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PREPARATION AND CHARACTERIZATION OF IPN MICROSPHERES CONTAINING MIGLITOL BY USING *IN HOUSE* SYNTHESISED ACRYLAMIDE GRAFTED HPMC K100 GUM

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ABSTRACT
Objective: The intention of the study is to formulate a miglitol-loaded microsphere of HPMC K-100 and polyvinyl alcohol by emulsion crosslinking method using glutaraldehyde as a crosslinker. **Method:** Emulsion crosslinking method was employed in the synthesis where firstly Preparation of Acrylamide grafted HPMC gum was done with optimization of full factorial design and later Miglitol was entrapped in IPN microspheres of PVA and Am-g-HPMC. **Results and Discussion:** The drug-loaded IPN microspheres released 60-92% at 12 hours of release studies and drug release follows a diffusion-controlled release pattern. Prolonged time drug release was observed without collapsing the particle matrix. **Conclusion:** IPN microspheres-based delivery system can be a superior approach for controlled delivery of highly water-soluble drugs like Miglitol.

KEYWORDS: Miglitol, Acrylamide HPMC K-100, Polyvinyl alcohol, Interpenetrating polymer network.

INTRODUCTION
Drug delivery research is primarily focussed on targeted delivery of the drug to the desired organ system to minimise toxicity and maximise therapeutic efficacy. As oral route is the most popular route of administration, a large emphasis is given on the development of controlled oral drug delivery systems.[1] Drug substances with high water solubility and short half life get readily absorbed and eliminated, thus requiring frequent dosing.[2] This may lead to decrease in patient compliance and increase chances of side effects due to dose dumping. Thus, the drugs having high water solubility and short half-life warrants extensive research to reduce frequent dosing and dose related side effects by controlling their release rate. Fabrications of drug delivery devices like microparticles or nanospheres are some of the approaches to control the release of highly water soluble drugs.[3]

Interpenetrating polymer network (IPN) is one such formulation which is considered to be promising in delivery of bioactive molecules, particularly in controlled release applications. The excellent biocompatibility and safety due to its physical characteristics such as impart stability of the drug in the formulations, improves 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 specially for the controlled release drug delivery systems.

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 HPMC gum may provide the system with better stability and improved mechanical strength to meet the major objectives of controlled release drug delivery.[4]

Hydroxypropyl methylcellulose (HPMC) or Hypromellose refers to soluble methylcellulose ethers. HPMC is used as a thickening agent, binder, film former, and hydrophilic matrix material. HPMC polymers for fabricating hydrophilic matrix systems are available in various viscosity grades ranging from 4000–100,000 mPa's. HPMC is a popular matrix material in oral controlled delivery systems and HPMC matrices show sustained release pattern by two mechanisms, i.e., diffusion and erosion of the gel layer. The viscosity of the polymer affects the diffusion pathway. HPMC can be employed as a matrix for controlling the release of both hydrophilic and hydrophobic drugs.

In the present study, HPMC 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 considered to be the most efficient in estimating the influence of individual variables and their interactions using nominal experiments.[6]

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. With the help of the full factorial design, the IPN microspheres of Am-g-HPMC 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.[7]

MATERIALS

Miglitol, HPMC 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 HPMC gum

1gm of HPMC was dissolved in 120ml of distilled water and stirred for about 30 minutes using magnetic stirrer. Dissolve specified amount (0.16 - 0.25mol) of acrylamide (Table 2) in 15ml of distilled water and then add to the HPMC solution and stirred for 1 hour. Add {5 mg (1.85×10^{-5} mol) – 14 mg (5.5×10^{-5} mol)} of KPS in 5ml of distilled water and mixed with HPMC solution. The grafting reaction was irradiated using microwave at 560W with alternate 1 min heating and 1 min cooling for specified time period and left-over night to reach ambient temperature. After the completion of irradiation, a saturated solution of hydroquinone was added to stop the copolymerization reaction. Then 250ml of acetone was added to form precipitation reaction. Then washed with the absolute and 30% of ethanol and dried at 60^0c in hot air oven, converted into fines.[8]

The grafting efficiency (% GE) was then calculated by using the formula.

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

Table 1: Formulation details of Acrylamide grafted HPMC gums.

Formulation Code	Potassium persulfate-KPS (in mg)	Irradiation Time (in mins)	Acrylamide (in g)
H1	5	2.5	17.77
H2	5	5	17.77
H3	5	2.5	11.37
H4	14	2.5	11.37
H5	14	5	17.77
H6	14	2.5	17.77
H7	5	5	11.37
H8	14	5	11.37

Full factorial design for the preparation of acrylamide grafted HPMC

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 HPMC gum. The amount of acrylamide, potassium persulfate and microwave irradiation time were selected as the independent variables and % Grafting efficiency was selected as the dependent variable[9](Table 2).

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

Independent factors	Levels	
	Low level (-1)	High level (+1)
Potassium persulfate-KPS (in mg)	5	14
Irradiation time (in minutes)	2.5	5
Acrylamide (in g)	11.37	17.77

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 HPMC-PVA IPN microspheres containing Miglitol

Miglitol entrapped IPN microspheres of PVA and Am-g-HPMC 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-HPMC in aqueous PVA solution. The required amount of Miglitol will be added to the polymeric dispersion. (Table 3)

Table 3: Full factorial design for the Acrylamide grafted HPMC gums - Independent variables with their levels.

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

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.[10]

Full factorial design for the preparation of Am-g-HPMC-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 HPMC, here, it was used to find out the optimized formula for the preparation of Am-g-HPMC-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. HPMC:PVA ratio, Glutaraldehyde and drug loading % were selected as Independent variables (Table 4).

Table 4: Formulation details of Am-g-HPMC-PVA IPN microspheres containing Miglitol.

Formulation Code	Am-g-HPMC:PVA	Drug loading (in %)	Glutaraldehyde (in mL)
F1	1:1	2.5	2.5
F2	1:2	2.5	5.0
F3	1:2	5	2.5
F4	1:1	2.5	5.0
F5	1:1	5	5.0
F6	1:2	2.5	2.5
F7	1:2	5	5.0
F8	1:1	5	2.5

The dependent variables (responses) selected were % Drug entrapment efficiency, % Swelling (pH 1.2 & pH 6.8) and % 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 analysed by factorial models using Design Expert software (Trial version 11.1.2.0 64-bit, Stat-Ease, Inc., Minneapolis, USA).

Fourier transform infrared spectroscopy (FTIR) Studies

The FTIR spectrums of Miglitol, PVA, Am-g-HPMC, drug-polymer physical mixture, blank microspheres, and drug loaded IPN microspheres were carried out by to confirm the formation of Am-g-HPMC 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.

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

Drug entrapment efficiency (% 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 drug entrapment efficiency (%) was calculated by the formula given below.

% 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 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 analysed in triplicate. The swelling index was calculated by using the following equation:

% Swelling = $(W_2 – W_1 / W_1) \times 100$

Where, W_1 and W_2 are the dry weight and swollen weight of the IPN microspheres.[11]

***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 37.5 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.[12]

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.[13]

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 aluminium 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 HPMC (% GE)

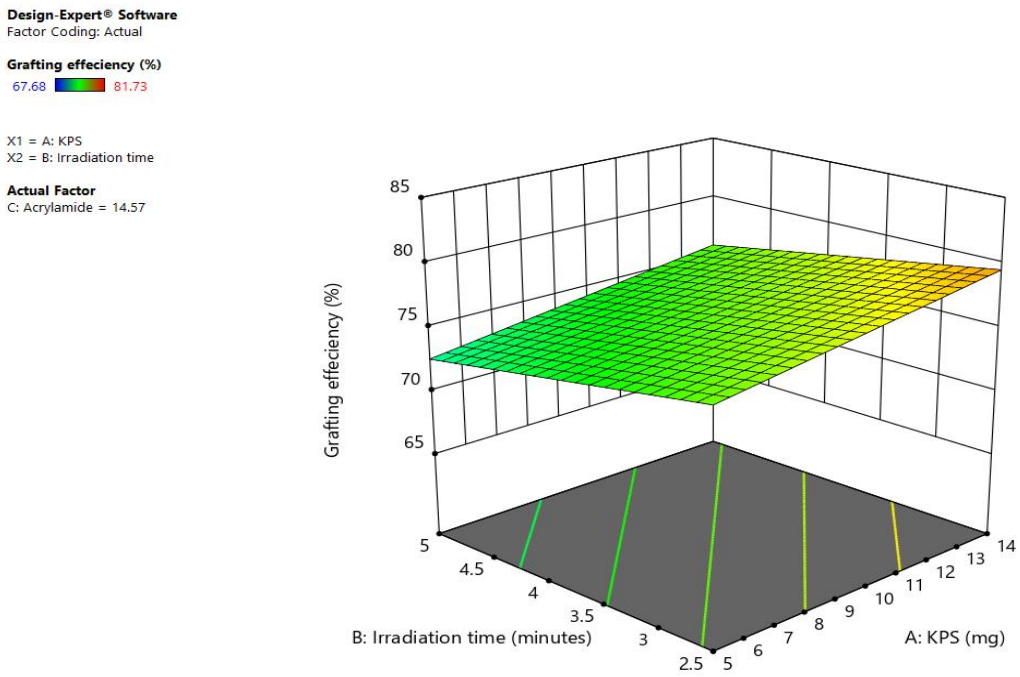
Table 5: Grafting efficiency percentage of prepared Acrylamide grafted gums.

Formulation Code	Grafting efficiency (in %) ± SD, n = 3
H1	79.39 ± 0.32
H2	78.24 ± 0.21
H3	72.29 ± 0.66
H4	78.06 ± 0.08
H5	80.29 ± 0.13
H6	81.73 ± 0.11
H7	67.68 ± 0.53
H8	70.88 ± 0.39

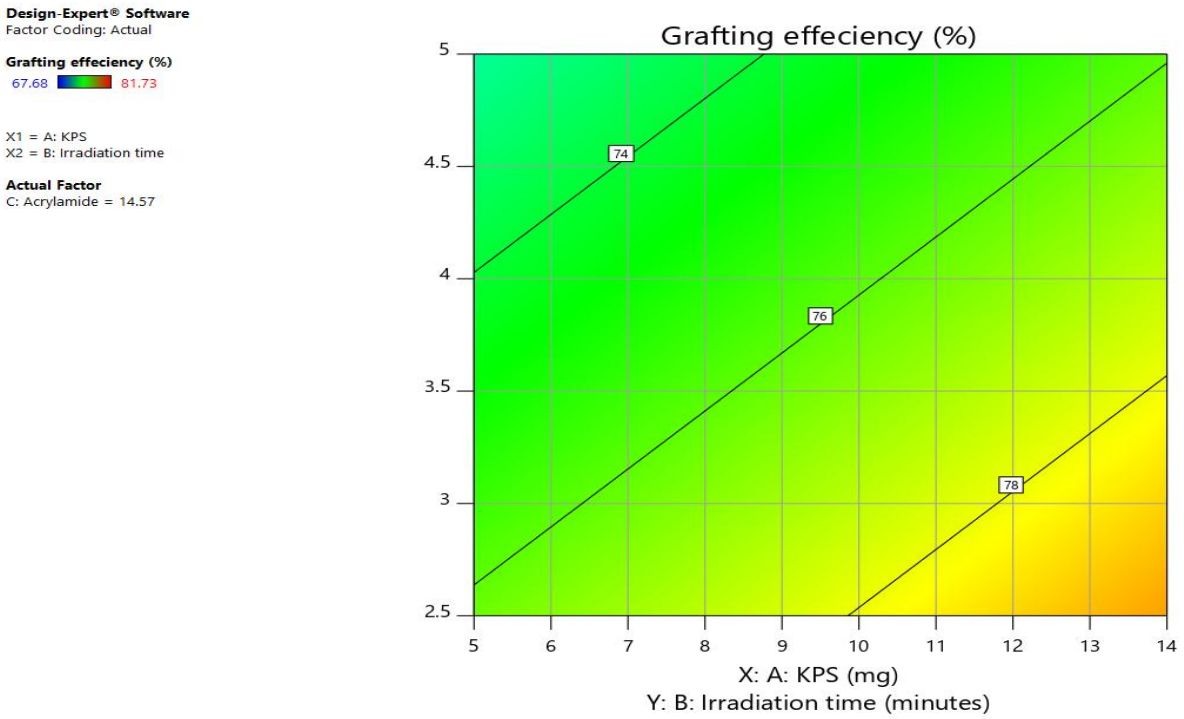
Table 5 represents the grafting efficiency of different prepared acrylamide grafted gum. The grafting efficiency of the prepared formulations (H1-H8) ranges from 67.68 ± 0.53 (H7) to 81.73 ± 0.11 (H6). As said earlier in the methodology, factorial design was used to optimize the GE using three different variables at 2 levels. Their mathematical relationship with GE was generated using multiple linear regression analysis (MLRA) and was expressed as.

% GE = 76.07 + 1.67 A – 1.80 B + 3.84 C -0.5725 AC + 1.15 BC

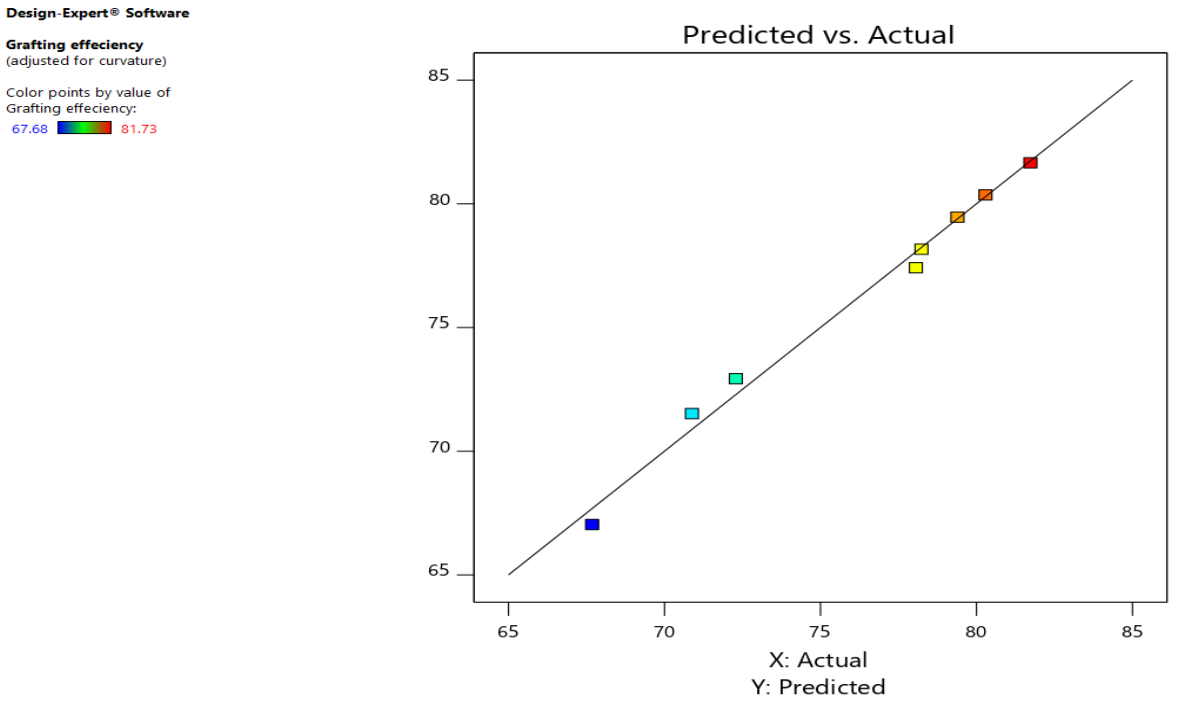
where, A is amount of CAN (mg), B is irradiation time (min) and C is amount of Acrylamide (g). The three-dimensional plot is shown in figure 1.



(a)



(b)



(c)

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.

The impact of KPS 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.

ANOVA analysis indicated that the factorial model was significant ($P = 0.0229$, i.e. $P < 0.05$) having R^2 value of 0.9908. The Predicted R^2 of 0.8523 is in reasonable agreement with the Adjusted R^2 of 0.9677; i.e. the difference is less than 0.2. (Table 6)

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

	Sum of squares	df	Mean square	F value	Significance F (p value)
A. Acrylamide grafted HPMC gums					
Grafting efficiency (in %)					
Model	179.48	5	35.90	42.93	0.0229
Residual	1.67	2	0.8361	-	-
Cor total	181.15	7	-	-	-
B. Am-g-HPMC-PVA IPN microspheres containing Miglitol					
Swelling at pH 1.2 (in %)					
Model	2206.75	4	551.69	12.65	0.0320
Residual	130.79	3	43.60	-	-
Cor total	2337.54	7	-	-	-
Swelling at pH 6.8 (in %)					
Model	2094.73	5	418.95	49.15	0.0201
Residual	17.05	2	8.52	-	-
Cor total	2111.78	7	-	-	-
Drug entrapment efficiency (in %)					
Model	273.72	5	54.74	94.14	0.0105
Residual	1.16	2	0.5815	-	-
Cor total	274.88	7	-	-	-
Cumulative Drug release (CDR) at 12 hours (in %)					
Model	832.90	4	208.22	9.54	0.0470
Residual	65.49	3	21.83	-	-
Cor total	898.38	7	-	-	-

Adequate precision measures the signal to noise ratio of the model. A ratio greater than 4 is desirable. The ratio of the model 18.46 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 7.803 mg of KPS, 2.5 minutes of irradiation time and 15.48 grams of acrylamide can satisfy the required conditions, which was in good and close agreement with the H1, which had good desirability of 0.747.

Hence, the formulation H1 was considered for the preparation of the IPN microspheres containing Miglitol.

FTIR Studies

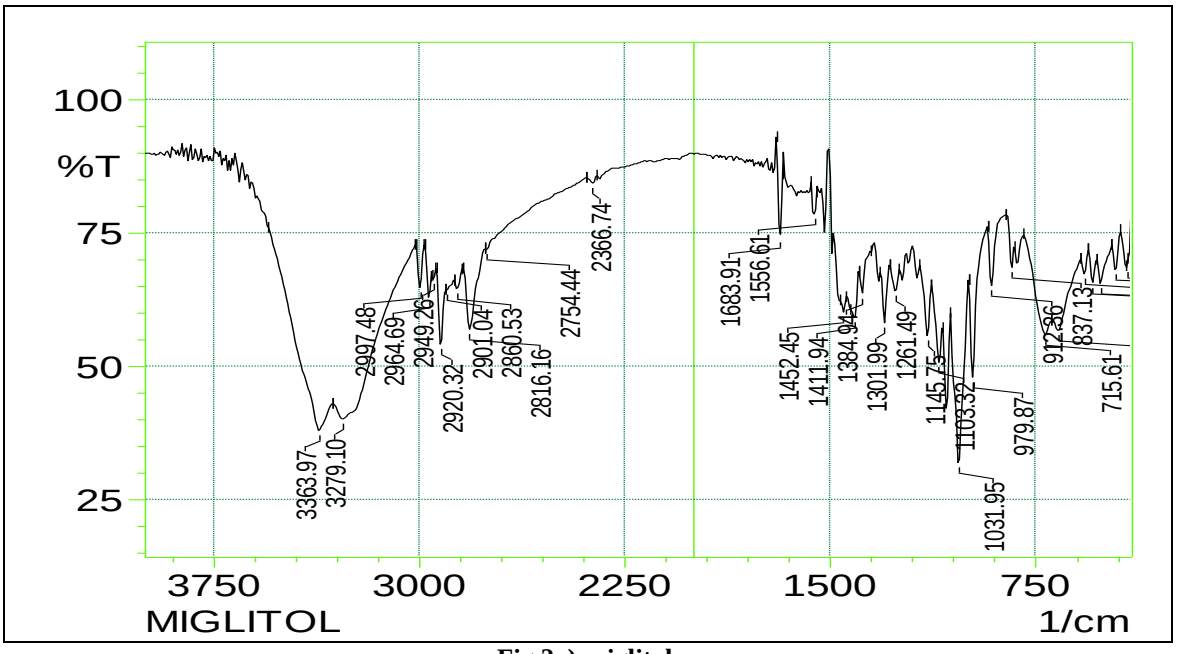


Fig.2a)-miglitol.

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.

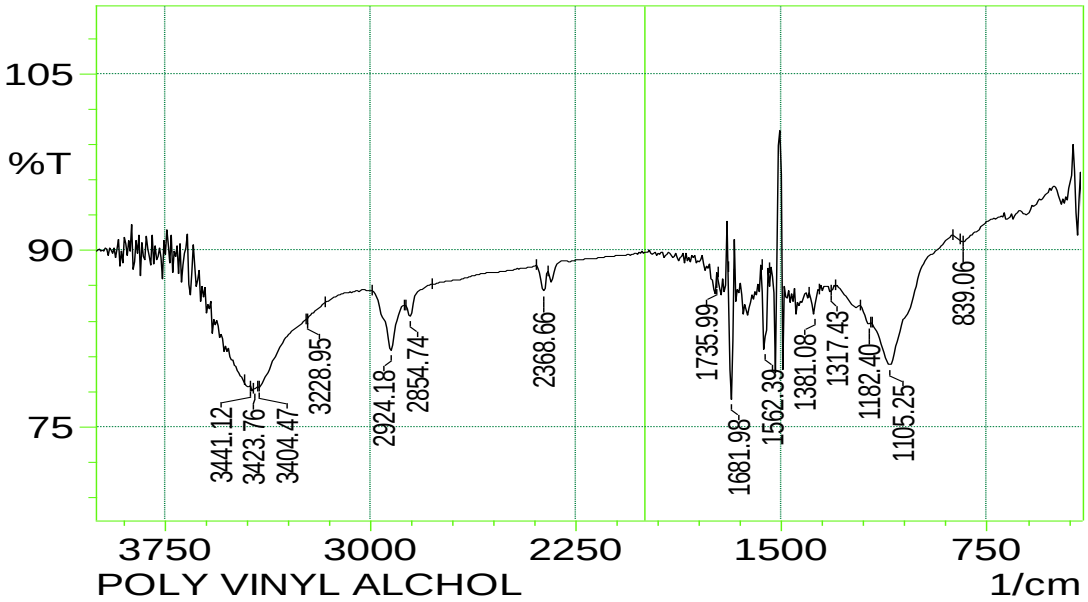


Fig 2b): PVA.

- 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.

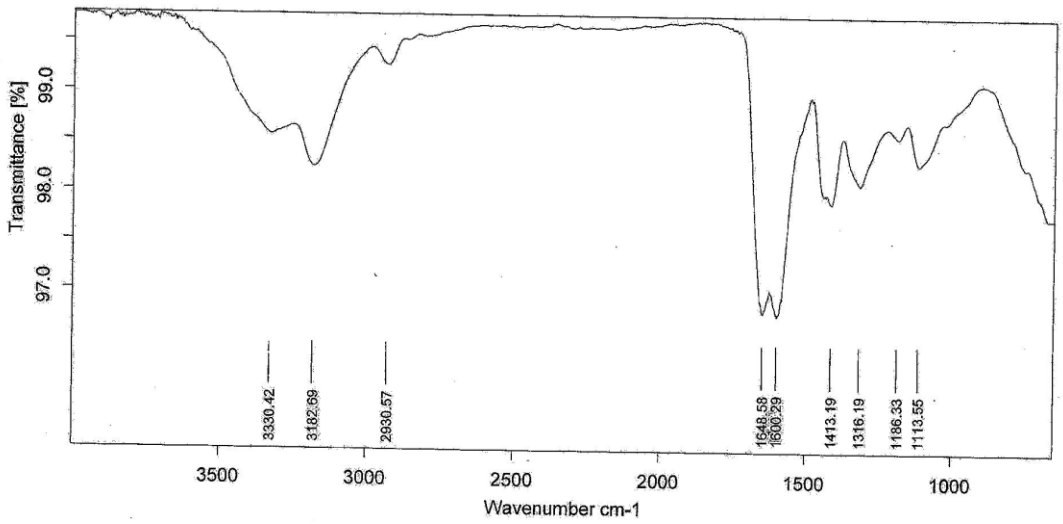


Fig 2c): HPMC.

- At 3330.42 cm^{-1} OH stretching peak of HPMC can be seen.
- From 2930.57 cm^{-1} to 3182.69 cm^{-1} a C-H stretching of HPMC
- From 1413.19 cm^{-1} to 1316.19 cm^{-1} another stretching are there in the spectrum, which might be due to there C-C stretching and C-OH stretching in HPMC.
- From 1113.55 cm^{-1} to 1186.33 cm^{-1} another stretching are there, these are because of C-O-C in the HPMC.

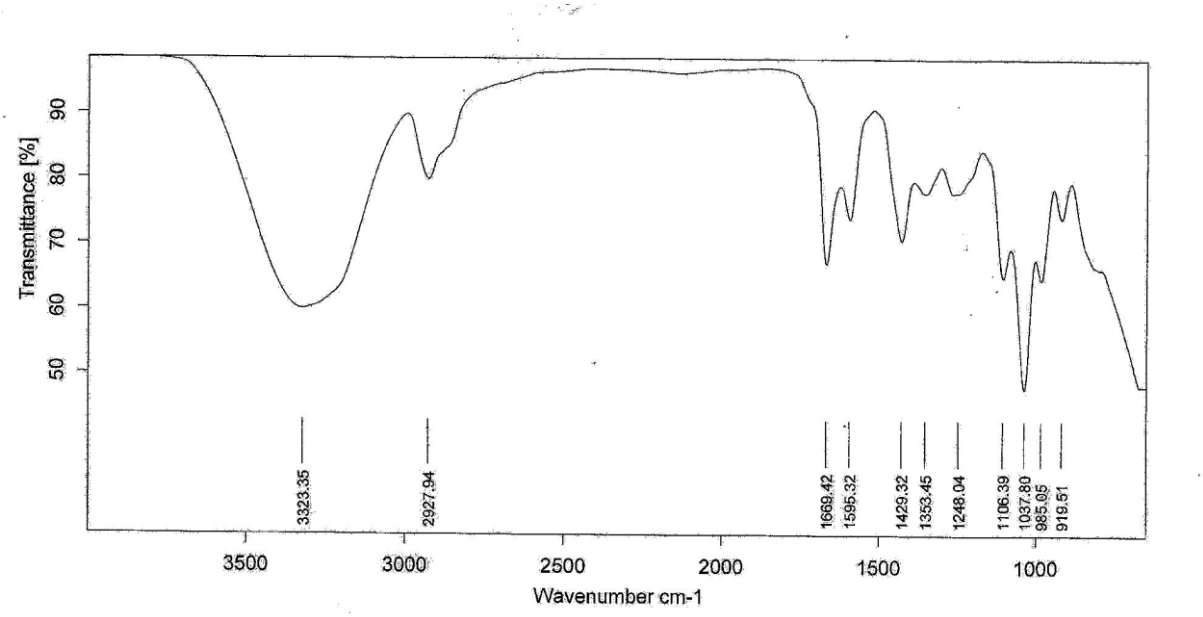


Fig 2d): M-HPMC.

- At 3323.35 cm^{-1} OH stretching peak of Miglitol and HPMC can be seen.
- At 2927.94 cm^{-1} a stretching is there, due to C-H stretching of both HPMC and Miglitol
- From 1413.19 cm^{-1} to 1248.04 cm^{-1} another stretchings are there in the spectrum, which might be due to the C-N stretching in miglitol, C-OH stretching in both and C-C stretching in both.
- From 1106.39 cm^{-1} to 1037.80 cm^{-1} another stretchings are there, this are because of C-O-C in the HPMC.

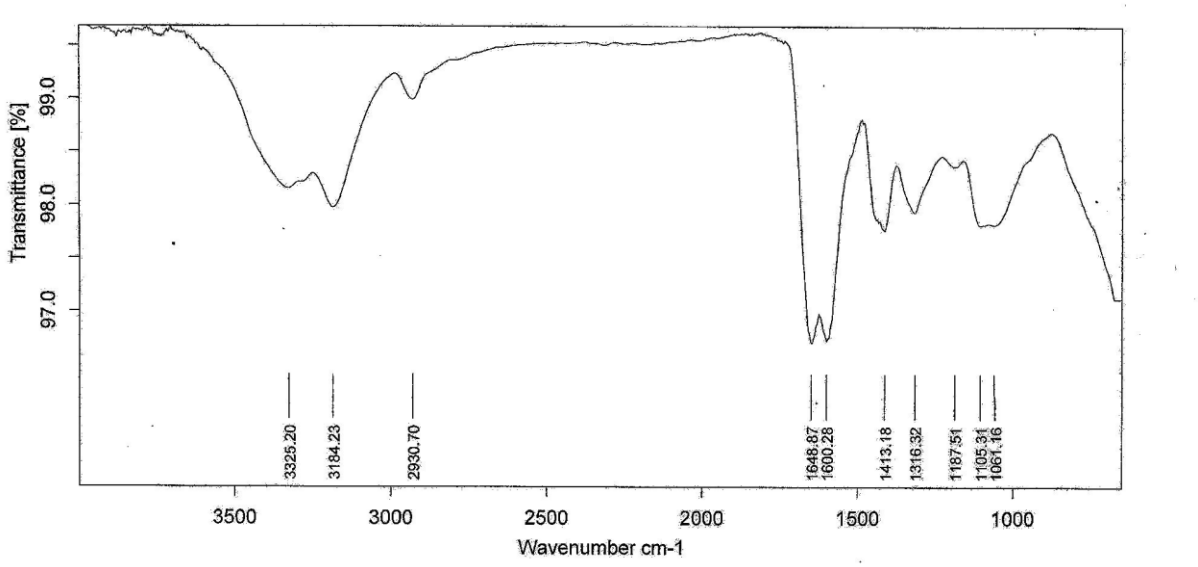


Fig 2e): Am-g-HPMC.

- At 3325.20 cm^{-1} OH stretching peak of HPMC can be seen.
- At 3184,23 cm^{-1} due to NH stretching of acrylamide.
- At 2930.70 cm^{-1}CH stretching of HPMC can be seen.
- At 1648.87 cm^{-1} one stretching can be seen, it may be because of amide carbonyl group present in acrylamide.
- At 1600.28 cm^{-1} another stretching is there in spectrum, which is due to the c=c in acrylamide.
- From 1100 cm^{-1} to 1400 cm^{-1} range few stretching are these are due to presence of C-N stretching in acrylamide and C-C stretching in both HPMC and acrylamide.
- Specifically, C-O stretching in HPMC can be seen at 1150 cm^{-1} range.

Scanning Electronic Microscopy

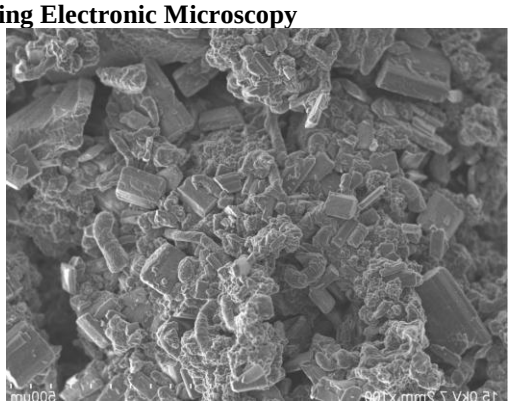


Fig. 3a

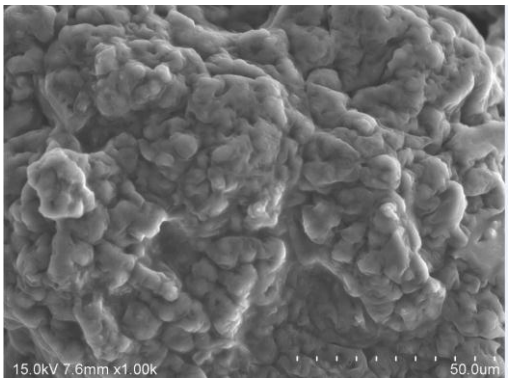


Fig 3b

The surface morphology of the particles prepared by acrylamide grafted Hydroxy propyl methyl cellulose (Am-g-HPMC) having (GA) glutaraldehyde in the lower concentration displayed porous nature on the surface fig 3a but in case of particles prepared with higher amount of GA fig 3b. showed the absence of pores.

X-ray Diffraction

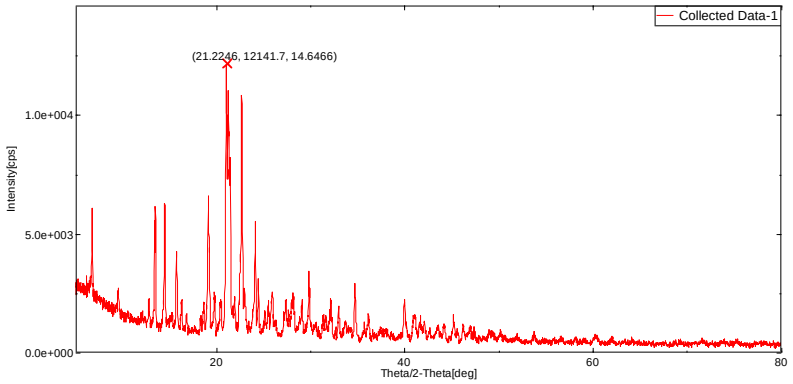


Fig 4a) miglitol.

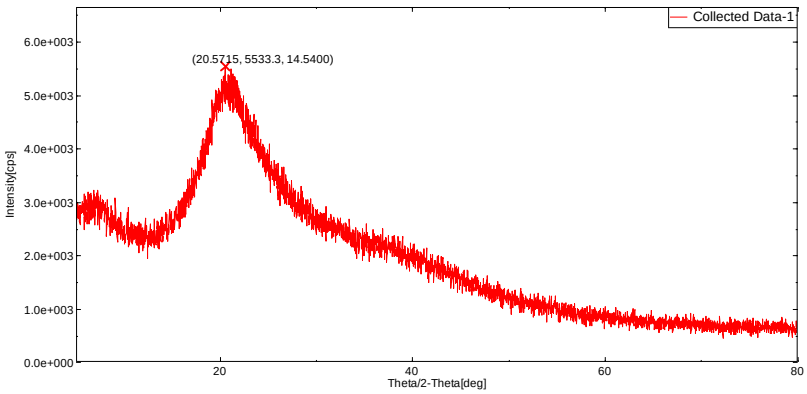


Fig.4b) Miglitol acrylamide grafted HPMC

A peak at 21.22^0 of miglitol XRD report exhibited indicated its nature of crystallinity i.e., fig 4a, the microspheres loaded with drugs shown a peak at 20.57^0 though microspheres with miglitol exhibited the disappearance of other peaks in the fig 4b.

Percentage yield of IPN microspheres

The percentage yield of prepared Am-g-HPMC-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 85.36 (F4) to 94.36 (F6). (Table 7)

Table 7: % yield of Am-g-HPMC-PVA IPN microspheres containing Miglitol.

Formulation code	Percent yield (%)
F1	92.22
F2	87.55
F3	91.28
F4	85.36
F5	91.78
F6	94.36
F7	92.58
F8	91.68

Drug entrapment efficiency: (% DEE)

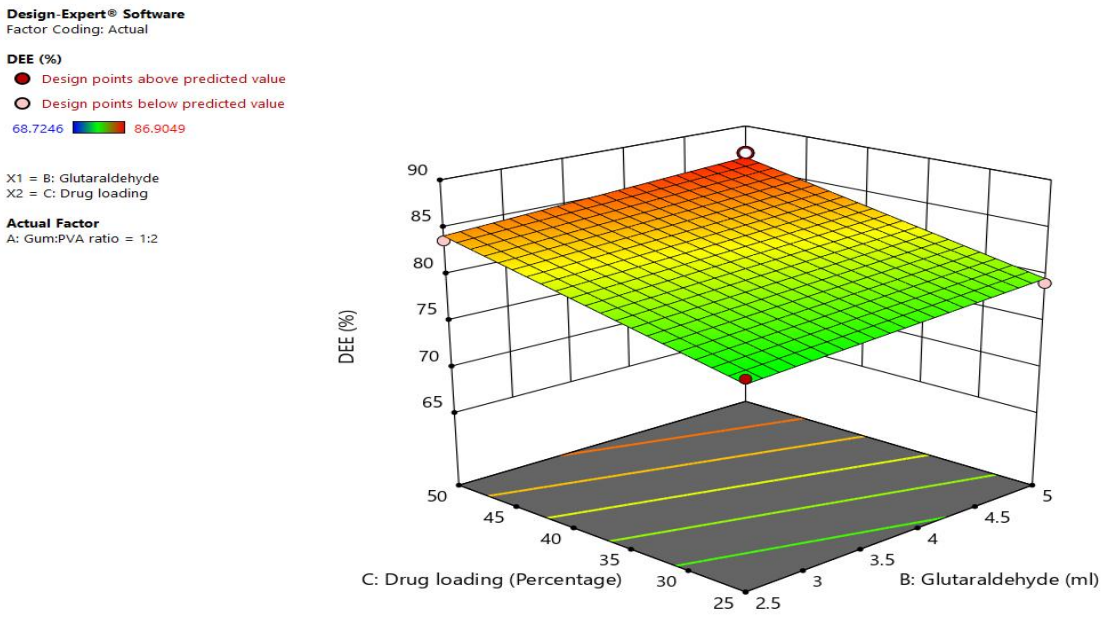
Entrapment of drug in any matrix system is considered as important criteria for selection of suitable batch formula as amount of drug retained in matrix indicates the overall efficiency of drug delivery system showing sustainability and ability to prolong drug availability in site of action. In the present study, the drug entrapment efficiency percentage ranges from 68.72 ± 0.85 (F1) to 86.90 ± 0.84 (F7) (Table 8).

Table 8: Drug entrapment efficiency percentage of Am-g-HPMC-PVA IPN microspheres containing Miglitol.

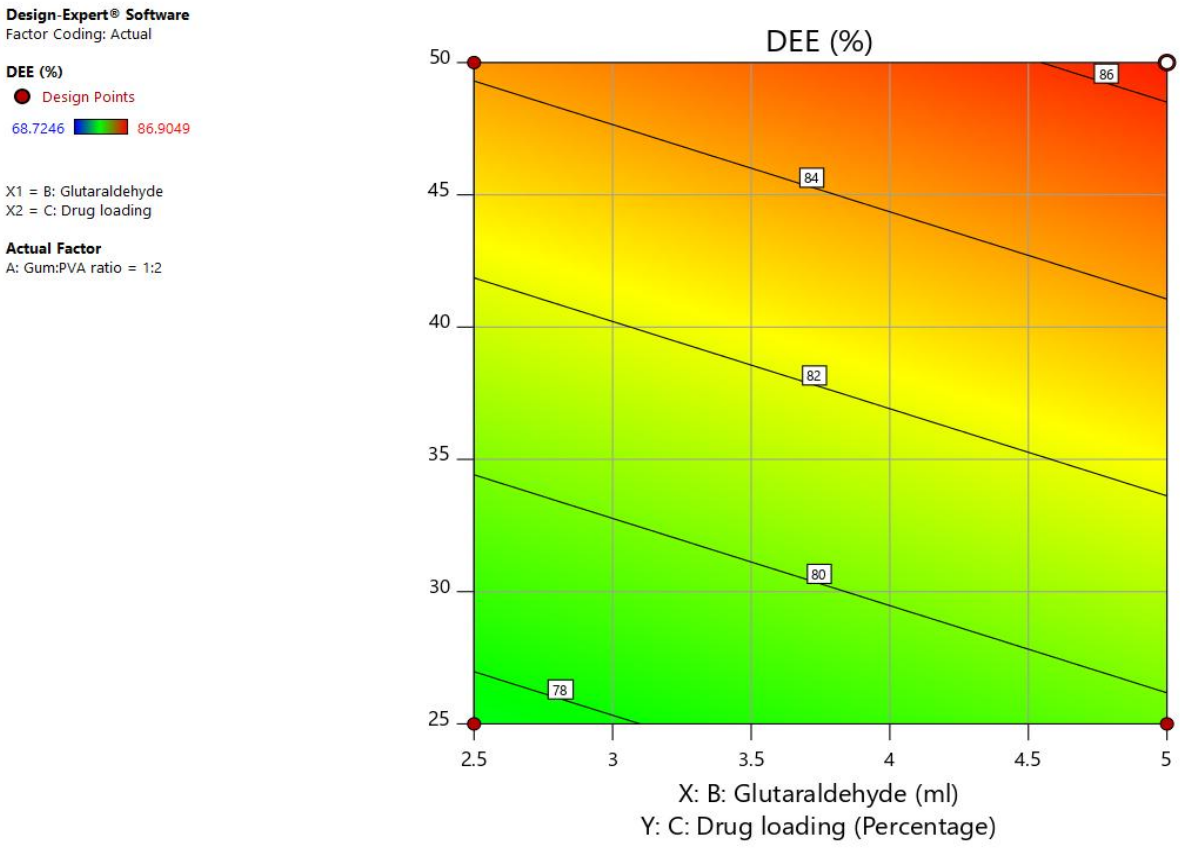
Formulation Code	Drug entrapment efficiency (in %) ± SD, n = 3
F1	68.72 ± 0.85
F2	83.68 ± 0.29
F3	79.17 ± 1.12
F4	73.36 ± 0.76
F5	75.82 ± 0.54
F6	77.97 ± 1.08
F7	86.90 ± 0.84
F8	70.41 ± 0.08

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.

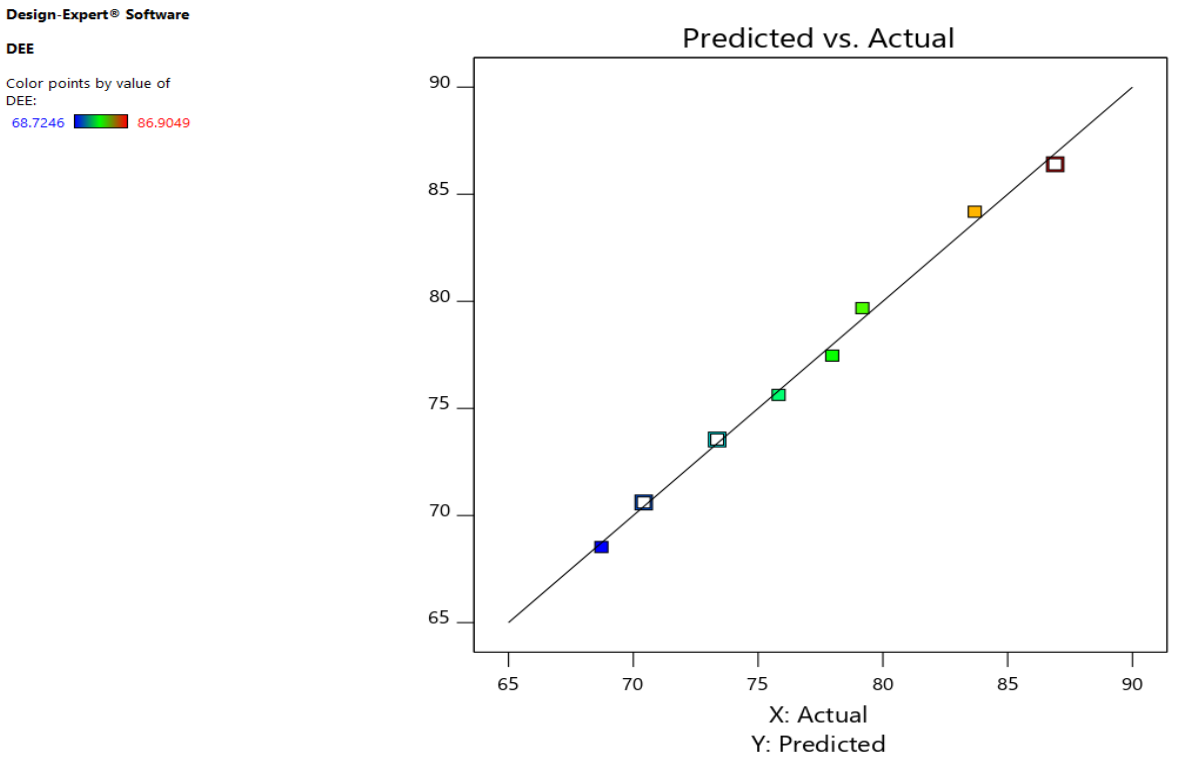
The three-dimensional plot of the effects of different independent variables on %DEE is shown in figure 5.



(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 %DEE with the independent variables (MLRA) was generated and expressed as:
% DEE = 77.01 + 4.93 A + 1.07 B + 2.93 C + 0.0339 AB + 0.4242 AC where, A is Am-g-HPMC:PVA ratio, B is glutaraldehyde amount and C is % drug loading. Here we observed that all the independent variables were having impact on %DEE as was observed experimentally.

ANOVA analysis indicated that the model was significant (P= 0.0105 < 0.0001) with R^2 value 0.9958. The Predicted R^2 of 0.9323 was in reasonable agreement with the Adjusted R^2 of 0.9852; i.e. the difference is less than 0.2. (Table 6)
Adequate precision ratio with >4 is desirable. The ratio of 27.05 indicates an adequate signal. Thus, the present model can be used to navigate the design space.

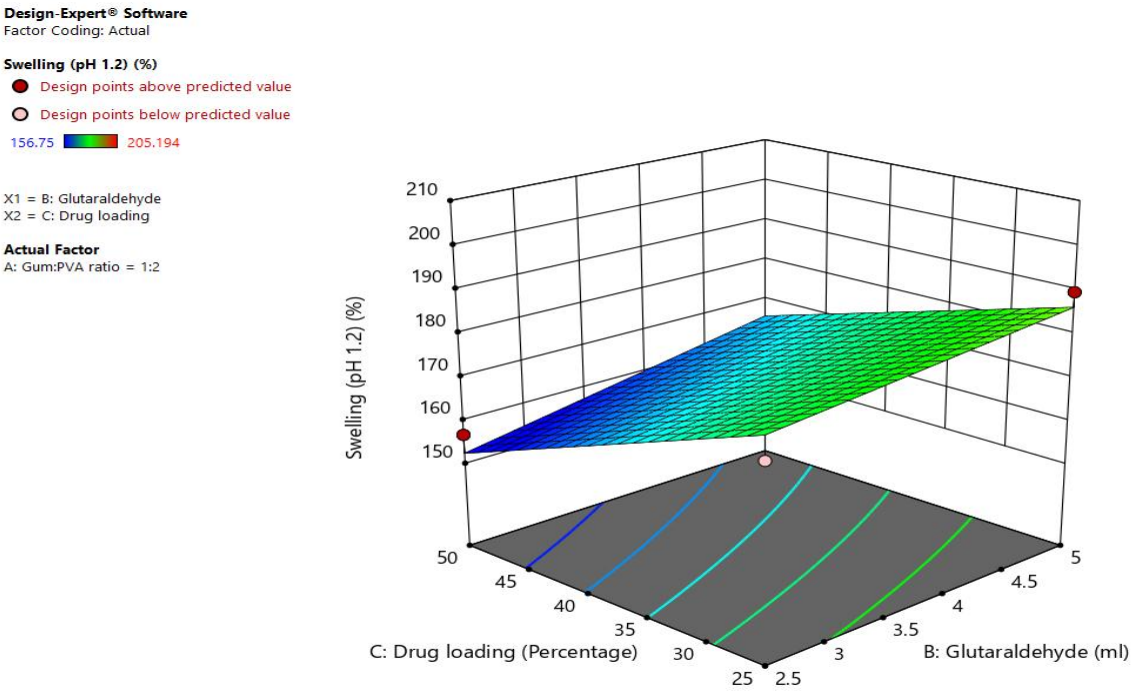
Swelling Studies
Swelling study is also considered as important criteria for selection of suitable batch formula for the optimum drug release. The % equilibrium water uptake data of the IPN microspheres (Table 9)

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

Formulation Code	Swelling / Equilibrium water uptake (in %) ± SD, n = 3	
	At pH 1.2	At pH 6.8
F1	205.19 ± 2.33	238.43 ± 0.85
F2	156.75 ± 1.87	192.89 ± 0.66
F3	189.71 ± 0.99	223.76 ± 0.58
F4	168.66 ± 1.25	211.04 ± 1.22
F5	188.93 ± 1.36	223.74 ± 1.56
F6	173.86 ± 1.54	212.26 ± 0.89
F7	163.28 ± 0.85	201.89 ± 0.55
F8	203.56 ± 0.47	243.66 ± 0.26

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 156.75 ± 1.87 (F2) to 205.19 ± 2.33 (F1) and at the pH 6.8 it ranges from 192.89 ± 0.66 (F2) to 243.66 ± 0.26 (F8).

The effect of independent variables on Swelling is shown in the figure 6 & 7.entrapment efficiency.



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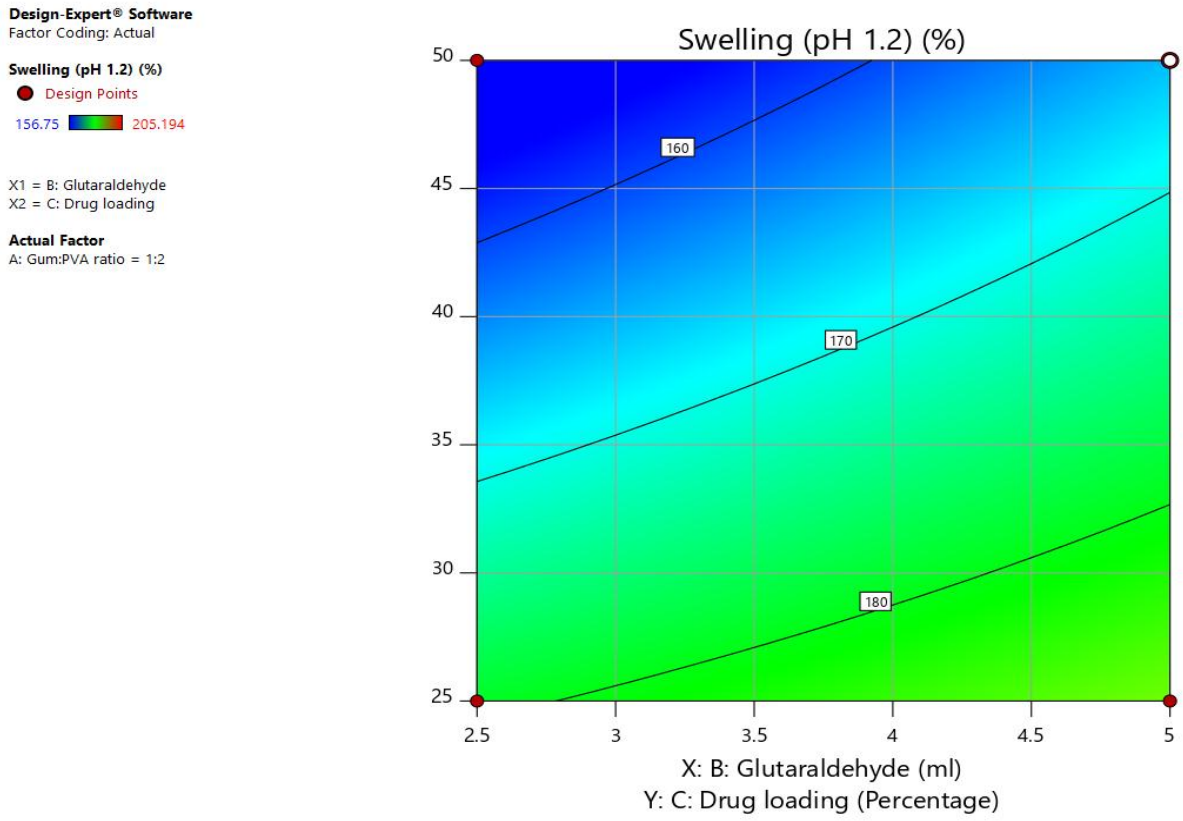
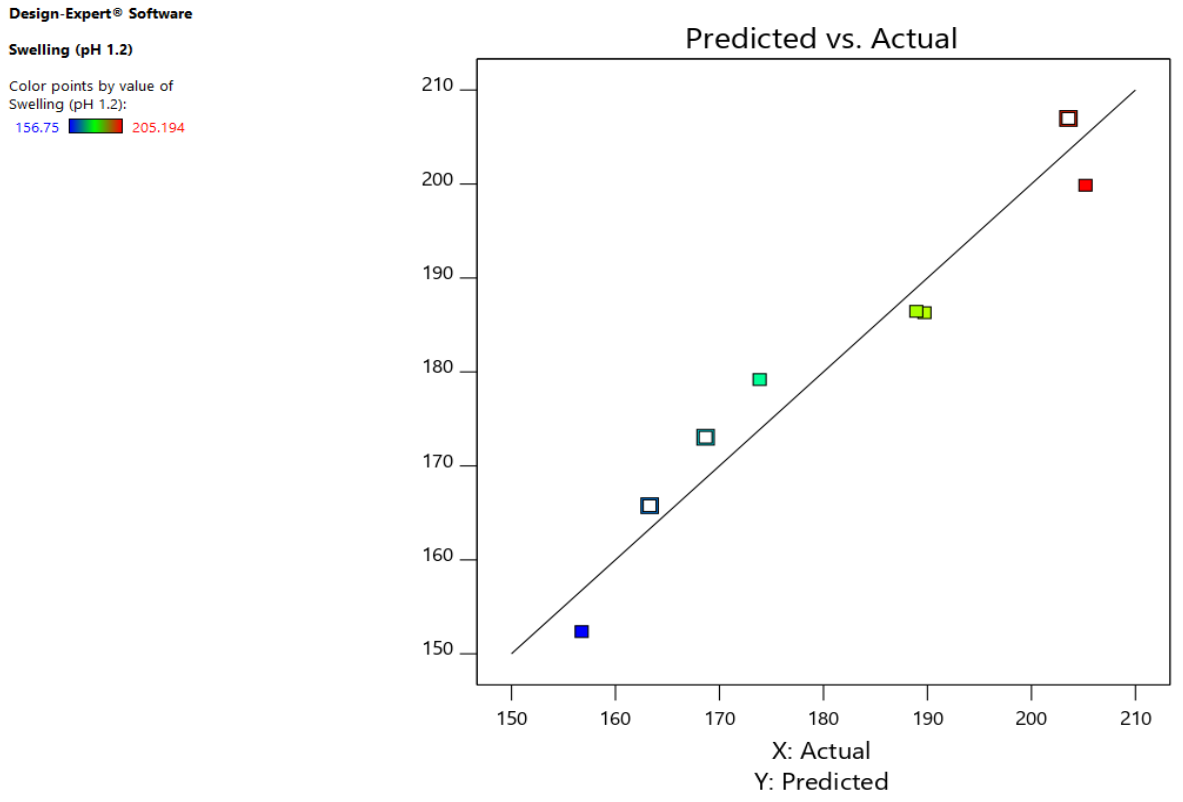
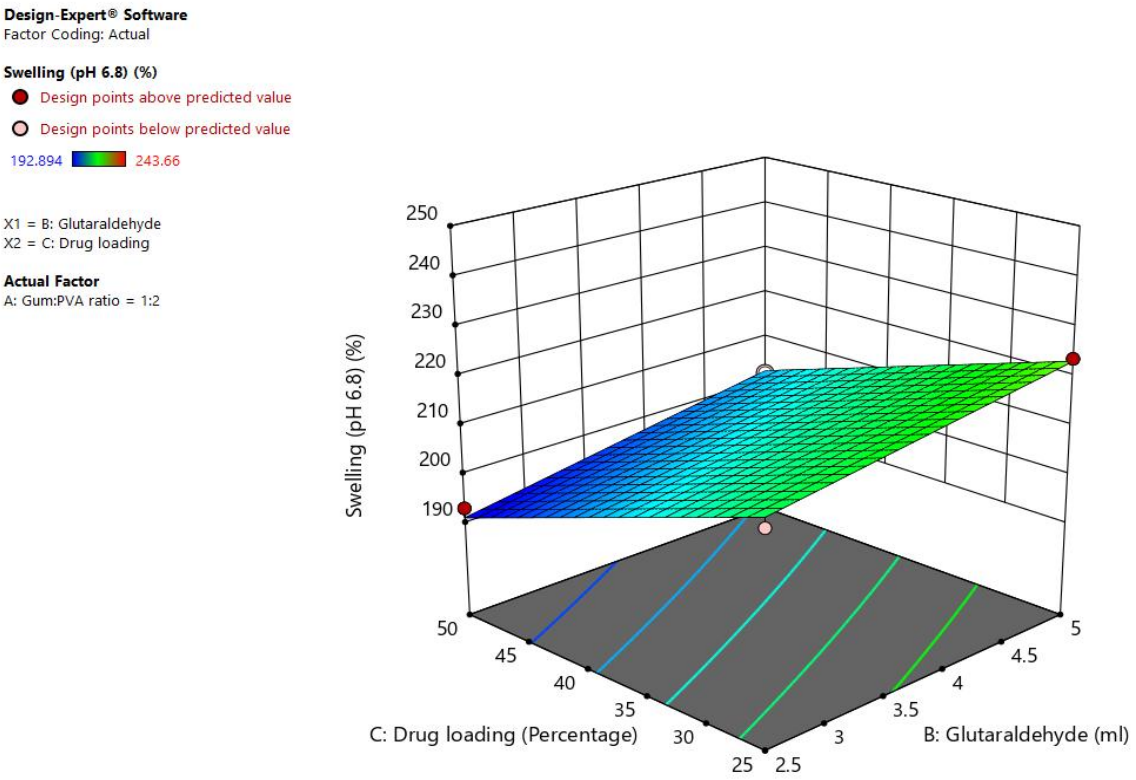


(b)

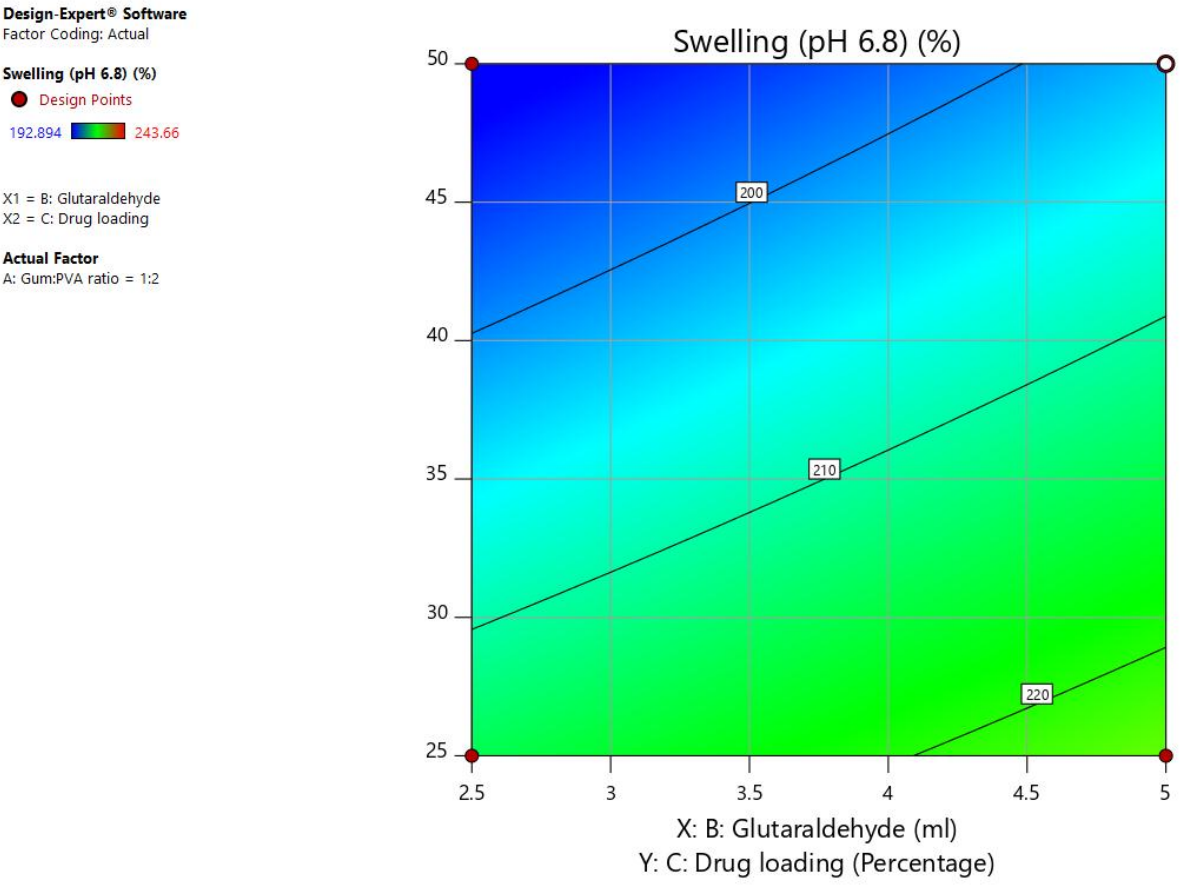


(c)

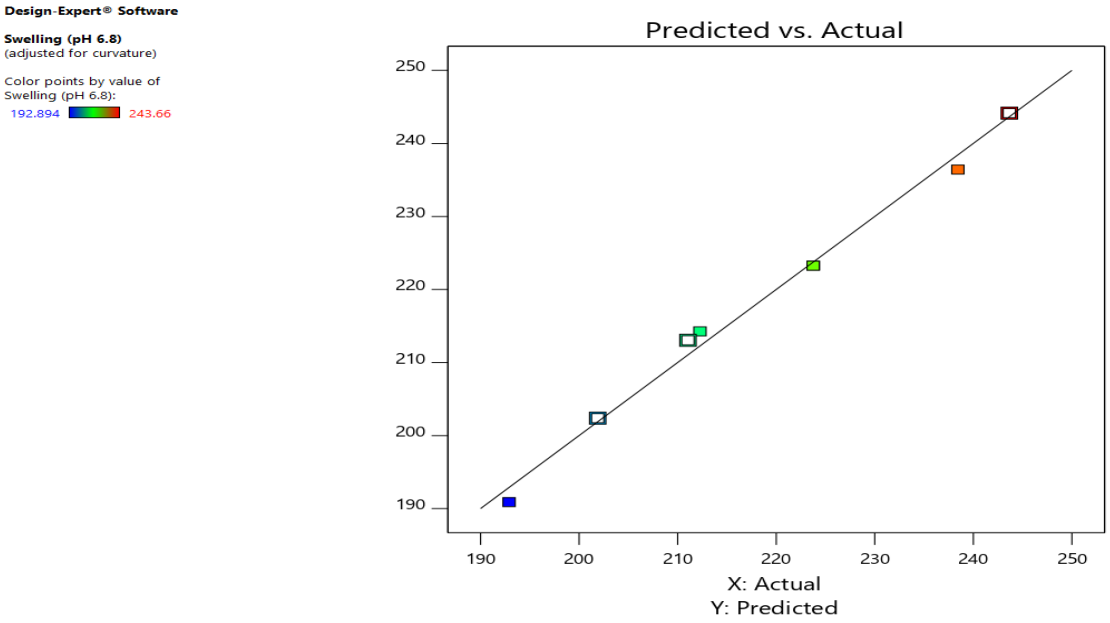
Figure 6: (a) Three dimensional response surface plots: showing the effects of synthetic condition on Swelling at pH 1.2 (b) Corresponding contour plot showing the relationship between various levels of the factors, (c) Plot between observed and predicted values of Swelling at pH 1.2.


Design-Expert® Software
Factor Coding: Actual
Swelling (pH 6.8) (%)
Design points above predicted value
Design points below predicted value
192.894
243.66
X1 = B: Glutaraldehyde
X2 = C: Drug loading
Actual Factor
A: Gum:PVA ratio = 1:2
Swelling (pH 6.8) (%)
250
240
230
220
210
200
190
C: Drug loading (Percentage)
50
45
40
35
30
25
B: Glutaraldehyde (ml)
2.5
3
3.5
4
4.5
5

(a)


Design-Expert® Software
Factor Coding: Actual
Swelling (pH 6.8) (%)
Design Points
192.894
243.66
X1 = B: Glutaraldehyde
X2 = C: Drug loading
Actual Factor
A: Gum:PVA ratio = 1:2
Swelling (pH 6.8) (%)
200
210
220
50
45
40
35
30
25
2.5
3
3.5
4
4.5
5
X: B: Glutaraldehyde (ml)
Y: C: Drug loading (Percentage)

(b)



(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 % (pH 1.2) = 181.25 – 10.34 A + 5.13 B – 11.84 C + 1.57 BC

Swelling % (pH 6.8) = 218.46 – 10.76 A + 4.80 B - 11.07 C + 0.321 AB + 0.622 BC

where, A is Am-g-HPMC: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 0.0320 & 0.0201 ($P < 0.05$) with R^2 value 0.9440 & 0.9919 respectively. (Table 6)

The predicted R^2 (0.7021, 0.8708), is in reasonable agreement with the adjusted R^2 (0.8694, 0.9717); 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 10.46 (pH 1.2) & 21.06 (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-HPMC-PVA IPN microspheres are presented in the figure 6.

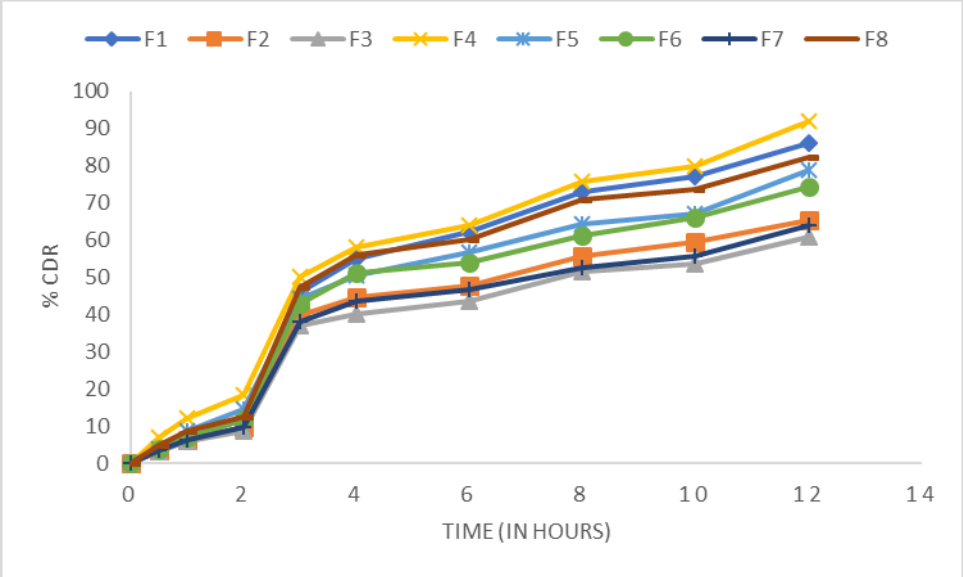


Figure 8: *In vitro* release characteristics of various formulations of Am-g-HPMC-PVA IPN Microspheres containing Miglitol.

In the present study, after 12 hours drug dissolution study, the drug release percentage ranges from 60.84 (F3) to 91.94 (F4) (Table 10).

Table 10: % Cumulative drug release of Am-g-HPMC-PVA IPN microspheres containing Miglitol.

TIME (in hours)	Cumulative Drug release (CDR) (in %)							
	F1	F2	F3	F4	F5	F6	F7	F8
0.5	4.09	3.63	3.08	6.89	3.9	3.7	3.5	4.79
1	7.01	6.33	5.79	12.19	8.48	7.01	6.07	8.8
2	11.65	9.72	8.76	18.27	14.45	11.85	9.6	12.61
3	46.13	39.84	36.95	50.19	44.07	42.96	37.88	47.25
4	54.77	44.41	40.19	58.22	50.32	51.2	43.39	56.11
6	62.07	47.65	43.68	63.99	56.52	53.79	46.52	60.26
8	72.87	55.49	51.54	75.77	64.33	61.09	52.42	70.93
10	77.21	59.47	53.54	79.9	66.98	65.9	55.66	73.72
12	86.13	65.3	60.84	91.94	78.94	74.27	63.86	82.38

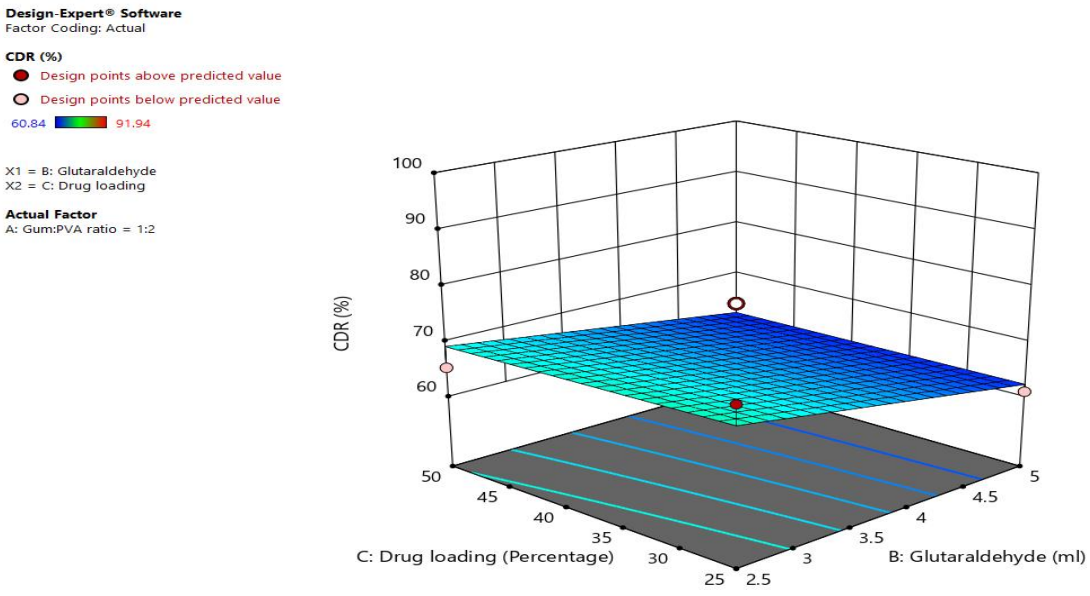
It was observed that the drug release from the IPN microspheres were dependent upon major factors like crosslinker amount, polymeric blend ratio and on the amount of drug loading.

Crosslinker effect: Formulations, having different drug loading in a fixed gum: PVA ratio and % drug loading, showed different extent of drug release. F2 (2.5 mL, 65.3% drug release) showed increased drug release property than F7 (5 mL, 63.86 % 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.

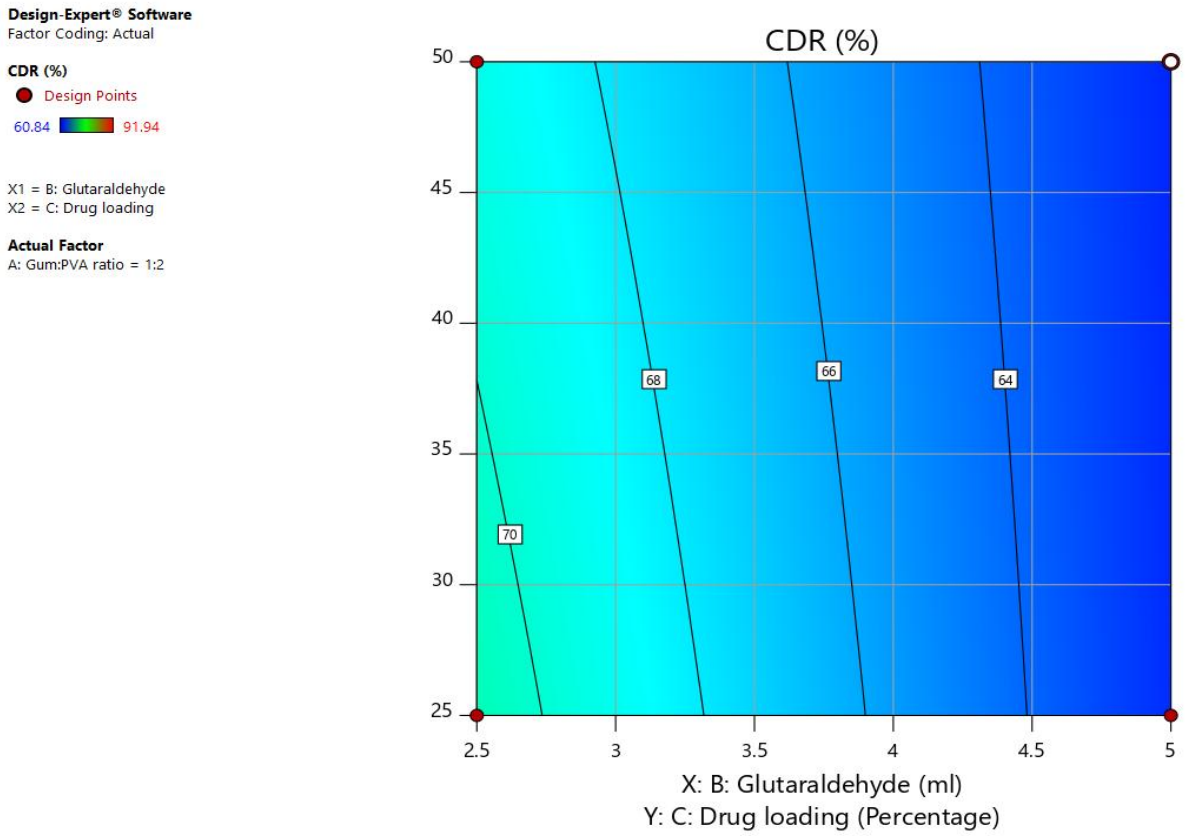
Effect of drug loading: In fixed formulating parameters, when the amount of drug loading was increased from 25% to 50%, the amount of drug release was decreased. In formulation F1 and F2 polymer composition (1:1) and glutaraldehyde (5 mL) was same but amount of drug loading was different. F8 (82.38%, 25% drug loading) showed more drug release than F5 (78.94 %, 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-HPMC: 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 74.27% (F6, 1:2) to 86.13% (F1, 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.

The 3-dimensional plot of the effects of different formulation variables on % CDR (at 12 hours) is shown in the figure 9.



(a)



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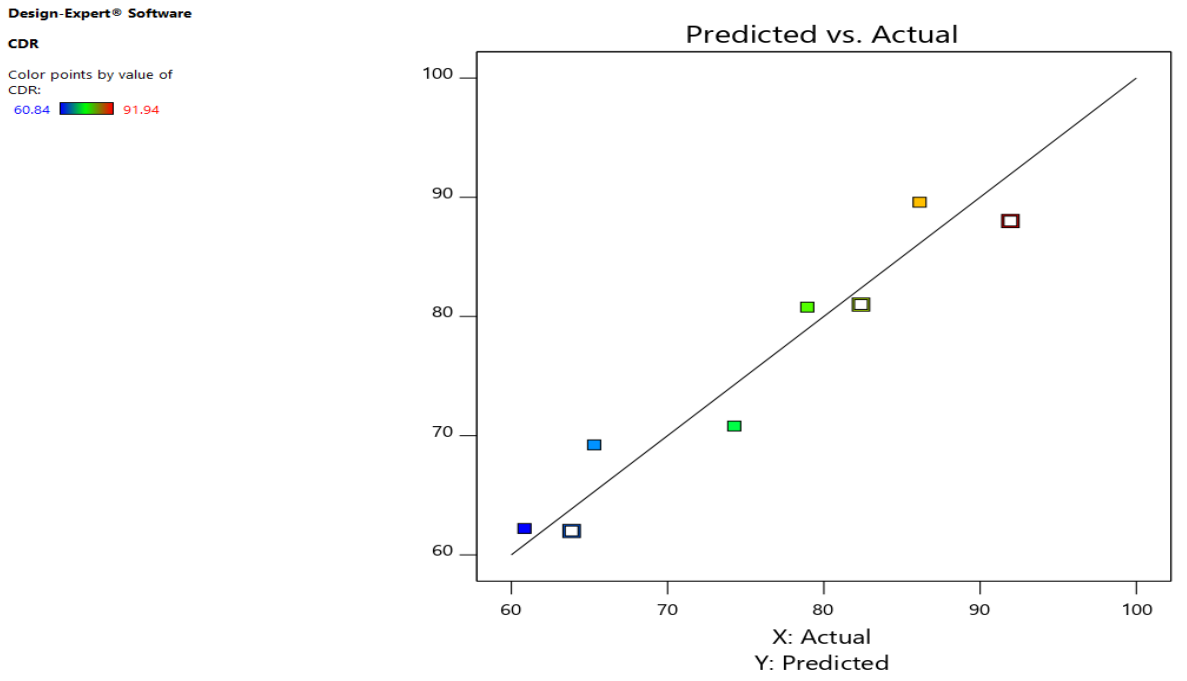


(c)

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 mathematical relationship of % CDR (at 12 hours) with the independent variables was generated and expressed as:

$$\% CDR = 75.46 - 9.39\,A - 3.95\,B - 0.4475\,C + 0.3425\,BC$$

where, A is Am-g-HPMC:PVA ratio, B is glutaraldehyde amount and C is % drug loading.

ANOVA analysis indicated that the model was significant with P values 0.0470 ($P < 0.05$) with R^2 value 0.9271. (Table 6).

The Predicted R^2 of 0.6816 is in reasonable agreement with the Adjusted R^2 of 0.8299; i.e. the difference is less than 0.2. Adequate Precision ratio greater than 4 was desired. The ratio of 7.4699 indicates an adequate signal. Hence, this model can be used to navigate the design space.

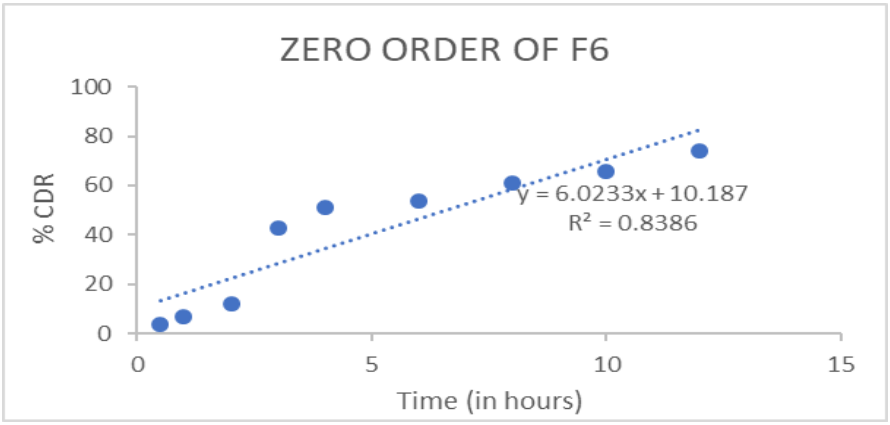
Drug release Kinetic Study

The *in vitro* release data were fitted into various empirical kinetic equations and presented in the Table 11.

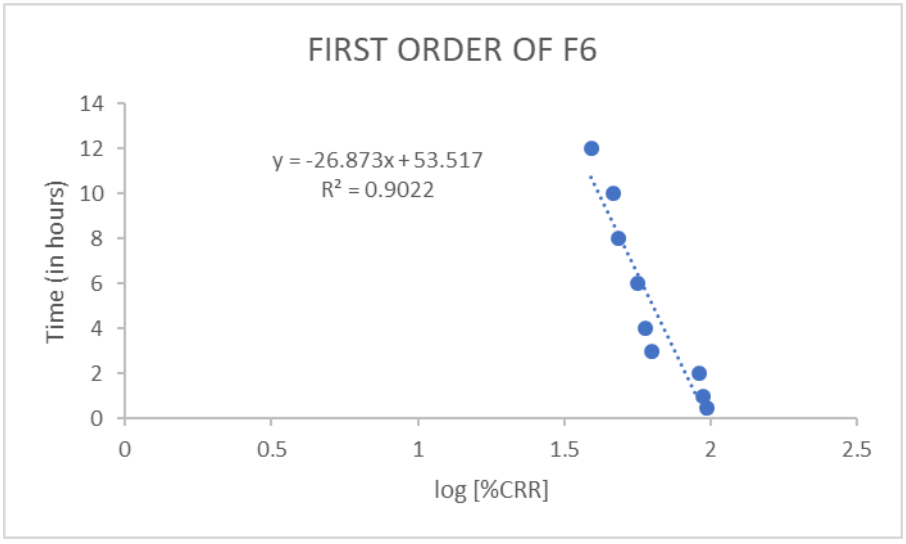
Table 11: Drug release kinetics data of Am-g-HPMC-PVA IPN microspheres containing Miglitol.

Formulation Code	Zero Order	First Order	Higuchi model	Korsmeyer- Peppas	
	R^2	R^2	R^2	R^2	n value
F1	0.8694	0.9576	0.9382	0.9259	1.0387
F2	0.8331	0.9817	0.913	0.9127	0.9792
F3	0.8347	0.9155	0.9131	0.9133	0.9975
F4	0.8831	0.9715	0.9497	0.9447	0.8482
F5	0.862	0.9107	0.9372	0.9347	0.9652
F6	0.8386	0.9022	0.9185	0.9212	0.9961
F7	0.8316	0.9571	0.9114	0.9135	0.9763
F8	0.8444	0.9464	0.9223	0.9201	0.9556

After plotting zero order, first order, higuchi plots for the optimized formulation F6, it was observed that the best fit was with the Higuchi model, which suggests the release of drug from matrix was diffusion controlled. (Figure 8)



(a)



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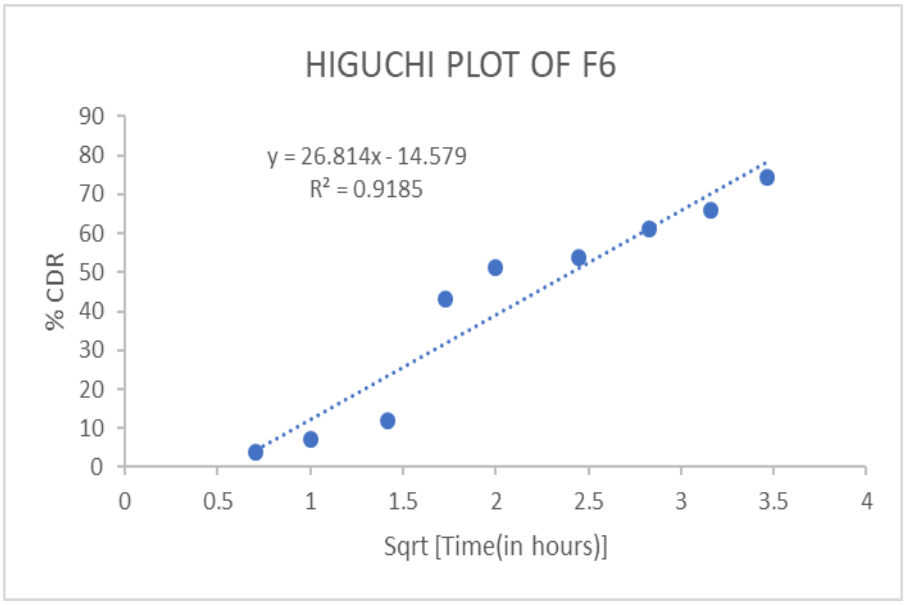

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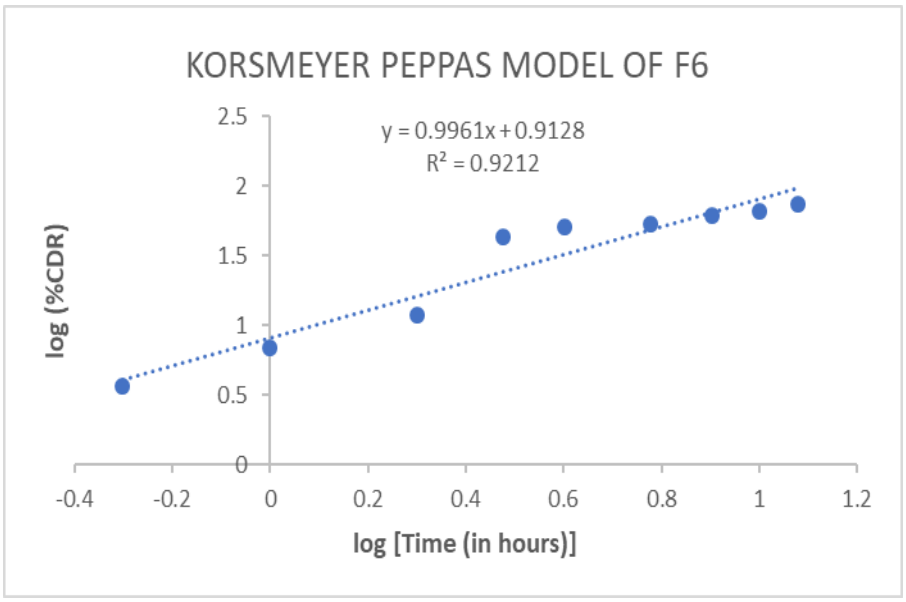

(d)

Figure 10: Release kinetics of the optimized formulation (F8) - (a) Zero Order (b) First order (c) Higuchi model (d) Korsmeyer peppas model.

The Korsmeyer peppas equation was also used for the kinetics study, except F1, the n value ranges from 0.8482 to 0.9975, which shows that all the formulations follows Super case-II transport, where as, F1 have n value of 1.0387, 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 F6 fulfilled nearly all the criteria set from the desirability search (Figure 11).

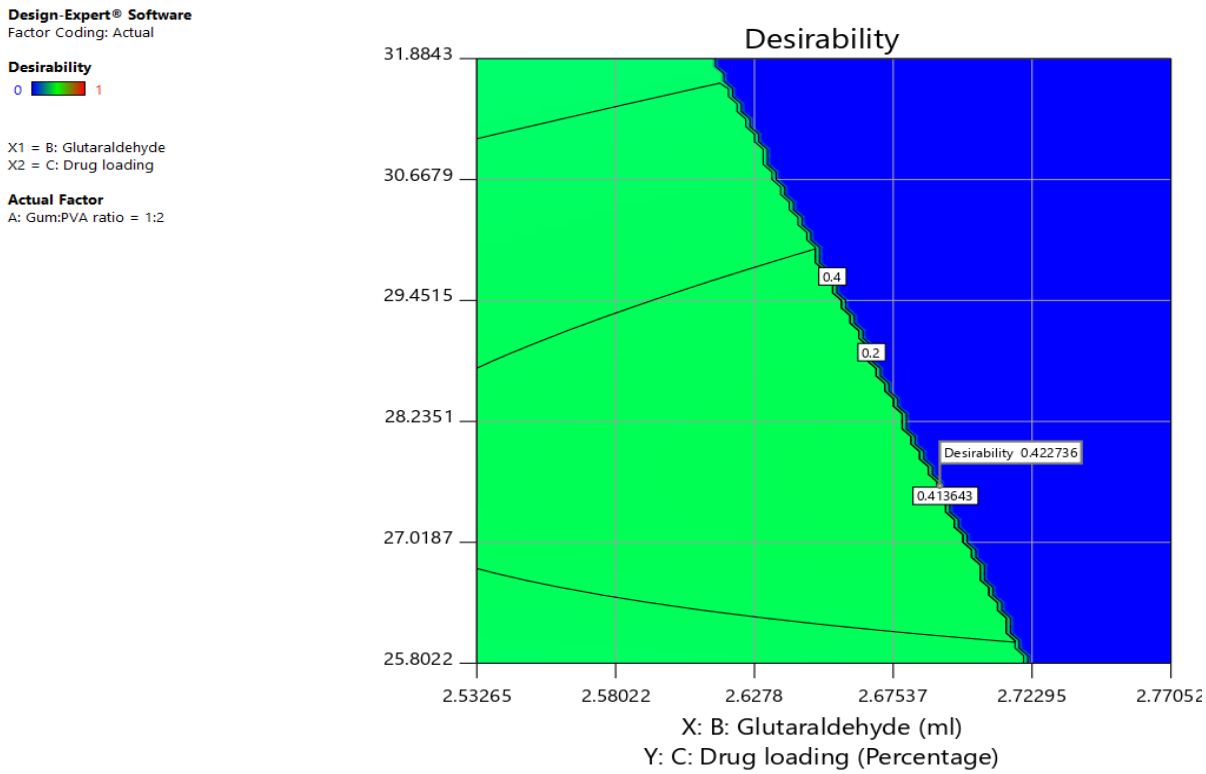

Figure 11: Contour plot showing the optimization procedure depending on numerical method.

The low % prediction error of 1.0087 to 8.4170 indicated the high prognostic ability of the factorial model (Table 12).

Table 12: Predicted and observed response variables of the optimal Am-g-HPMC-PVA IPN microspheres containing Miglitol.

Response variables	Predicted ± SD (Software suggested)	Observed (H1 & F6)	Predicted error (in %)
A. Acrylamide grafted HPMC gums			
Grafting efficiency (in %)	79.46 ± 0.91	79.39	1.2096
B. Am-g-HPMC-PVA IPN microspheres containing Miglitol			
Swelling at pH 1.2 (in %)	179.19 ± 6.60	173.867	8.4170
Swelling at pH 6.8 (in %)	214.27 ± 2.91	212.266	3.8622
Drug entrapment efficiency (in %)	77.46 ± 0.76	77.9726	1.0087
Cumulative Drug release (CDR) at 12 hours (in %)	70.81 ± 4.67	74.27	5.9558

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 (F6) was stable. The results (Table 10) suggests that the developed IPN microspheres containing Miglitol were stable for storage for long period of time.

Table 13: Stability study of Am-g-HPMC-PVA IPN microspheres containing Miglitol.

Trial No.	Drug entrapment efficiency (%) of F6			
	1st Day	After 4 weeks	After 6 weeks	After 12 weeks
I	82.33	82.51	81.23	80.25
II	81.25	80.52	81.56	80.66
III	81.58	80.69	80.22	81.25
Average	81.72 ± 0.45	81.24 ± 0.90	81.00 ± 0.57	80.72 ± 0.41

CONCLUSION

In conclusion, Am-g-HPMC-PVA IPN microspheres containing Miglitol were successfully prepared by emulsion crosslinking method by using glutaraldehyde as a crosslinker. The IPN microspheres were produced following design of experiment and optimized with the

help of response surface methodology involving the independent factors and variable responses. The formulation coded as F6 was found to be optimized with desirable controlled release property with moderate pH sensitivity and had the better drug entrapment effeciency. These findings when taken together suggest that the present formulated IPN micropheres containing Miglitol can be reproduced with high predictability and shall be potentially useful to patients with hyperglycaemia . This type of formulations may be also usefull as a promising biomaterial to overcome the major problems of controlled release of highly water soluble drugs with shorter half life.

ACKNOWLEDGMENT

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