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EFFECT OF PROCESS AND FORMULATION VARIABLES: MUPIROCIN LOADED BIODEGRADABLE POLYMERIC MICROSPHERES

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ABSTRACT

Mupirocin is an antibacterial agent used as topical agent in the treatment of superficial infections by gram positive bacteria, particularly staphylococcus aureus. The purpose of the study was to formulate and *invitro* exploration of mupirocin encumbered biodegradable microspheres using simple orthogonal factorial design. The concentration of retardant material, volume of external and internal phase, stirring speed and time and polymer-drug ratio as independent variables and the effects were analyzed on dependent variables like particle size, morphology, percentage drug loading and percentage drug entrapment efficiency. The function of desirability (DF) (d_i) was produced to optimize the formulation by design expert software. Data analysis showed that microspheres with optimum diameter, shape, drug loading and drug release were 70.4µm, round, 32.5%(w/w) and 86.5% respectively with 3% chitosan, 75mg mupirocin, 10ml internal phase, 50ml external phase containing 2% span 80 emulsified at 1500 rotations per minute for 2hrs stabilization. The *invitro* release of mupirocin from chitosan microspheres was suggested to be controlled by the dissolution and diffusion from the matrix. The independent variables had great significant effects on dependent variables and a significant burst release effect and sustained delivery of mupirocin for more than 24 hrs.

INTRODUCTION:

Mupirocin calcium is a salt of pseudomonic acids which is having antibacterial activity produced by the growth of pseudomonas fluorescens inhibiting bacterial isoleucyl transfer ribonucleic acid–tRNA synthetase and blocking protein synthesis (R Sutherland et al 1985). The clinical effectiveness of mupirocin and impairing of wound healing in infected tissues is the result of its antibacterial action (F Jacobsen et al 2011). Mupirocin itself does not form a depot in the skin; its metabolites (biologically inactive monic acid) are present in the skin for up to 168 hours after application (Reilly GD et al 1984). In an invitro study, mupirocin with occlusion achieved a fivefold greater penetration than without occlusion (Radika O et al 2014). The applications of biodegradable polymeric carrier provide a

process of controlled and localized delivery of drugs. Nevertheless the biodegradable polymer dissolves/degrades rather rapidly in aqueous biological systems (Suping Lyu et al 2009). Hence the difficulty in polymer modification and production of a long period drug delivery carrier. These problems require the use of cross linking agent to form hydrophilic lipophilic networks to avoid rapid dissolution (Enas M.A. et al 2015). The selection of an encapsulation technique is primarily determined by the solubility uniqueness of the drug and polymer (Rainer Alex et al., 1990). The biodegradable mupirocin microspheres (MM) developed with suitable microencapsulation method should ideally produce high yields microparticles and free of agglomeration, high entrapment of drug substance, a reproducible release profile characteristics (M Jelvehgari et al., 2006).

The study was to explorate the mupirocin loaded biodegradable polymeric microspheres by the use of simple orthogonal design. The effects of internal and external phase volume ratio, stirring speed and time, drug: polymer ratios were analyzed on morphology, size of the particle, drug entrapment efficiency and drug release was studied. The orthogonal factorial design is an efficient tool for optimization of formulation design with an appropriate mathematical model.

MATERIALS AND METHODS

Mupirocin is a gift sample from Fourrts India laboratories, Chennai. Gelatin, Chitosan and sodium alginate were supplied by Merck; all other chemicals and reagents in this study used were of pharmaceutical and analytical grade.

Formulation of Mupirocin Loaded Microspheres (Table no 1)

Preparation of mupirocin loaded sodium alginate microspheres

Mupirocin (100mg) was previously dissolved in 25ml water was suspended sodium alginate (2%w/v) solution, homogenized for 15 min. The mixture was carefully added drop wise to the dispersion medium (100 ml) consisting of a mixture of light and heavy liquid paraffin in a specific ratio (2:1) and 2 ml of span 80. The dispersion was stirred for 15 min to obtain w/o emulsion and was cross linked by the addition of $CaCl_2$ (5%w/v) solution (2ml) of specific strength (Shanmugam V et al., 2016) and continued stirring for 10 minutes. Microspheres were rigidized by adding 10ml of petroleum ether (10 ml) after 5 minutes. The microspheres were vacuum filtered and removed the adhered liquid paraffin by multiple washings with cold pet. Ether and freeze dried (M.C. Gohel et al 1998).

Preparation of mupirocin loaded gelatin microspheres

100mg mupirocin was dissolved in water and added to 25ml of preheated gelatin solution (5% *m/V*, in water) and added drop-wise to 100 ml) consisting of a mixture of light and heavy liquid paraffin in a specific ratio (2:1) with 2ml of Span 80 (Jayvadan patel et al., 2010). The biphasic system was stirred using an overhead stirrer to form a w/o emulsion. 2 ml of gluteraldehyde(GTA)-saturated toluene was added to the w/o emulsion (Majeti NV et al 2000) and stirred at 1000 rpm. Microspheres were rigidized, washed free of oil ice cold with isopropyl alcohol and dehydrated with 20 ml of acetone. Microspheres were freeze dried to obtain a yellow to yellowish orange colored free flowing material. (Rassoul D et al 2005).

Preparation of mupirocin loaded chitosan microspheres

Mupirocin was dispersed into the chitosan (2.5% w/v) solution previously prepared with acetic acid (3%, v/v) (Tarek Ahmed et al., 2016) and stirred for 1 hour to homogeneity. The mixture was then emulsified into 100ml of light: heavy liquid paraffin oil (2:1) containing span 80 (0.1%w/w) at 37°C. The ionic gellification of the microspheres was achieved by adding 2ml sodium tripoly phosphate solution (Analava Mitra et al 2011) in water (pH 8.5±0.2) in the oily suspension. After 1 hour of prefixed cross-linking time, rigidized with 10ml of acetone (B. Venkateswara Reddy et al 2015) and the microspheres were vacuum filtered (0.45µm PFTE membrane

filters), (S. Senthil kumar et al 2012) washed with equal volume of *n*-hexane (non solvent) (Jelvehgari et al., 2010) and freeze dried (Anil K. Anal et al 2006).

Process variables in preparation of microspheres

Effect of polymer on physical appearance and particle size

For the selection of polymer, various investigations were carried out using different polymers like gelatin, sodium alginate and chitosan (M.Jelvehgari et al 2010) with constant drug to polymer ratio of 2:1 and the stirring speed of 2000 rpm for a period of 5 hrs. Initial selections of both the phases were based on the solubility of mupirocin and biopolymers (Gelatin, Sodium alginate, Chitosan). The formed microspheres (P1 to P3) were evaluated for physical appearance and particle size.

Effect of concentration of retardant material

To optimize the concentration of polymers in the internal phase, microspheres were prepared using 10ml internal phase and 100 ml of external phase with varying concentration of retardant material. The minimum concentration of retardant material required for the formation of microspheres was determined. The microspheres (R1 to R9) were characterized for physical appearance; particle size (P.S) and production yield (P.Y) (Y M Jagtap et al 2012).

Effect of volume of external phase

Different volumes of internal phase i.e. distilled water were evaluated using 25, 50, 75, 100, 125 and 150 ml of external phase but the drug to polymer ratio was kept constant at 1:1 as internal phase, stirring speed of 1000 rpm for the period of 5 hours with surfactant concentration of 1% w/v of the external phase. The formed microspheres (MM1-MM6) were evaluated for their particle size, drug content (DC), percentage drug entrapment (DE) and free drug (Jelvehgari M et al 2006).

Effect of volume of internal phase

Different volumes of external phase were evaluated using 5 ml, 10 ml, 15 ml, 20 ml, 25 ml and 30 ml of the internal phase, but the drug to polymer ratio was kept constant at 1:1 in 100 ml of external phase, stirring speed of 1000 rpm for a period of 5 hours with surfactant concentration of 1.0%w/v of external phase. The formed microspheres (MM7-MM12) were evaluated for their particle size, drug content, entrapment efficiency and free drug (Jelvehgari M et al 2010).

Effect of stirring speed on formation of microspheres

Micro particles were prepared with different rpm of 500, 1000, 1500, 2000, 2500 and 3000 keeping the other entire variables constant (Utsav C. Rathod et al 2012) and the formed microspheres (MM13-MM18) were evaluated for their particles size, drug content, entrapment efficiency and free drug.

Effect of stirring time on the formation of microspheres

Microspheres were prepared at different time intervals of 1, 2, 3, 4, 5 and 6 hours keeping the other entire variables constant. The microspheres (MM19-MM24) were evaluated for particle size, drug content, entrapment efficiency and free drug (Nokhodchi A et al 2007).

Effect of Drug: Polymer ratio

In order to evaluate the effect of drug on the microspheres and its release characteristics, different polymers to mupirocin ratios (1:1, 2:1, 3:1, 4:1, 5:1 and 6:1) were used to prepare microspheres. The formed microspheres (MM25-MM30) were evaluated for their production yield, particle size, drug content, entrapped drug and free drug (Jelvehgari M et al 2006).

Evaluation of Mupirocin Microspheres

Percentage yield of microspheres *(Shovarani KN et al 1994)* The yield of microspheres was calculated by the following formula:

% Yield of microspheres = Practical weight / Theoretical weight × 100

Drug content

Microspheres equivalent to 100 mg of mupirocin were dissolved in 100 ml of methanol, further dilutions were made to Beer's range and absorbance was measured at 220nm by UV spectroscopy (Ghosh A et al 2007).

Free drug content

Microspheres equivalent to 100mg of mupirocin were washed with 100ml of Polyethylene glycol 400 (PEG 400). Filtrate was suitably diluted to get a concentration within Beer's range and measured the absorbance at 220nm using UV spectrophotometer against blank (Ghosh A et al 2007).

Determination of entrapment efficiency

Weigh accurately microspheres equivalent to 100mg of mupirocin and dissolved in dilute acidic condition and vortexed. After 15 minutes of agitation, centrifuge the solution and measure the supernatant liquid for absorbance at 228nm by UV spectroscopy (Gohel MC et al 2005). The percentage of entrapment efficiency was calculated by the following formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Theoretical drug/ Released drug}}{\text{Theoretical drug}} \times 100$$

Particle size analysis

The dried microspheres were dispersed in isopropyl alcohol and vortexed for 10 seconds to reduce the inter particle interactions; the particle size of microspheres were measured using a Microtrac S3500, USA particle size analyzer. (Shovarani KN et al 1994).

Morphology by scanning electron microscopy

The external morphology of microspheres was visualized at an accelerating voltage of 0.3–30 kV and identified by using SE, BSE detectors (B.Sree Giri Prasad et al 2011) using scanning electron microscopy (SEM, Hitachi, Japan).

In vitro drug release from microspheres: Mupirocin microspheres equivalent to 10mg of mupirocin were added to dissolution medium (100ml of pH 7.4 PBS) and kept at 37 ± 5^0C for 72 hours. At predetermined time intervals aliquots were drawn and maintained the sink condition. After suitable dilutions, the samples absorbance was analyzed spectrophotometrically at 220nm and compared with the stock solution. The experiment was carried out in triplicate (MG Sankalia et al 2005).

Experimental design and optimization (Ya Min Wang et al 2006)

An orthogonal design was used to optimize the formulation of microspheres with different levels as given in the table 2. Seven factors of mupirocin microsphere formulation and 3 levels for each factor were selected based on our preliminary data and simple orthogonal experimental table was arranged accordingly. To compare each of the formulation quantitatively and qualitatively, the function of desirability (d_i) was calculated according to Hassan et.al (1992).

$$d_i = Y_1 - Y_{min}/Y_{max}-Y_{min}$$

d_i values were calculated from percentage of particles, drug content, drug entrapment efficiency, morphology and free drug in each formulation batch respectively. These factors were not considered for optimization because prolong time to differentiate required drug release profiles for several preparations, although drug release properties is an important goal of microsphere formulation. The normalization of 5 levels of mupirocin microspheres by Y_{max} and Y_{min} values respectively are given in table 3. The function of desirability (FD) of various formulations was compared by a statistic analytical system to identify the optimal formulation mixture of optimum levels of each factor. (Hassan E.E et al 1992).

Simplification of model and analysis of data: The relationships between the three indices and variables were determined by multiple regression method. Model simplification was done by a gradual linear regression program. Variables were introduced into the model gradually while significant variables ($p>0.05$) were

eliminated out of the model. The experimental results were analyzed the influence of variables on the particle size distribution, drug content and drug loading efficiency by utilizing the simplified models.

RESULTS AND DISCUSSION

Effect of Process Variables

The minimum concentration of retardant material required in the internal phase was found to be in case of sodium alginate, % in case of gelatin and % in case of chitosan. At these concentrations the microspheres formation was initiated and showed good physical characteristic like proper shape, size, particle size (figure 1) and did not collapse even after removal from the solvent and subsequent drying. It was found that by decreasing the internal phase volume increased the particle size of microspheres (Li X, et al., 1999). When the viscosities of the internal phase of these formulations were studied, it was found that the particle sizes of microspheres were directly proportional to the viscosities of the dispersed phase. When the dispersed phase with higher viscosity was poured into continuous phase, due to the higher viscosity of the internal phase, the formed emulsion globules could not be divided into smaller particles and bigger droplets were formed with increased mean particle size (M.Jelvehgari et al., 2010). Table 6 also shows that when the amount of internal phase volume was increased from 25 to 150, % entrapment and drug content of microspheres decreased. This could be probably due to the lower concentration of the drug in the higher volume of internal phase (Rainer Alex et al., 2008).

The volume of external phase plays a crucial role in the formation of microspheres with reduction in free drug concentration and particle size. The results of the study show that the particle size ranged from 58µm to 112µm. The free drug concentration varied from 3.76% to 5.14%, when the volume of external phase varied from 25ml to 150 ml. It was found that was the optimum volume necessary and resulted in the mean particle size of 87 µm with 83.16% entrapment efficiency.

The effect of stirring rate on the physical characteristics of microspheres was investigated on the mean particle size of microspheres, drug content, % entrapment and % free drug is shown in table 6. The results revealed that, increasing stirring rate from 500 to 3000 rpm decreased the % entrapment (Rainer Alex et al., 2008) from 84.40% to 77.60%. It was also observed that the turbulence at higher rate of stirring employed, (Jelvehgari M et al 2006) within the external phase had a distinct effect on diameter of particle size. Therefore, a suitable stirring rate to optimize particle size and subsequent drug release from microspheres was needed. An increase in rate of stirring resulted in decrease in particle size. Any increase in mean particle size at lowering stirring rates (M C Gohel et al., 1998) could be attributed to the increased tendency of globules to coalescence and aggregate (Navneet Sharma et al 2016). Hence, on the other side at higher stirring rates, a vigorous, uniform, increased mechanical shear is imposed resulting a rapid distribution of formed droplets which may have less ability of coalescence into bigger droplets. The effect of stirring time on microspheres was studied with drug polymer ratio 2:1 at stirring speed of 2000 RPM and at various duration of stirring. It was found that stirring time also played a major role in the formation of microspheres with reduced free drug content and particle size. The results of the experiment showed that the particle size ranged from 92µm to 196µm with a free drug concentration ranging from 13.33% to 30.32%. Stirring for 6hours at 200RPM speed resulted in better microspheres formation (Navneet Sharma et al 2016). It was found that drug to polymer ratio on production yield, drug loading efficiency; particle size and free drug are shown in table 4. The mean particle size ranged from 78µm to 188µm respectively when the drug to polymer ratio was increased from 1:1 to 6:1.

Table 1: Formulation of mupirocin microspheres

Ingredients	F1	F2	F3
Mupirocin (mg)	100	100	100
Sodium alginate (%)	2.0	-	-
Gelatin (%)	-	5.0	-
Chitosan(%)	-	-	1.0
Glutaraldehyde saturated toluene (ml)	2	-	-
Trisodium polyphosphate (ml)		2	
Calcium chloride (5%(w/v)) (ml)	-	-	2
Heavy/ Light liquid paraffin (ml)	100	100	100
Span 80 (ml)	2	2	2
Cold pet. ether	q.s	q.s	q.s
Isopropyl alcohol		qs	-
Acetone+ n-Hexane+3% Acetic acid	-	-	qs
Water	q.s	-	-

Table 2: Experimental optimal design – Orthogonal

Factor	LEVEL					
	1	2	3	4	5	6
Polymer	G	C	S A	-	-	-
Concentration of polymer (% w/v)	a b c 5, 10, 15	a b c 0.5, 1, 1.5	a b c 2.5, 5, 7.5	-	-	-
Volume of Internal phase (ml)	5	10	15	20	25	30
Volume of External phase (ml)	25	50	75	100	125	150
Stirring rate (RPM)	500	1000	1500	2000	2500	3000
Stirring Time (hrs)	1	2	3	4	5	6
Drug : Polymer ratio	1:1	2:1	3:1	4:1	5:1	6:1

Table 3: Normalization of 5 indices by Y_{max} and Y_{min} values

Source factors	Y_{max}	Y_{min}
1. % of particle size in 50-150µm Y_1	100	10
2. Drug content (%w/w) Y_2	100	30
3. Drug trapping efficiency (%) Y_3	80	20
4. Morphology Y_4	Round	Irregular
5. Free drug (%) Y_5	30	5

Table 4: Effect of polymer(s) on formation and particle size of microspheres

S.No	Polymer	IP:EP	Concentration	Physical appearance	Average particle size in µm
P1	Gelatin	10:50	5	Spherical agglomerates	180
P2	Sodium Alginate	10:50	5	Spherical and smooth	160
P3	Chitosan	10:50	2	Spherical and rigid	85

Table 5: Effect of concentration of retardant material (polymer) in the internal phase (I.P)

S.No	Polymer	I.P (%w/v)	Physical appearance	Particle size (50-00µm)	Average P.S(µm)	% yield
R1	Gelatin	5	Spherical agglomerates	33.47	125	89.6
R2	Gelatin	10	Spherical agglomerates	36.87	132	88.4
R3	Gelatin	15	Uniform sized	41.05	144	89.7
R4	Sodium alginate	2.5	Spherical and smooth	11.24	118	82.3
R5	Sodium alginate	5.0	Spherical and smooth	12.70	120	83.8
R6	Sodium alginate	7.5	Spherical agglomerates	14.12	112	82.6
R7	Chitosan	0.5	Not uniform	30.91	94	88.2
R8	Chitosan	1.0	Spherical agglomerates	65.85	58	88.0
R9	Chitosan	1.5	Spherical and rigid	78.70	68	88.5

Table 6: Effect of process and formulation variables on microspheres

Variables	Quantity/ Rate	Code	Particle size (µm)	Drug content (%)	% Entrapment	% Free drug	DF
Internal phase (ml)	5	MM1	82	73.72±0.27	68.32 ± 1.14	21.45±0.94	0.071
	10	MM2	76	78.27±0.31	65.40 ± 1.08	20.15±1.04	0.085
	15	MM3	73	84.45±0.82	61.10 ± 0.74	18.20±2.34	0.241
	20	MM4	65	94.60 ±0.48	56.85 ± 0.44	16.14±1.85	0.081
	25	MM5	59	88.18±0.50	50.56 ± 1.24	14.11±1.13	0.356
	30	MM6	44	73.30±1.04	48.33 ± 1.06	10.23±1.26	0.293
External phase (ml)	25	MM7	58	67.35±1.75	62.40 ± 1.51	5.14 ± 0.32	0.105
	50	MM8	71	76.48±1.20	71.27 ± 1.40	4.87 ± 0.74	0.426
	75	MM9	79	84.16±0.98	80.88 ± 0.84	4.13 ± 1.04	0.724
	100	MM10	87	94.29±1.32	83.16 ± 1.33	3.76 ± 1.51	0.132
	125	MM11	96	89.08±1.65	74.07 ± 1.56	4.54 ± 0.98	0.176
	150	MM12	112	80.2±0.92	70.19 ± 0.62	4.04 ± 1.26	0.085
Stirring speed (RPM)	500	MM13	-	-	-	-	
	1000	MM14	204	93.40±1.5	84.40 ± 1.04	8.55 ± 1.45	0.219
	1500	MM15	175	90.48±1.24	82.16 ± 0.96	8.21 ± 1.07	0.107
	2000	MM16	120	90.05±1.33	81.04 ± 1.03	8.15 ± 3.04	0.276
	2500	MM17	85	89.65±0.94	78.27 ± 1.18	8.05 ± 1.44	0.645
	3000	MM18	54	89.12±0.86	77.60 ± 1.25	7.90 ±1.02	0.428
Stirring time (hours)	1	MM19	-	-	-	-	
	2	MM20	-	-	-	-	
	3	MM21	196	63.34±0.74	38.44 ± 1.11	30.32±1.21	0.326
	4	MM22	145	68.26±1.08	47.36 ± 1.74	24.74±1.38	0.284
	5	MM23	112	76.0 ±1.22	56.25 ± 1.32	18.65±0.96	0.417
	6	MM24	92	80.33± 1.38	69.84 ± 1.40	13.33±0.74	0.251
Drug : Polymer ratio	1:1	MM25	78	41.74±1.21	32.36±1.16	9.45±0.98	0.159
	2:1	MM26	91	49.36±1.38	44.18±1.38	6.17±1.21	0.384
	3:1	MM27	103	64.18±1.17	57.56±1.41	7.04±1.81	0.587
	4:1	MM28	120	72.20±1.45	69.04±0.86	4.33±2.16	0.742
	5:1	MM29	156	83.51±1.08	77.15±1.92	6.32±0.64	0.104
	6:1	MM30	188	89.73±1.15	82.11±1.30	5.15±0.55	0.653

Table 7: The model – predicted and observed values of the 4 indices under optimum conditions

Index	Predicted values	Observed values
Average particle size (µm)	50-90	85
Drug content (%w/w)	70-95	94.60
Entrapment efficiency (%)	60-80	83.16
% Free drug	5-15	7.04

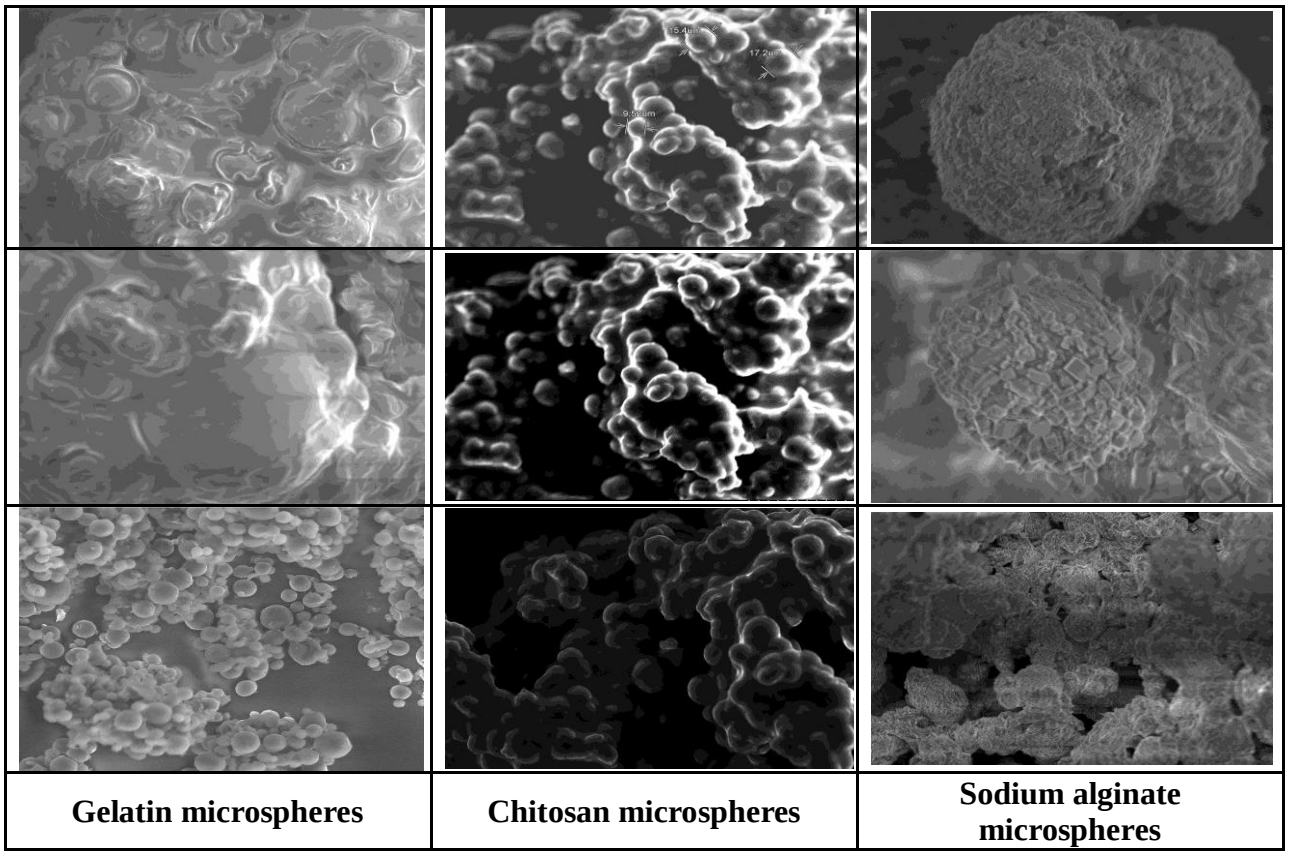

Figure 1: Scanning electron microscopical images of biodegradable microspheres based on different concentrations of retardant material

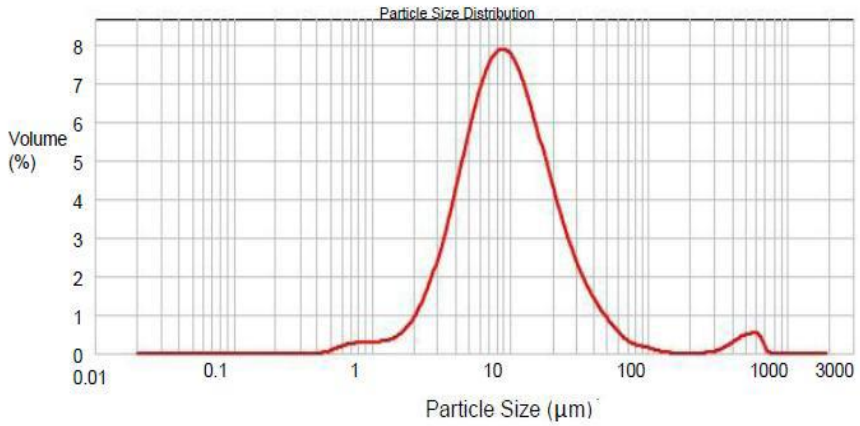

Figure 2: Particle size report of MM9 formulation

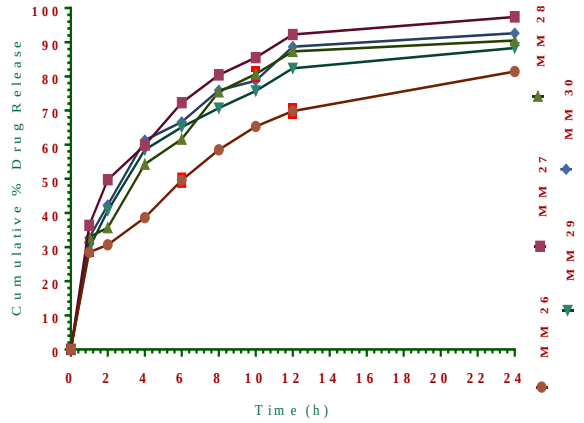

Figure 3: Graph showing the effect of drug polymer ratio on *invitro* release profiles of mupirocin loaded biodegradable polymeric microspheres (data represent mean ± standard deviation, n=3).

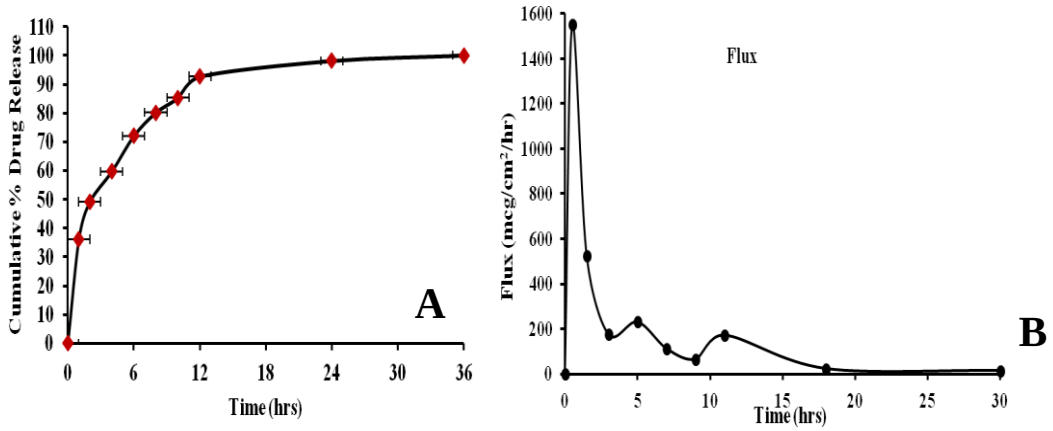

Figure 4: (A) Cumulative percentage drug release, (B) Drug release rate as a function of flux(mcg/cm^2/hr)

The encapsulation efficiency gradually increased with increase in the drug to polymer ratio till ratio 6:1 ratio but no further increase in encapsulation efficiency was found with increasing drug to polymer ratio. But the mean particle size decreased and the particle size distribution become narrower. This effect may be due to higher viscosity and faster diffusion of the internal phase from the emulsion system.

Particle size and size distribution

The size distribution of microspheres were ranged from 10 to 80µm and the average particle size were found to be 280µm for gelatin microspheres, 800µm for sodium alginate microspheres and 12.5µm for chitosan microspheres as shown in figure 2. The particle size should be within the range which can be penetrated through skin when applied topically (Cross S E et al., 2007) and should be accommodated in the pores. Particles in the range of 1 to 100µm shown better retention time/sustained effect through topical and transdermal delivery systems. Based on this investigation, we have attempted to formulate chitosan microspheres with further variables for their intended use.

***Invitro* drug release**

The invitro drug release studies were performed in buffer saline (pH 7.2) close to the physiological skin conditions. Incorporation of mupirocin into the microspheres had a significant effect on the rate of drug release. Various variables like phase volume ratio, stirring speed and time,

drug polymer ratio were illustrated in table 6. From the results, it was found that as concentration of polymer increases, the drug release percentage decreases (Analava Mitra et al., 2011). An initial burst release of drug was observed from all the batches that could be due to two reasons: the drug near or on the surface of the microsphere is the primary reason and secondly, well known leaching of drug with a faster ingress of the dissolution medium followed by diffusion of drug from porous nature of the microspheres (S. Senthil kumar et al., 2012), the pore providing a channel for release of the drug. The microspheres differ from micro sponges with their highly porous surface. The characteristic gives property control the drug release rate than that of the porous micro sponges. Among 30 formulations, MM8 showed a beneficial drug release effect of 40-50% burst release in 1st hour followed by controlled release observed till 24 hrs (figure 3 and figure 4(A). This release effect could support the therapeutic benefit with collagen scaffold.

Optimization of formulation by orthogonal design

The average particle size in µm, drug content, entrapment efficiency, free drug of microspheres of 27 formulations and whole desirability function are tabulated in table 6. The optimum combination of factors and levels for the preparation of mupirocin microspheres was established as A2; B2b; C4; D4; E5; F3 and G3 respectively. To 20ml of 1% (w/v) chitosan solution, 100mg of mupirocin is added, and the mixture is homogenized to obtain the desired dispersion. The mixed solution is added to 100ml of heavy/light liquid paraffin oil (1:1 ratio) containing 2ml of span 80 and emulsified at 2500 RPM for 3 hrs followed by stabilization with 2ml of 10% sodium tripolyphosphate solution for 1 hr. The reproducibility of this procedure was good, since no significant differences were observed in the mupirocin content, average particle size, entrapment efficiency, free drug content in all microspheres formulation prepared with optimum procedure. The average diameter was determined to be 65-85µm, drug content, %EE and %FD were 94.60±0.48, 83.16±1.33 and 7.04±1.81% (mean±S.D, n=3), respectively. The values of the 4 indices (Table 7) of the optimized formulation of mupirocin microspheres by this method appear to be close to the observed data. The rate of flux (figure 4(B)) of drug out of the microsphere per mass of formulation will increase with decreasing particle size (Neelesh K Varde et al., 2005).

CONCLUSION

Mupirocin loaded biodegradable microspheres were prepared using emulsification method followed by solvent removal. The polymer(s), concentration of retardant material, volume of internal and external phase, stirring speed and time, drug: polymer ratio influenced the agglomeration free microspheres. The entrapment efficiency was good for all formulations and the drug load was less unbiased with speed of stirring and time, volume of internal and external phase volume (dispersed and continuous phase volume). It was observed that the higher drug concentration, the mean particle size of the microspheres is elevated but smaller particle size with increasing the rate of stirring resulted here. The variation in the release profiles can be recognized to the difference in the surface of the microspheres regardless of the difference of particle size.

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