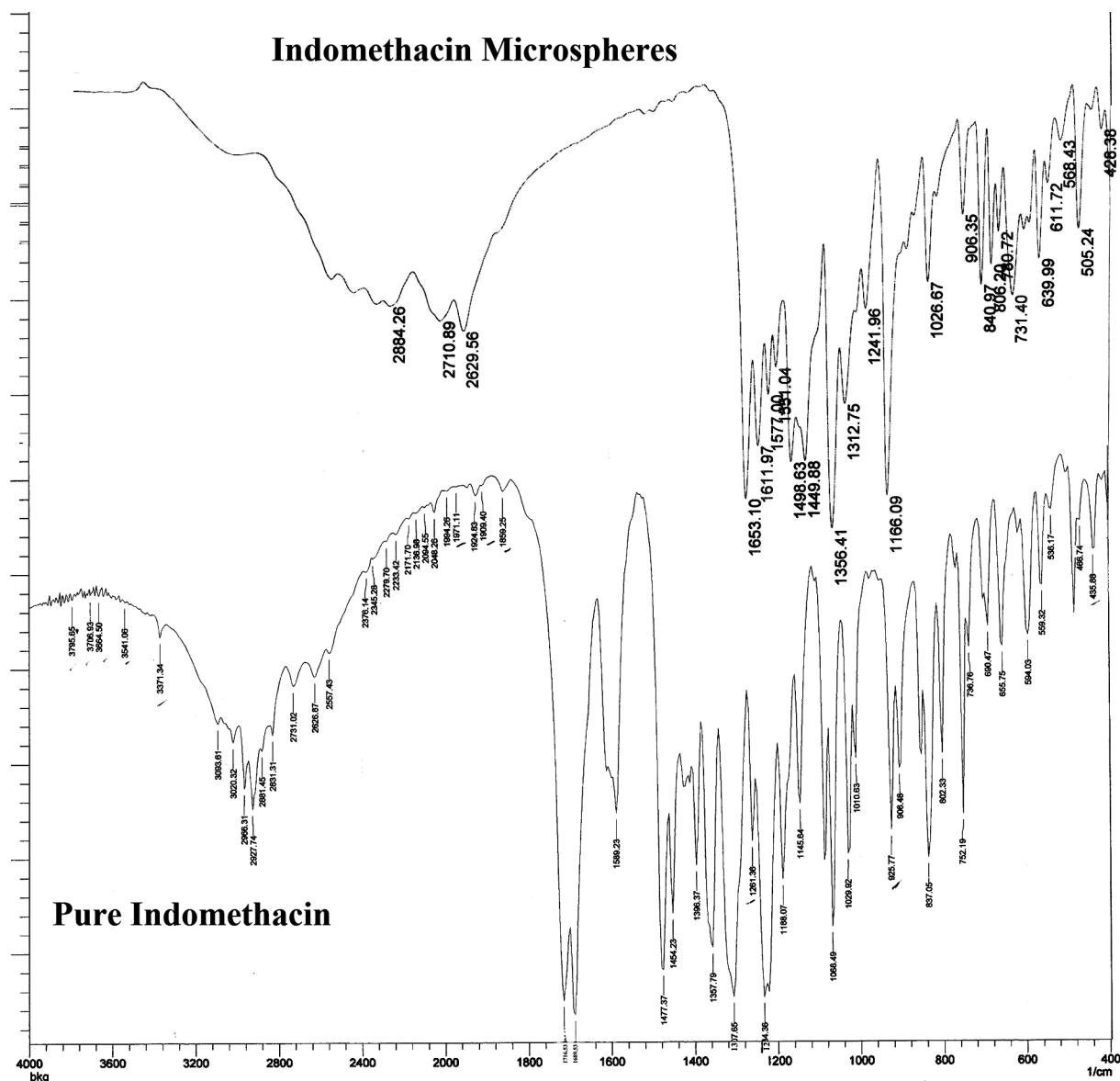


Fig. 1. FTIR spectra of pure indomethacin and indomethacin microspheres

in the formulations F1, F2, F3, F4 and F5 and sodium alginate and methylcellulose in the formulations F6, F7 and F8. This could be probably attributed to an increase in the relative viscosity at higher concentration of sodium alginate and formation of large droplets during addition of the polymer solution to the cross-linking agents²³.

Fourier Transform Infrared Spectroscopy

The FTIR spectra for pure indomethacin and indomethacin-loaded alginate microspheres are shown in Figure 1. Pure indomethacin showed characteristic bands at 3400–2500 cm⁻¹ (aromatic C-H stretching), 1716.53 cm⁻¹ and 1689.53 cm⁻¹ (C = O stretching), 1589.23 cm⁻¹ (aromatic C = C stretching), 1454.23 cm⁻¹ (O-CH₃ deformation), 1234.36 cm⁻¹ (C-O stretch plus O-H deformation), 925.77 cm⁻¹ (carboxylic O-H out of plane deformation) and 900–600 cm⁻¹ (C-H out of plane deformation for the substituted aromatic ring), whereas indomethacin microspheres showed characteristic bands with lower intensity indicating the chemical stability of indomethacin in alginate microspheres²⁴.

Differential Scanning Calorimetry

The DSC thermograms of pure indomethacin and indomethacin-loaded microspheres are shown in Figure 2. Indomethacin showed a sharp endothermic peak at 159.88 °C indicating the melting of the drug, whereas

indomethacin-loaded alginate microspheres showed a small endothermic peak with low intensity at 142 °C which demonstrated the reduction in drug crystallinity and molecular dispersion of the drug in the polymer matrix^{24, 25}.

XRD Studies

XRD spectra of pure indomethacin and indomethacin-loaded microspheres are shown in Figure 3. Pure indomethacin showed many peaks due to its crystalline nature, whereas XRD spectrum of indomethacin-loaded sodium alginate microspheres did not show any peaks indicating the amorphous molecular dispersion of indomethacin in microspheres^{24, 26}.

Scanning Electron Microscopy

SEM photograph showed that the indomethacin microspheres were spherical in nature with smooth surfaces with inward dents and shrinkage due to the collapse of the wall of the microspheres²⁷ (Fig. 4). The sphericity factor calculated for the microspheres are nearer to value 1 (1.024 ± 0.02, 1 ± 0.007, 0.994 ± 0.005, 1.004 ± 0.005, 0.994 ± 0.005, 0.996 ± 0.005, 1.002 ± 0.004 and 1.016 ± 0.02 for the formulations F1 to F8), which confirms the prepared formulations are spherical in nature.

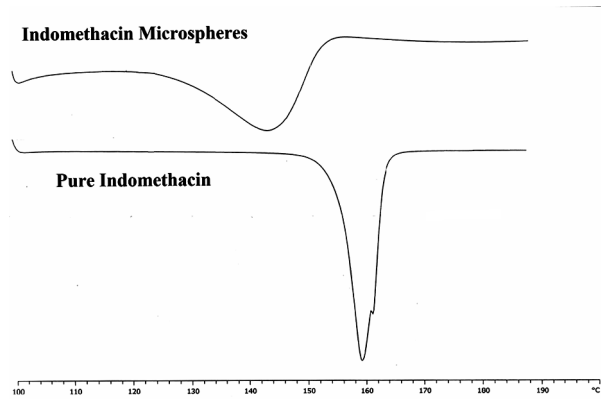


Fig. 2. DSC thermograms of pure indomethacin and indomethacin microspheres

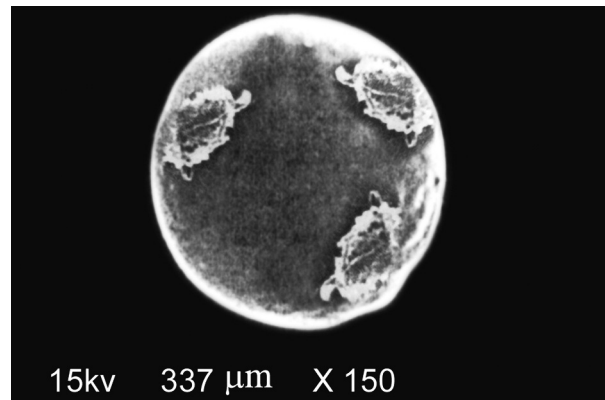


Fig. 4. SEM photograph of indomethacin microspheres

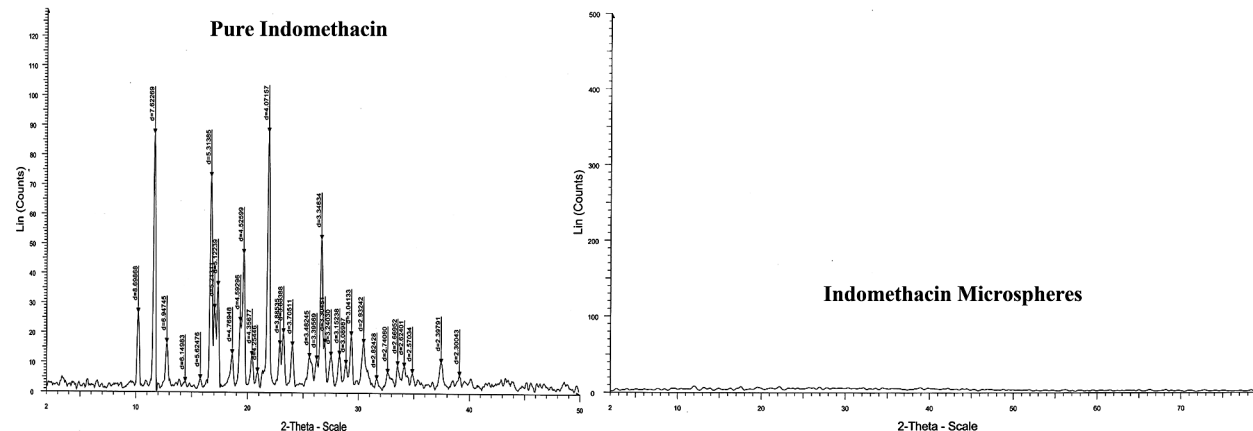


Fig. 3. XRD spectra of pure indomethacin and indomethacin microspheres

Dissolution Studies

Faster release observed in some formulations could be related to the lesser amount of the polymer in formulation as well as small size of microspheres which might be due to the fact that smaller particles offered more surface area to release the drug^{28, 29}. A slower release pattern was observed for formulation containing higher amounts of the polymer and larger size^{30, 31}. The effect of sodium alginate and methylcellulose concentration on indomethacin release from microspheres is shown in Figure 5. It can be observed that with increasing polymer concentration, the rate of drug release from the microspheres decreases dramatically. The formulations containing relatively higher polymer contents showed less initial drug release due to the unavailability of drug molecules at the surface of microspheres. Moreover, an increase in the concentration of the polymer in microspheres retarded the formation of pores or channels by drug particles in the matrix. This in turn affected the leaching and diffusion of drug from the matrix, and thus the drug release rate was lowered. Based on controlled drug release (over a period of 12 hr) and good entrapment efficiency, F8 was selected as optimized formulation and was used for pharmacokinetic evaluation.

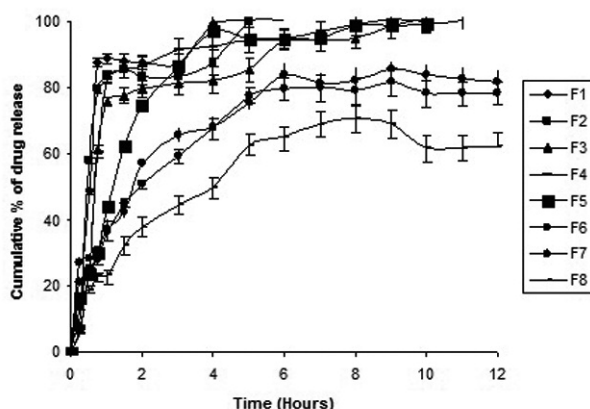


Fig. 5. *In vitro* dissolution profile of indomethacin microspheres

To study the release kinetics of optimized formulation, obtained *in vitro* release data were fitted in various kinetic models such as zero order, first order, Higuchi model, Hixson-Crowell model and Korsmeyer-Peppas model (Table 3). The *in vitro* release profile of F8 could be best expressed by Higuchi kinetic model, as the plot showed highest linearity (R^2 : 0.9029) indicating that the release of indomethacin was diffusion-controlled. During the

Table 3. Determination coefficient (R^2) of different kinetic models of F8

Model	R^2
Zero order	0.7329
First order	0.8102
Higuchi	0.9029
Hixson-Crowell	0.7229
Korsmeyer-Peppas	0.4074
Korsmeyer-Peppas (n value)	0.4152

dissolution studies microspheres did not burst, but extended swelling occurred and thus good conditions for diffusion of the drug from microspheres were created^{32, 33}.

Pharmacokinetic evaluation of the optimized formulation¹⁸⁾

The elimination rate constant K_e of indomethacin when given in microspheres (0.092 hr^{-1}) was found to be decreased in comparison with pure indomethacin (0.184 hr^{-1}) (Table 4). When microspheres were administered, about a two-fold decrease in elimination rate was observed indicating a slow elimination of the drug from the body. The elimination half life ($t_{1/2}$) of the indomethacin from microspheres was found to be 6.861 hours indicating a slow elimination and longer residence of the drug in the body which was further evidenced by the increased AUC value of the drug when given in microspheres. Almost a two-fold increase in AUC was observed when the drug was loaded in microspheres. The absorption rate constant K_a of indomethacin when given in microspheres was found to be decreased when comparing to pure indomethacin indicating a slower absorption of the drug because of slow release of the drug from the cross links of the polymer matrix.

Table 4. Pharmacokinetic parameters of indomethacin

Pharmacokinetic parameter	Pure Indomethacin	Indomethacin microspheres
K_e (hr^{-1})	0.184 ± 0.004	0.092 ± 0.006
K_a (hr^{-1})	1.17 ± 0.12	0.161 ± 0.1
$t_{1/2}$ (hr)	3.766 ± 1.24	6.861 ± 1.8
AUC ($\mu\text{g}\cdot\text{hr}/\text{ml}$)	154.275 ± 1.98	339.705 ± 5.66
C_{max} ($\mu\text{g}/\text{ml}$)	21.0794 ± 0.22	21.956 ± 0.27
t_{max} (hr)	2	2.5

However, there was no significant change in time required to reach the maximum drug concentration in plasma observed between pure indomethacin and indomethacin-loaded microspheres (t_{max} for pure indomethacin, 2 hours and t_{max} indomethacin-loaded microspheres, 2.5 hours). But the C_{max} was found to be the same for pure indomethacin as well as indomethacin loaded in controlled release Microspheres (Fig. 6). Significant changes in K_e , $t_{1/2}$, K_a and AUC values of the drug when administered as microspheres clearly

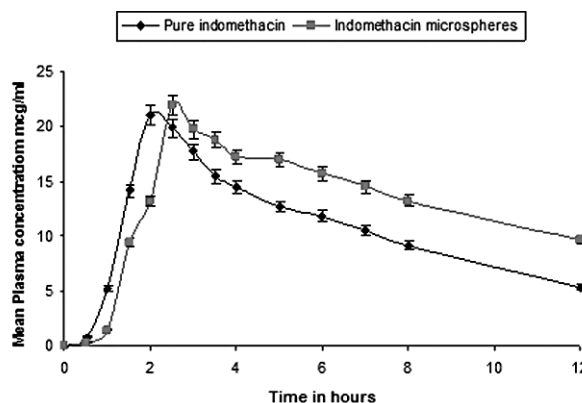


Fig. 6. Mean plasma concentration-time profiles of indomethacin

indicated that the microspheres developed in the present study showed a controlled release of the drug confirming the results of *in vitro* studies.

Conclusion

From the present study it can be concluded that indomethacin controlled release microspheres can be formulated using sodium alginate, methyl cellulose and calcium chloride. Prepared microspheres exhibited a spherical shape, good entrapment efficiency up to 87% and controlled drug release over a period of 12 hours with no drug-excipient interactions. Based on *in vivo* pharmacokinetic data, it can be concluded that the indomethacin microspheres released the drug in controlled manner leading to prolonged drug residence and therapeutic effect. Both *in vitro* and *in vivo* studies confirmed 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.

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Conflict of interest: none.

References

1. **Salib S., Donney S., Doyle D.** Therapy and drugs in the control of osteoarthritis. *Prescr.* 1996; 8, 41–59.
2. **Brahmankar M., Sunil Jaiswal B.** Controlled Release Medication. In: Biopharmaceutics and Pharmacokinetics A treatise. 1st ed. New Delhi: Vallabh Prakashan 2005.
3. **Jackson Roberts L., Jason D. M.** Analgesic antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Joel Hardman G., Lee Limbird E., Alfred Goodman Gilman. The Pharmacological Basis of Therapeutics, 10th ed. London: Mc Graw-Hill 2001.
4. **Gowda D. V., Shivakumar H. G.** Comparative Bioavailability studies of Indomethacin from Two-controlled release formulations in healthy albino sheep. *Ind. J. Pharm. Sci.* 2006; 68, 760–763.
5. Monographs. In: Indian Pharmacopoeia 1996, Volume I (A–O), The controller of Publications, Delhi, 393–395.
6. **Gunnar A., Orme M., Bertilsson L., Ekstrand R., Palmer L.** Pharmacokinetics of Indomethacin. *Clin. Pharm. Ther.* 1975; 18, 364–373.
7. **Mallikarjun Reddy K., Ramesh babu V., Krishna Rao K. S. V., Subha M. C. S., Chowdoji Rao K., Sairam Aminabhavi T. M.** Temperature sensitive Semi-IPN microspheres from sodium alginate and N-iso propylacrylamide for controlled release of 5-fluorouracil. *J. Appl. Polym. Sci.* 2007; 107, 2820–2829.
8. **Bodmeier R., Chen H.** Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen, and ketoprofen. *J. Control. Release.* 1989; 10, 167–175.
9. **Anande N. M., Jain S. K., Jain N. K.** Con-A conjugated mucoadhesive microspheres for the colonic delivery of Diloxanide furoate. *Int. J. Pharma.* 2008; 359, 182–189.
10. **Babay D., Hoffman A., Bentia S.** Design and release kinetic pattern evaluation of indomethacin microspheres intended for oral administration. *Biomaterials* 1988; 9, 482–488.
11. **Malamataris S., Avgerinos A.** Controlled release indomethacin microspheres prepared by using an emulsion solvent-diffusion technique. *Int. J. Pharm.* 1990; 62, 105–111.
12. **Basu S. K., Kunchu K., Mani R.** Evaluation of ketorolac tromethamine microspheres by chitosan/gelatin B complex coacervation. *Sci. Pharm.* 2010; 78, 79–92.
13. **Paradhkar A. R., Pawar A. P., Chordia J. K., Patil V. B., Ketkar A. R.** Spherical crystallization of celecoxib. *Drug Dev. Ind. Pharm.* 2002; 28, 1213–1220.
14. Indian Pharmacopoeia, Ministry of Health and Family welfare, The Indian Pharmacopoeia commission, Ghaziabad, 6th edition 2010, PP 559, 560, 1482, 1495, 1758.
15. **Brazel C. S., Peppas N. A.** Modelling of drug release from swellable polymers. *Eur. J. Pharm. Biopharm.* 2000; 49, 47–58.
16. **Peppas N. A.** Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta. Helv.* 1985; 60, 110–111.
17. **Boon V., Glass B., Nimmo A.** High-performance liquid chromatographic assay of indomethacin in porcine plasma with applicability to human levels. *J. Chromatogr. Sci.* 2006; 44, 41–44.
18. **Riew K. D., Long J., Rhee J., Lewis S., Kuklo T., Kim Y. J., Yukawa Y., Zhu Y.** Time-dependent inhibitory effects of indomethacin on spinal fusion. *J. Bone. Joint. Surg. Am.* 2003; 85, 632–634.
19. **Ahregart L., Lindgren U., Reinholt F. P.** Comparative study of the effects of radiation, Indomethacin, prednisolone, and ethane-1-hydroxy-1, 1-diphosphonate (EHDP) in the prevention of ectopic bone formation. *Clin. Orthop. Relat. Res.* 1988; 229, 265–273.
20. **Johnson A. G., Ray J. E.** Improved high-performance liquid chromatographic method for the determination of indomethacin in plasma. *Ther. Drug. Monit.* 1991; 14, 61–65.
21. **El-Kamel A. H., Al-Ghoray O. M. N., Hosny.** Alginate-diltiazem hydrochloride beads: Optimization of formulation factors, *in vitro* and *in vivo* availability. *J. Microencapsulation* 2003; 2, 211–225.
22. **Das M. K., Senapati P. C.** Evaluation of furosemide-loaded alginate microspheres Prepared by ionotropic external gelation technique. *Acta Pol. Pharm.* 2007; 64, 253–262.
23. **Das M. K., Senapati P. C.** Furosemide-loaded Alginate Microspheres Prepared by Ionic Cross-linking Technique: Morphology and Release Characteristics. *Ind. J. Pharm. Sci.* 2008; 70, 77–84.
24. **Madani F., Jean-claude C.** Coating of indomethacin-loaded embolic microspheres for a successful embolization therapy. *J. Microencapsul* 2008; 25, 121–133.
25. **Ofokansi K. C., Okorie O., Adikwu M. U.** Biodegradable microspheres based on gelatin-porcine mucin admixtures: *in vitro* and *in vivo* delivery studies. *Biol. Pharm. Bull.* 2009; 32, 1754–1759.
26. **Hafner A., Filipovic J., Voinovich D., Jalsenjak I.** Development and *in vitro* characterization of chitosan-based microspheres for nasal delivery of promethazine. *Drug Dev. Ind. Pharm.* 2007; 33, 427–436.
27. **Gowda D. V., Shivakumar H. G.** Preparation and evaluation of waxes/fat microspheres loaded with indomethacin for controlled release. *Ind. J. Pharm. Sci.* 2007; 69, 251–256.
28. **Kilicarslan M., Baykara T.** The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. *Int. J. Pharm.* 2003; 252, 99–109.
29. **Gibaud S., Bonneville A., Astier A.** Preparation of 3,4-diaminopyridinemicroparticles by solvent-evaporation methods. *Int. J. Pharm.* 2002; 242, 197–201.
30. **Venkatesan P., Muralidharan C., Manavalan R., Valliappan K.** Selection of better method for the preparation of microspheres by applying analytic hierarchy process. *J. Pharm. Sci. Res.* 2009; 1, 64–78.
31. **Sahin S., Selek H., Ponchel G., Ercan M. T., Sargon M., Hincal A. A., Kas HS.** Preparation, characterization and *in-vivo* distribution of terbutaline sulfate loaded albumin microspheres. *J. Cont. Rel.* 2002; 82, 345–358.
32. **Sanghvi S. P., Nairn J. G.** Effect of viscosity and interfacial tension on particle size of cellulose acetate trimellitate microspheres. *J. Microencapsulation* 1992; 9, 215–227.
33. **Perumal D.** Microencapsulation of ibuprofen & Eudragit RS 100 by the emulsion solvent diffusion technique. *Int. J. Pharm.* 2001; 218, 1–11.