Design, Development and Characterization of Valsartan Microsponges by Quasi Emulsion Technique and the Impact of Stirring Rate on Microsponge Formation

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ABSTRACT

Microsponges are highly cross linked, patented, porous polymeric microparticles and each particle consists of a myriad of interconnected voids within a non-collapsible structure. Moreover, they may enhance stability, reduce side effects and modify drug release favourably. They are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability reduce side effects and modify drug release. The rationale behind present work was to formulate valsartan entrapped micro porous micro particles (microsponges) to control the release of the drug. Valsartan microsponges was prepared using quasi emulsion solvent diffusion method. In order to optimize the micro sponge formulation, factors affecting the physical properties of micro sponges were determined. Compatibility of the drug with excipients was studied by FT-IR, Production yield, loading efficiency and surface morphology of microsponges were performed. It was shown that the drug: polymer ratio and stirring rate influenced the particle size and drug release behaviour of micro sponges. The results showed that, generally an increase in the ratio of the drug: polymer and stirring rate resulted the cumulative percentage drug release up to 8 hrs for M7, M8, M9 were 72%, 89% and 94% respectively and entrapment efficiency values of 33.8 - 84.5 respectively.

INTRODUCTION

Microsponges are polymeric delivery system. They are highly cross linked, porous polymeric microparticles which contain countless interconnected voids within a non-collapsible structure (D'souza *et al*., 2014) They are usually between 20 and 200 microns in diameter that acquire the flexibility to entrap a wide variety of active ingredients such as emollients, fragrances, sunscreens, essential oils, anti-infective, antifungal and anti-inflammatory agents etc., that are mostly used for prolonged topical administration (Jain *et al*., 2011). Recently their use is also being investigated for oral drug delivery. In oral applications, the microsponge system has been shown to increase the rate of solubilisation of poorly water soluble drugs by entrapping such drugs in the microsponge system's pores. As these pores are very small, the drug is in effect reduced to microscopic particles and the significant increase in the surface area thus greatly increases the rate of solubilisation (Karthika *et al*., 2013). Polymers are important in the field of drug delivery for their use as binders, viscosity, flow controlling, film coatings, to modify drug release characteristics. Controlled drug delivery systems include the maintenance of drug levels, administrations, increased patient compliance (Parikh *et al*., 2010).

The development of valsartan microsponge formulation of a drug is to enhance its therapeutic benefits and minimize its side effects, while improving the management of the disease condition. Valsartan is anti hypertensive drug belongs to BCS class II which is poorly soluble in water. In our present work Valsartan microsponges were prepared by quasi emulsion solvent diffusion technique with different stirring rates.

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MATERIALS AND METHODS

Materials

Valsartan as an endowment sample from Lobachemie Pvt. Ltd., Mumbai, Dichloromethane was procured from Merck specialities Pvt. Ltd., Mumbai, Ethyl cellulose and Polyvinyl alcohol also procured from Merck specialities Pvt. Ltd., Mumbai and Distilled water. All the reagent and materials were of analytical or pharmacopoeial grade.

Methods for preparation of Valsartan microsponges

The microsponges enclosing valsartan were fabricated by quasi-emulsion solvent diffusion method which is seemed to be promising for the preparation of valsartan microsponges as it is easy, reproducible, rapid and has the advantage of avoiding solvent toxicity. In the present study, dichloromethane (DCM), which is capable of dissolving both the drug and the polymer, was selected as the internal solvent and inner phase comprises of Ethyl cellulose (as mentioned in table 1) dissolved in 20 ml of dichloromethane. Further valsartan was put in and dissolved through ultrasonication at 35 °C for 15 min. Followed by drop-wise addition of this mixture into aqueous solution of PVA (outer phase) with different stirring rates 1000, 1500, 2000 rpm for 120 min. Subsequently, microsponges were formed due to dichloromethane removal from system by evaporation (Singh and vikas., 2010) Prepared microsponges were then filtered, washed with distilled water and subjected to drying at 40 °C for 24 h in hot air oven. Lastly microsponges obtained were weighed to determine production yield. Various formulation batches were prepared as per Table 1.

Table 1: Formulation of valsartan microsponges prepared by Quasi Emulsion Solvent diffusion technique.

| Formulation	Drug: Polymer ratio	Drug(mg)	EC(mg)	DCM(ml)	PVA(mg)	Distilled Water(ml)	Stirring rate (RPM)
F1	1:4	100	400	20	400	150	1000
F2	1:4	100	400	20	400	150	1500
F3	1:4	100	400	20	400	150	2000
F4	1:5	100	500	20	500	150	1000
F5	1:5	100	500	20	500	150	1500
F6	1:5	100	500	20	500	150	2000
F7	1:6	100	600	20	600	150	1000
F8	1:6	100	600	20	600	150	1500
F9	1:6	100	600	20	600	150	2000

* DMC- Dichloromethane.
* EC- Ethyl cellulose.
* PVA- Polyvinyl alcohol.

CHARACTERIZATION OF PREPARED MICROSPONGES

Fourier transform infrared analysis

The Physicochemical interaction between valsartan and ethyl cellulose polymer and polyvinyl alcohol used in the research were carried out by subjecting to IR spectral studies using Fourier Transform infrared Spectroscopy (Chadawar and Shaji, 2007). The

samples were prepared by mixing the 100 mg of the drug with 100 mg of polymer used in the preparation of the microsponges. These samples were scanned under diffuse reflectance mold and plotted the graph by KBr pellet method (Amrutiya *et al.*, 2007). The spectra obtained for pure drug was compared with that of the physical mixtures of the drug with polymer and shown in figure 1, 2 and 3.
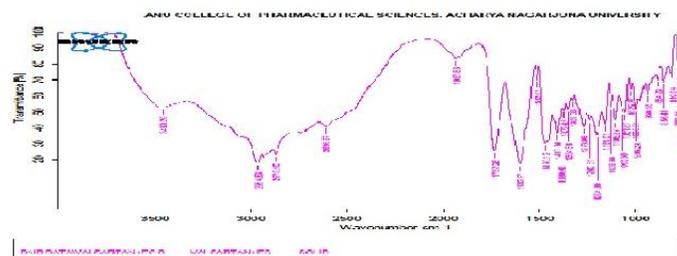
Fig. 1: IR spectra of pure valsartan.
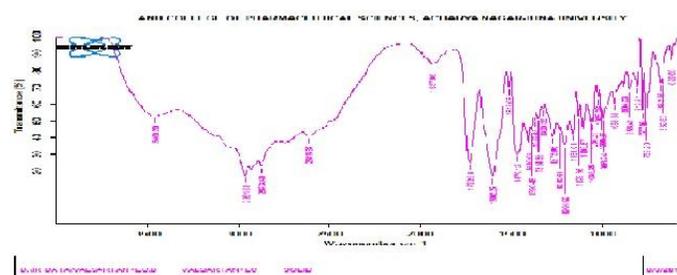
Fig. 2: IR spectra of pure valsartan and Ethylcellulose.
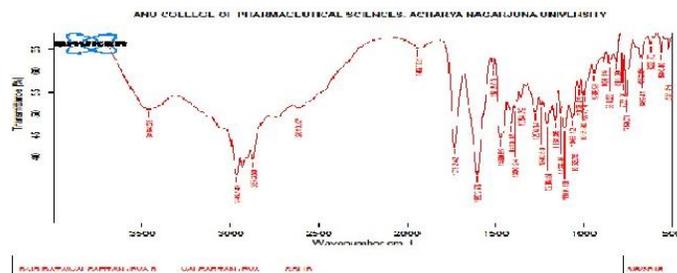
Fig. 2: IR spectra of pure valsartan and polyvinylalcohol.

Particle Size Analysis

Particle size and size distribution of microsponge particles was determined using optical microscope (Subhash *et al.*, 2009). A minute quantity of microsponges was spread on a clean glass slide and average size of 100 microsponges was determined in each batch. The values were given in Table 2 for the formulations in the form of mean particle size.

Determination of the production yield:

The Microsponge production yield of valsartan was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponges (Mahajan *et al.*, 2011). Obtained values were given in Table 2.

$$\text{Production yield} = \frac{\text{Production mass of microsponge} \times 100}{\text{Theoretical mass (Drug + Polymer)}}$$

Table 2: Production yield, actual drug content, Entrapment efficiency, and mean particle size of various microsponge formulations.

| S .No	Formulation code	Production yield±*SD	% Drug content±*SD	% Drug release±*SD	Particle size±*SD	Entrapment efficiency±*SD
1	F1	51.3±0.05	33.7±0.65	12.70±1.05	83.2±0.08	33.8±0.85
2	F2	58.6±0.08	42.6±0.93	23.66±0.11	79.3±0.93	42.7±0.38
3	F3	61.3±0.38	51.4±0.92	28.50±0.93	65.2±0.75	51.5±0.92
4	F4	68.3±0.75	45.3±0.49	29.40±0.66	61.6±0.92	45.4±0.38
5	F5	67.2±1.05	53.12±0.32	34.42±1.36	54.8±1.35	53.2±0.44
6	F6	62.8±0.69	70.1±0.83	44.46±0.85	45.3±0.56	70.2±0.76
7	F7	79.2±0.75	68.9±0.23	71.35±0.98	48.5±0.68	69.1±0.82
8	F8	80.5±1.05	71.4±0.91	88.02±1.25	39.2±0.85	71.6±0.42
9	F9	83.2±0.82	84.4±1.05	93.40±0.08	29.6±0.93	84.5±0.19

mean±SD, n = 3.
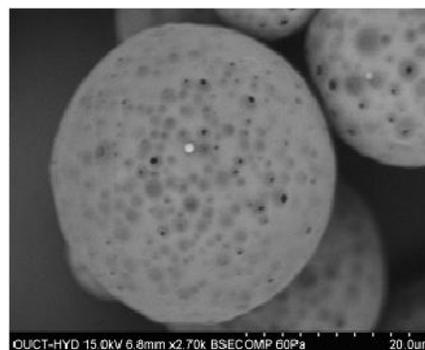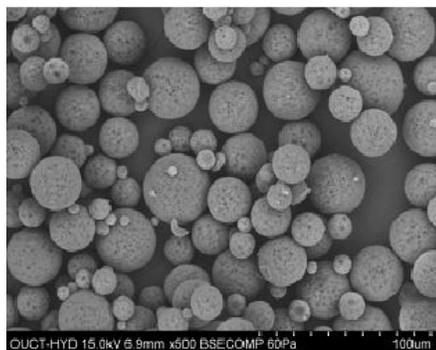
Fig. 4 & 5: SEM images of Drug loaded microponges at 1000 rpm.
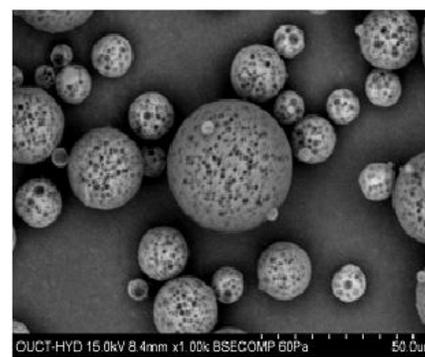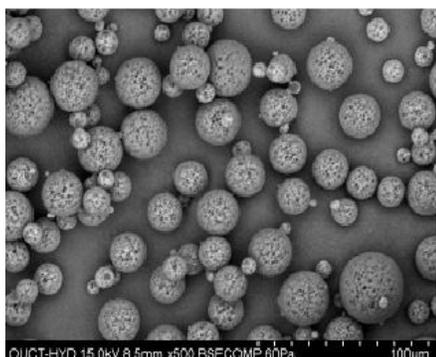

Fig. 6 & 7: SEM images of Drug loaded microponges at 2000 rpm.

Determination of loading efficiency (Percentage entrapment efficiency analysis)

Precisely weighed quantity (100 mg) of microsponges containing drug was kept in 100 ml phosphate buffer solution (pH 6.8) for 12 h with continuous stirring 21. Filtered samples (using 0.45 lm membrane filter) were further analyzed at 248 nm next to blank using UV spectrophotometer (Pharmaspec 1700, Shimadzu, Japan). Estimation of entrapment efficiency for all batches were done (Malamataris and Avgerinos, 1990) using following expressions and values were given in Table 2.

Encapsulation Efficiency= $(M_{act}/M_{the})_x$ 100

Scanning Electron Microscopy analysis

The morphology and surface characteristics of the microsponges were studied using scanning electron microscopy

(SEM) (Anderson *et al*., 1994). All the samples were coated with gold palladium alloy under vaccum. Coated samples were then examined using LEO 430 SEM analyzer. The dried samples were placed on NEM TAPE adhesive paper and photographed and shown in figures 4-7.

***In Vitro* Drug release Studies**

The microsponges, equivalent to a 100 mg drug were subjected to *In-vitro* release studies (Devrim and Canefe, 2006) The in vitro release of valsartan from the microsponges was studied using USP Paddle Type II apparatus with a stirring rate of 50 rpm.

The temperature was maintained at 37 ± 0.5°C. Drug release was carried out in 900ml of phosphate buffer (pH 6.8). An aliquot of 5ml sample was collected over a period of 8 h and was assayed spectrophotometrically for the drug at λ max 248 nm.

Each time sample was withdrawn, a fresh buffer was added to keep the dissolution medium volume constant. The Percent drug release values were given in Table 3. A kinetic treatment of the data was performed to determine the mechanism of the release of the drug was given in Table 4.The cumulative release profiles of the formulations were represented in figures 8-10

RESULTS AND DISCUSSION

Physical appearance

Microsponge particles which are fairly white were obtained by quasi-emulsion solvent diffusion method; with good flow properties than as compared with pure drug.

FTIR studies

FTIR of drug and excipients revealed that there was no interaction between the selected drug and polymers.

Particle size

Visual inspection results of all batches done using optical microscope for particle size discovered decreased particle size with increase in drug:polymer ratio. Optimized batch has been found with more percent of intact, uniform, spherical particles during optical microscopy. In quasi emulsion solvent diffusion method affinity between good solvent and drug is stronger than the affinity between good solvent and poor solvent. Drug solution in the good solvent formed emulsion droplets (quasi) upon pouring into the poor solvent and the organic phase then diffused out in to the external phase resulting in the formation of pores in the micron-sized particles, known as microsponges that have taken a spherical shape due to constant stirring (Pande *et al.*, 2015). The particle size of valsartan microsponge formulation were given in Table no 2.The formulations with ethyl cellulose F1-F9 shows the average particle sizes of 83.2,79.3, 65.2, 61.6, 54.8, 45.3, 48.5, 39.2 and 29.6 respectively. It was found that particle size is decreased upon increasing the RPM.

Table 3: *In-vitro* Drug release Profile.

S.No.	Formulation code	% Drug release							
		1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr
1	F1	0.3585	0.7171	1.6135	2.8685	5.0199	5.5577	9.6812	12.7290
2	F2	0.8964	1.4342	1.9721	3.7649	7.7091	7.8884	15.418	23.665
3	F3	1.7928	2.1513	3.4065	5.3784	9.8605	10.577	23.665	28.505
4	F4	2.3306	2.6892	3.944	6.8127	10.577	11.294	12.72	29.402
5	F5	5.199	6.274	8.0677	8.247	10.936	12.729	15.776	34.422
6	F6	7.709	9.681	10.936	12.191	12.37	15.418	23.84	44.46
7	F7	14.34	18.10	27.25	32.98	36.93	39.08	54.60	71.35
8	F8	16.314	27.60	34.42	38.007	54.14	58.26	73.68	88.02
9	F9	29.4	34.42	44.46	71.35	75.29	81.39	86.77	93.40

Table 4 : Kinetic data from in vitro Drug release models for the microsponge formulations.

| S.No	Formulation code	Zero order correlation coefficient R^2	First order correlation coefficient R^2	Higuchi correlation coefficient R^2	Peppas plot Diffusional exponent n
1	F1	0.783	0.820	0.586	0.977
2	F2	0.751	0.831	0.511	0.909
3	F3	0.833	0.849	0.545	0.879
4	F4	0.694	0.703	0.546	0.871
5	F5	0.741	0.882	0.576	0.751
6	F6	0.789	0.869	0.586	0.683
7	F7	0.845	0.853	0.842	0.934
8	F8	0.887	0.923	0.834	0.971
9	F9	0.880	0.958	0.724	0.939
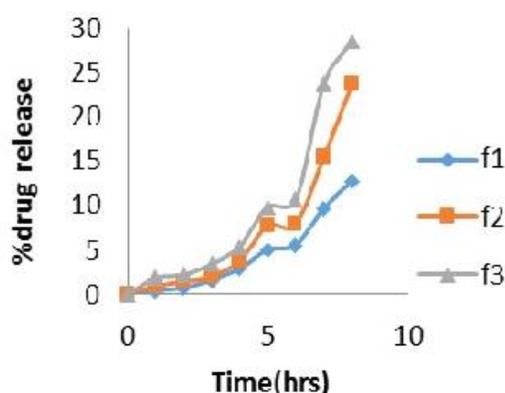
Fig. 8: In vitro Drug release profile of microsponges at 1000 RPM.
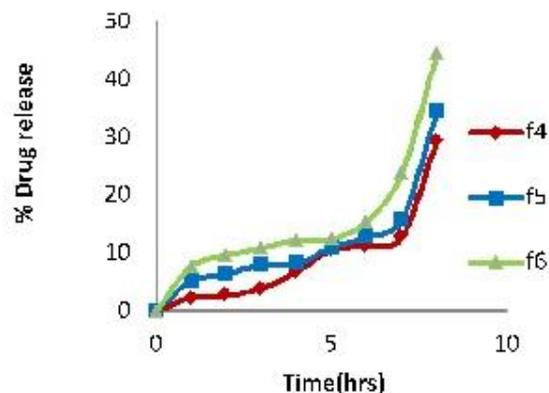
Fig. 9: In vitro Drug release profile of microsponges at 1500 RPM.

Scanning Electron Microscopy Analysis

Scanning electron microscopy confirms the outer surface of M1 and M9 formulations was smooth and dense, while theshape of microsponges prepared by quasi-emulsion solvent diffusion method were spherical, and uniform with no drug crystals on surface. The higher the rate of stirring, the higher the early solvent drag for diffusion and accordingly the agglomeration of microsponge. It was hypothesized that with the increase in speed, the precipitation of polymer solution droplets gradually became slower, allowing more time for formation of agglomeration. Hence for batch F9 desired porous micro sponges with maximum entrapment of drug was observed.

Drug content

The drug content of valsartan microsponge formulation are given in Table 2. It was found the drug content increased with increase in the drug: polymer ratio as well as increase in rotation per minute. The drug content in formulations with ethyl cellulose from F1-F9 ranges between 33.7-84.4

Production yield

The production yield of valsartan microsponge formulation are given in Table 2.It was found that production yield increased with increase in the drug : polymer ratio. The formulations F1-F9 shows the production yield values of 51.3, 58.6, 61.3, 67.2, 62.8, 79.2, 80.5 and 83.2 respectively

Entrapment efficiency

The entrapment efficiency of valsartan microsponge formulation are given in Table 2.The Entrapement efficiency in formulations with ethyl cellulose from F1-F9 ranges between 33.8-84.5

Effect of drug to polymer ratio

Production yield, drug content and entrapment efficiency was found to be increase with increase in drugpolymer ratio while drug:polymer ratio has reverse effect on particle size, as drug:polymer ratio increase, particle size decreases.

Effect of stirring rate on the morphology and yield of microsponges

The effect of stirring rate on the morphology of microsponges is shown in Fig 4-7. The formulation with higher drug to polymer ratio (i.e., 1:6) was chosen to investigate the effect of stirring rate on the morphology of microsponges. The stirring rate was varied in the range of 1000 to 2000 rpm. The dispersion of the drug and polymer into the aqueous phase was found to be dependent on the agitation speed. As the speed was increased the size of microsponges was reduced and the microsponges were found to be spherical and uniform. A decrease in particle size was observed with increases in the drug: polymer ratio, which could be correlated with the kinetics of microsponge formation in the presence of comparatively lower concentrations of the polymer (Niray Patel *et al*., 2016). When the rate of stirring was increased

up to 2000 rpm the spherical microsponges were formed with mean particle size of 29.6 ± 0.86 μm. It was also noted that at higher stirring rate, the production yield was increased.

Dissolution profile

The dissolution rates are inversely related to particle size as would be expected from surface area relationships. The drug polymer ratio showed significant effect on the encapsulation efficiency of microsponges. The increased concentration of polymer showed the increased drug encapsulation efficiency. It was found that after 8hrs of dissolution study the formulations F1, F2, F3, F4, F5, F6, F7, F8 and F9 (figure 10) were showing 12.70, 23.66, 28.50, 29.40, 34.42, 44.46, 71.35, 88.02 and 93.40% of drug release in 8 hrs. The study indicated that the amount of drug release is increased. Formulation F9 shows better result with 93.40 (% drug release) than other formulations.
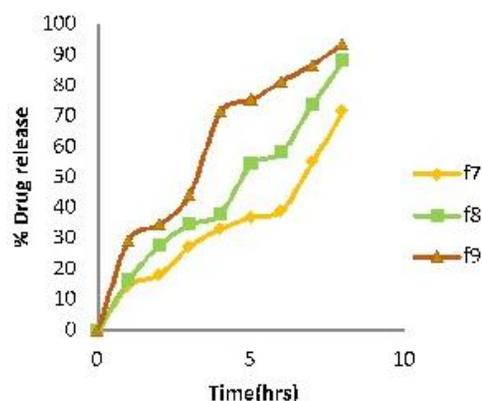

Fig. 10: *In-vitro* Drug release profile of microsponges at 2000 RPM

Drug release kinetics

The mechanism of drug release from the formulations during the dissolution was determined using the zero order, first order, higuchi equation and Peppas equation. All formulations were best fitted to Zero order and peppas plot. The best formulation follows Zero order and release mechanism is non fickian diffusion

CONCLUSION

Controlled drug delivery via the polymer based systems has been proposed to be prevailing both in present and in future; as having numerous potential advantages for scientific as well as economic reasons. The thought behind developing polymeric microsponge delivery system was to deliver active ingredient in a continual manner for extensive time period to reduce application frequency, hypersensitive reactions and to improve bioavailability, safety than marketed conventional formulation and precisely control the release rates or target drugs to a specific body site have a vast impact on the health care system. The method implemented was quasi-emulsion solvent diffusion; found to be simple, reproducible and rapid and was evaluated for its different parameters which revealed many interesting results for efficient preparation of the microsponges. The formulation F9 have better

results than other formulations. FT-IR spectroscopy analyses indicated the chemically stable, amorphous nature of the drug in these microsponges. SEM photographs revealed the spherical nature of the micro sponges in all variations. Varied drug–polymer ratio and stirring rate reflected remarkable effect on particle size, drug content and encapsulation efficiency. It was observed that as the drug to polymer ratio was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratios, the amount of polymer available per microsponge was comparatively less. Hence fewer polymers surrounded the drug resulting in smaller microsponges. The effect of stirring rate on the morphology of microsponges was also investigated. The dispersion of the drug and polymer into the aqueous phase was found to be dependent on the agitation speed. As the speed was increased, the size of microsponges was reduced and uniform spherical microsponges were formed .It was also noted that at higher stirring rate, the production yield was decreased. Possibly at the higher stirring rates the polymer adhered to paddle due to the turbulence created within the external phase, and hence production yield increased. A microsponge delivery system can release its active ingredient on a timer mode. Therefore, micro sponge has got a lot of potential and is a very emerging field which is needed to be explored. Microsponges constitute a significant part by virtue of their small size and efficient carrier characteristics.

ACKNOWLEDGEMENTS

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