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Preparation and Characterization of IPN microspheres containing Miglitol by using *in house* synthesized acrylamide grafted ghatti gum

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ABSTRACT

The main objective of this study is to improve the physicochemical stability, swelling and drug release pattern of the polymers in biological condition by Hybridization. In this study, interpenetrating polymer network (IPN) of acrylamide grafted ghatti gum (Am-g-GG) and poly vinyl alcohol (PVA) was developed by emulsion crosslinking method. Glutaraldehyde was used as the crosslinking agent. Experiments were performed according to a 2^3 factorial design to evaluate the effects of GG:PVA ratio, Glutaraldehyde and drug loading percentage on the percent Drug entrapment efficiency, percentage of Swelling at pH 1.2 & pH 6.8 and percentage Cumulative drug release. The effect of the three independent variables on the response variables was studied by response surface plots and contour plots generated by the Design-Expert software. The desirability function was used to optimize the response variables. The compatibility between Miglitol and the excipients was confirmed by differential FTIR spectroscopy analysis. The prepared IPN microspheres showed well controlled release characteristics and continued to drug release following a diffusion-controlled release pattern. The drug release was for a prolonged time without collapsing the IPN matrix. The observed responses taken were in good agreement with the experimental values. Thus, Miglitol IPN microspheres were produced with fewer experimental trials, and a patient compliant product with good stability was achieved with the concept of formulation by design.

Keywords: Miglitol, Acrylamide grafted ghatti gum, Poly vinyl alcohol, Interpenetrating polymer network, Microsphere.

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INTRODUCTION

Over the past decades, blends have been investigated to satisfy the need of specific sectors of polymer industry. Such polymeric blends showed superior performances over the conventional individual polymers and consequently, the range of applications have grown rapidly for such class of materials. In the recent years, carbohydrate and biodegradable hydrophilic polymers have been extensively used to develop the controlled release formulations of drugs having short plasma life. Among the various polymers employed, hydrophilic biopolymers are quite suitable because they are non-toxic and acceptable by the regulating authorities.

Interpenetrating polymer network (IPN) is regarded as one of the most useful novel biomaterials[1]. The excellent biocompatibility and safety due to its physical characteristics such as impart stability of the drug in the formulations improves the solubility of hydrophobic drugs, excellent swelling capacity, and its biological characteristics, like biodegradability, impart bioavailability, drug targeting in a specific tissue, and very weak antigenicity, made IPN the primary resource in pharmaceutical applications. The potential applications of IPN as drug delivery systems especially for the controlled release drug delivery systems.

Controlled release drug delivery employs drug-encapsulating systems from which therapeutic agents may be released at controlled rates for long periods of time, ranging from days to months. Such systems offer numerous advantages over traditional methods of drug delivery, including tailoring of drug release rates, protection of fragile drugs and increased patient comfort and compliance[2].

Recently, a large number of IPN microspheres have been developed using different polymer combinations for drug delivery purpose. Among them, Poly vinyl alcohol (PVA) based IPN systems are extensively studied. A combination of PVA with natural polymer such as Ghatti gum (GG) may provide the system with better stability and improved mechanical strength to meet the major objectives of controlled release drug delivery[3].

Ghatti gum is a water-soluble complex polysaccharide exuded from the bark of the tree *Anogeissus latifolia* of the family Combretaceae. Ghatti gum is almost odourless and has a bland taste. The commercial powder is 140 mesh and varies from buff to dark brown. The lightest colour has the least impurities and the greatest effectiveness in most applications. Ghatti gum is a calcium-magnesium salt composed of L-arabinose, D-galactose, D-mannose, D-xylose, and D-glucuronic acid in a molar ration of 10:6:2:1:2 and less than 1% of 6-deoxyhexose[4].

In the present study, Ghatti gum was grafted with Acrylamide to enhance the aqueous solubility of the resultant polymer and this combination with PVA was used for the hybridization in order to

develop IPN based microspheres. The hybridization helps to provide sufficient integrity and make the IPN matrix cross linkable to the delivery device during its GI residence[5].

Miglitol is commonly prescribed to diabetic patients, as it reduces postprandial hyperglycaemia by inhibiting alpha-glucosidase in the small intestine, and thereby prolongs carbohydrate absorption. Miglitol was approved as an anti-diabetic drug in 1996 and has since been sold in Japan, the USA, Australia, France, Germany, Spain, Switzerland, and Mexico. Furthermore, there is growing evidence that Miglitol also exerts an anti-obesity effect based on both animal and human studies.

In the development of a dosage form, a critical issue is to design an optimized pharmaceutical formulation in a short time period with marginal trials. Due to the complication in the development of pharmaceutical formulations, some computer-based optimization techniques based on response surface methodology (RSM) representing the use of appropriate experimental designs and applying polynomial equation have been widely used.

Factorial designs, dealing with factors in all possible combinations, are the most efficient in estimating the influence of individual variables and their interactions using nominal experiments. The applicability of factorial design in the development of pharmaceutical formulation has helped in understanding the link between the independent variables and the responses to them. The independent variables are manageable, whereas responses are dependent. The technique needs minimum experimentation and time, thus establishing far more cost-effective formulation than the conventional methods of formulating dosage form[6].

With the help of the full factorial design, the IPN microspheres of Am-g-GG and PVA containing Miglitol were developed by emulsion crosslinking method and the microspheres were evaluated for their drug entrapment efficiency, swelling, Fourier transform infrared spectroscopy (FTIR) profile. An *in vitro* drug release study [in both acidic media (pH 1.2) and phosphate buffer (pH 6.8)] and kinetic modelling were performed to understand the drug release mechanism. The effect of all the independent variables on the dependent variables was studied by response surface plots and contour plots generated by the Design-Expert software. To optimize the response variables, the desirability function was used.

MATERIALS AND METHOD

Miglitol, Ghatti gum, poly vinyl alcohol (PVA), Hydrochloric acid (HCl), Light liquid paraffin, Glycine, Acetone, Span 80 and Glutaraldehyde were purchased from Yarrow chemicals. All other chemicals and reagents used were of analytical grade.

METHODS:

Preparation of Acrylamide grafted Ghatti gum:

1 gm of Ghatti gum was dissolved in 120ml of distilled water and stirred for half an hour using magnetic stirrer. The specified amount of acrylamide was dissolved in 30ml of distilled water and then added to aqueous dispersion of ghatti gum and stirred for about one hour. 300 mg/150 mg of CAN was dissolved in 30ml of water and mixed with the gum and acrylamide solution. The dispersion was irradiated at 480W using microwave for specified time period (1 min heating, 1 min cooling and 1 min heating). Then irradiated samples were left overnight at ambient temperature and then precipitated using acetone (200-250ml). Then it was washed with absolute and 30% ethanol for the removal of unreacted acrylamide. The prepared copolymer was dried in hot air oven at 60 ^{0}C and converted into fines[7,8,9].

The grafting efficiency (percent GE) was then calculated by using the formula:

Percentage Grafting efficiency = [{Mass of graft co polymer} / {Mass of (Acrylamide + GG)}] x 100

Table 1: Preparation details of Acrylamide grafted Ghatti gums

Preparation Code	Ceric ammonium nitrate(in mg)	Irradiation Time(in mins)	Acrylamide (in g)
GG1	150	2.5	5
GG2	300	2.5	5
GG3	150	5	5
GG4	300	5	5
GG5	150	2.5	10
GG6	300	2.5	10
GG7	150	5	10
GG8	300	5	10

Full factorial design for the preparation of acrylamide grafted GG:

Factorial design is a popular and widely used experimental design in which, different levels of a variable factor are combined with all other factors of every other variable in the experiment. In the present study, two-level, three-factor, full factorial design (8 batches) was used for the optimization of acrylamide grafting onto Ghatti gum. The amount of acrylamide, ceric ammonium nitrate and microwave irradiation time were selected as the independent variables and percentage of Grafting efficiency was selected as the dependent variable [10] (Table 2).

Table 2: Full factorial design for the Acrylamide grafted Ghatti gums - Independent variables with their levels

Independent factors	Levels	
	Low level (-1)	High level (+1)
Ceric ammonium nitrate (in mg)	150	300
Irradiation time (in minutes)	2.5	5
Acrylamide (in g)	5	10

Each dependent factor was studied at two levels, high level (+1) and low level (-1). Polynomial models including interactions and quadratic terms were generated for the dependent variable using multiple linear regression analysis (MLRA) approach. The results of response generated using the experimental designs were analysed by factorial models using Design Expert software (Trial version 11.1.2.0 64-bit, Stat-Ease, Inc., Minneapolis, USA).

Preparation of Acrylamide grafted GG-PVA IPN microspheres containing Miglitol:

Miglitol entrapped IPN microspheres of PVA and Am-g-GG was developed by water-in-oil (w/o) emulsion-crosslinking method. 20 mL of 2% (w/v) aqueous polymeric solution (total polymer amount was kept constant) was prepared by dispersing varying amounts of Am-g-GG in aqueous PVA solution. The required amount of Miglitol will be added to the polymeric dispersion. (Table 3)

Table 3: Formulation details of Am-g-GG-PVA IPN microspheres containing Miglitol

Formulation Code	Am-g-GG:PVA	Drug loading (in %)	Glutaraldehyde (in mL)
F1	1:1	2.5	25
F2	1:2	2.5	25
F3	1:1	5	25
F4	1:1	2.5	50
F5	1:2	5	25
F6	1:2	2.5	50
F7	1:1	5	50
F8	1:2	5	50

The drug-polymer blend must be slowly emulsified with light liquid paraffin containing 1% (w/w) Tween-80 under constant mechanical stirring at 500 rpm. A milk white emulsion (w/o) will be obtained. To this emulsion, Glutaraldehyde (GA) (2.5 and 5 mL) containing 0.5 mL of 1 N HCl shall be added slowly and stirring must be continued for 3 hours.

The crosslinked microspheres then filtered and washed with acetone, 0.1 M glycine solution and water to remove excess amount of liquid paraffin, unreacted GA and surfactant, respectively. Complete removal of unreacted GA was confirmed by treating the filtrate with Fehling's reagent. A negative result assured the absence of unreacted GA. Hardened microspheres were vacuum-dried at 40°C for 24 hours and stored in desiccator until further use. The absence of unreacted GA was confirmed in dried particle matrix by making an aqueous dispersion of crushed dried particles and treating it in similar way as said earlier.

Full factorial design for the preparation of Am-g-GG-PVA IPN microspheres containing Miglitol:

As like the previous factorial design used in the experiment, where it was used for the optimization of acrylamide grafting onto GG, here, it was used to find out the optimized formula for the preparation of Am-g-GG-PVA IPN microspheres containing Miglitol. In the present study, two-level [High level (+1) & Low level (-1)], three-factor, full factorial design (8 batches) was used for the optimization process. GG: PVA ratio, Glutaraldehyde and drug loading percentage were selected as Independent variables[11,12] (Table 4). The dependent variables (responses) selected were percentage Drug entrapment efficiency, percentage of Swelling (pH 1.2 & pH 6.8) and percent Cumulative drug release at 12 hours. Polynomial models including interactions and quadratic terms were generated for the dependent variable using multiple linear regression analysis (MLRA) approach. The results of response generated using the experimental designs were analyses by factorial models using Design Expert software (Trial version 11.1.2.0 64-bit, Stat-Ease, Inc., Minneapolis, USA).

Table 4: Full factorial design for the Acrylamide grafted Ghatti gums - Independent variables with their levels

Independent factors	Levels	
	Low level (-1)	High level (+1)
Am-g-GG:PVA	1:1	1:2
Drug loading (in %)	25	50
Glutaraldehyde (in mL)	2.5	5

Fourier transform infrared spectroscopy (FTIR) Studies:

The FTIR spectrums of Miglitol, PVA, Am-g-GG, drug-polymer physical mixture, blank microspheres, and drug loaded IPN microspheres were carried out by to confirm the formation of Am-g-GG and compatibility of different ingredients of the IPN formulations. A small amount of each material was mixed with potassium bromide (KBr) (1% w/w sample content), taken into sample holder and scanned in the range of 600-4000 cm^{-1}.

Percentage yield of Microspheres:

The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by total amount of drug and polymers which were used for the preparation of the microspheres to obtained percentage yield.

Percentage Yield = (weight of microspheres/ weight of drug+ weight of polymer) x 100

Drug entrapment efficiency (percentage of DEE):

IPN microspheres were crushed in mortar and pestle and a required amount (10 mg) was taken into 50 ml of phosphate buffer solution (pH 6.8), heated at 50 ºC for effective drug extraction. After 24 hours, the suspension was allowed for filtration and centrifugation for the removal of polymeric

debris. The supernatant was then analysed with a spectrophotometer (UV-1800, Shimadzu, Japan) at λ_{max} of 282 nm. All samples were analysed in triplicate. The percentage drug entrapment efficiency was calculated by the formula given below [12]:

Percentage of DEE = (Actual drug content / Theoretical drug content) x 100

Equilibrium swelling studies:

Equilibrium swelling study of IPN microspheres was done in different media. An accurately weighed amount of microspheres (W_1) was immersed in 50 mL buffer (pH 1.2 and pH 6.8) and allowed to swell for 24 hours at 37± 0.5 ºC. The swollen microspheres were collected and the adhered liquid droplets on the surface of the particles was removed carefully with tissue paper and reweighed (W_2) to an accuracy of ±0.01 mg on an electronic microbalance. All the samples were analyzed in triplicate. The swelling index was calculated by using the following equation [13]:

Percentage Swelling = ($W_2 – W_1$ / W_1) x 100

Where, W_1 and W_2 are the dry weight and swollen weight of the IPN microspheres.

***In vitro* drug release study:**

In vitro drug release was performed in triplicate in a dissolution tester equipped with eight baskets (glass jars) at the stirring speed of 50 rpm. The drug release from the IPN microspheres were investigated in acidic medium (pH 1.2) for the initial 2 hours, to be followed by using phosphate buffer of pH 6.8. Throughout the experiment an accurately weighed quantity of each sample (equivalent to 100 mg Miglitol) was placed in 900 mL of dissolution medium maintained at 37± 0.5 ºC. At regular intervals of time, sample aliquots were withdrawn and analysed using UV spectrophotometer (UV-1800, Shimadzu, Japan) at the fixed λ_{max} of 282 nm.

Release kinetics:

To understand the mechanism of drug release, *in vitro* drug release data have been analysed using the empirical kinetic equations. The regression factor (R^2) of zero order, first order, Higuchi & Korsmeyer peppas plot was calculated along with the n value for Korsmeyer peppas plot.

Optimization data analysis:

Various response surface methodology computations for the current optimization study were performed employing Design-Expert software (Trial version 11.1.2.0 64-bit, Stat-Ease, Inc., Minneapolis, USA). The polynomial equation was used to draw conclusion after considering the intensity of coefficient and the mathematical sign it carries, that is, positive or negative. A positive sign signifies synergesis. Statistical validity of the polynomials was established on the basis of ANOVA provided in the Design Expert software. Level of significance was considered at $P <$

0.05. Also, three-dimensional response surface graphs and contour plots were generated by the Stat-Ease Design-Expert software.

Stability study:

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors. To assess the drug and formulation stability, stability studies were done as per ICH guidelines. The formulated IPN microspheres were wrapped in aluminum foil and stored at 45 ± 0.5ºC for period of twelve weeks. After the period of three month, the prepared IPN microspheres were tested for drug entrapment efficiency [14].

RESULTS AND DISCUSSION

Grafting efficiency of prepared Acrylamide grafted GG (percentage GE):

Table 5: Grafting efficiency percentage of prepared Acrylamide grafted gums

Preparation Code	Grafting efficiency (in %) ± SD, n = 3
GG1	78.12 ± 1.23
GG2	84.35 ± 0.68
GG3	73.89 ± 1.56
GG4	76.91 ± 0.42
GG5	86.15 ± 0.66
GG6	89.05 ± 1.03
GG7	80.75 ± 0.97
GG8	82.78 ± 0.55

Table 5 represents the grafting efficiency of different prepared acrylamide grafted gum. The grafting efficiency of the prepared formulations (G1-G8) ranges from 73.89 ± 1.56 (G3) to 89.05 ± 1.03 (G6). As said earlier in the methodology, factorial design was used to optimize the GE using three different variables at 2 levels. Their mathematical relationship with GE was generated using multiple linear regression analysis (MLRA) and was expressed as:

Percentage GE = 81.50 + 1.77 A – 2.92 B + 3.18 C – 0.54 AC +0.08 BC

Where, A is amount of CAN (mg), B is irradiation time (min) and C is amount of Acrylamide (g). The three-dimensional plot is shown in figure 1. The impact of CAN and Acrylamide were positive, which was due to the formation of a greater number of free radical sites and attachment of Acrylamide side chains. While irradiation time had the negative impact on GE, which was due to frequent breakage of chain under microwave irradiation.

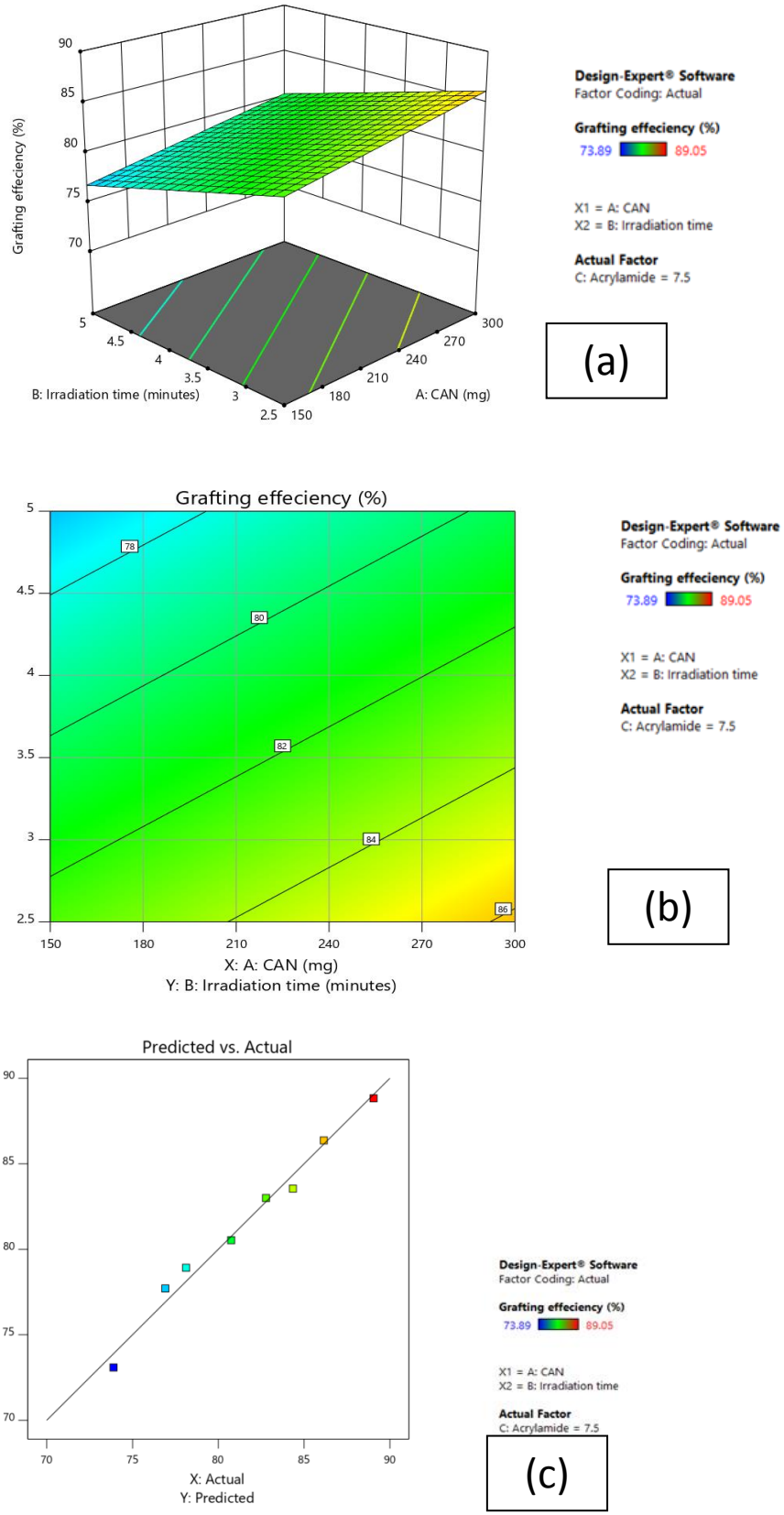


Figure 1: (a) Three-dimensional response surface plots: showing the effects of synthetic condition on Grafting efficiency, (b) Corresponding contour plot showing the relationship

between various levels of the factors, (c) Plot between observed and predicted values of Grafting efficiency.

ANOVA analysis indicated that the factorial model was significant (P = 0.0381, i.e. P < 0.05) having R^2 value of 0.9846. The Predicted R^2 of 0.7533 is in reasonable agreement with the Adjusted R^2 of 0.9460; i.e. the difference is less than 0.2. (Table 6)

Table 6: Results of analysis of variance (ANOVA) for measured responses

Parameter	Sum of squares	df	Mean square	F value	Significance F (p value)
A. Acrylamide grafted Ghatti gums					
Grafting efficiency (in %)					
Model	176.59	5	35.32	25.54	0.0381
Residual	2.77	2	1.38	-	-
Cor total	179.35	7	-	-	-
B. Am-g-GG-PVA IPN microspheres containing Miglitol					
Swelling at pH 1.2 (in %)					
Model	2297.68	4	574.42	22.62	0.0141
Residual	76.17	3	25.39	-	-
Cor total	2373.85	7	-	-	-
Swelling at pH 6.8 (in %)					
Model	2139.47	4	534.87	166.49	0.0007
Residual	9.64	3	3.21	-	-
Cor total	2149.11	7	-	-	-
Drug entrapment efficiency (in %)					
Model	247.85	4	61.96	27.03	0.0109
Residual	6.88	3	2.29	-	-
Cor total	254.72	7	-	-	-
Cumulative Drug release (CDR) at 12 hours (in %)					
Model	749.66	4	187.41	25.20	0.0121
Residual	22.31	3	7.44	-	-
Cor total	771.97	7	-	-	-

Adequate precision measures the signal to noise ratio of the model. A ratio greater than 4 is desirable. The ratio of the model 15.46 indicates an adequate signal. Thus, model can be used to navigate the design space.

After generating the model polynomial equations to relate the dependent and independent variables the best optimized amount of independent variables to achieve the maximum grafting efficiency. The software generated solution shows the formulation having 150 mg of CAN, 2.5 minutes of irradiation time and 8.605 grams of acrylamide can satisfy the required conditions, which was in good and close agreement with the GG5, which had good desirability of 0.704.

Hence, the formulation GG5 was considered for the preparation of the IPN microspheres containing Miglitol.

FTIR Studies:

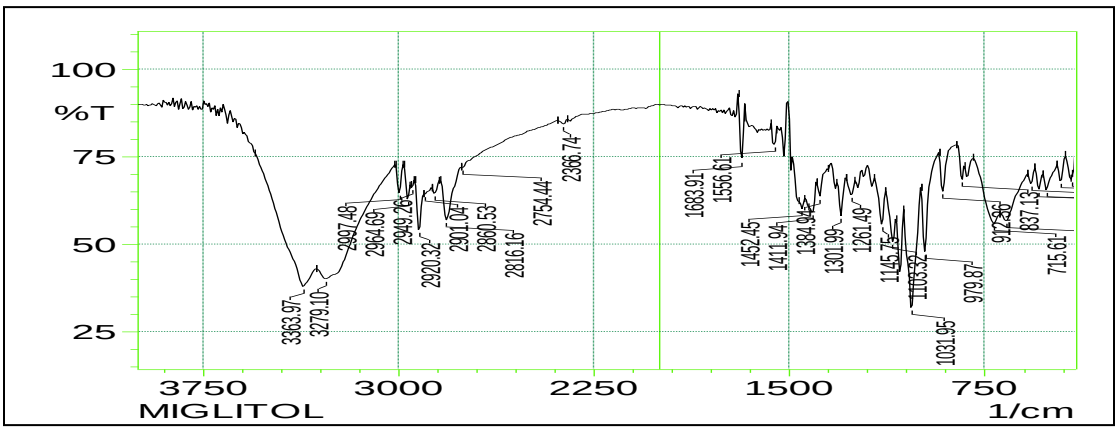


Figure 2a) Miglitol

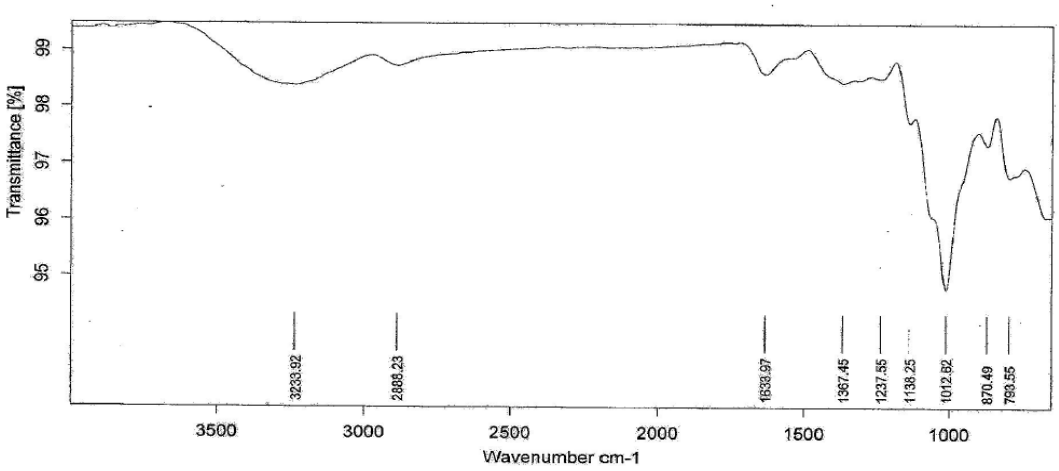


Figure 2b) GG- Ghatti Gum

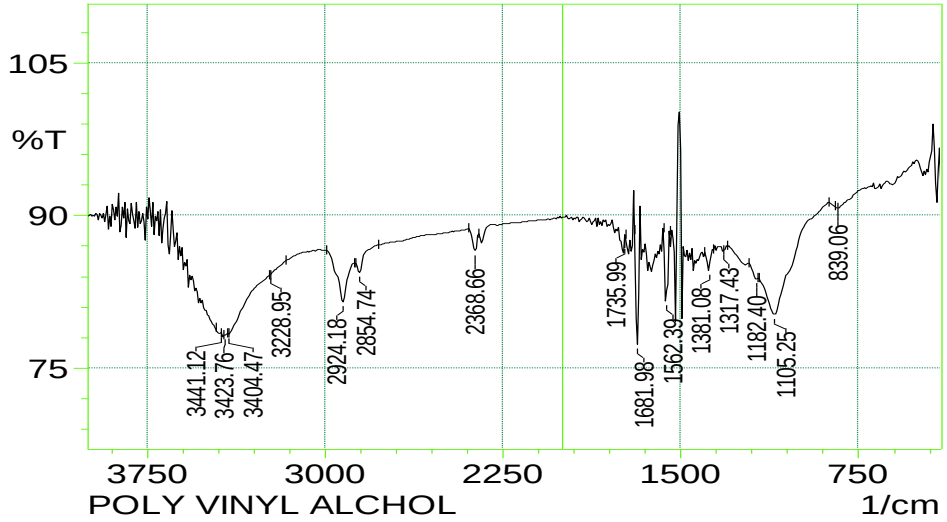


Figure 2d): Poly vinyl alcohol

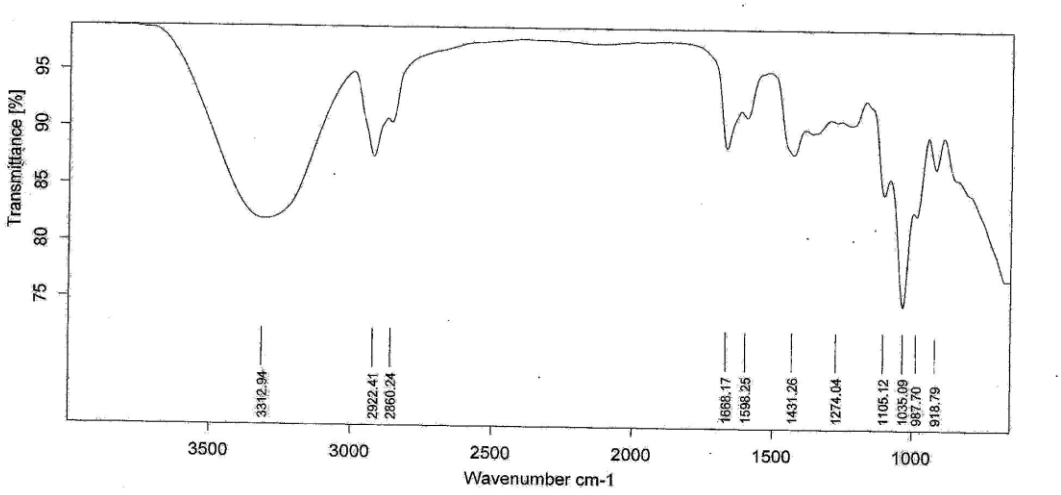


Figure 2e) M-GG-Miglitol grafted ghatti gum

Figure 2a)-miglitol:

The FTIR spectrum of miglitol standard consists of characteristic band values at 3279 cm^{-1} due to C-H stretching and 1261cm^{-1}due to C-O stretching. It was confirmed as miglitol.

Figure 2b) GG- ghatti gum:

- At 3233.92 cm^{-1} OH stretching peak of Ghatti gum can be seen.
- At 2888.23 cm^{-1} a stretching is there due to C-H stretching of of Ghatti gum
- At 1654.41 cm^{-1} one stretching can be seen, it is because of carbon carbon double bond present in Ghatti gum.
- At 1012.62 cm^{-1} another stretching is there in the spectrum, which might be due to the carbon carbon or C-O stretching in the gum.

Figure 2c) Am-g-GG-acrylamide grafted ghatti gum:

- At 3330.96 cm^{-1} -OH stretching peak of Ghatti gum can be seen.
- At 3196.97 cm^{-1} - N-H stretching of acrylamide and Ghatti gum can be seen.
- At 2920.88 cm^{-1} -C-H stretching of Ghatti gum can be seen.
- At 1654.41 cm^{-1} one stretching can be seen, it may be because of amide carbonyl group present in acrylamide and Ghatti gum.
- At 1604.15 cm^{-1} another stretching is there in the spectrum, which is due to the carbon carbon double bond in acrylamide.
- From 1100 to 1400 cm^{-1} range few stretching are there, those may be presence of C-N stretching in acrylamide (1300) and C-C stretching in both Ghatti gum and acrylamide.
- There is an another possibility of C-OH stretching at 1350 cm^{-1}which is present in ghatti gum in the spectrum.

- Specifically C-O stretching in ghatti gum can be seen at 1000 to 1150 cm^{-1} range in the spectrum.

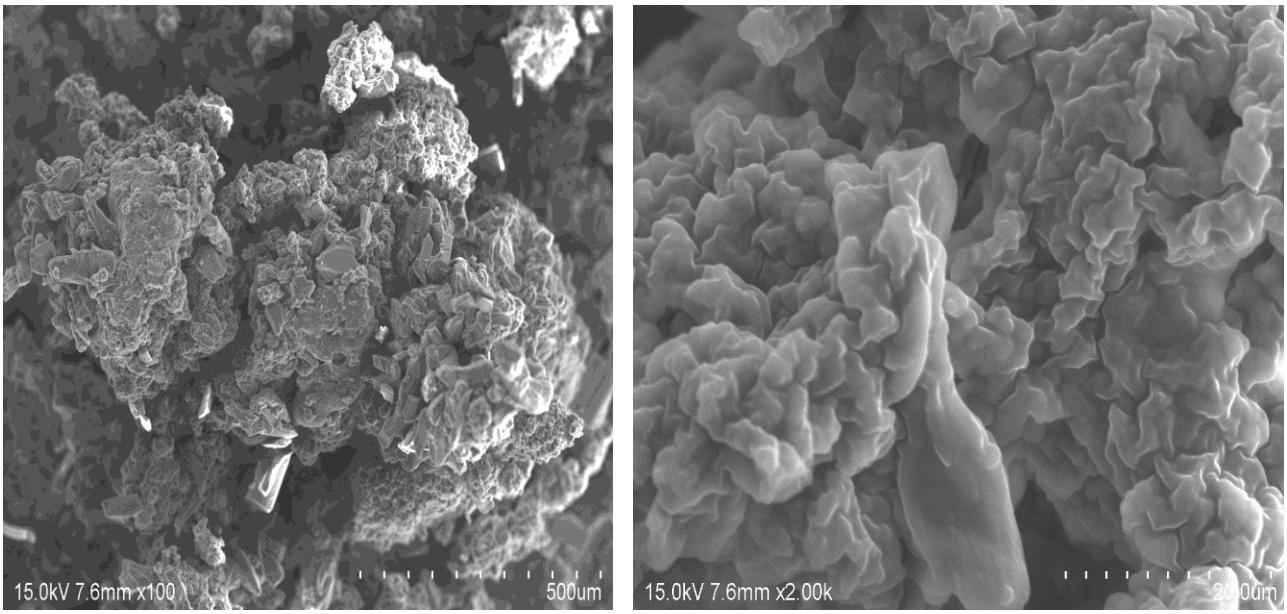
Figure 2d): Poly vinyl alcohol:

- 3423 cm^{-1} is due to -OH stretching of the hydroxyl group
- 2924 cm^{-1} is due to -CH stretching
- 1681.98 cm^{-1} is due to C=O stretching
- 839 cm^{-1} is due to carbon-carbon stretching.

Figure 2e) M-GG-miglitol grafted ghatti gum:

- At 3312.94 cm^{-1} Stretching vibration can be seen, due to NH group.
- At 2922.41 cm^{-1} another stretching is there which is due to CH stretching of miglitol and Ghatti gum.
- At 1688.17 cm^{-1} another amide linkage stretching of ghatti gum can be seen.
- Then C-C , C-O & C-N stretching can be seen at 1100 – 1300 cm^{-1} range in spectra.

SEM ANALYSIS:



In the figure 3. surface morphology of the particles prepared by acrylamide grafted ghatti gum(Am-g-GG) having (GA) glutaraldehyde in lesser amount displayed porous on the surface but in case of particles prepared with more amount of GA figure 3. The absence of porous was observed.

XRD ANALYSIS:

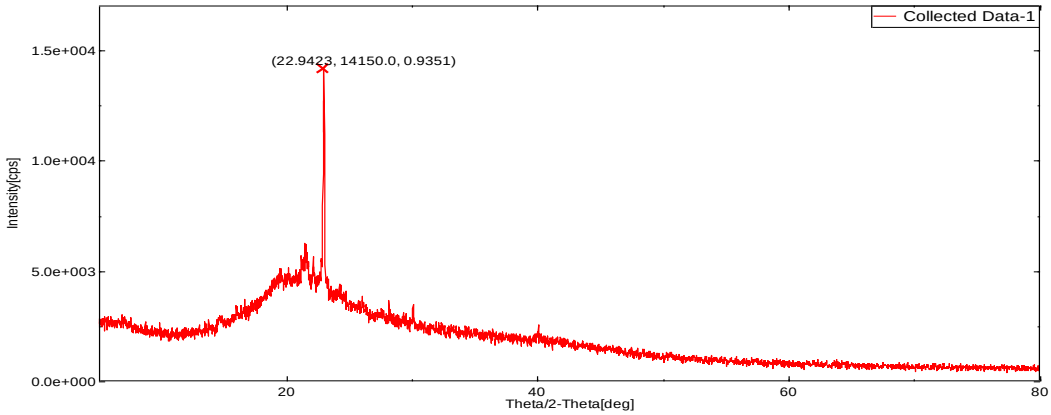


Figure 4a: Miglitol

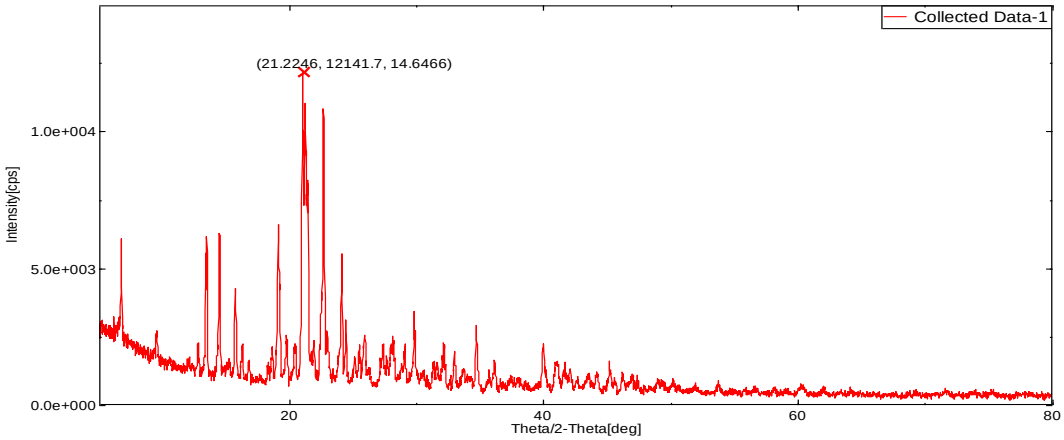


Figure 4b: Miglitol grafted ghatti gum microsphere

In the fig 4a the miglitol XRD report exhibited a peak at 21.22^0 evidenced by it's nature of crystallinity, the microspheres loaded with drug i.e., fig 4b exhibited a peak at 22.94^0 though microspheres with miglitol displayed the disappearance of other peaks was observed.

Percentage yield of IPN microspheres:

The percentage yield of prepared Am-g-GG-PVA IPN microspheres containing miglitol was calculated by using the formula, as described above in the methodology. The percentage yield of IPN microspheres ranges from 95.22 ± 0.05 (F3) to 98.22 ± 0.12 (F4). (Table 7)

Table 7 : Percentage yield of Am-g-GG-PVA IPN microspheres containing Miglitol and: Drug entrapment efficiency percentage of Am-g-GG-PVA IPN microspheres containing Miglitol

Formulation code	Percent Yield (%)	Drug entrapment efficiency (in %) ± SD, n = 3
F1	95.36 ± 0.21	75.33 ± 1.02
F2	96.22 ± 0.16	82.33 ± 0.84
F3	95.22 ± 0.05	74.33 ± 0.22
F4	98.22 ± 0.12	79.22 ± 0.87

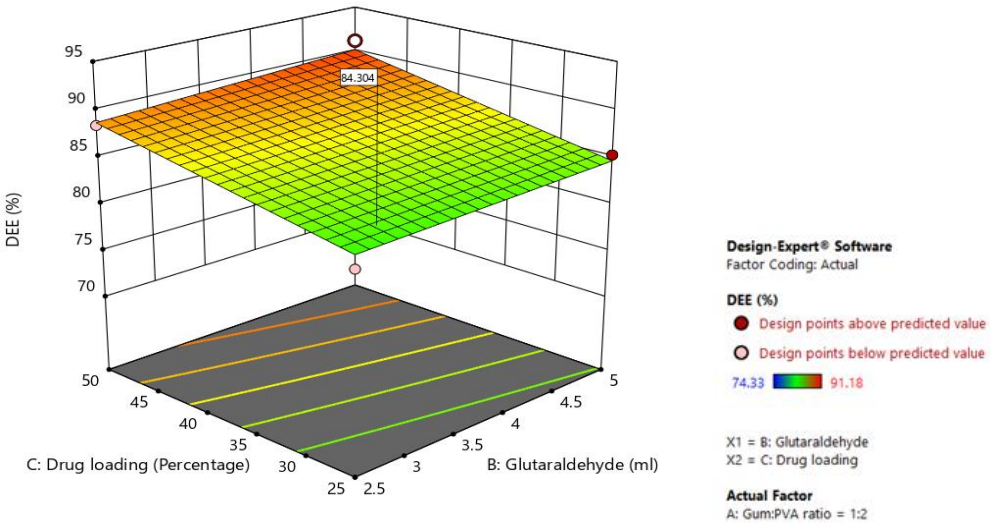
F5	97.51 ± 0.09	85.33 ± 0.33
F6	96.44 ± 0.35	88.42 ± 0.54
F7	97.10 ± 0.26	79.35 ± 0.61
F8	95.23 ± 0.18	91.18 ± 0.08

Drug entrapment efficiency: (percent DEE)

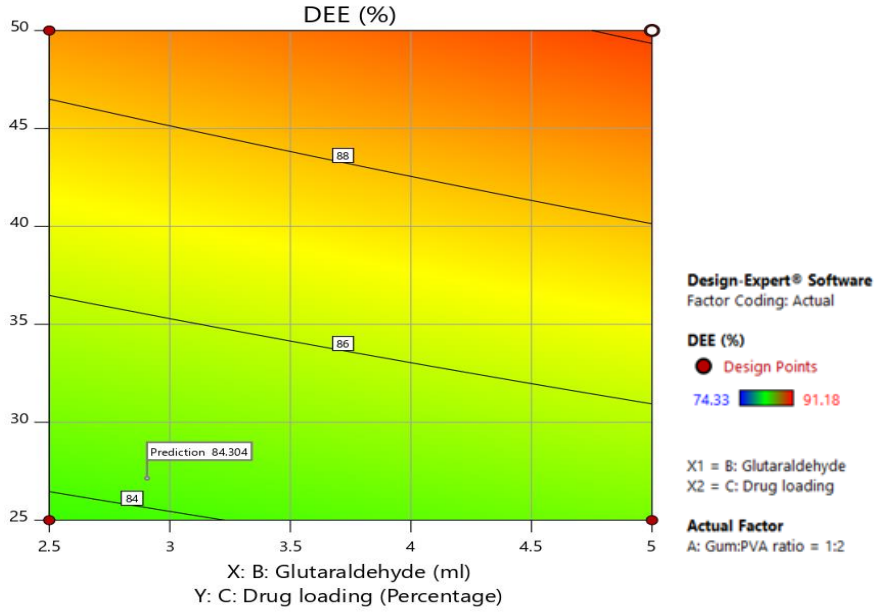
Entrapment of drug in any matrix system is considered as important criteria for selection of suitable batch formula as amount of drug retained in matrix indicates the overall efficiency of drug delivery system showing sustainability and ability to prolong drug availability in site of action. In the present study, the drug entrapment efficiency percentage ranges from 74.33 ± 0.22 (F3) to 74.33 ± 0.22 (F8) (Table 8).

The percentage of entrapment efficiency of Miglitol was increased with the increase in polymer concentration as shown in present study. Drug entrapment capacity had a strong dependence on particular proportion of polymeric complex.

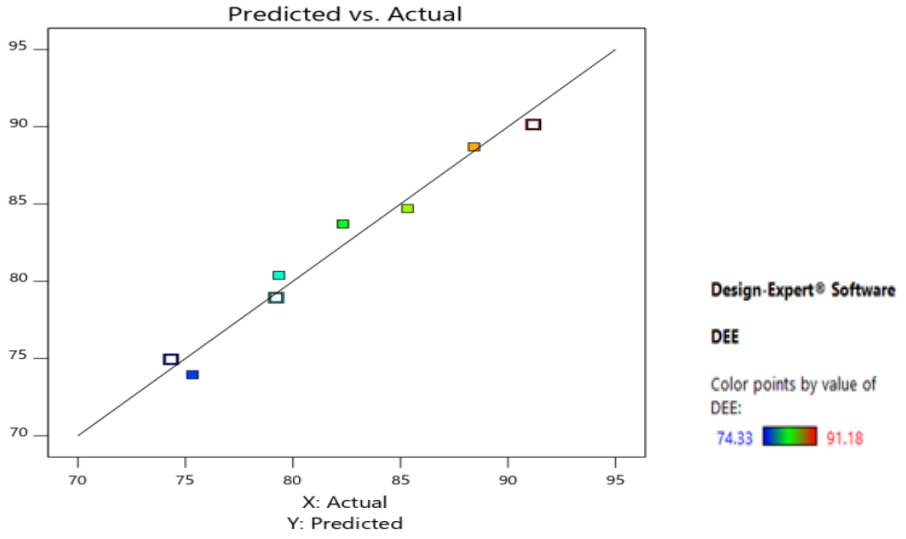
The three-dimensional plot of the effects of different independent variables on percent DEE is shown in figure 5.



(a)



(b)



(c)

Figure 5: (a) Three dimensional response surface plots: showing the effects of synthetic condition on Drug entrapment efficiency (b) Corresponding contour plot showing the relationship between various levels of the factors, (c) Plot between observed and predicted values of Drug entrapment efficiency.

The mathematical relationship of percentage of DEE with the independent variables (MLRA) was generated and expressed as:

Percentage of DEE = 81.94 + 4.88 A + 0.61 B + 2.61 C + 0.1113 BC

where, A is Am-g-GG:PVA ratio, B is glutaraldehyde amount and C is percent drug loading. Here we observed that all the independent variables were having impact on percent DEE as was observed experimentally.

ANOVA analysis indicated that the model was significant (P=0.0109 < 0.0001) with R^2 value 0.9730. The Predicted R^2 of 0.8080 was in reasonable agreement with the Adjusted R^2 of 0.9370; i.e. the difference is less than 0.2. (Table 6)

Adequate precision ratio with >4 is desirable. The ratio of 13.52 indicates an adequate signal. Thus, the present model can be used to navigate the design space.

Swelling Studies:

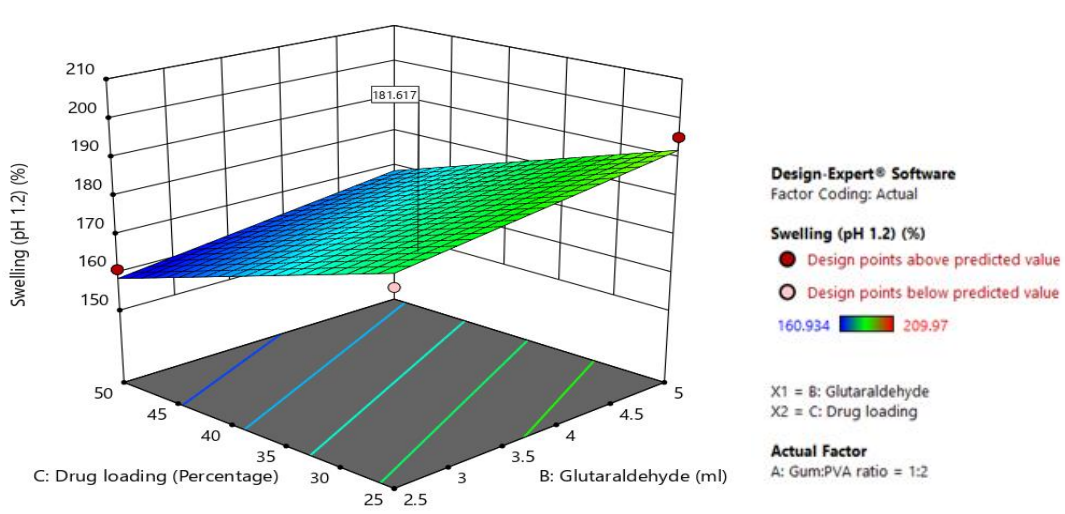
Swelling study is also considered as important criteria for selection of suitable batch formula for the optimum drug release. The percent equilibrium water uptake data of the IPN microspheres (Table 9)

Table 9: Swelling / Equilibrium water uptake of Am-g-GG-PVA IPN microspheres containing Miglitol

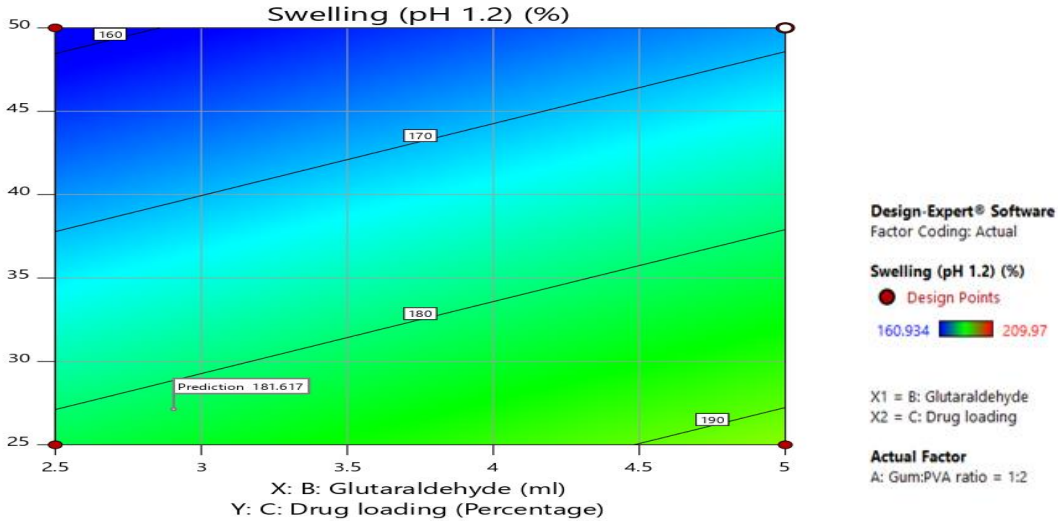
Formulation Code	Swelling / Equilibrium water uptake (in %) ± SD, n = 3	
	At pH 1.2	At pH 6.8
F1	207.27 ± 2.15	240.85 ± 2.56
F2	178.69 ± 1.08	218.16 ± 1.85
F3	209.97 ± 1.29	250.18 ± 2.21
F4	171.93 ± 0.85	215.13 ± 1.02
F5	195.38 ± 1.33	230.45 ± 0.85
F6	160.93 ± 2.36	198.04 ± 1.52
F7	187.65 ± 3.02	228.08 ± 2.38
F8	166.28 ± 1.95	205.59 ± 3.01

From the IPN microspheres suggests that it was dependent upon major factors like crosslinker amount, polymeric blend ratio and on the amount of drug loading. In the present study, the swelling at pH 1.2 ranges from 160.93 ± 2.36 (F6) to 209.97 ± 1.29 (F3) and at the pH 6.8 it ranges from 198.04 ± 1.52 (F6) to 250.18 ± 2.21 (F3).

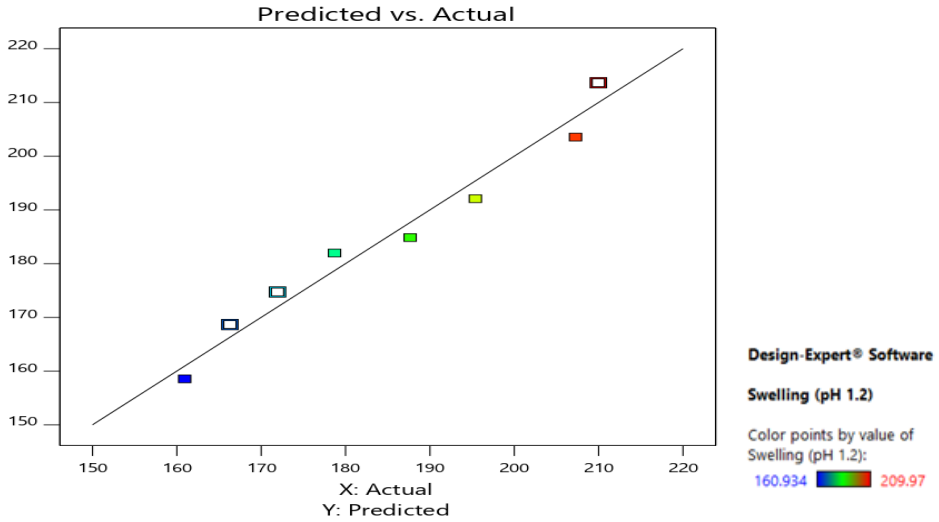
The effect of independent variables on Swelling is shown in the figure .


Swelling (pH 1.2) (%)
210
200
190
180
170
160
150
181.617
50
45
40
35
30
25
C: Drug loading (Percentage)
2.5
3
3.5
4
4.5
5
B: Glutaraldehyde (ml)
Design-Expert® Software
Factor Coding: Actual
Swelling (pH 1.2) (%)
Design points above predicted value
Design points below predicted value
160.934
209.97
X1 = B: Glutaraldehyde
X2 = C: Drug loading
Actual Factor
A: Gum:PVA ratio = 1:2

(a)

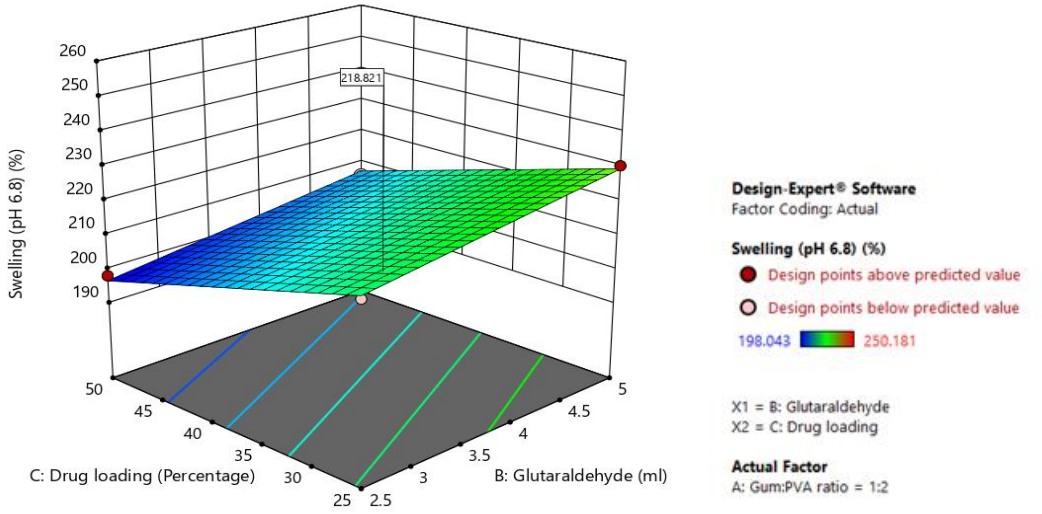

Swelling (pH 1.2) (%)
160
170
180
190
Prediction 181.617
50
45
40
35
30
25
2.5
3
3.5
4
4.5
5
X: B: Glutaraldehyde (ml)
Y: C: Drug loading (Percentage)
Design-Expert® Software
Factor Coding: Actual
Swelling (pH 1.2) (%)
Design Points
160.934
209.97
X1 = B: Glutaraldehyde
X2 = C: Drug loading
Actual Factor
A: Gum:PVA ratio = 1:2

(b)

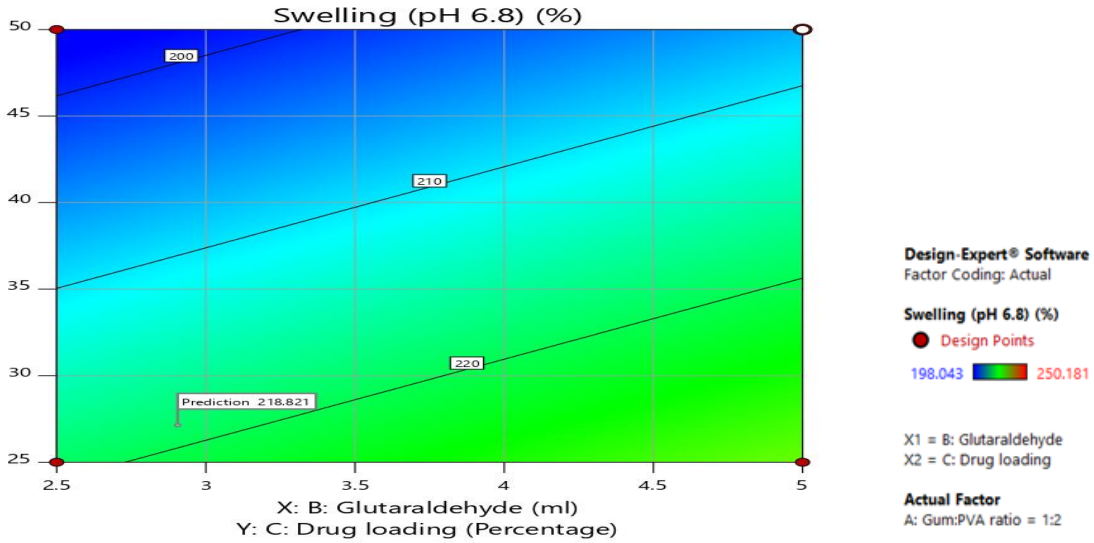

Predicted vs. Actual
220
210
200
190
180
170
160
150
150
160
170
180
190
200
210
220
X: Actual
Y: Predicted
Design-Expert® Software
Swelling (pH 1.2)
Color points by value of
Swelling (pH 1.2):
160.934
209.97

(c)

Figure 6: (a) Three-dimensional response surface plots: showing the effects of synthetic condition on Swelling at pH 1.2 (b) Corresponding contour plot showing the relationship between various levels of the factors, (c) Plot between observed and predicted values of Swelling at pH 1.2.



(a)



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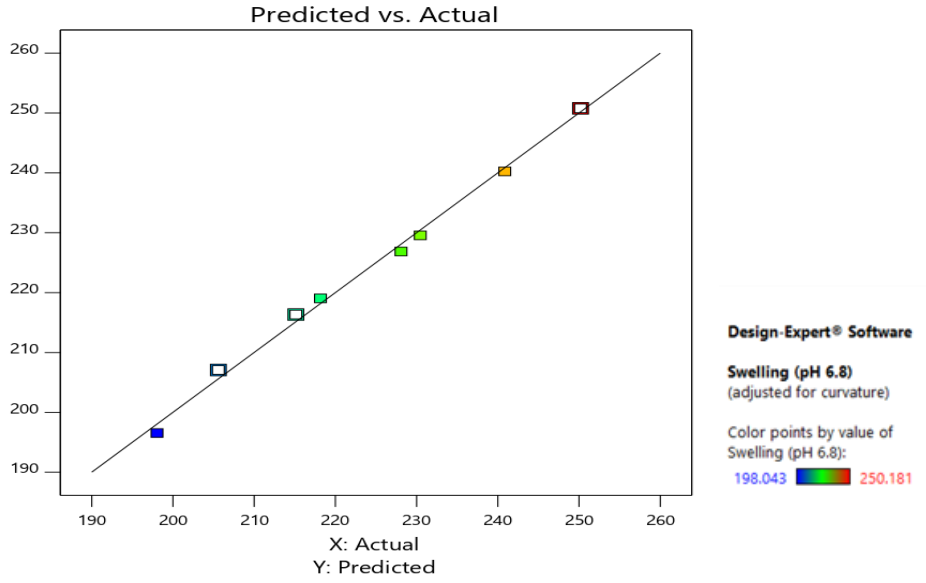


(c)

Figure 7: (a) Three dimensional response surface plots: showing the effects of synthetic condition on Swelling at pH 6.8 (b) Corresponding contour plot showing the relationship between various levels of the factors, (c) Plot between observed and predicted values of Swelling at pH 6.8.

The mathematical relationship is expressed as:

Swelling percentage (pH 1.2) = 184.76 – 9.44 A + 5.06 B – 13.06 C + 1.35 AC

Swelling percentage (pH 6.8) = 223.31 – 10.25 A + 5.27 B – 11.60 C + 0.36 AC

where, A is Am-g-GG:PVA ratio, B is glutaraldehyde amount and C is % drug loading. ANOVA analysis indicated that the both models (Swelling at pH 1.2 & 6.8) were significant with P values 0.0141 & 0.0007 ($P < 0.05$) with R^2 value 0.9679 & 0.9955 respectively. (Table 6)

The predicted R^2 (0.7718, 0.9681), is in reasonable agreement with the adjusted R^2 (0.9251, 0.9895); i.e., the difference is less than 0.2. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 was required. The ratio of 13.83 (pH 1.2) & 38.26 (pH 6.8) indicates an adequate signal. Thus, the model can be used to navigate the design space.

***In vitro* drug release studies:**

The cumulative percentage drug release vs. time plot of different batches of Miglitol loaded Am-g-GG-PVA IPN microspheres are presented in the figure 6. In the present study, after 12 hours drug dissolution study, the drug release percentage ranges from 65.71 (F5) to 92.55 (F4) Figure 8: *In vitro* release characteristics of various formulations of Am-g-GG-PVA IPN Microspheres containing Miglitol

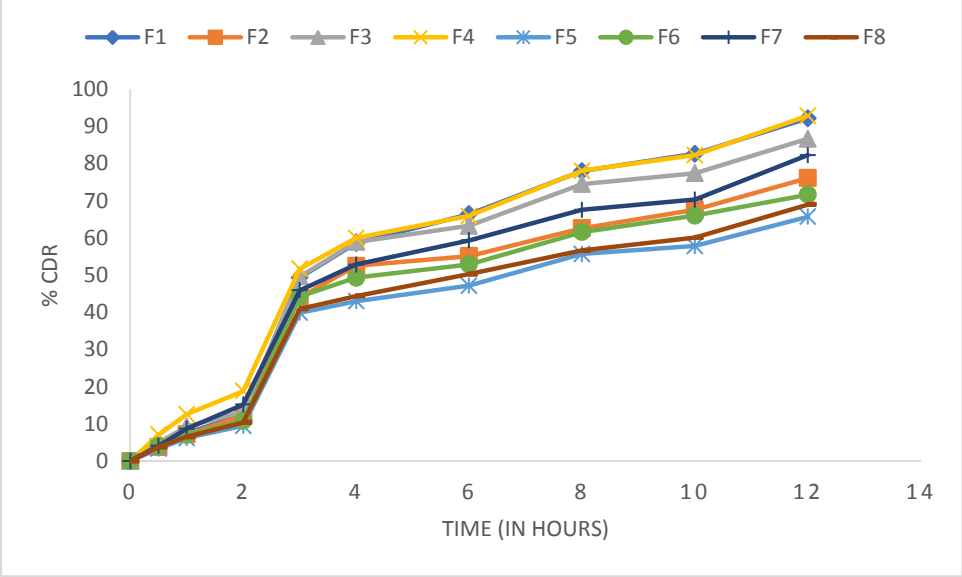


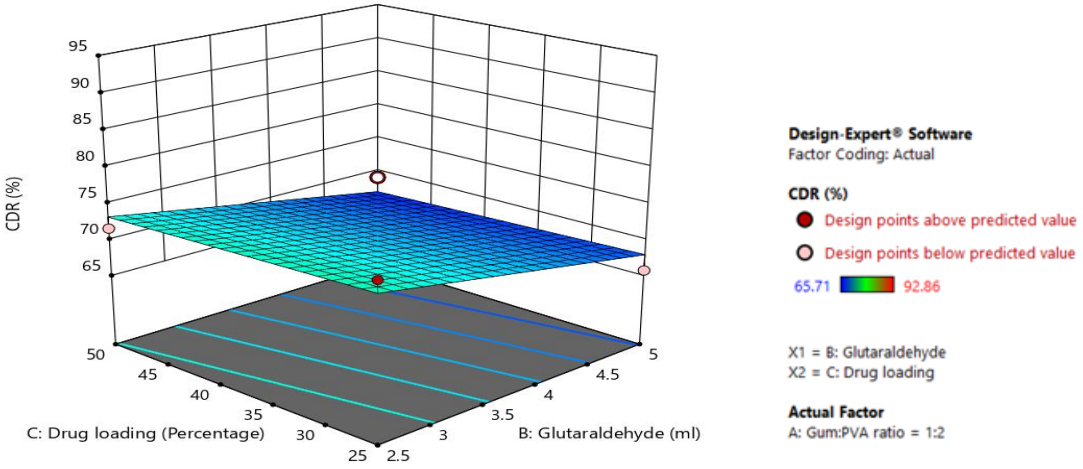
Figure 8: *In vitro* release characteristics of various formulations of Am-g-GG-PVA IPN Microspheres containing Miglitol

It was observed that the drug release from the IPN microspheres were dependent upon major factors like crosslinker amount, polymeric blend ratio and on the amount of drug loading.

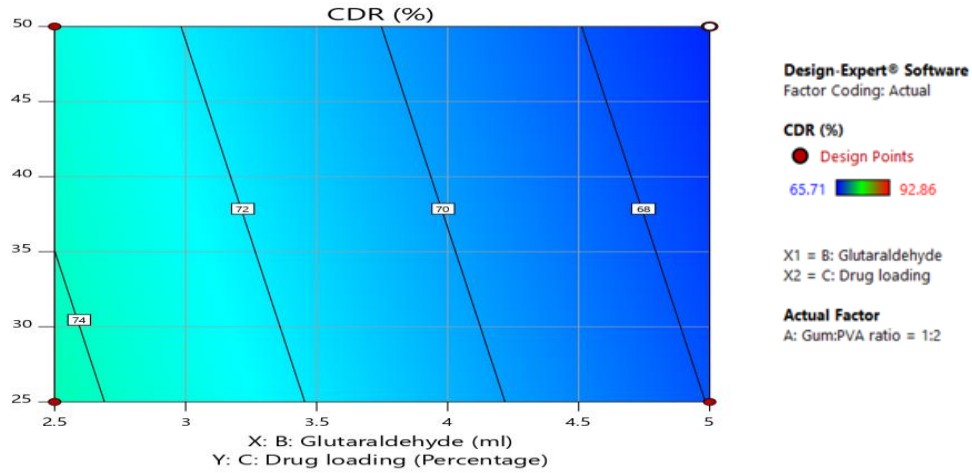
Effect of drug loading: In fixed formulating parameters, when the amount of drug loading was increased from 25% to 50%, the amount of drug release was decreased. In formulation F3 and F7 polymer composition (1:1) and glutaraldehyde (5 mL) was same but amount of drug loading was different. F8 (86.66 %, 25% drug loading) showed more drug release than F1 (82.26 %, 50% drug loading). It may be due to the concentration gradient & the driving force, will be more in high drug load formulations and promoted faster drug release. Moreover, low drug load matrix would have a greater gum and polymer fraction to act as the barrier to drug release.

Effect of gum: PVA blend ratio: When the Am-g-GG: PVA ratio of IPN particles were changed from 1:2 to 1:1 in fixed crosslinker and drug loading percentage, at 12 hours, the drug release increased from 65.71% (F5, 1:2) to 86.66% (F3, 1:1). This was due to the hydrophilic nature of the grafted gum which interacted with media to swell and erode in a faster rate and helped in faster drug release.

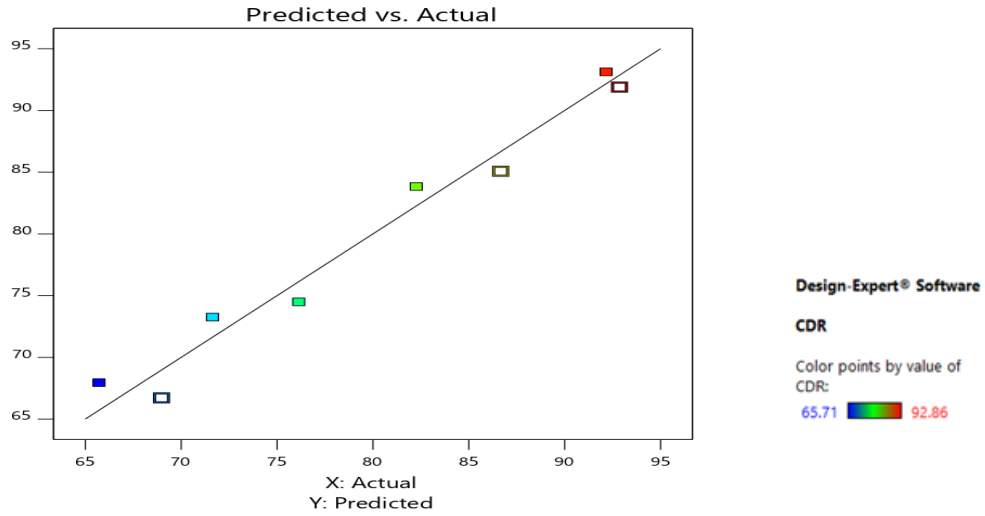
Crosslinker effect: Formulations, having different drug loading in a fixed gum: PVA ratio and percent drug loading, showed different extent of drug release. F1 (2.5 mL, 92.16% drug release) showed increased drug release property than F3 (5 mL, 86.66% drug release) though other parameters for both the formulations were same. This may be due to the higher crosslinking of the IPN matrix which prevent solvent imbibition leading to less release retardant property.



(a)



b)



(c)

Figure 9: (a) Three-dimensional response surface plots: showing the effects of synthetic condition on Cumulative drug release at 12 hours (b) Corresponding contour plot showing

the relationship between various levels of the factors, (c) Plot between observed and predicted values of Cumulative drug release at 12 hours.

The 3-dimensional plot of the effects of different formulation variables on percent CDR (at 12 hours) is shown in the figure 9. The mathematical relationship of percent CDR (at 12 hours) with the independent variables was generated and expressed as:

Percentage of CDR = 79.55 – 8.94 A – 3.65 B – 0.6175 C + 0.3775 AB

where, A is Am-g-GG:PVA ratio, B is glutaraldehyde amount and C is percentage drug loading. ANOVA analysis indicated that the model was significant with P values 0.0121 ($P < 0.05$) with R^2 value 0.9711. (Table 6)

The Predicted R^2 of 0.7945 is in reasonable agreement with the Adjusted R^2 of 0.9326; i.e. the difference is less than 0.2. Adequate Precision ratio greater than 4 was desired. The ratio of 12.24 indicates an adequate signal. Hence, this model can be used to navigate the design space.

Drug release Kinetic Study:

The *in vitro* release data were fitted into various empirical kinetic equations and presented in the Table 10.

Table 10: Drug release kinetics data of Am-g-GG-PVA IPN microspheres containing Miglitol

Formulation Code	Zero Order	First Order	Higuchi model	Korsmeyer- Peppas	
	R^2	R^2	R^2	R^2	n value
F1	0.8694	0.972	0.9383	0.9259	1.0385
F2	0.8386	0.9326	0.9185	0.9213	0.9961
F3	0.8451	0.9579	0.9226	0.9203	0.9558
F4	0.8773	0.9635	0.9471	0.9431	0.8379
F5	0.8367	0.9122	0.9145	0.9139	0.9978
F6	0.8287	0.9204	0.9106	0.9118	0.9975
F7	0.8612	0.9513	0.9372	0.9351	0.9672
F8	0.8444	0.9236	0.9204	0.9176	0.9754

After plotting zero order, first order, Higuchi plots for the optimized formulation F5, it was observed that the best fit was with the Higuchi model, which suggests the release of drug from matrix was diffusion controlled.

The Korsmeyer peppas equation was also used for the kinetics study, except F1, the n value ranges from 0.8379 to 0.9978, which shows that all these formulations follows Super case-II transport, whereas, F1 have n value of 1.0385, which indicated that it follows non fickian diffusion.

Optimization data analysis:

An optimum setting for the formulation was generated by the numerical optimization technique following desirability approach. The process was optimized for the dependent (response) variables,

and the optimized formula was reached by keeping the goal to maximize swelling at both pH 1.2 & pH 6.8 and Drug entrapment efficiency percentage, and cumulative drug release at 12 hours was kept in range of 70 to 80 %. The formulation F5 fulfilled nearly all the criteria set from the desirability search (Figure 10).

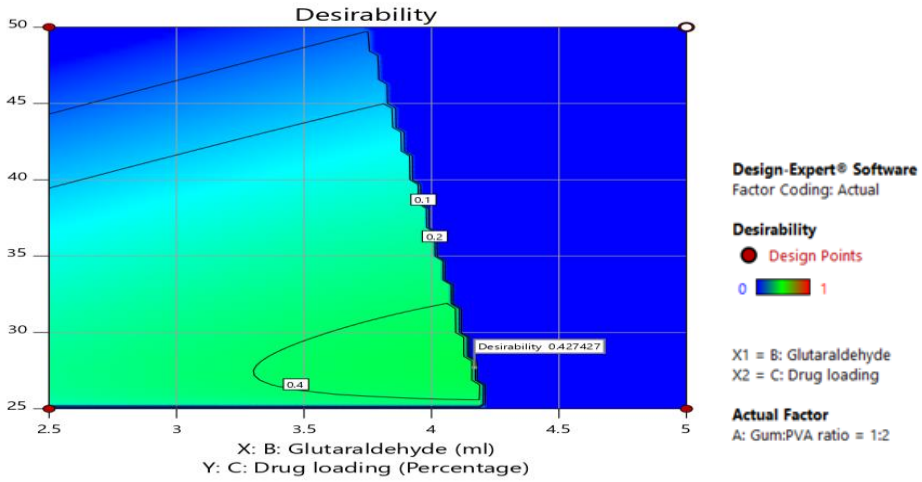


Figure 10: Contour plot showing the optimization procedure depending on numerical method.

The low percentage of prediction error of 1.7586 to 5.9991 indicated the high prognostic ability of the factorial model (Table 11)

Table 11: Predicted and observed response variables of the optimal Am-g-GG-PVA IPN microspheres containing Miglitol

Response variables	Predicted ± SD (Software suggested)	Observed (G5 & F5)	Predicted error (in %)
A. Acrylamide grafted Ghatti gums			
Grafting efficiency (in %)	86.37 ± 1.18	86.15	1.5555
B. Am-g-GG-PVA IPN microspheres containing Miglitol			
Swelling at pH 1.2(in %)	192.09 ± 5.03	195.38	6.4233
Swelling at pH 6.8(in %)	229.56 ± 1.79	230.44	2.2848
Drug entrapment efficiency (in %)	84.70 ± 1.51	85.33	1.9300
Cumulative Drug release (CDR) at 12 hours (in %)	67.95 ± 2.72	65.71	3.4765

Stability studies:

Stability studies were conducted for the optimized formulation as per ICH guidelines for a period of 90 days which revealed that the formulation (F5) was stable. The results (Table 12)

Table 12: **Stability study of Am-g-GG-PVA IPN microspheres containing Miglitol**

Trial No.	Drug entrapment efficiency (%) of F5			
	1st Day	After 4 weeks	After 6 weeks	After 12 weeks
I	84.22	84.96	84.08	84.22

II	85.17	84.68	84.66	85.02
III	84.51	85.02	84.16	85.14
Average	84.63 ± 0.39	84.89 ± 0.14	84.30 ± 0.25	84.79 ± 0.40

The above data shows that the developed IPN microspheres containing Miglitol were stable for storage for a long period of time.

CONCLUSION:

In conclusion, Am-g-GG-PVA IPN microspheres containing Miglitol were successfully prepared by the emulsion crosslinking method by using glutaraldehyde as a crosslinker. The IPN microspheres were produced following the design of experiment and optimized with the help of response surface methodology involving the independent factors and variable responses. The formulation coded as F5 was found to be optimized with desirable controlled release property with moderate pH sensitivity and had the better drug entrapment efficiency. These findings when taken together suggest that the present formulated IPN microspheres containing Miglitol can be reproduced with high predictability and shall be potentially useful to patients with hyperglycaemia. This type of formulation may be also useful as a promising biomaterial to overcome the major problems of controlled release of highly water-soluble drugs with a shorter half-life.

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