Development and Characterization of Hydrogel Containing Resveratrol by using 3^2 Factorial Design

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ABSTRACT:

The primary goal of this study was to create a controlled-release dosage form utilizing a cellulose-based hydrogel that was cross-linked with propylene glycol. Hydrogels were made by cross-linking the polymer Chitosan Hydrochloride with propylene glycol, a suitable cross-linking agent. According to an IR and DSC analysis, there is no indication of interaction between the medication, polymers, and other excipients. The hydrogel gave good swelling and controlled release properties due to the cross-linking process. Design-Expert 11.0 was used in this study to create a 32 complete factorial design with two central points. This study chose the concentration of Polyethylene Glycol(X1) and reaction time (X2) as independent factors.
In contrast, drug content, swelling index, and t75 % cumulative drug release were chosen as dependent variables. The effect of Polyethylene Glycol concentration on drug content and t75 % CDR was non-significant based on the findings. However, the influence of Polyethylene Glycol concentration on the swelling index was substantial, indicating that as Polyethylene Glycol concentration rose, the swelling index of the hydrogel decreased. Again, the effect of response time on drug content and t75 of % CDR was substantial, implying that as reaction time rose, drug content and t75 of % CDR of hydrogel increased. However, the effect of response time on the swelling index was not statistically significant. Drug content, swelling index, and t75 of % CDR of run BB1 are the best based on all responses. This run's drug content, swelling index, and t75 % CDR are 99.5 %, 276.64 %, and 3.5 hrs. So BB1 was used to test the improved formulation, which produced the best in vitro release of 94.24 % in 6 hrs. Different kinetic models were fitted to the in vitro data, which showed the best model was Higuchi with the non-friction mode of drug release. Stability data showed that the formulations were stable during the study period. From the study, it was concluded that the prepared hydrogel could provide a sustained release effect with better bioavailability which will surely enhance its absorption throughout the body

KEY WORDS: Resveratrol, Hydrogel, Factorial Design, Cross-linking, Controlled-Release Dosage Form

I. INTRODUCTION:

There is no precise control over drug release in traditional dosage forms, and the supplied dose of medication reaches the systemic circulation immediately. Designing oral controlled medication delivery systems should be to provide more predictable and improved drug bioavailability. Among all the methods that have been investigated for the systemic delivery of medicines via various pharmaceutical products of varied dose forms, the oral drug delivery system has made significant progress as the most commonly used drug administration. Hydrogels are hydrophilic, three-dimensional polymeric networks capable of absorbing vast quantities of water or biological fluids. The networks are made up of homopolymers or copolymers and are insoluble owing to chemical crosslinks (tie-points, junctions) and physical crosslinks such as entanglements and crystallites. The latter is responsible for network structure and physical security.

Resveratrol (3, 5, 4′-trihydroxy-trans-stilbene) is a member of the stilbenoids category of polyphenols, with two phenol rings connected by an ethylene bridge. Resveratrol (trans-3,5,4′-trihydroxystilbene) is having two isomeric forms: cis- and trans-resveratro5. Different biological actions are attributed to the Transform, including triggering cellular responses such as cell cycle arrest, differentiation, and apoptosis and enhancing cancer cell anti-proliferation. Resveratrol is an antioxidant and anti-inflammatory that can help to avoid illnesses including cancer, diabetes, and Alzheimer's. Resveratrol's anti-inflammatory properties make it an effective treatment for arthritis and skin irritation.

II. MATERIALS AND METHODS:

Resveratrol was procured from Yarrow Chem Pvt. Ltd, Mumbai, Polyethylene Glycol was obtained from Rankem Chemicals Ltd. Ahmadabad and Chitosan Hydrochloride was obtained as a gift sample from Colorcon India, Goa. All other chemicals and reagents used were of analytical grade and were used as obtained.

2.1 Standard Calibration Curve:

The standard calibration curve of resveratrol was carried out on a UV spectrophotometer by using a phosphate buffer of pH 7.4 as the solvent. The solution, which is now having a concentration of 100 µg/ml samples of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml were pipette out into 10ml volumetric flasks. The volume was made up to the mark with Phosphate buffer 7.4 to get the final concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/ml, respectively. The absorbance of concentration was measured at 304nm[7-8].

2.2 Drug-Excipients Interaction Study:

The drug and excipients compatibility study used FTIR and DSC resp[9-10].

2.3 Fourier Transformation Infra-Red Spectroscopy:

The study by FTIR of the drug and excipient was carried out by conventional KBr plate method to study the interaction of the drug and polymer to determine the physical and chemical changes that can occur during the formulation. The powder and excipient mixture and the pure drug were mixed in a 1:1 ratio with potassium bromide, and the small pellet was created by pressing the mixture in a hydraulic press. The FT-IR was performed in the frequency range of 400-4000 cm^{-1}.

2.4 Differential Scanning Calorimeter:

The DSC study was carried out by studying thermo-grams of pure drugs and their physical mixture with polymers to investigate any possible interaction between the drug and the utilized polymer. The selected heating rate is from 50°C to 400°C at an increase of 20°C per minute using Differential Scanning Calorimeter.

2.5 Formulation of Hydrogel:

The resveratrol hydrogel formula was created utilizing Chitosan Hydrochloride as a polymer at a concentration of 2% w/v and Polyethylene Glycol as a cross-linking agent at a concentration of 1-3 % w/v. Initially, the correct weights of resveratrol, propylene glycol, and chitosan hydrochloride were used. The water was then measured and put into a beaker with a motorized stirrer. The drug was dissolved in the minimum quantity of Polyethylene Glycol. The polymer was dissolved in the required amount of water, and both the solution was mixed, followed by the remaining amount of propylene glycol. This solution is then poured onto a china dish set in an 80±2°C thermostatic water bath. The reaction was kept going for another two hours until the water evaporates from the prepared hydrogel. The hydrogel is then placed in the oven to dry. The formulation chart of hydrogel preparation is depicted in Table 2. The dried hydrogel is then wrapped in an aluminium foil and stored in a desiccator for further use[11-13].

2.6 Experimental Design:

In this project, Design-Expert 11.0 was utilized to create a 32 factorial design enhanced for two central points. Two variables were assessed at three levels each, and experimental trials were conducted in eleven different combinations, including two augmentation experiments that were repeated twice, as shown in Table 1. Polyethylene Glycol content (X1) and reaction time (X2) were chosen as independent variables. Drug content, swelling index, and t75 of % cumulative drug release were used as dependent variables.

Table 1: formulation of batches in a 3^2 full factorial design

Variable Levels in Coded Form		
Batch code	**X_1**	**X_2**
BB1	-1	1
BB2	1	-1
BB3	0	-1
BB4	0	1
BB5	-1	-1
BB6	0	0
BB7	1	1
BB8	1	0
BB9	-1	0
BB10	0	0
BB11	0	0

Coded values	**Actual values**	
	X_1	**X_2**
-1	1%	60 min
0	2%	90 min
1	3%	120 min

Table 2: formulation chart of hydrogel

Batch	Chitosan Hydrochloride (%w/v)	Polyethylene Glycol (%w/v)	Stirring Time (min)	Distilled Water (ml)
BB1	2%	1%	120	10ml
BB2	2%	3%	60	10ml
BB3	2%	2%	60	10ml
BB4	2%	2%	120	10ml
BB54	2%	1%	60	10ml
BB6	2%	3%	90	10ml
BB7	2%	3%	120	10ml
BB8	2%	3%	90	10ml
BB9	2%	1%	90	10ml
BB10	2%	2%	90	10ml
BB11	2%	2%	90	10ml

2.7 Evaluation of Hydrogels:

2.7.1 Percent Yield:

The evaluation parameter of percentage yield calculates the amount of practical yield obtained after the experiment of a particular batch. This parameter gives the efficiency of the process involved[14]. In the current experiment, the parameter is calculated by the following formula:

$$\text{Percent Yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

2.7.2 Drug Content:

1gm of hydrogel was weighed and transferred to a beaker containing phosphate buffer pH 7.4, which was previously mounted on a mechanical stirrer. Continued the stirring for 12 hrs. to dissolve the hydrogel completely into the solvent. The obtained solution was filtered studied under a double beam UV spectrophotometer at 304nm[15].

Drug content can be determined by using the formula:

$$\text{Drug Content (\%)} = \frac{\text{Practical Value}}{\text{Theoretical Value}} \times 100$$

2.7.3 Swelling Index:

The swelling index of hydrogel was determined by placing the weighed hydrogel in the basket. For the first 2 hrs. the dissolution medium was 0.1N HCl followed by phosphate buffer pH 7.4 for further study, at 37ºC ± 0.5ºC. Hydrogel samples were withdrawn at a time interval of 30 min, blotted with tissue paper to remove the excess water, and weighed on the analytical balance16. The swelling index was calculated by using the following formula:

$$\text{Swelling Index (\%)} = \frac{\text{Weight of swollen hydrogel} - \text{Weight of dry hydrogel}}{\text{Weight of dry hydrogel}} \times 100$$

2.7.4 in Vitro Dissolution Studies:

In vitro dissolution tests were conducted in triplicate for all formulations in a USP type II dissolution test apparatus. The dissolution medium used was 900 ml 0.1N HCl for 2hr, followed by phosphate buffer of pH 7.4 at 370C ± 0.50C. The speed of rotation was maintained to 50 RPM. The prepared hydrogel was tied in the dialysis membrane, which was previously soaked in the phosphate buffer of pH 7.4. The dialysis membrane was then knotted on both ends to keep the hydrogel in the membrane's center. To keep the sink condition, 5ml of the sample was taken at regular intervals and 5ml of dissolution media was replaced with a freshly prepared medium. The withdrawn samples were filtered through Whatman filter paper and diluted to 10 ml with the same dissolution medium. The absorbance of resultant samples of all intervals was measured at 304nm using a double beam UV- spectrophotometer[17-18].

2.7.5 Determination of t_{75} of Drug Release Study:

It is necessary to determine a drug release of 75% for the optimization research. The time it takes for 75% of the drug in a formulation to be released is "t75." This data contains information on the drug's formulation release pattern. Finally, it aids in the research of hydrogel optimization[19].

2.7.6 Scanning Electron Microscopy:

SEM was used to evaluate the surface morphology of the cross-linked hydrogel produced with three different doses of citric acid: 1%, 2%, and 3%. The samples were strewn onto double-sided tape, sputter-coated with platinum, and inspected under a 10 kV microscope[20].

2.7.7 Release Kinetic Studies:

The analysis of drug release from swellable hydrogel requires a flexible model that identifies the contribution to overall kinetics. The dissolution profiles of all batches were fitted to different models such as zero-order, first-order, Korsmeyer Peppas, and Higuchi[21-22].

2.7.8 Stability Study:

For stability, testing is to test the product and provide evidence on how the quality of a drug substance or product varies with time underneath the influence of assorted environmental factors like temperature, light, humidity and allows suggested storage conditions, retest periods, and shelf lives to be established[23-24].

In the present study, stability studies were carried out at Room Temperature at 25°C ± two °C / 60 % RH ± 5% RH and Accelerated testing at 40°C ± two °C / 75 % RH ± 5% RH for three months for the optimized formulation. The optimized formulation was analyzed for the swelling ratio, % drug content, % drug release, and t75.

III. RESULTS AND DISCUSSIONS:

3.1 Standard Calibration Curve:

The results of the standard calibration curve, as shown in Figure 1, revealed that it follows the beers lamberts law as the equation obtained was linear with the values of y =0.014x + 0.005 and the regression value of R2 = 0.999.

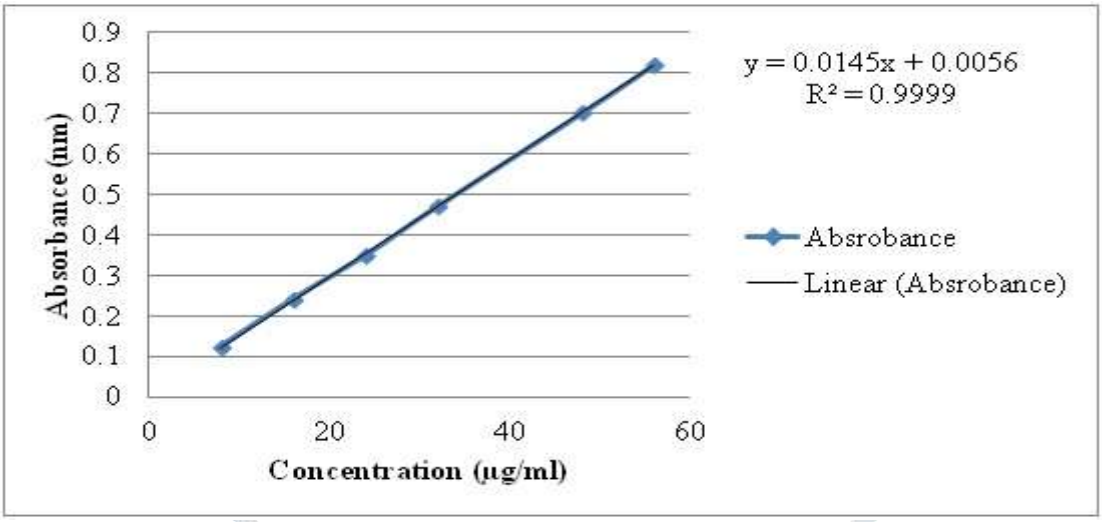

Figure 1: standard calibration curve of resveratrol

3.2 Drug –Excipient Interaction Study:

FTIR results of pure resveratrol represented the following band characteristics at 3309cm-1 of Free O-H stretching vibration, 1458, 1504, 1597cm-1 exhibited benzene skeleton vibrations, and 995cm-1 represented bending vibration of C=C-H, the typical transolefinic band. The results of FTIR were compared with the standard. It was found that the pure drug had the same peaks like that of the standard, which confirmed that the drug was pure. The optimized formulation was then matched with the peaks of pure drug, and it was seen that there was no new formation, disappearance, mismatching of peaks which can be seen in Figure 2 and 3 respectively.

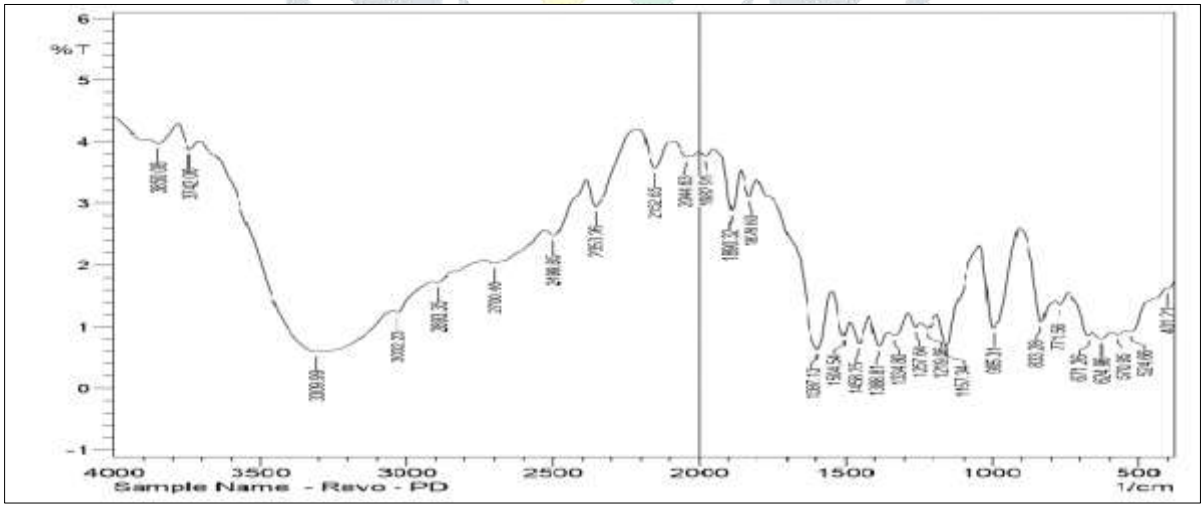

Figure 2: FTIR of pure drug

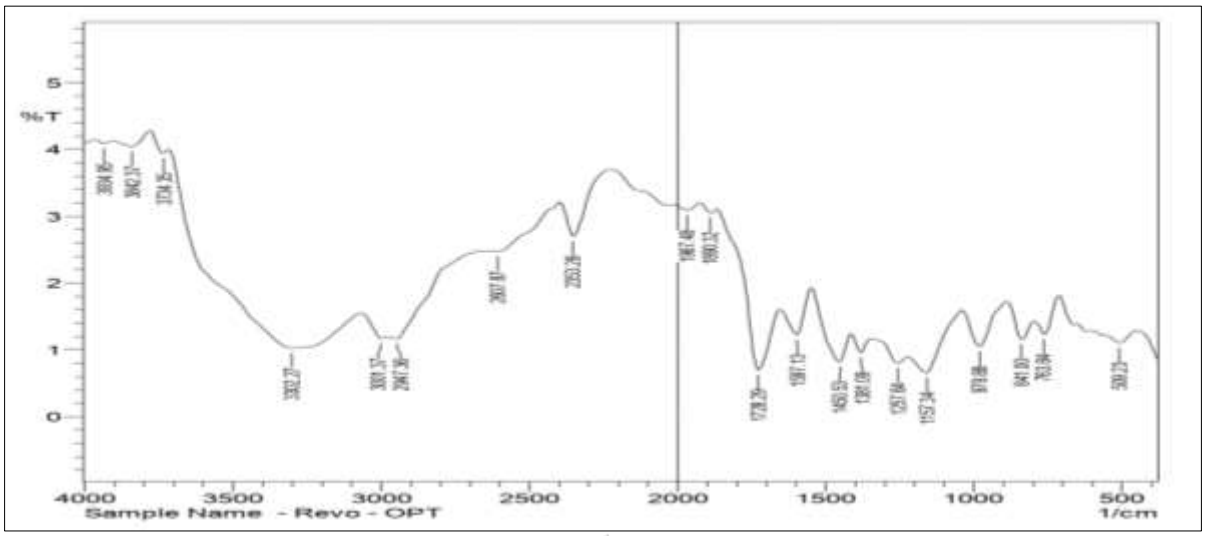

Figure 3: FTIR of optimized formulation

3.3 Thermal Study:

The DSC study of pure drugs shows the melting point of 267.24oC, which indicates its purity, and the optimized formulation reveals the melting point of 281.56oC. The thermal study of the pure drug and the optimized formulation revealed that the drug was pure. When mixed with the excipients, it showed a slight difference in the melting point, which was not a significant difference in the temperature and therefore, it was concluded that the drug was pure.

It shows less physical or chemical interaction with the excipients. The results of the DSC study are shown in Figures 4 and 5.

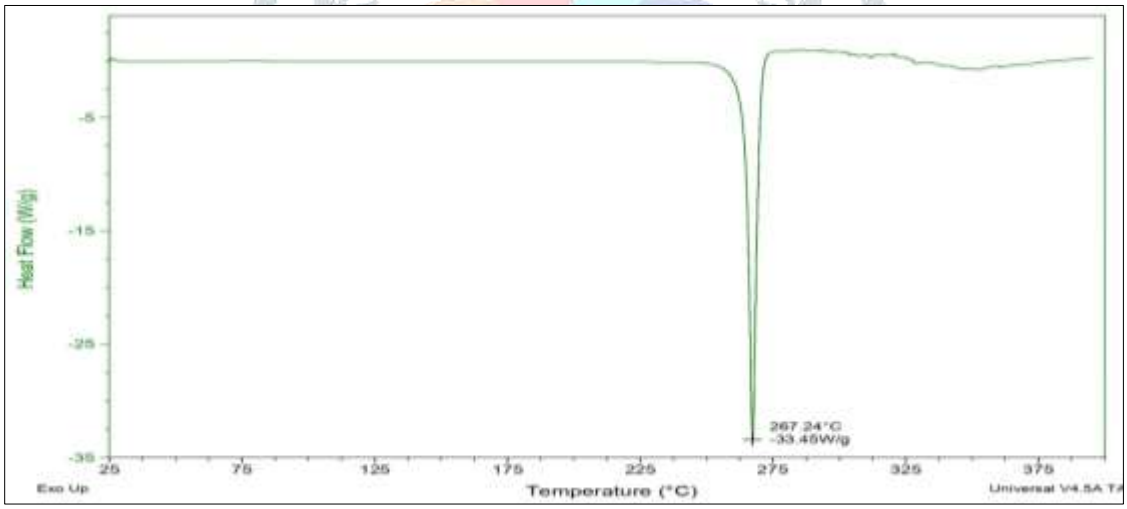

Figure 4: DSC of pure drug

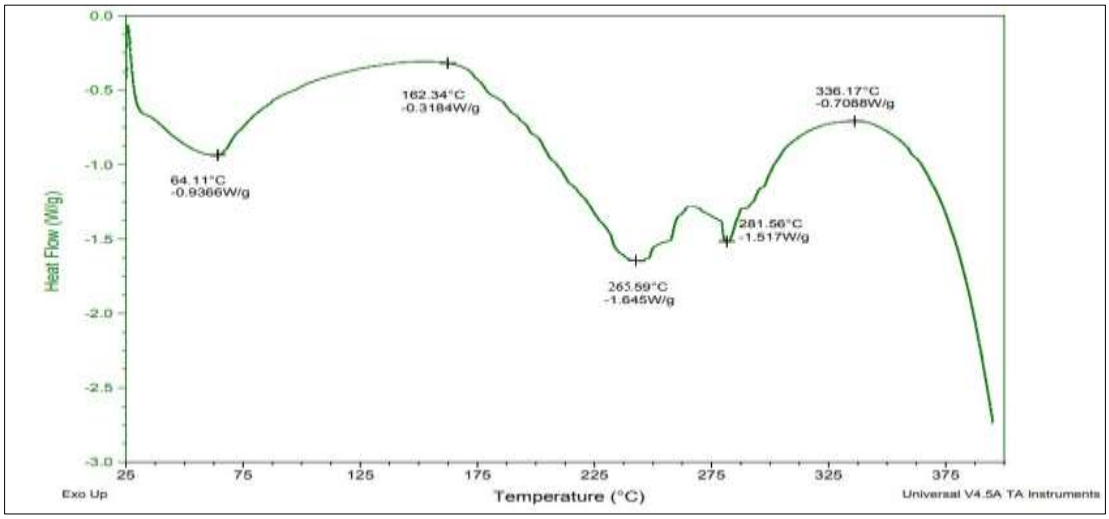

Figure 5: DSC of optimized formulation

3.4 Evaluation Parameters:

The percentage yield, drug content and swelling ratio of the prepared hydrogel were found to be within the range of 71.25±0.28-85.14±1.27%, 87.98±1.82-99.54±0.87%, and 158.21±0.14-267.74±0.25%. From the evaluated results, it can be concluded that the prepared hydrogel had a better drug content efficiency. This suitable swelling property can hold a more significant amount of water for sustained release action. Lastly, the percentage yield was quite excellent, which shows a minimum wastage of the materials used. From all the batches, it was seen that the batch of BB1 had higher drug content with better yield and better swelling ratio than compared with the others, which is depicted in Table 3 and Figure 6.

Table 3: evaluation of hydrogel

Batch	Percent Yield (%)	Drug Content (%)	Swelling Ratio (%)
BB1	85.14±1.27	99.54±0.87	267.74±0.25
BB2	81.64±0.78	94.28±0.47	158.21±0.14
BB3	77.41±1.85	97.34±0.74	195.92±0.87
BB4	79.96±0.76	89.87±0.72	208.98±0.65
BB5	80.12±1.31	85.63±0.29	247.08±0.68
BB6	78.35±0.11	88.37±1.58	205.02±0.17
BB7	77.25±1.38	87.98±1.82	169.94±0.87
BB8	71.25±0.28	96.87±0.31	164.56±1.84
BB9	82.37±0.15	92.58±0.31	255.42±0.58
BB10	81.24±0.25	90.28±0.68	202.74±1.87
BB11	83.47±1.82	89.67±0.42	208.84±1.11

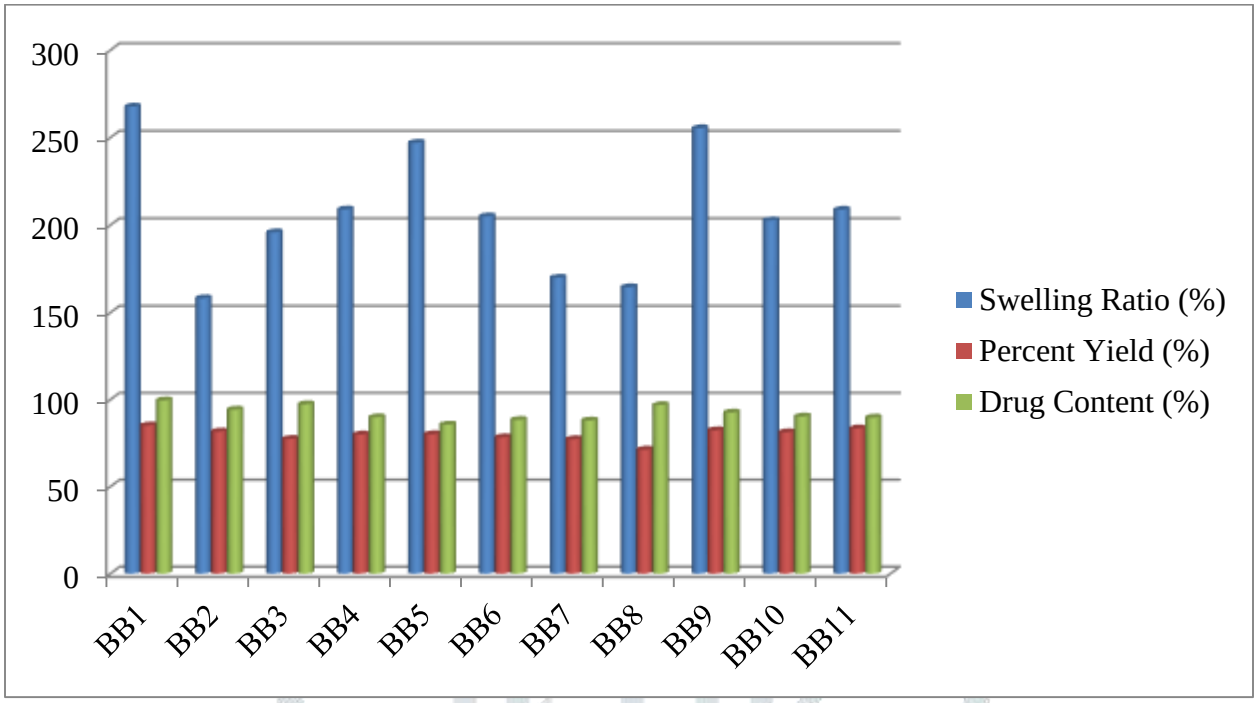

Figure 6: results for evaluation parameters of prepared hydrogel batches

3.5 *In-vitro* Drug Release:

The drug release study of the prepared hydrogel revealed that the drug release ranges between of 23.98±2.3-97.24±0.7%. From this data, it was seen that the batch of BB1 as having a better amount of drug release, i.e. 97.24±0.7% in 6 hours, than compared with the other batches, as shown in Figure 6. From this study, it can be concluded that cross-linking affects the rate of drug release, which is not significant. Nevertheless, to a certain extent, we can say that the more cross-linking, the more the drug release is sustained. So, finally, it can be concluded that the batch BB1 is the optimized batch. This optimized batch was further studied for the stability study.

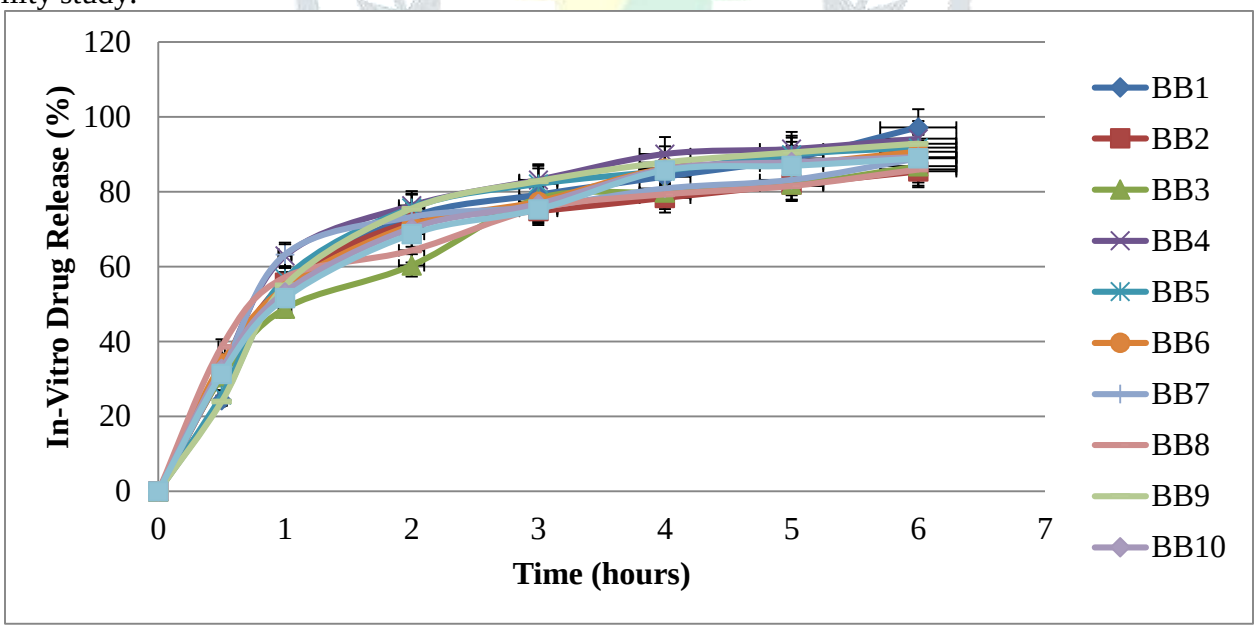

Figure 7: *In-vitro* drug release of prepared hydrogel

3.6 Statistical Design:

Drug content, swelling index, and t75 of % CDR of run BB1 are best from all responses obtained. Drug content, swelling index and t75 of % CDR of this run are 99.54±0.87%, 267.74±0.25% and 3.46 hrs,

respectively. So finally, it can be concluded that the optimized formulation is run BB1, which gave the best in vitro release up to 97.24±0.7% in 6 hrs of the period.

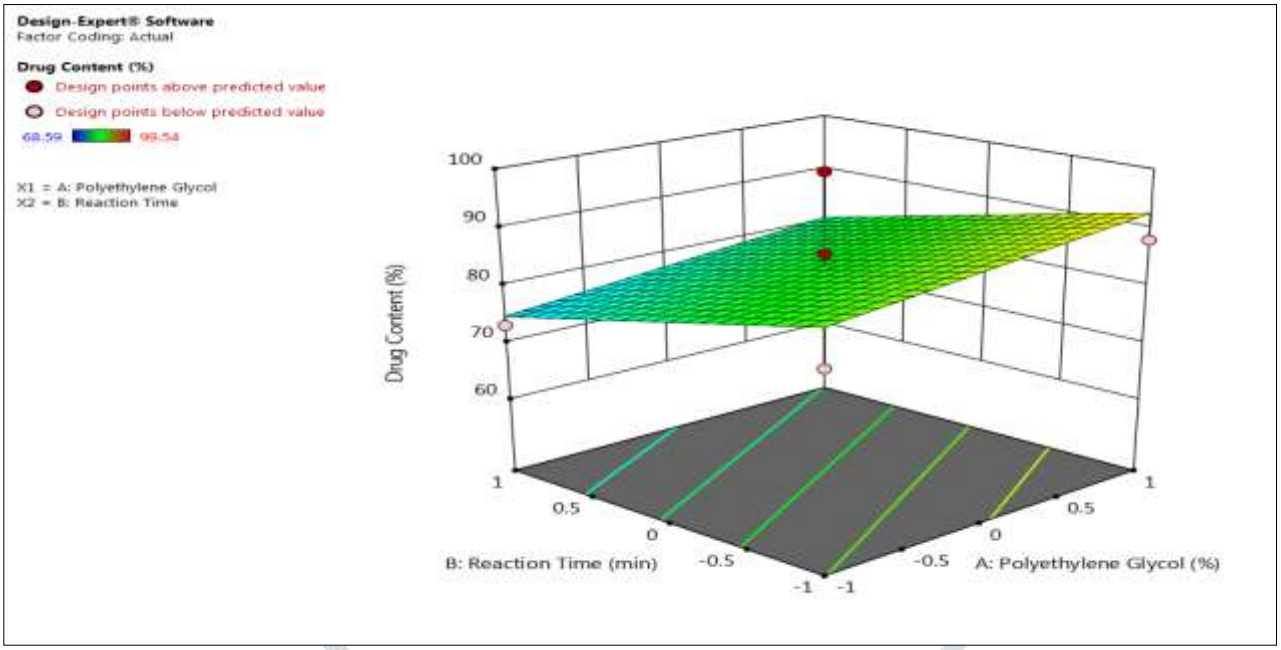

Figure 8: response surface plot of drug content

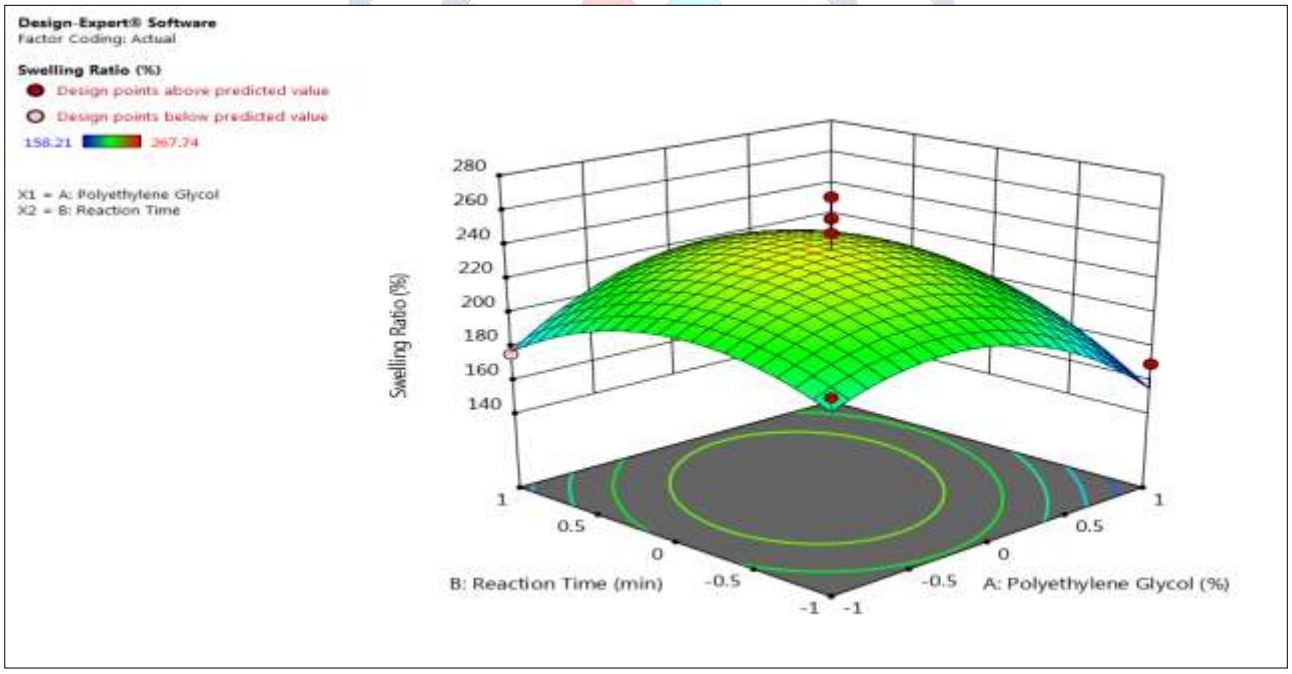

Figure 9: response surface plot of swelling ratio

3.7 t_{75} of Percent Cumulative Drug Release:

The graphical representation depicts the 75% of drug release from the formulation concerning time in Figure 10. This graphical representation shows that the hydrogel has a good % of drug release for enhancing the bioavailability of the drugs. The in-vitro drug release helps to identify the t75 value of the formulation, which was in the range of 2.11-3.46 hrs. respectively.

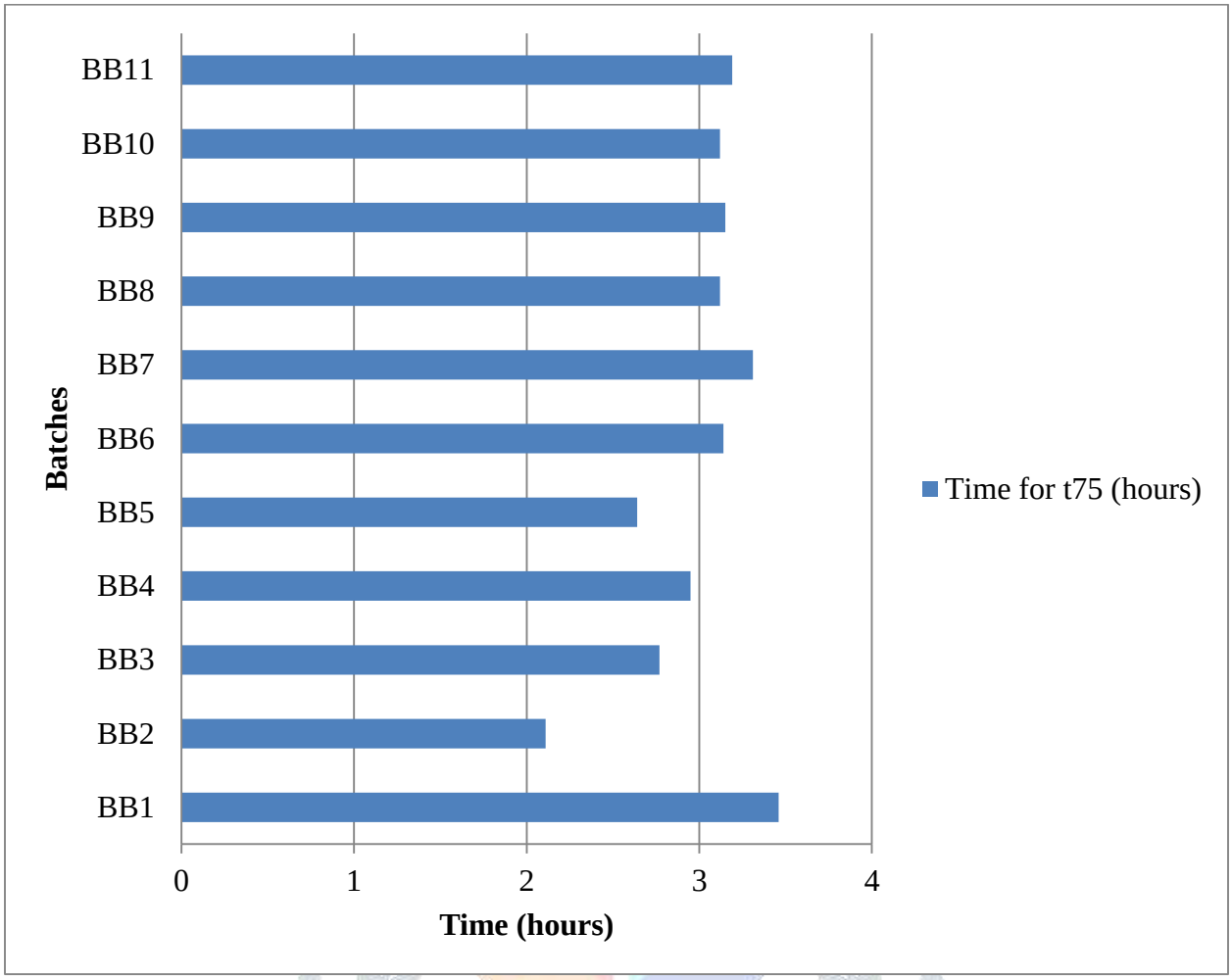

Figure 10: t_{75} of the prepared hydrogel batches

3.8 Scanning Microscopy:

The surface morphology of the crosslinked polymer was studied by employing SEM. The results showed more crosslinking for hydrogel formulation BB1 than BB4, which is far more than BB7 revealed as the rough surface of morphology shown in Figure 11. Crosslinking occurs much higher at a low citric acid concentration as the hydrogel formulations BB1, BB4 and BB7 have been formulated at reaction time 120min by varying the polyethylene glycol 1, 2 and 3%, respectively.

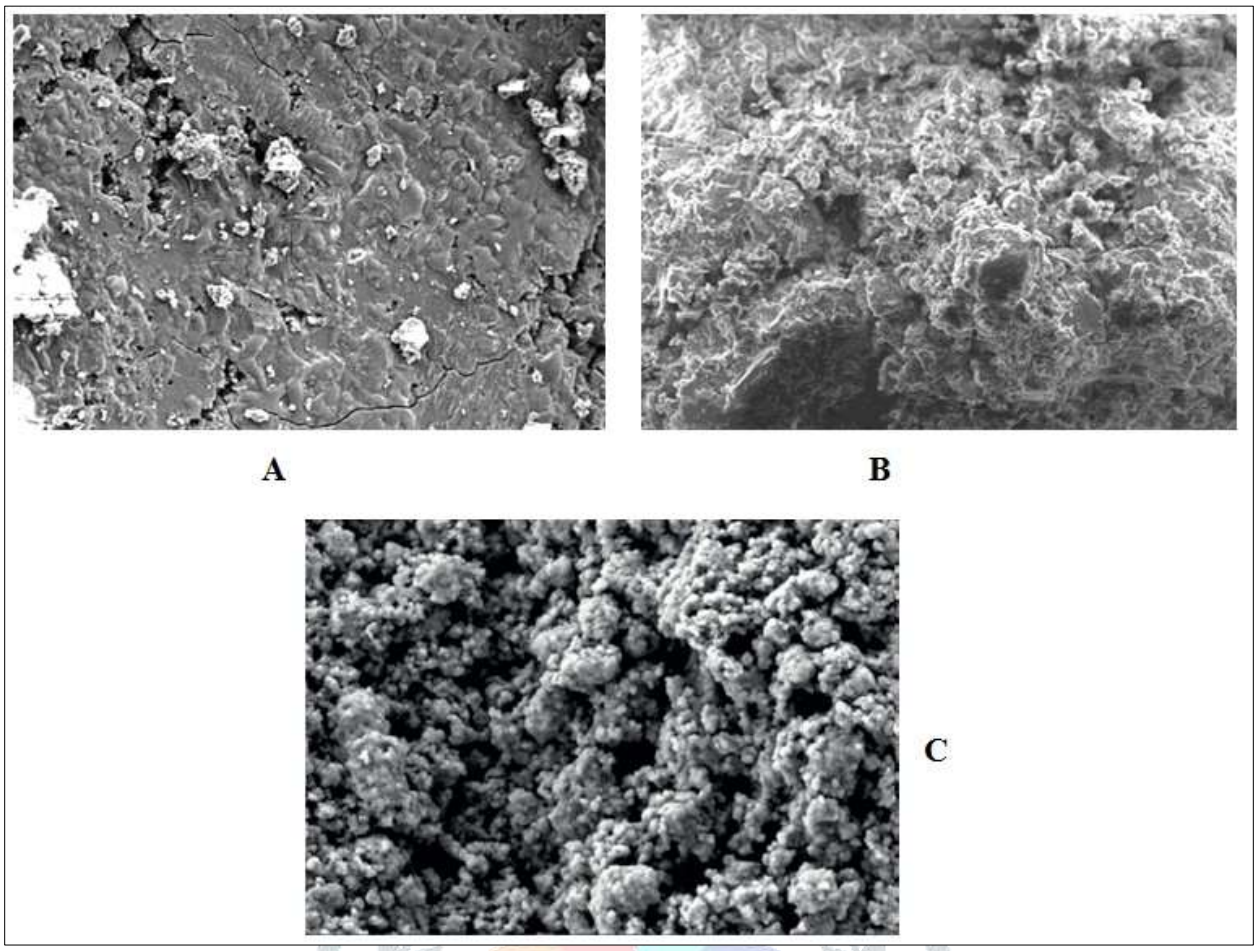

Figure 11: SEM Study of BB1 (A), BB4 (B), BB7 (C)

3.9 Kinetics Study:

The in vitro data were fitted to different kinetic models. The drug release of almost all batches, including resveratrol hydrogels, followed the Higuchi model of release kinetics, as shown in Table 4. The mechanism of drug release from hydrogel formulation was anomalous (non-friction) diffusion.

Table 4: kinetics study of the prepared hydrogel

Batch	Zero order (R^2)	First order (R^2)	Korsmeyer Peppas (R^2)	Higuchi (R^2)	Release Exponent (n)
BB1	0.852	0.740	0.907	0.939	0.624
BB2	0.907	0.823	0.947	0.964	0.574
BB3	0.897	0.883	0.954	0.955	0.473
BB4	0.807	0.720	0.890	0.909	0.485
BB5	0.837	0.716	0.892	0.926	0.613
BB6	0.858	0.824	0.957	0.945	0.436
BB7	0.737	0.640	0.831	0.854	0.440
BB8	0.821	0.794	0.908	0.908	0.315
BB9	0.848	0.717	0.892	0.933	0.640
BB10	0.868	0.812	0.952	0.952	0.458
BB11	0.856	0.797	0.943	0.944	0.470

3.10 Stability Study

The stability study of the prepared hydrogel for the optimized batch BB1 revealed that the prepared batch was quite stable during the time of study at both room temperature and accelerated study. During this study, the optimized batch was evaluated for the parameters such as swelling ratio, % drug content, % drug release and t75, respectively. These evaluation parameters showed a slight decrease in the swelling ratio, drug content and drug release pattern. However, the t75 remains the same in both studies, i.e. room temperature and accelerated stability study. The data obtained from these evaluations had minor changes, i.e. the changes observed during the studies were negligible. Therefore, it can be concluded that the optimized batch was stable during the study period.

Table 5: stability of BB1 at room temperature (25°C ± 2°C / 60 % RH ± 5% RH)

Day(s)	Swelling Ratio (%)	Drug Content (%)	Drug Release (%)	t_{75} (hours)
00	267.74±0.25	99.54±0.87	97.24±0.70	3.46
15	267.74±0.25	99.54±0.87	97.24±0.81	3.46
30	267.74±0.74	99.54±0.87	97.24±1.74	3.46
45	267.74±0.87	99.54±0.15	97.24±1.98	3.46
60	265.74±0.28	99.45±0.28	97.15±0.89	3.46
75	265.15±0.18	99.21±0.31	96.95±1.21	3.46
90	264.98±1.38	98.99±0.84	96.84±1.98	3.46

Table 6: stability of BB1 for accelerated testing (40°C ± 2°C / 75 % RH ± 5% RH)

Day(s)	Swelling Ratio (%)	Drug Content (%)	Drug Release (%)	t_{75} (hours)
00	267.74±0.25	99.54±0.87	97.24±0.70	3.46
15	267.74±0.25	99.54±0.87	97.24±0.7	3.46
30	267.74±0.25	99.25±1.89	97.07±0.51	3.46
45	266.58±1.15	99.18±0.28	96.87±1.87	3.46
60	266.47±0.48	98.94±0.21	96.34±0.82	3.46
75	266.38±1.85	98.54±1.26	96.19±0.47	3.46
90	266.28±1.15	98.27±1.98	96.08±0.31	3.46

IV. CONCLUSION:

Hydrogels containing resveratrol are prepared to increase its release time and better absorb the drug throughout the body by increasing its bioavailability. Hydrogels are known to sustain the release rate of the drug, which employs various types of hydrophilic polymers like chitosan hydrochloride, which is used in this experiment. Chitosan hydrochloride was cross-linked with polyethylene glycol, and this cross-linked polymer was then utilized to prepare hydrogel utilizing design expert 11.0. The prepared hydrogel was then evaluated for various parameters, and it found that from the prepared batches of hydrogel, the BB1 was the optimized formulation as it had maximum drug content, the maximum amount of drug release, and all the other parameters were in favor of the same batch for optimization

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ABBREVIATIONS:

CDR (Cumulative Drug Release), FTIR (Fourier Transformation Infrared Spectroscopy), DSC (Differential Scanning Calorimetry), UV (Ultra-Violet Spectroscopy), SEM (Scanning Electron Microscopy), HCL (Hydrochloric Acid), RPM (Revolutions per Minute), RH (Relative Humidity)

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