Biosciences, Biotechnology Research Asia Vol. **7**(1), 219-230 (2010)

Formulation and evaluation of flurbiprofen loaded microspheres for transdermal delivery

SANTHOSHI POLA, B. VAMSHI KRISHNA, V. VAIJAYANTHI, S.K. UMA DEVI, S. PADMAPRIYA and V. RAVICHANDIRAN

Vel's college of Pharmacy, Department of Pharmaceutics, Pallavaram, Chennai - 600 117 (India).

(Received: January 01, 2010; Accepted: February 02, 2010)

ABSTRACT

The conventional treatment for osteoarthritis and rheumatoid arthritis involves the use of Non steroidal anti inflammatory drugs (NSAIDs), which are having many side effects, there by limiting their use. The aim of the present study is to prepare flurbiprofen-loaded microspheres and incorporate into a transdermal patch for sustained release of flurbiprofen. The microspheres were prepared by emulsification method using sodium alginate (concentration 2.5%, 5%, 7.5%) as a polymer and evaluated for entrapment efficiency, loading efficiency, particle size, drug content, surface morphology and *in-vitro* drug release studies. The microspheres with least percentage release were incorporated into transdermal patch, which was prepared by using combination of polymers hydroxy propyl methyl cellulose (HPMC): ethyl cellulose (EC): EUDRAGIT L100. The patches were evaluated for drug content, thickness, tensile strength and *invitro* permeation studies. The *invitro* release studies of the prepared flurbiprofen transdermal patch loaded microspheres were compared and the results showed microspheres(MS2) loaded transdermal patch exhibited controlled release with non- fickian diffusion as release mechanism.

Key words: Flurbiprofen microspheres, sodium alginate, transdermal patches, emulsification method.

INTRODUCTION

The word arthritis literally means inflammation of the joints characterized by pain, swelling and redness, heat and some times, structures changes. Joints can become inflamed for many reasons, but arthritis is usually of two kinds, osteoarthritis (OA) and rheumatoid arthritis (RA). There are huge sources of discomfort and disability. The main goal in the treatment of rheumatoid arthritis is to reduce joint inflammation and pain, their by maximizes joint function, prevents joint destruction and deformity. First line medication for treatment of rheumatoid arthritis includes NSAIDs and Corticosteroids. The NSAIDs which are used for the treatment of rheumatoid arthritis are Aspirin, Naproxen, Ibuprofen, Etodolac. Flurbiprofen is also used as non steroidal anti inflammatory drug which undergoes first pass metabolism to overcome that transdermal route is being selected to minimize side effects. Transdermal route was attempted to deliver flurbiprofen into systemic circulation[1, 2]. Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints. Rheumatoid arthritis can also cause inflammation of the tissue around the joints, as well as in other organs in the body[3].

Aspirin doses higher than that used in treating headache and fever, is an effective anti inflammatory medication for rheumatoid arthritis The most common side effects of aspirin and other NSAIDs includes stomach upset, abdominal pain, ulcers, and even gastrointestinal bleeding. In order

to reduce stomach side effects, NSAIDs are usually taken with food[4].

Flurbiprofen is one of the most potent NSAID currently available. *Invitro* studies indicate that the prostaglandin inhibitory activity of flurbiprofen on a molar basis is approximately 1–20 times that of indomethacin, 10–200 times that of ibuprofen, and 200–5600 times that of aspirin [5].

The novelty of the present study includes preparing Flurbiprofen loaded microspheres which is incorporated into transdermal patch for sustained release of an Flurbiprofen a cox-2 selective inhibitor which is used orally/topically in the treatment of rheumatoid arthritis and Osteo arthritis

For the rheumatoid arthritis Alginate microspheres of Flurbiprofen was prepared and incorporated into transdermal patch, prepared with various polymers such as Hydroxy propyl methyl cellulose, Ethyl cellulose, and Eudragit-L100 in order to provide a prolonged effect and relatively less side effects. Combination of polymers retards the drug release there by achieving controlled action.

MATERIAL AND METHODS

Materials

Flurbiprofen was obtained as a gift sample from FDC Mumbai. Sodium alginate, Hydroxy propyl methyl cellulose, Ethyl cellulose, EudragitL-100, Light liquid paraffin, Span-80, Calcium chloride, Propylene glycol, n-Hexane, Isopropyl alcohol were purchased from Loba chemie Pvt. Ltd Chennai.

Methods

Pre-formulation studies like melting point, loss on drying, residue on ignition, solubility studies, UV analysis, Viscosity, FTIR studies were conducted for drug and polymers. Emulsification method was utilized for the preparation of various microspheres followed by cross linking with calcium chloride. The drug loaded microspheres were incorporated into transdermal patch which is prepared by using solvent casting technique.

Fourier Transform Infrared spectroscopy

The Fourier transform infrared analysis was conducted for the structure characterization. FTIR spectra of the formulated microspheres and drug were recorded. Microspheres were taken in KBr pellets using BOMEN MB FTIR instrument. Approximately 5mg of sample mixed with 50mg of spectroscopic grade KBr pellet and scanned in the IR range of 400-4000cm^{-1}, with a resolution of 4cm^{-1}.

FTIR Spectroscopy was carried out to ensure that no chemical interactions between the drug and polymer. From the FTIR spectra interpretations the following results were obtained. The FTIR of flurbiprofen showed intense band at 2354cm^{-1}, 1416cm^{-1}, 1216cm^{-1},698cm^{-1}. The same peak was observed in FTIR of MS2 Formulation 2322 $cm^{-1,}$ 1411cm^{-1},1201cm^{-1}, 694 cm^{-1} from the above interpretations the results ensured that there is no chemical interaction. Results were shown in figure 1, 2, table 1

Preparation of microspheres

Emulsification method[6, 18]

The emulsification method was utilized for the preparation of microspheres followed by cross linking with calcium chloride. Core material, flurbiprofen (100mg) was dispersed in specific concentrations like 2.5%,5%,7.5% aqueous solution of sodium alginate(10ml).The aqueous phase was emulsified in light liquid paraffin in the ratio 1:10 containing 2%(V/V) span 80 using a mechanical stirrer at 1500-2000 rpm for 60min, and to it 5ml of 0.2M calcium chloride dissolved in a mixture of methanol and isopropyl alcohol (2:3) was added slowly to the emulsion and stirred to ensure efficient cross linking. Microspheres were collected by filtration in vacuum, and washed with isopropyl alcohol thrice and finally air dried at room temperature. Formulations are given in table 2.

Method of preparation of transdermal patch[7,14]

Method used for the preparation of film was solvent casting technique employing a glass substrate .The polymers were dissolved in suitable solvent at room temperature, the polymeric solution was obtained by stirring the solution on a magnetic stirrer for 30min, the prepared microspheres equivalent weight to 100mg of flurbiprofen was added to the polymeric solution. The solution was poured in Petri dish and dried at 40º for 6hrs in an oven, Films of 20mm diameter were cut, packed in

an aluminum foil and stored in dessicator. The formulations are given in table 3

Characterization of microspheres

Microspheres dried at room temperature were weighed and the yield of microspheres prepared were calculated using the formula

$$\text{Percentage (\%) yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Determination of drug content in microspheres[8,13]

The Flurbiprofen microspheres were analyzed UV spectrophotometrically. 25mg of microspheres were crushed in mortor and added to 50 ml of ethanol was suitably diluted for the absorbance measured at 248nm. The drug content of microspheres were determined spectrophotometrically (λ_{max} = 248nm). The alginate microspheres (10mg) loaded with Flurbiprofen were dissolved in 10ml of phosphate buffer pH 7.4 under sonication for 20min. The solutions were filtered and the amount of flurbiprofen was measured

$$E = \frac{Qp}{Qt} \times 100$$

Where,

E = Percentage of entrapment efficiency of microspheres.

Qp = Percentage of drug loaded in the microspheres

Qt = quantity of drug added for loading (gms)

Determination of drug loading in microspheres[9, 15]

The flurbiprofen loaded in the microspheres was estimated by using the formula

$$L = Qm/Wm \times 100$$

Where,

L = percentage of drug loaded in the microspheres

Wm = weight of microspheres (gms)

Qm = quantity of flurbiprofen present in Wm grams of microspheres

Determination of mean particle size of microspheres

Particle size determination of microspheres was carried out by optical microscopy. Required quantity of dried microspheres were suspended in glycerin and the particle size of 100 microspheres were determined in each batch and the mean particle size was calculated

Scanning electron microscopy (SEM)

For the external morphology studies, air dried particles were visualized using scanning electron microscopy (SEM Jeol JSM-6400, JAPAN) operating at 15.0kv. The samples were mounted on a metal stub with double adhesive tape and coated with platinum/palladium alloy under vacuum.

***invitro* release studies**

The *in-vitro* drug release studies were conducted in pH 7.4 buffer for 12 hours using USPXXIII, type-II dissolution apparatus under sink conditions. Containing equivalent weight of Flurbiprofen weighed samples were added to the dissolution medium kept at 37º±5ºC. At present time intervals aliquots were withdrawn and replaced by equal volume of dissolution medium to maintain constant volume. After suitable dilution, samples were analysed spectrophotometrically at 248nm.

Evaluation of transdermal patch

Thickness [10, 11, 17]

The thickness of the films were measured by dial caliper (Mituotyo).The mean of the three observations were calculated.

Tensile strength

The tensile strength of the films were determined by using the method reported by Seth *et al* the drug reservoir film was fixed to the assembly, the weights required to break the films were noted and simultaneously film elongation was measured with the help of pointer mounted on the assembly and calculated the tensile strength of the drug reservoir film using the formula delivered by Allen *et al*

TS= (Break force/a**×**b) **×** (1+L/I)

Where,

a = Width

b = Thickness

L = Length of the film

I = Elongation of the film at break point

Drug content

The drug content was determined by

performing assay, the area of the patch was 3.14 square cm and dissolved in the respective solvents, samples were analyzed spectrophotometrically at 248nm[11].

***In vitro* diffusion study[12, 16]**

Permeability of ingredients was evaluated using Franz-type diffusion cell. The diffusion cell consists of two parts, the upper part is the donor compartment which contains the patch, and the bottom part contains the receptor solution; here phosphate buffer saline (pH7.4) was used. Dialysis membrane was placed above the receptor solution .The whole assembly was placed on a magnetic stirrer in order to maintain the temperature at 32±2^{0}C by a thermostat fitted in the magnetic stirrer. A magnetic Teflon coated rod shaped bar was placed at the bottom of the receptor solution, which ensures consistent mixing in the receptor solution. 2ml of sample withdrawn from the receiver compartment at different time intervals at 1,2,3, 4,5,6, 8,10, 12,15,24 hrs. Fresh receptor fluid (buffer pH7.4) was added to the receiver compartment to maintain constant volume. The samples were assayed according to the formulation by UV analysis at respective wavelengths.

RESULTS AND DISCUSSION

Characterization of microspheres

Percentage of drug loading and entrapment efficiency

Table 1: Ranges of FTIR spectra of flurbiprofen

S.No	Vibrations	Range (cm^{-1})
1	C-H Stretching	2962-2853
2	Carboxylate anion stretching	1610-1550
3	C-F Stretching	1400-1000
4	Aryl stretching	800-600

Table 2: Formulation of flurbiprofen loaded alginate microspheres

S. No	Ingredients	Batches of SA microspheres prepared		
		MS-1	MS-2	MS-3
1	Drug(flurbiprofen)	100mg	100mg	100mg
2	Sodium alginate	2.5%	5%	7.5%
3	Light liquid paraffin	100ml	100ml	100ml
4	Span 80	2%v/v	2%v/v	2%v/v
5	Calcium chloride	5ml	5ml	5ml
6	Isopropyl alcohol	2ml	2ml	2ml
7	Methanol	3ml	3ml	3ml

Table 3: Formulation of Flurbiprofen loaded alginate microspheres of Transdermal patches

S. No	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	Flurbiprofen (equivalent wt)	I00	100	100	100	100	100	100	100	100	100	100	100	100	100
2	HPMC	100	200	400	-	-	200	100	300	-	-	-	200	100	300
3	EC	-	-	-	400	-	200	300	100	200	100	300	-	-	-
4	EL-100	-	-	-	-	400	-	-	-	200	300	100	200	300	100

Table 4: Percentage loading and percentage entrapment of microspheres

Batches	Drug content (mg)	Yield (%)	Loading (%)	Entrapment (%)
MS1	5.78	74.2	33.33	61.2±0.6
MS2	6.93	78.3	52.1	73.1±0.8
MS3	4.92	81.1	49.2	78.27±0.5

Mean ± Standard deviation (n=3)

Table 5: Mean particle size of microspheres

S.No	Batches	Mean particle size (µm)
1	MS1	60.72±0.8
2	MS2	70.51±0.6
3	MS3	78.76±0.4

Mean ± Standard deviation (n=3)

The percentage of drug loading and percentage entrapment for all batches are given in table 4. The entrapment efficiency of all the batches increased with increase in Polymer concentration. 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 active calcium binding sites in the polymeric chains and, consequently, the greater degree of cross linking as the quantity of sodium alginate increased. The batch MS3 (78.27%) showed better entrapment when compare with MS1 (61.2%) and MS2 (73.1%).The results are shown graphically in figure 3 and values are given in table 4

Mean particle size

Mean particle size was determined by optical microscopy and the average particle size. All batches of microspheres were prepared by keeping the drug amount, concentration of cross linking agent constant. The result indicates that the mean particle size increases with increase in polymer concentration. The results are shown in table 5 and figure 4

Scanning electron microscopy

The morphology of the optimized

Table 6*: invitro* release studies of microspheres

S. No	Time (hrs)	Cumulative % Drug Release MS1	MS2	MS3
1	1	4.3±0.3	4.1±0.1	3.4±0.2
2	2	10.8±0.2	12.2±0.2	12.3±0.2
3	3	22.2±0.1	22.3±0.2	18.8±0.1
4	4	38.1±0.2	35.1±0.2	21±0.1
5	5	47.2±0.3	52±0.3	33±0.1
6	6	55.1±0.4	58±0.2	48±0.3
7	8	76.3±0.2	63±0.2	57.1±0.1
8	12	82±0.1	67±0.1	69.2±0.1

Mean ± Standard deviation (n=3)

Table 7: Thickness of patch, tensile strength, drug content

S. No	Thickness (mm)	Tensile strength (Kg/mm^2)	Drug content (mg)
1	0.040	2.16	14.34
2	0.038	2.60	14.02
3	0.041	2.72	14.08
4	0.036	2.74	14.00
5	0.036	1.70	14.34
6	0.038	3.12	14.32
7	0.037	3.74	14.35
8	0.037	3.92	14.54
9	0.040	1.82	14.73
10	0.040	1.87	14.52
11	0.041	2.34	14.31
12	0.038	2.45	14.58
13	0.035	2.65	14.88
14	0.032	2.80	14.90

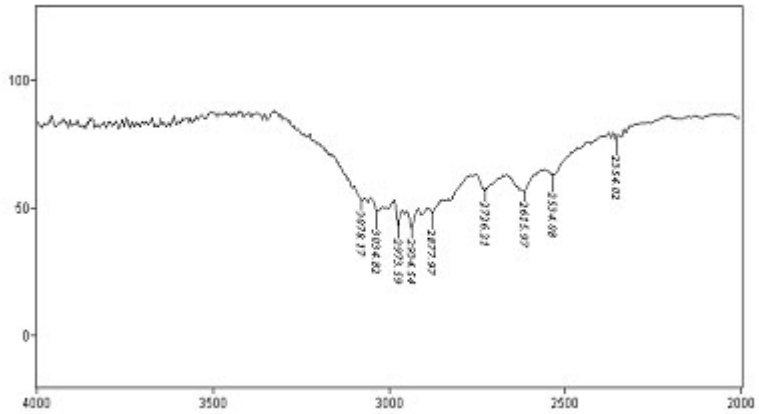

Fig. 1: FTIR spectra of flurbiprofen

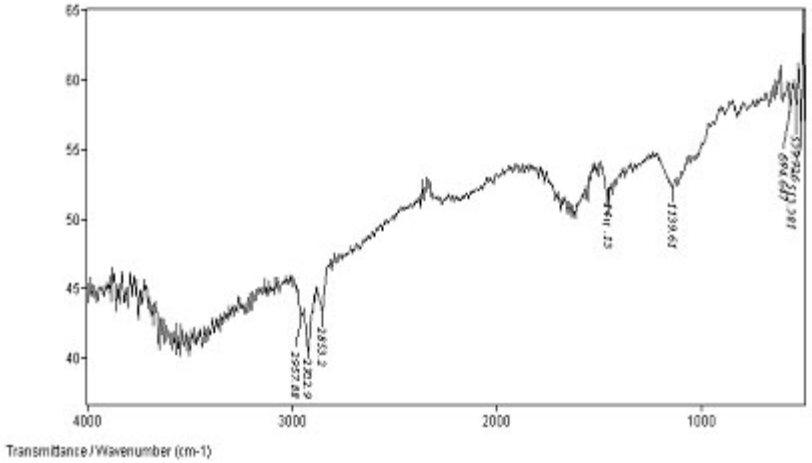

Fig. 2: FTIR spectra of flurbiprofen microspheres (ms2)

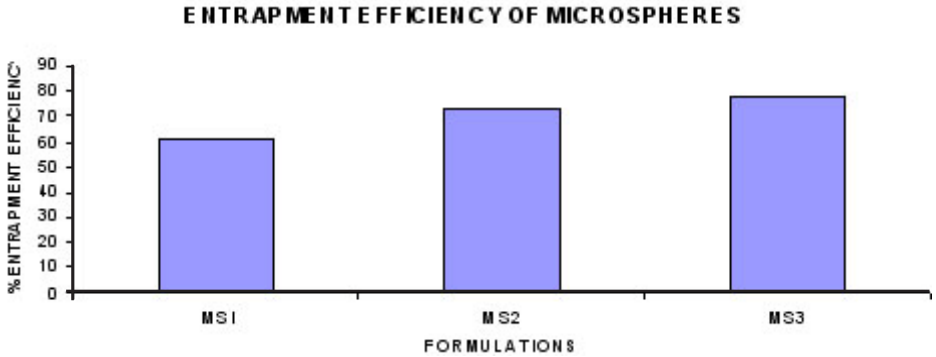

Fig. 3: Entrapment efficiency of microspheres

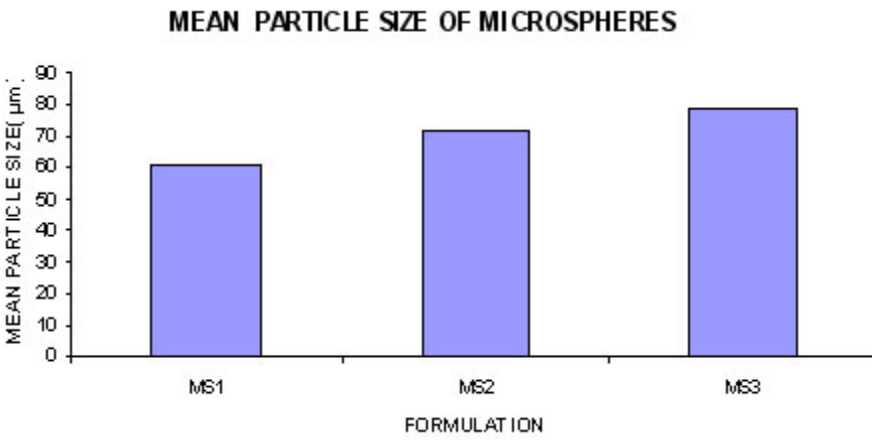

Fig. 4: Mean particle size of microspheres

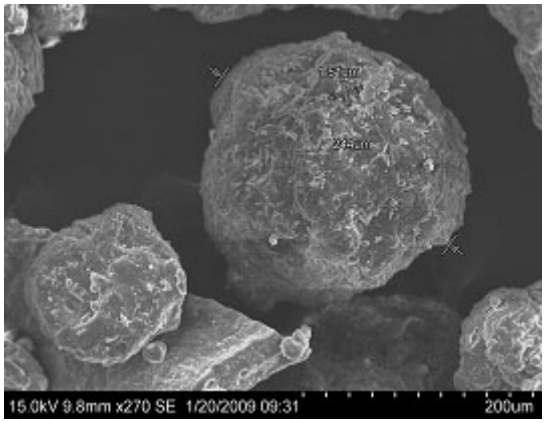

Fig. 5: SEM picture of ms2 formulation

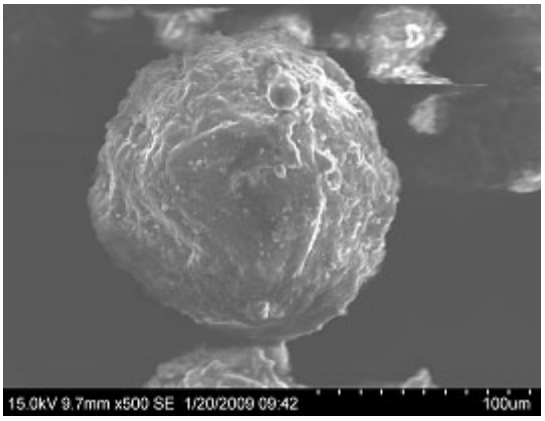

Fig. 6: SEM picture of ms2 formulation

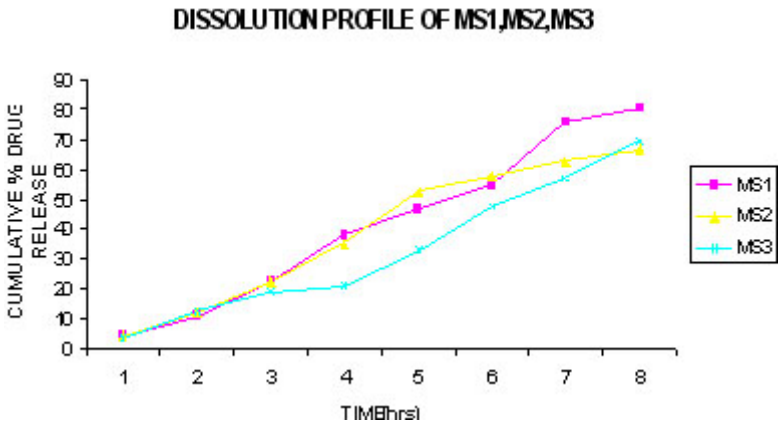

Fig. 7: Dissolution profile of ms1, ms2, ms3

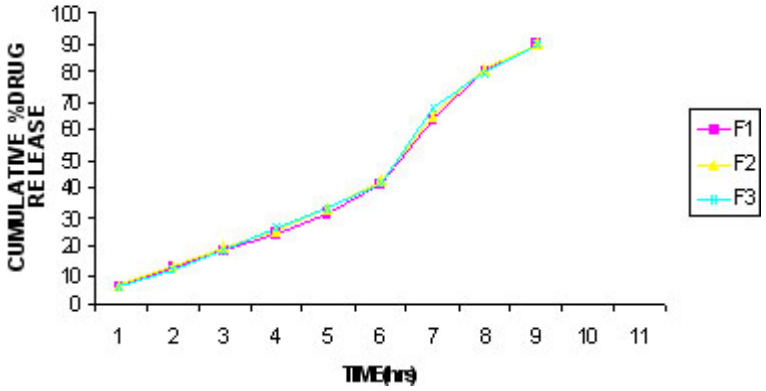

Fig. 8: Diffusion profile of f1, f2, f3

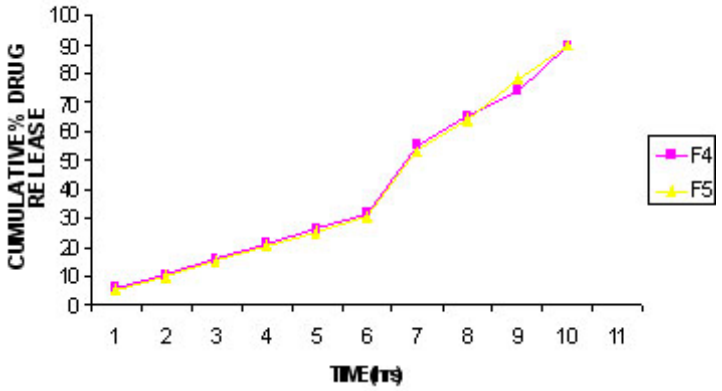

Fig. 9: Diffusion profile of f4, f5

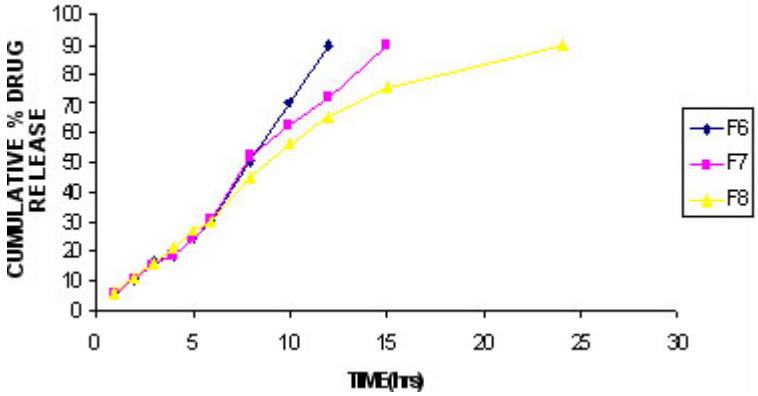

Fig. 10: Diffusion profile of f6, f7, f8

formulation of alginate microspheres was found to be discrete and spherical in shape, the surface of the alginate microspheres was rough due to the higher concentration of drug uniformly dispersed at the molecular level in the alginate matrices. The SEM picture of alginate microspheres are shown in fig. 5, 6

***invitro* release studies**

The dissolution profile of alginate microspheres are shown in table 6 and Figure 7. The release profile of MS2 less when compare toMS1 and MS3

Thickness of patch, tensile strength, drug content

All the formulations, thickness measured with low standard deviation values and ensured uniformity of the films prepared by solvent casting method .The tensile strength of EC: HPMC (F6, F7, F8) patches found to be better. Results are shown in table 7

***invitro* diffusion study**

Formulations containing EC: HPMC patches showed higher drug release rate. F6 shows 90% of drug releases at 12th hour which could be due to the less polymer concentration. F7 shows 90% of drug release at 15thhour, F8 Shows 90% of drug release at 24hour which could be due to the increase in polymer concentration.

Formulations containing EC

EL100 (F9, F10, F11) increase in polymer

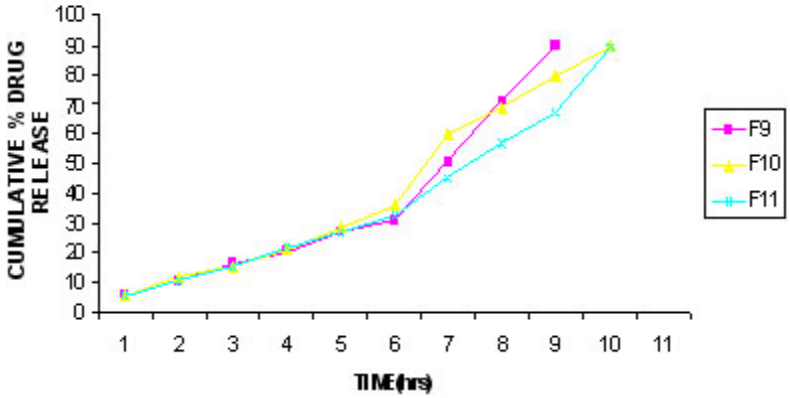

Fig. 11: Diffusion profile of f9, f10, f11

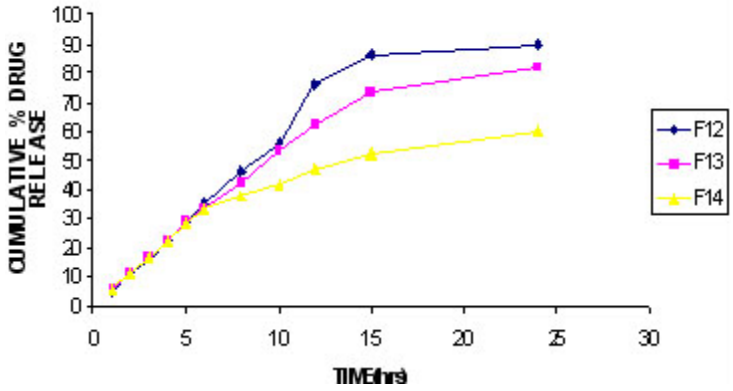

Fig. 12: Diffusion profile of f12, f13, f14

Table 8: *invitro* diffusion study of single polymers

S.No	Time (hrs)	F1	F2	F3	F4	F5
1	1	6.2±0.1	6.1±0.1	5.9±0.1	5.8±0.1	5.1±0.1
2	2	12.3±0.4	13.3±0.1	12.2±0.1	10.2±0.1	10±0.1
3	3	18.1±0.4	19.2±0.2	18.3±0.2	15.4±0.4	15.1±0.1
4	4	24.2±0.3	25.2±0.1	26.1±0.1	21.2±0.3	20.8±0.2
5	5	31.2±0.3	33.2±0.1	33±0.1	26.3±0.2	25.1±0.2
6	6	41.3±0.4	42.3±0.1	41.8±0.3	31.2±0.1	30.1±0.1
7	8	63.2±0.3	64.8±0.4	67.2±0.1	55.2±0.1	53.2±0.2
8	10	80.1±0.2	80.8±0.3	79.3±0.2	65.3±0.3	64.1±0.1
9	12	90±0.2	90±0.1	90±0.1	74.1±0.1	78.2±0.2
10	15	-	-	-	90±0.2	90±0.2
11	24	-	-	-	-	-

Mean ± Standard deviation (n=3)

Table 9: *invitro* diffusion study of combination of polymers

S. No	Time (hrs)	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	1	5.5±0.2	5.1±0.1	5.8±0.2	5.2±0.1	4.9±0.3	5.3±0.2	4.8±0.2	5.3±0.2	5.5±0.1
2	2	10.2±0.1	10.3±0.1	10.7±0.3	10.1±0.1	11.2±0.3	10.2±0.1	10.8±0.2	10.9±0.2	10.7±0.3
3	3	16.2±0.1	15.1±0.2	15.3±0.2	15.8±0.2	15±0.1	15.1±0.1	16.1±0.1	16.3±0.1	16.8±0.3
4	4	18.3±0.1	18.2±0.1	20.6±0.2	20.1±0.2	20.4±0.1	21.2±0.3	22.1±0.3	22.2±0.1	22.1±0.2
5	5	24.1±0.3	24.2±0.1	26.8±0.1	26.6±0.2	28.4±0.2	26.2±0.2	28.8±0.3	28.3±0.3	28±0.2
6	6	30.2±0.4	30.8±0.2	30.1±0.1	30.8±0.1	36.2±0.2	32.3±0.1	34.8±0.3	33.1±0.3	33±0.1
7	8	50.1±0.2	52.1±0.3	45.1±0.1	50.2±0.1	59.3±0.1	45.2±0.1	46.1±0.1	42.1±0.4	38±0.1
8	10	70.3±0.1	62.3±0.3	56.2±0.2	70.8±0.1	68.8±0.1	56.6±0.1	55.3±0.1	53.2±0.3	42±0.2
9	12	90±0.1	72.2±0.2	65.3±0.4	90±0.3	79.3±0.1	67.1±0.2	76.2±0.2	62.1±0.2	47±0.2
10	15		90±0.2	75.2±0.2		90.1±0.2	89±0.2	86.1±0.2	73.2±0.1	52±0.1
11	24			90±0.1				90±0.1	82±0.1	60±0.2

Table 10: Stability studies

S. No	Time (hrs)	Diffusion profile0f F14	Diffusion profile ofF14 after 2 months
1	1	5.5	5
2	2	10.7	10.1
3	3	16.8	16
4	4	22.1	21.8
5	5	28	27.7
6	6	33	32.3
7	8	38	38.3
8	10	42	42
9	12	47	47.3
10	15	52	52.3
11	24	60	60.4

Table 11: Kinetic analysis of diffusion data

Batch	Zero order	First order	Higuchi	Korsmeyer peppas
F8	0.9478	0.857	0.996	0.737
F11	0.987	0.893	0.983	0.704
F14	0.876	0.901	0.974	0.668

Table 12: Comparative studies

S.No	Time(hrs)	MS2	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	12 hour	67%	90%	72.2%	65.3%	90%	79.3%	67.1%	76.2%	2.1%	47%
2	24 hour	-	-	-	90%	-	-	-	90%	82%	60%

concentration decreases the drug release rate. F11 shows 89% of drug release at 15th hour.

The results are shown in table 8, 9 and Figure 8, 9, 10, 11, 12.

Formulation containing HPMC

EL100 (F12, F13, F14) showed that increasing polymer concentration will decrease the drug release rate. F14 showed 60% drug release at 24 hours.

Stability studies

Stability studies were carried out, for diffusion data of F14 at 40^{0}C after two months, the results ensure that there is no considerable change in the diffusion profile which indicates that the prepared patch was stable. The results are shown in table 10

CONCLUSION

There has been considerable interest in recent years in developing controlled or sustained drug delivery systems using drug loaded microspheres for transdermal drug delivery. The present study was aimed to formulate and evaluate flurbiprofen loaded alginate microspheres as transdermal drug delivery for topical treatment of arthritis.

By analyzing the results, it was observed that MS2 formulation showed sustained release of drug from microspheres which could be due to the optimum polymer concentration. In MS3, the mean particle size was greater due to the increased polymer concentration. Among all the formulations, MS2 showed satisfactory release profile; hence MS2 was incorporated into trasdermal patch for controlled release of Flurbiprofen.

The comparative studies (table12) of microspheres and transdermal patch at 12 hour showed that MS2 (67%), F14(47%) hence concluded that the release of microspheres alone showed faster release, after incorporated into transdermal patch the release is sustained, so the prepared patch is used for prolonged therapy. The formulated transdermal patch was evaluated for thickness, tensile strength, drug content and *in vitro* diffusion study, all evaluation studies shown satisfactory results. Among all the formulations EC: HPMC patches showed higher drug release rate, more permeability which could be due to hydrophilic nature of the polymer which increases the thermodynamic activity of drug. Kinetic data (table11) showed microspheres (MS2) loaded transdermal patch exhibited controlled release with non- fickian diffusion as release mechanism.

REFERENCES

1. Srivastava, KC., Mustafa, T., Med Hypothese. Rheumatic Disorders. 25-28, 29 (1989).
2. http:// www.arthritis.org
3. WB Saunders Co, edited by shaun Ruddy et al, Kelley's text book of Rheumatology, (2000).
4. Lippincott Williams & Wilkens, Clinical Primer of Rheumatology edited by William Koopman, et al.
5. Hubstier, JA., Ballard, IM., Walker, BR., *J. Int. Med. Res*. **7**: 69-76 (1979).
6. Rastogi, R., Sultana, Y., Aquil. M., *Int. J. Pharm. Sci.* **334**: 71-77 (2007).
7. Manvi,F.V., Dandagi, P.M., Gadad, A.D., Mastitiolimath V.S., Jagadeesh, J., *Indian. J. Pharm. Sci*. **65**: 239-243(2003).
8. Das, M.K., Senapati P.C., *Indian. J. Pharm. Sci*. **70**: 77-84 (2008).
9. Das, A., Sridhar, B.K., *Indian. J. Pharm Sci*. 7**0**: 422-428 (2008).
10. Agarwal, S.S., priya Majal. *Indian. J. Pharm. Sci*. **69**: 535-539 (2007)
11. Sadhana, P., Gupta, S.K., Jain, *Indian. J. Pharm. Sci*. **67**: 346-350 (2005)
12. Chandra shekar, M.S., Shoba Rani, R.H., *Indian. J.Pharm. Sci*. **70**: 91-94 (2008).
13. Ting Li, Ctiangstium Rem, R.L., *Indian J. Pharm. Sci*. **69**: 55-58(2007).
14. Rathore RPS*., Indian. J. Pharm. Sci.* **68**: 55-58 (2006).
15. Jian Ping wang, Jinlan ruan, Changgongztang, *J. Pharm Sci*. 21: 407-410(2008).
16. Srinivas, A., Mautalik. M., **63**, 418-424 (2006).
17. Watid, A., Sridhar, B.K., Shivakumar, S., *Indian J. Pharm. Sci*. **70**: 455-460 (2008).
18. Kapoor, A., Lewis. S., Venkatesh and Udapa,N., *Indian drugs.* **39**: 415-418 (2002).