

Study on effect of combination of sodium alginate and xanthan gum on drug release from Tacrolimus microbeads

Mullaicharam Bhupathyraaj*, Sushama Pole.

College of Pharmacy, National University of Science and Technology, Muscat, Sultanate of Oman.

For Correspondence:

Prof. Dr. Mullaicharam Bhupathyraaj,

mullaicharam@nu.edu.om

00968-91021307

Abstract

Tacrolimus micro beads are promising pharmaceutical dosage forms by providing sustained release drug delivery systems. The drug Tacrolimus has low bioavailability, hence to improve its bioavailability. Tacrolimus loaded microbeads were prepared by Ionotropic gelation and cross linking technique by using sodium alginate as the hydrophilic carrier in combination with xanthan gum as release modifier. Micro beads with different ratio of sodium alginate and xanthan gum were formulated and evaluated for percentage yield and Percentage of drug entrapment efficacy. An optimized batch was selected from the previous stages and four batches with same concentrations of Tacrolimus and different concentration of calcium chloride and stirring time were loaded. The drug loaded batches were evaluated for percentage yield, drug entrapment efficiency, in vitro release, and release kinetics. Particle size distribution of beads was measured by SEM. No significant drug-polymer interactions were observed in FT-IR studies. In-vitro drug release profile of Tacrolimus micro beads in phosphate buffer pH 6.8 exhibited zero order release with kinetics of super case II-transport. The sustained release effect of microbeads depends on the polymer concentration and type of polymer used in the formulation. Hence the formulated Microbeads of sodium alginate with xanthan gum as release modifiers could be used as an alternative and cost effective carrier.

Keywords: *Microbeads, Tacrolimus, Sodium Alginate, Xanthan Gum, Ionic Gelation*

Introduction:

The goal in designing delayed release sustained or controlled delivery system is to reduce the frequency of dosing or to increase the effectiveness of the drug by localization at the site of action, reducing the dose required, or providing uniform drug delivery (Rajininath et al.2003).Sustained release, sustained action, prolonged action, extended action are the terms used to identify drug delivery system that are designed to achieve a prolong therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose (H.C.Kiran et al .2019). The design of effective drug delivery systems has recently become an integral part of the development of new medicines. Microparticulate drug delivery

systems have various well-known advantages over single unit dosage form. Multiple unit dosage forms such as microspheres or micro beads have gained in popularity as oral drug delivery systems because of more uniform distribution of the drug in the gastrointestinal tract, more uniform drug absorption, reduced local irritation and elimination of unwanted intestinal retention of polymeric material, when compared to non-disintegrating single unit dosage form (Singh et al. 2013). Microbeads are nearly spherical, small with diameter of 0.5- 1000 μm . The solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form allow a sustained release or multiple release profiles of treatment with various active agents without major side effects (Pavan Kumar et al 2011).

The advantages of microbeads are Limited fluctuation within therapeutic range, reduced side effects, less dosing frequency, improved bioavailability and patient compliance.

Research shows that micro beads are prepared by Inotropic Gelation technique to improve bioavailability, and also to reduce dose frequency thereby achieving an oral controlled release of the drug. In future by combining various other strategies, micro beads will find the central and significant place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted, specific and effective in-vivo delivery and supplements as miniature versions of diseased organ and tissues in the body (Shreegiri et al. 2014).

Immunosuppressive drugs are used to dampen the immune response in organ transplantation and autoimmune disease. Tacrolimus (FK 506) is a highly potent immunosuppressive agent and has proven activity in both in-vivo and in-vitro experiments, also calcineurin inhibitor. Because of its poor solubility (Sherina et al 2012) . Tacrolimus has a large inter-/intra-patient variability in pharmacokinetics profile.

Tacrolimus is used most frequently in comparison to other immunosuppressant because it offers better safety profile with increased long-term survival in patients (Toatous et al. 2005). Tacrolimus has become an important therapeutic option for the optimal individualization of immunosuppressive therapy especially.

- Tacrolimus has proved to be a promising drug since 19th century especially as an immunosuppressant in organ transplantation and has been explored in different areas of medication but its efficacy and toxicity issues needs to be dealt properly with more precision and accuracy.
- The majority of work has been focused on enhancement of in vitro solubility and absorption.
- There is no doubt that development of new formulations or analogues of tacrolimus with better bioavailability and having low inter/intrasubject variability will be critical for future development of tacrolimus formulation. This will make the use of drug more effective and safe.

Polymers have played an integral role in the advance drug delivery technology by providing controlled release of therapeutic agents in constant doses over long periods, cyclic dosage, and constant release of both hydrophilic and hydrophobic drugs (Mohan et al. 2014). When formulating challenging molecules into solid oral dosage forms, polymeric pharmaceutical excipients permit masking undesired physicochemical properties of drugs and consequently, altering their pharmacokinetic profiles to improve the therapeutic effect. The main role of polymer is to protect drug from physiological environment and prolong release of drug to improve its stability (Badrinath et al. 2010). Sodium alginate micro Beads are one of the multiparticulate drug delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites (Thulasi V.M. et al. 2013).

Xanthan gum has important pharmaceutical and economical advantages i.e. less susceptible to erosion during drug release, higher drug retarding ability, absence of initial burst release. (Rajat et al. 2014) Reported that xanthan gum shows a sustainable drug release pattern to reduce dosing frequency. (Yongzhen et al 2016) suggested that chemical crosslinking with xanthan gum, hydrogels show the stronger elastic

property, tough to resist deformation, good swelling and sustained drug release properties. (Fareez et al 2015) reported that the inclusion of three polymers i.e xantan gum ,sodium alginate shows good miscibility for microencapsulation due to molecular interaction of xantan gum and sodium alginate, which led to the formation of a matrix structure.

Materials and Methods

Materials

Tacrolimus was a sample from concord biotech Ltd, Ahmadabad, India. Sodium alginate, Xanthan gum and calcium chloride were purchased from Muscat,Oman.All other reagents and solvents used were of analytical grade.

Preparation of Placebo Microbeads:

The microbeads were prepared by the ionotropic gelation technique. Microbeads were prepared by using sodium alginate alone with the calcium chloride used as counter ion. 25 ml of a 2% w/v aqueous solution of sodium alginate was introduced drop wise from a glass syringe with a size-23G syring with needle into 100 ml of an aqueous calcium chloride solution being stirred at 100 rpm. The concentration of CaCl₂ in the solution should be 2%w/ allowed the beads to be formed by running the stirrer for 15 min. checked the beads under microscope. After stirring for 15 min, filter the solution and collect the beads. Obtained microbeads were washed with water and dried at room temperature for 24 hrs. Total three sets of placebo microbeads were prepared for the selection of best concentration of polymer solution by using sodium alginate and calcium chloride used as counter ion. Composition data of placebo microbeads given in table 1.

Table 1: Composition of Placebo microbeads.

Formulation code	Sodium Alginate	Calcium chloride	Curing Time
P1	2%	2%	15 min
P2	1.5%	2%	15 min
P3	1%	2%	15 min

Preparation of formulation drug with Alginate–Xanthan Gum Microbeads:

Study on effect of different concentration of Xanthan gum as a release modifier on sodium alginate microbeads: three batches of microbeads were prepared with same concentration of drug Tacrolimus by using sodium alginate and Xanthan Gum as coating polymer. To 25 ml of deionized water, different percentages of sodium alginate was added and stirred with magnetic stirrer to form uniform dispersion then added tacrolimus and 1ml acetonitrile for good solubility of tacrolimus in the solution after 10 min stirring added different concentration of Xanthan gum in the solution. The resulting dispersion was dropped through syringe with needle slowly into a 50 ml of 2% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer. Allow the beads to be formed by running the stirrer 1 hours. Checked the beads under microscope. After stirring for one hours, filter the solution and collect the beads. Obtained microbeads were washed with water and dried at room temperature for 24 hrs.

Table 2: Composition of various formulations of tacrolimus microbeads containing xanthan gum polymer.

Formulation Code	Drug	Sodium alginate	Xanthan gum	Calcium chloride	Curing Time
F1	5 mg	2%	1%	2%	1 hrs
F2	5 mg	1.5%	1.5%	2%	1 hrs
F3	5 mg	1%	2%	2%	1 hrs

Factorial design (2²) used in the optimization of micro beads formulation

Four optimization batches of factorial design of micro beads were prepared with same concentration of drug with two different factors. The different concentration of calcium chloride and various stirring time were used in selected F1 batch based on the high drug entrapment and delayed drug release properties of previous study, shown in Table 3,4and 5.

Procedure for optimization of micro beads formulations (Batch F1a, F1b,F1c and F1d)

Batch F1a

To 25 ml of deionized water, 2 % of sodium alginate was added and stirred with magnetic stirrer to form uniform dispersion then added 5 mg tacrolimus and 1ml acetonitrile for good solubility of tacrolimus in the solution after 10 min stirring added 1% of xanthan gum in the solution. The resulting dispersion was dropped through syringe with needle slowly into a 50 ml of 2% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer. Allow the beads to be formed by running the stirrer 30 min. After stirring, filter the solution and collect the beads. Obtained micro beads were washed with water and dried at room temperature for 24 hrs.

Batch F1b

To 25 ml of deionized water, 2 % of sodium alginate was added and stirred with magnetic stirrer to form uniform dispersion then added 5 mg tacrolimus and 1ml acetonitrile for good solubility of tacrolimus in the solution after 10 min stirring added 1% of xanthan gum in the solution. The resulting dispersion was dropped through syringe with needle slowly into a 50 ml of 2% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer. Allow the beads to be formed by running the stirrer 1 hr. After stirring for one hours, filter the solution and collect the beads. Obtained microbeads were washed with water and dried at room temperature for 24 hrs.

BatchF1c

To 25 ml of deionized water, 2 % of sodium alginate was added and stirred with magnetic stirrer to form uniform dispersion then added 5 mg tacrolimus and 1ml acetonitrile for good solubility of tacrolimus in the solution after 10 min stirring added 1%of xanthan gum in the solution. The resulting dispersion was dropped through syringe with needle slowly into a 50 ml of 4% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer.

Allow the beads to be formed by running the stirrer 30 min .After stirring, filter the solution and collect the beads. Obtained microbeads were washed with water and dried at room temperature for 24 hrs.

BatchF1d

To 25 ml of deionized water, 2 % of sodium alginate was added and stirred with magnetic stirrer to form uniform dispersion then added 5 mg tacrolimus and 1ml acetonitrile for good solubility of tacrolimus in the solution after 10 min stirring added 1% of xanthan gum in the solution. The resulting dispersion was dropped through syringe with needle slowly into a 50 ml of 4% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer. Allow the beads to be formed by running the stirrer 1 hr. After stirring for one hours, filter the solution and collect the beads. Obtained micro beads were washed with water and dried at room temperature for 24 hrs.

Table 3: No. of trials

Batch no.	Factor A	Factor B
F1a	-1	-1
F1b	-1	+1
F1c	+1	-1
F1d	+1	+1

Level	Code	CLA quantity (%)	Stirring Time
Low level	-1	2%	30 min
High level	+1	4%	1 hr

Table 4: Code for level

Factor A: CLA (calcium chloride as a cross linking agent)
Factor B : Stirring time

Table 5: 2 Factorial design used in the optimization of micro beads formulation

Batch no	Composition			Variable Parameters	
	Tacrolimus	Sodium Alginate (w/v)%	Xanthan gum(w/v)%	Calcium chloride (w/v)%	Stirring Time (hrs)
F1a	5 mg	2%	1%	2	30
F1 b	5 mg	2%	1%	2	30

F1c	5 mg	2%	1%	4	1
F1d	5 mg	2%	1%	4	1

Evaluation of placebo and drug loaded microbeads:

1. Yield of production

The yields of production of micro beads of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microbeads and percent production yields were calculated as per the formula mentioned below(UmeshShivdharet al.2013).

$$\text{Percentage yield} = \frac{\text{Mass of microbeads obtained}}{\text{Total weight of drug and polymer used}} \times 100$$

2) Percentage Drug Entrapment Efficacy (%DEE):

Accurately weighed microbeads equivalent to 100mg were triturated in mortal and pestle and added 1 ml acetonitrile for good solubility were suspended in 100ml of simulated intestinal fluid of pH 6.8 and kept for 24hrs. Next day it was stirred for 10 min and filtered. After suitable dissolution, the drug content in the filtrate was analyzed spectrophotometrically at 297nm using UV spectrophotometer (S.Bashir et al 2014). Finally, drug encapsulation efficiency is calculated by-

$$\text{Percentage Drug Entrapment Efficiency} = \frac{\text{Test Absorbance}}{\text{Standard absorbance}} \times 100$$

Scanning Electron Microscopy:

The surface morphology of drug-loaded beads obtained from various percentages of polymers, CaCl₂ and drug were studied by using a scanning electron microscope (Bindu Madhavi et al.2009).

In-vitro Dissolution Study:

Dissolution studies of Tacrolimus microbeads was performed according to USP type I dissolution apparatus in phosphate buffer of pH 6.8 for 24 hrs. The temperature was maintained at 37±0.5°C and the rotation speed was 100 rpm. The 5 ml of sample was withdrawn at various time intervals and replenished with an equal volume of fresh dissolution media(AnurajaKunduet al.2012). The drug content in the sample was analyzed spectrophotometrically at 297nm.

Result and Discussion

The aim of the study is to investigate possibility of using sodium alginate microbeads coated with xanthan gum as drug release modifiers as a sustained release system. We prepared microbeads containing Tacrolimus by ionotropic gelation and cross linking method and examined the effects of various factors (concentration of sodium alginate, concentration of coating polymer xanthan gum and concentration of drug tacrolimus) xanthan gum and sodium alginate were used because of good miscibility in microencapsulation

technique due to molecular interaction of xanthan gum and sodium alginate, which led to the formation of a matrix structure.

Compatibility Study

The compatibility of Tacrolimus with various polymers such as sodium alginate, xanthan gum was investigated by IR-spectroscopy study. The spectra of the drug and polymer combination were compared with the spectra of the pure drug (Smdrel P et al.2008). In which no shifting of peaks was significantly found, indicating the stability of the drug during encapsulation process. The spectra are included as figure 1.

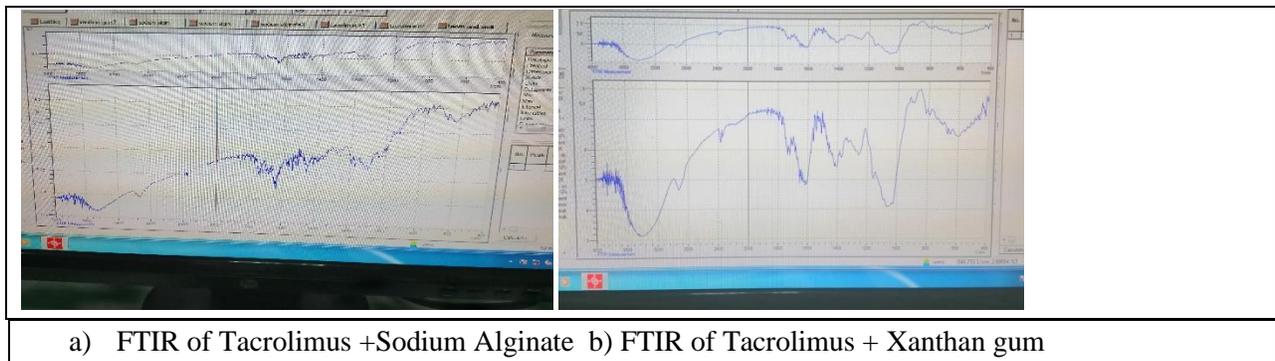


Figure 1: FTIR spectra of Tacrolimus with polymers

Standard calibration curve of tacrolimus:

Calibration curve plotted by concentration (mg/ml) on x-axis vs. absorbance on y-axis

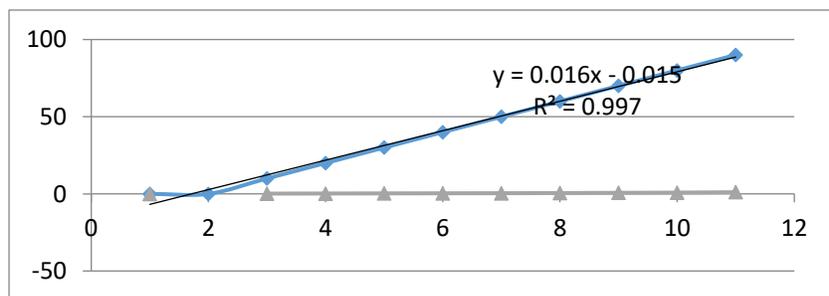


Figure 2. Calibration curve of tacrolimus.

Evaluation of drug loaded microbeads

Percentage yield and drug entrapment efficacy:

Chemical reaction between sodium alginate and calcium chloride to form calcium alginate was utilized for the microencapsulation of tacrolimus core material. For slowing the drug release hydrophilic polymers were added in different concentration so that the drug will release constantly for 24 hrs.by increasing the concentration of sodium alginate, the mean particle size of microbeads increased. From results it can be seen that larger microbeads were obtained by increasing the concentration of sodium alginate. Percentage yield of placebo batches was found to be in range of 68% to 78%, P1 batch with higher 78% yield was selected for further formulation. Percentage yield of drug loaded batches was found to be in range of 69% to 76% . Maximum percentage yield was found to be 76% for F1 formulation is summarized in table 4. Percentage yield increases with increase in concentration of the polymer added to formulation.

The drug entrapment efficacy result was found within 75.12%-77.35% is summarized in table 6. Maximum drug entrapment efficacy found to be 77.35 % for F1 batch. The encapsulation efficiency determines the percentage of encapsulated drug with respect to the total drug introduced into polymer solution.

Table 6: Characteristics of drug loaded microbeads

Formulation Code	%yield	%DEE
F1	76±0.09%	77.35±0.07%
F2	74±0.1%	76.39±0.05%
F3	69±0.12%	75.12±0.1%

Dissolution studies

Dissolution profile of Tacrolimus loaded microbeads using xanthan gum Polymer with different concentration was shown in following figure 3.

The in-vitro drug release study was performed using dissolution test apparatus in phosphate buffer pH 6.8. The in-vitro drug release studies of microbeads (F1 to F3) were observed in the range of 95.06 ±1.09% to 92.6±0.72%. The formulations F1 containing 2% of sodium alginate and 1 % of coating polymers xanthan gum showed a release 92.6±0.72%. This indicates that the release rate is further retarded due to addition of coating polymer and drug release was delayed due to increasing concentration of sodium alginate.

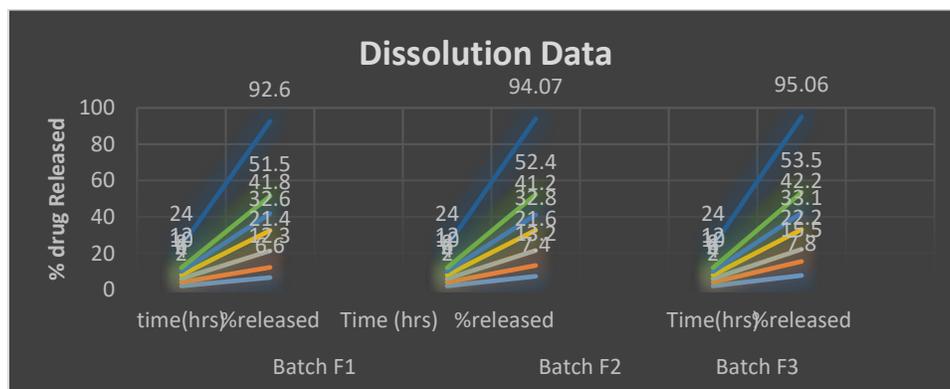


Figure 3: % Cumulative drug release of Tacrolimus loaded microbeads using Xanthan gum Polymer with different concentration batch F1,F2 and F3.

Release kinetic study

All the release data was fitted into various kinetic models like, zero order, first order, Higuchi, and Korsmeyer's Peppas, in order to find out the mechanism of drug release from polymeric spheres. The value of 'n' gives an indication of the release mechanism; when $n = 1$, the release rate is independent of time (zero-order) (case II transport), $n = 0.5$ for Fickian diffusion and when $0.5 < n < 1.0$ super case II transport is apparent. Regression coefficient and 'n' were calculated and is given in table 7. From the table, the value showed that, prepared microbeads exhibited zero order kinetics followed by super case -II transport.

Table 7: Data for release kinetics for formulation microbeads

Formulation code	Zero order (R ²)	Higuchi Matrix (R ²)	Korsmeyer's peppas (R ²)	'n' value
F1	0.9900	0.9991	0.9805	1.042
F2	0.9906	0.9892	0.9732	0.976
F3	0.9920	0.9760	0.9939	1.104

The formulations F1 containing 2% of sodium alginate and 1 % of coating polymers xanthan gum showed a slow release $92.6 \pm 0.72\%$ than other batches. This indicates that the release rate is further retarded due to addition of coating polymer and drug release was delayed due to increasing concentration of sodium alginate. F1 batch was selected for further formulation of optimization batches.

Evaluation of Factorial design batches:

% Yield and drug entrapment efficacy

Percentage yield of factorial design batches was found to be in range of $65.96 \pm 0.12\%$ to $74.80 \pm 0.08\%$ which is shown in table 6. F1c batch shown higher $77.88 \pm 0.12\%$ yield. The drug entrapment efficacy result was found within the range of $72.19 \pm 0.12\%$ to $84.87 \pm 0.1\%$ is summarized in table 8. Maximum drug entrapment efficacy found to be for F1d batch i.e. $84.87 \pm 0.1\%$. The encapsulation efficiency determines the percentage of encapsulated drug with respect to the total drug introduced into polymer solution.

Table 8: Evaluation Data for Optimized batches of microbeads

Formulation Code	%yield	%DEE
F1a	$74.43 \pm 0.09\%$	$74.39 \pm 0.05\%$
F1b	$71.39 \pm 0.06\%$	$76.09 \pm 0.06\%$
F1c	$77.88 \pm 0.12\%$	$72.19 \pm 0.12\%$
F1d	$70.59 \pm 0.04\%$	$84.87 \pm 0.1\%$

Dissolution studies of optimized batches:

The in-vitro drug release study of Optimized batches was performed using dissolution test apparatus in phosphate buffer pH 6.8. The in-vitro drug release studies of microbeads (F1a to F1d) was observed in the range of $89.86 \pm 0.34\%$ to $93.06 \pm 0.66\%$ is shown in figure 4.. The formulations F1d containing 2% of sodium alginate and 1 % of coating polymers xanthan gum and maximum % of cross linking agent i.e. 4% CaCl₂ with higher 1 hr. stirring which shows a slow release $89.86 \pm 0.34\%$ This indicates that the release rate is further retarded due to addition of coating polymer and drug release was delayed due to increasing

concentration of sodium alginate and calcium chloride. The result was indicate that the rate and extent of drug release decreased with increase of concentration of calcium chloride. F1d batch was selected for study of scanning electron microscopy.

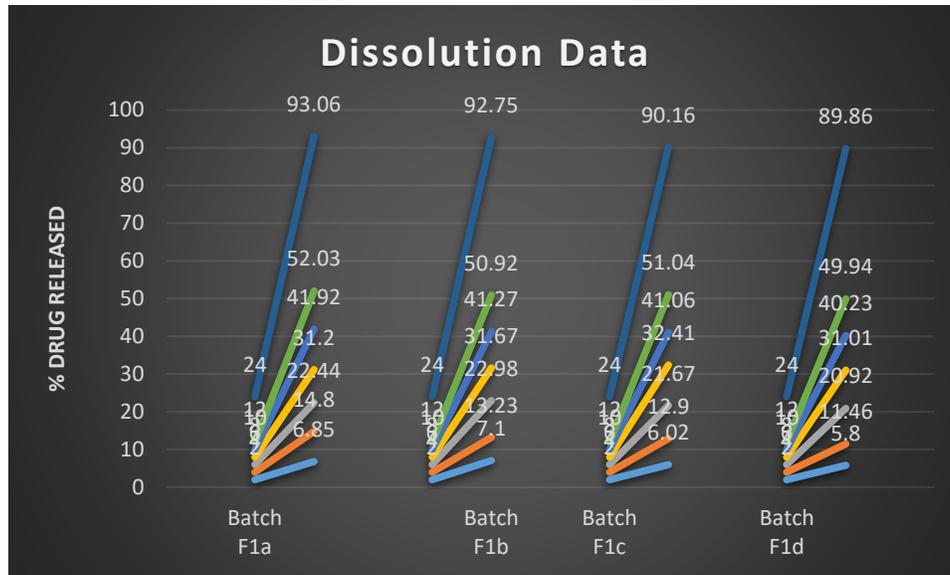


Figure4: % Cumulative drug release of optimizes batches containing tacrolimus loaded microbeads.

Release kinetic study of optimized batches

All the release data was fitted into various kinetic models like, zero order, first order, Higuchi, and Korsmeyer's Peppas, in order to find out the mechanism of drug release from polymeric spheres. Regression coefficient and 'n' were calculated and is given in table 9. From the table, the value showed that, prepared microbeads exhibited zero order kinetics followed by super case -II transport.

Table 9: Data for release kinetics of optimized batches.

Formulation code	Zero order (R ²)	Higuchi Matrix (R ²)	Korsemeyer's peppas (R ²)	'n' value
F1a	0.9904	0.9780	0.9711	1.1800
F1b	0.9941	0.9760	0.9806	1.0994
F1c	0.9890	0.9835	0.9724	1.1568
F1d	0.9910	0.9800	0.9812	1.0912

Scanning electron microscopy study of optimized selected batch.

The morphological evaluation of the optimized micro beads formulation (sodium alginate micro beads coated with xanthan gum) was done by scanning electron microscopy. SEM study revealed that the microbeads were almost spherical in shape with rough outer surface. Tacrolimus loaded microbeads of selected optimization batches were found to be discrete, almost spherical, and of uniform in size. The average size of microbeads for two batches was found to be 1.14 mm to 1.50mm which is under the range.

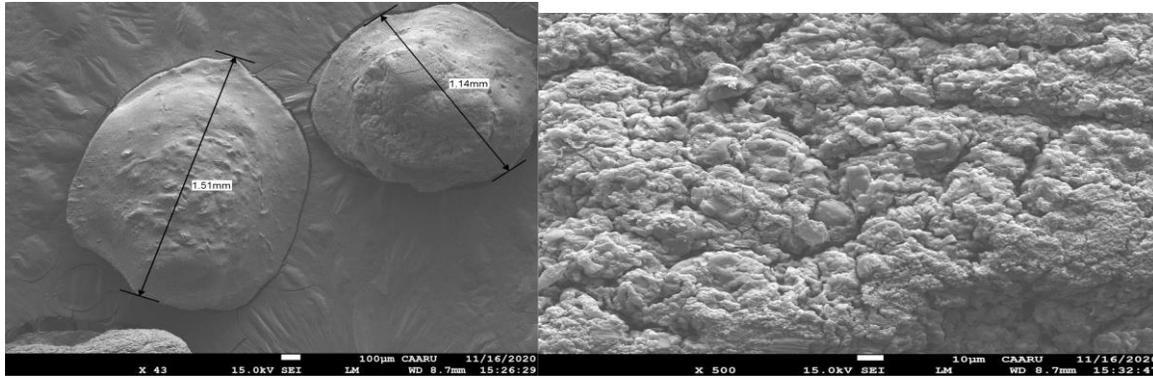


Figure 5: SEM photos of selected optimized batch containing tacrolimus loaded microbeads.

Conclusion:

Tacrolimus microbeads were prepared for the sustained release using the sodium alginate as a major polymer and in later it combined with hydrophilic polymer xanthan gum to improve entrapment and sustained release of the drug from alginates beads by using ionotropic gelation and cross linking technique. It also depends on using different concentration of cross-linking agents to improve drug entrapment efficiency, maximum drug release from the beads at extended period of time. Tacrolimus release from microbeads was influenced by alginate and coating polymer concentration. The microbeads formed have a spherical shape with rough surface as evidenced by SEM. The FTIR Studies didn't reveal any significant drug interactions with polymers. The result of in-vitro study indicated sustained release kinetics and exhibited zero order drug release followed by super case II- transport. It was concluded that tacrolimus micro beads are promising pharmaceutical dosage forms by providing sustained release drug delivery systems and improving bioavailability.

Acknowledgement

This study had been funded by The Research Council, Oman

Funding Support

The authors declare that they have funding support for this study from The Research Council, Oman.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

References

1. Michael E. Aulton, Kevin M. G. Taylor. 2013. Aulton's Pharmaceutics, The design and manufacture of medicines. International Elsevier publication. 4th edition. 465-550
2. Singh C, Purohit S., Singh M, Pandey B.L., 2013. Design and evaluation of microspheres: A Review. Jddr. 2(2): 18-27.
3. Pavan Kumar B, Chandiran I. S., Bhavya B, Sindhuri M. 2011. Microparticulate drug delivery system: A Review. Indian journal of pharmaceutical science & research. 1(1):19-37.
4. Gibaldi M, Parrier D. 1984. Bio pharmaceutics and clinical Pharmacokinetics. Philadelphia: Lea and Febiger, 3rd edition. 15:64-82

5. Smrdel P, Marija Bogataj, Ales Mrhar. 2008. The influence of selected parameters on the Size and Shape of Alginate beads prepared by ionotropic gelation. *Sci Pharm*. 76 (1):77-89.
6. SreeGiri Prasad B, Gupta V. R. M, Devanna N, Jayasurya K. 2014. Microspheres as drug delivery system – A review. *JGTPS*. 5 (3):1961-72.
7. Mohan M, Sujitha H, Dr. Rao V, U. M, Ashok M, Arun Kumar B. 2014. A brief review on mucoadhesive microspheres. *IJRRPAS*. 4(1):975-86.
8. Badarinath A.V, Reddy J.R, Mallikarjuna R.K, Alagusundaram M, Gnanaprakash K, Chetty M.S. 2010. Formulation and characterization of alginate microbeads of flurbiprofen by ionotropic gelation techniques. *Int J Chem Tech Res*. 2(1):361-36.
9. Thulasi VM, Sajeeth CI. 2013. Formulation and evaluation of sustained release sodium alginate microbeads of Carvedilol. *Int J Pharm Tech Res*. 5(4):746-53.
10. Bhanja S, Sudhakar M, Neelima V, Roy H. 2013. Development and evaluation of mucoadhesive microspheres of Irbesartan. *Int J Pharm Res Health Science*. 1:17-26.
11. Hardenia SS, Jain A, Patel R, Kaushal A. 2011. Formulation and evaluation of mucoadhesive microspheres of Ciprofloxacin. *Adv Pharm Edu Res*. 1(4): 214-24
12. Brahmaiah B, Sasikanth K, Sreekanth N, Krishna C. 2013. Formulation and evaluation of extended release mucoadhesive microspheres of Rosuvastatin. *Int J Bio Pharm Res*. 4(4):271-81.
13. Ghareeb MM, Issa AA, Hussein AA. 2012. Preparation and characterization of cinnarizine floating oil entrapped calcium alginate beads. *Int J Pharm Sci Res*. 3:501-8.
14. Umesh D, Shivhare, Vijay B. Mathur, Chandrashekhar G. Shrivastava, Vivek I. Ramteke. 2013. Preparation of microbeads by different techniques and study of their influence on evaluation parameters. *J. Adv. Pharm. Edu. & Res*. 3(3): 279-288.
15. Klaus Florey, Harry Brittain, David Mazzo. 1991. Analytical profiles of drug substances. Elsevier publications 1st edition. 20: 397-427.
16. Rajinikanth PS, Sankar C, Mishra B. 2003. Sodium alginate microspheres of metoprolol tartarate for intranasal systemic delivery: development and evaluation. *Drug Delivery*. 10(1):21-8.
17. Verma A, Sharma M, Verma N, Pandit JK. 2013. Floating alginate beads: studies on formulation factors for improved drug entrapment efficiency and *in vitro* release. *Farmacia*. 61(1): 143-61.
18. Fareez IM, Lim SM, Mishra RK and Rama samy K. 2015. Chitosan coated alginate-xanthan gum bead enhanced pH and thermotolerance of *Lactobacillus plantarum* LAB12. *International Journal of Biological Macromolecules*. 72: 1419-28.
19. Tao Y, Zhang R, Xu W, Bai Z, Zhou Y, Zhao S, Xu Y and Yu D. 2016. Rheological behavior and microstructure of release-controlled hydrogels based on xanthan gum cross-linked with sodium trimetaphosphate. *Food Hydrocolloids*. 52: 923-33.
20. Higuchi WI. 1962. Analysis of data on the medicament release from ointments. *J Pharm Sci*. 51(8):802-4.
21. Sherina VM, Santhi K, Sajeeth CI. 2012. Formulation and evaluation of sodium alginate microbeads as a carrier for the controlled release nifedipine. *Int J Pharm Chem Sci*. 1:699-710.
22. Farhana Y, Talukdar MU, Islam MS, Laila S. 2008. Evaluation of aceclofenac loaded agarose beads prepared by ionotropic gelation method, *Stamford J Pharm Science*. 1 (1):10-7.
23. Follonier N and Doelkar E. 1992. Biopharmaceutical comparison of oral multiple-unit and single-unit sustained release dosage forms. *STP Pharmaceutical Sciences*. 2:141-158.

24. Manna A, Ghosh I, Goswami N, Ghosh LK and Gupta BK. 1999. Design and evaluation of an oral controlled release microparticulate drug delivery system of nimesulide by ionotropic gelation technique and statistical optimization by factorial analysis *J Sci Ind Res.* 58 (9):717-722.
25. Somashree R, Sabyasachi Maiti, Biswanath Sa. 2010. Preliminary investigation on the development of diltiazem resin complex loaded carboxymethyl xanthan beads. *AAPS Pharm Sci Tech*; 9(1): 574-576.
26. Divya Dheer, Jyoti, Prem N. Gupta, Ravi Shankar. 2018. Tacrolimus: An updated review on delivering strategies for multifarious diseases. *European Journal of Pharmaceutical Sciences*, 114: 217–227.
27. Nayak AK, Khatua S, Hasnain MS, Sen K.K. 2011. Development of diclofenac sodium-loaded alginate-PVP K 30 microbeads using central composite design. *Daru.* 19 (5):356-66.
28. M. S. Khan, B. K. Sridhar, A. Srinatha. 2010. Development and evaluation of pH-dependent micro beads for colon targeting. *Indian J. Pharm. science.* (1):18-23.
29. Toutous-Trellu L, Abraham S, Pechere M. 2005. Topical tacrolimus for effective treatment of eosinophilic folliculitis associated with human immunodeficiency virus infection. *Arch Dermatol.* 141:1203–1208.
30. Sajid Bashir. 2014. Development of Sustained-Release Microbeads of Nifedipine and In vitro Characterization. *Trop J Pharm Res.* 13 (4): 505-510.
31. Anuranjita Kundu. 2012. Preparation and evaluation of Sustained release microbeads of norfloxacin using sodium alginate. *IJRPC.* 2 (3): 647-65.
32. Kanika Nayak, Manoj Kumar Mishra. 2017. Formulation and Evaluation of ibuprofen loaded agar microbeads. *International Journal of Advanced Pharmaceutics.* 7 (1):11-15.
33. H. C. Kiran, Dhandapani Nagasamy Venkatesh, Raman Rajesh Kumar. 2019. Formulation and characterization of sustained release microbeads loaded with zaltoprofen. *International Journal of Applied Pharmaceutics.* 11(5): 173-180.