Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

for

Response surface methodology and optimized synthesis of guar gum-based hydrogels with enhanced swelling capacity

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Table of contents

Table S1: Resolution-IV design for screening of important process variables in Phase-1

S. no.	Point type	A Initiator conc. x 10^{-3}	B Reaction time	C pH	D Monomer conc. x 10^{-6}	E Reaction temp	F Crosslinker conc. x 10^{-3}	G Vacuum level	Response Swelling rate (Ps)	
	Units →	mol / L	min	-	mol / L	°C	mol / L	mm of Hg	%	
									Actual	Predicted
1	Factorial	35.0	60	6.50	145.0	40	64.1	800	3939	4088
2	Center	26.3	75	7.00	217.5	60	53.4	600	4613	4623
3	Factorial	17.5	90	7.50	145.0	40	64.1	800	4399	4414
4	Factorial	35.0	60	7.50	145.0	80	64.1	400	2874	2818
5	Factorial	35.0	90	7.50	290.0	80	64.1	800	2721	2780
6	Factorial	35.0	90	6.50	145.0	80	42.7	800	4193	4088
7	Factorial	17.5	60	6.50	145.0	40	42.7	400	4388	4420
8	Factorial	17.5	90	6.50	290.0	40	42.7	800	3279	3169
9	Factorial	17.5	60	7.50	145.0	80	42.7	800	4469	4414
10	Factorial	17.5	60	6.50	290.0	80	64.1	800	3098	3169
11	Factorial	17.5	90	7.50	290.0	80	42.7	400	3131	3163
12	Center	26.3	75	7.00	217.5	60	53.4	600	4724	4623
13	Factorial	17.5	60	7.50	290.0	40	64.1	400	3156	3163
14	Factorial	35.0	90	6.50	290.0	40	64.1	400	4024	4050
15	Factorial	17.5	90	6.50	145.0	80	64.1	400	4413	4420
16	Factorial	35.0	90	7.50	145.0	40	42.7	400	2806	2818
17	Center	26.3	75	7.00	217.5	60	53.4	600	4531	4623
18	Factorial	35.0	60	7.50	290.0	40	42.7	800	2794	2780
19	Factorial	35.0	60	6.50	290.0	80	42.7	400	4120	4050

Table S2: Factorial design with fitted ANOVA statistics in Phase-2

S. no.	Point type	**A** Initiator conc. x 10^{-3}	**C** pH	**D** Monomer conc. x 10^{-6}	**Response** Swelling rate (Ps)	
	Units →	mol/L	-	mol/L	%	
		Coded (Actual)			Actual	Predicted
1	Factorial	-1 (17.5)	-1 (6.25)	-1 (116.0)	3353	3482
2	Factorial	-1 (17.5)	-1 (6.25)	+1 (217.5)	3516	3482
3	Factorial	-1 (17.5)	+1 (7.00)	-1 (116.0)	5569	5593
4	Factorial	-1 (17.5)	+1 (7.00)	+1 (217.5)	5712	5593
5	Factorial	+1 (35.0)	-1 (6.25)	-1 (116.0)	3072	3093
6	Factorial	+1 (35.0)	-1 (6.25)	+1 (217.5)	3208	3093
7	Factorial	+1 (35.0)	+1 (7.00)	-1 (116.0)	5032	5205
8	Factorial	+1 (35.0)	+1 (7.00)	+1 (217.5)	5283	5205
9	Center	0 (26.3)	0 (6.63)	0 (166.8)	4628	4472
10	Center	0 (26.3)	0 (6.63)	0 (166.8)	4464	4472
11	Center	0 (26.3)	0 (6.63)	0 (166.8)	4324	4472

ANOVA statistics of reduced linear model

Source	F-value	p-value Prob > F
Model	224.050	< 0.0001 *
A: Initiator conc.	14.691	0.0050 *
C: pH	433.409	< 0.0001 *
Lack of Fit	0.852	0.628 #
Model statistics:	R^2 = 0.982 Adj R^2 = 0.978	Pred R^2 = 0.968

* significant at $p<0.05$

\# not-significant at $p<0.05$

Table S3: Center composite design with fitted ANOVA statistics in Phase-3

S. no.	Point type	**A** Initiator conc. x 10^{-3}	**C** pH	**Response** Swelling rate (Ps)	
	Units →	mol/L	-	%	
		Coded (Actual)	Coded (Actual)	Actual	Predicted
1	Factorial	-1 (19.4)	-1 (6.75)	4770	4675
2	Factorial	-1 (19.4)	+1 (7.25)	4345	4116
3	Factorial	+1 (28.7)	-1 (6.75)	4219	4085
4	Factorial	+1 (28.7)	+1 (7.25)	3559	3526
5	Axial	0 (24.1)	-1.41 (6.65)	4029	4140
6	Axial	0 (24.1)	+1.41 (7.35)	3216	3350
7	Axial	-1.41 (17.5)	0 (7.00)	4695	4873
8	Axial	+1.41 (30.6)	0 (7.00)	3971	4038
9	Center	0 (24.1)	0 (7.00)	5170	5223
10	Center	0 (24.1)	0 (7.00)	5325	5223
11	Center	0 (24.1)	0 (7.00)	5031	5223
12	Center	0 (24.1)	0 (7.00)	5390	5223
13	Center	0 (24.1)	0 (7.00)	5202	5223

ANOVA statistics of reduced quadratic model

	F-value	p-value
Source		Prob > F
Model	50.55	< 0.0001*
A: Initiator conc.	24.70	0.0011*
C: pH	22.12	0.0015*
A^2	36.31	0.0003*
C^2	134.69	< 0.0001*
Lack of Fit	1.88	0.2781#
Model statistics:	R^2 = 0.961 Adj R^2 = 0.942	Pred R^2 = 0.864

* significant at $p<0.05$

not-significant at $p<0.05$

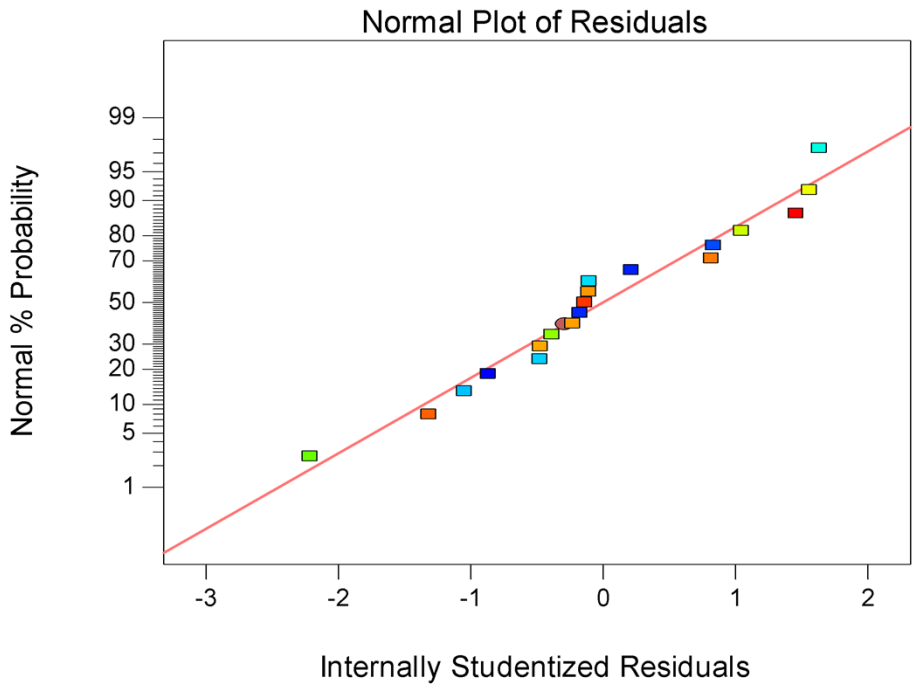

Fig. S1. Normal plot of residuals of Resolution-IV design as per Phase-1

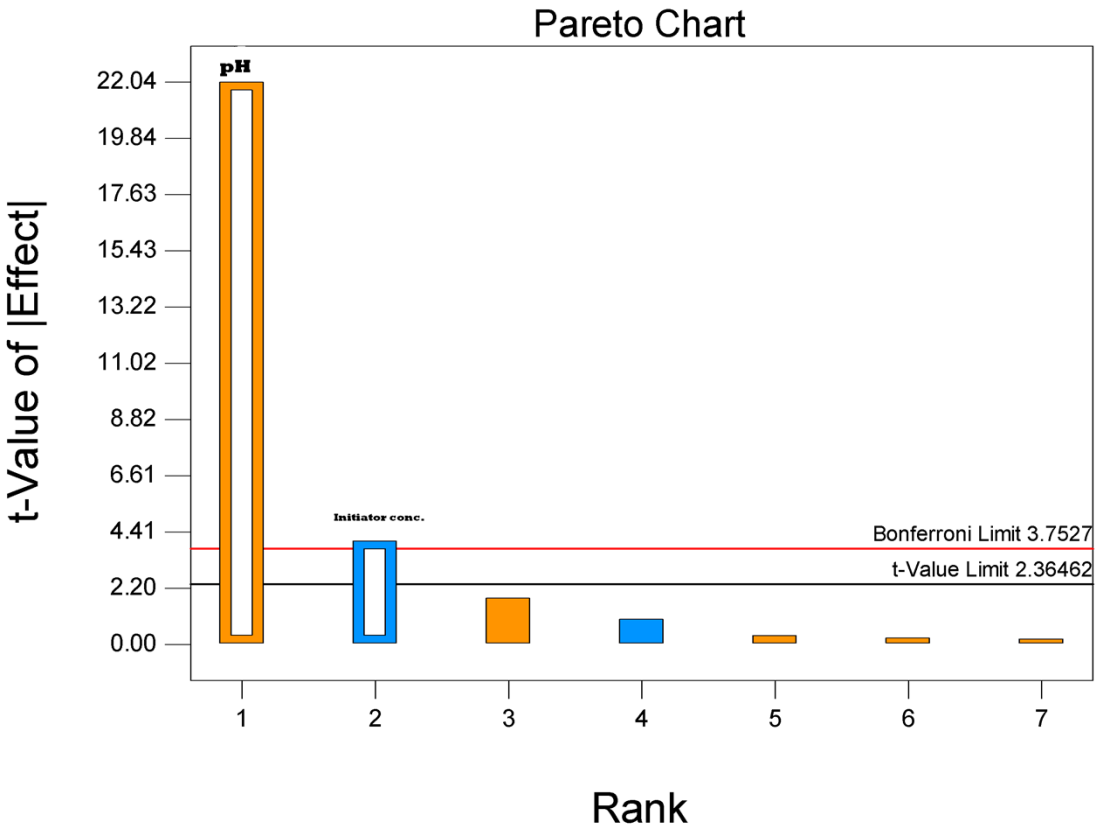

Fig. S2. Pareto chart showing significant process variables as per Phase-2

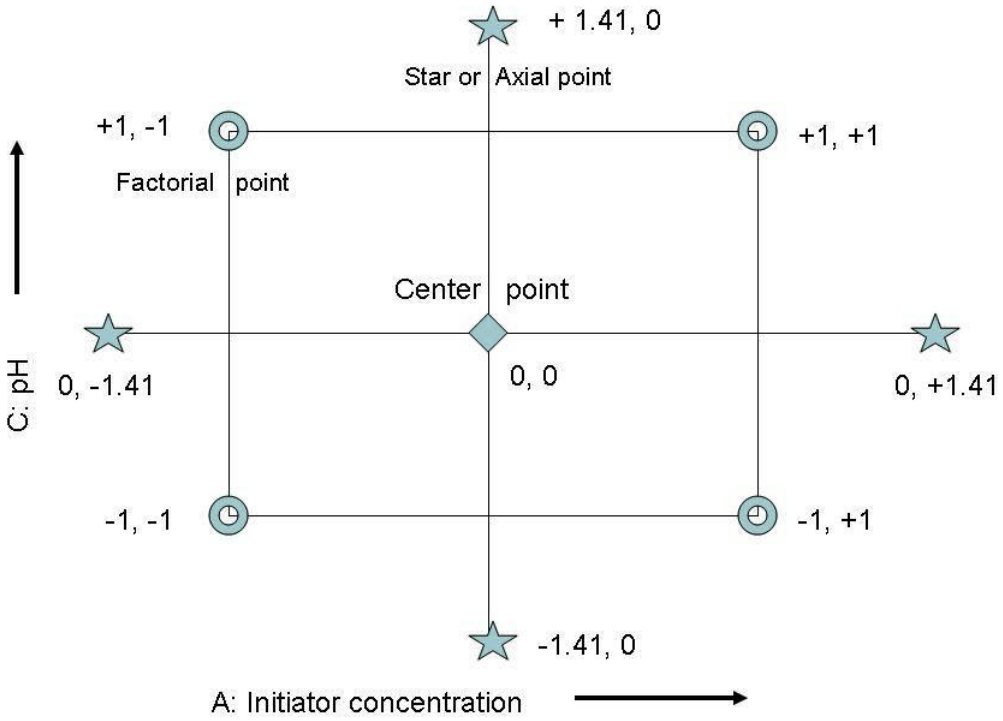

Fig. S3. Center composite design showing design points as per Phase-3

Statistical modelling details:

Experimental design and model development for Ggum-cl-poly(AA)

Process optimization using design of experiments (DOE) yield good results when significant process variables were selected in model building. Addition of non-significant or redundant variables added model noise and thus model generated has poor prediction capabilities. Robust experimental design strategy suggests that first applying either OFAT or fractional factorial design (Resolution-IV) to screen important variables using t-test or stringent significance testing through Boneferroi limits. Resolution-IV is slightly cautious design but at the same time, it saves the experimental runs (2^{7-3}) from 128 runs (2^7) and is the choice for screening variables. This design allows all main effects to be estimated and along with few two factor interactions (2FI). Although, 2FI is aliased with each other, but this will be taken care using subject matter knowledge. Response Surface Optimization using central composite design (CCD) is the best choice for fine tuning of the process conditions. The methodology of sequential experimentation explained above will result in sound and robust DOE, wherein, experimentation will be carried with significant variables only, eliminating all noise variables at preliminary stage and the analysis will yield a model with good predictability and explained excellent variance of the response data. Model fitting and graphical analyses were carried out using the Design-Expert software v9.0 (Stat-Ease Inc., Minneapolis, USA).

Phase-1: Screening of significant variables

Experimental data (Table S1) was modeled to screen the important process variables (Table S1). ANOVA model was highly significant at 99.99% (F=189.4, degree of freedom (df)=5) with not-significant *lack of fit* (p=0.7029) (table not shown). The fitted model explained 72% variance (R^2=0.720) with coefficient of variation (CV) of 12% and signal to noise (S/N) ratio of 6.45 (> 4 is recommended). The selected models were generated both in terms of coded factors (standardized equations) and actual factors (unstandardized equations) as given in Eq.(1) and Eq.(2) respectively. The regression constants in the coded equation are unitless coefficients and are used for process understanding.

$$P_s = + 3772.19 - 178.79\ A - 319.10\ C - 322.34\ D - 316.18\ A \times C + 303.16\ A \times D \quad (1)$$

Ps= Percentage Swelling (%) and A= initiator, C=pH and D=monomer are in coded units.

P_s = - 808.244 + 381.516 A + 1258.892 C - 16.990 D - 72.270 A x C + 0.477 A x D (2)

Ps= Percentage Swelling (%) and A= initiator conc. (mol L^{-1}), C=pH and D=monomer concentration (mole L^{-1}) are in actual units.

Phase-2: Full factorial design

In the Phase-2, significant process variables (initiator concentration, pH and monomer concentration) are taken with modified range selection so as to find optimum process conditions (Table S2). The fixed process conditions are 75 min reaction time, 60°C reaction temperature, 53.4 x 10^{-3} mol L^{-1} of crosslinker and vacuum of 600 mm of mercury. Full factorial design (2^3) with 3 center runs were performed (Table S2) and Pareto chart gave pH and initiator concentration as significant process parameters. Pareto chart predicted pH and initiator concentration as highly significant variables exceeding Bonferroni limits (Fig. S2). ANOVA model was generated using highly significant parameters (Table S2). ANOVA model was highly significant at 99.99% (F=224) with not-significant *lack of fit* (p=0.628) (Table S2). The model high very high prediction capability (Pred. R^2=0.968). Final equation in terms of unitless regression coefficient for fitted model is given below in eq. (3) and in actual factors is given in eq. (4). Finally, highly significant variables were selected for second order model building in phase-3.

P_s = + 4378.38 - 194.37 A + 1055.75 C (3)

Ps= Percentage Swelling (%) and A=initiator concentration, C=pH are in coded units.

P_s = - 13689.992 - 22.214 A + 2815.320 C (4)

Ps= Percentage Swelling (%) and A= initiator conc. (mol L^{-1}), C=pH and are in actual units.

Phase-3: Center composite design

The effect of pH was studied in the range of 6.65 to 7.35 and initiator concentration between 17.5 x 10^{-6} to 30.6 x 10^{-6} mol L^{-1} with fixed monomer concentration of 166.8 x 10^{-6} mol L^{-1}. Each variable was varied at 5 levels (-1.41, -1, 0, +1, +1.41) having four factorial points, four axial points and five center runs to check the reproducibility of results (Table S3 and Fig. S3). ANOVA statistics suggested quadratic model best fitted the response data. The reduced quadratic model using backward elimination methods removed 2FI term between pH and initiator concentration based on high alpha to exit (p>0.1). Reduced ANOVA model was highly significant at 99.99% (F=50.5) with not-significant *lack of fit* (p=0.278) (Table S3).

The fitted model explained 96.1% variance (R^2=0.961). The model passed the diagnostic tests for any outliers including normal probability plot. Final equation in terms of unitless regression coefficient for fitted model is given below in eq. (5) and in actual factors is given in eq. (6)

$$P_s = +\ 5223.45 - 295.18\ A - 279.35\ C - 383.80\ A^2 - 739.21\ C^2 \quad (5)$$

Ps= Percentage Swelling (%) and A=initiator concentration, C=pH and are in coded units.

$$P_s = -\ 5.753 \times 10^5 + 1.644\ 105\ C + 797.414\ A - 11827.430\ C^2 - 17.903\ A^2 \quad (6)$$

Ps= Percentage Swelling (%) and A= initiator conc. (mol L^{-1}), C=pH and are in actual units.