DOI https://doi.org/10.5281/zenodo.10049652#28

TWIST, 2023, Vol. 18, Issue 4, pp. 76-79

TWIST

Journal homepage: www.twistjournal.net

Fabrication of *Tamarindus indica* Seed and *Basella alba* Seed Mucilage-Based Microspheres for Ocular Delivery of Ketorolac Tromethamine: HET-CAM Test

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Abstract
The present study was initiated with aim to fabricate ketorolac tromethamine loaded *Tamarindus indica* seed and *Basella alba* seed mucilage based alginate microspheres for ocular drug delivery. The ionotropic gelation method was used for preparation of drug loaded microspheres. The formulated mucilage-alginate based microspheres showed acceptable particle diameter and good stability as predicted from surface charge. The *Tamarindus indica* and *Basella alba* mucilage based microspheres revealed better swelling and mucoadhesive potential compared to both *Tamarindus indica* as well as *Basella alba* mucilage microspheres. All three formulation showed slow drug release for 12 hours in simulated tear fluid. In addition to this, the hen egg chorioallantoic membrane test revealed minimum irritation potential of formulation. Thus mucilage obtained from seeds of *Tamarindus indica* and *Basella alba* could be promising alternative for preparation of drug loaded microspheres.

Keywords
Tamarindus indica, *Basella alba*, Ketorolac, Ocular microspheres

INTRODUCTION

The controlled mucoadhesive drug delivery is recently investigated approach for effective as well as prolonged delivery of medicaments (Hou et al., 2014). The micropolymeric carrier particles with mucoadhesion potential have been explored for efficient drug delivery by many formulation experts. The mucoadhesion potential of various natural mucilages have been investigated by many scientist for prolonged delivery of drugs. Akin-Ajani et al. (2022) have recently utilized *Talinum triangulare* leaves mucilage for controlled delivery of ibuprofen. Ghumman et al. (2022) have fabricated cefixime loaded Quince seeds mucilage- sodium alginate microspheres for sustained oral drug delivery. Kurra et al. (2022) formulated jackfruit mucilage based microspheres of curcumin. Ghumman et al. (2019) utilized Taro corn mucilage for fabrication of pregabalin loaded microbeads. However, none of the investigator have attempted to fabricate *Basella alba* seed mucilage (BASM) based microspheres. Thus, present study was planned to formulate ketorolac tromethamine loaded *Tamarindus indica* seed mucilage (TSM) and *Basella alba* seed mucilage (BASM) based microspheres.

MATERIALS AND METHODS

Materials

KTM was kindly gifted by Saymed Laboratories, India. Sodium alginate and sodium hydroxide were purchased from SDFC Ltd, India. Spinach and tamarind seeds were purchased locally. All other solvents, reagents and chemicals were obtained locally.

Extraction of BASM and TSM

The BASM was extracted according to method mentioned by Sandaruwan et al. (2022). TSM was extracted using tamarind kernel powder according to technique mentioned by Pal and Nayak (2012).

Design of ketorolac tromethamine loaded microspheres

The ionotropic gelation technique was utilized for fabrication of KTM loaded BASM TSM-alginate microspheres (Nayak et al., 2013). Briefly, aqueous dispersions of BASM: TSM (1:1) and alginate were separately prepared and mixed with the aid of stirring. The KTM was added to the resulting mixture. The drug to polymer ratio was selected as 1:1. The resulting medicated mucilaginous solution was injected in $CaCl_2$ solution through needle at the rate of 1 ml/min and stirred for 20 minutes. At last, microspheres were filtered, washed with double distilled water and dried at 40ºC for 10 hours. Similarly KTM loaded BASM microspheres and TSM microspheres were formulated separately.

Evaluation of formulated microspheres

Particle diameter and surface charge of formulated KTM loaded microspheres were assessed using Zetasizer Nano ZS (Malvern instruments, UK). The percent entrapment of KTM in microsphere matrix was assessed according to technique reported by Pal and Nayak (2012). The KTM release *in vitro* in simulated tear fluid was assessed using specially fabricated cylindrical polyvinyl chloride tube (length: 5 cm & diameter: 1 cm) according to technique reported by Rajawat et al. (2016). Mucoadhesion ability on goat intestinal mucosa and swelling potential were assessed according to method reported by Rajawat et al. (2016). The ocular irritation of formulated microspheres was assessed using HET-CAM test according to method reported by McKenzie et al. (2015).

RESULTS

Evaluation of microspheres

The BASM and TSM microspheres showed 5217 ± 317 nm and 3847 ± 382 nm diameter respectively. Whereas BASM-TSM microspheres showed mean particle diameter of 3455 ± 266 nm as highlighted in **Fig. 1.** All three formulations showed zeta potential in the range of +29.17 to +32.7. KTM entrapment was in the range of 69.84 to 76.17 %.

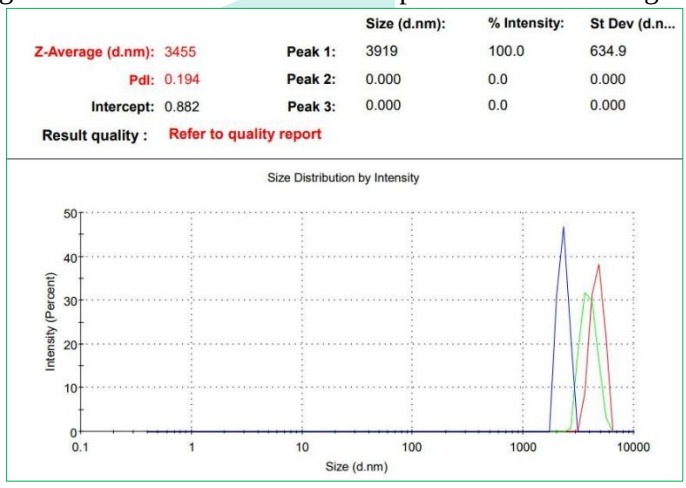

Fig. 1 Particle size distribution curve of BASM TSM microspheres

KTM showed initial burst release from all three microspheres in simulated fluid and then sustained release was observed for next 12 hours (Fig. 2.). BASM microspheres and TSM microspheres revealed 39% and 37% of KTM release respectively in first one hour. Whereas, BASM-TSM microspheres released 30% of encapsulated KTM in 1 hour. At end of 12 hour the percent cumulative release of KTM from BASM and TSM microspheres were 89% and 85% respectively. Whereas SSM-TSM microspheres showed 82% of drug release at the end of 12 hours.

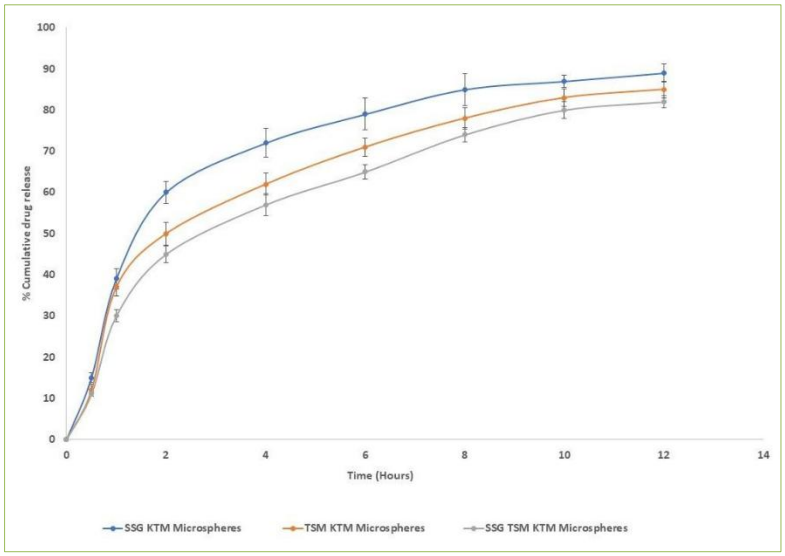

Fig. 2 *In vitro* release behavior of formulated microspheres

The microspheres exhibited swelling in first 20 minutes. The swelling index of 1.93 and 1.47 was observed for BASM and TSM microspheres respectively. The BASM-TSM microsphere revealed 0better swelling behavior with swelling index of 2.18. The percent of mucoadhesion to intestinal mucosa was found to be 62.95±2.63 % and 75.17±3.41 % for BASM and TSM microspheres respectively. However, BASM-TSM microspheres exhibited 83.74±2.61 % mucoadhesion (Table 1). The result was found to be significantly better ($p<0.05$) compared to both BASM and TSM microspheres. The irritation potential of developed microspheres was assessed using HET-CAM test. The 300 µL of isotonic solution produced intact blood vessels with no haemorrhage (Fig. 3A). In contrast, 1 M NaOH showed instant haemorrhage and coagulation (Fig. 3E). The BASM microspheres, TSM microspheres and BASM TSM microspheres (Fig. 3B, 3C and 3D) showed intact blood vessels with absence of haemorrhage for five minutes. Thus all three microspheres based systems are found to have minimum irritation potential to ocular tissues.

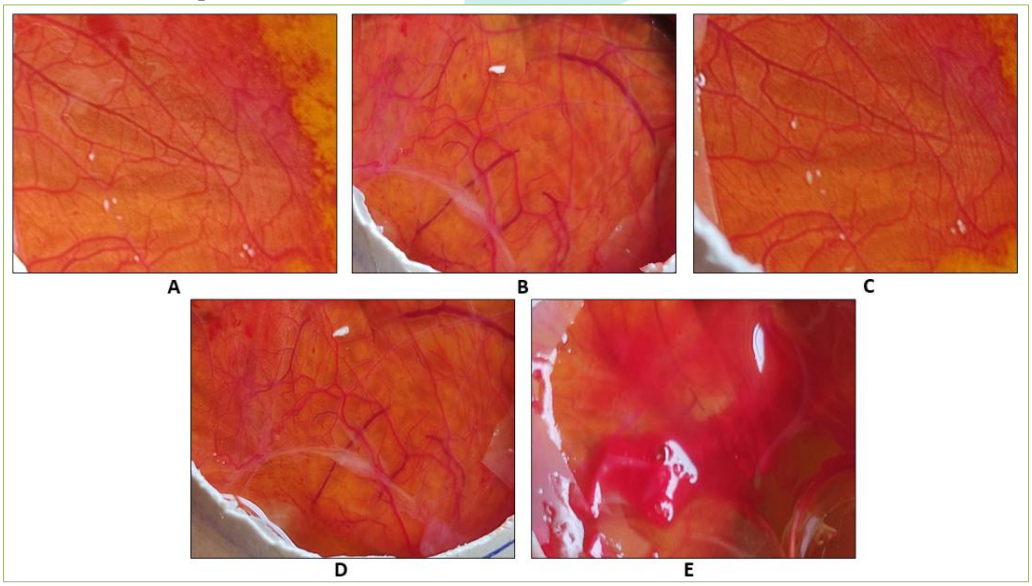

Fig. 3 HET-CAM images on treatment with A. Isotonic solution, B. BASM microspheres, C. TSM microspheres, BASM TSM microspheres and E. 1M NaOH

Table 1 Overview of evaluation parameters of formulated microspheres (n=3)

Microsphere	Evaluation parameters				
	Particle size (nm)	Zeta potential (mV)	Entrapment (%)	Swelling index	Mucoadhesion (%)
BASM-KTM	5217±317	+31.81±2.71	69.17±1.64	1.93	62.95±2.63
TSM-KTM	3847±382	+29.17±9.4	71.84±2.74	1.47	75.17±3.41
BASM TSM-KTM	3455±266	+32.7±6.4	75.93±1.65	2.18	83.74±2.61

DISCUSSION

Ketorolac tromethamine is anti-inflammatory drug mainly administered in ocular cul-de-sac for management of pain and swelling after cataract surgery. Conventionally dug is administer in the form of eye drop. However, nasolacrimal drainage and poor precorneal residence time are major hurdles for effective ocular delivery of drugs. Thus there stringent need to develop the drug delivery system which can prolong the precorneal residence time of drug. The mucoadhesive

microspheres are promising carriers for ocular delivery of drugs. Thus in present study, the natural mucilage based microspheres were utilized for effective ocular delivery drugs. The formulated microspheres showed acceptable particle diameter, which reflects its suitability for ocular administration. The positive zeta potential confirmed physical stability of microspheres. The combination of BASM and TSM revealed better swelling and mucoadhesion potentials compare to individual mucilage based microspheres. The results could be due to crosslinking of two mucilages in microsphere matrix. In addition to this, the HET-CAM test revealed minimal ocular irritation potential of formulated microspheres. Thus combination of two natural mucilages i.e. BASM and TSM could be viable alternative for formation of drug loaded microspheres.

CONCLUSION

The present landmark investigation has proved use of BASM and TSM for formulation of KTM loaded microspheres. The BASM TSM coloaded microspheres exhibited better physicochemical characteristics, swelling index, mucoadhesive potential and minimal irritation potential. Thus combination of BASM and TSM could be promising mucoadhesive carrier for ocular delivery of KTM.

DECLARATION OF CONFLICT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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