Design Development and Evaluation of Acyclovir Loaded Compressed Microsponge

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Abstract— *The Microsponge Drug Delivery System (MDS) is a patented technology has been successively used for the controlled release of topical agents which consist of macro porous beads, typically 10-25 microns in a diameter, which are loaded with active agent. Allowing a sustained flow of substances out of the sphere, the outer surface is typically porous, This system can suspend or entrap a wide variety of substances, and incorporated into a formulated product such as a liquid, gel, cream, or powder. The Microsponge shows time mode release when applied to the skin and they also response to other stimuli like rubbing, pH, etc. MDS technology is currently used in different dosage forms like cosmetics, over the counter (OTC) skin care, sunscreens and prescription products. Microsponge technology allows entrapment of ingredients and it also shows reduced side effects, more stability, increased elegance and enhanced formulation flexibility. In addition, various studies have showed that microsponge systems are non-irritating, non-mutagenic, non-allergenic, and non-toxic. Microspheres can be prepared by different methods using emulsion system or by suspension polymerization in liquid system.*

Keywords— Microsponge, Porous-beads, controlled-release, Quisi-emulsion-solvent- diffusion method, Liquid-liquid-suspension-method.

I. INTRODUCTION

1.1 The Microsponge Delivery System

A Microsponge drug delivery system (MDDS) is a patented, highly cross-linked, porous, polymeric microspheres polymeric system (10-25 µ) consisting of porous microspheres particles consisting of a myriad of inter connecting voids within non-collapsible structures with a large porous surface that can entrap wide range of actives (cosmetics, over-the-counter (OTC) skin care, sunscreens and prescription products). A typical 25 µm sphere can have up to 250000 pores and an internal pore structure equivalent to 10 ft in length providing a total pore volume of about 1ml/g. Microsponge technology offers entrapment of ingredients and is believed to contribute towards reduced side effects, increased efficacy, improved stability, increased elegance and enhanced formulation flexibility.[3,31,32] In addition, numerous studies have confirmed that microsponge systems are non- irritating, non- mutagenic, non-allergenic and non-toxic.

The microsponge drug delivery system (MDS) releases its active ingredient on a time mode and also in response to other stimuli (rubbing, temperature, pH, etc.) Microsponges have the capacity to absorb or load a high degree of active materials into the particle or onto its surface. Its large capacity for entrapment of actives up to 3 times its weight differentiates microsponges from other types of dermatological delivery systems. The MDS has advantages over other technologies like microencapsulation and liposomes. Microcapsules cannot usually control the release rate of actives. Once the wall is ruptured the actives contained within microcapsules will be released. Microsponges are stable over range of pH 1 to 11, temperature up to 130^{o}C, compatible with most vehicles and ingredients, self sterilizing as average pore size is 0.25 µm where bacteria cannot penetrate, higher payload (50 to 60%), still free flowing and can be cost effective. Most liquid or soluble ingredients can be entrapped in the particles.

The Microsponges are prepared by different methods using emulsion systems as well as by suspension polymerization in a liquid–liquid system. The most common emulsion system used is oil-in-water (o/w), with the microsponges which are produced by the emulsion solvent diffusion method.[33]

II. MATERIAL AND METHODS

2.1 Analytical Method for Identification of Acyclovir

1. **λ max** by determination of UV spectroscopy.
2. Fourier transformed infrared spectrometry.

3. Differential Scanning Calorimetry. (DSC)

2.2 Authentication of Drug

2.2.1 λ_{max} by determination of UV spectroscopy

- **Preparation of stock solution**

10 mg of Acyclovir was accurately weighed and transformed to 100 ml clean and dry volumetric flask and 70 ml of solution (i.e. Ph 1.2, pH 6.8 and pH 7.4 phosphate buffer) and sonicate to dissolve the drug completely and make up the volume with same solvent (i.e, pH 1.2, pH 6.8 and pH 7.4 phosphate buffer).

- **Preparation of Sub-stock solution**

Sub-stock solution of Acyclovir is prepared by taking aliquot from stock solution and dilute them using same solvent (i.e, pH 1.2, pH 6.8 and pH 7.4 Phosphate buffer). Take the absorbance at 301.8 nm, 330.8 nm and 330.0 nm, respectively.

- **Preparation of calibration curve**

For the calibration curve of the Acyclovir standard stock solution and sub stock solution of Acyclovir was prepared in solution of different pH, pH 1.2, pH 6.8 and pH 7.4 Phosphate buffer and plot the graph between concentration v/s absorbance.

2.2.2 Fourier transmission Infrared (FT-IR) Spectral analysis (Drug & Drug-exipient Compatibility study)

FTIR spectroscopy was performed on Fourier transformed infrared spectrophotometer (Jasco International) The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 mint on KBr press and the spectra were scanned in the wave number range of 600-4000 cm^{-1}. FTIR study was carried on Acyclovir.

- **IR values of Acyclovir**

TABLE 1
IR VALUES OF ACYCLOVIR.

Functional Group Gro	Wave Number cm -1m^{-1})
O-H Stretching mode associated with the hydroxyl group	3600-3200
C-H stretch of the aromatic group	~3000
C = C stretch of the aromatic group; N-H bond scissoring	1619
C-C stretching mode	1449, 1490
O-H deformation of the hydroxyl groups	1355, 1378
In plane bending mode	1190- 1267
C-O stretching mode	1131
C-H bond Out of plane bending mode; ring deformation of the aromatic group	685-808

2.2.3 Differential Scanning Calorimetry (DSC) studies

The sample of Acyclovir was scanned at 10˚C/min from 30˚C to 305˚C and thermal behavior of the drug was studied by recording the thermogram. The DSC thermogram of Acyclovir exhibited sharp endothermic peak. This is same as that of melting point of Acyclovir.

2.3 Formulation of Acyclovir Microsponges

Microsponges were prepared by **Quasi Emulsion Solvent Diffusion Technique** which requires two immiscible phases internal and external phase with a surfactant which aids formation of an emulsion by reducing the interfacial tension.

2.4 Method of preparation of Acyclovir Microsponges using Eudragit RS-100 and Eudragit ES-100

The required amount of Acyclovir and Eudragit polymers were weighed accurately and dissolved in 20 ml of DCM: IPA (1:1) under sonication. The surfactant PVA was weighed accurately and dissolved in distilled water. The surfactant mixtures were allowed to cool to room temperature. The internal phase containing Acyclovir and Eudragit was added drop wise with the aid of syringe with stirring at 1500 rpm until the complete diffusion of the external phase i.e. about 8 hrs. After complete diffusion of the external phase the microsponges were filtered and dried overnight at room temperature.

2.5 Formulation Batches

TABLE 2
DIFFERENT BATCHES OF THE MICROSPONGE FORMULATIONS

SR No	Ingredients	Different Formulation Batches									
		MMS1	MMS2	MMS3	MMS4	MMS5	MMS6	MMS7	MMS8	MMS9	MMS10
1	Drug:Polymer ratio	1:1	1:1	1.5:1	1.5:1	2:1	2:1	2.5:1	2.51	3:1	3:1
	Internal Phase										
2	Acyclovir (mg)	500	500	750	750	1000	1000	1250	1250	1500	1500
3	Eud RS 100 (mg)	500	_	500	_	500	_	500	_	500	_
4	Eud ES 100 (mg)	_	500	_	500	_	500	_	500	_	500
5	Dichloromethane (DCM)	10	10	15	15	20	20	25	25	30	30
7	Iso-propyl Alcohol (ml)	10	10	15	15	20	20	25	25	30	30
8	Di-Butyl Pthalate (ml)	5	5	5	5	5	5	5	5	5	5
	External Phase										
9	PVA (mg)	50	50	75	75	100	100	125	125	150	150
10	Water (ml)	75	75	100	100	125	125	150	150	175	175

III. RESULTS AND DISCUSSION

3.1 Construction of calibration curve by UV-Visible Spectrophotometer

3.1.1 Calibration curve of Acyclovir in HCL pH 1.2

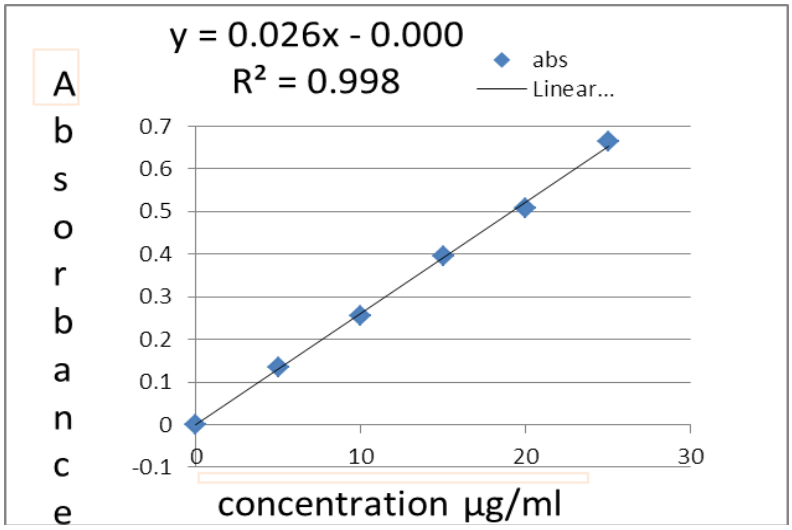

FIGURE 1: Calibration curve of Acyclovir in HCL pH 1.2.

3.1.2 Calibration curve of Acyclovir in PBS pH 6.8

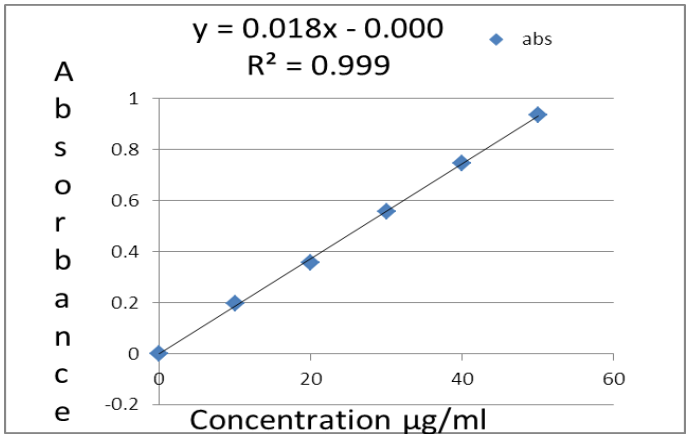

FIGURE 2: Calibration curve of Acyclovir in PBS pH 6.8.

3.1.3 Calibration curve of Acyclovir in PBS pH 7.4

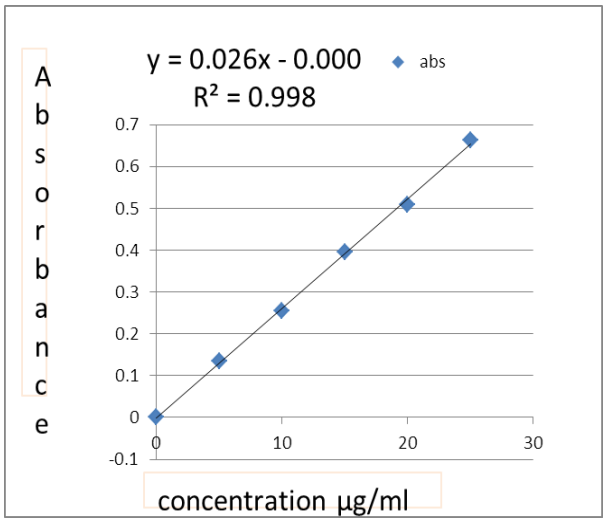

FIGURE 3: Calibration curve of Acyclovir in PBS pH 7.4.

3.1.4 Fourier transmission Infrared (FT-IR) Spectroscopy

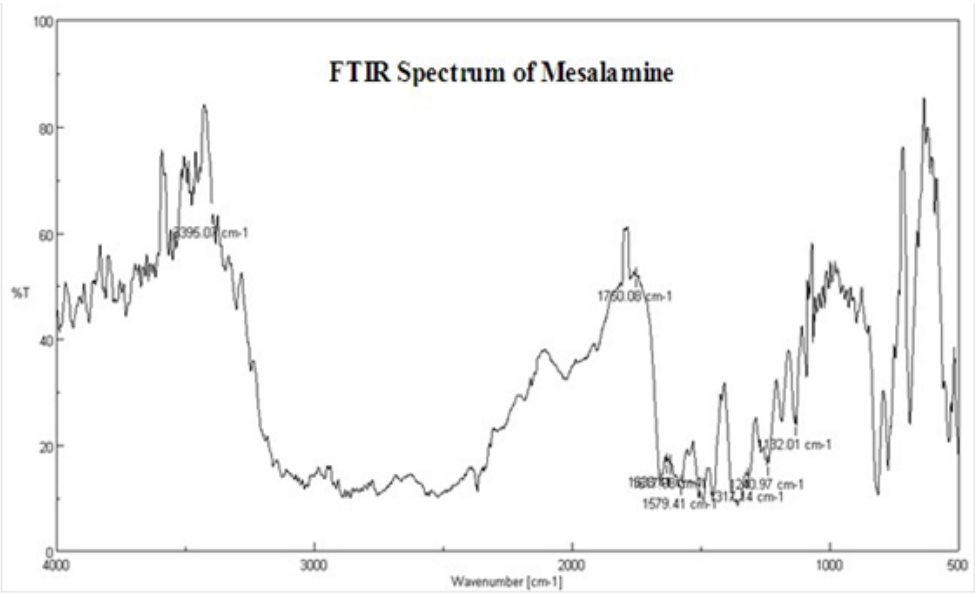

FIGURE 4: FTIR spectra of the Acyclovir.

3.1.5 Differential scanning colorimetry (DSC)

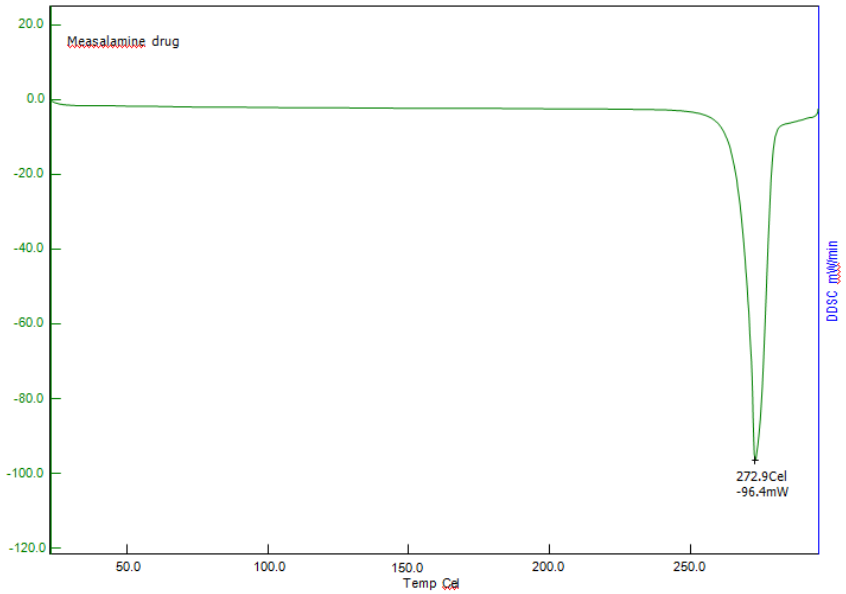

FIGURE 5: DSC plot of the Acyclovir.

3.1.6 Characterization of microsponges

TABLE 3
CHARACTERIZATION OF POWDERED MICROSPONGES.

Sr no	Formulation Code	Evaluation Parameters				
		Angle of Repose (θ)	Bulk Density (gm/cm3)	Tapped Density (gm/cm3)	Carr's Index (%)	Hausners Ratio
1	MMS1	23.75	0.51±0.01	0.57±0.01	11.86	1.11
2	MMS 2	24.46	0.52±0.01	0.55±0.02	12.00	1.09
3	MMS 3	25.20	0.51±0.01	0.53±0.01	10.53	1.12
4	MMS 4	25.24	0.52±0.01	0.57±0.01	11.48	1.13
5	MMS 5	25.35	0.52±0.01	0.59±0.01	11.53	1.16
6	MMS 6	23.56	0.50±0.01	0.57±0.01	11.80	1.10
7	MMS 7	24.35	0.52±0.01	0.55±0.02	12.05	1.09
8	MMS 8	25.18	0.50±0.01	0.53±0.01	11.15	1.11
9	MMS 9	25.45	0.52±0.01	0.57±0.01	11.42	1.10
10	MMS 10	25.38	0.53±0.01	0.59±0.01	11.55	1.13

3.1.7 Characterization of coated tablets [27]

TABLE 4
CHARACTERIZATION OF COATED TABLETS

Coated Tablets Of Batch MMS 5	Evaluation Parameters			
	Average Weight (mg)	Average Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)
1	629.88	6.11	10.26	0.73
2	630.20	6.11	10.35	0.75
3	629.50	6.12	10.39	0.77
4	629.76	6.10	10.45	0.76
5	631.20	6.12	10.52	0.76
6	631.35	6.12	10.56	0.78

3.2 Trial Batches for the Selection of Drug as to polymer ratio

3.2.1 Selection of Drug: Polymer ratio in initial trials:

TABLE 5
SELECTION OF DRUG: POLYMER RATIO IN INITIAL TRIALS.

SR No		Different Formulation Batches									
	Ingredients	**MMS1**	**MMS2**	**MMS3**	**MMS4**	**MMS5**	**MMS6**	**MMS7**	**MMS8**	**MMS9**	**MMS 10**
1	**Drug:Polymer ratio**	1:1	1:1	1.5:1	1.5:1	2:1	2:1	2.5:1	2.5:1	3:1	3:1
	Internal Phase										
2	**Acyclovir (mg)**	500	500	750	750	1000	1000	1250	1250	1500	1500
3	**Eud RS 100 (mg)**	500	_	500	_	500	_	500	_	500	_
4	**Eud ES 100 (mg)**	_	500	_	500	_	500	_	500	500	500
5	**Dichloromethane (DCM)**	10	10	10	10	10	10	10	10	10	10
7	**Iso-propyl Alcohol (ml)**	10	10	10	10	10	10	10	10	10	10
8	**Di-Butyl Pthalate (ml)**	5	5	5	5	5	5	5	5	5	5
	External Phase										
9	**PVA (mg)**	100	100	100	100	100	100	100	100	100	100
10	**Water (ml)**	100	100	100	100	100	100	100	100	100	100

3.2.2 IN-VITRO drug release studies of Acyclovir loaded microsponges

In-vitro release studies were carried out in USP basket apparatus with stirringrate 50 rpm at 37±0.5^{o}C. Initial drug release was carried out in 900 ml of 0.1 N hydrochloric acid for 2 hours followed by phosphate buffer pH 6.8 for next 2 hours followed by phosphate buffer PH 7.4 for next 8 hours. Samples were withdrawn at regular intervals and analyzed spectrophotometrically, at 301.8, 330.8, 330 nm respectively.

3.2.3 IN-VITRO drug release data for Eudragit RS-100 and ES-100 based colon specific

TABLE 6
DISSOLUTION READINGS OF THE FORMULATED BATCHES.

SR no	Media	Time (Hr)	% Cumulative Drug Release									
			MMS1	**MMS2**	**MMS3**	**MMS4**	**MMS5**	**MMS6**	**MMS7**	**MMS8**	**MMS9**	**MMS10**
			Eud RS 100	**Eud ES 100**	**Eud RS 100**	**Eud ES 100**	**Eud RS 100**	**Eud ES 100**	**Eud RS 100**	**Eud ES 100**	**Eud RS 100**	**Eud ES 100**
			1:1	**1:1**	**1.5:1**	**1.5:1**	**2:1**	**2:1**	**2.5:1**	**2.5:1**	**3:1**	**3:1**
1	**HCL PH 1.2**	1	0.02	0.034	0.0126	0.06	0.07	0.01	0.02	0.07	0.02	0.02
2		2	0.03	0.05	0.26	0.06	0.08	0.02	0.03	0.08	0.03	0.03
3	**PBS PH 6.8**	3	0.12	0.17	0.12	0.17	0.19	0.11	0.12	0.10	0.12	0.12
4		4	0.12	0.17	0.12	0.17	0.20	0.12	0.12	0.10	0.13	0.13
5	**PBS PH 7.4**	5	15.14	16.50	16.44	15.55	19.00	21.48	20.80	16.90	18.40	17.28
6		6	20.90	19.00	20.35	21.63	23.45	22.50	22.38	17.80	22.60	19.66
7		7	20.49	20.50	29.15	27.55	32.16	30.10	29.50	20.65	30.15	24.20
8		8	36.10	32.15	41.44	38.83	43.66	39.80	44.52	33.32	40.16	33.18
9		9	51.50	46.80	56.30	48.37	61.28	50.62	50.34	39.88	54.68	43.10
10		10	63.00	50.38	67.85	57.90	70.22	67.36	62.65	48.12	61.90	45.75
11		11	68.66	62.68	72.30	64.95	79.12	73.44	65.50	58.15	62.12	57.40
12		12	77.50	71.65	78.30	74.15	84.50	82.40	75.26	72.20	72.28	68.35

3.3 Results

Total 10 formulation were formulated MMS 1- MMS 10. The formulation batches **MMS 5** and **MMS 6** shows highest drug release, 84.50 and 82.40% respectively, having the Drug: Polymer ratio of **2:1**

3.3.1 Selection of Internal Phase for the Formulation of Microsponges

TABLE 7
SELECTION OF INTERNAL PHASE.

SR No.	Concentration of Polymer in Internal phase (mg)		Formation of Microsponges		Physical Appearance of microsponges		Particle Size (in µm)	
	RS 100	ES100	RS 100	ES 100	RS 100	ES 100	RS 100	ES 100
1	300	300	+	+	Irregular Spherical	Irregular Spherical	14.28	16.25
2	350	350	+	+	Spherical	Spherical	14.83	16.56
3	400	400	+	+	Spherical	Spherical	15.56	17.25
4	450	450	+	+	Spherical	Spherical	16.52	19.18
5	**500**	**500**	**+**	**+**	**Spherical**	**Spherical**	16.99	20.0
6	550	550	+	+	Irregular	Irregular	17.35	21.88
7	600	600	+	+	Spherical microsponges which Collapses after some time	Irregular microsponges	19.60	24.17
8	650	650	+	+	Spherical	Spherical microsponges which Collapses after some time	28.25	30.46
9	700	700	+	+	Spherical	Spherical	33.16	533.89
10	750	750	+	+	Spherical Rigid	Spherical Rigid	35.80	36.42

3.3.2 Selection of Concentration of Polymer in the Internal Phase

TABLE 8
SELECTION OF CONCENTRATION OF POLYMER IN THE INTERNAL PHASE.

SR NO	DRUG:POLYMER RATIO	INTERNAL PHASE (ml)	EXTERNAL PHASE (ml)	PVA (mg)	DRUG CONTENT (%)		FREE DRUG CONTENT (%)		% ENTRAPMENT	
					RS 100	ES 100	RS 100	ES 100	RS 100	ES 100
1	1:1	20●	125	100	32.42	30.14	27.80	28.26	20.42	19.25
2	1:1	20▲	125	100	36.64	35.90	10.70	10.89	33.94	32.3
3	1:1	20*	125	100	28.75	27.09	32.65	32.20	17.15	16.1
4	1:1	20♦	125	100	36.48	35.55	9.86	10.85	35.32	34.5
5	1:1	20▲●	125	100	52.60	52.10	12.90	13.15	72.70	71.60
6	**1:1**	**20♦▲**	**125**	**100**	**65.75**	**65.00**	**5.10**	**5.68**	**83.02**	**80.47**
7	1:1	20♦●	125	100	36.48	35.35	9.56	10.85	35.32	34.50
8	1:1	20▲*	125	100	41.87	40.56	12.22	13.15	28.67	28.30
9	1:1	20♦*	125	100	50.15	49.35	15.7	17.8	29.3	25.75
10	1:1	20●*	125	100	18.30	17.38	22.00	22.45	25.6	24.10

●Ethanol, ▲ Dichloromethane, ♦ IPA, * Methanol

3.3.3 Selection of Surfactant Concentration in the External Phase:

TABLE 9
SELECTION OF SURFACTANT CONCENTRATION IN THE EXTERNAL PHASE

S. No	Drug: Polymer	PVA (mg)	External phase (Water) (ml)	Physical appearance		Particle Size in µm		Drug Content (%)		Free Drug Content (%)		% Entrapment	
				RS 100	ES100	RS 100	ES 100	RS 100	ES100	RS 100	ES 100	RS 100	ES 100
1	2:1	50	125	Large Clumps	Large Clumps	-	-	-	-	-	-	-	-
2	2:1	75	125	Irregular Large	Irregular Large	16.86	18.56	35.50	32.22	7.30	8.60	81.50	80.20
3	**2:1**	**100**	**125**	**Uniform Spherical Rigid**	**Uniform Spherical**	16.99	20.0	**65.75**	**65.00**	**5.10**	**5.68**	**83.02**	**80.47**
4	2:1	125	125	Uniform Spherical Rigid	Uniform Spherical Rigid	20.25	22.39	65.47	65.22	6.15	6.11	82.88	80.15
5	2:1	150	125	Uniform Spherical Rigid	Uniform Spherical Rigid	21.56	24.67	63.20	62.11	7.50	7.92	80.50	79.45
6	2:1	175	125	Uniform Spherical Rigid	Uniform Spherical Rigid	25.22	29.32	62.4	62.05	7.90	8.30	77.45	76.65
7	2:1	200	125	Uniform Spherical Rigid	Uniform Spherical Rigid	27.46	31.28	59.88	59.65	7.70	8.48	73.90	72.55
8	2:1	225	125	Uniform Spherical Rigid	Uniform Spherical Rigid	27.98	29.54	58.36	58.21	10.50	11.26	70.45	70.12
9	2:1	250	125	Irregular Big	Irregular Big	30.75	32.22	55.84	55.36	13.55	15.30	68.05	67.00
10	2:1	275	125	Irregular Big	Irregular Big	33.16	34.57	52..2	51.56	16.60	17.11	64.80	60.50

3.3.4 Effect of External Phase Volume on Microsponge

TABLE 10
EFFECT OF EXTERNAL PHASE VOLUME ON MICROSPONGE.

SR No	External phase (ml)	PVA(mg)	Physical appearance		Particle Size in µm		Drug Content (%)		Free Drug Content (%)		% Entrapment	
			RS 100	ES100	RS 100	ES100	RS 100	ES100	RS 100	ES100	RS 100	ES 100
1	75	100	Irregular	Irregular	34.12	35.36	45.76	42.83	9.10	11.5	47.60	44.10
2	100	100	Spherical	Spherical	32.62	34.91	51.53	48.20	5.60	5.85	61.83	58.48
3	**125**	**100**	**Spherical**	**Spherical**	16.99	20.0	**65.75**	**65.00**	**5.10**	**5.68**	**83.02**	**80.47**
4	150	100	Spherical Uniform	Spherical Uniform	15.56	17.25	65.05	62.40	4.30	4.82	79.16	81.10
5	175	100	Spherical Uniform	Spherical Uniform	15.35	16.86	63.90	60.20	7.65	8.38	66.50	69.5
6	200	100	Spherical Uniform	Spherical Uniform	14.92	15.48	60.32	56.30	10.30	11.5	56.60	55.74
7	225	100	Spherical Uniform	Spherical Uniform	13.56	13.98	57.43	52.45	15.50	17.2	50.25	48.55
8	250	100	Irregular Shape	Irregular shape	12.67	13.32	53.90	48.60	17.22	20.5	48.20	36.82
9	275	100	Irregular Nonuniform	Non-Uniform	12.56	13.12	48..20	42.78	18.60	23.1	37.90	33.15
10	300	100	Irregular Large size	Irregular Large size	12.15	12.63	44.56	40.88	19.95	25.8	33.88	29.40

3.3.5 Effect of Internal Phase Volume on Microsponges

TABLE 11
EFFECT OF INTERNAL PHASE VOLUME ON MICROSPONGES.

SR No.	Internal phase (ml)	Particle Size (in µm)		Drug Content (%)		Free Drug Content (%)		% Entrapment	
		RS 100	ES 100	RS 100	ES 100	RS 100	ES 100	RS 100	ES 100
1	10.00	32.10	33.55	47.61	45.76	9.10	11.5	47.60	44.10
2	15.00	31.53	32.65	51.53	47.07	5.60	5.85	58.48	55.50
3	**20.00**	16.99	20.0	**65.75**	**65.00**	**5.10**	**5.68**	**83.02**	**80.47**
4	25.00	16.24	17.36	62.23	60.45	4.25	5.15	82.14	78.89
5	30.00	**15.35**	**16.58**	60.95	58.20	10.60	9.23	79.00	74.20

3.3.6 Effect of Rate of Stirring on Microsponges

TABLE 12
EFFECT OF RATE OF STIRRING ON MICROSPONGES.

SR No	Stirring in rpm (For 8 Hrs)	Physical Appearance		Mean Particle Size in µm		Drug Content (%)		Free Drug Content (%)		% Entrapment	
		RS 100	ES 100	RS 100	ES 100	RS 100	ES 100	RS 100	ES100	RS 100	ES 100
1	500	-	-	-	-	-	-	-	-	-	-
2	800	Irregular large	Irregular	98	109	61.30	58.65	5.50	5.65	65.10	64.20
3	1000	Spherical	Spherical	65	78	63.53	61.12	4.10	4.55	70.49	67.50
4	1200	Uniform Spherical	Uniform Spherical	35.98	39.40	65.47	65.22	2.95	2.78	75.16	71.10
5	**1500**	**Uniform Spherical**	**Uniform Spherical**	16.99	20.0	**65.75**	**65.00**	**5.10**	**5.68**	**83.02**	**80.47**
6	1700	Uniform Spherical	Uniform Spherical	15.56	17.25	65.47	65.22	3.65	3.98	79.16	77.10
7	1900	Uniform Spherical	Uniform Spherical	15.35	16.86	59.88	59.45	5.75	6.05	62.25	59.40
8	2000	Uniform Spherical	Uniform Spherical	14.92	15.48	57.36	58.11	6.70	7.11	56.20	54.80
9	2500	Uniform Spherical	Uniform Spherical	13.56	13.98	54.80	55.46	8.09	10.95	45.55	43.20
10	3000	Uniform Spherical	Uniform Spherical	12.67	13.32	50..25	50.50	11.02	13.45	43.26	42.50

3.3.7 Effect of Stirring Time on the Formation of Microsponge

TABLE 13
EFFECT OF TIME OF STIRRING ON MICROSPONGES.

S No	Drug / Polymer	Time of Stirring in Hrs (at 1500 rpm)	Physical Appearance		Particle Size		Drug Content (%)		Free Drug Content (%)		% Entrapment	
			RS 100	ES 100i	RS 100	ES 100	RS 100	ES 100	RS 100	ES 100	RS 100	ES 100
1	2:1	1	Suspension filtered as such	Suspension filtered as such	-	-	-	-	-	-	-	-
2	2:1	2	Suspension filtered as such	Suspension filtered as such	-	-	-	-	-	-	-	-
3	2:1	4	irregular shape	irregular shape	45.25	48.80	58.50	55.12	8.10	9.55	65.49	62.50
4	2:1	6	spherical	spherical	32.68	35.00	65.47	65.22	2.95	2.78	75.16	71.10
5	2:1	8	spherical rigid	spherical rigid	16.99	20.0	65.75	65.00	5.10	5.68	83.02	80.47
6	**2:1**	**10**	**spherical rigid**	**spherical rigid**	**15.30**	**55.86**	**66.47**	**64.22**	**4.85**	**5.26**	**83.85**	**81.80**

3.3.8 Effect of Drug / Polymer Ratio on Physical Properties of Microsponges

TABLE 14
EFFECT OF DRUG / POLYMER RATIO ON PHYSICAL PROPERTIES OF THE MICROSPONGES

SR No	Drug: Polymer	Production Yield (%)		Mean Particle Size in µm		Drug Content (%)		Free Drug Content (%)		% Entrapment	
		RS100	ES 100	RS 100	ES 100	RS 100	ES 100	RS 100	ES100	RS 100	ES 100
1	1:1	49.52	47.80	32.00	33.42	47.61	45.76	13.68	15.80	47.60	45.94
2	1.5:1	67.92	67.54	31.53	32.65	51.53	47.07	7.60	9.85	61.83	56.17
3	2:1	78.00	77.37	16.99	20.0	65.75	65.00	5.10	5.68	83.02	80.47
4	2.5:1	84.48	86.56	16.24	17.36	66.53	64.23	5.25	5.69	85.48	83.39
5	**3:1**	**89.30**	**88.32**	**15.37**	**16.20**	**67.46**	**65.84**	**4.43**	**5.05**	**86.34**	**83.91**

3.4 In-Vitro Drug Release Studies[18,26]

3.4.1 IN-VITRO drug release studies of Acyclovir loaded microsponges

In-vitro release studies were carried out in USP basket apparatus with stirring rate 50 rpm at 37±0.5^{o}C. Initial drug release was carried out in 900 ml of 0.1 N hydrochloric acid for 2 hours followed by phosphate buffer pH 6.8 for next 2 hours followed by phosphate buffer PH 7.4 for next 8 hours. Samples were withdrawn at regular intervals and analyzed spectrophotometrically, at 301.8, 330.8, 330 nm respectively.

3.4.2 IN-VITRO drug release data for Eudragit RS-100 and ES-100 based colon specific formulations

TABLE 15
DISSOLUTION READINGS OF THE FORMULATED BATCHES.

SR no	Medium	Time (Hr)	% Cumulative Drug Release									
			MMS1	MMS2	MMS3	MMS4	MMS5	MMS6	MMS7	MMS8	MMS9	MMS10
			Eud RS 100	Eud ES 100	Eud RS 100	Eud ES 100	Eud RS 100	Eud ES 100	Eud RS 100	Eud ES 100	Eud RS 100	Eud ES 100
			1:1	1:1	1.5:1	1.5:1	2:1	2:1	2.5:1	2.5:1	3:1	3:1
1	HCL PH 1.2	1	0.02	0.034	0.0126	0.06	0.07	0.01	0.02	0.07	0.02	0.02
2		2	0.03	0.05	0.26	0.06	0.08	0.02	0.03	0.08	0.03	0.03
3	PBS PH 6.8	3	0.12	0.17	0.11	0.17	0.19	0.10	0.12	0.015	0.12	0.12
4		4	0.12	0.17	0.13	0.17	0.20	0.12	0.13	0.10	0.12	0.12
5	PBS PH 7.4	5	15.14	15.09	17.44	17.80	19.60	21.48	24.83	21.76	20.10	19.22
6		6	20.90	18.07	21.25	22.30	26.33	23.63	24.52	27.82	24.30	23.48
7		7	20.49	22.40	28.09	25.88	36.76	33.95	33.14	35.90	32.99	33.60
8		8	36.19	34.17	41.44	39.38	45.69	39.23	48.92	39.55	43.45	38.66
9		9	52.79	50.33	59.60	48.35	69.88	51.46	61.88	56.49	62.52	43.40
10		10	65.46	52.42	70.28	57.90	67.81	66.15	70.20	60.44	68.88	56.10
11		11	76.06	64.98	79.30	68.95	83.81	75.55	78.72	67.46	75.15	64.98
12		12	79.90	73.43	81.88	79.10	92.12	87.82	83.26	75.35	79.40	71.99

3.5 Results

Total 10 formulation were formulated MMS 1- MMS 10. The formulation **MMS 5** shows highest drug release, 92.12%.

3.6 STABILITY STUDIES [25,30]

Stability studies of the developed formulations were carried out according to ICH and WHO guidelines. The formulations MMS 1- MMS 10 sealed in aluminum foils were kept in the stability chamber maintained at 40^0 C ± 2^0C and 75% ± 5% RH for 3 months. The samples were analyzed for the drug content for 2 month period (ie- 60 days study).

Stability data is presented in Table.

3.7 Stability studies of different formulations. Mean ± S.D. Dissolution study of all the batches

TABLE 16
STABILTY STUDY DATA OF THE ALL BATCHES FOR TWO MONTH.

SR NO.	FORMULATION CODE	DRUG CONTENT (%)	
		AFTER 30 DAYS	AFTER 60 DAYS
		ACYCLOVIR	ACYCLOVIR
1	MMS 1	79.71	79.54
2	MMS 2	73.35	73.20
3	**MMS 3**	**81.68**	**81.56**
4	MMS 4	79.00	78.92
5	MMS 5	92.08	92.00
6	MMS 6	87.74	87.65
7	MMS 7	83.20	83.05
8	MMS 8	75.15	75.00
9	MMS 9	79.30	79.19
10	MMS 10	71.90	71.76

IV. CONCLUSION

1) Microsponge containing Acyclovir was prepared by Quasi-emulsion diffusion method using Eudragit RS 100 and ES100 polymers.

2) All the microsponge formulations were subjected for the drug content estimation and loading efficiency. The drug content was uniform and reproducible in all the formulations.

3) The **IR spectral analysis** suggested that there were no interaction between the drug and formulation additives.

4) **Internal Phase Volume:** As there is increase in the internal phase volume, there is decrease in the Particle Size, Drug Content and Entrapment Efficiency and there is Increase in the free drug content.

5) **Polymer Concentration:** As Polymer concentration increases, the drug release decreases.

6) **Surfactant Concentration:** As surfactant concentration increases, there is increase in the particle size and, decrease in the encapsulation efficiency and the production yield and larger microsponges.

7) **External Phase Volume:** As there is increase in the external phase volume, there is decrease in drug content, drug entrapment and increase in the free drug content and particle size.

8) **Rate of stirring:** As stirring speed increases there is increase in the free drug content and there is decrease in the drug content, entrapment efficiency and particle size.

9) **Time of stirring:** As stirring time increases there is decrease in the free drug content, particle size and there is increase in the entrapment efficiency and drug content.

10) **Drug: Polymer Ratio:** As there is increase in the drug: polymer ratio, there is increase in the encapsulation efficiency and the production yield, decrease in the particle size.

11) The dissolution was carried out of all the batches.

REFERENCES

[1] Raymond C R, Sheskey P J and Marian E Q., Editors. Handbook of Pharmaceutical Excipients, 6th edition 2006 Pharmaceutical Press and the American Pharmacists Association.

[2] Vyas SP, Khar RK, Targeted and Controlled Drug Delivery-Novel Carrier System: New Delhi: CBS Publication, 2002; 453-95.

[3] Kawashima Y, Niwa T, Handa T, Takeuchi H, Iwamoto T, Itoh K., Preparation of controlled-release microspheres of ibuprofen with acrylic polymer by a novel quasi- emulsion solvent diffusion method. J Pharm Sci., 1989; 78: 68-72.

[4] Perumal D., Microencapsulation of ibuprofen and Eudragit RS 100 by the emulsion solvent diffusion technique. Int. J. Pharm., 2001; 218: 1-11.

[5] Mandal TK, Bostanian LA, Graves RA, Chapman SR, Idodo TU., Porous biodegradable microparticles for delivery of Pentamidine. European Journal of Pharmaceutics and Biopharmaceutics, 2001; 52: 91-96.

[6] Tansel C. Nursin G, Tamer B., The effects of pressure and direct compression on tabletting of microsponges. Int. J. Pharm., 2002; 242: 191–95.

[7] Beruto DT, Botter R, Fini M., The effect of water in inorganic microsponges of calcium phosphates on porosity and permeability of composites made with polymethylmethacrylate. Biomaterials., 2002; 23: 2509-17.

[8] Tansel Comoglu, Nursin Gonul, Tamer Baykara., Preparation and in vitro evaluation of modified release ketoprofen microsponges. II Farmaco, 2003; 58: 101-106.

[9] Kilicarslan M, Baykara T., The effect of drug/polymer ratio on the properties of the Verapamil Hydrochloride loaded microspheres. Int. J. Pharm., 2003; 252: 99-109.

[10] United state Pharmacopoeia 32 and National Formulary, 2009; 27(III): 2894.

[11] Orlu M, Cevher E and Araman A., Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. Int. J. Pharm., 2006; 318: 103-17.

[12] British Pharmacopoeia, 2009; II: 1311-1313.

[13] Chadavar V and Shaji J., Microsponge Delivery System. Current Drug Delivery., 2007; 4: 123-29.

[14] Patel G, Patel JK. Use of a Microsponge in Drug Delivery Systems. Available from. Kilicarslan M, Baykara T. The effect of the drug/polymer ratio on the properties of Verapamil HCl loaded microspheres. Int J Pharm, 2003; 252: 99-109.

[15] American journal of pharmatech research. Microsponge Drug Delivery System: A novel dosage form Rahul Shivali Patil 1, Vishnu uddhav kemar 2, s.s. patil 1. Ashokraomane college of pharmacy, peth-vadgaon. Ichalkaranji, Kolhapur, MS.2 Appasaheb Birnale college of pharmacy, south shivaji nagar, sangali, MS.

[16] Microsponges for colon targeted drug delivery system: an overview rajendra jangde*issn- 2231–5705 (print) issn- 2231–5713 (online) university institute of pharmacy, pt. ravishankar shukla university, raipur.

[17] USP dissolution medium database; updated feb 2016.

[18] Martin A, Swarbrick J, Cammarrata A. (1991) Chapter 19, In: Physical Pharmacy- Physical Chemical Principles in Pharmaceutical Sciences. 3rd Ed., pp 527.

[19] Acyclovir [Internet] 2009 (UPDATED 2011 Jan 27) Available from: http://en.wikipedia.org/wiki/Acyclovir.

[20] http://www.drugs.com/drug-interactions/Acyclovir.html.

[21] Raymond C R, Sheskey P J and Marian E Q., Editors. Handbook of Pharmaceutical Excipients, 6th edition 2006 Pharmaceutical Press and the American Pharmacists Association