

FORMULATION AND EVALUATION OF MARAVIROC MUCOADHESIVE MICROSPHERES BY IONOTROPIC GELATION METHOD

SELLAPPAN VELMURUGAN^{a,b,*}, MOHAMED ASHRAF ALI^{a,c}

^aDepartment of Pharmaceutics, Sunrise University, Alwar, Rajasthan, ^bDepartment of Pharmaceutics, KLR Pharmacy College, Palvoncha, Andhra Pradesh, India, ^cInstitute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang, Malaysia.
Email: willard_cbe@rediffmail.com

Received: 29 Aug 2013, Revised and Accepted: 06 Oct 2013

ABSTRACT

Objective: The objective of the present study was to prepare mucoadhesive microspheres of Maraviroc by Ionotropic gelation technique by using sodium alginate as mucoadhesive polymer in various proportions for retaining the dosage form remain in vicinity of absorption site for prolonged period of time to treat HIV infection and to avoid first pass metabolism. Maraviroc is an antiretroviral drug that can precisely control viral load in HIV/AIDS patients.

Methods: Fourteen batches of Maraviroc loaded mucoadhesive microspheres were formulated to investigate the effect of certain process variables, such as different crosslinking agent (Ca chloride, barium chloride, aluminium sulphate), Maraviroc to sodium alginate polymer ratio (0.5, 1, 1.5 and 2), concentration of cross-linking agent (5,10 and 15%), curing time (15, 30 and 60 min), Maraviroc ratios (0.5, 1.0 and 1.5) and stirring rate (200,400 and 600 rpm) on the mean particle size, yield, drug entrapment efficiency, mucoadhesion , percentage swelling index and in-vitro drug release.

Results: The formulated mucoadhesive microspheres were discrete, free flowing and SEM studies indicated that the Maraviroc microspheres were almost spherical in shape. The prepared mucoadhesive microsphere formulations having entrapment efficiency of 66.66%- 89.78% and mucoadhesion of 02-37% at the end of 8 hours. In-vitro drug release studies of Maraviroc mucoadhesive microspheres showed a controlled release of 10 hours. The best batch F9 exhibited drug entrapment efficiency of 84.22%, and the drug release from the microspheres was also sustained for more than 10 hrs (96.48%). There were no compatibility issues and the crystallinity of maraviroc drug was found to be reduced in prepared maraviroc mucoadhesive microspheres, which were confirmed by IR, DSC and XRD studies .The Stability of Maraviroc mucoadhesive microspheres was determined in 40°C/75% RH; it was found that both Maraviroc and mucoadhesive microspheres were stable in 40°C/75% RH for 3 months.

Conclusion: The results obtained in this present work suggest that mucoadhesive microspheres of an anti-retroviral drug Maraviroc can be successfully designed to give controlled drug delivery, minimizing the drug related side effects and improved oral bioavailability.

Keywords: Maraviroc, Ionotropic gelation; Sodium alginate, Mucoadhesive microspheres; Controlled drug delivery.

INTRODUCTION

Mucoadhesion has been a topic of interest for last two decades in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption [1]. Mucoadhesive micro carrier systems utilizing bioadhesive property of some polymers, which become adhesive on hydration, and hence can be used for localizing the bioactives to a particular region of gastrointestinal tract for extended periods of time[2, 3]. Bioadhesion is an interfacial phenomenon in which one may be synthetic or biological macromolecules and second materials is biological surface (epithelial tissue or the mucus coat on the surface of tissue) are held together by means of interfacial forces, when the associated biological surface is mucin layer of a mucosal tissue, it is called mucoadhesion. Mucoadhesive microspheres delivery system is an attractive concept, due to their ability to adhere to the mucosal surface and release the entrapped drug in a sustained manner [4, 5]. Mucoadhesive microspheres have advantages like efficient absorption, enhanced bioavailability of the bioactives due to high surface to volume ratio, much more intimate contact with the mucin layer of a mucosal tissue and site specific targeting of the bioactives to absorption site can be achieved by using suitable plant lectins, bacteria and antibodies on the surface of mucoadhesive micro carriers [6-8].

Maraviroc is one of a new class of antiretroviral drug known as CCR5 antagonists and only oral entry inhibitor approved for the treatment of HIV1 infection. These acts as a human immuno deficiency virus type 1 (HIV 1) co receptor [9,10]. Maraviroc is practically insoluble in water and belongs to BCS class III drug. Maraviroc poorly absorbed from lower gastrointestinal tract and the oral bioavailability has been reported to be 33% with short biological half life of 10.6-2.7 h. Administration of conventional dosage form of Maraviroc has been

reported to exhibit fluctuation in the plasma drug level resulting either in manifestation of side effect [11,12].

All the drawbacks necessitate the development of an effective drug delivery system which could utilize all the potential of efficacy of the Maraviroc drug and reduce the dosing frequency of Maraviroc, improve patient compliance and enhance bioavailability. Therefore, the development of mucoadhesive microspheres of Maraviroc could protect Maraviroc from first hepatic pass degradation and maintain a constant drug plasma level for extended period of time.

MATERIAL AND METHODS

Materials

Maraviroc was a gift sample from Hetro Pharma Ltd, Hyderabad. Sodium alginate polymers were received as gift sample from Cadila Pharma, Ahmedabad, India. Calcium chloride, barium chloride and aluminium sulphate from S.D. fine chemicals Pvt. Ltd. All other ingredients used were of analytical grade.

Preparation of Maraviroc mucoadhesive microspheres

The Maraviroc mucoadhesive microspheres were prepared by Ionotropic external gelation technique the composition of the various Maraviroc mucoadhesive microspheres formulations were mentioned in Table 1. Maraviroc and sodium alginate polymers were individually passed through sieve # 60. The required quantities of sodium alginate were dissolved in purified water to form a homogenous polymer solution. The drug maraviroc was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was sonicated for 30 min to remove any air bubbles. The bubble free dispersion was then

added manually drop wise into crosslinking ion solution using polyethylene syringe (needle size 22 G) and stirred at 200-600 rpm.

Fourteen batches of Maraviroc loaded mucoadhesive microspheres were prepared to investigate the effect of certain formulation and process variables, such as different crosslinking agent (Calcium chloride, barium chloride, aluminium sulphate), Maraviroc to polymer ratio (0.5, 1, 1.5 and 2), concentration of cross-linking agent (5,10 and 15%), curing time (15, 30 and 60 min), Maraviroc ratios (0.5, 1.0 and 1.5) and stirring rate (200-600 rpm) on the mean particle size, yield, drug entrapment efficiency, mucoadhesion and in-vitro drug release. The obtained microspheres were collected

by decantation, washed repeatedly with distilled water and dried at 45°C for 12 hour.

Determination of ideal cross linking agent

Different cross linker solution i.e. calcium chloride, barium chloride and aluminum sulphate were prepared and used to formulate Maraviroc mucoadhesive microspheres while keeping sodium alginate concentration, cross linker concentration, curing time and stirring speed at fixed values i.e. 1:1 drug polymer ratio, 10%w/v cross linker concentration, 30 min curing time and 400 rpm respectively [13].

Table 1: Composition of Maraviroc mucoadhesive microspheres

Formulation code	Drug : Alginate	Electrolyte	Percentage electrolyte	Curing time	Stirring speed
F1	1.0 : 1.0	CaCl ₂	10	30	400
F2	1.0 : 1.0	BaCl ₂	10	30	400
F3	1.0 : 1.0	Al ₂ (SO ₄) ₃	10	30	400
F4	1.0 : 1.0	Al ₂ (SO ₄) ₃	5	30	400
F5	1.0 : 1.0	Al ₂ (SO ₄) ₃	15	30	400
F6	1.0 : 1.0	Al ₂ (SO ₄) ₃	10	15	400
F7	1.0 : 1.0	Al ₂ (SO ₄) ₃	10	60	400
F8	1.0 : 0.5	Al ₂ (SO ₄) ₃	10	30	400
F9	1.0 : 1.5	Al ₂ (SO ₄) ₃	10	30	400
F10	1.0 : 2.0	Al ₂ (SO ₄) ₃	10	30	400
F11	0.5 : 1.0	Al ₂ (SO ₄) ₃	10	30	400
F12	1.5 : 1.0	Al ₂ (SO ₄) ₃	10	30	400
F13	1.0 : 1.0	Al ₂ (SO ₄) ₃	10	30	200
F14	1.0 : 1.0	Al ₂ (SO ₄) ₃	10	30	600

Determination of Optimum Cross-linker Concentration

Serial concentrations i.e. 5%, 10% ,15% w/v, of cross linker solution (aluminum sulphate) were prepared and used to formulate Maraviroc mucoadhesive microspheres while keeping sodium alginate concentration, curing time and stirring speed at fixed values i.e. 1:1 drug polymer ratio, 30 min curing time and 400 rpm respectively [13].

Determination of Optimum Curing Time

Sodium alginate, drug Maraviroc 1:1 ratio were mixed and stirred well till homogenous solution formed. This solution was added drop wise to cross linker solution (i.e. aluminum sulphate 10% w/v) using polyethylene syringe (needle size 22 G) and kept for 15, 30, 60 minutes in cross linking solution while keeping cross linking agent concentration, sodium alginate polymer concentration, and stirring speed at fixed values i.e. 1:1 drug polymer ratio, 10%w/v cross linker solution and 400 rpm respectively [14].

Determination of Optimum sodium alginate Concentration

Serial ratios i.e. 0.5, 1.0, 1.5 and 2.0 , of sodium alginate solution were prepared and used to formulate mucoadhesive microspheres while keeping cross linker concentration, curing time and stirring speed at fixed values i.e. 10% w/v ,30 min curing time and 400 rpm, respectively. Sodium alginate solutions were added to cross linker solution drop wise using polyethylene syringe (needle size 22 G) [15].

Determination of Optimum Maraviroc drug Concentration

Serial ratios i.e. 0.5, 1.0, 1.5 and 2.0 , of drug Maraviroc and sodium alginate solution were prepared and used to formulate mucoadhesive microspheres while keeping cross linker concentration, curing time and stirring speed at fixed values i.e. 10% w/v ,30 min curing time and 400 rpm, respectively. Sodium alginate solutions were added to cross linker solution drop wise using polyethylene syringe (needle size 22 G) [16].

Determination of Optimum Stirring Speed

Stirring of prepared droplets in the crosslinking medium was made at different speeds i.e. 200,400,600 rpm, while keeping sodium alginate solution, cross-linker concentration and curing time at fixed

values i.e. 1:1 drug polymer ratio, 10%w/v cross linker solution, 30 min curing time respectively [17].

Percentage yield

The percentage yield of Maraviroc microsphere was calculated by weighing after drying. The weight of dried microspheres (W1) was divided by the total amount of all initial dry weight of starting materials (W2) used for the preparation of the Maraviroc microspheres, which gave the total percentage yield of Maraviroc microspheres [18].

Particle Size

Particle size of the Maraviroc micro particles was determined by using an optical microscope method and the mean particle size was calculated by measuring 50-100 particles in each batch with the help of a precalibrated ocular micrometer. The mean Maraviroc microspheres particle size and standard deviation values were calculated and reported [19].

Morphology of Microspheres

The surface morphology and shape of the Maraviroc microspheres was examined by scanning electron microscopy. The sample was mounted on to an aluminum stub and sputter-coated with platinum particles in an argon atmosphere [20].

Drug Entrapment Efficiency

The amount of drug entrapped was estimated by crushing 100mg of maraviroc mucoadhesive microspheres and extracting with 100 ml of 0.1 N HCl for 24 hr in rotary shaker. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (LABINDIA UV-3092 PC) at 210 nm against 0.1 N HCl as a blank. The amount of Maraviroc entrapped in the microspheres was calculated by the following formula [21].

Percentage entrapment efficiency = Observed Drug Content x 100 / Calculated Drug content.

Swelling study of Microsphere

A 100mg of Maraviroc mucoadhesive microspheres from each batch was placed in 500ml of 0.1 N HCL and allowed to swelled for the required

period of time, at $37 \pm 0.5^\circ\text{C}$ using USP dissolution apparatus 2 at 100rpm. The Maraviroc microparticles were removed every hour interval up to 8 hour, blotted carefully with filter paper and their changes in weight were measured during the swelling until equilibrium was obtained [22]. Finally, the swelling ratio (SR) of each microsphere formulation was calculated according to the following equation

$$\text{SR} = (W_e - W_0) / W_0$$

Where W_0 is the initial weight of the dry Maraviroc microparticles and W_e is the weight of swollen Maraviroc microparticles at equilibrium swelling in the media.

Mucoadhesive Test

The mucoadhesive property of Maraviroc microspheres was evaluated by in vitro wash off test. A Piece of goat intestinal mucosa was tied on the glass slide using a thread. About 100 microspheres were spread onto each wet rinsed tissue specimen and immediately therefore the support was hung onto the arm of USP disintegration apparatus. Now operating the disintegration test machine, the goat intestinal mucosa was given a slow regular up and down movement in 900ml of 0.1N HCL buffer at $37 \pm 0.5^\circ\text{C}$. At the end of 1 hr and at hourly intervals up to 8 hrs the equipment was stopped and the number of Maraviroc microspheres still sticking onto the intestinal mucosa was counted [23]. Percent mucoadhesion was calculated by the using following formula

$$\% \text{ MUCOADHESION} = (\text{No. of particle remains on mucosa} / \text{No. of applied microsphere}) \times 100$$

In Vitro Dissolution

The in vitro dissolution studies of prepared Maraviroc microspheres were carried out using USP type II (paddle) dissolution test apparatus. Weighed amount of Maraviroc loaded microspheres were introduced into 900 ml dissolution medium of 0.1N HCl for 8 hrs at $37 \pm 0.5^\circ\text{C}$ at a rotation speed of 50 rpm. 5ml of aliquots were withdrawn at predetermined time intervals and an equivalent volume of fresh 0.1N HCl was replaced to maintain volume constant. The samples were analyzed spectrophotometrically at 210 nm after suitable dilution to determine the Cumulative percentage of Maraviroc release [24].

Release kinetic and mechanism of Maraviroc drug release

The Maraviroc release data from all the mucoadhesive microspheres formulation were fitted in various kinetic models like zero order; first order, Higuchi's model and korsmeyer-peppas equations to determine the corresponding release rate and mechanism of drug release. A criterion for selecting the best fit model was based on goodness of fit, high R^2 (regression coefficient) value [25].

FTIR Studies

Compatibility study of Maraviroc with the excipients was determined by I.R. Spectroscopy (FTIR) using Shimadzu FT-IR

spectrometer model. The pellets were prepared with KBr using pure drug Maraviroc, polymers and crushed mucoadhesive microspheres formulations and the scanning was done between wave numbers 4000 to 400 cm^{-1} at 4 cm^{-1} resolution [26].

Thermal Analysis (DSC)

Differential scanning calorimetry were carried out for pure drug Maraviroc and Maraviroc loaded microspheres using a Shimadzu DSC 60 to evaluate any possible Maraviroc drug- sodium alginate polymer interaction. Samples (5mg each) were accurately weighed into aluminum pans and sealed. The measurements were conducted over a temperature range $40-400^\circ\text{C}$ at a heating rate of $10^\circ\text{C} / \text{min}$ under nitrogen atmospheres.

X-Ray Diffraction study (XRD)

The crystallinities of Maraviroc, Maraviroc loaded microspheres and Physical mixture were evaluated by XRD measurement using an X-ray diffractometer. XRD studies were performed on the samples by exposing them to $\text{CuK} \alpha 1$ radiation (40 kV, 30 mA) and the scanning rate was $5^\circ / \text{min}$ over a range of $4-90^\circ$ and with an interval of 0.1 [27].

Stability Study

To assess the Maraviroc and mucoadhesive formulation stability, accelerated stability studies were done according to ICH guidelines. The optimized mucoadhesive microspheres formulation (F9) was selected for stability study on the basis of in vitro drug dissolution studies; drug entrapment efficacy and invitro wash off test. In the investigation, stability studies were carried out at $40 \pm 2^\circ\text{C} / 75 \pm 5\%$ in closed high density polyethylene bottles for 3 months. The samples were removed every month interval up to 3 months and evaluated for physical changes, drug release, entrapment efficiency, during the stability studies [28].

RESULT AND DISCUSSION

Maraviroc loaded mucoadhesive microspheres were prepared by Ionotropic gelation technique employing calcium chloride, barium chloride and aluminium sulphate as cross linking agent. The obtained Maraviroc microspheres were discrete, spherical in shape and freely flowing. The percentage yield of the different alginate mucoadhesive microsphere formulations were found to be 87.96% for calcium-alginate microspheres and 87.13 % for barium alginate and 87.25% to 92.75 % for aluminium alginate (Table 2). It was observed that as the Maraviroc to sodium alginate concentration increases, the product yield also increases. The particle size were found to be $730.67 \pm 13 \mu\text{m}$ for calcium-alginate microspheres and $715.33 \pm 14 \mu\text{m}$ for barium-alginate microspheres whereas in case of aluminium-alginate, particle size were found within the range of $642.33 \pm 36 \mu\text{m}$ to $806.67 \pm 38 \mu\text{m}$ respectively. The mean particle size of the prepared Maraviroc microspheres in presented in Table 2.

Table 2: Physicochemical properties of Maraviroc mucoadhesive microspheres

Formulation code	Percentage yield	Entrapment efficiency	Particle size [μm]	Angle of Repose
F1	87.96	66.66 ± 0.46	730.67 ± 13	23.98
F2	87.13	73.89 ± 1.39	715.33 ± 14	24.50
F3	87.25	82.83 ± 1.45	694.33 ± 19	22.49
F4	88.18	72.43 ± 1.08	732.33 ± 20	24.40
F5	87.54	82.40 ± 2.46	671.67 ± 22	20.96
F6	86.91	67.77 ± 0.46	732.33 ± 19	19.35
F7	87.69	83.71 ± 1.07	684.67 ± 23	25.66
F8	87.48	79.43 ± 1.09	672.33 ± 17	23.00
F9	90.33	84.22 ± 0.75	753.67 ± 28	20.41
F10	92.75	87.60 ± 1.13	806.67 ± 38	17.72
F11	91.27	78.81 ± 0.82	682.33 ± 19	19.04
F12	90.32	89.78 ± 1.41	717.00 ± 24	23.98
F13	86.14	80.86 ± 1.43	794.33 ± 29	21.69
F14	88.41	83.69 ± 1.78	642.33 ± 36	24.79

Determination of ideal cross linking agent

Drug entrapment of the prepared Maraviroc mucoadhesive microspheres was determined and it ranged from 66.66 to 89.78. The results tabulated in Table 2 indicate that the encapsulation efficiencies were more than 70 % for Maraviroc microspheres cross-linked with, Al^{3+} and Ba^{2+} . This may be due to the formation of nonporous alginate Maraviroc microspheres due to an increase in the apparent cross-linking density in presence of, Al^{3+} and Ba^{2+} which prevent the diffusion of the Maraviroc out of the microspheres at the time of curing. The low encapsulation efficiency of alginate Maraviroc microspheres cross-linked with Ca^{2+} could be due to the formation of porous microspheres ensuring the diffusion of the Maraviroc out of the microspheres at the time of curing.

Different ions had been used as cross-linking agents for preparation of Maraviroc microspheres. The microspheres prepared from Ba^{2+} and Al^{3+} ions were found to be more appropriate in terms of shape, and entrapment efficiency. We had to use Ba^{2+} and Al^{3+} ions for screening. However, due to the toxicity of Ba^{2+} , these crosslinking ions were not used in the following steps. The best one is Al^{3+} ion due to the size and ability of Al^{3+} ion to form continuous Al-alginate film, suitable swelling kinetic of Al -alginate Maraviroc microspheres, lack of toxicity we chosen to use this ion for Maraviroc mucoadhesive microspheres formulations.

Optimum Cross linker Concentration

Table 2 shows that the mean particle size of the Maraviroc microspheres decreased with increase in the cross-linker concentration, when crosslinking agent concentration increased from 5% w/v to 15% w/v, the mean particle size (mean diameter) was decreased from $732.33 \pm 20 \mu m$ to $671.67 \pm 22 \mu m$. However, concentration of the cross-linker above 15% w/v caused formation of irregular shapes due to extensive crosslinking of the guluronic acid unit of alginate polymeric chain. Increase in the concentration of crosslinking ion (aluminium) from 5% w/v to 15% w/v in formulations F3 to F5 led to an increase in the Maraviroc encapsulation efficiency which may be explained by the increase in the gel strength as the aluminium ion increased. Consequently the cross linking of the sodium alginate and compactness of the formed mucoadhesive microspheres also increased. This would result in more Maraviroc encapsulation in the microspheres. However, further increase in concentration of crosslinking agent above 15%w/v did not enhance the Maraviroc encapsulation due to possible saturation of aluminium binding sites of the sodium alginate polymer. Increase in Aluminium sulphate cross linking agent concentration led to a decrease in the mean particle size. The higher amount of Al^{3+} ion appears to favor better cross linking forming spherical Maraviroc microspheres [29].

Optimum Curing Time

From Table 2, it was observed that increasing crosslinking time from 15 to 60 minutes, the drug entrapment efficiencies were found to be in the range of 67.77 % to 83.71 % for Maraviroc. It was found that optimum drug entrapment was achieved at 30 minutes, with no

higher increase in entrapment efficiency after this curing time i.e. 30 minutes. Increase in curing time from 15 to 60 minutes increases the degree of cross linking of the sodium alginate polymer, which ultimately results in shrinking of the particles, which leads to decrease in particle size.

Optimum Drug Concentration

Table 2 shows that an increase of the initial Maraviroc concentration caused the increasing of entrapment efficiency from 78.81 % to 89.78 % for drug concentration of 0.5 and 1.5 ratios, respectively. It was also noticed that as, the concentration of Maraviroc was increased particle size also increased which could be attributed to the increased drug content of the dispersion droplet at higher Maraviroc concentration [30].

Optimum Polymer Concentration

When sodium alginate ratio was increased from 0.5 to 2.0 the particle diameter increased from ($672 \pm 17 \mu m$) to ($806 \pm 38 \mu m$). From Table 2, for Maraviroc microspheres prepared with sodium alginate ratio (0.5), showed a unimodal distribution ($SD = \pm 17 \mu m$) with a mean particle diameter of ($672 \mu m$), indicating relatively homogeneous size. It was also found that percentage entrapment efficiency increased with increase in sodium alginate polymer concentration (Table 2) which may be due to higher concentration of the polymer may increases the viscosity of the medium and hence, increases availability of cross-linking binding sites in the alginate polymeric chains, as a result the degree of cross-linking may be improved and larger droplets were formed which entrapping greater amount of Maraviroc in the microspheres [31].

Optimum Stirring Speed

Table 2 shows that the effect of the stirrer rotational speed during the crosslinking step on microsphere particle size was evaluated, and it was shown that increasing the stirring speed decreased the mean particle size of Maraviroc microspheres reaching a minimum at 600 rpm.

At 600 rpm, a lower particle size was obtained, but a broader distribution of microspheres increased the mean size. At 400 rpm, the standard deviation relative to the mean was ($SD = 19 \mu m$) and at 600 rpm this value increased ($SD = 36 \mu m$) [32].

Release behavior

The Maraviroc release behavior of alginate mucoadhesive microspheres, produced by ionotropic internal gelation with different cross-linking agents depend upon the valency and size of the cations of the respective cross-linking agent. Their release profiles in 0.1N HCl pH 1.2 were depicted in Figure 1-2. Calcium alginate and barium-alginate microspheres (F1 and F2) were able to sustain the maraviroc release up to 8 hours whereas aluminium-alginate microspheres were able to sustain the drug released up to 10 hours. It has been observed that calcium, barium-alginate microsphere showed comparatively rapid Maraviroc release as compared to aluminium-alginate formulations.

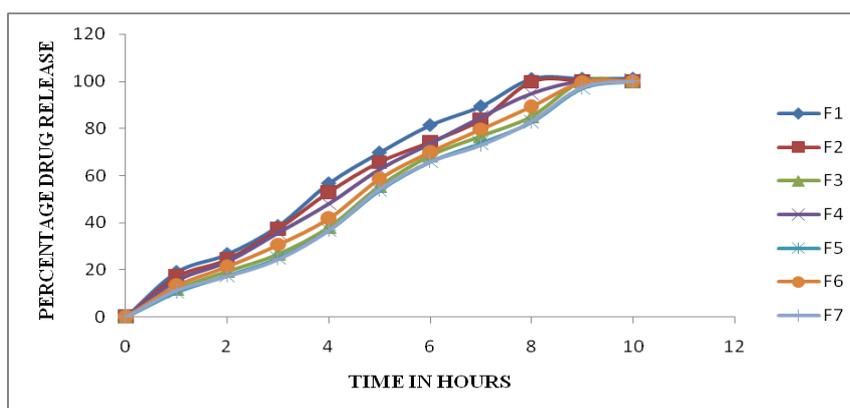


Fig. 1: Comparative release profile of formulation F1 to F7

The results obtained can be explained on the basis of the extent of cross-linking in the microspheres. Ca²⁺ and Ba²⁺ being divalent, form two-dimensional bonding structure with sodium alginate inside the alginate matrices. But since Ba²⁺ has the largest size as compared to the other two cations (Ca²⁺ and for Al³⁺), it is expected to form strong alginate mucoadhesive microspheres with smaller voids and low water uptake. Therefore, the exchange of larger Ba²⁺ in the

microspheres with Cl⁻ of dissolution medium (Hydrochloric acid, pH 1.2) and also their removal was hindered, thus resulting in delayed swelling where as in case of Ca²⁺ alginate microspheres, the smaller size of Ca²⁺ as compared to Ba²⁺ ensure rapid removal of Ca²⁺ from the microspheres due to ion exchange process with Cl⁻ of hydrochloric acid buffer medium and thus leading to greater water uptake and rapid release.

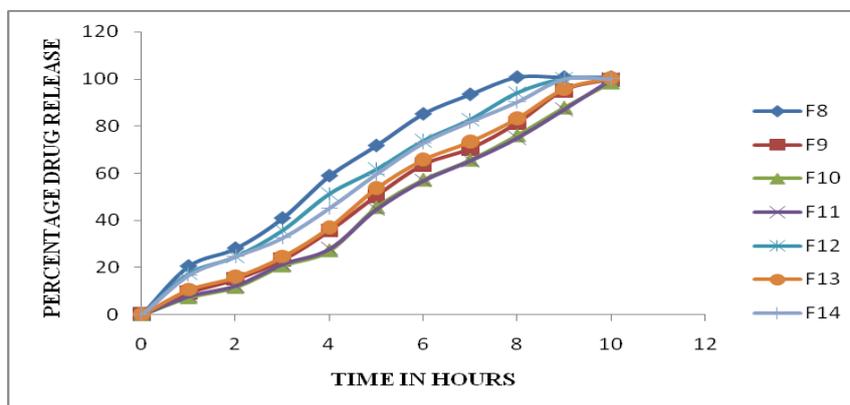


Fig. 2: Comparative release profile of formulation F8 to F14

In case of Al³⁺ alginate Maraviroc microspheres, the delay was due to the capacity of Al³⁺ ion to form three-dimensional bonding structure with the sodium alginate inside the mucoadhesive microspheres. This strong three dimensional bonding results in an extended cross linking throughout the mucoadhesive microspheres, producing hard alginate mucoadhesive microspheres with low water uptake and thus leading to slow removal of Al³⁺ due to ion exchange with Cl⁻ in the hydrochloric acid. As a result, the swelling of the microsphere are delayed leading to slow disintegration as well as slow dissolution. Consequently increasing the concentration of alginate and Al³⁺ ion as cross-linking agent, prolonged the maraviroc release was observed up to 10 hours because alginate could form more rigid coat with trivalent (Al³⁺) ion as compared to divalent (Ba²⁺).

The order of decreasing Maraviroc release rate observed with different cross linking agents was as follows

Aluminum sulphate > Barium chloride > Calcium chloride

Drug release kinetic data for maraviroc mucoadhesive microspheres was shown in Table No. 3. All the formulations (F1 to F14) follow zero order release kinetics with regression values ranging from 0.950 to 0.992. All the formulations were subjected to Korsmeyer-Peppas plots, 'n' value ranges from 0.811 to 1.208 indicating that the maraviroc drug release was from the microspheres followed the anomalous transport and super case-II transport mechanism.

Swelling study

The swelling study of the prepared Maraviroc microspheres was carried out in hydrochloric acid buffer pH 1.2 and the results are

presented in Figure 3-4. The swelling of mucoadhesive microspheres depends upon the concentration of sodium alginate and extent of Al³⁺ cross linking in the Maraviroc microspheres. The swelling of the Maraviroc microspheres increased with an increasing amount of sodium alginate polymers and swelling decreased with an increasing amount of AlCl₃.

Mucoadhesion studies of maraviroc microspheres

The results of *in vitro* wash off studies are shown in Table 4. Percentage mucoadhesion increased with the increase in concentration of sodium alginate mucoadhesive polymer. The rank order of percentage mucoadhesion of all the maraviroc formulations after 8 h was found to be as follows.

F10 > F9 > F11 > F14 > F3 > F2 > F7 > F5 > F13 > F1 > F12 > F8 > F4

Surface morphology

The SEM photographs of the optimized Maraviroc mucoadhesive microspheres formulation (F9) taken by scanning electron microscope are depicted in the Figure 5. The SEM photographs revealed that the Maraviroc microspheres were discrete and spherical in shape with a rough outer surface morphology which could be due to the surface association of the Maraviroc with the sodium alginate.

FT-IR spectra of pure Maraviroc and drug loaded microspheres were compared and shown in Figure 6. The FT-IR spectra of the Maraviroc loaded Maraviroc showed the characteristic peaks of the pure drug Maraviroc indicating that there was no interaction between the Maraviroc and sodium alginate polymer.

Table 3: Kinetic parameter of Maraviroc mucoadhesive microspheres

Formulation code	Zero order	First order	Higuchi	Korsmeyer pepas	n-value	Hixson crowell
F1	0.950	0.886	0.972	0.977	0.811	0.742
F2	0.966	0.728	0.977	0.983	0.847	0.555
F3	0.985	0.79	0.967	0.985	1.004	0.601
F4	0.976	0.875	0.981	0.989	0.885	0.666
F5	0.989	0.637	0.968	0.986	1.047	0.42
F6	0.985	0.746	0.976	0.989	0.947	0.509
F7	0.988	0.8	0.965	0.980	1.030	0.566
F8	0.934	0.913	0.966	0.973	0.778	0.766
F9	0.992	0.669	0.966	0.987	1.110	0.405
F10	0.990	0.743	0.952	0.983	1.208	0.575
F11	0.991	0.626	0.95	0.983	1.169	0.368
F12	0.978	0.662	0.981	0.985	0.834	0.492
F13	0.990	0.806	0.969	0.982	1.065	0.523
F14	0.982	0.637	0.975	0.983	0.851	0.477

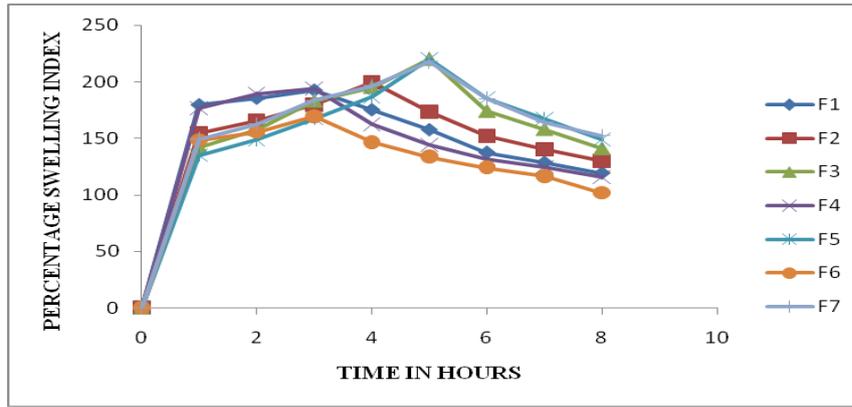


Fig. 3: Swelling behavior of formulation F1 to F7 in 0.1 N HCL

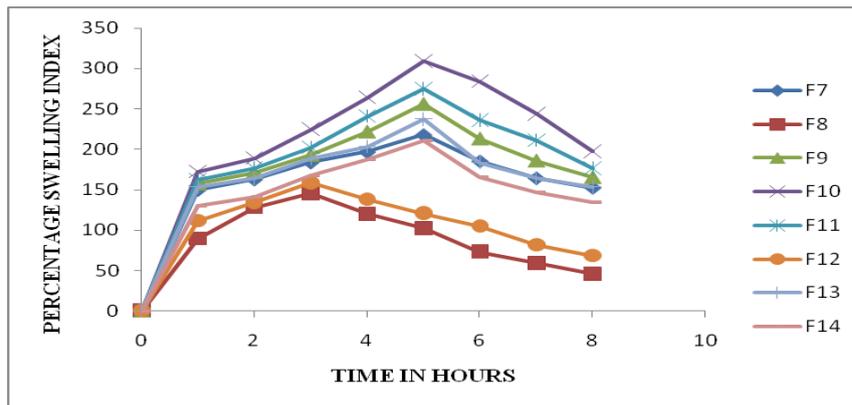
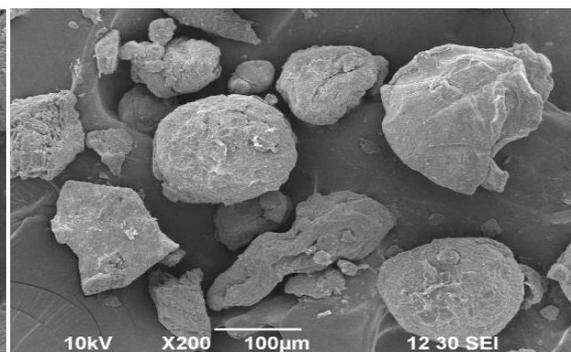
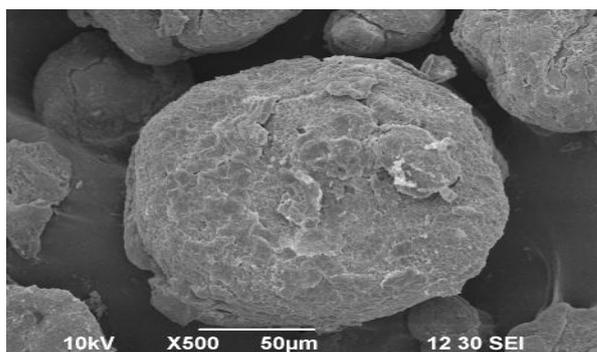


Fig. 4: Swelling behavior of formulation F8 to F14 in 0.1 N HCL

Table 4: Results of *in vitro* wash off test in 0.1N hydrochloric acid

Hours	1	2	3	4	5	6	7	8
F1	92 ± 1.53	72 ± 2.52	60 ± 2.08	47 ± 1.53	38 ± 2.52	25 ± 2.08	12 ± 1.53	2 ± 0.58
F2	94 ± 0.58	73 ± 2.08	61 ± 1.53	50 ± 0.58	42 ± 1.53	30 ± 2.52	19 ± 1.53	5 ± 0.588
F3	96 ± 1.53	75 ± 2.08	61 ± 2.52	55 ± 3.51	51 ± 2.52	31 ± 1.53	22 ± 2.52	6 ± 1.53
F4	97 ± 0.58	64 ± 2.52	32 ± 3.06	29 ± 2.08	21 ± 2.52	03 ± 2.08	0	0
F5	95 ± 1.53	73 ± 2.52	58 ± 2.08	45 ± 3.51	33 ± 2.52	21 ± 1.53	16 ± 2.52	3 ± 2.08
F6	94 ± 2.52	56 ± 3.06	28 ± 3.51	14 ± 3.06	06 ± 2.08	03 ± 2.31	0	0
F7	97 ± 1.53	80 ± 4.51	73 ± 3.51	45 ± 2.52	32 ± 1.53	25 ± 2.52	17 ± 1.53	4 ± 0.57
F8	91 ± 2.52	56 ± 3.06	38 ± 3.51	23 ± 3.06	15 ± 3.21	06 ± 2.65	0	0
F9	97 ± 1.53	86 ± 2.52	74 ± 2.89	64 ± 3.06	55 ± 3.06	45 ± 2.52	34 ± 3.06	25 ± 2.52
F10	99 ± 0.58	94 ± 2.08	86 ± 3.06	78 ± 2.08	62 ± 2.52	54 ± 1.53	43 ± 2.08	37 ± 2.52
F11	96 ± 1.53	82 ± 2.52	74 ± 3.51	64 ± 2.52	56 ± 3.06	41 ± 3.51	35 ± 2.08	20 ± 2.52
F12	92 ± 2.52	63 ± 3.51	45 ± 2.52	34 ± 1.53	26 ± 2.08	17 ± 25.6	03 ± 1.53	0
F13	95 ± 2.52	72 ± 2.08	60 ± 2.52	51 ± 3.51	45 ± 3.06	27 ± 2.08	13 ± 2.52	2 ± 1.15
F14	97 ± 1.53	78 ± 3.06	67 ± 1.53	57 ± 2.52	50 ± 3.21	35 ± 3.51	29 ± 2.08	11 ± 1.53



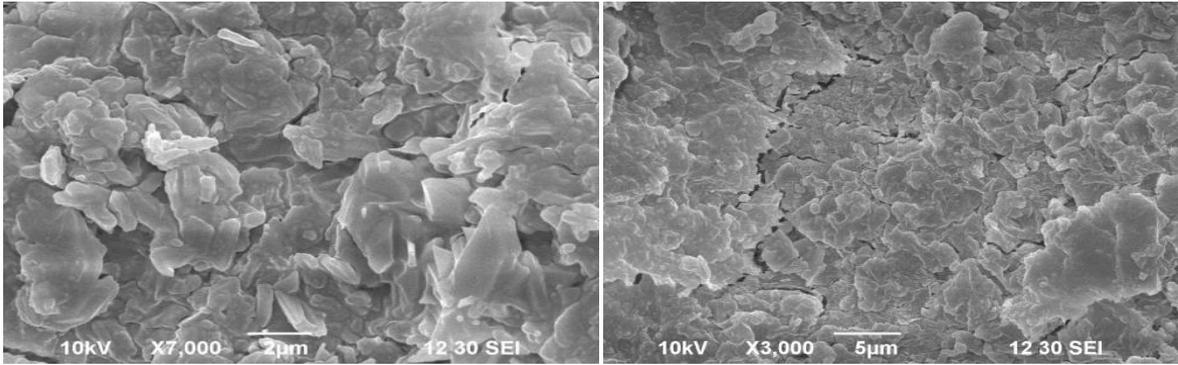


Fig. 5: Scanning electron photomicrographs of the Maraviroc alginate microspheres (F9): a) 200 X, b) 500 X, c) 3000 X, d) 7000 X.

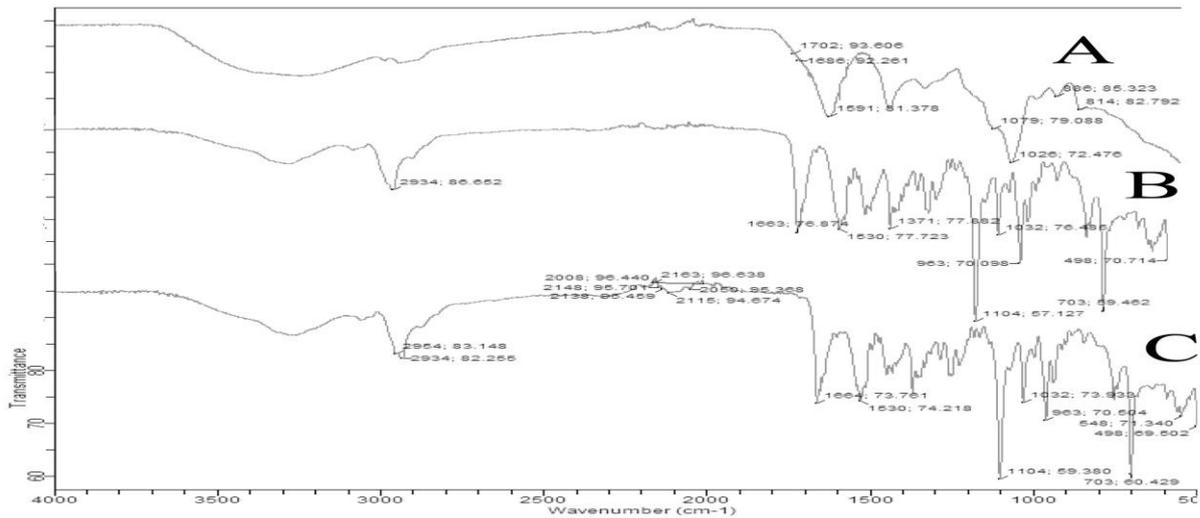


Fig. 6: FTIR spectra of A. Pure Drug Maraviroc B. Sodium alginate polymer C. Optimized formulation F9

DSC study of pure drug Maraviroc and Maraviroc loaded Maraviroc were compared to study the stability of the Maraviroc during the formulation and any drastic change in the thermal behavior of either the Maraviroc or polymers. DSC curve of Maraviroc showed a sharp endothermic peak at 196°C, corresponding to its melting point. Similar

endothermic peaks were obtained at 196.2°C for the formulations prepared with sodium alginate (F9). Presence of all peaks indicates that all ingredients are compatible with maraviroc and there is no incompatibility between the selected ingredients. Thermogram of F9 formulations and maraviroc drug are shown in figure 7.

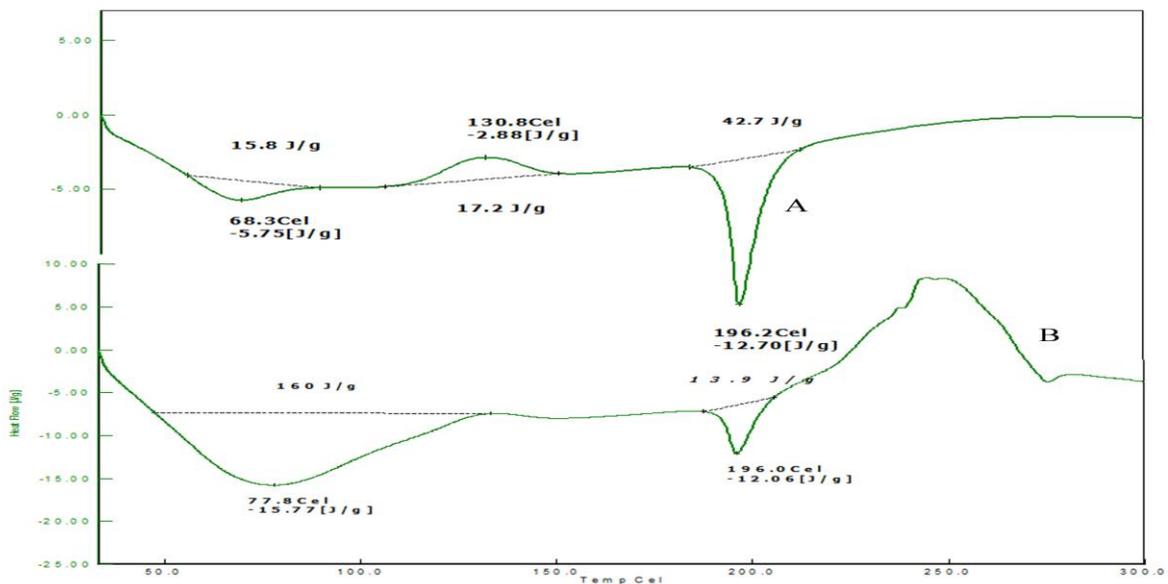


Fig. 7: DSC Thermograms of A. Pure Drug Maraviroc B. Optimized formulation F9

The X-ray diffractograms of pure Maraviroc, physical mixture and Maraviroc loaded Maraviroc are shown in Figure 8. Maraviroc has shown characteristic intense peaks due to its crystalline nature. Whereas, in case

of Maraviroc loaded mucoadhesive microspheres, no intense peaks related to drug were noticed. It indicates the amorphous dispersion of the Maraviroc after entrapment into mucoadhesive microspheres.

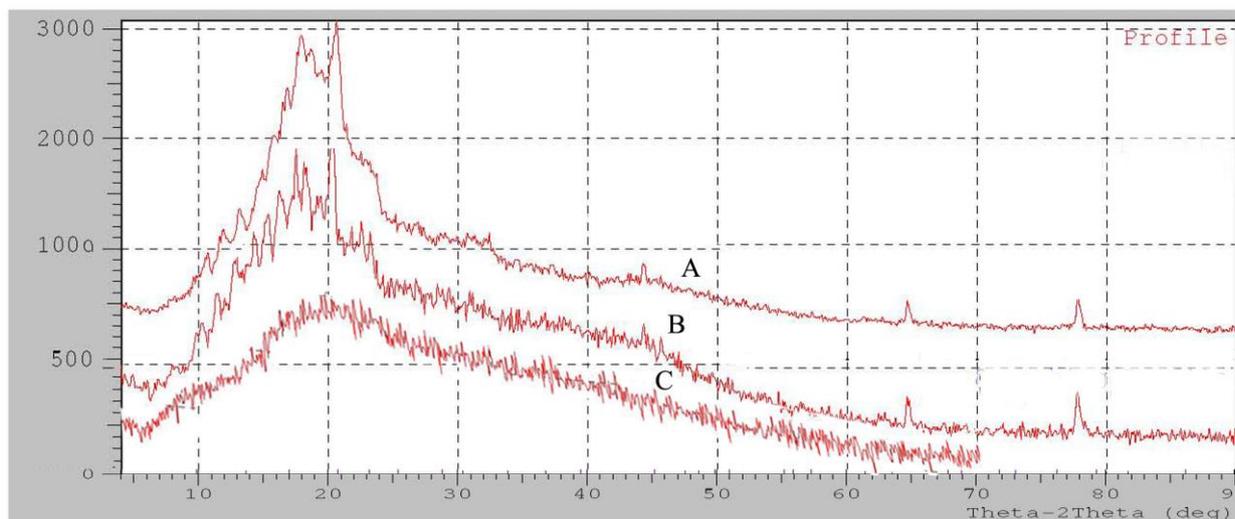


Fig. 8: XRD pattern of A. Pure Drug Maraviroc B. Physical mixture of Maraviroc and sodium alginate C. Optimized formulation F9.

Table 5: Stability data of F9 maraviroc microspheres formulations

Formulation code	Months	Entrapment efficiency	Percentage drug release
F9	0	84.22 ± 0.75	99.84 ± 0.21
	1	83.82 ± 0.09	97.31 ± 2.01
	2	82.71 ± 0.41	97.56 ± 0.36
	3	81.84 ± 0.24	96.48 ± 0.36

Stability studies of the prepared maraviroc mucoadhesive microspheres were carried out by storing the best formulation F9 at $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{RH}$ for 3 month. For optimized formulation F9 show negligible change in percentage drug release, and entrapment efficiency as shown in table 5.

CONCLUSION

The sodium alginate based mucoadhesive microspheres were prepared by Ionotropic gelation method for the controlled release of Maraviroc. The swelling of microsphere and drug release depends upon the polymer concentration and extent of crosslinking in the polymer matrix. The effect of polymer, cross linking agent and its concentration and curing time on *in vitro* release of sodium alginate mucoadhesive microspheres was well investigated. The results show that as the concentration of sodium alginate and cross linking agent increases, entrapment efficiency increases and Maraviroc release rate decrease. Drug release followed the anomalous transport and super case-II transport mechanism. Thus, it can be concluded that this technique could be used to prepare multiparticulate drug delivery system for oral controlled release of Maraviroc.

ACKNOWLEDGMENT

The authors are thankful to Hetro Lab, Hyderabad for providing gift samples. Authors are also thankful to The Principal K.L.R Pharmacy College, Paloncha, Andhra Pradesh for permitting to carry out research work.

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