INTRODUCTION

Drug delivery systems (DDS) that can precisely control the release rates or target drug to a specific body site have had an enormous impact on the health care system. The last two decades there has been a remarkable improvement in the field of novel drug delivery systems.

Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc. which modulates the release and absorption characteristics of the drug. Microspheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics [1].

Solvent evaporation technique is one of the several methods that is used for production of microspheres. Although this way may not be the main method, but it is the simplest one that several variables can affect the outcome, as well. Kilicarsan and Baykara (2003) investigated the effect of the drug/polymer ratio on properties of the verapamil loaded microspheres that were made by solvent evaporation method and found that the drug release profile could be sustained by increasing polymer amount, and the particle size and surface characterization of microsphere could be modified through the variation of drug/polymer ratio. Diaminopyridine microparticles by solvent evaporation method were prepared by Gibaud et al. (2002) [2-4].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation. NSAIDs produce their therapeutic effect by inhibiting the cyclooxygenase (COX) enzymes, which are involved in the biosynthesis of prostaglandins (PGs) [5, 6]. Loxoprofen, 2-[4-(2-oxocyclopentylmethyl)phenyl]-propionate with two chiral centers, is marketed as an equal parts mixture of four stereoisomers. Loxoprofen sodium is an important non-steroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid group used for the treatment of rheumatoid arthritis and osteoarthritis. Loxoprofen is a prodrug which produces effects after being absorbed from the gastrointestinal tract rather than the sodium salt, which causes just weak irritation of the gastric mucosa, and is then converted to an active metabolite by reduction of the ketone carbonyl to the trans-OH form. The active isomer has the 2S, 1R, 2S configuration, which potently inhibits prostaglandin biosynthesis [7]. Loxoprofen has an activity to treat inflammatory rheumatoid diseases and relieve acute pain. It is effective against period pains, pain after surgery and fever. Loxoprofen available in pharmaceutical formulations as Tablets and Transdermal patches [8, 9].

The aim of present study was to formulate and evaluate the loxoprofen loaded sustained release microspheres by emulsion solvent evaporation technique.

Sustained release microspheres may be produced by several methods utilizing emulsion system (oil-in-water, oil-in-oil, water-in-oil-in-water), as well as by spray drying. The common emulsion system used oil-in-water (o/w), with microspheres being produced by the emulsion solvent evaporation method. This relatively simple method enables the entrapment of a wide range of hydrophobic drugs [10]. Ethyl cellulose is non-biodegradable, bio-compatible, non-toxic natural polymer and widely used in oral and topical formulation [11,12].

The main objective of this work was to investigate the possibility of obtaining a sustained release formulation of Loxoprofen microspheres by using ethylcellulose in various drugs, polymer ratios (1:1, 1:2, and 1:3). The various physicochemical characteristics and the invitro release rates from these microspheres were thus examined.

MATERIALS AND METHODS

Working standards of Loxoprofen (99.79%) and Ethylcellulose (viscosity grade, 100 cp) were donated by M/S Micro labs limited, Hourer, India. Dichloromethane and Tween 80 were of analytical-reagent grade supplied by M/S SD Fine chemicals, Mumbai, India. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

PREPARATION OF LOXOPROFEN MICROSHERES

This is the method widely used in the microencapsulation process. Calculated quantity of polymer was dissolved in Dichloromethane to form a homogenous polymer solution. Then calculated quantity of drug was added to the polymer solution and mixed thoroughly. The resulting mixture was then added in a thin
stream of 300ml of aqueous solution containing 1%(v/v) tween80, while stirring at 1000rpm to emulsify the added dispersion as fine droplets. The solvent, dichloromethane was then removed by evaporation during continuous stirring at room temperature for 3 hours to produce spherical microspheres. Here dichloromethane was used as polymer solvent, aqueous solution as the microencapsulating vehicle, tween80 as the dispersing agent. During 3hours stirring period, dichloromethane was completely removed by evaporation[12-18]. The microspheres were collected by vacuum filtration and washed repeatedly and dried in room temperature over a night to get free flowing microspheres. By varying this drug: polymer ratio, three batches of microspheres were prepared (Table 1).

**PHYSICOCHEMICAL CHARACTERIZATION OF THE MICROSPHERES:**

**Percentage yield**

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula [10, 12].

\[
\text{Percentage yield} = \left( \frac{\text{Weight of microspheres}}{\text{Weight of polymer + drug}} \right) \times 100
\]

**Drug content**

The various batches of the microspheres were subjected for drug content analysis. Accurately weighed microsphere samples were mechanically powdered. The powdered microspheres were dissolved in adequate quantity of phosphate buffer PH 7.4 then filter. The UV absorbance of the filtrate was measured using a UV spectrometer at 220nm.

**Drug loading and encapsulation efficiency**

Drug loading and encapsulation efficiency was determined for all batches using the following formulas [9,11] Values are expressed as percentage.

| S.No | Formulation code | Polymer: Drug Ratio | Theoretical drug content(mg) | Actual drug content(mg) | Percentage Yield. |
|------|------------------|---------------------|------------------------------|------------------------|-------------------|
| 01   | F1               | 1:1                 | 200                          | 154.21                 | 77.11             |
| 02   | F2               | 1:2                 | 400                          | 324.80                 | 81.20             |
| 03   | F3               | 1:3                 | 600                          | 524.04                 | 87.34             |

**RESULTS AND DISCUSSION**

**Percentage yield**

The percentage yield of three formulations was ranging from 77.11 to 84.34 respectively (Table 2). This higher percentage yields indicates that this method was very useful for adoption in the formulation of loxoprofen microsphere.

**Drug content**

The results of the determination of microsphere drug content for various polymers: drug ratios are shown in table 2. From the three formulations F3 has the
highest milligram of the drug content following by other formulations. Because it may be due to the highest amount of theoretical drug content and highest percentage yield in this ratio.

**Drug loading and encapsulation efficiency**

The results of the variation in drug loading and encapsulation efficiency with polymer: loxoprofen ratio is shown in table 3. Higher percentage of loading was obtained by increasing the amount of loxoprofen with respect to ethyl cellulose. The encapsulation process was found to be good and 74.96 to 84.57 of the drug employed in the process were encapsulated by the microsphere.

**Particle size**

The particle size of loxoprofen loaded microsphere was analysed by optical microscopy. All the batches of microspheres show uniform size distribution. The average particle size of loxoprofen loaded microspheres was found to be in the range of 266.16 to 329.18μm. As the polymer: drug ratio was increased, the microspheres size was also found to be increased (table 4).

**Scanning Electron Microscopy**

The microspheres had good spherical geometry as evidenced by the SEM photographs. The surface of the microspheres was quite smooth (figure1).

**In vitro drug release studies**

Microspheres of all batches had faster initial drug release approximately 25 percentages within 15 minutes. Then the release was slow and sustained over 8 hours, depending upon the polymer: drug ratio. By the end of 8th hour the percentage of drug release was found to 76.84, 80.32 and 86.17 for F1, F2 and F3 formulation respectively (figure 2). The formulation F3 showed better sustained release
The average particle size was found to be in the range of 266.16-329.18 μm. With respect to polymer, the particle size of a microsphere was determined by optical microscopy. Higher percentage of loading was obtained by increasing the amount of loxoprofen. The encapsulation of three formulations was found to be in the range of 74.96 to 84.57. The percentage of elution from its higher percentage yield. The formulation F3 has highest milligram of drug content followed by other formulations. The percentage of encapsulation of three formulations was found to be in the range of 74.96 to 84.57. Higher percentage of loading was obtained by increasing the amount of loxoprofen with respect to polymer. The particle size of a microsphere was determined by optical microscopy. The in vitro dissolution studies showed that loxoprofen microspheres formulation F3 showed better sustained effect (86.17%) over a period of 8 hours than other formulations.

**REFERENCES**

1. Vasir JK, Tambwekar K, Garg S, et al. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm. 2003;255:13-22.
2. Kilicarslan M, Baykara T, et al. The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. Int J Pharm. 2003;252:99-109.
3. Gilbau S, Bonneville A, Astier A, et al. Preparation of 3, 4- dianiminopyridine microparticles by solvent-evaporation methods. Int J Pharm. 2002;242:197-201.
4. Venkatesan P, Muralidharan C, Manavalan R, et al. Selection of better method for the preparation of microspheres by applying Analytic Hierarchy Process. J Pharm Sci & Res. 2009;1(3):61-78.
5. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol. 1971;231:232-235.
6. Vane JR, Bakile YS, Botting RM, et al. Cyclooxygenases 1 And 2. Annu Rev Pharmacol Toxicol. 1998;38:97-120.
7. Nagamma H, Mochizuki Y, Kawahara Y, et al. Study of pharmacokinetics following oral administration of loxoprofen sodium (CS-600) in humans. J Clin Therap Med. 1986;2:1219.
8. Tohru Araki, Teruhiko Yokoyama, Motoo Araki, Seiji Furuya et al. A Clinical Investigation of the Mechanism of Loxoprofen, a Nonsteroidal Anti-inflammatory Drug, for Patients with Nocturia. J Acta Med Okayama. 2008;62:373-378.
9. Shinichi Yoshikawa, Ryo Murata, Shigenari Shida, Koji Uwai, Tsuneo Yuki Suzuki, Shunji Katsumata, Mitsuhiro Takeshita, et al. Evaluation of Correlation between Dissolution Rates of Loxoprofen Tablets and Their Surface Morphology Observed by Scanning Electron Microscope and Atomic Force Microscope. Int J Chem Pharm Bull. 2010;52:34-37.
10. Thompson CJ, Hansford D, Higgins S, Rostron C, Hutechon, Munday DL, et al. Evaluation of ibuprofen-loaded microspheres prepared from novel copolymers. Int J Pharm. 2007;329:53-61.
11. Ainey wade & Paul J Weller. Handbook of pharmaceutical excipients, second edition, The Pharmaceutical Press, London, 1994, 116-190.
12. Sudhamani T, Novenekumar reddy K, Ravi Kumar VR, Revathi R, Ganesan V, et al. preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery, Int J Pharma Res and Dev. 2010;1:119-125.
13. Saravanan M., Dhanaraju D, SRidhar SK, Ramachandran S, kishore Gran sam A, Anand P, Bhaskar K, Srinivasarao G, et al. Preparation, Characterization and in vitro release kinetics of ibuprofen polystyrene microspheres, Ind J Pharm Sci, 2004;66(3): 287-292.
14. Sengel CT, Haceiek G, Gould N, et al. Development and In-vitro evaluation of modified release tablets enclosing ethyl cellulose microspheres loaded with diltiazem hydrochloride, Microencapsule, 2005;23(2):135-152.
15. Das MK and Rao KR, et al. Evaluation of Zidovudine encapsulated ethyl cellulose microspheres prepared by water in oil in oil (w/o/w) double emulsion solvent diffusion technique. Acta Pol Pharm. 2006; 63(2):141-148.
16. Zinnuti C, Kedzierszewski F, Hoffman M, Mainent P, et al. preparation and Characterisation of ethyl cellulose microspheres containing 5- fluorouracil. Microencapsule, 1994;11(5):555-563.
17. Sathiya Sundar R, Murugesan A, Venkatesan P, Manavalan R, et al. Formulation Development and Evaluation of Carprofen Microspheres. Int J Pharm Tech Research. 2010;2(3):1674-1676.
18. Venkatesan P, Manavalan R, Vallippan K, et al. Microencapsulation: A Vital Technique In Novel Drug Delivery Systems. J Pharm Sci & Res. 2009;1(4):26-36.
19. Trivedi P, Verma AML, Garud N, et al. Preparation and Characterization of Acclofenac Microspheres. Asian Journal of pharmaceutics. 2008;2(2): 110-115.
20. Chowdery KPR, Vijaya Ratna J, et al.20. Preparation and evaluation of cellulose acetate microcapsules of diclofenac for sustained release. The Indian Drugs. 1992; 29 : 491.
21. Lachman, Liberman, Kaing “Theory and practice of Industrial Pharmacy”, 3rd ed.Varghese Publishing House; Bombay, India, 1987
22. Rastogi R, Sultana Y, Agil M, Ali A, Kumar S, Chuttani K, Mishra AK, et al. Alginate microspheres of Isonizaid for oral sustained drug delivery. Int J Pharm Sci & Res. 2009;1(4):26-36.
23. Perumal D, Microencapsulation of ibuprofen & Endragit RS 100 by the emulsion solvent diffusion technique, Int. J.Pharrn.2001;210:1-11.
24. Sanghvi SP, Naik JG, et al. Effect of viscosity and interfacial tension on particle size of cellulose acetate trimellitate microspheres. J Microencapsulation.1992(9(2):215-227.