Pharma Science Monitor 7(3), Jul-Sep 2016

PHARMA SCIENCE MONITOR

AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

Journal home page: http://www.pharmasm.com

FORMULATION AND EVALUATION OF FLOATING MICROSPONGES OF ALLOPURINOL

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ABSTRACT

The aim of the study is to develop a floating microsponges of allopurinol to minimize the frequency of dosing by increasing half life and sustained release action up to 12 hrs by single unit dosage form. Allopurinol is xanthine oxidase inhibitor drug that leads to decrease in the production of uric acid from xanthine and hypoxanthine. It is BCS class I drug. It shows absorption window in upper gastro intestinal track, which makes it suitable candidate for gastro retentive dosage forms. Floating microsponges of allopurinol was formulated with quasi emulsion solvent diffusion method using Ethyl cellulose and Eudragit EPO as a polymer. A 3^2 factorial design was applied to optimize the formulation, it was found that formulation containing Ethyl cellulose 175 mg and of Eudragit EPO 75 mg, % drug release of allopurinol in 12 hrs with desired % buoyancy and % entrapment and best formulation was selected. The final optimized formulation shows entrapment 90.61%, buoyancy 86.52% and *in vitro* drug release 94.23% up to 12 hrs. Particle size was measured by optical microscopy. By using FTIR analysis drug and polymer compatibility was observed and using SEM prepared microsponges shape and surface morphology was determined. Accelerated stability study was carried for a period of 1 month. It was found that there was no significant change in formulation.

KEYWORDS Allopurinol, Ethyl cellulose, Eudragit EPO, Quasi emulsion solvent diffusion method.

INTRODUCTION

A Microsponges Delivery System (MDS) is “Patented, highly cross-linked, porous, polymeric microspheres that can entrap wide range of actives and then release them onto the skin over a time and in response to trigger” (Kilicarslan,2003). By Won in 1987, microsponges technology was developed and the original patents were assigned to highly developed polymer systems. Microspongess are porous microspheres having many of consistent voids of particle size ranging between 5-300 µm. To control the release rate of active agents to a programmed site in human body has been one of the biggest challenges faced by drug industry. Microsponges polymers have the flexibility to load a wide range of actives providing the benefits of improved product efficacy, tolerability, mildness and extended wear to a wide range of skin therapies. Improved in

formulation stability to ensuring long term product efficacy and extended shelf life. These microspongess have ability to entrap broad range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti-infectives and are used as a topical drug delivery system. These porous microspheres consist of active ingredients can be incorporated into formulations like creams, lotion, powders, and tablet for drug delivery. Microsponges is one of the modern and new approach to deliver a drug for longer period of time in a sustained manner.[1, 2] In 1956 allopurinol was first synthesized and reported by Roland K. Robins (19261992), in a search for antineoplastic agents. Allopurinol is a white or off white powder. Allopurinol is an ideal drug for treatment of gout. It is potent xanthine oxidase inhibitor. Chemically it is analogue of hypoxanthine. Formation hypoxanthine and xanthine is reducing due to inhibition of enzyme xanthine oxidase by allopurinol so that of xanthine and hypoxanthine excreted in unchanged form in urine. Thus, deposition of crystals in joints reduces by decrease in the precipitation of uric acid, and monosodium urate crystals.

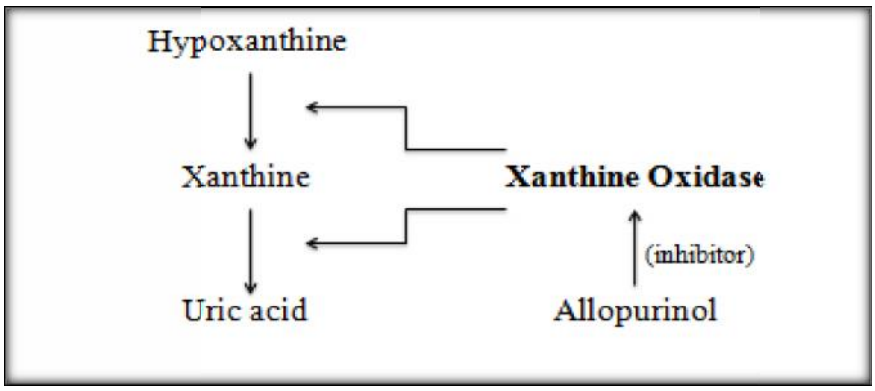

Figure 1: Mechanism of allopurinol

Whereas xanthine cannot be converted in to purine ribotide, hypoxanthine can be salvaged to purine ribotide adenosine and guaoside monophosphate, so increase in level of ribotide may cause inhibition of amidophosphoribosyltransferase which is the enzyme for biosynthasis of purine therefore, reduction in uric acid formation also reduce purine synthesis.[3, 4]

MATERIALS AND METHOD

Allopurinol was obtained from Indoco remedies Ltd. Eudragit EPO, Ethyl cellulose, Tween 80, Poly Vinyl Alcohol (PVA), $CaCl_2$, Dichlor methane (DCM) and 0.1N HCl were procured from Sulab Laboratory, Vadodara.

Quasi emulsion solvent diffusion method is a two step process consist of two phase internal phase and external phase. Internal phase consist of dichloromethane in which first drug was dispersed into it and after that polymer Ethyl cellulose and Eudragit EPO polymers were added in to it. External phase consist of PVA (polyvinyl alcohol) 1% (w/v), 5 ml (10mg in 10 ml) of

calcium chloride solution (pore forming agent), and tween 80. After that internal phase was slowly added in to the external phase and the system was continuously stirred till the dichloromethane was totally evaporated from the emulsion. That leads to formation of a highly porous microparticles called 'Microsponges'. Then mixture was filtered, washed and dried for 24 hours and microspongess were collected.[5, 6]

Micromeritic properties

Bulk density

Bulk density of a microsponges is dependent on particle packing and changes in the consolidation. Apparent bulk density (gm/ml) was determined by pouring dried microsponges into a graduated cylinder via funnel and measuring the volume and weight and bulk density was calculated by the following formula.

Bulk density = Weight of microsponges/ Bulk volume

Tapped density

Tapped density is the bulk density of a microsponges which has been compacted by tapping. Tapped density was determined by placing a graduated cylinder containing known mass of microspongess on a mechanical tapping apparatus. Which wad operated for a fixed number of taps (100) or the microspongess' bed volume was reached to the minimum level and tapped density was computed by taking the weight of microspongess in the cylinder and final volume. Calculation was done by using following formula in gm/ml.

Tapped density = Weight of power / Tapped volume

Carr's index

The compressibility index of the microspongess was determined by Carr's compressibility index and it was simple test to evaluate the BD and TD of a microspongess and the rate at which it packed down. For the calculation of Carr's index following formula was used.

Carr's index (%) = [(TD-BD) × 100]/TD

Hausner's ratio

The Hausner's ratio is a number that is correlated to the flow ability of the microspongess. Formula for calculation of hausner's ratio is as below.

Hausner's ratio = TD/BD

Angle of repose

Angle of repose of microspongess was determined by the fixed funnel method. Accurately weighted microspongess were taken in the funnel and height of funnel was adjusted in such way that the tip of the funnel just touched the apex of the microspongess. The Microspongess were

allowed to flow through the funnel freely on to the surface and diameter of the microspongess' cone was measured. Calculation of angle of repose was done by using following formula.

$$\tan^{-} = h/r$$

Where, h and r the height and radius of the microspongess cone respectively.

CHARACTERIZATION OF MICROSPONGES[7-10]

Fourier Transform Infrared Spectroscopy (FTIR)

The microsponges were subjected to Fourier Transform Inferred Spectroscopy (FTIR) studies using (Shimadzu 8400 s). The potassium bromide (KBr) disk method was used for preparation of sample. The spectrum was compared with the infrared spectra of plain drug and polymer and checked for the drug-polymer interaction.

Percentage Practical Yield

The prepared microsponges were collected and weighed. The measured weight was devided by total amount of all non-volatile components which were used for preparation of microsponges.

$$\%\ \mathbf{PracticalYield} = \frac{\mathbf{Weightofdriedmmicrosponge}}{\mathbf{Weightofsolidused\ (eexcipients + drug)}} \times \mathbf{100}$$

Percentage entrapment efficiency

50 mg of dried drug loaded microsponges were dissolved in 10 ml of DCM. After that 10 ml of 0.1 N HCl was added in to that and mixed well. Than aqueous phase was separated and the dissolved amount of drug was measured at 250 nm with UV spectrophotometer. Drug content of microsponges was calculated by following equation.

% drug entrapment = Actual drug content / Theoretical drug content x 100

Floating time

The 100 mg of floating microspongess were placed in the 500 ml of 0.1 N HCl and examined for the duration of time till they float.

In vitro drug release

Drug release from the microsponges was studied using USA dissolution test apparatus I. In the dissolution vessels fitted with basket capsules with microsponges placed in to it. Capsule was placed inside the basket and immersed in a dissolution vessel containing 900 ml 0.1 N HCl (pH) used as dissolution medium with temperature 37 and stirred at a 100 rpm speed. A 5 ml of sample was withdrawn at predetermined time interval and fresh dissolution medium was replaced and collected samples were filtered and analyzed for drug content by using UV visible spectrophotometer at 250 nm. After that %drug release at various time intervals was calculated.

Particle size analysis

The eye piece of micrometer was calibrated and the spherical crystal agglomerates were transferred on clean slide. One or two drops of water were added. The sample was dispersed uniformly with the help of a brush. The slide was placed on the stage of the microscope. The slide was focused in low magnification (10X). The presence of individual particle was observed. The size of each particle in terms of eye-piece division was measured.

Particle morphology

For the description of shape and surface morphology of microspongess was done by using scanning electron microscopy (SEM) (JSM- 5610LV, JEOL, Japan). Microspongess were dusted onto double side carbon dust, which was placed onto a sample carrier and after fixing the sample on stubs, capture a photomicrograph of microspongess.

In vitro *buoyancy*

100 mg microspongess were dispersed in 0.1 N HCl solution containing Tween 80 (0.02 w/v %) to stimulate gastric fluid and the mixture was stirred with a paddle at 75 rpm. After 12 hours, the layer of the buoyant particles was pipetted and the floating particles were separated by filtration and particles in sinking particulate layer were separated by filtration. Both particles were dried overnight and each weight was measured and buoyancy was determined by the weight ratio of the floating particles to the sum of floating and sinking particles.

$$\textbf{Buoyancy (\%)} = Q_f/Q_f + Q_s \times 100$$

Where Q_f and Q_s are the masses of floating and settled microsponges.

Residual solvent determination

Gas Chromatography of optimized batch DP was done. Gas chromatography was performed to residual solvent determination of dichloromethane. A volume of 1 ml standard and sample solution was injected into the GC injection port. The temperature of the injection port was maintained at 170^{o}C at a split ratio of 1:10, with nitrogen as a carrier gas. The pressure was maintained at 14 psi with flow of 1ml/min. The temperature of the detector was set at 250^{o}C for twelve min and then increased at a rate of 10^{o}C min^{-1} up to 220^{o}C to a final temperature of 220^{o}C and maintained for 5 min.

Release kinetic

For the analyses of mechanism of drug release from all the floating microsponges formulations, *In vitro* drug release data of all formulations were subjected to the kinetic analysis. Various models such as zero order, first order, higuchi model, and korsemeyer and Pappas dissolution data of all batches were fitted in to it. The model for best fit was predicted from the R^2 value.

RESULTS AND DISCUSSION

Drug Excipient compatibility study FTIR

The pure allopurinol and its mixture with Ethyl cellulose and Eudragit EPO was mixed separately with IR grade KBr and were scanned over a range of 400-4500 cm^{-1} using FTIR instrument. It was observed that there were no changes in the main peaks of allopurinol in the FTIR spectra of a mixture of drug and polymers as well as in the physical mixture of all ingredients used in the formulation of capsule. The FTIR study revealed no physical or chemical interactions of allopurinol with ethyl cellulose and Eudragit EPO, as well as with any other excipients.

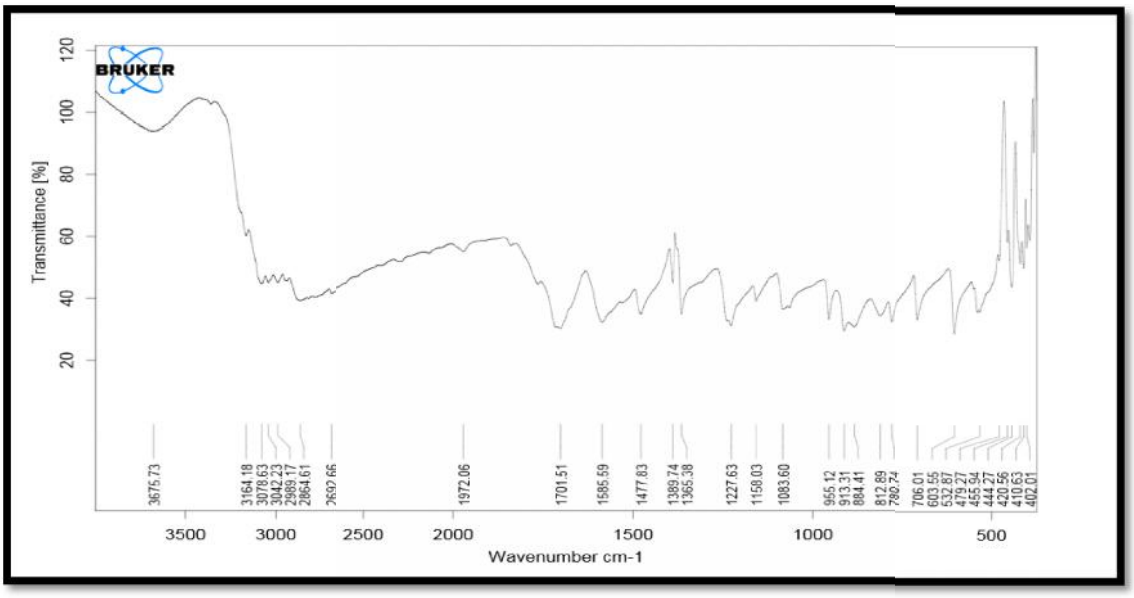

Figure 2: FTIR spectra of Allopurinol

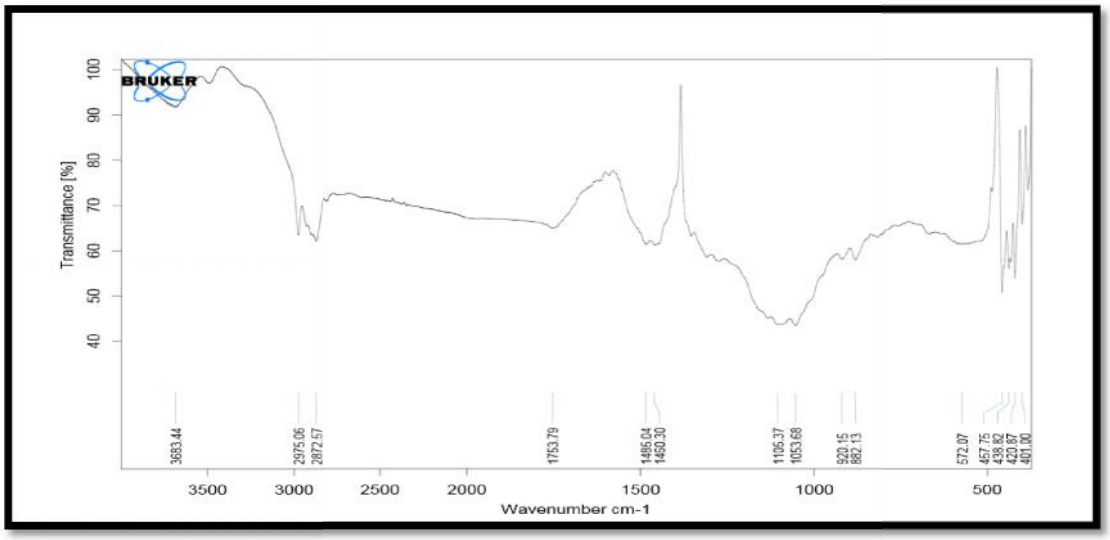

Figure 3FTIR spectra of Ethyl cellulose

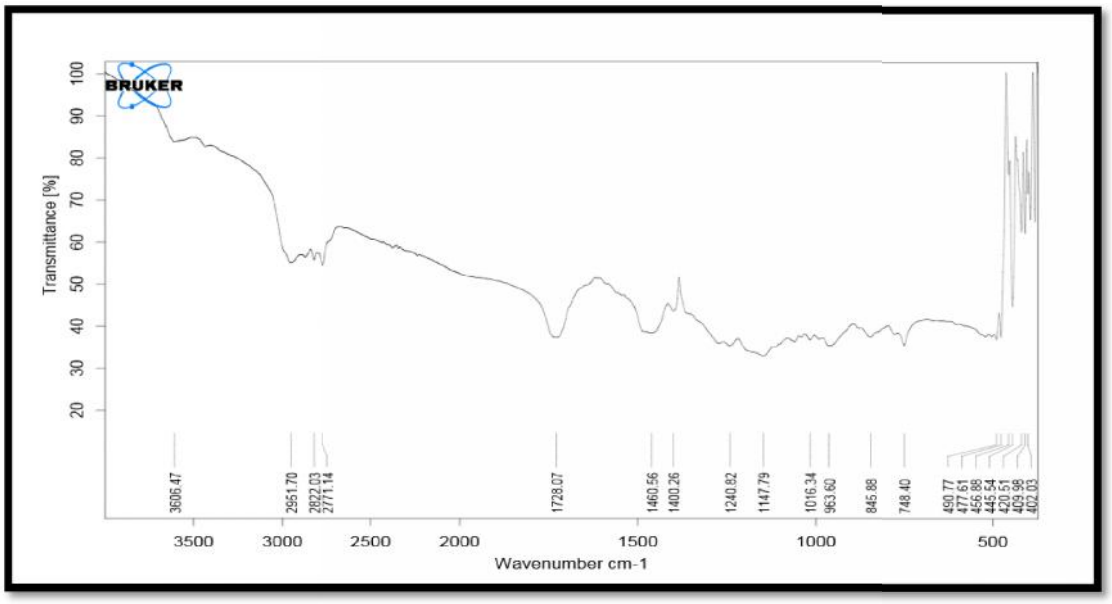

Figure 4: FTIR spectra Eudragit EPO

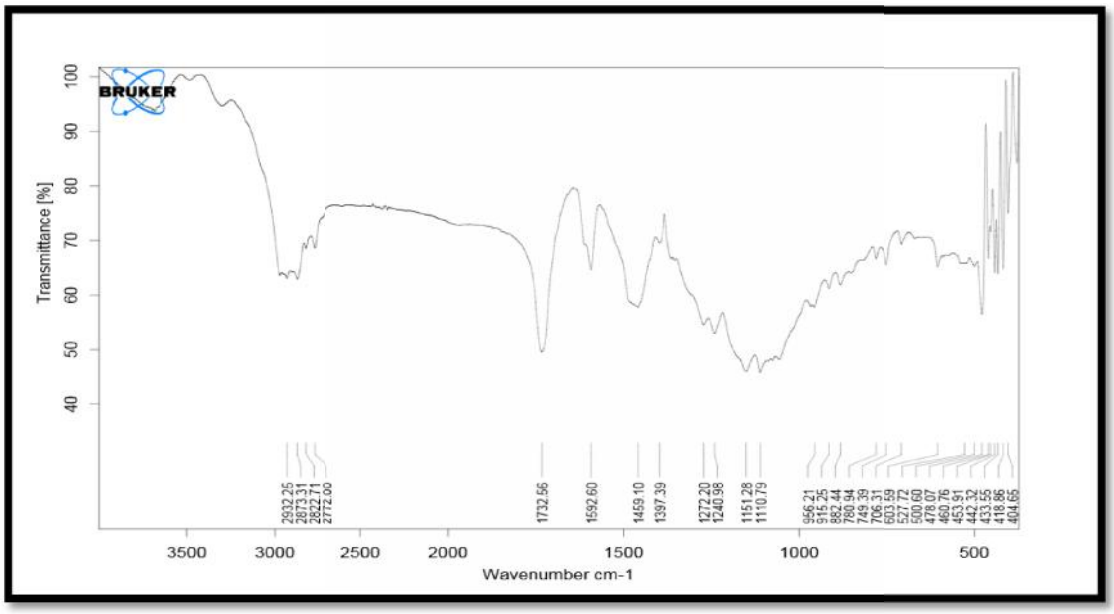

Figure 5: FTIR spectra of drug + physical mixture

Optimization of variables using 3^2factorial design

Optimization was carried out by using 3^2 factorial design using the Design Expert® software (Version 10.0.0, Stat-Ease Inc., Minneapolis,USA) by taking amount of ethyl cellulose (X_1) and amount of Eudragit EPO (X_2) as the independent variables and % entrapment efficiency (Y_1) , % buoyancy (Y_2), and % drug release (Y_3) at 12 hour dependent variables respectively.

Table 1: Optimization of variables using 3^2full factorial design

Batch Code	Factor 1: A Ethyl cellulose (mg)	Factor 2: B Eudragit EPO (mg)	Response 1 % entrapment efficiency	Response 2 % buoyancy	Response 3 % drug release
DP1	150	50	64.29	71.43	72.54
DP2	150	75	77.40	75.06	82.16
DP3	150	100	72.21	70.54	75.39
DP4	175	50	81.05	82.24	85.09
DP5	**175**	**75**	**89.78**	**85.97**	**93.88**
DP6	175	100	84.61	83.74	89.57
DP7	200	50	60.97	63.07	65.09
DP8	200	75	68.80	69.33	73.18
DP9	200	100	63.28	65.14	68.23

Analysis of micromeritic properties of microsponges

Table 2:Micromeritic properties of formulation

Formulation code	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Hausner's ratio	Carr's index	Angle of repose
DP5	0.302	0.354	1.062	5.73	21.56

Evaluation of factorial batches

Table 3: Results of factorial batches

Formulation code	%Practical yield	Particle size	Floating time (hr)
DP1	82.04	26.87	>10
DP2	81.36	29.06	>11
DP3	71.25	59.17	>11
DP4	79.95	34.06	>11
DP5	**91.74**	**43.89**	**>12**
DP6	76.81	62.19	>11
DP7	85.08	37.56	>11
DP8	86.47	57.02	>11
DP9	88.20	70.09	>10

***In vitro* drug release study from factorial formulations (DP1 to DP9)**

All the formulations were evaluated for drug release pattern. The data and curves obtained from *in vitro* release test are as follows.

Table 4: Drug release data of DP1 to DP9 formulation

Time	***In-vitro* dissolution data of batches DP1 to DP9**								
	DP1	**DP2**	**DP3**	**DP4**	**DP5**	**DP6**	**DP7**	**DP8**	**DP9**
0	0	0	0	0	**0**	0	0	0	0
0.5	14.08	17.05	20.78	15.01	**15.25**	15.85	14.95	15.25	14.87
1	17.02	21.55	25.50	17.12	**19.05**	21.52	18.94	17.58	17.50
2	23.17	33.85	38.65	21.56	**28.82**	32.55	20.21	25.69	26.35
3	31.05	41.54	44.33	28.54	**36.55**	38.52	26.33	32.56	33.85
4	38.16	47.04	49.65	34.75	**41.45**	42.44	32.94	38.32	39.15
5	41.30	49.91	52.98	38.58	**49.34**	50.45	39.14	42.58	47.54
6	48.58	52.18	55.55	44.11	**53.27**	56.15	47.58	49.74	50.85
7	60.20	58.56	58.04	58.72	**64.11**	65.65	52.33	52.12	61.09
8	63.18	61.85	61.17	65.26	**76.07**	73.49	55.74	58.25	55.69
9	65.01	64.65	64.08	72.68	**80.05**	80.33	58.19	62.36	58.58
10	71.69	68.50	69.41	78.58	**85.21**	82.82	60.34	66.78	61.25
11	76.65	76.36	73.55	81.22	**89.29**	85.62	62.55	68.28	65.84
12	79.03	82.40	75.20	84.25	**93.88**	89.13	65.71	73.69	69.39

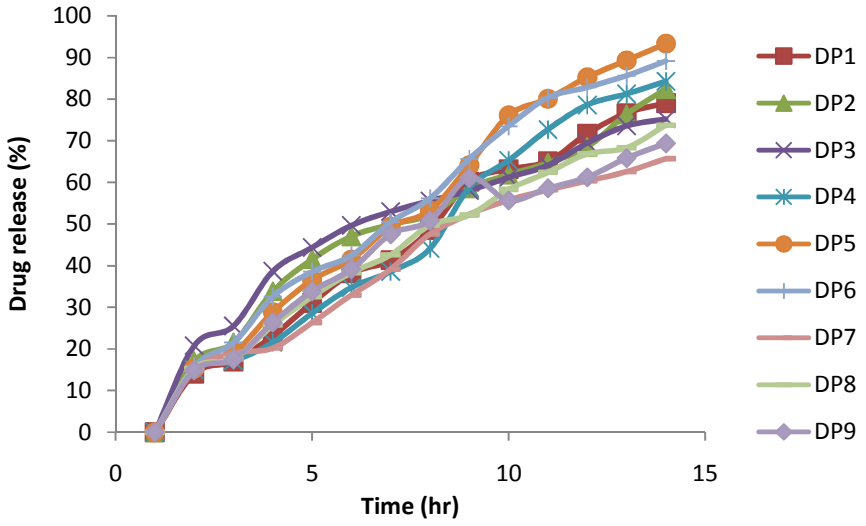

Figure 6: Comparative % drug release profile of DP1 to DP9 formulation

Dissolution is a most important parameter to check out the drug release. Here, Table 5.14 shows dissolution data of all DP1 to DP9 formulation. DP1 to DP3 formulation showed drug release in

short time of duration as these formulations contain low concentration of Ethyl cellulose with different concentration of Eudragit EPO which give faster drug release because of incomplete formulation. DP7 to DP9 formulation showed slow drug releases which contain higher concentration of ethyl cellulose with different concentration of Eudragit EPO. Due to higher concentration of polymer shows agglomeration of formulation, it showed slow drug release from the formulation. DP4 to DP6 formulation contained moderate amount of ethyl cellulose and Eudragit EPO which impart moderate viscosity to formulation and thus satisfactory drug release, entrapment, and buoyancy was observed.

Contour Plots

Contour plots are diagrammatic representation of the values of the response and they are helpful in explaining the relationship between independent and dependent variables. The reduced models were used to plot two dimension contour plots using Design Expert® software. Two dimensional contour plots were established for all the responses as shown in Figure 7, 8 and 9. The contour plots were found to be non-linear for all the responses. So the relationship between independent variables and all the responses are not linear.

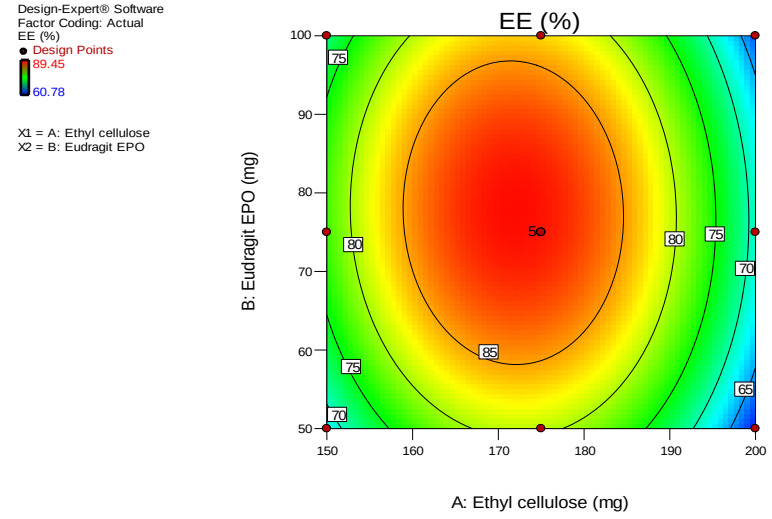

Figure 7:Contour plot showing the effect of Amount of Ethyl cellulose (X_1) and Amount of Eudragit EPO (X_2) on response Y_1

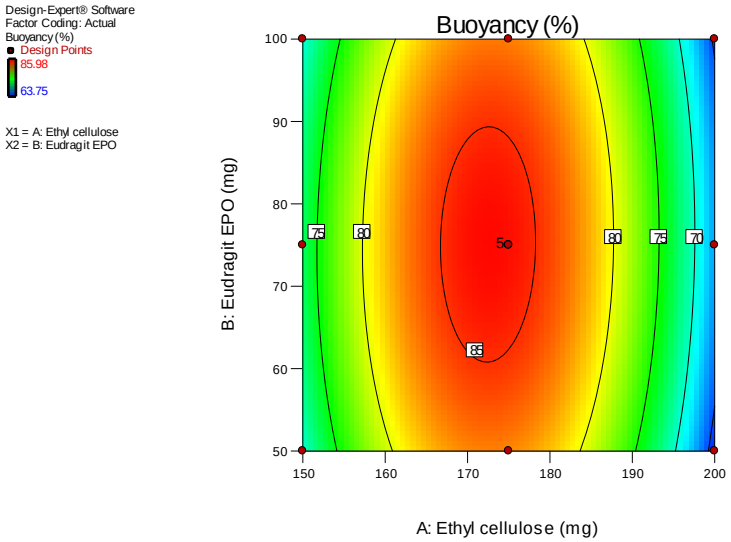

Figure 8: Contour plot showing the effect of Amount of Ethyl cellulose (X_1) and Amount of Eudragit EPO (X_2) on response Y_2

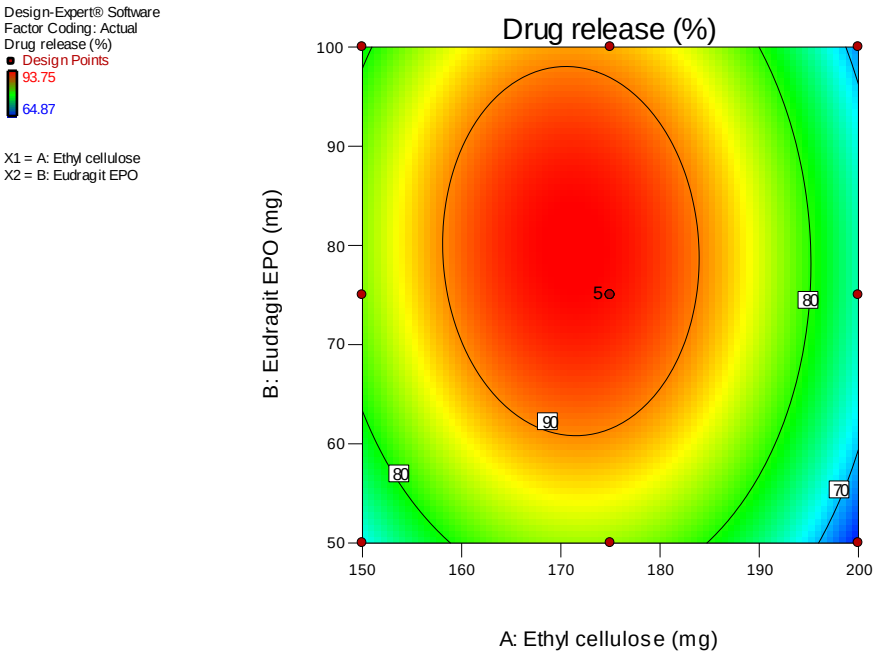

Figure 9: Contour plot showing the effect of Amount of Ethyl cellulose (X_1) and Amount of Eudragit EPO(X_2) on response Y_3

Response Surface Plots

Response surface plots are more helpful in understanding both the main and the interaction effects of variables. The effects of different levels of independent variables on the response

parameters can also be predicted from the respective response surface plots depicted in Figure10, 11 & 12.

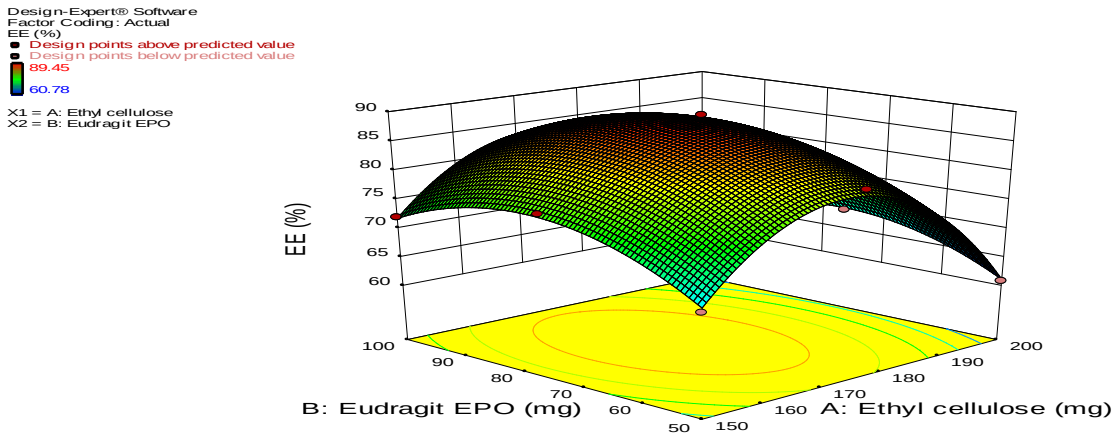

Figure 10: Response surface plot showing the effect of Amount of Ethyl cellulose (X_1) and Amount of Eudragit EPO (X_2) on response Y_1

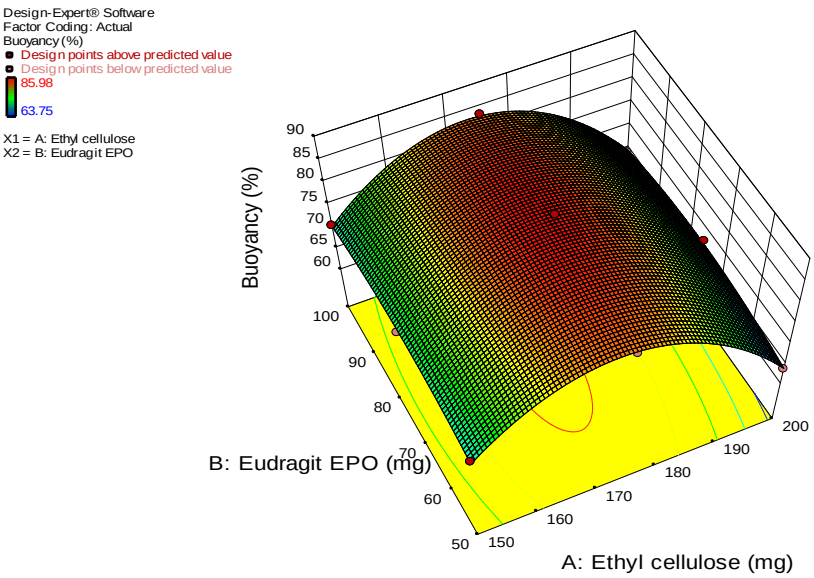

Figure11: Response surface plot showing the effect of Amount of Ethyl cellulose (X_1) and Amount of Eudragit EPO (X_2) on response Y_2

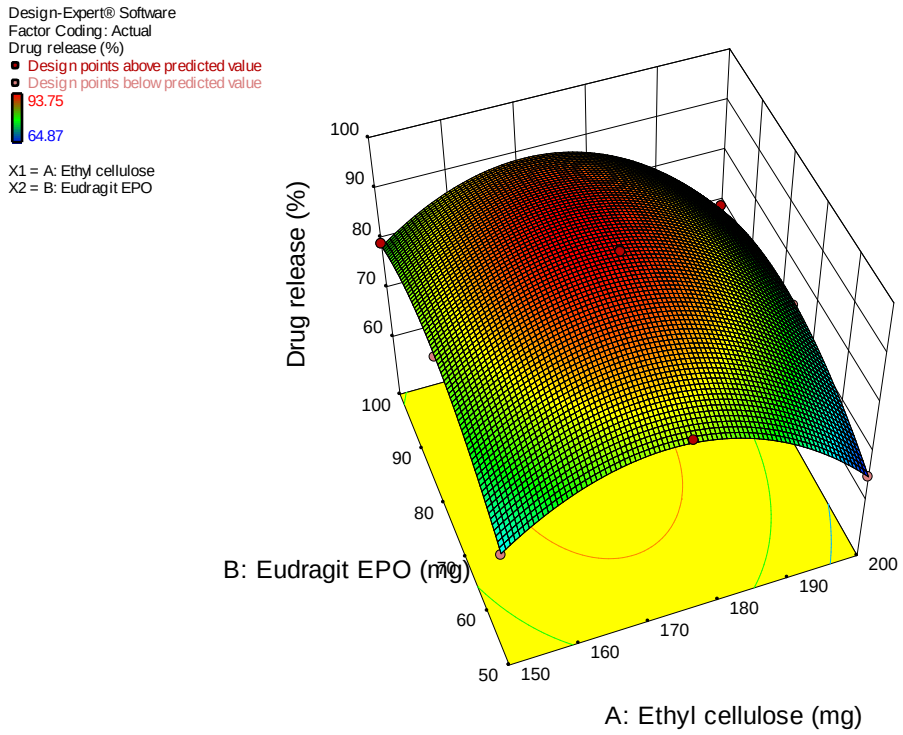

Figure 12: Response surface plot showing the effect of Amount of Ethyl cellulose (X_1) and Amount of Eudragit EPO (X_2) on response Y_3

Optimized Formula

After generating the reduced model polynomial equations to relate the dependent and independent variables, the process was optimized for all three responses. Optimum formulation was selected based on the constraints set on independent variables (Table 5).

Table 5:Constraints for selection of optimum formulation

Name	Goal	Lower Limit	Upper Limit
X_1 = Amount of Ethyl cellulose (mg)	In range	150	200
X_2 = Amount of Eudragit EPO (mg)	In range	50	100
Y_1 = % Entrapment efficiency	Maximum	60.78	89.78
Y_2 = % Buoyancy	Maximum	63.75	85.97
Y3 =% Drug release	Maximum	64.87	93.88

The final optimal experimental parameters were calculated using the extensive grid search and feasibility search provided in the Design Expert software (Table 6).

Composition of final optimized formulation

Table 6: Composition of Final optimized formulation

Ingredients	**Quantity**
Allopurinol	150 mg
Ethyl cellulose	172.18mg
Eudragit EPO	76.65mg
$CaCl_2$	50mg in 5ml water
Tween 80	2ml
DCM	10ml
PVA	1gm in 100ml dis. Water

Evaluation of Optimized batch

Table 7: parameters of optimized formulation

Parameters	**Results**
%Practical yield	95.06%
Particle size	35.24 µm
Bulk density	0.567gm/cm^3
Tapped density	0.502gm/cm^3
Compressibility index	12.5%
Hausner's ratio	1.135
Angle of repose	21.04
Floating time	>12 hr
% entrapment	90.61%
% buoyancy	86.52%
% drug release at 12 hours	94.23%

Microscopy

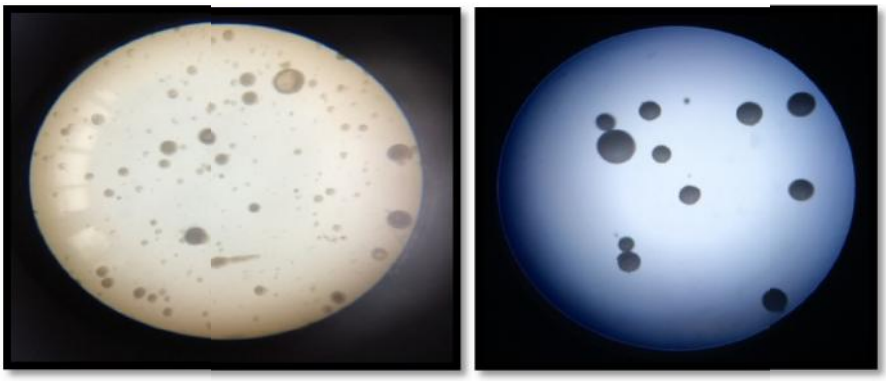

Figure 13: Microscopy of microsponges (a) before drying (B) after drying

Optical microscopy of floating microsponges before drying and after drying is shown in figure 13. It shows that microsponges having spherical shape before and after drying and no significant change in particle size.

Scanning electron microscopy

Optimized floating microsponges formulation surface morphology was evaluated by scanning electron microscope. SEM study at x1000 and x1500 magnification shows that drug is entrapped in to the microsponges with extreme spherical shape and very tiny numerous pores on the surface.

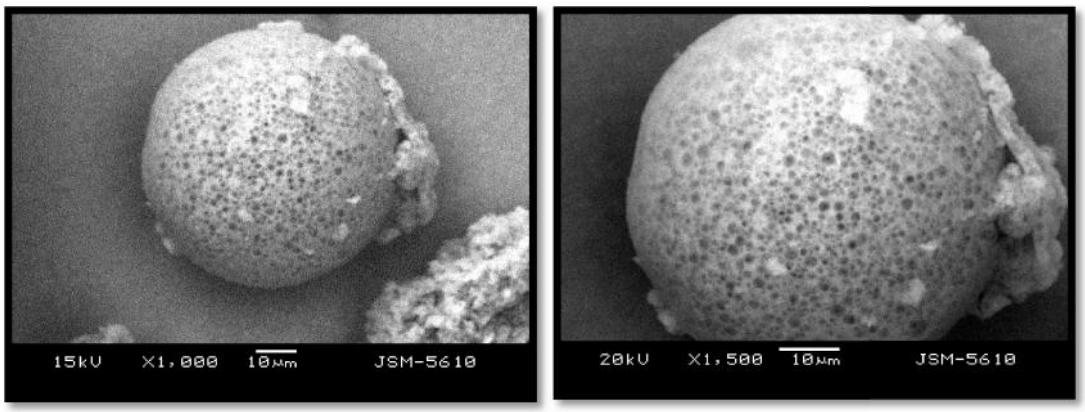

Figure 14: SME image of microsponges

Residual solvent determination

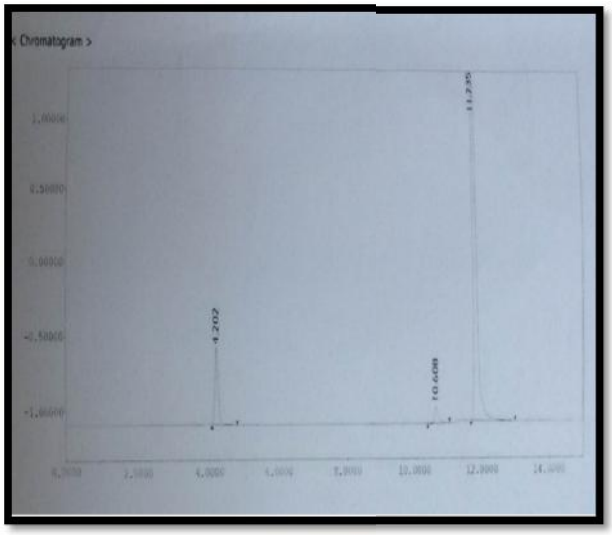

Figure15: GC of Dichloromethane (Reference chromatogram)

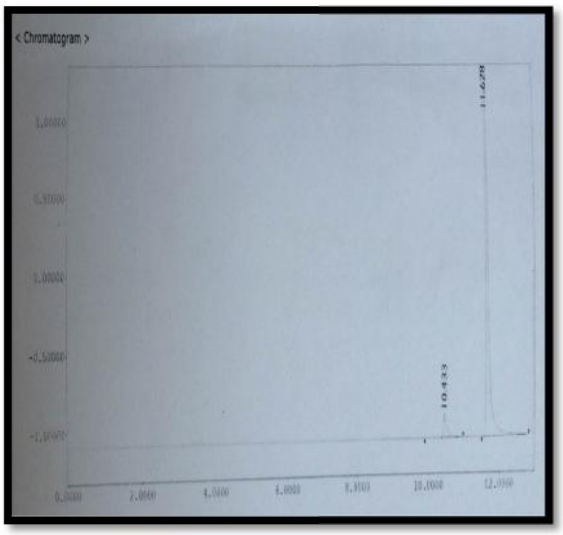

Figure 16: Chromatogram of floating microsponge

Floating microsponges of allopurinol was not containing DCM residue in formulation. Chromatogram of floating microsponges is shown in Figure 16.

Release kinetic of dissolution data

The *in-vitro* dissolution data were analyzed by different kinetic models in order to understand drug release mechanism. The Correlation coefficient (r^2) values of the optimized batches for fit of different kinetic models are given in Table 18.

Table 8: I*n-vitro* dissolution data of different kinetic models

Optimized formulation	**Higuchi (R^2)**	**Zero order (R^2)**	**First order (R^2)**	**Korsmeyerpeppas (R^2)**
PD1	**0.994**	0.884	0.814	0.964

From the above results, the R^2 values indicated that the release data was best fitted with Higuchi kinetic model.

Weight variation

Table 9: Weight variation of capsule containing microsponges

Sr. no	Weight of Filled capsule (mg)	Weight of Empty capsule (mg)	Net content	% deviation of average weight (%)
1	460	99	361	1.0
2	464	98	366	0.4
3	466	100	366	0.4
4	467	99	368	1.0
5	459	100	359	1.5
6	461	99	362	0.7
7	463	99	364	0.1
8	458	98	360	1.2
9	467	97	370	1.5
10	366	100	366	0.4
11	465	99	366	0.4
12	464	98	366	0.4
13	462	98	364	0.1
14	460	99	361	1.0
15	461	99	362	0.7
16	465	99	366	0.4
17	467	97	370	1.5
18	459	98	361	1.0
19	465	100	365	0.1
20	466	99	368	1.0
			Avg=364.50 mg	

As per I.P. weight variation limit for less than 365 mg capsule is 10 %. Table showed that none of the tablets showed % deviation greater than 10%. Thus, the batch passes the variation test.

Content uniformity

Drug content of floating microsponges capsule was found to be 92.36%. For more than 300 mg of capsule drug content is not less than labeled amount of 85% and not more than 110% of labeled amount. Microsponges complies as per the IP limit of content uniformity of Allopurinol.

In vitro drug release of capsule

Table 10: In-vitro drug release of capsule

Time (Hr)	Drug release (%)	Time (Hr)	Drug release (%)
0.25	4.88	6	42.58
0.5	8.30	7	52.05
1	13.17	8	56.47
2	17.27	9	64.77
3	21.58	10	79.98
4	24.36	11	85.34
5	38.70	12	95.53

Table 10 showed 95.53% drug release within 12 hr so % drug release from the capsule of floating microsponges was show excellent sustained drug delivery from the microspongess.

Stability studies

Stability study was carried out for 1 month as shown in Table 11.

Table 11: stability study data

Sr. No.	Parameter	Before storage	After 1 month storage
1	Particle size	37.56µm	37.04µm
2	%Entrapment efficiency	89.25%	86.25%
3	% Buoyancy	85.36%	83.02%
4	% Drug release	94.23%	92.57%

On the bases of this study it was considered that there was no significant change in the formulation and so we can conclude that formulation was stable after 1 month study at accelerated stability study.

CONCLUSION

Allopurinol was successfully formulated in floating microsponges to deliver drug up to 12 hr. From the screening of process parameter and preliminary study conclude that three main prerequisites of floating microsponges of allopurinol were % entrapment efficiency, % buoyancy, and %drug release, and should have optimum result for proper formulation of floating microsponges for sustained drug delivery for 12 hr. From the above study floating microsponges

containing maximum drug content, % buoyancy, %entrapment efficiency and % drug release was found in ethyl cellulose and eudragit EPO as polymer and $CaCl_2$ as pore forming agent and 1% (w/v) concentration of polyvinyl alcohol were selected for appropriate formulation of floating microsponges. For the proper formulation of floating microsponges sufficient concentration of ethyl cellulose and eudragit EPO was used for longer floating and sustained drug delivery. From the factorial design it can be concluded that as the concentration of polymer decreases shows decrease in yield and drug release. As the concentration of polymer increases shows lump formation of the polymer so decrease in entrapment, drug release, and also affect the floating property. Thus, in moderate concentration of polymer shows excellent results of buoyancy and sustained drug delivery. From 3^2 factorial design observed that 172.18 mg of ethyl cellulose and 76.65 mg of eudragit EPO formulation imparts good result for formulation. Evaluations were performed for optimized batch shows 86.52% buoyancy, 90.61% drug entrapment, 94.23 % drug release at 12 hr and floating time up to 14 hr.

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