Formulation and Evaluation of Acyclovir by Quality and Design based on Microsponges

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Abstract—

Objective*: The proposed study is focussed at developing acyclovir microsponges for oral drug delivery systems. QbD was applied for better understanding of the process and to generate design space, using quality target product profile, critical quality attributes, and risk assessment. The aim of the experiment is to prepare a safe, efficacious, stable and patient compliant microsponge dosage form of Acyclovir.*

Materials and methods*: Pre-formulation studies were carried out which helped in developing a suitable dosage form. UV, FTIR, DSC, and SEM studies were done for pre-formulation and post-formulation evaluations. QbD was applied to generate design space, using QTPP, CQA, and risk assessment. Microsponges of acyclovir were developed by 2^3 factorial designs. Three variables Drug: Polymer ratio (X1), Concentration of surfactant (X2) and Stirring speed (RPM) (X3) at two levels low and high were selected and response surface plots were generated. The microsponges were prepared by Quassi-emulsion solvent diffusion method. Various characterizations that were carried out include entrapment efficiency, percentage yield, particle size determination, in-vitro drug release studies and kinetic modelling of drug release. Statistical analyses of batches and surface response studies were done to understand the effect of various independent variables on the dependent variables.*

Results and Discussions*: The λmax was confirmed at 251 nm by UV spectroscopy. The melting point was determined experimentally to be 246^0C which confirms the drug to be Acyclovir. FTIR and DSC studies confirmed that the drug is Acyclovir. Eight trials were taken as per the by 2^3 factorial designs.*

Conclusion*: The study indicates that microsponges of Acyclovir by QbD approach were successfully developed.*

Keywords— Microsponge, Acyclovir, DoE, QbD.

I. INTRODUCTION

Acyclovir is a potent, specific antiviral drug which is active against herpes simplex viruses' types I and II and varicella zoster virus [1]. Literature studies indicate that the oral bioavailability of acyclovir is relatively less, which is around 20-30%. Hence there is a need for enhancement of oral bioavailability of the acyclovir drug by employing various approaches. Acyclovir is available as various dosage forms in the market which includes capsules, creams, ointments, tablets and suspension. For all oral dosage forms the limiting factor of bioavailability which is poor. In order to overcome this limitation of oral delivery of Acyclovir, attempts have to be made to develop novel drug delivery systems of the same drug. The underlying aim of the proposed investigation is to augment the oral bioavailability of acyclovir by developing a microsponge drug delivery system of acyclovir which will attempt to increase the oral bioavailability of the drug. Microsponges are spherical small structures having large void spaces where there can be entrapment of the drug.

These voids are non-collapsible; hence it is better for drug entrapment and the entrapment efficiency of microsponges would be very high. The release of the drugs from the microsponges involves the movement of the drug from these non-collapsible void spaces to outside. The presence of such void spaces may enable the micrsoponges to deliver the drug slowly over a period for prolonged time2. As such drug delivery systems are devoid of much irritation and are capable of prolonged activity; they can enhance the patient compliance. Quality by design (QbD) is an intelligent way to bring quality into both product and process. QbD can be achieved by constructive planning of all the previous data that is accessible. Although it is based on certain amount of risks, it provides results that minimizes the risk of end product failure and enhances the chances of regulatory acceptance3. ICH Q8, ICH Q9 and ICH Q10 do explain the principles of QbD in the best way. They provide guidelines on science and risk based assessment, life cycle of product and various approaches in its development. It is also well known fact that there can be a great deal of unpredictability in scale up of a product from research and development, although the reason for failure is not generally understood. QbD is an approach to be applied in all stages of drug discovery, production and delivery [4-6].

II. MATERIALS AND METHODS

2.1 Materials

The drug Acyclovir was obtained as gift sample from Aurobindo Pharma, Hyderabad. All other chemicals that were used in the experiment were of the analytical grade.

2.2 Methods

2.2.1 Pre-formulation studies

- Determination of melting point of Acyclovir:

Melting point of Acyclovir was determined by open capillary method.

Determination of wavelength maxima (λmax) of Acyclovir: Determination of wavelength maxima (λmax) was done for Acyclovir.

- Preparation of calibration curve for Acyclovir:

The calibration curve of Acyclovir was plotted by taking 0.1N HCl as the solvent.

- Identification of Acyclovir by FT-IR Spectroscopy:

FTIR study was carried for Acyclovir.

Identification of Acyclovir by DSC Study:

The thermograph of Acyclovir was obtained by DSC.

2.2.2 Method of preparation of Acyclovir microsponges:

Microsponges are prepared by quasi-emulsion solvent diffusion technique. In this method external phase and internal phases are used. The internal phase is organic phase was containing drug (acyclovir), Dichloromethane, Eudragit RS100 and triethyl citrate (TEC) which is added in order to facilitate the plasticity. The external phase consisted of distilled water and polyvinyl alcohol (PVA) which acts as surfactant. Measured amounts of drug and polymer are dissolved in measured quantity of DCM. The formed solution is poured into water containing polyvinyl alcohol. Internal phase and external phases were properly mixed. This results in the solidification of the drug and its diffusion out of the liquid phases. Finally the solidified microsponges are collected by filtration. Then they are subjected to washing and drying.

2.3 Characterization of Acyclovir microsponges:

2.3.1 Drug content:

Drug content is determined by using the UV Visible spectrophotometer.

2.3.2 Average particle size analysis:

Malvern apparatus was used for particle size analysis

2.3.3 In-vitro drug release study of microsponges:

Dissolution test was carried out to determine the in-vitro drug release profile of the prepared batches of microsponges.

2.3.4 Kinetics of drug release:

Kinetic release study was performed to find drug release mechanism from dissolution parameter by using different various kinetic model equations like Zero order, First order, Higuchi, Hixon-Crowell and Korsemeyer-Peppas model.

2.3.5 Risk Assessment to identify CQAs affecting drug product quality:

Risk assessments was done to select formulation and process variable which may affect product quality for CQAs by process characterization that defines satisfactory changes in material and process parameters. As a final point, this can result in quality assurance by process design space to understand and develop control strategy. Critical quality attributes were categorized into high, medium and low risk parameters based on knowledge space. Usually high-risk parameters are considered important for Design of Experiments as they are having more effect than others and need to be in accepting multivariate ranges.

FIGURE 1: QbD approach

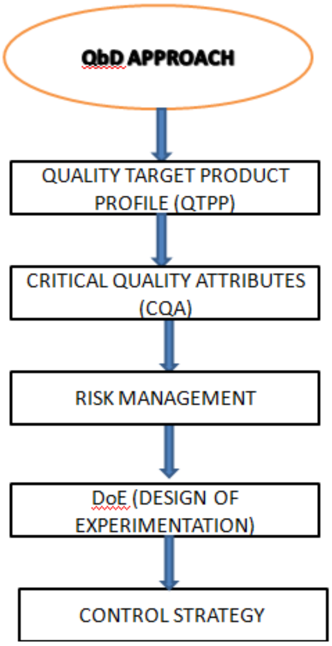

TABLE 1
INDEPENDENT AND DEPENDENT VARIABLES

Independent variables – X	Dependent Variables – Y
Polymer Type and Concentration	Particle Size
Drug: Polymer Ratio	
Internal Phase Type	Entrapment Efficiency (%)
Internal Phase Volume	
External Phase Volume	Drug content
Surfactant Type and Its Concentration	
Stirring Speed and Time	% Cumulative Drug Release

Effect of different independent variables were checked by evaluating particle size, entrapment efficiency (%), particle size and % cumulative drug release of Acyclovir microsponges formulated in preliminary trial batches. Based on that characterization, CQAs were selected which have greater effect on microsponges formulations.

2.3.6 Design of Experimentation (DoE) of Acyclovir microsponges by using QbD approach:

A design space can signify formulation and process variables that affects attributes which are related to drug substance, materials, equipments and finished product quality. For this purpose, risk assessment was done based on understanding of process and formulation related parameters on microsponges' quality. Preliminary studies and later Design of Experimentation (DoE) was carried out for high risk parameters. Based on effect of critical quality attributes of target product profile, design space for obtaining robust formulation was proposed.

III. RESULTS AND DISCUSSIONS

3.1 Pre-formulation studies

3.1.1 Determination of melting point of Acyclovir:

- The melting point of Acyclovir was found to be 256.5 °C.
- Determination of wavelength maxima (λmax) of Acyclovir:
- The wavelength maxima (λmax) of Acyclovir were found to be 251 nm.

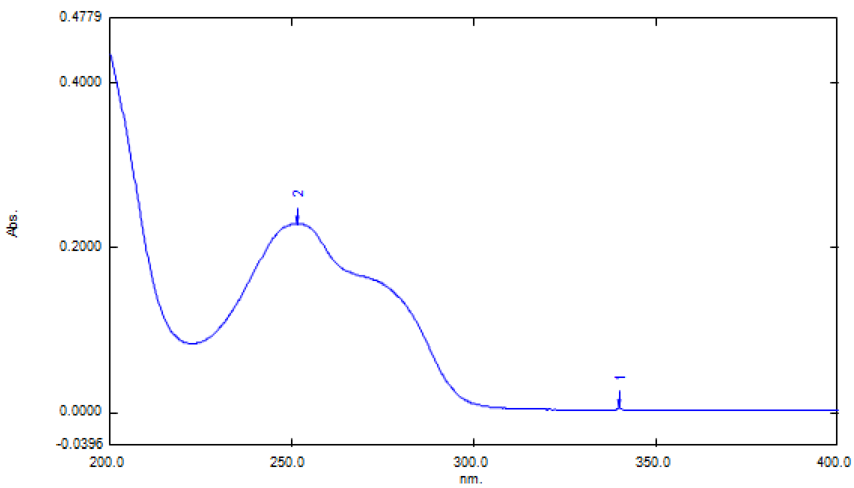

FIGURE 2: Wavelength max (λmax) of Acyclovir

3.2 Preparation of calibration curve for Acyclovir:

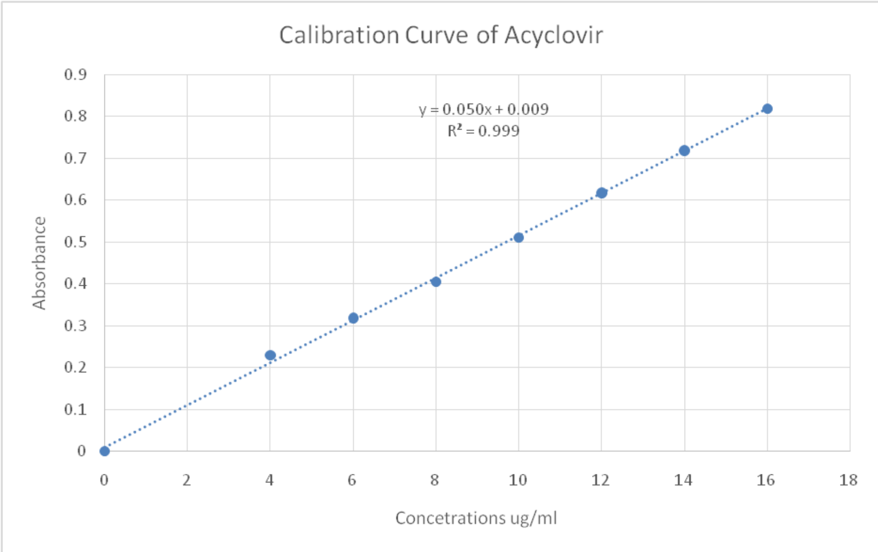

FIGURE 3 Calibration curve for Acyclovir

3.3 Identification of Acyclovir by FT-IR Spectroscopy:

The recorded IR spectrum of Acyclovir is shown in following figure.

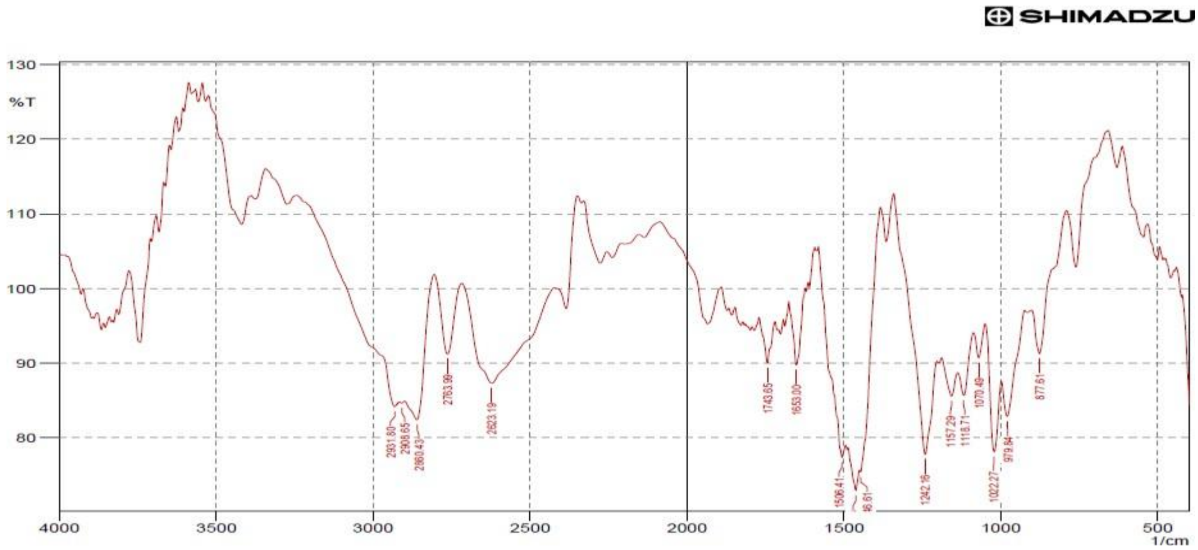

FIGURE 4: FTIR spectrum of Acyclovir

TABLE 2
FT-IR PEAKS OF ACYCLOVIR

Type of Vibration	Standard Wave number(cm-1)	Observed Wave number(cm-1)
C=C Stretching of Aromatic	1600-1475	1465.90
- tretching of amine	3500-3300	3417.88
N-O Stretching of N - Oxide	1300-1200	1222.87
C-H Stretching of Piperidines	2850-3000	2937.59
C-N Stretching of C-NH2	860-766	761.88

3.4 Identification of Acyclovir by DSC Study

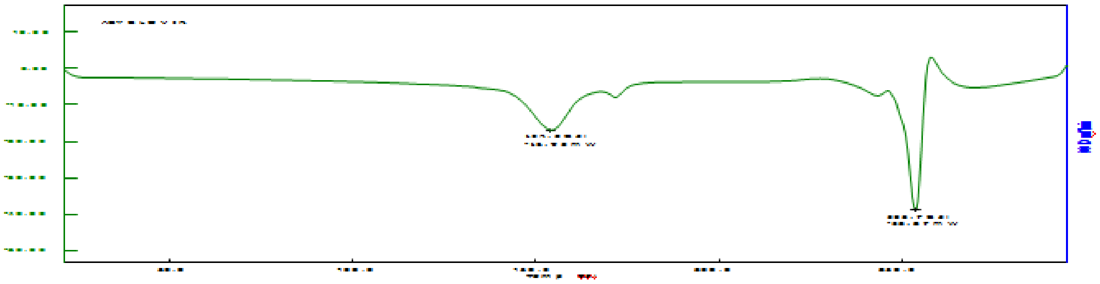

FIGURE 5: DSC thermograph

3.5 2^3 Factorial Design for Acyclovir microsponges

Various batches of Acyclovir microsponges by DoE Using QbD approach were prepared according to 23 factorial designs which are as shown in Table 3.

TABLE 3
2^3 FACTORIAL DESIGN

Independent Variables	Low (-)	High (+)
Drug: Polymer ratio (X1)	1:1	2:1
Concentration of surfactant (X2)	0.75%	1%
Stirring speed (RPM) (X3)	1500	2000
Dependent Variables		
Y1 = % Yield		
Y2 = % Entrapment efficiency		
Y3 = Particle Size		
Y4 =% CDR		

3.6 Compositions of Factorial Batches in Coded Form

Various batches of Acyclovir microsponges with Eudragit RS 100 were prepared according to 23 factorial designs which are as shown in Table 4.

TABLE 4
COMPOSITIONS OF FACTORIAL BATCHES IN CODED FORM

2^3 =8 Batches			
Batch No.	**Variable level in coded form**		
	Drug: Polymer Ratio (X1)	**Concentration of surfactant (X2)**	**Stirring speed (RPM) (X3)**
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1

3.7 Formulation Design by 2^3 Factorial Design

TABLE 5
FORMULATION DESIGN BY 2^3 FACTORIAL DESIGNS

Batch	Drug: Polymer Ratio (X1)	Concentration of surfactant (X2)	Stirring speed (RPM) (X3)
ACVMS1	1:1	0.75	1500
ACVMS2	2:1	0.75	1500
ACVMS3	1:1	1	1500
ACVMS4	2:1	1	1500
ACVMS5	1:1	0.75	2000
ACVMS6	2:1	0.75	2000
ACVMS7	1:1	1	2000
ACVMS8	2:1	1	2000

3.8 Characterization of Acyclovir microsponges:

TABLE 6
CHARACTERIZATION OF BATCHES ACVMS1- ACVMS8

Batch No	Yield-% (Y1) (Mean ± S.D.) (n = 3)	E.E.-% (Y2) (Mean ± S.D.) (n = 3)	P. Size-µm (Y3) (Mean ± S.D.) (n = 3)	Drug Content (Y4) (Mean ± S.D.) (n = 3)
ACVMS1	71.45±1.15	86.25±1.82	19.42±2.54	82.66±1.55
ACVMS2	75.52±1.66	87.28±1.97	26.46±2.76	80.46±1.20
ACVMS3	72.48±1.85	88.56±1.54	17.25±1.18	85.38±2.33
ACVMS4	81.49±2.24	92.28±1.77	15.23±1.87	88.57±1.44
ACVMS5	67.38±1.52	86.66±1.65	18.29±1.60	81.39±1.56
ACVMS6	77.13±1.38	93.14±1.44	7.4±1.74	87.63±1.75
ACVMS7	84.18±2.28	88.19±1.89	17.22±1.67	83.82±2.65
ACVMS8	86.17±1.49	90.52±2.73	21.51±2.23	86.32±1.78

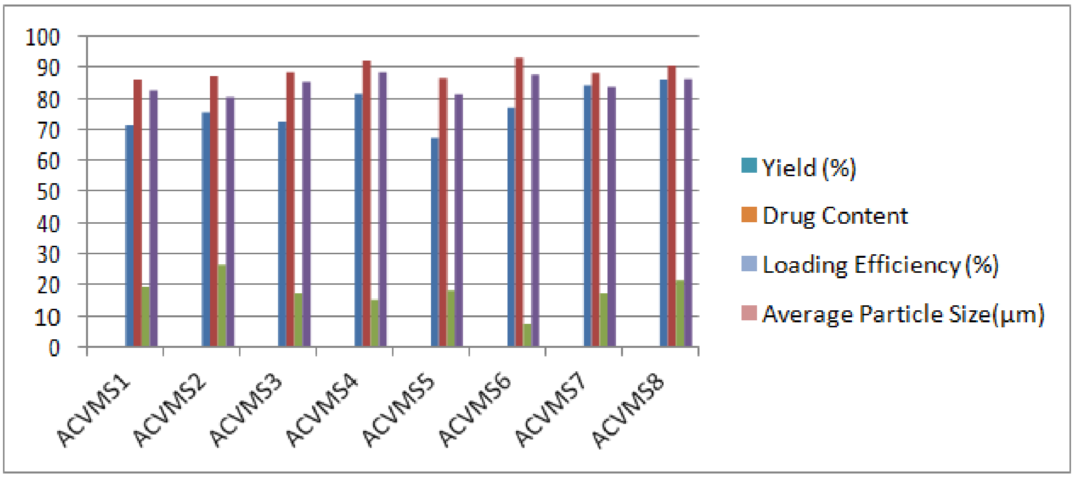

FIGURE 6: Characterization of Batches ACVMS1 – ACVMS8

3.9 % Cumulative Drug Release profile of batches ACVMS1- ACVMS8

TABLE 7
% CUMULATIVE DRUG RELEASE PROFILE OF BATCHES ACVMS1 – ACVMS4

Time	ACVMS1 (Mean ± SD) (n=3)	ACVMS2 (Mean ± SD) (n=3)	ACVMS3 (Mean ± SD) (n=3)	ACVMS4 (Mean ± SD) (n=3)
0	0	0	0	0
1	28.01±1.13	14.18±1.78	15.16±1.45	21.25±1.49
2	37.81±1.74	18.21±1.46	17.30±1.17	27.81±1.65
3	44.95±1.56	26.84±1.29	22.85±1.59	36.38±1.37
4	56.25±1.93	32.59±1.66	31.37±1.17	46.90±1.58
5	63.45±1.63	43.20±1.52	42.61±1.79	51.48±1.48
6	76.11±1.28	54.01±1.68	53.33±1.62	66.37±1.75
7	85.26±1.82	65.01±1.84	66.08±1.28	81.42±1.81
8	90.32±1.25	78.06±1.94	75.91±1.56	88.96±1.26

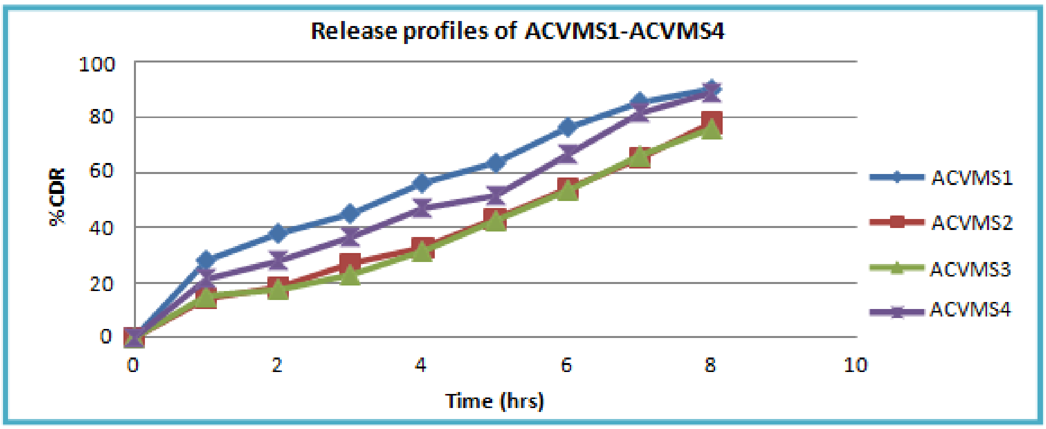

FIGURE 7: % Cumulative Drug Release profile of Batches ACVMS1 – ACVMS4

TABLE 8
% CUMULATIVE DRUG RELEASE PROFILE OF BATCHES ACVMS5 – ACVMS8

Time	ACVMS5 (Mean ± SD) (n=3)	ACVMS6 (Mean ± SD) (n=3)	ACVMS7 (Mean ± SD) (n=3)	ACVMS8 (Mean ± SD) (n=3)
0	0	0	0	0
1	16.33±1.17	28.01±1.87	21.73±1.54	17.88±1.93
2	21.44±1.67	37.84±1.64	30.35±1.71	26.45±1.56
3	31.76±1.65	44.95±1.92	46.31±1.95	36.97±1.76
4	40.67±1.39	56.25±1.66	56.34±1.62	38.72±1.82
5	51.18±1.36	63.45±1.25	61.80±1.48	60.95±1.19
6	61.48±1.82	76.11±1.86	71.82±1.26	73.38±1.17
7	74.94±1.28	85.26±1.48	84.87±1.82	84.87±1.42
8	85.55±1.52	90.32±1.49	93.24±1.65	91.74±1.62

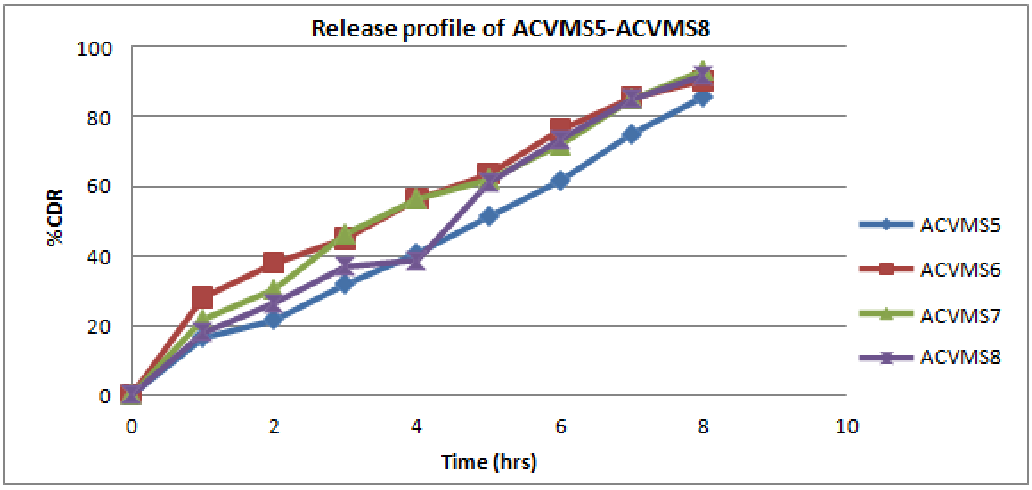

FIGURE 8: Cumulative Drug Release profile of Batches ACVMS5 – ACVMS8

TABLE 9
RELEASE KINETIC OF BATCHESACVMS1 – ACVMS4

Model	Parameter	ACVMS1	ACVMS2	ACVMS3	ACVMS4
Zero Order	R^2	0.9468	0.9829	0.9609	0.9733
	Slope	10.299	8.4804	8.2129	10.202
	Intercept	11.45	2.4204	2.0214	5.7064
First Order	R^2	0.9688	0.9654	0.9338	0.9673
	Slope	-0.0956	-0.0532	-0.0513	-0.073
	Intercept	1.9886	2.0048	2.0067	1.9988
Higuchi Model	R^2	0.9856	0.9899	0.9859	0.9865
	Slope	30.03	21.123	20.267	25.136
	Intercept	- 1.8141	- 4.825	- 4.7016	- 6.3716
Hixon Crowell	R^2	0.9757	0.9752	0.9462	0.9775
	Slope	0.2699	0.1669	0.1611	0.2183
	Intercept	0.0997	0.0055	- 0.0014	0.042
Korsmeyerpe ppas equation	R^2	0.8786	0.8938	0.8432	0.9089
	Slope	74.833	54.858	52.188	66.878
	Intercept	14.108	5.4689	5.3575	9.0138

TABLE 10
RELEASE KINETIC OF BATCHESACVMS5 – ACVMS8

Model	Parameter	ACVMS5	ACVMS6	ACVMS7	ACVMS8
Zero Order	R^2	0.9867	0.9467	0.97	0.9679
	Slope	10.049	11.451	11.735	11.413
	Intercept	2.1189	10.306	6.5582	2.8125
First Order	R^2	0.964	0.9688	0.9935	0.9103
	Slope	-0.0687	-0.0956	-0.0915	-0.0889
	Intercept	2.0155	1.9886	2.0019	2.03
Higuchi Model	R^2	0.9968	0.9856	0.9938	0.9827
	Slope	24.896	30.029	30.221	28.326
	Intercept	- 6.2585	- 1.8083	- 5.0004	- 6.7818
Hixon Crowell	R^2	0.9767	0.9757	0.9934	0.9386
	Slope	0.2089	0.2699	0.2651	0.2573
	Intercept	- 0.0177	0.0999	0.0439	- 0.0387
Korsmeyerpeppas equation	R^2	0.899	0.8785	0.9429	0.8798
	Slope	65.068	74.827	78.487	73.807
	Intercept	5.7057	14.115	9.7267	6.9227

3.10 Statistical analysis of batches ACVMS1- ACVMS8

In factorial design, amount of drug (ACV): polymer (Eudragit RS100) ratio (X1), amount of PVA Concentration (X2), and Stirring Speed (X3) were taken as independent variables. % Yield (Y1), % E. E (Y2). Particle sizes (Y3), % CDR (Y4) were selected as dependent variables.

3.11 Effect on % Yield (Y1) - Surface Response Study

Y1 (%Yield) = 77.73 + 5.52*X1 + 1.44 *X2 – 2.86*X3

Positive value for coefficient of X1 in equation indicates Increase in yield with Drug Concentration. Positive value of coefficient of X2 PVA concentration indicates increase in response of Y1 i.e. % yield. Negative value of coefficient X3, time indicates decrease in yield.

3.12 Effect on % Entrapment Efficiency (Y2) - Surface Response Study

3.12.1 Entrapment Efficiency (Y2) =

84.75 + 0.875 * X1 + 0.55 * X2 + 1.05 * X3

Positive value for coefficient of X1 in equation indicates increase in Entrapment Efficiency with Drug Concentration. Positive value of coefficient of X2 PVA concentration indicates increase in response of Y2 i.e. % E.E. Positive value of coefficient X3, time indicates increase in yield.

3.13 Effect on Particle Size (Y3) - Surface Response Study P.S. (Y3) = 18.85 – 2.51 * X1 + 2.26 * X2 – 3.31 * X3

Negative value for coefficient of X1 in equation indicates decrease in particle Size with Drug Concentration. Positive value of coefficient of X2 PVA concentration indicates increase in response of Y3 i.e. P.S. Negative value of coefficient X3, time indicates decrease in Particle size.

3.14 Effect on % CDR (Y4) - Surface Response Study

%**CDR (Y4) = 87.03 + 4.54 * X1 - 2.86 * X2 – 0.84 * X3**

Positive value for coefficient of X1 in equation indicates Increase in CDR with Drug Concentration. Negative value of coefficient of X2 PVA concentration indicates decrease in response of Y4 i.e. % CDR. Negative value of coefficient X3, time indicates decrease in CDR.

IV. CONCLUSION

The focus of the current study was to develop microsponge drug delivery system of acyclovir using QbD approach. . Literature studies indicate that the oral bioavailability of acyclovir is relatively less, which is around 20-30%. The underlying objective of the proposed investigation is to augment the oral bioavailability of acyclovir by developing a microsponge drug delivery system of acyclovir. Pre-formulation studies were carried out which helped in developing a suitable dosage form. UV, FTIR, DSC, and SEM studies were done for pre-formulation and post-formulation evaluations. QbD was applied to generate design space, using QTPP, CQA, and risk assessment. Microsponges of acyclovir were developed by 2^3 factorial designs. Three variables Drug: Polymer ratio (X1), Concentration of surfactant (X2) and Stirring speed (RPM) (X3) at two levels low and high were selected and response surface plots were generated. The microsponges were prepared by Quassi-emulsion solvent diffusion method. Various characterizations that were carried out include entrapment efficiency, percentage yield, particle size determination, in-vitro drug release studies and kinetic modeling of drug release. Statistical analyses of batches and surface response studies were done to understand the effect of various independent variables on the dependent variables. Lastly it was concluded that microsponges of Acyclovir using QbD approach were successfully developed.

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