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Formulation, Optimization and Evaluation of Allopurinol Loaded Nanospoges for the Treatment of Gout

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ABSTRACT
Allopurinol is a poorly soluble drug with a short half life. Allopurinol loaded Nanosponges were prepared by Emulsion Solvent Diffusion method by using Ethyl cellulose as a polymer, Polyvinyl alcohol is a stabilizer and dichloromrthane as a solvent. Box-Behnken Design were used for the optimization of Allopurinol loaded Nanosponges. The prepared Nanosponges were evaluated for Percentage Yield, Entrapment Efficiency, Drug content, Particle Size, In-vitro drug release, Zeta Potential. SEM images showed morphology of Allopurinol loaded Nanosponges. Allopurinol loaded nanosponges would be a alternative delivery system to a conventional oral formulation to improve its bio availability. Scanning Electron Microscopic studies confirmed their porous structure of nanosponges. The spectrum Fourier Transform Infrared Spectroscopy showed the stable character of Allopurinol in a mixture of polymers and revealed the absence of drug-polymer interactions. The mean particle size and Z average of optimized formulation was found to be 181.4 nm. Polydispersity of optimized formulation was found out to be 0.327, indicating uniformity of particle size within formulation. The zeta potential for the optimized formulations was found to be be -25.5 mV which showed that the formulation is stable. In vitro release of the drug of Allopurinol loaded Nanosponges follows the Peppas model and showed the controlled release behavior of 8 hours. The optimized Nanosponges were subjected to stability studies. The purpose of this research was to prepare Allopurinol Nanosponges for controlled release of drug, to improve the solubility, reduce dose dependent, side effects and improve the patient compliance.

Key words - Nanosponges, Box-Behnken Design, Allopurinol, Emulsion Solvent Diffusion method, Release kinetics.

I. INTRODUCTION

Encapsulation of a Drug in Vesicular structure, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity. Nanosponge Drug Delivery system are superior to other system, provides the controlled release pattern with a targeted drug delivery. Nanosponges are nono-sized drug carrier with a three- dimensional structure created by a cross linking polymer, able to hold a variety of drugs at various sizes. The three dimensional structure allows capture, transporatation and selective release of a variety of substances. These sponges are porous particles having high aqueous solubility used mainly to encapsulate the poor soluble drugs[1]. The sponges are self sterilizing as their pore size is about 0.25 µm, where bacteria cannot penetrate. It consists of multiple openings in their structure available in their core, which allow free passage of the drug molecule. Gouty arthiritis, one of the oldest disease was first identified by Egyptians in 2640 B.C. historically, it was termed the "disease of kings", because it was associated with eating rich foods and consumes excessive alcohol beverages. Gout is a disorder of purine metabolism and occurs when its final metabolite, Uric acid crystallizes in the form of Mono Sodium Urate, precipitating and forming deposits (tophi) in joints and surrounding tissues. Joint Fluid Test, Dual-energy computerized Tomography (DECT) are the tests which help to diagnose, Gout. Allopurinol, a Xanthine-Oxidase inhibitor, is a Urate lowering medication, that is FDA approved for managing Gout, preventing Tumour lysis Syndrome and Calcium Nephroliathisis in patients with Hyper uricosuria. Allopurinol inhibits an enzyme of Xanthine-Oxidase that is vital role to form an Uric acid[2]. It is one of the safe medication less adverse effects can occur. The drug is about 90% absorbed from the GI tract. The maximum plasma levels of about 3mcg/ml. It reduces the amount of uric acid in the body and the solid

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

deposits of Mono Sodium Urate crystals in joints, thus improving the joint function.

II. MATERIALS AND METHODS

Allopurinol was gift sample from Par Formulations Private Limited, Chennai. Ethyl cellulose, Poly vinyl alcohol, Dichloromethane were purchased from Loba Chemie Private Limited, Mumbai.

Drug-Excipient Compatability Study

The drug and excipients selected for the formulation were evaluated for chemical compatibility studies by using FT-IR.

Solubilty Studies

Solubility studies of allopurinol was established by different solvent systems such as methanol and purified water as per the standard procedure.

Determination of λ max

100 mg of Allopurinol was weighed and transferred to 100 ml of volumetric flask. The drug was dissolved in 10 ml of methanol and volume was made up to 100 ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000 µg/ml (stock solution I). 10 ml of this stock solution was again diluted with phosphate buffer pH6.8 up to 100 ml to obtain a solution of 100 µg/ml (Stock solution II). From stock solution-II, 10 ml was pipette out in 100 ml volumetric flask. The volume was made up to 100 ml using phosphate buffer pH 6.8 get a concentration of 10µg/ml[3]. This solution was then scanned at 200-400 nm in UV-Visible spectrophotometer to attain the absorption maximum (λ max).

Standard curve for Allopurinol

Preparation of stock solution

100 mg of Allopurinol was weighed and transferred to 100 ml of volumetric flask. The drug was dissolved in 10 ml of methanol and volume was made up to 100ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000 µg/ml (Stock solution I). 10 ml of this stock solution was again diluted with phosphate buffer pH6.8 up to 100 ml to obtain a solution of 100µg/ml (Stock solution II). From stock solution II 5, 10, 15, 20, 25 ml were transferred to series of 100 ml volumetric flasks. The volume was made up with phosphate buffer pH 6.8.The procedure was repeated for 0.1N HCl The absorbance of these solutions was measured at 250 nm against blank.

Preparation of Allopurinol loaded Nanosponges

Allopurinol loaded Nanosponges were prepared by Emulsion solvent diffusion method. The polymer used in the formulation of Allopurinol loaded Nanosponge is Ethyl cellulose. External phase consists of Polyvinyl alcohol (0.4%-0.9%w/v) in distilled water (50ml) was used as the aqueous phase. Internal phase consists of Specified amount of drug and polymer (0.3%-1.5% w/v) which was dissolved in an organic solvent dichloromethane was used as dispersed phase (organic phase). Disperse phase was added drop by drop into aqueous phase by stirring on a magnetic stirrer at 1000-2000 rpm for about 2 hours to remove the solvent dichloromethane from the mixture. The formed Nanosponges were collected by filtration and dried in an oven at 40ºC for about 24 hours to remove the residual solvent dichloromethane[4].

Formulation by Box Behnken Statistical Design

A Box Behnken statistical design was developed to statistically optimize the formulation factors and evaluate the main effects, interaction effects and quadratic effects on the independent variables[5]. The amount of Ethyl cellulose, Polyvinyl alcohol, and Stirring speed were the three factors studied for Allopurinol loaded Nanosponges. The two responses were Entrapment Efficiency, and cumulative percentage drug release.

Percentage Yield

The percentage yield (PY) can be determined by calculating the following formula

Percentage Yield = Practical weight of Nanosponge/ Theoretical mass*100

Determination of Drug Entrapment Efficiency

For the determination of drug entrapment, the nanosponge dispersion with known amount of drug was centrifuged at 4000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 100 ml of phosphate buffer solution pH 6.8 and the absorbance was measured using UV-Visible spectrophotometer at 250 nm using of phosphate buffer solution pH 6.8 as blank. The amount of drug un entrapped was calculated. The percentage of entrapment efficiency was determined according to the following equation given below.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

Entrapment Efficiency (%) = Total amount of drug – Amount of unbound drug/ Total amount of drug*100.

Drug Content Determination

Equivalent to 100 mg of the prepared formulation were weighed and dissolved in minimum quantity of methanol and made up to 100 ml with phosphate buffer pH 6.8. The solution is placed for 24 hours and filter to separate fragments. Drug content was analyzed after suitable dilution by UV- Visible spectrophotometer at a wave length 250 nm against phosphate buffer pH 6.8 as blank. From the absorbance the drug content in the batches were calculated.

Solubility determination of optimized Allopurinol Nanosponges

Solubility of the Allopurinol loaded Nanosponge formulation were tested in various medium (distilled water and phosphate buffer pH 6.8) by adding an excess amount of fomulations. The mixtures were stirred in a mechanical shaker at speed 50 rpm for 24 hours at room temperature. Visual inspection was carefully made to ensure that there were excess Allopurinol solids in the mixture, indicating saturation had been reached. Then the mixtures were filtered using 0.45µm filter and filtrates were suitably diluted with same media. The absorbance of the solution was measured at 250 nm in UV-Visible spectrophotometer.

IN VITRO drug release studies

The in vitro release of Allopurinol from Nanosponges was evaluated using USP Type-I (Basket) dissolution test apparatus. Allopurinol loaded Nanosponges were filled in capsule and placed in a dissolution jar containing 900ml of Hydrochloric acid and Phosphate buffer pH 6.8 as dissolution medium maintained at 37±0.5 ^{0}C and rotated at 50 rpm. 1ml of samples were withdrawn at predetermined intervals up to 8 hrs and replaced with equal amount of Hydrochloric acid and phosphate buffer pH 6.8 for further dissolution testing. The absorbance was determined spectrophotometrically at 250nm.

Morphology of Nanosponge by Scanning Electron Microscopy

SEM analysis is significant for determination of surface characteristics and size of the particle. Scanning Electron Microscopy was operated at 15kV as an acceleration voltage. A aqueous suspension was spread in an equipment cell receiver and dried under vacuum. The sample was shown on a 20 mm thickened gold layer cathodic evaporator attached with a computers which represents the images of the sample[6]. The represented images were recorded and individual Nanosponge diameter was measured.

Particle Size and Polydispersity Index

Particle size (z-average diameter) and Poly Dispersity Index (as a measure of particle size distribution) of Allopurinol loaded Nanosponge dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 nano S (Malvern Instruments, UK) at 25°C[7].

Zeta Potential

For Zeta Potential determination, 1ml of sample of Allopurinol suspension was filled in clear disposable zeta cell, without air bubble within the sample, the system was set at 25^{o}C temperature, an electric field of about 15 V/cm and results was recorded[8]. The more negative zeta potential indicates more stable the Nanosponge formulation[9].

Preformulation study of optimized Nanosponges
Flow property measurements

The flow properties of powders are crucial for an efficient tableting and capsule filling operation. The flow property measurements include bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio[9].

Release kinetics of the optimized formulations

To study the in vitro release kinetics of the optimized Allopurinol loaded Nanosponges, data obtained from dissolution study were plotted in various kinetics models. Based on the highest r^2 values for correlation coefficients, the best-fit model was decided[10].

III. RESULTS AND DISCUSSIONS

Physical Compatability studies were done for 30 days. It shows the Drug and excipients were compatabile with each other. FT-IR spectrum of Allopurinol shows the characteristic peak of 3446.92 cm^{-1} for N-H Stretching; 1589.92 cm^{-1} for C=N Stretching; 1704.70 cm^{-1} for C=O Stretching; 1604.70 cm^{-1} for C=C Stretching (figs.6). The maximum absorbance of Allopurinol was found at 250 nm. The Ultraviolet Spectroscopic method was used to analyze standard Calibration curve of Allopurinol in 0.1 HCl and phosphate buffer pH 6.8. the absorbance of drug in concentration

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

ranging from 5-15 µg/ml was measured at 250 nm. Box Behnken Design are used to optimize the Allopurinol loaded Nanosponges. A total of 15 experimental runs were generated and evaluated using Design Expert Software (version 13; Stat-Ease Inc; Mineapolis, Minnesota) (figs. 7, 8, 9, and 10). Series of nanosponges were prepared By using Emulsion Solvent Diffusion method. Variables used in this design were shown in (Table No.1). The Entrapment Efficiency and In-vitro drug release were shown in (Table No.2). The optimized Allopurinol Loaded Nanosponges prepared by using the Polymer Ethyl Cellulose (1.04 %w/w), polyvinyl alcohol (0.8539 %w/v) and at Stirring Speed of 1272 RPM. The optimized Allopurinol loaded Nanosponges were subjected to FT-IR, it shows the characteristic peak of Allopurinol. Percentage yield of Allopurinol loaded Nanosponges found to be 96.98%. Drug content and Entrapment Efficiency of Allopurinol loaded Nanosponges was found to be 91.25 % and 91.18%. In-vitro release kinetics of optimized formulations was found to be 96.3% at 8 hours, where the pure drug was found to be 64.38% at 30 mins shown in graphical presentation in (figs 1). The Scanning Electron Microscopy images of the Allopurinol loaded Nanosponges were shown in (figs. 2). SEM images revealed that the Allopurinol loaded Nanosponges were Spherical with Numerous pores on surfaces. The PDI of the Optimized Allopurinol loaded Nanosponge was found to be 0.327, indicating the Particle Size uniformity in (figs 3). The Zeta Potential of the optimized Allopurinol loaded nanosponge was found to be -25.5 mV, shows the formulation is stable (figs 4). Preformulation studies of optimized formulation were studied. The optimized formulation have good flow property compared the results. Thus the Nanosponges are filled in hard gelatin capsules. The post formulation studies for capsules of Uniformity of weight was found to be 0.8106±0.001779, Drug content was found to be 91.26±0.374767, and the Disintegration time of the capsules are 8.45 minutes. The data from In vitro release of Optimized formulations were fit into various kinetic models, thus the release kinetics of the optimized formulations was best fitted into Zero order drug release with anomalous diffusion mechanism (Table 3). The optimized Allopurinol loaded Nanosponges was subjected to a Stability study for 6 months the results were shown in (Table 4).

IV. CONCLUSIONS

The purpose of this research was to prepare Allopurinol loaded Nanosponges for controlled release of drug, to improve the solubility, reduce dose dependent side effects and improve the patient compliance. The foregoing results attempt to suggest that for poorly soluble drugs like Allopurinol, Nanosponge approach would be a possible alternative delivery system to conventional oral formulation to improve its bioavailability.

V. ACKNOWELDGEMENT

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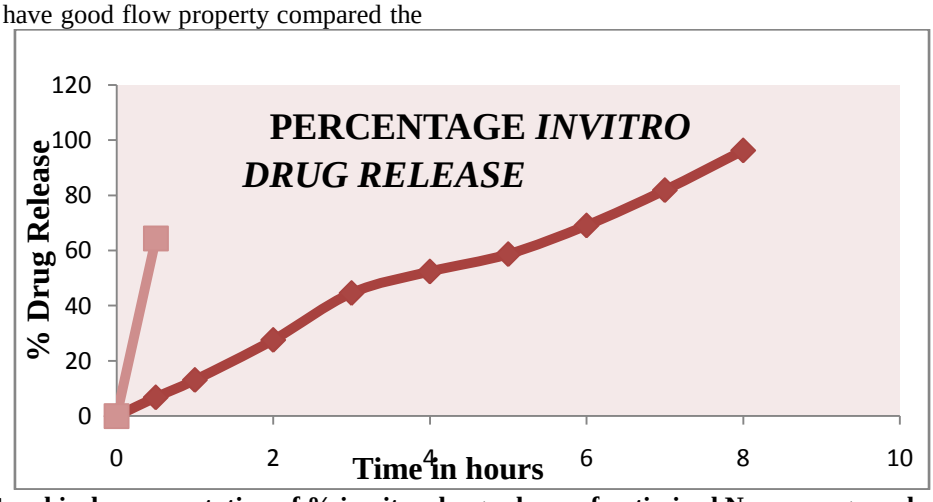

Fig. 1. Graphical representation of % in vitro drug release of optimized Nanosponge and pure drug

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

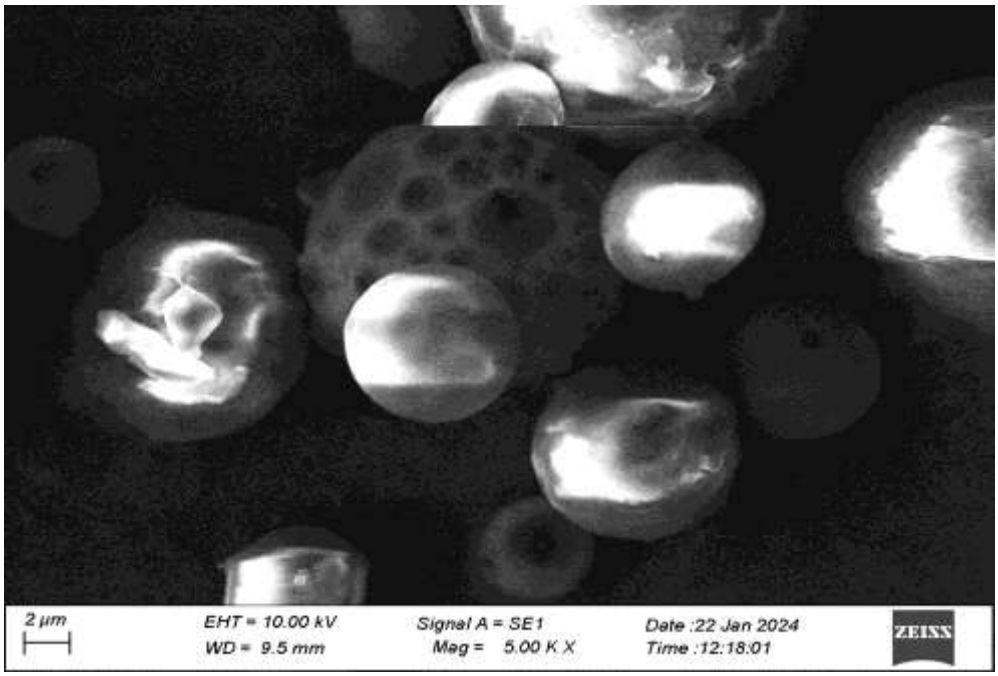

Fig. 2. SEM image of Optimized formulations at 5.0 KX

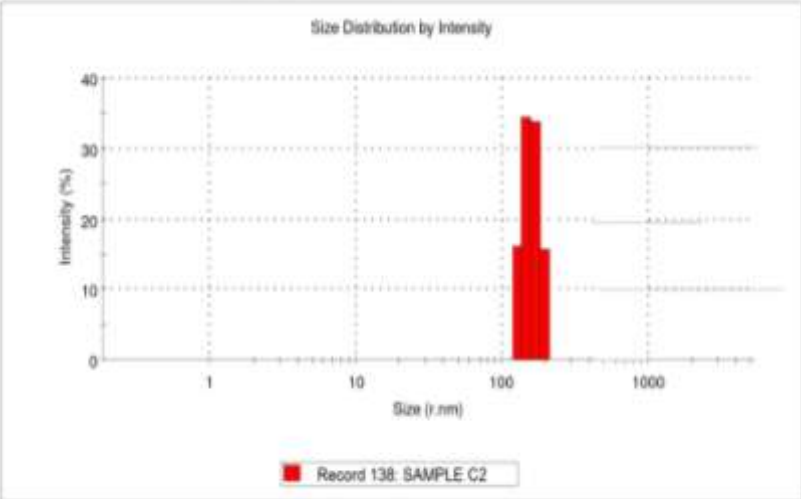

Fig. 3. Particle size distribution of optimized formulation

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

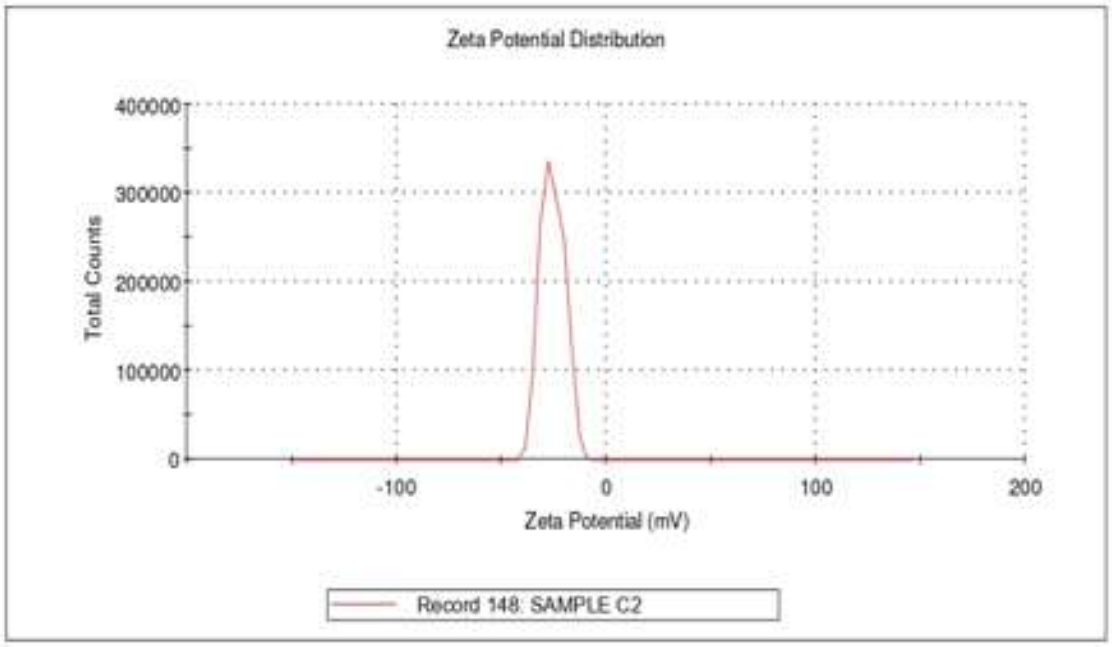

Fig. 4. Zeta Potential of optimized formulation

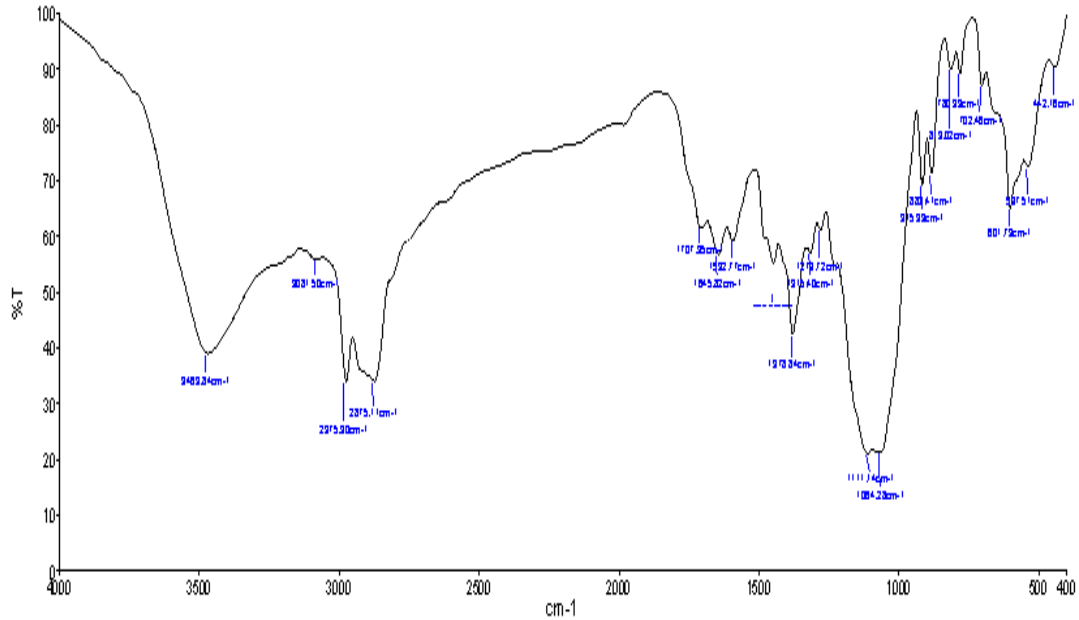

Fig. 5. FT-IR spectrum of optimized formulation

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

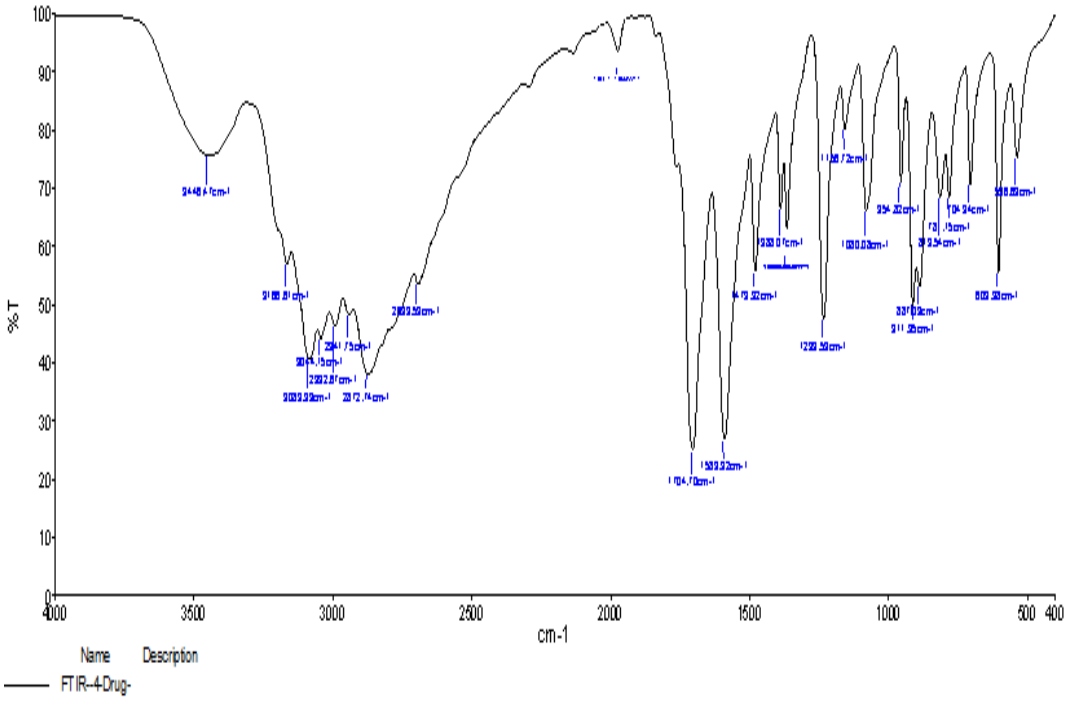

Fig. 6. FT-IR Spectrum of Allopurinol drug

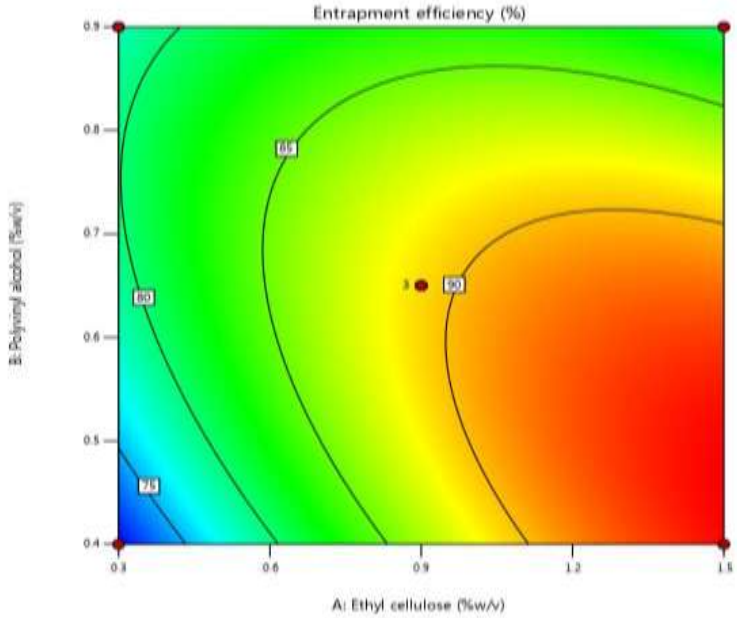

Fig. 7. Contour Plot Ethyl cellulose and polyvinyl alcohol of Response 1 (Entrapment Efficiency)

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

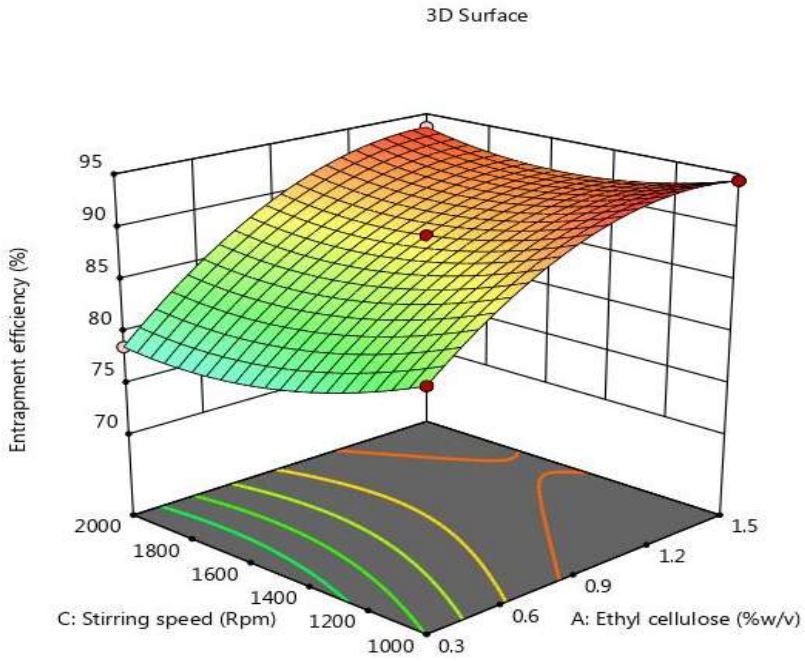

Fig. 8. 3D Plot Ethyl cellulose and stirring speed of Response 1 (Entrapment Efficiency)

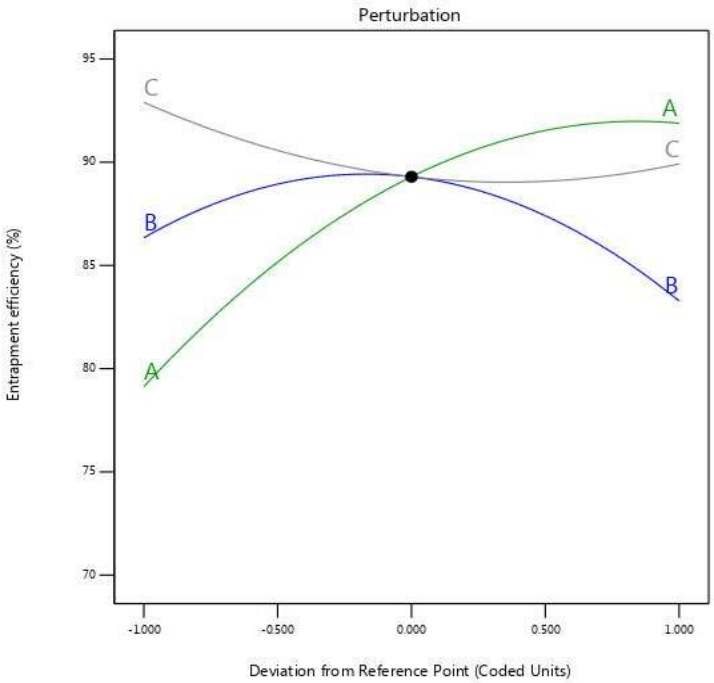

Fig. 9. Perturbation of Response 1 (Entrapment Efficiency)

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

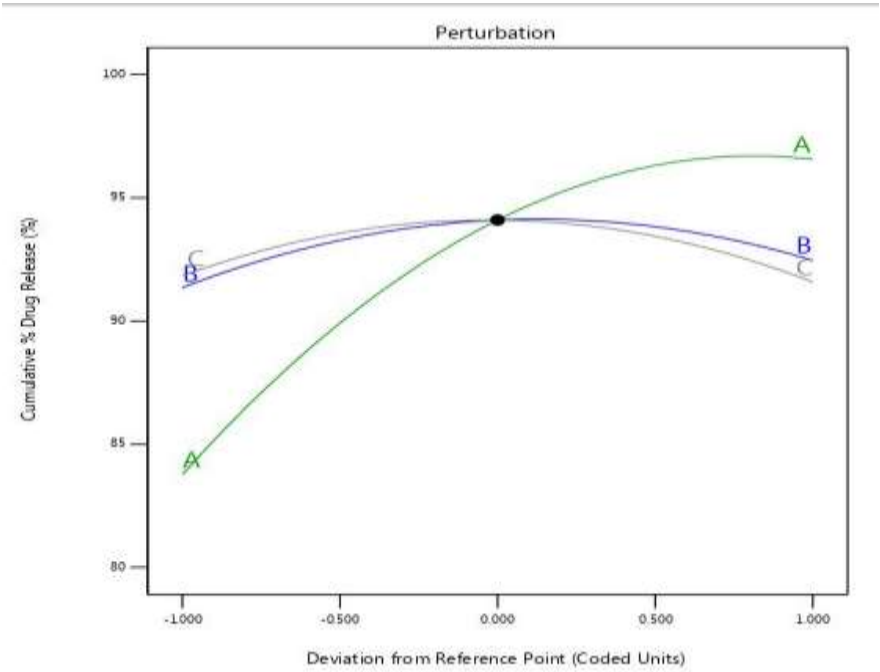

Fig. 10. Perturbation of Response 2 (% CDR)

Table No.1. Variables used in BBD

Variable	Low	Medium	High
A: Amount of ethyl cellulose (%w/v)	**0.3**	**0.9**	**1.5**
B: Amount of Polyvinyl alcohol (%w/v)	**0.4**	**0.65**	**0.9**
C: Stiring speed(rpm)	**1000**	**1500**	**2000**

Table No. 2. Actual summary of Box Behnken design for Allopurinol loaded Nanosponges

Formulation Code	Response 1	Response 2
	Entrapment Efficiency (%)	Cumulative %Drug release (%)
F1	89.3	92.7
F2	80.6	94.9
F3	84.88	91.1
F4	94.2	93.8
F5	78.4	81.7
F6	85.9	89.5
F7	90.92	89.98
F5	93.63	94.3
F9	86	87.5
F10	83.87	81.5
F11	94.38	94.1
F12	89.29	94.8
F13	89.3	94.8
F14	78.62	81.2
F15	70.9	81.5

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1772-1782 www.ijprajournal.com ISSN: 2456-4494

Table No.3. Kinetic Models

Kinetic Models	Coefficient of determination ($R^{2)}$ of optimized formulations
First order	0.919
Zero order	0.984
Higuchi	0.851
Korsmeyer- peppas kinetics	0.986
Hixson Crowell	0.769

Table No.4. Stability studies of optimized formulation

Stability conditions	Entrapment Efficiency (%)			Drug content (%)		
Optimized formulations	**Initial**	**After 15 days**	**After 30 days**	**Initial**	**After 15 days**	**After 30 days**
At room temperature	91.18	90.87	90.57	97.64	97.61	97.27
40±2^{0}C/75±5%RH	91.18	91.12	91.02	97.64	97.58	97.45
4±2^{0}C	91.18	91.17	91.15	97.64	97.61	97.61

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Tables and figure titles: