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Invitro Characterization of Metformin Okra Alginate Microspheres: CRDDS

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Abstract— *Metformin is an oral hypoglycemic agent which reduce the basal and post prandial plasma glucose without effecting insulin secretions, but it may cause freezing of arms & legs, muscles pain, stiffness &weakness due to its adverse effects it is recommended as a modified release dosage form. In this work ionically gelled modified alginate beads are prepared by using SA and plant derived natural polysaccharide (OKRA/Abelmoschus officinalis) blends with different concentration by using calcium chloride ($CaCl_2$) as a cross -linker in an aqueous environment.*

In present work we prepared microspheres with different concentrations of OKRA mucilage and SA (sodium Alginate) was dropped into the 10 % Calcium chloride solution and prepared alginate spheres were evaluated for micrometric properties i.e. Bulk density, C.I, angle of repose and characterized by SEM, swelling Index, %yield, Drug entrapment.

Keywords— *SA, OKRA, $CaCl_2$, SEM, C.I, Cross Linking Agent*.

I. INTRODUCTION

Microspheres are small spherical particles, with diameters 1μm to 1000μm. They are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall. And micro matrices in which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microspheres play an important role to improve bioavailability of conventional drugs and minimizing side effects. [1,2]

Alginate beads are approved as safe CRDDS by the FDA. Sodium alginate is the sodium salts of alginic acids, which is a co-polymer of β-d mannuronic acid (M) and α-1-guluronic acid (G) having 1,4 glycoside linkage between them. It has been used in various pharmaceutical applications, it produce cured gel matrices in the present of multivalent cations like Ca^{+2} , Cd^{+2}, due to intermolecular ionic – gelation cross linking between COO- groups located on the sodium alginate (SA) to prepare alginate gel for the delivery of various drugs. Alginate beads can be prepared easily through simple procedure but low drugs encapsulation &poor mechanical property in the intestinal P^H are the draw backs of alginate beads which leads to rapid drug release. To minimize these limitations in this work ionically gelled modified alginate beads are prepared by using SA and plant derived natural polysaccharide blends with different concentration by using calcium chloride ($CaCl_2$) as a cross -linker in an aqueous environment.[3.5]

Okra (Hibiscus esculent, family – Malvaceae) is an annual plant cultivated throughout the tropical and subtropical areas of the world. The immature okra fruit is also used in folk medicine composed with d-galactose, 1-rhamnose and 1-galacturonic acid. It is chemically inert, nonirritant, biodegradable and biocompatible, water soluble and produce highly viscous solution with a slimy appearance. Okra gum is ready investigated as usefulness of its drug release controlling polymer. Excipients in the development of various pharmaceutical formulations. The highly viscous property of okra gum leads the usefulness of it as a drug release. [4]

II. MATERIALS

Okra is collected from local market, metformin was purchased from BMR chemicals & remaining all reagents and chemicals collected from laboratory.

III. RAW MATERIAL ANALYSIS OF METFORMIN

Description:

White or almost white crystalline powder.

Solubility test: by Shake flask method [3]

Solubility studies done by using shake flask method; An excess amount of Metformin was transferred to a 250 ml of conical flask containing 100ml of dissolution media. The solubility study was performed at a temperature of 25^0C , flask was shaken for 24 hrs by using rotary shaker at 200 RPM. A portion of drug solution dissolved in buffer solution was filtered and absorbance was measured at 233 nm using UV-visible double beam spectrophotometer the test was prepared in triplicate in the selected buffer (pH 1.2, 4.4, 6.8 and 7.4 buffer solutions) results were shown in table 2.

Melting point determination: [8]

Melting point of Metformin was determined by capillary method. Melting point of a drug sample is a first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by lowering as well as widening in the melting point range. And results were shown in table 3.

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Percentage purity[8]

Weigh 60 mg of Metformin dissolve in 4ml of anhydrous formic acid, add 50 ml of acetic anhydrate. Titrate with 0.1 M Perchloric acid, determine the end point potentiometrically carry out blank titration and results were shown in table 3.

Standard calibration curve of Metformin: [7]

Stock solution-I:

A weighed amount of metformin (500mg) was taken in a 100 ml volumetric flask and dissolved in 100 ml of Hcl (4.25 gm dissolved in 500 ml of water) Final volume was made up to the mark with Hcl solution.

Stock solution-II:

From the I stock solution 5 ml was withdrawn and dilute to 50ml Hcl solution to get a concentration of 10 mcg/ml. From the standard stock solution samples of 1, 2, 3, 4, 5, ml were pipette out into 25 ml volumetric flasks. The volume was made up to the mark with NaOH solution to get final concentration 4,8,12,16,20 mcg/ml. the absorbance was measured at 233m results were shown in table 3 & fig. 3.

Incompatibility study (drug – excipients) (FTIR):

The physicochemical compatibility between etodolac and the excipients used in the research was tested by Infrared (IR) Spectroscopy using ABB Bomem IR spectroscopy. The Fourier-Transformed Infrared (FTIR) spectra of the sample were obtained, using an FTIR spectrophotometer. About 2mg of the samples were mixed with potassium bromide of equal weight and compressed to form a KBr disc. The samples were scanned from 500 to 4000 cm^{-1} the results were shown in the fig. 4.

Isolation of Okra gum: [4-7]

Okra (Abelmoschus esculentus) fruits were purchased from a local market. About 1 kg of fresh immature fruits of Okra was purchased from local market. After removal of the seeds, the fresh immature fruits were sliced, homogenized and extracted with cold water containing 1% w/v of sodium meta bi sulphate. The crude mucilage was centrifuged at 3000 rpm for 5 min. The gum was precipitated from the supernatant with acetone. The precipitated gum was washed several times with acetone. The obtained cream-colored product was dried under vacuum in desiccators. A light brown colured powder was obtained after complete removal of moisture.

The dried gum was pulverized using end runner mill and screened with a 0.25 mm stainless steel sieve. This was stored in a well closed amber colored specimen bottle till ready for use.

Identification tests of mucilage:

Ruthenium Red test:

Take a small amount of sample on slide add little amount of ruthenium red solution and observe under microscope. Pink color observed.

Molisch's test:

100 mg dried mucilage powder Molisch's reagent Add conc. H_2SO_4 on the side of a test tube Violet green color observed at the junction of the two layers Carbohydrate present.

Iodine test:

100mg dried mucilage powder And 1 ml 0.2 N iodine solution Side of a test tube No color observed in solution.

Fehling's test:

Take small amount of sample in test tube add Fehling's reagent to the test tube. Blue colour is observed.

Formulation of microspheres: [4-7]

The Microspheres were prepared by Ionotropic gelation technique. Accurate weight quantity of Drug and Polymer were added to 50 ml of Sodium alginate solution thoroughly mixed with a magnetic stirrer to form homogenous polymer Okra solution (1:1). The Resulting solution was sonicated for 30 Min to remove any Air Bubbles. For the formation of Microspheres, the dispersion was added dropwise from a needle of 22 G in diameter from height of about 5 cm into to 100ml of Calcium Chloride (CaCl2) solution stir at 300 rpm. The added droplet was retained in the calcium chloride solution for 30 min to complete then the solution and to produce Spherical rigid microspheres by continuous stirring.

Then the solution containing formed Microspheres was filtered by using Whatmann filter paper. The Obtained microspheres were allowed to dry at 40°C for 6 hrs and stored in well closed container

Fig. 1. Microspheres

TABLE 1. Formulation of beads.

S.No	Formulation Code	Drug (mg)	Okra gum (mg)	Na alginate (mg)	Calcium chloride (in%)
1	F1	200	50	100	10
2	F2	200	100	100	10
3	F3	200	200	100	10
4	F4	200	300	100	10
5	F5	200	400	100	10
6	F6	200	500	100	10

IV. Evaluation Of Microspheres

Micromeritic Properties of Microspheres [9]

Bulk density

Apparent bulk density (g/ ml) was determined by pouring bulk blend into a graduated cylinder via a large funnel and measuring the volume and weight, as its bulk density was calculated using the formula. Results are shown in table 5.

$$\text{Bulk density}(\rho o) = W/ V b$$

Tapped density:

Tapped density was determined by placing a graduated cylinder containing a known mass of blend on a mechanical tapper apparatus until the powder bed has reached a minimum.

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Tapped density was calculated using the formula and results are shown in table 5.

Tapped density (ρt) = W/ V t

Carr's compressibility index

Compressibility is the ability of powder to decrease in volume under pressure. Using untapped density and tapped density the percentage compressibility of granules were determined, which is given as Carr's compressibility index and results were shown in table 5.

Cl = Vi – Vo / Vi x100

Hausner's ratio

It is measurement of frictional resistance of the drug. The ideal range should be 1.0 – 1.5. It was determined by the ratio of tapped density and bulk density using the formula for determination of Hausner's ratio & results were shown in table 5.

Hausner's ratio = Vo / Vi

Angle of repose

Angle of repose was determined by using funnel method. Accurately Weighed blend was poured from funnel which was raised vertically until a maximum cone height (h) was obtained and diameter (d) was measured. The angle of repose was calculated by formula. Results were shown in table 5.

Φ = tan-1 (h/r)

V. Characterization of Microspheres

Particle Size

The size of microspheres of each formulation was determined using a microscope fitted with an ocular micrometer, and stage micrometer and average particle size was determined. Results were shown in table 6.

Scanning electron microscopy (SEM):

The shape and surface morphology of microspheres were examined using a Scanning Electron Microscope JSM-6360 (JEOL, Japan). The microspheres were previously coated with a thin layer of gold under vacuum so as to make them electrically conductive. Surface morphology of Bora rice powder, the DI mucilaginous substance and the AE mucilaginous substance, metformin hydrochloride loaded microspheres were examined by photomicrographs at an excitation voltage of 20 kV under different magnification and results were shown in fig. 5

Determination of percentage yield: [1]

The prepared microspheres were collected and weighed. The weight of microspheres was divided by the total weight of all the non- volatile components that were used for the preparation of the microspheres and multiplied by 100 gives the % yield of microspheres &results were shown in table 7.

$$\% \text{ Yield} = \frac{\text{Actual weight of the product}}{\text{Total weight of excipients and drug}} \times 100$$

Drug entrapment efficiency: [1]

Drug entrapment efficiencies of dried microspheres were approximated by mashing dried microspheres and extracting the drug into aqueous medium in phosphate buffer of pH 7.4 by dynamic shaking with a magnetic stirrer for 24 h; the drug content was then examined. Entrapment efficiency of microspheres was calculated. Results were shown in table 8.

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Swelling index: [1]

Swelling indices of different batches of microspheres were analyzed by determining the percentage of water retained by the microspheres after 12 hours. About 25 mg of microspheres were placed on an electronic balance and weighed. The microspheres were then dispersed in 20 mL of distilled water, and also phosphate buffer of pH 7.4 at a temperature of 37 ± 1 °C. After 12 hours, the microspheres were taken out from their respective media, air dried and weighed. The swelling index was determined by percent water uptake. Results were shown in table 9.

Swelling index (%) = (C/I)100 (1)

C = weight gain

I = initial weight of the microbeads.

Before drying of microspheres

After Drying of microspheres

Fig. 2. Swelling index.

VI. Invitro Dissolution Studies

Dissolution test by USP paddle apparatus. The in vitro dissolution study is carried out using apparatus II (paddle).

The dissolution jars are cleaned with a mild detergent and then rinsed with distilled water and dry to room temperature.

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900 mL of dissolution medium is transferred into the dissolution jars and are placed in the test assembly which is maintained at 37 degree Celsius which is given an allowance of 0.5 degrees Celsius. The medium is allowed to attain the set temperature. The rpm is set to 100. 10mL of the samples are withdrawn at various time intervals such as 0 minutes, 10 minutes, 20 minutes, 30 minutes, 45 minutes, and 60 minutes using a graduated pipette and transfer it immediately lean, dried and labeled test tubes. The equal volume of fresh dissolution medium is replaced after each sampling and maintained at the correct temperature. The sample withdrawn is diluted 10 by 10 times and the absorbance is measured at 233nm. 10 mL of sample is withdrawn at the end of 30 minutes from each of the test jar, using a graduated pipette and it is filtered if necessary. It is then transferred to a cleaned, dried and labeled test tube. The sample is diluted by 10 times and the absorbance is measured at 233nm. The cumulative percentage of released is calculated using the given formula Results were shown in table 10 & fig. 6.

VII. RESULTS

Raw material analysis of Metformin:

Solubility Analysis

TABLE 2. Solubility analysis.

S No.	Pure Drug	Solubility
1	Ethanol	Very slightly soluble
2	Buffer PH 1.2	185
3	Buffer PH 6.8	200
4	Buffer PH 7.4	218 mg / ml

TABLE 3. Identification test of metformin.

S. No	Test	Method
1	Melting point	223-226 °C
2	Sulphated ash	NMT 0.1 per cent
3	Assay	NLT 98%, & NMT102%

Standard calibration curve of Metformin:

TABLE 4. Standard calibration curve of Metformin.

S. No	Concentration (µg/ ml)	Absorbance
1	0	0
2	1	0.033
3	2	0.059
4	3	0.081
5	4	0.109
6	5	0.132
7	6	0.172
8	7	0.185
9	8	0.218
10	9	0.256
11	10	0.275

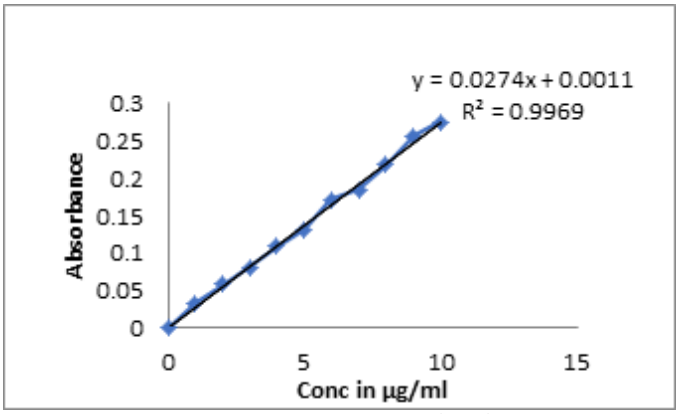

Fig. 3. Standard calibration curve of Metformin.

Drug - excipient incompatibility study (FT-IR)

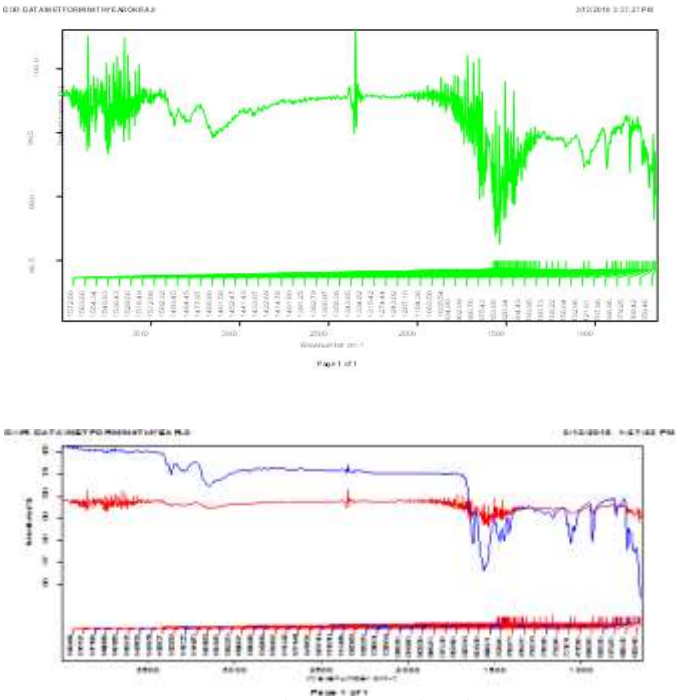

Fig. 4. FTIR Spectrum of combination of Metformin and Okra.

Micrometric Evaluation Results:

TABLE 5. Micrometric results.

Formulation code	Bulk density	Tapped Density Gm/cm3)	Corr's Compresibility	Hausner's Ratio	Angle of repose
F1	0.41 ± 0.03	0.44 ±0.02	7.31 ± 0.03	1.08	24˚58'
F2	0.48 ± 0.14	0.58±0.10	17.24 ± 0.12	1.23	35˚48'
F3	0.54 ± 0.18	0.59±0.11	8.48 ± 0.21	1.08	41˚54'
F4	0.47 ± 0.03	0.52±0.02	10.63±0.03	1.10	25˚41
F5	0.49 ± 0.01	0.54±0.01	10.20± 0.02	1.11	33˚50'
F6	0.52 ± 0.12	0.61±0.08	14.76±1.09	1.17	38˚67'

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Particle Size Evaluation Results:

TABLE 6. Particle size evaluation results.

S. No	Formulation Code	Particle Size (µm)
1	F1	79.46±0.07
2	F2	88.17±0.09
3	F3	79.74±0.08
4	F4	127.91±0.05
5	F5	94.18±0.10
6	F6	110.4±0.07

Each value is average of three separate determinations ±SD

Surface Morphology:

Scanning Electron Microscopy (SEM):

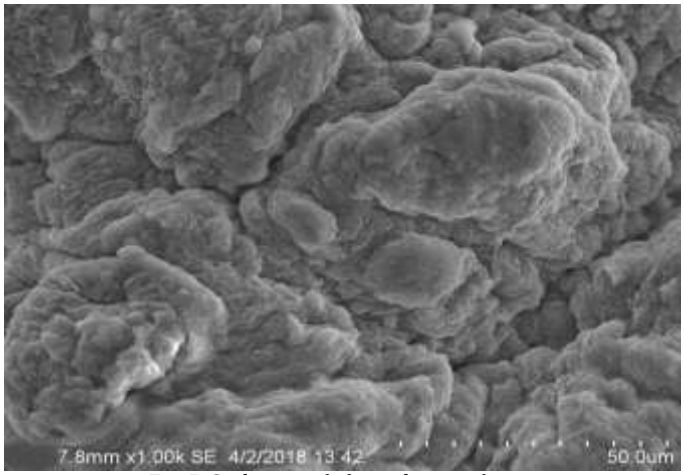

Fig. 5. Surface morphology of microspheres.

Determination of percentage yield:

TABLE 7. Percentage yield of formulated microspheres.

S. No	Formulation Code	Percentage Yield (%)
1	F1	44.45±0.15
2	F2	58.76±0.17
3	F3	80.45±0.13
4	F4	88.84±0.18
5	F5	77.64±0.11
6	F6	79.16±0.14

Each value is average of three separate determinations ±SD

Entrapment Efficiency (%):

TABLE 8. Drug loading entrapment efficiency of Metformin microspheres formulation.

S. No	Formulation Code	Entrapment Efficiency (%)
1	F1	65.14±0.09
2	F2	70.63±0.09
3	F3	72.94±0.08
4	F4	85.63±0.07
5	F5	72.45±0.08
6	F6	80.27±0.09

Swelling index:

TABLE 9. Swelling index.

S. No	Formulation Code	Swelling Index
1	F1	42.43 ± 0.21
2	F2	63.28 ± 1.82
3	F3	68.84 ± 0.82
4	F4	80.19 ± 0.63
5	F5	77.52 ± 2.04
6	F6	72.65 ± 1.04

Invitro drug release study of microspheres

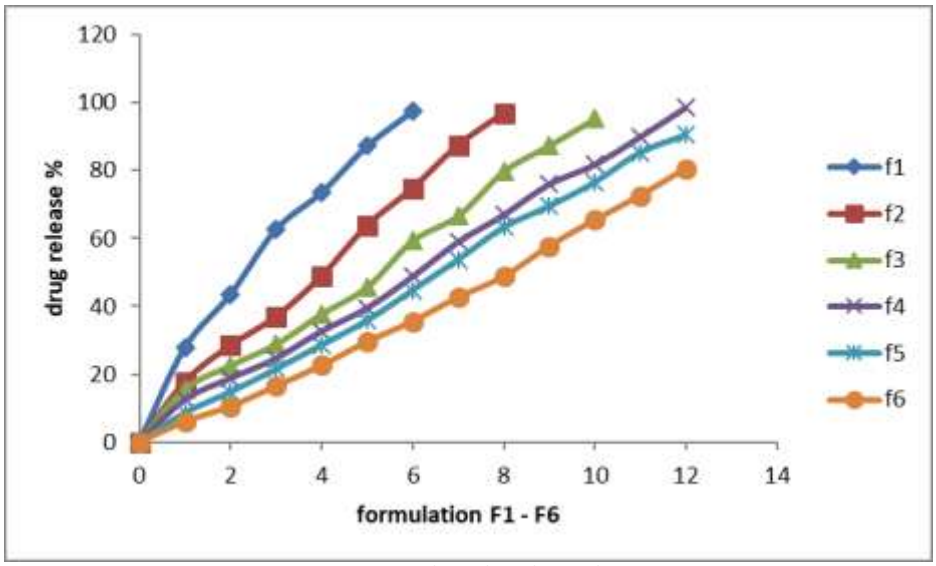

Fig. 6. Invitro drug release profiles of Metformin formulation (F1-F6).

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TABLE 10. Invitro drug release formulation (F1 to F6).

Time in hr	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	27.88 ± 0.5	17.78±0.30	15.53±0.12	12.50±0.50	8.59±0.23	6.00±0.12
2	43.54 ± 0.2	28.45±0.41	22.53±0.51	18.79±0.44	14.78±0.45	10.53±0.23
3	62.65±0.5	36.93±0.52	28.78±0.80	24.67±0.23	21.54±0.12	16.46±0.15
4	73.54±0.09	48.89±0.66	37.72±0.03	32.67±0.52	28.60±0.23	22.56±0.23
5	87.19±0.3	63.76±0.5	45.67±0.64	39.49±0.33	35.85±0.12	29.53±0.45
6	97.45±0.06	74.78±0.02	59.34±0.02	48.76±0.55	44.69±0.22	35.56±0.12
7	-	87.45±0.05	66.73±0.04	58.96±0.41	53.76±0.24	42.67±0.15
8	-	96.84±0.04	79.78±0.02	66.95±0.04	63.26±0.22	48.75±0.23
9	-	-	87.46±0.04	75.98±0.20	69.54±0.15	57.74±0.45
10	-	-	95.32±0.60	81.79±0.0.41	76.58±0.45	65.42±0.21
11	-	-	-	89.95±0.23	85.34±0.12	72.62±0.24
12	-	-	-	98.58±0.45	90.52±0.65	80.64±0.67

VIII. DISCUSSION

Raw material analysis of Metformin

The given drug metformin is tested for the given test as per the IP standard & limits .Assay value obtained by procedure as per I.P showed a purity of 98 % which was found to be in the range of I.P standard .Thus the evaluation of the drug ensures its quality as per standard of the Indian pharmacopeia and thus it can be included for further study for the formulation. Table is enlisted in table 3.

Solubility soluble in methanol, soluble in phosphate buffer &sparingly soluble in water

Standard calibration curve of Metformin

The calibration curve was constructed with phosphate 7.4buffer solution and results were shown in table 4 and figure 3. The regression coefficient obtained was 0.99 which shows better correlation between both axis.

Drug - excipient incompatability study FT-IR (Fourier transforms Infrared Spectroscopy):

Drug-polymer compatibility studies were carried out using FTIR spectroscopy to establish any possible interaction of etodolac with the polymer used in the formulation. Thus results indicate that the characteristic absorption peak due to pure etodolac have appeared in the formulated microspheres without any significant change in their position indicating no chemical interaction between etodolac and polymers. Results were shown in figure 4.

Micromeritic Properties:

Micromeritic Properties Bulk density, Angle of Repose, Tapped density & C.I are evaluated for all batches and all the values are showing prepared microspheres are good in flow &compressibility as per the results shown in table 5.

Characterization of microspheres:

Particle Size of micro balloons:

As per the results (table 10 & fig. 6) of the study the average particle size of Micro balloons were found to be for F1, F2, F3 formulation and and for F4, F5 and F6 formulations, respectively. The particle size increased with increasing polymers concentration. This is due to the increase viscosity of the solution and the decrease in stirring efficiency. Also with increasing polymer concentration, the hardening time of the microspheres was shortened. Therefore, a shorter time was provided for the breakup of droplets, and large microspheres were formed.

Surface Morphology:

Scanning Electron Microscopy (SEM):

The scanning electron microscope showed that the developed Microspheres were spherical with porous surface which facilitate diffusion of drug as shown in fig. 5.

Determination of percentage yield:

Production yield was more for F3 & F4 it was found to be 80.45±0.13, 88.84±0.18 respectively due to increase in concentration of okra mucilage.

Entrapment Efficiency (EE):

The percentage loading efficiencies were found to be good for F1, F2, F3, F4, F5 and F6 formulations, out of 6 formulation F4 is 85.63±0.07 is good due the viscosity of polymer entrapment is improved.

In vitro drug release studies: -

The Invitro drug release of formulations for F1 at 6 th hour is 97.45±0.06, F2 at 8 th hr 96.84±0.04, F3 at 10 hr 95.32±0.60, for F4, F5 and F6 at 12 hr is 98.58±0.45 90.52±0.65, 80.64±0.67 respectively, Results indicate that proportion of polymers in formulation was the key factor governing the release of drug from microspheres. As the concentration of polymer increased, there was an increase in diffusional path length. This may decrease the overall drug release from the polymer matrix. Formulations comprised of okra gum300 mg & sodium Alginate (SA) 100mg (F4) is good in release

IX. CONCLUSION

As per the study we are concluding okra mucilage is a potential polymer to prepare Metformin Microspheres to increase the Bioavailabity and sustained action. CRDDS, Targeted Drug Delivery system & pulsatile Drug Delivery system. Isolation of mucilage is very easy, economic and beneficial, Due to the viscosity of Okra mucilage is can be used in various stages of formulation

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