



FORMULATION, EVALUATION AND OPTIMIZATION OF SUSTAINED RELEASE MICROCAPSULES OF LORNOXICAM PREPARED WITH *GUM DIKAMALI* & PECTIN EXTRACTED FROM *DILLENIA INDICA*

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ABSTRACT

The present research work was focused on the development of microcapsules using natural polymers like *gum dikamali* and pectin extracted from *Dillenia indica* by employing ionotropic gelation method with Lornoxicam as a model drug. The microcapsules were prepared with combination of different concentration of gum and pectin and the formulations were optimized on the basis of drug release up to 24 hrs. The drug and polymers were subjected for compatibility studies using FTIR & DSC. The formulated microcapsules were characterized for particle size, percentage drug entrapment efficiency, surface morphology, mucoadhesivity and *in vitro* drug release study. The microcapsules exhibited good mucoadhesive property in the *in vitro* wash-off test and also showed high percentage drug entrapment efficiency. The optical microscopic studies revealed that mean particle size of all the formulations was found in the range of 912.16 ± 16.33 to $998.14.12 \mu\text{m}$ and percentage of drug entrapment was between 39.28% - 88.92%. The study has revealed that natural materials can be used for formulation of controlled release microcapsules and will provide ample opportunities for further study.

Key words: Lornoxicam, *Gum dikamali*, *Dillenia indica*, Pectin, Ionotropic gelation method, Microcapsules.

INTRODUCTION

Lornoxicam, a congener of tenoxicam, is a new NSAID belonging to the oxicam class. It is a strong analgesic and antiinflammatory properties, which belongs to biopharmaceutical class II (Low solubility and high permeability). The bioavailability of

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Lornoxicam is 90% – 100%. Because of its relatively short plasma half-life (3-5 hr), it is prescribed to take lornoxicam in divided daily doses either twice or thrice daily in order to maintain the therapeutic plasma concentration. These characteristics make lornoxicam a suitable candidate for developing into sustained release microcapsules¹. In the present study, it is aimed to formulate, evaluate & optimize the sustained release microcapsules prepared with hydrophilic polymers like *gum dikamali* & pectin extracted from *Dillenia indica*. *Gum dikamali* is *Gardenia gummifera* belonging to the family Rubiaceae, are medium sized trees growing all over India. The gum-resin oozing out from the leaf buds of these trees is called Dikamali. *D. indica* is commonly known as Elephant-apple, Indian catmon in English, *Ou tenga* in Assamese, *Lisora* in Hindi and *Bahubar* in Sanskrit². Extracts of *D. indica* contain mainly pectous polysaccharides, which can be extracted by precipitation with acetone. Pectin is a very promising biopolymer to construct drug carriers for controlled drug delivery because of its gelling, film forming, binding properties, biocompatibility and stability towards acidic media, and non-toxicity. The natural polymer selected for the present study was *Dikamali*. *Dikamali* is a hydrophilic matrix forming agent³. Natural polysaccharides extracted from *D. indica* and *gum dikamali* were used to formulate microcapsules.

EXPERIMENTAL

Materials used

Lornoxicam was obtained from Inventia Health Care Pvt. Ltd. The Girijan Co-operative Corporation Ltd. (Visakhapatnam, India) has supplied Gum Dikamali. Sodium alginate (having a viscosity of 5.5 cps in a 1% w/v aqueous solution at 25 OC), calcium chloride and heavy liquid paraffin were procured from SD Fine Chemicals Pvt. Ltd., Mumbai, India. The fruit of *Dillenia indica* was procured from local market of Balasore (Odisha) and was confirmed by local people. All other chemicals were of analytic grade.

Methods

Extraction of pectin

At first the fruits were collected and were cut into small pieces with knife. The chopped pieces of the fruits were kept in a beaker containing distilled water with ratio 1:1.5. The beaker was then placed on the heating mantle with temp. 60°C for 5-6 hrs. After about 6 hours the slurries were strained through a Buchner funnel and the filtrate was kept in refrigerator in a beaker for overnight for sedimentation. The decanted filtrate was taken out of refrigerator and the supernatant was poured into a clean and dry beaker of 1 L size. The

supernatant was evaporated to 1/5th of its volume by heating mantle at 60°C. The concentrated samples were washed with acetone and dried at 50 to 60°C in a hot air oven for 4 hrs. On drying, the sample became hard and brownish in colour. The powdered samples were passed through sieve No. 120 & were stored in desiccators under sealed conditions for further study⁴.

Preformulation studies

Drug excipient compatibility studies

FTIR Studies⁵

The FTIR-8400S spectrophotometer (Shimadzu, Japan) was used to obtain the infrared spectra of pure drug (Lornoxicam) and physical mixture containing drug and natural polymers. Samples were prepared in KBr (Potassium bromide) disks of 2 mg sample in 200 mg of KBr with the Scanning range was 400-4000 cm⁻¹.

DSC Studies

The DSC thermograms of the pure drug and optimized formulation were obtained using the Perkin Elmer JADE DSC system, to identify any interaction between the components of drug and natural polymers⁶.

Preparation of Lornoxicam microcapsules

Aqueous dispersion of Pectin, Dikamali & Sodium alginate was prepared and was kept for overnight. Appropriate amount of Lornoxicam (2:1 polymer: drug) was dispersed in the above dispersion until a uniform dispersion was obtained. The bubble free dispersion was added drop wise, through a disposable syringe (nozzle of 1.0 mm inner diameter) to a 200 mL of a gently agitated solution of the cross linking agent (CaCl₂) with different concentration i.e. 2%, 4% & 6% at room temperature separately as shown in Table 1. The distance of falling of the drops was 5 cm. The gelled particles thus formed were allowed to remain in the cross linking solution up to time period of 15 mins. The particles were subsequently washed with purified water, in order to remove Cl⁻ ions and separated by filtration. The particles were air dried for 24 hrs and stored in a desiccator at room temperature. Similarly Dikamali-pectin microcapsules were prepared without using sodium alginate by the above gelation technique⁷.

UV Spectrum analysis of Lornoxicam

The solution (pH 1.2 & pH 6.8) was scanned in the range of 200 to 400 nm to fix the maximum wavelength and UV spectrum was obtained by using UV Spectrophotometer⁸.

Characterization of microcapsules

Percentage yield⁹

Percent yield of microspheres was determined by the amount of material recovered in the form of microspheres in relation to total amount of particulate matter taken in the form of drug and polymers by using following Eq. –

$$\text{Percent yield} = \frac{\text{Microspheres recovered (mg)}}{\text{Initial amount of drug \& polymers (mg)}} \times 100$$

Entrapment efficiency¹⁰

The drug entrapment efficiency of microcapsules was estimated by dispersing the microspheres in 100 mL of phosphate buffer at 6.8 by vigorous shaking on mechanical shaker (Remi Motors, Mumbai) for 12 hr. Then, the solution was filtered, and the Lornoxicam content was assayed by an UV spectrophotometer. The entrapment efficiency of microcapsules was calculated using the following formula.

$$\text{Entrapment efficiency} = \frac{\text{Estimated percentage drug loading}}{\text{Theoretical percentage drug loading}} \times 100$$

Particle size

The particle size of the microspheres was determined by using optical microscopy method. Approximately 50 microspheres were counted for particle size using a calibrated optical microscope¹¹.

Swelling studies

The swelling studies of uncoated beads were performed in aqueous swelling media with pH 6.8 buffer at $37.5 \pm 0.5^\circ\text{C}$. The swelling ratio, S_{wt} , was calculated from the following expression.

$$S_{wt} = [(W_t - W_0) / W_0] 100$$

Where W_t and W_0 are weight of sample swollen at time t (10 hrs) and weight of the original sample, respectively^{10,11}.

Wash-off test for microcapsules

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test. A 1 cm by 1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch by

1 inch) using thread. Microcapsules (50) were spread onto the wet rinsed tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus is operated such that the tissue specimen was given regular up and down movements in beaker containing the phosphate buffer (pH 6.8). At the end 10th hrs, the number of microspheres still adhering onto the tissue was counted¹².

***In vitro* release study**

In vitro dissolution of all the formulations was conducted by using USP Dissolution apparatus I. Microspheres equivalent to 16 mg of Lornoxicam were filled in the capsules of size 2. Filled capsules were placed in the baskets of dissolution apparatus. The dissolution media used were 0.1 N HCl for first 2 hr and phosphate buffer pH 6.8 for remaining time. The temperature was maintained at $37 \pm 0.5^\circ\text{C}$ and rotation was set at 50 rpm. 10.0 mL of sample was taken, filtered, and analyzed at 373.6 nm using double beam spectrophotometer to get percentage drug release versus time profile. Fresh medium was replaced for each sample taken out of the apparatus

RESULTS AND DISCUSSION

Calibration curve of Lornoxicam

The λ_{max} of Lornoxicam at 10 $\mu\text{g}/\text{mL}$ concentration was found to be 373.6 nm. The standard calibration curve was found to be linear in the range of 2-14 $\mu\text{g}/\text{mL}$.

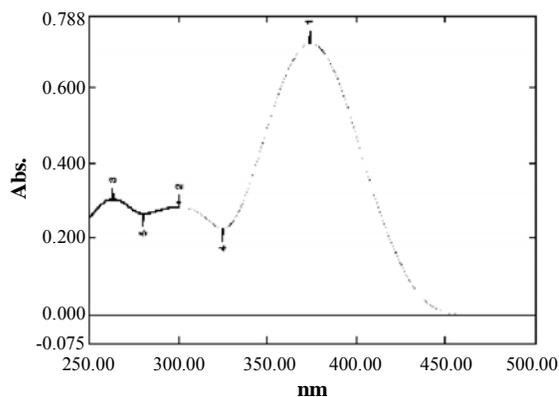


Fig: 1 (a): Spectrum in 0.1 N HCl

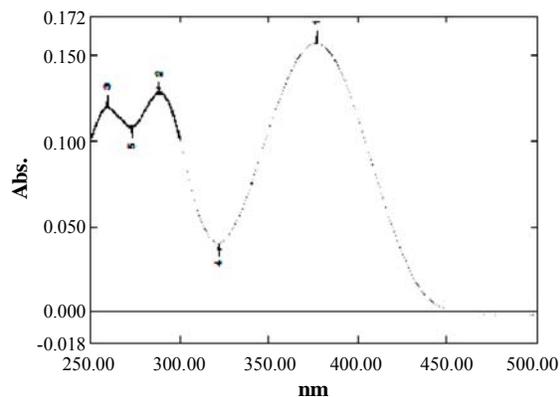


Fig: 1(b): Spectrum in pH-6.8 phosphate buffer

FT-IR studies

The FT-IR spectra of optimized formulation was compared with the FT-IR spectrum of pure drug. The pure drug Lornoxicam exhibited its characteristic absorption bands at 3099.61 cm^{-1} due to aromatic NH stretching and at 2924.1 cm^{-1} due to C-H stretching of CH_3 group. The other prominent absorption bands appeared at 1642.5 cm^{-1} due to stretching vibrations of C=O of CONH, 1546.4 due to aromatic C=C and C=N stretching. The peaks at 1425.8 cm^{-1} and 1331.7 cm^{-1} corresponds to O=S=O group, 769.3 cm^{-1} due to C-Cl stretching vibrations. The IR spectra of optimized formulation exhibited all the characteristic absorption bands as that of pure drug Lornoxicam without any significant variations. It is clear from the spectra that, the drug has not undergone any kind of interactions with the excipients used and retained its identity in its formulation.

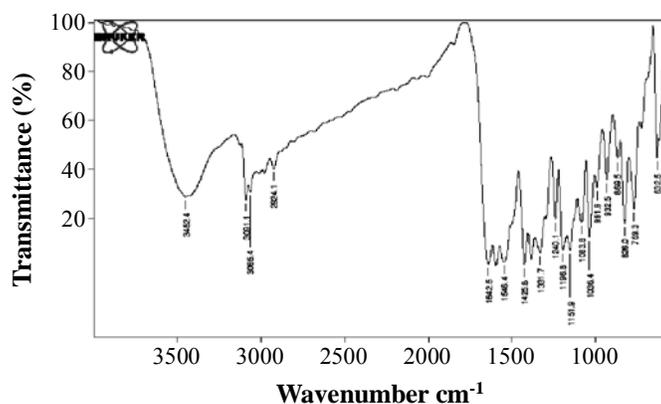


Fig. 2 (a): FT-IR spectrum of pure Lornoxicam

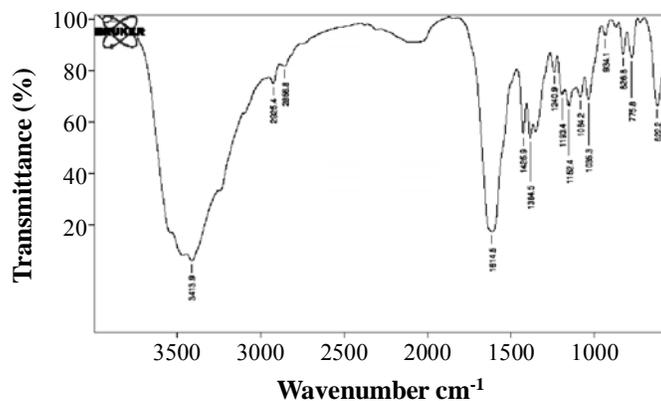


Fig. 2 (b): FT-IR spectrum of optimized formulation

DSC Studies

Differential scanning calorimetric analysis was also performed in order to establish the identity and integrity of drug in its pure form and also in the optimized formulation. The thermogram of the pure drug Lornoxicam showed an exothermic peak exhibiting its sharp melting point at 234.81°C and thermogram of optimized formulation showed exothermic peak at 224.77°C, indicating the absence of interaction between the drug and the excipients.

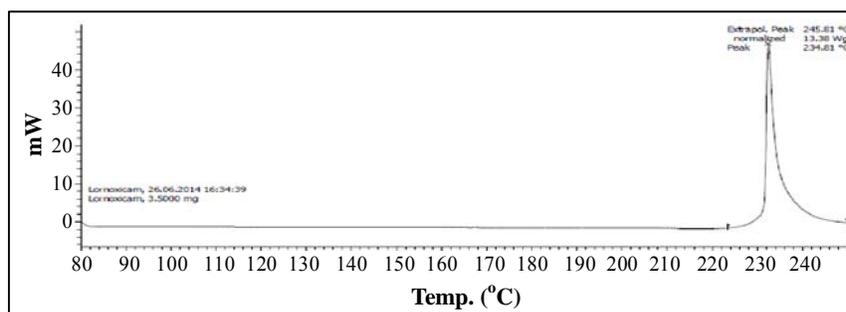


Fig. 3(a) DSC of pure Lornoxicam

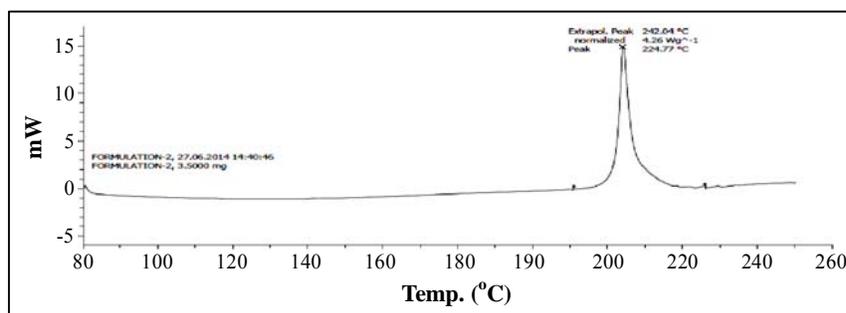


Fig. 3 (b): DSC of optimized formulation

Particle size analysis

Optical microscopy

The mean particle size of all the formulations was found to be in the range of 998.10 ± 14.12 to 912.16 ± 16.33 μm showed in (Table 1), and it was observed that the size increased with the increase in the concentration of *gum dikamali* and pectin in different ratio.

Yield of Lornoxicam microcapsules

The percentage yields of all the formulations were calculated and the yield was found to be in the range of 68.1-82.42% showed in (Table 1).

Drug entrapment efficiency

The percentage drug entrapment efficiency of microcapsules in all the formulations was found to be in the range of 39.28 ± 1.58 - $88.92 \pm 1.37\%$. The formulation LX-13, which showed maximum drug entrapment efficiency of $88.92 \pm 1.37\%$.

Table 1: Particle size, yield, entrapment efficiency, swelling studies & % mucoadhesion of lornoxicam loaded microcapsules

| Formulation code | Particle size (μm) | Yield (%) | Drug entrapment efficiency (%) | Swelling studies | % Mucoadhesion |
|------------------|---------------------------------|-----------|--------------------------------|------------------|------------------|
| LXM 1 | Irregular | - | - | - | - |
| LXM 2 | Irregular | - | - | - | - |
| LXM 3 | 912.1 ± 16.33 | 68.2 | 55.2 ± 1.2 | 32.6 | 41.4 ± 0.5 |
| LXM 4 | 902.7 ± 12.22 | 72.7 | 61.9 ± 0.89 | 32.91 | 41.6 ± 0.53 |
| LXM 5 | 918.7 ± 14.1 | 72.1 | 61.4 ± 1.21 | 35.71 | 42.3 ± 0.44 |
| LXM 6 | 922.6 ± 10.91 | 82.6 | 78.9 ± 2.10 | 79.7 | 55.2 ± 0.21 |
| LXM 7 | 927.2 ± 11.2 | 91.2 | 90.1 ± 0.89 | 80.1 | 56.3 ± 0.5 |
| LXM 8 | 920.4 ± 13.76 | 90.7 | 89.6 ± 0.54 | 79.9 | 55.9 ± 0.25 |
| LXM 9 | 911.9 ± 12.8 | 89.7 | 85.7 ± 0.21 | 69.24 | 45.3 ± 0.5 |
| LXM 10 | 926.9 ± 9.61 | 92.7 | 91.9 ± 0.54 | 86.31 | 54.7 ± 0.21 |
| LXM 11 | 919.8 ± 11.23 | 90.1 | 87.3 ± 0.11 | 76.7 | 47.32 ± 0.15 |
| LXM 12 | 929.7 ± 14.72 | 93.6 | 87.8 ± 1.01 | 88.72 | 58.82 ± 0.11 |
| LXM 13 | 944.0 ± 8.21 | 96.9 | 97.3 ± 2.21 | 89.1 | 59.2 ± 0.48 |
| LXM 14 | 949.6 ± 7.98 | 96.1 | 92.4 ± 0.53 | 88.9 | 58.9 ± 0.33 |
| LXM 15 | 946.2 ± 10.61 | 95.9 | 90.7 ± 1.02 | 91.34 | 56.32 ± 0.18 |

In vitro drug release

The *in vitro* drug release studies revealed that the formulation LX 13 showed a slow and sustained drug release of 100.2% at the end of 24 hr. The results indicated that increasing the concentration of pectin & dikamali with respect to the drug resulted in decreasing the drug release. The optimized formulation (LX 13) was compared with marketed formulation Lofecam SR (16 mg) showed almost same drug release.

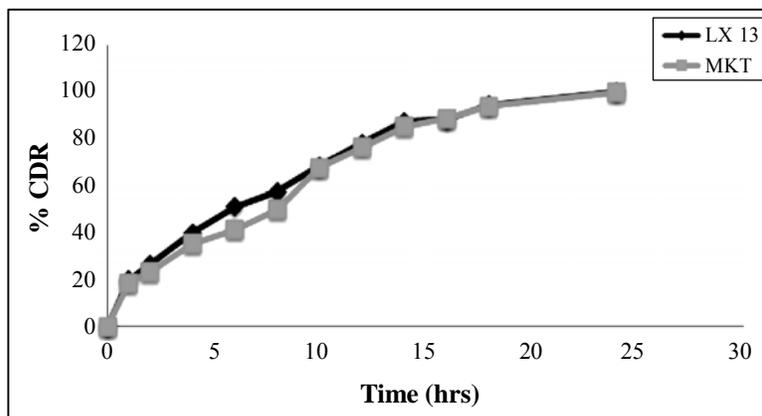


Fig. 4: % Cumulative drug release with marketed formulation

CONCLUSION

The formulation LXM 13 containing drug: polymer ratio 1:2 was found to be the best formulation prepared by orifice ionic gelation technique. Regarding all properties evaluated in order to achieve objective of this study the novel formulation design facilitated the optimization and successful development of Lornoxicam microcapsules. Lornoxicam release from the mucoadhesive microcapsules was slow and extended over longer periods depending on composition of the polymers. These mucoadhesive microcapsules are thus, suitable for oral controlled release of Lornoxicam.

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REFERENCES

1. A. Mahmood, A. Iqbal and R. M. Sarfraz, Fabrication and Optimization of Novel Lornoxicam Matrix Tablets Using 3-Factor 3-Level Box-Behnken Statistical Design: *In vitro* and *In vivo* Evaluation, Indian J. Pharm. Biol. Res., **2(2)**, 87-98 (2014).
2. T. Aparuba and T. Nayan, *Dillenia Indica* (Outenga) as Anti-Diabetic Herb Found In Assam: A Review, Int. J. Ph. Sci. Res., **3(8)**, 2482-2486 (2012).

3. M. Sunitha and A. Navatha, Formulation and Evaluation of Zidovudine Matrix Tablets using Dikamali, *Int. J. Pharm Bio.*, **4(3)**, 581-590 (2013).
4. S. Hemant and L. K. Nath, Extraction, Characterisation and Compatibility Study of Polysaccharides from *Dillenia Indica* and *Abelmoschus Esculentus* with Metformin Hydrochloride for Development of Drug Delivery System, *Int. J. Pharm. Tech. Res.*, **5(1)**, 275-283 (2013).
5. S. S. Patil and V. R. M. Gupta, Formulation and Characterization of tpp Cross-linked Chitosan Microspheres Loaded with Lornoxicam, *J. Biomed. Pharmaceut. Res.*, **3(3)**, 51-58 (2014).
6. A. L. Patil and S. S. Patil, Modification of Physicochemical Characteristics of Lornoxicam using Cyclodextrin as Modulator, *Int. J. Pharm. Tech. Res.*, **4(1)**, 201-207 (2012).
7. K. Ramteke and L. K. Nath, Formulation, Evaluation and Optimization of Pectin-Bora Rice Beads for Colon Targeted Drug Delivery System, *Advanced Pharmaceutical Bulletin*, **4(2)**, 167-177 (2014).
8. A. S. Tapkir, H. Bhimashankar and B. Shailaja, Development and Validation of a Stability Indicating Assay Method of Lornoxicam by using UV-Visible Spectrophotometer, *Int. J. Pharmaceut. Chem. Sci.*, **2(1)**, 468-476 (2013).
9. T. Buranachai, N. Praphairaksit and N. Muangsin, Chitosan/Polyethylene Glycol Beads Cross Linked with Tripolyphosphate and Glutaraldehyde for Gastrointestinal Drug Delivery, *AAPS Pharm. Sci. Tech.*, **11(3)**, 1128-1137 (2010).
10. S. Srinatha, J. K. Pandit and S. Singh, Ionic Cross-linked Chitosan Beads for Extended Release of Ciprofloxacin: *In Vitro* Characterization, *Indian J. Pharm. Sci.*, **70(1)**, 16-21 (2008).
11. D. Kaushik, S. Sardana and D. Mishra, *In vitro* Characterization and Cytotoxicity Analysis of 5-Fluorouracil Loaded Chitosan Microspheres for Targeting Colon Cancer, *Indian J. Pharm. Edu. Res.*, **44(3)**, 274-282 (2010).
12. A. Senthil, T. Sivakumar and D. A. Jain, Formulation and Evaluation of Mucoadhesive Glipizide Microspheres using Carboxy Methyl Cellulose, *Int. J. Preclinical Pharmaceut. Res.*, **2(1)**, 45-51 (2011).

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