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Quality-by-design Approach for Development of Sustained-release Microbeads of Aceclofenac Based on Ionically Cross-linked Composite of Xanthan Gum and Sodium Alginate: An *In Vitro* Evaluation and Mathematical Kinetic Modeling

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Abstract

In the present research work, colon-targeted aceclofenac hydrogel microbeads were formulated by an ionotropic gelation method using a quality-by-design (QbD) approach. A 2^3 factorial design was used to optimize the xanthan gum-sodium alginate (Xg-Sa) microbeads by considering the effect of three factors: Sodium alginate, Xg, and calcium chloride on response percent yield, entrapment efficiency (EE), drug content (DC), Particle size, and swelling degree. The results of the 3D response surface plot and contour plot identified an optimized microbead formulation batch with high yield, EE, and DC, with a comparatively small particle size. Swelling studies showed pronounced expansion at pH 7.4 but negligible swelling at acidic pH, confirming protection against pre-mature release. The *in vitro* cumulative drug release study exhibited minimal drug release in simulated gastric fluid (SGF) at pH 1.2, simulated intestinal fluid (SIF) at pH 6.8, and sustained drug release at simulated colonic fluid (SCF) at pH 7.4, attributed to electrostatic repulsion between ionized carboxyl groups within the Xg-Sa polymeric matrix. Drug release followed the Higuchi model (R^2 = 0.984–0.996) and Korsmeyer-Peppas (R^2 = 0.982–0.997) with an anomalous non-Fickian transport mechanism (n = 0.59–0.79). Thus, aceclofenac release was governed by combined erosion and diffusion mechanisms. These findings confirm that Xg-Sa microbeads, developed through ionic gelation, constitute an effective colonspecific platform. They safeguard aceclofenac against upper gastrointestinal degradation and provide sustained release in the colon.

Key words: Colon-targeted drug delivery, controlled release, factorial design, hydrogel microbeads, ionotropic gelation, quality-by-design

INTRODUCTION

Oral drug administration is considered the most preferred route for the delivery of drugs due to its convenience and patient compliance. However, most drugs undergo degradation or get metabolized in the upper gastrointestinal (GI) tract, thereby reducing their therapeutic effectiveness. Colon-targeted drug delivery system (CDDS) overcomes these limitations by protecting the drug during GI transit and releasing it directly to the colonic region, thereby enhancing therapeutic effectiveness and reducing systemic side effects in disease conditions, such as inflammatory bowel disease (IBD).[1] The colon has unique physiological characteristics, such as neutral pH, long transit time, and high microbial presence, which make it a suitable site for delivery of drugs. However, differences in gastric pH, transit time,

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and microbial presence may interfere with the efficacy of conventional colon-targeted systems. Thus, recent research has focused on developing multi-triggered drug delivery systems to achieve site- specific drug release.[2] Colon-targeted drug delivery has gained considerable attention because of the potential of achieving site-specific drug release, which increases efficiency and decreases toxicity. The use of CDDS is particularly advantageous in the management of colonic diseases, such as IBD and colorectal cancer.[3]

Biocompatible natural polymers are preferred in the design of CDDS due to their biodegradation into physiological metabolites and their ability to serve as effective excipients. Sodium alginate and xanthan gum (Xg) are the most widely used polymers for developing hydrogel polymeric matrix that enable sustained and controlled drug release.

Sodium alginate, an anionic polymer extracted from brown algae, is widely used in drug delivery due to its biocompatibility, biodegradability, non-toxicity, and pH responsiveness. It forms a hydrogel matrix through ionotropic gelation through metal ions, preventing premature release of drug in the upper GI tract and enabling colon targeting.[4] Structurally, it consists of β-D-mannuronic and α-L-guluronic acid units linked by (1→4) glycosidic bonds, which readily crosslink with divalent cations to form 3 dimensional "eggbox" hydrogel network.[5] The Xg, which is produced from *Xanthomonas campestris*, is a biocompatible, biodegradable, and non-toxic polysaccharide widely applied in oral and colon-targeted drug delivery.[6] It forms viscous, stable hydrogels, resists upper GI degradation, and is partially fermented in the colon, making it ideal for controlled release systems, such as beads and nanoparticles. Combined with polymers, such as chitosan or sodium alginate, it offers improved strength and site-specific release.[7]

Due to the short half-life of aceclofenac (approximately 4 h), controlled-release delivery systems are considered an effective approach to reduce dosing frequency, stabilize plasma concentrations, and reduce side effects. By promoting gradual release, these systems maintain therapeutic levels while minimizing GI tract damage.[8] Microbead systems, composed of multiple drug-loaded subunits, offer distinct advantages over single-unit forms: Their small particle size ensures uniform distribution, prevents peak plasma levels, and enhances dissolution and bioavailability. Drug entrapment within a polymeric matrix further reduces direct mucosal contact, thereby limiting GI irritation.[9] The present study focused on the development of dual cross-linked aceclofenac-loaded Xg-Sa hydrogel microbeads through ionic gelation, utilizing a QbD approach. Experimental design was structured using a 2^3 factorial design with DesignExpert® version 13.5.0 (StatEase Inc., USA). Key parameters, including yield, entrapment efficiency (EE), drug content (DC), swelling, and particle size were evaluated, and release mechanisms were studied through *in vitro* dissolution study.

MATERIALS AND METHODS

Aceclofenac was procured from Animus Healthcare, Ahmedabad, Gujarat, India, as a gift sample. Xg, sodium alginate (Sa), calcium chloride anhydrous (HiMedia Pvt. Ltd., India), and sodium sulphate (Central Drug House Pvt. Ltd., India) were used without any modifications. All other procured and utilized chemicals and reagents were belonging to A.R. grade.

Experimental design

The present experimental protocol was designed through a 2^3-factorial design utilizing Design-Expert® version 13.5.0 (Stat-Ease Inc., USA). Independent variable factors for optimization of the design were the concentrations of Sa, Xg, and $CaCl_2$ as first cross-linker while, $NaSO_4$ as second cross-linker and drug were remained constant for all respective batches. The factorial design provided eight different experimental composition formulations with two different levels (+1 high level and -1 low level) of each factor as depicted in Table 1. All those prepared formulations were evaluated for %EE, %DC, % Yield, particle size and swelling degree in SCF pH 7.4, respectively. Experimental data obtained from the formulations were analyzed using design-expert software to develop polynomial equations in coded factors, perform analysis of variance (ANOVA), fit statistics and determine the significance of the model terms. The software was further used to generate 3D response surface plots, contour plots, and optimization solutions for identifying the optimal combination of Sa, Xg, and calcium chloride concentrations that produced microbeads with desirable response.

Development of dual crosslinked aceclofenac loaded Xg-Sa microbeads utilizing ionic gelation technique

Aceclofenac-loaded Xa-Sa microbeads were developed using ionic gelation technique in laboratory under the ambient condition. For the development of Xg-Sa microbeads with different compositions [Table 1], Xg solution was prepared in distilled water and, separately, Sa solution was prepared by dissolving the weighed amounts specified in distilled water. Aceclofenac (20% w/w of the total polymer weight) was added into the prepared Sa solution and homogenize for 30 min using a homogenizer. The prepared Xg solution was transferred into the Sa solution with continue stirring using mechanical stirrer at a speed of 1000 rpm for 60 min at room temperature (25 ± 2°C) and to prepare blend mixture and pH was adjusted up to 5 using 0.1N NaOH. For development of microbeads, this blend mixture was transferred dropwise into the specified concentration of the first cross-linking solution using a 10 mL syringe (#22G) and maintained a falling distance of 6 cm with a stirring rate of 200 rpm, respectively. These

Table 1: 2^3 factorial designs with dependent and independent variable of aceclofenac loaded Xg-Sa dual cross-linked microbeads

Formulations	Independent variables			Dependent variable				
	Factor A Concentration of Sodium alginate (% w/v)	Factor B Concentration of xanthan gum (% w/v)	Factor C Concentration of $CaCl_2$ (% w/v)	Response 1 Yield (%)	Response 2 EE (%)	Response 3 DC (%)	Response 4 Particle size (µm)	Response 5 Swelling degree (pH 7.4)
AAXF1	1.5 (−1)	0.3 (−1)	4 (−1)	80.42±0.16	70.53±0.35	69.18±0.07	792±33.09	8.31±0.38
AAXF2	1.7 (+1)	0.3 (−1)	4 (−1)	82.92±0.31	86.96±1.72	83.62±0.34	741±55.25	7.64±0.13
AAXF3	1.5 (−1)	0.5 (+1)	4 (−1)	85.33±0.24	74.52±1.68	74.37±0.15	825±47.55	8.73±0.43
AAXF4	1.7 (+1)	0.5 (+1)	4 (−1)	83.92±0.05	83.91±0.74	79.98±0.32	830±50.73	8.07±0.53
AAXF5	1.5 (−1)	0.3 (−1)	6 (+1)	81.50±0.30	81.81±0.71	78.32±0.14	748±32.38	8.65±0.49
AAXF6	1.7 (+1)	0.3 (−1)	6 (+1)	83.17±0.09	85.47±0.69	84.82±0.14	809±29.91	7.79±0.56
AAXF7	1.5 (−1)	0.5 (+1)	6 (+1)	85.45±0.20	86.73±1.22	85.70±0.23	752±24.89	8.97±0.46
AAXF8	1.7 (+1)	0.5 (+1)	6 (+1)	84.98±0.31	84.81±0.48	81.82±0.09	760±36.23	8.22±0.40

Each value represents the mean±S.D; (*n*=3). DC: Drug content, EE: Entrapment efficiency

obtained microbeads were washed triplicate with distilled water to remove excess surface adsorbed calcium ions. The obtained microbeads were soaked into second cross-linking solution for 30 min to obtained dual cross-linked microbeads followed by washing with distilled water. These developed dual cross-linked microbeads were dried at ambient temperature for 48 h, followed by oven drying at 50°C for 10 h and preserve it in air tight container for further evaluations. Each batch was prepared in triplicate to ensure statistical reproducibility.

Characterizations

Percent yield, EE and drug content determination

To determine EE and DC, 100 mg (equivalent) of drug-loaded microbeads were immersed in 100 mL of SCF and kept for 24 h with occasional shaking. This obtained mixture was sonicated for 30 min, followed by centrifugation at 10000 rpm for 30 min; the supernatant was filtered using membrane filter (#0.45), this filtered solution was used to analyzed entrapped drug and drug content using a UV-visible spectrophotometer (Shimadzu 1800, Japan) at λmax of 276 nm against blank. The yield percentage, EE and DC were calculated using the given equations:[10]

$$\text{Yield}(\%) = \frac{\text{Weight of microbeads}}{\text{Weight of drug and polymers}} \times 100$$

$$\text{EE}(\%) = \frac{\text{Experimental amount of drug}}{\text{Theoritical amount of drug}} \times 100$$

$$\text{DC}(\%) = \frac{\text{Amount of drug in microbeads}}{\text{Amount of microbeads}} \times 100$$

Particle size determination

Mean Feret diameter determination of the Xg-Sa microbeads was assessed using a fluorescence microscope (Leica DM IL LED, Germany). Feret diameters were determined for 100 randomly selected microbeads per batch under ×4 magnification, with mean ± SD calculated from three replicates.[11]

Degree of swelling

The swelling degree of prepared microbeads was investigated at different simulated GI fluids (SGF, SIF, and SCF), following Wang *et al*., 2016 with slight modifications.[12] Dried Xg-Sa microbeads (100 mg) (Wi) were separately immersed into various simulated GI fluids with continuous stirring by a magnetic stirrer at 100 rpm at room temperature for 2 h. Thereafter, these swelled microbeads were removed and blotted gently with tissue paper, and then weighed our carefully (Ws). Swelling degree of Xa-Sa dual cross-linked microbeads was calculated using the equation:[13]

$$S.D. = \frac{Ws - Wi}{Wi}$$

In vitro drug release study and release kinetic model fitting

The *in vitro* release study was carried out for all batches of Xg-Sa microbeads SGF, SIF and SCF using the USP-II dissolution apparatus (Electrolab TDT-06P, Mumbai, India).[14] Initially, the developed microbeads 100 mg equivalent weight of aceclofenac were placed into dissolution vessel containing 900 mL SGF for dissolution at 37 ± 0.5°C and 50 rpm rotational speed for initial 2 h. After that, the test was repeated in SIF for 3 h and SCF for 12–24 h under similar conditions. During the study simulation of GI conditions was achieved by sequentially adjusting the dissolution medium pH. Initially, the medium was maintained at pH 1.2 for 2 h with 0.1 N HCl. It was then adjusted to pH 6.8 for 3 h using KH_2PO_4 (1.7 g), $Na_2HPO_4.2H_2O$ (2.225 g), and 1.0 N NaOH. Finally, the medium was set to pH 7.4 and maintained for 12–24 h. At regular intervals, 5 mL samples were withdrawn and replaced with fresh medium to maintain sink conditions. Aliquoted samples were filtered through membrane filter (# 0.45) and diluted then analyzed by UV-Visible spectroscopy at λ_{max} of 276 nm respectively.

In vitro release data from all formulation batches were fitted to mathematical kinetic models (zero-order, first-order, Higuchi, HixsonCrowell, and Korsmeyer-Peppas) to elucidate drug release mechanisms. Model suitability was assessed using correlation coefficients (R^2), while the Korsmeyer-Peppas release exponent (n) classified transport mechanism as Fickian ($n \leq 0.43$), non-Fickian (0.43–0.85), or case-II ($n \geq 0.85$).[15] The bestfit model was identified based on R^2 values.

Statistical optimization of experimental design using 2^3 factorial designs

The effect of independent variables on dependent variables was studied by 2^3 factorial designs. The obtained data sets of dependent variables were entered into the response column of Design-Expert® software and proceeded for analysis. To validate the accuracy of the model, analysis of variance (ANOVA) along with several statistical indices were examined, including the probability value (*P*-value), the lack-of-fit (F value), degree of freedom, sum of square and various fit statistic parameters, such as correlation coefficient R^2, predicted R^2 and adjusted R^2. The relationships between independent and dependent variables were effectively illustrated through 3D surface plots, 2D contour plots, Pareto charts, and predicted versus actual values of responses were also presented. For each response variable, factorial models incorporating both interaction and quadratic terms were developed using multiple linear regression. The equation for multiple linear regression in terms of coded factors is depicted below:

$$Y = \beta 0 + \beta 1A + \beta 2B + \beta 3C + \beta 12AB + \beta 13AC + \beta 23BC + \beta 123ABC$$

Where Y is calculated dependent variable; β_0 is the intercepts; A, B, and C are the coded level of independent variables; β_1, β_2, β_3, β_{12}, β_{13}, β_{23} are the estimated coefficients, AB, AC, BC are the two-factor interactions and ABC are the three factor interactions, respectively.

RESULTS AND DISCUSSION

Percent yield, EE and drug content

Aceclofenac-loaded Xg-Sa microbeads batch formulations were evaluated for the micromeritic properties as presented in Table 1, respectively. The prepared formulations exhibited EE ranging from 70.53 ± 0.35 to 86.73 ± 1.72%, with drug content between 69.18 ± 0.07 and 85.70 ± 0.23%. The percent yield was varied between 80.42 ± 0.16 to 85.45 ± 0.20. EE and drug content increased significantly ($P < 0.05$) with mass proportions Sa and Xg in the core composition. This effect arises because elevated polymer concentrations provide additional Ca^{2+} binding sites within the reaction system and strong electrostatic interactions, which lead to the formation of a strong polymeric network, thereby facilitating higher drug entrapment. An increase in mass proportion of Xg significantly increases EE, DC, and yield ($P \leq 0.05$), attributable to the formation of a denser, more viscous polymeric network that restricted drug diffusion, consistent with the inverse relationship between matrix viscosity and diffusion rate. These observations are in concordant with the reports of.[16] The ANOVA result [Table 2] indicated for the model significancy with P = 0.0185, 0.0104, and 0.0054; F-value of 11.87, 27.98, and 43.65 for EE, DC and yield, respectively. Model adequacy was confirmed with R^2, adjusted R^2, predicted R^2, and adequate precision value obtained from fit statistics [Table 2]. The impact of the independent variables on responses was presented in a 3D response surface plot, a contour plot, and a Pareto chart, respectively, in Figure 1-3. The predicted versus actual values for all those responses were presented in Figures 1c-3c. The model equation for responses Y1, Y2, and Y3 shows the influence of the factors A, B, and C.

$$Y1\ (Yield) = 85.46125 + 0.28625A + 1.45875B + 0.31375C - 0.75625AB$$

$$Y2\ (\%EE) = 83.7175 + 3.82A + 2.7375C - 2.635AC$$

$$Y3\ (\%DC) = 81.60125 + 2.45875A + 2.81375C - 2.77625AB - 2.55375AC$$

Particle size

The particle size of Xg-Sa microbeads was determined using a fluorescent microscope as depicted in Table 1. Results

Table 2: Various parameters of the models obtained from ANOVA for the various responses

Response	Sum of square	Degrees of freedom	Mean square	F-value	*P*-value
Yield (%)	23.04	4	5.76	43.65	0.0054
Entrapment efficiency (%)	232.24	3	77.41	11.87	0.0185
Drug content (%)	225.53	4	56.38	27.98	0.0104
Particle size (μm)	11230.00	4	2807.50	35.46	0.0074
Swelling degree	1.51	4	0.3768	262.86	0.0004

ANOVA: Analysis of variance

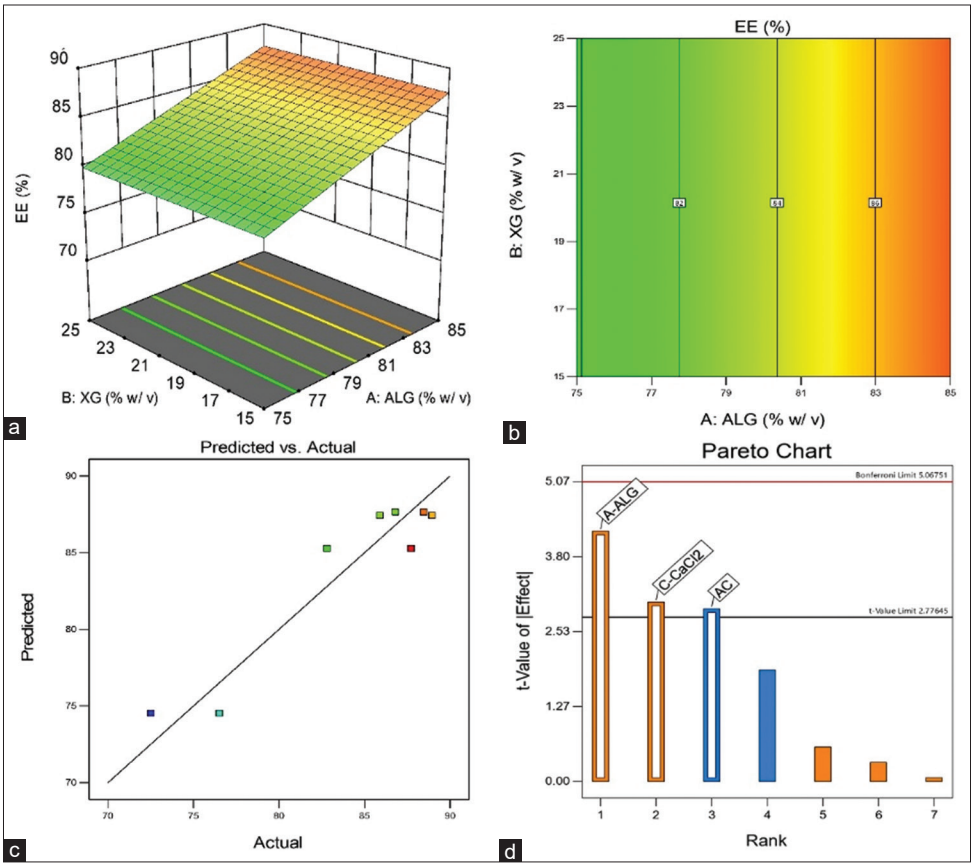


Figure 1: 3D response surface plot (a) 2D contour plot (b) predicted versus actual value plot (c) and pareto chart (d) for the %EE of aceclofenac loaded Xg-Sa microbeads

revealed that the formulation batch MAXF7 exhibited the lowest particle size with a value of 752 ± 24.89 μm, while formulation batch MAXF4 showed the greater particle size with a value of 830 ± 50.73 μm. It was found that the particle size of microbeads was proportional to the composition of total polymers. An increase in Xg concentration significantly increased the mean particle diameter ($P < 0.05$), whereas on increments for mass proportion of Sa resulted into decreased particle sizes. This suggests that Sa cross-linked networks are more compact than those formed by Xg, thereby resulting in reduced particle sizes; the observed results are found concord with the report of Sonkar and Lanjhiyana (2020).[17] The ANOVA results with model F value of 662.41 and $P = 0.0074$ implies the model is significant [Table 2]. The obtained fit statistics parameters are depicted in Table 3. The 3D response surface, contour plot, and Pareto chart demonstrated the impact of the independent variable on particle size [Figure 4]. Figure 4c represents the predicted versus actual value of particle size. The recorded final equation in terms of coded factors is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Y4 (Particle size) = 778.75–3.25A+10.25B–35.25C+6.75AB

Swelling degree

The swelling degree was carried out for all batches of Xg-Sa microbeads loaded with aceclofenac in SGF, SIF, and SCF as depicted in Table 1. The results were found to be 0.29 ± 0.12 to 0.83 ± 0.17 in SGF, 6.30 ± 0.10 to 7.84 ± 0.10 in

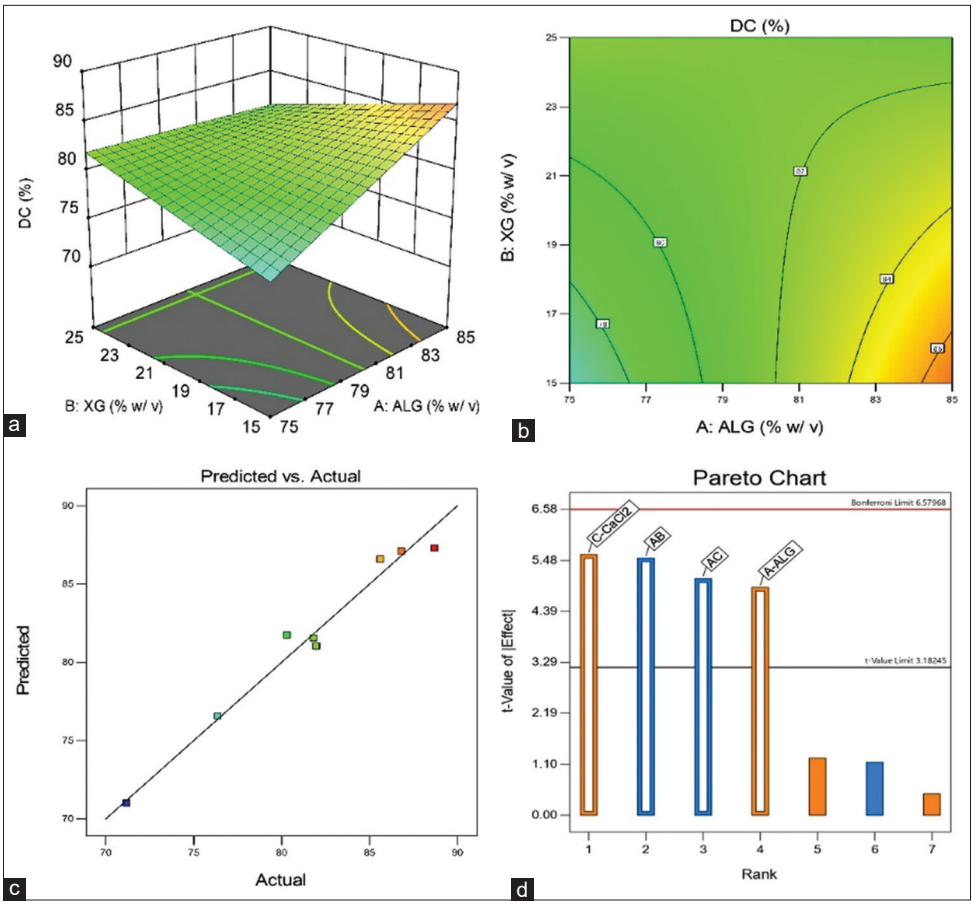


Figure 2: 3D response surface plot (a) 2D contour plot (b) predicted versus actual value plot (c) and pareto chart (d) for the DC of aceclofenac loaded Xg-Sa microbeads

Table 3: Summary of model fit statistics (standard deviation, coefficient of variation, R^2, adjusted R^2, predicted R^2, and adequate precision) for the selected responses

Responses	Std. Dev.	Mean	C.V.%	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
Yield (%)	0.3633	85.46	0.4251	0.9831	0.9606	0.8799	17.6093
Entrapment efficiency (%)	2.55	83.72	3.05	0.8990	0.8232	0.5960	7.2620
Drug content (%)	1.42	81.60	1.74	0.9739	0.9391	0.8144	14.5142
Particle size (μm)	8.90	778.75	1.14	0.9793	0.9517	0.8527	14.8561
Swelling degree	0.0379	8.30	0.4563	0.9972	0.9934	0.9798	45.2716

SIF, and 7.64 ± 0.13 to 8.97 ± 0.46 in SCF. Based on the obtained results, microbeads exhibited pH-responsive behavior, demonstrating the lowest swelling degree in SGF pH 1.2, moderate swelling in SIF pH 6.8, and maximum swelling in SCF pH 7.4, respectively. In acidic pH, water uptake was restricted due to carboxyl group protonation and compact network formation under acidic conditions. At alkaline pH, swelling increased markedly as partial ionization of polysaccharide chains enhanced matrix hydration. The pronounced swelling observed at colonic pH resulted from ionization of carboxylate groups in Xg and Sa, which induced electrostatic repulsion between polymer chains and enhanced matrix hydration through increased fluid penetration. The swelling behavior of pH-sensitive gel systems was explained by ionization and ion-exchange theories associated with polyelectrolytes, such as chitosan, Sa, and Xg. Protonation, deprotonation, and chelation of polyions contributed to swelling under acidic or alkaline conditions. In addition, Donnan equilibrium (Gibbs-Donnan effect) accounted for swelling through ionic concentration gradients across gel phase boundaries.[18-20] The model's significance was confirmed by ANOVA [Table 2] with $P = 0.0004$ and lack of fit F = 662.86. In addition, fit statistics confirmed the model adequacy as the data represented in Table 2. 3D response surface, contour plot, and Pareto chart [Figure 5] depict the impact of factors on swelling degree, and Figure 5c represents the predicted versus actual values of swelling degree, respectively. The final equation in terms of

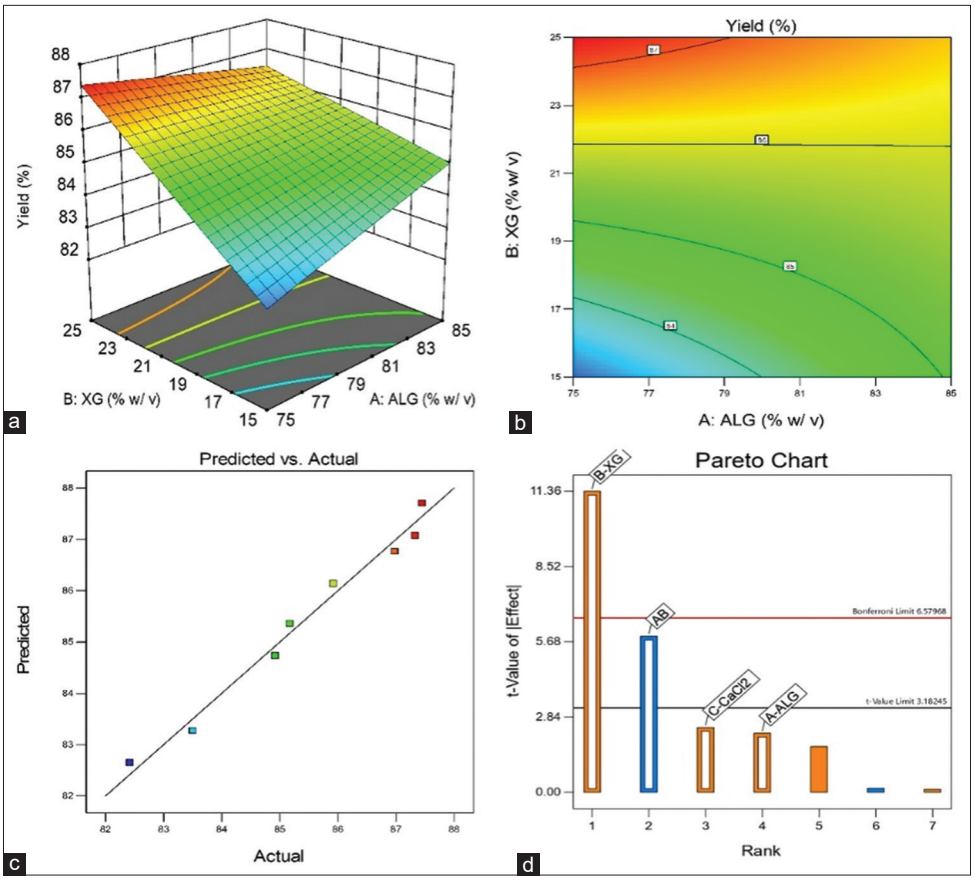


Figure 3: 3D response surface plot (a) 2D contour plot (b) predicted versus actual value plot (c) and pareto chart (d) for the yield of aceclofenac loaded Xg-Sa microbeads

coded factors demonstrates the influence of the independent variable factors on swelling degree.

$$Y5 \text{ (Swelling degree)} = 8.2975 - 0.3675A + 0.2000B + 0.1100C - 0.0350AC$$

Statistical optimization of experimental design using 2^3 factorial designs

Statistically significant relationships between independent variable factors and dependent variable factors were determined using 2^3 factorial designs. A polynomial equation model was selected based on the various statistical parameters, such as sum of squares, degrees of freedom, mean square, F-value, *P*-value, and various fit statistic parameters, such as correlation coefficient R^2, predicted R^2, and adjusted R^2 depicted in Tables 2 and 3. The statistical and fit parameters confirmed the significance of each model for yield, EE, DC, swelling behavior and particle size. The predicted R2 was found to be in reasonable agreement with the adjusted R^2 for all dependent variable factors (responses), that is, the difference is <0.2. While the Adequate Precision measures the signal-to-noise ratio, which is >4, it is desirable and also indicates that the selected model equations hold in all cases. ANOVA studies confirmed that the independent variables had individual effects on the dependent variable; this was also confirmed by the experimental design model, which was found to be statistically significant ($P < 0.05$) and discussed in the respective section. The Pareto chart identified which independent variables significantly affected the response, with bars showing standardized effects and reference lines (t-value limit 2.776, Bonferroni limit 5.067) marking statistical significance at 95% confidence. Numerical optimization was carried out to obtain optimized formulation factors. In the optimization, the targets for yield percentage, entrapment, drug loading capacity, and selling behaviour were set at maximum and for particle size at minimum. Based on numerical optimization, AACF7 (−1, +1, and +1 concentrations of Sa, chitosan, and $CaCl_2$, respectively) was selected as the optimized formulation with maximum desirability (0.814) from 51 different solutions provided by the software. In the case of aceclofenac-loaded Xg-Sa microbeads formulation, batch AAXF7 were selected as optimized batch from numerical optimization with maximum EE, drug content, yield, swelling degree and desirability of 85.270, 87.286, 87.704, 9.010 and 0.916, respectively, and minimum particle size of 750.250 from 38 different solution provided by software. The 3D surface and contour plots in Figure 1-5 showed the influence of the interaction between the dependent and independent variables, as obtained from ANOVA.

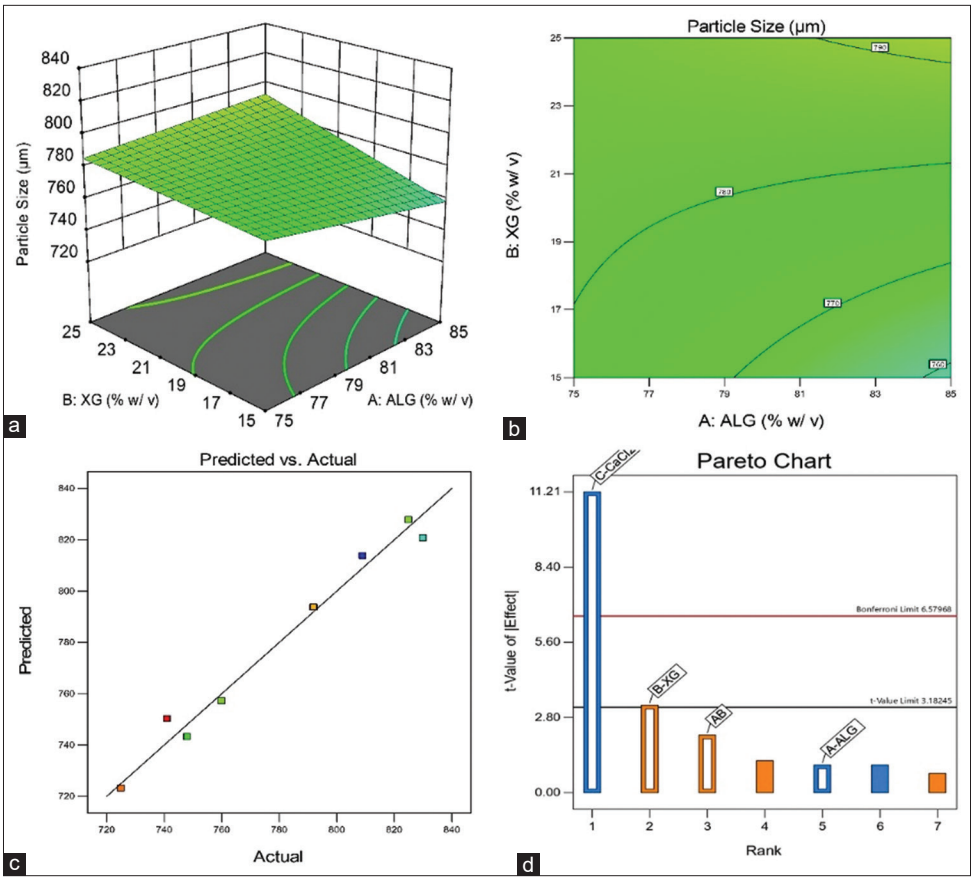


Figure 4: 3D response surface plot (a) 2D contour plot (b) predicted versus actual value plot (c) and pareto chart (d) for the particle size of aceclofenac loaded Xg-Sa microbeads

Table 4: Correlation coefficient and release exponent of various release kinetic models for dual cross-linked aceclofenac loaded Xg-Sa microbeads

Formulations	Zero order	First order	Higuchi	Hixon Crowell	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	R^2	n
AAXF1	0.986	0.973	0.991	0.987	0.997	0.59
AAXF2	0.979	0.983	0.995	0.973	0.982	0.62
AAXF3	0.967	0.941	0.987	0.968	0.996	0.69
AAXF4	0.966	0.964	0.994	0.953	0.983	0.67
AAXF5	0.972	0.939	0.984	0.967	0.992	0.79
AAXF6	0.965	0.945	0.992	0.960	0.991	0.69
AAXF7	0.983	0.968	0.986	0.984	0.994	0.60
AAXF8	0.972	0.947	0.996	0.964	0.996	0.63

In vitro release profile of aceclofenac from microbeads

The drug release of aceclofenac from Xg-Sa microbeads was assessed in SGF, SIF, and SCF. The cumulative release profiles of all batches are represented in Figure 6. The results demonstrated that all formulations exhibited minimal drug release in the SGF, with cumulative release ranging from 4.83 ± 0.98% to 7.53 ± 1.17% during the initial 2 h. This limited release indicates that the dual cross-linked hydrogel network effectively protected aceclofenac from premature release in the stomach. The restricted release can be attributed to the compact hydrogel structure formed by the ionic cross-linking of Sa and Xg with calcium ions, which minimized water penetration, polymer swelling, and drug diffusion under acidic conditions.[21] Upon transferring the formulation to SIF, a gradual increase in drug release was observed from 3 to 5 h. At 5 h, cumulative drug release ranged between 18.64 ± 2.13% to 25.67 ± 2.75%. A substantial increase in drug release was observed in SCF, with cumulative values ranging from 66.10 ± 1.57% to 81.06 ± 1.89% at 24 h. Among the formulations, AAXF4 exhibited the highest release (81.06 ±

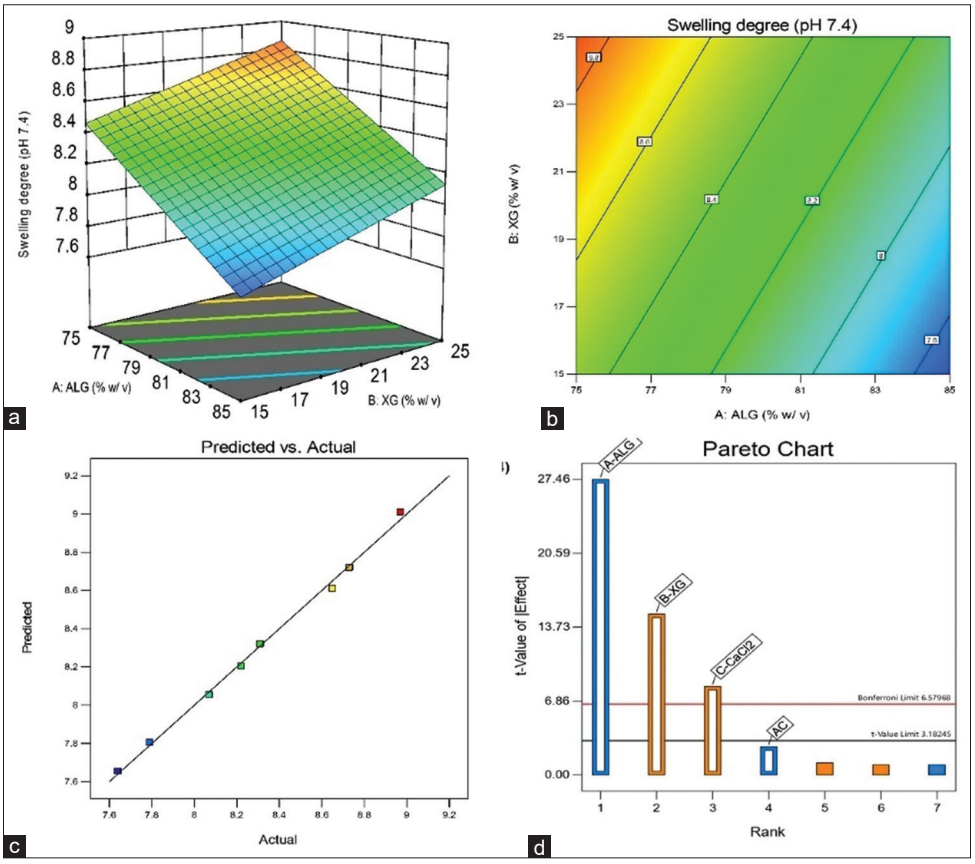


Figure 5: 3D response surface plot (a) 2D contour plot (b) predicted versus actual value plot (c) and pareto chart (d) for the swelling degree of aceclofenac loaded Xg-Sa microbeads

1.89%), followed by AAXF6, AAXF2, and AAXF8, whereas AAXF1, AAXF7, and AAXF5 showed comparatively lower release. The increased release observed at SCF, attributed to the increased ionization of the carboxyl groups of both Sa and Xg, resulted in greater electrostatic repulsion within the polymer chains, increased hydration, and extensive swelling of the hydrogel matrix. This structural expansion permitted greater water uptake and diffusion of aceclofenac, while partial relaxation and erosion of the polymer chains under alkaline conditions sustained release throughout the 24 h study.

The drug release profile of Xa-Sa microbeads was strongly influenced by cross-linker concentration. Higher $CaCl_2$/Na_2SO_4 levels produced a dense, stable hydrogel network with reduced swelling, smaller pores, and slower sustained release, while lower $CaCl_2$ yielded a loosely cross-linked matrix with greater swelling, faster diffusion, and higher cumulative release. Release behavior also varied with pH due to ionization and swelling of the polyionic matrix.

In vitro release data were analyzed using zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas models [Table 4]. Model accuracy was assessed by regression coefficients (R^2). Drug release best fit the Higuchi model (R^2 = 0.984–0.996), closely aligning with the Korsmeyer-Peppas model (R^2 = 0.982–0.997), indicating matrix diffusion. The

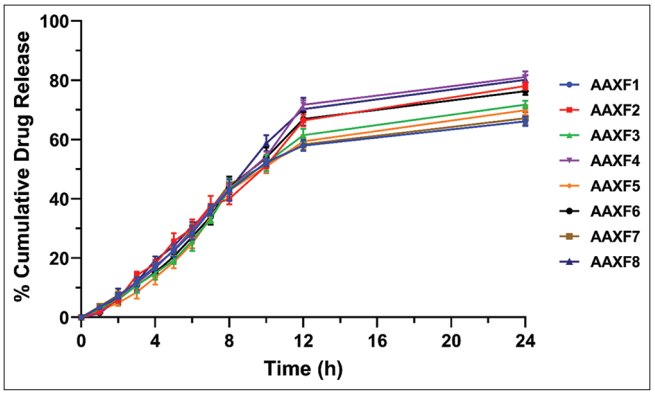


Figure 6: Cumulative percent drug release profile for all formulation batch of aceclofenac loaded dual cross-linked Xg-Sa microbeads

release exponent (n) ranged from 0.59 to 0.79, confirming anomalous transport ($0.45 < n < 0.89$). Thus, aceclofenac release was governed by combined erosion and diffusion mechanisms.

CONCLUSION

In the present study, aceclofenac-loaded Xg-Sa hydrogel microbead formulations were successfully developed using

the ionic gelation technique and optimized using a 2^3 factorial design. Through factorial design, the influence of independent formulation variables on dependent variables (responses) was elucidated. Concentrations of sodium alginate, Xg, and calcium chloride were found to significantly impact yield, EE, drug content, particle size, and swelling properties. The optimized formulation batch (AAXF7) from Design Expert® software exhibited high EE, satisfactory drug content, desirable swelling behavior in various simulated GI fluids, and appropriate particle size suitable for colon-specific delivery. *In vitro* release study of developed hydrogel microbeads exhibited minimum pre-mature drug release in SGF pH 1.2 and a sustained release profile in SCF pH 7.4 over a period of 24 h, following the Higuchi and Korsmeyer-Peppas models with non-Fickian transport, suggesting that both diffusion and polymer relaxation governed the release mechanism. In summary, the developed dual cross-linked Xg-Sa hydrogel microbeads constitute a promising colon-specific delivery platform for aceclofenac, offering controlled release that may improve therapeutic outcomes and minimize GI adverse effects. Future research will emphasize accelerated and long-term stability testing in accordance with ICH guidelines, scale-up of manufacturing processes, rheological profiling, and clinical evaluations. In addition, therapeutic efficacy will be assessed in experimental colitis models before clinical translation.

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