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FORMULATION AND *IN-VITRO* EVALUATION OF SUSTAINED RELEASED GLIBENCLAMIDE MICROSPHERES

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ABSTRACT

In the present investigation, an attempt has been made to prepare sustained release microspheres of Glibenclamide by using Eudragit RS 100 as rate retardant polymer. Microspheres were successfully prepared by non-aqueous emulsion solvent evaporation method. Magnesium stearate was used as hydrophobic dispersant and droplets stabilizer. The yields were varies from 90-97% and encapsulation efficacy is up to 94% which encourage the investigation. The desired sizes of microspheres were obtained when the stirring was carried out at 600 rpm. The *in-vitro* dissolution profile of optimized formulation batch i.e., F5 is resulted up 11.5 hours. The various parameters of model equation of microspheres containing Glibenclamide in vitro kinetic release were thoroughly investigated and it was seen that the statistically significant confined to Zero- order, Higuchi Model and Korsmeyer-Peppas Model. To establish the release kinetic, Korsmeyer-Peppas Model shows the prominent release characteristics and the release pattern is non-fickian diffusion controlled. The SEM photograph of microspheres confirmed good spheres and smooth surface of the microspheres. The IR and DSC studies used to of confirmed the interaction between drug and polymer.

INTRODUCTION: It is Needless to say that one of the most difficult problems of the new millennium is the management of vast majority of our population afflicted with diabetes specially the Type-2, which are not dependent on insulin production. It is feared that within few years India would have 50 million cases of diabetes especially among the younger generation among men and women including children will suffer from this destructive disease.

Extensive work is being taken up not only to develop newer more specific molecules for Type-2 diabetes but also develop proper delivery system to maintain the activity of the drug over a prolong period of time so the proper compliance of taking the drugs regularly. The principal aim of the investigation undertaken is to develop a Multi-Particulate Drug Delivery System for non-insulin dependent diabetes mellitus drug. This type of diabetes is rising exponentially even in developing country like India due to fast life style with concomitant stressful living condition. It is expected that within coming 5 years fifty million Indian of both sexes and different age group including children will suffer from this destructive diseases, keeping above view the investigation has undertaken as the topic of national importance.

As the objective of the investigation desires most important FDA approved type 2 diabetes second generation sulfonylurea drugs, for clinical use of oral non-insulin dependent diabetes mellitus, namely Glibenclamide. The drug in oral conventional dosage form has the dosage regime of three times a day due to having short elimination half life of 5 hour.

Thus, the development of controlled release dosage forms is to be designed considering the above factors of the model drug molecules. Furthermore the extended release single unit dosage form has the demerits of all and nothing effect, person to person variability and non uniform drug release. These complains certainly can be overcome by the sustained release multiunit dosage form like microspheres.

Behere *et al*., [1] 2008 prepared and characterized Glipizide loaded microsphere by emultion solvent evaporation method. They used Eudragit RS 100 polymer and solvent as methanol and acetone. As well as used light liquid paraffin and n hexane. They used phosphate buffer for dissolution study. Patel J.K and co-workers [2] in 2005 prepared chitosan microspheres containing Glipizide by simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent. *In vivo* testing of the mucoadhesive microspheres to albino Westar rats demonstrated significant hypoglycemic effect of glipizide.

K.P.R. Chowdary *et al.,* [3] 2004 prepared ethyl cellulose microspheres of glipizide were prepared by an industrially feasible emulsion solvent evaporation technique and the microspheres were investigated. The microspheres are spherical, discrete and free flowing. Encapsulation efficiency was in the range of 81-91%. Glipizide release from the microspheres was slow and diffusion controlled and extended over some days and the sustained hypoglycemic affect over six days in normal rabbits.

In the present study for the design and development of microspheres a suitable retardant polymer, Eudragit RS 100 is selected. Eudragit RS 100 is the copolymers of acrylic and meth acrylic acid esters with a low content in quaternary ammonium groups. The ammonium groups are present as salts and make polymers permeable. The average Molecular weight is approx. 150, 000. 1g of the substances dissolves in 7g aqueous methanol, ethanol and isopropyl alcohol (containing approx. 3% water), as well as in acetone, ethyl acetate and methylene chloride to give clear to cloudy solutions. The substances are practically insoluble in petroleum ether, 1 N sodium hydroxide and water.

MATERIALS AND METHOD

Materials:

Materials	Source
Glibenclamide IP	CADILA HEALTHCARE LTD, Ahmadabad. Batch no- GC LTM 1044. Mfg- Jan-09, Exp-Dec-2013
Eudragit RS100	CADILA HEALTHCARE LTD, Ahmadabad.
Magnesium stearate	CENTRAL DRUG HOUSE (P) LTD., Delhi.
Paraffin Liquid Light	YARROW CHEM PRODUCTS, Mumbai.
Acetone	MERCK P. LTD, Mumbai.
Methanol	MERCK P. LTD, Mumbai.
n-hexane	LOBA CHEM P. LTD, Mumbai.

Method of Preparation: Glbenclamide microspheres were prepared by non-aqueous solvent evaporation technique [4-6]. Different amount of polymer Eudragit RS 100 was dissolved in organic solvent containing 8.5 ml of acetone and 1.5 ml of methanol by using magnetic stirrer. Then the drug and magnesium stearate were dispersed in the polymer solution. The resulting dispersion was poured into a beaker of 500 ml containing the mixture of 90 ml light liquid paraffin (LLP) and 10-ml n-hexane while stirring with a mechanical stirrer at a stirrer speed of 600 rpm. Stirring was continued for 3 hours until all acetone evaporated completely.

After that, the rigidized microspheres formed were collected by filtration and washed three times with 50 ml of n-hexane each. Microspheres were dried at room temperature for 24 hrs. Repeated batches were prepared to obtain reproducible results and all the experiments were conducted in duplicate. The various formulations of glibenclamide microspheres are shown in **Table 1**.

TABLE 1: COMPOSITION OF VARIOUS FORMULATIONS OF GLIBENCLAMIDE MICROSPERES

Formulation code	Drug (mg)	Polymer (mg)	Magnesium stearate (mg)	LLP (ml)	n-hexane (ml)	Stirring Speed
F1	500	500	50	90	10	600
F2	500	500	100	90	10	600
F3	500	1000	50	90	10	600
F4	500	1000	100	90	10	600
F5	500	1500	50	90	10	600
F6	500	1500	100	90	10	600

Evaluation: The prepared glibenclamide- loaded microspheres was evaluated by studying the following parameters.

Percent Yield of Microspheres: Microspheres dried at room temperature were then weighed and the yield of microspheres preparation was calculated using the following formula [4];

$$\text{Percent Yield} = \frac{\text{The amount of microspheres obtained (g)}}{\text{The theoretical amount (g)}} \text{ X } 100$$

The theoretical amount is the sum of weight of all the non-volatile solid ingredients used in the process.

Size Distribution of Microspheres [7, 8]: Microspheres were separated into different size fractions by sieving for 10 minutes using mechanical sieve shaker (Cuprit Electrical Co. India) containing standard sieves having apertures of 1000, 710, 500, 355, 250 & 180 µm. The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula;

Mean Particle size=

$$\frac{\Sigma(\text{Mean particle size of the fraction X weight fraction})}{\Sigma \text{ Weight fraction}}$$

Flow Properties of Prepared Microspheres: The flow properties of the prepared microspheres were characterized by determining various parameters like bulk density, tapped density, Carr's Index and Hausner's Ratio.

- **Determination of Bulk Density and Tapped Density:** An accurately weight quantity of drug crystal and drug loaded microspheres were taken separately in a 10 ml graduated cylinder. Then, the initial volume was noted. The graduated cylinder was tapped for 1000 times and the final volume was measured. The bulk density and tapped density were determined from the following formula:

Bulk Density = W/V_B ,
Tapped Density = W/V_T

where, W = Weight of the drug or formulations, V_B = Bulk Volume, V_T = Tapped Volume.

- **Carr's Index or Compressibility Index:** The Carr's Index predicts the formulation which can be determined as

$$\text{Carr's Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \text{ X } 100$$

- **Packing Factor / Hausner's Ratio:** Packing factors is a measure of flow properties, which was calculated as the ratio of tapped density to bulk density.

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Drug Entrapment Efficiency: About 50 mg of accurately weighted drug loaded microspheres were added to 50 ml of phosphate buffer, pH 7.4. The resulting mixture was kept shaking on mechanical shaker for 24 h. Then the solution was filtered (0.45 µm pore size) and 1 ml of this solution was appropriately diluted to 25 ml using phosphate buffer, pH 7.4 and analyzed spectrophotometrically at 276 nm [9, 10] using Systronic 2101 UV-Visible Spectrophotometer. Entrapment efficiency was calculated by the following formula [73]:

$$\text{Entrapment Efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

Scanning Electron Microscopy (SEM): JEOL JSM – 6480LV, scanning electron microscope was used to characterize surface topography of prepared microspheres. The microspheres were placed on a metallic support with a thin adhesive tape and microspheres were coated with platinum under vacuum (fine coat, ion sputter JFC – 1110) to render them electron conductive. The surface was scanned and photomicrographs were taken at 15 kV accelerating voltage for the drug loaded microspheres.

Fourier Transform Infrared Spectroscopy (FTIR) [12]: Drug – polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and drug loaded microspheres using FTIR JASCO (Model No. 410). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm-1 and the resolution was 2 cm^{-1}.

Differential Scanning Calorimeter (DSC) Study: The DSC analysis of pure drug, drug-loaded microspheres were carried out using Perkin Elmer, USA (Diamond DSC) to evaluate any possible drug-polymer interaction. 5 mg drug loaded microspheres were triturated to get finely divided powder. The powder passed through No. 100 sieve. In a similar way, pure drug was also passed through No. 100 sieve. Sample [2-4mg] were accurately weighed using electronic microbalance and heated in sealed aluminum pans at a rate 500°C /min from 50°C to 200°C temperature range under nitrogen flow of 25 ml/min.

***In vitro* Release Studies:** United States Pharmacopoeia basket-type dissolution rate test apparatus (LABINDIA, DISSO-2000, and Mumbai, India) was used for all the *in vitro* release studies. A weighed quantity of the microspheres (500 µm size fraction) was taken in 500 ml of phosphate buffer of pH 7.4. The dissolution medium was stirred at 100 rpm and maintained at constant temperature (37±1^{o}C). At preset time intervals 2 ml aliquots were withdrawn and replaced by an equal volume of fresh pre warmed dissolution medium maintaining sink condition throughout the experiment.

After suitable dilution, the samples were analyzed for drug quantification at 276 nm using UV-VIS spectrophotometer. The concentrations of drug in samples were calculated using regression equation of the calibration curve of drug in phosphate buffer of pH 7.4. Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from microspheres. The kinetic models used were Zero order equation, first order equation, Higuchi, Hixon Crowell model and Korsemeyer-Peppas model.

RESULTS AND DISCUSSION: Microspheres are one of the important classes of microencapsulation technique, which also termed as multi-particulate delivery system and are prepared to obtain prolonged or controlled drug delivery to improve bioavailability, stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuations within therapeutic range; reducing site affects decreasing dosing frequency and improving patient's compliance [13]. Based on the above facts and circumstances, the present investigation yield the following outcomes.

Factor effecting Mean Particle Size:

- **Effect of Polymer Concentration:** One of the most important factors that affect the mean particle size is the drug-polymer ratio. An increase in total polymer concentration (keeping hydrophobic dispersant amount constant) increases the relative viscosity of the dispersed phase in the fixed amount of the solvent, and the sub-division of the dispersed phase into smaller one prevented by higher interfacial viscosity [14-16]. In this case same results were obtained, i.e. (F5 >F1 >F3), (F6> F2 >F4) which have been shown in **Fig. 7 and table 3**.

- **Effect of Stirring Speed:** Stirring speed had pronounced effect on the mean particle size of the microspheres. When the stirring speed was maintained at 1000 rpm for the formulation F5 the resulting microspheres were very small and were of uniform size and the mean particle size was found to be 305.27µm. This may have occurred as a result of segregation at high stirring speed. The stirring speed below 500 rpm resulted in formulation of large and aggregated microspheres (mean particle size 785µm) [8, 17, 18]. The desired sizes of microspheres were obtained when the stirring was carried out at 600 rpm.

- **Effect of Magnesium Stearate Concentration:** The effect of dispersant was found to have a negative effect on the mean particle size [19, 20] which has been summarized in **table 3**. It was found that with increasing dispersant concentration the mean particle size of the various formulations decreases as mentioned below (keeping the polymer concentration constant) i.e. (F2 < F1), (F5 < F3), (F6 <F5) as shown in **Fig. 6**.

TABLE 3: MEAN PARTICLE SIZE OF VARIOUS FORMULATIONS

Formulation code	Mean Particle Size (µm)
F1	536.63
F2	493.01
F3	518.29
F4	457.64
F5	604.19
F6	512.89

Study of flow properties of Microspheres: The result of flow properties shown by all the formulation summarized in **table 4** suggesting the Carr's index value between 5-15 and Hausner's Ratio between 0-1.2, indicating an excellent flow properties for all the formulation.

TABLE 4: FLOW PROPERTIES OF GLIBENCLAMIDE MICROSPHERES

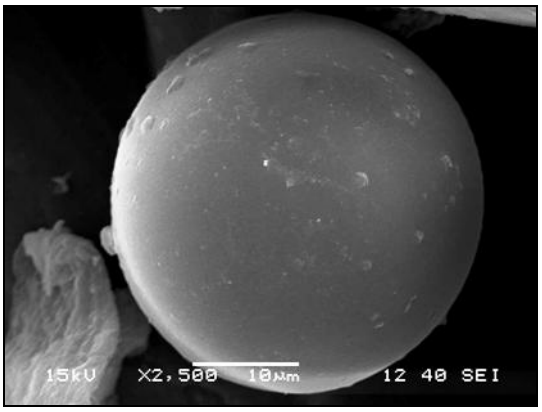
Formulation Code	Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index	Hausner's Ratio
Pure Drug Crystal	0.533	0.805	33.79	1.51
F1	0.581	0.637	8.79	1.10
F2	0.603	0.675	10.67	1.12
F3	0.577	0.631	8.56	1.09
F4	0.620	0.701	11.55	1.13
F5	0.561	0.600	6.50	1.07
F6	0.597	0.658	9.27	1.10

Factors Effecting Drug Entrapment Efficiency: The encapsulation efficiency of glibenclamide was found to be increased with increase in drug to polymer ratio (keeping dispersing agent constant) the encapsulation efficiency increases due to the fact that higher the drug-polymer ratio, the higher probability of drug surrounding by the polymer which acted as a barrier to prevent escape of the drug into the external medium [4, 21]. The drug entrapment efficiency was found to follow the order (F5> F3> F1), (F6> F4 >F2) which is shown in **Table 5**.

TABLE 5: PERCENTAGE YIELD AND ENTRAPEMENT EFFICIENCY OF VARIOUS FORMULATION

Formulation code	% Yield	Practical Drug Content (mg)	Theoretical Drug Content (mg)	Entrapment Efficiency (%)
F1	58.70	11.55	25	46.23
F2	89.10	11.44	25	45.76
F3	87.93	10.72	16.66	64.38
F4	85.2	9.85	16.66	59.14
F5	95.21	11.28	12.5	90.25
F6	97.47	11.48	12.5	91.84

Morphology of the Microspheres: The SEM photomicrographs are shown in **Fig. 8(A) to (G)** of formulations no. F5. Photomicrographs show that the microspheres are white, spherical with smooth surface. The SEM photograph of microspheres after dissolution shows pores over their surfaces, which may have provided channel for drug to release in a controlled manner into the surrounding medium [4, 21, and 32].



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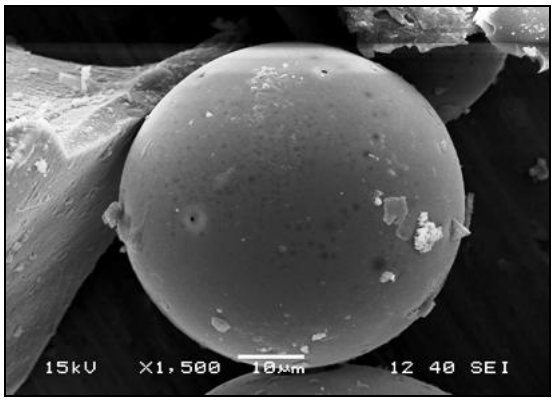

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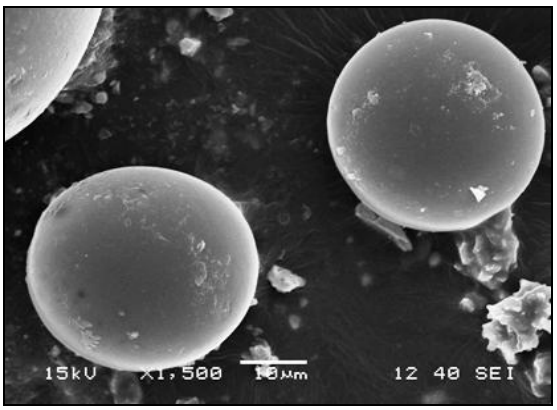

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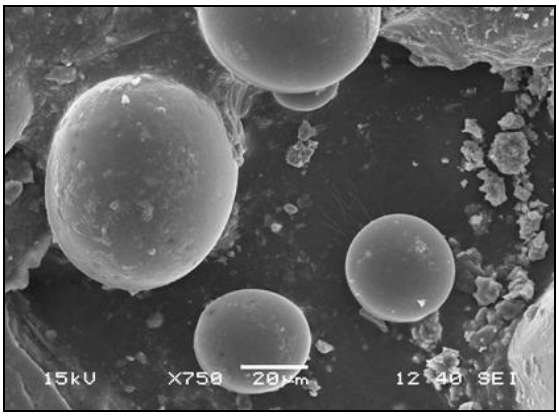

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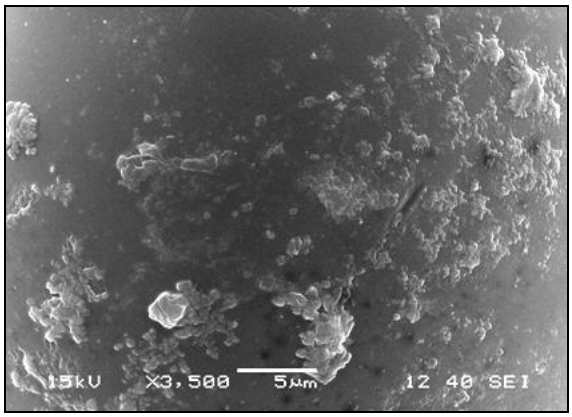

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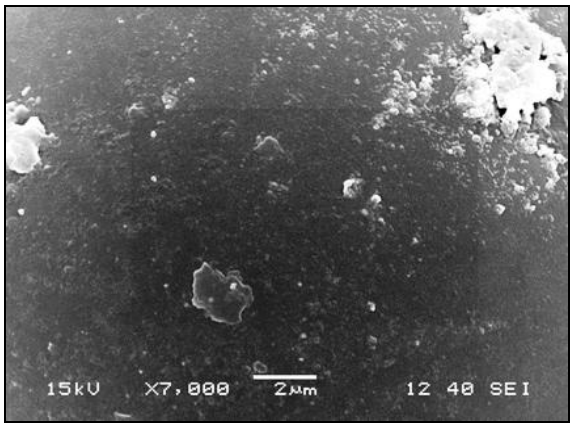

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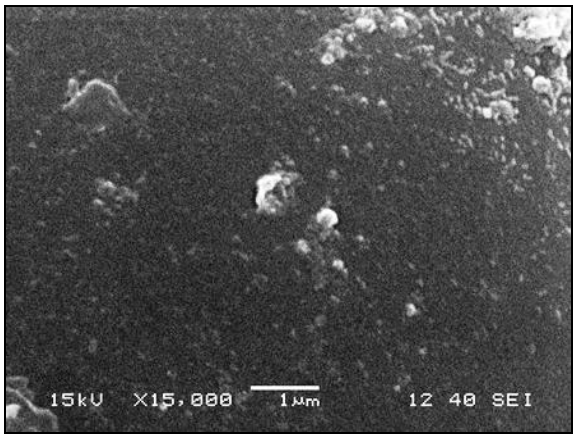


G

FIG. 8: SCANNING ELECTRON MICROGRAPH OF GLIBENCLAMIDE MICROSPHERES

Infrared Spectroscopic Study [17, 21]**:** The FTIR spectroscopy of pure drug glibenclamide shows prominent peaks at 3354.32 cm^{-1}, 1652.52 cm^{-1}, 1628.12 cm^{-1}, 1161.19 cm^{-1}, 1035.31 cm^{-1}, 1591.34 cm^{-1}, 2974.54 cm^{-1}, 2920.14 cm^{-1}, 1332.86 cm^{-1} and 666.68 cm^{-1} due to NH-stretching C=O stretching, NH-bending, C-N stretching (aliphatic), C-N stretching (aromatic), C=C stretching (aromatic), $-CH_3$ stretching, C-H stretching (aliphatic), S=O stretching, C-H bending (aliphatic) respectively. The C-Cl group is shown at 722.75 cm^{-1} and the C-O stretching shown at 1100.59 cm^{-1} which confirming the position of OCH_3. The IR spectrum of formulation also showed peaks in same region confirming no drug polymer interaction as shown in **Fig. 9**.

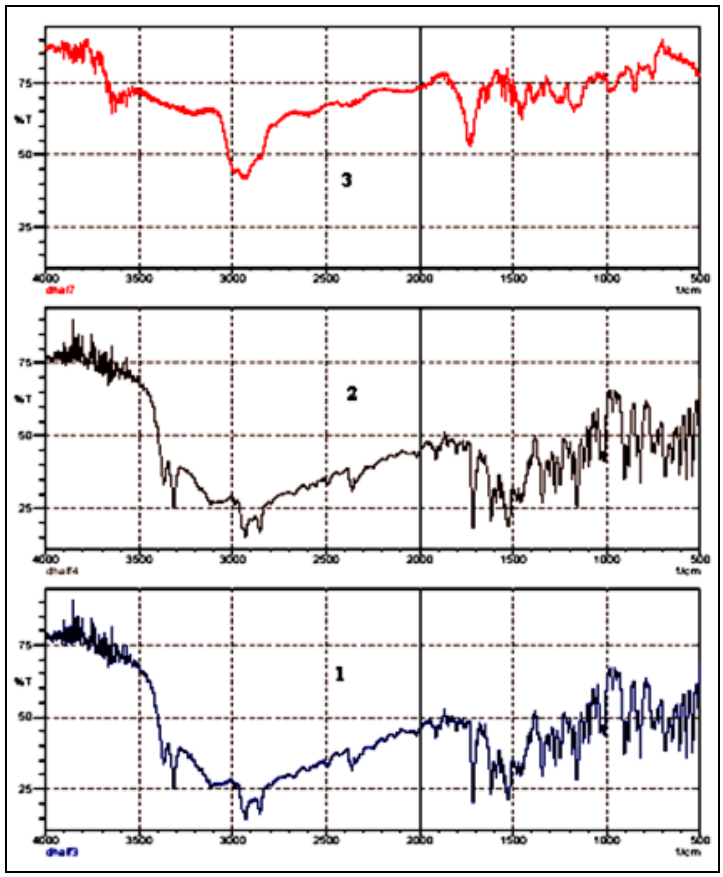
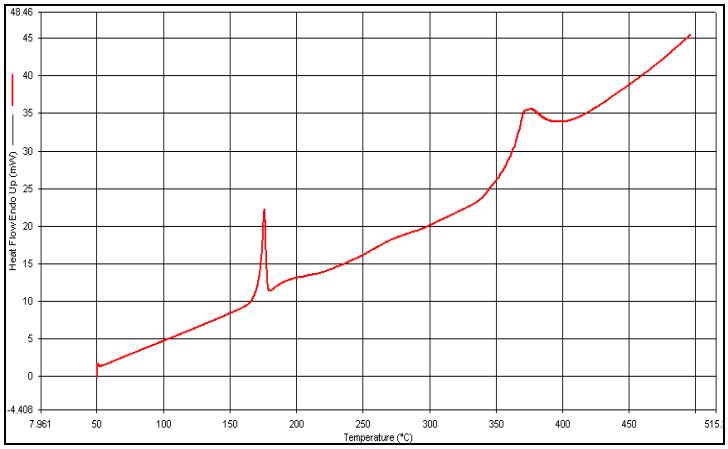


FIG. 9: FTIR SPECTRA OF PURE GLIBENCLAMIDE (1), DRUG AND EUDRAGIT RS 100 LOADED MICROSPHERES (2), EUDRAGIT RS 100 BLANK MICROSPHERE (3)

Differential Scanning Calorimetric (DSC) Study: The thermograph of DSC is shown in **Fig. 10** of glibenclamide pure drug showed a sharp endothermic peak at 174° C no such peaks were observed in the thermograph of the formulation indicating the drug has been dispersed thoroughly at molecular level in the formulation. Therefore, it may be concluded that the drug is in intact form within the formulated microspheres [17, 21, and 22].



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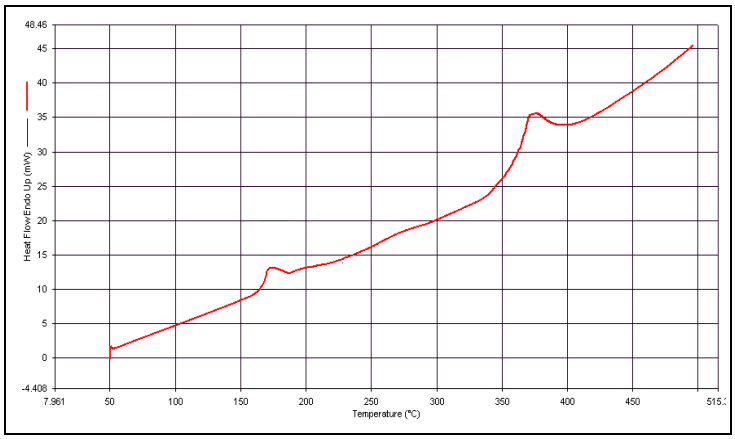

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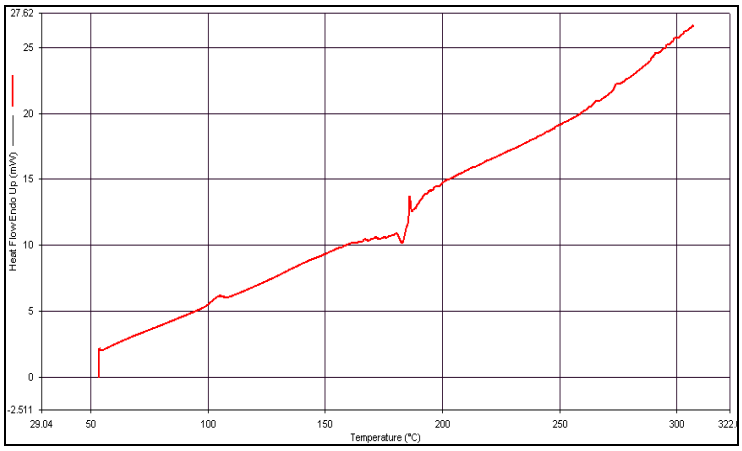


C

FIG. 10: DSC OF (A) GLIBENCLAMIDE, (B) FORMULATION F5 AND (C) EUDRAGIT RS 100

***In-Vitro* Drug Release Study:** The *in-vitro* release study was carried out with 500 µm size microspheres for all the formulations in order to keep the total area of microspheres constant and to get the comparable results [23]. **Table 6 and Fig. 3** shows the dissolution of pure drug glibenclamide at pH 7.4 phosphate buffer from the graph it was found that the t_{50} and t_{90} to be 19 min. and 37 min respectively for glibenclamide but when it was encapsulated into the microspheres the t_{50} and t_{90} all the formulations increases as shown in **table 8 and fig. 4**.

TABLE 6: RELEASE PROFILE OF GLIBENCLAMIDE (PURE DRUG)

Time (min.)	% Pure Drug Release
0	0
2	6.93
5	23.74
10	31.88
15	41.06
20	51.85
30	70.98
35	84.14
40	96.81

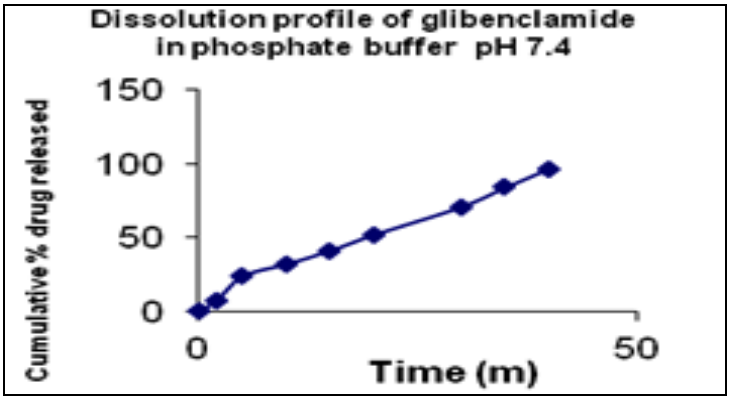


FIG. 3: DISSOLUTION PROFILE OF GLIBENCLAMIDE (PURE DRUG) IN PHOSPHATE BUFFER pH 7.4

TABLE 8:*IN VITRO* RELEASE PROFILE OF VARIOUS FORMULATION BASED ON t_{50} AND t_{90} OF GLIBENCLAMIDE

Formulation Code	$T_{50\%}$ (h)	$T_{90\%}$ (h)
F1	3.2	7.1
F2	3.1	7.0
F3	3.8	8.5
F4	3.4	7.1
F5	4.8	9.4
F6	4.1	8.7

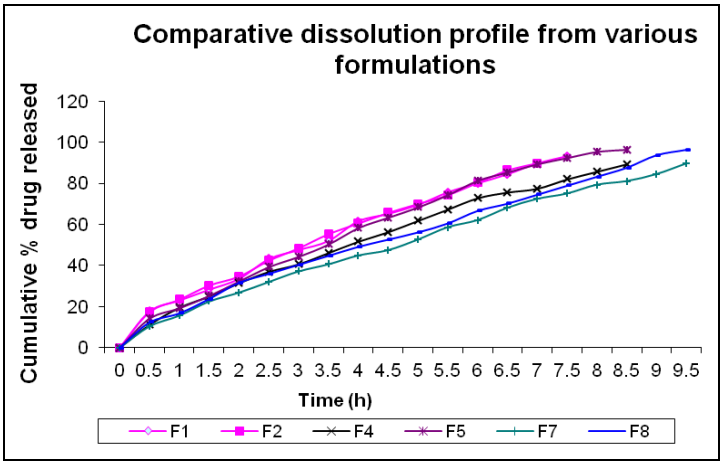
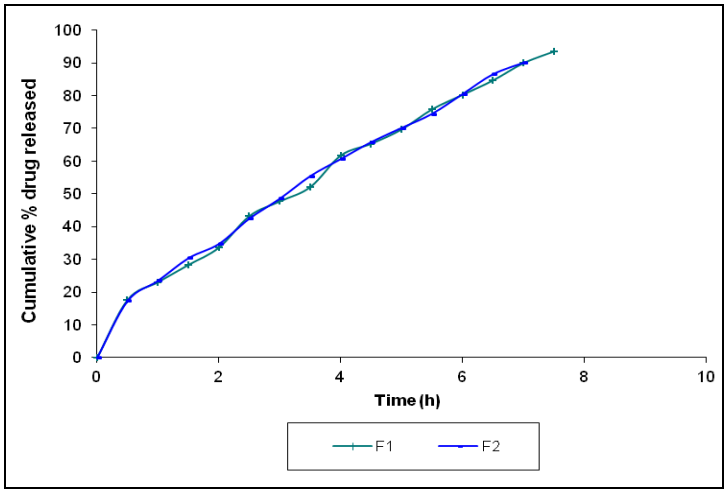


FIG. 4: COMPARATIVE DISSOLUTION PROFILE FROM VARIOUS FORMULATION OF GLIBENCLAMIDE

Effect of Drug- Polymer Ratio on Drug Release Rate: As seen in the **Fig. 7 (A), (B) and (C)**, the release of the drug from microspheres increases with increase in drug polymer ratio (keeping dispersant amount constant). This may have occurred due to the fact that with increase in drug polymer ratio the viscosity of the polymeric phase increases which act at a barrier and prevents the faster diffusion of the drug through the device and the sustaining effect was found to be following the order (F5>F3>F1), (F6>F4>F2) [24, 25]. The magnetic sterate also played a significant role on drug release rate. It was found that when the dispersant was used in low concentration (50 mg.) the drug release was more sustaining as compared to microspheres formulated at higher dispersant concentration which shown in **Fig. 6 (A) to (C)** which may have occurred due to attainment of optimum concentration of dispersant at 50 mg. After this, it releases the drug at faster manners.



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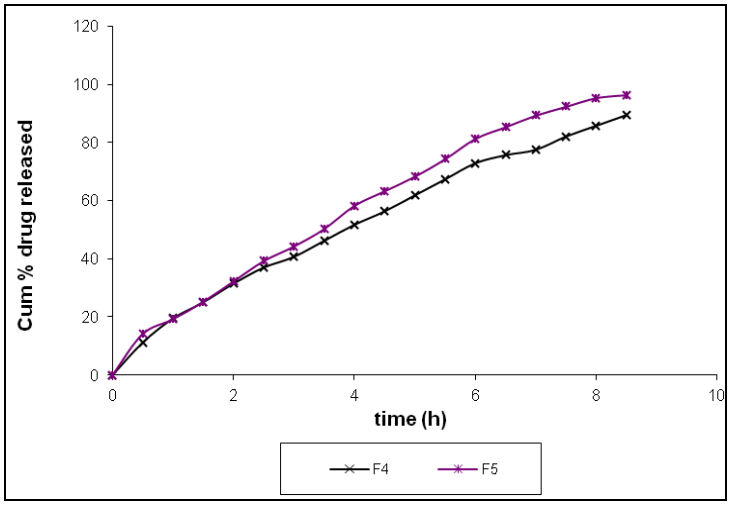

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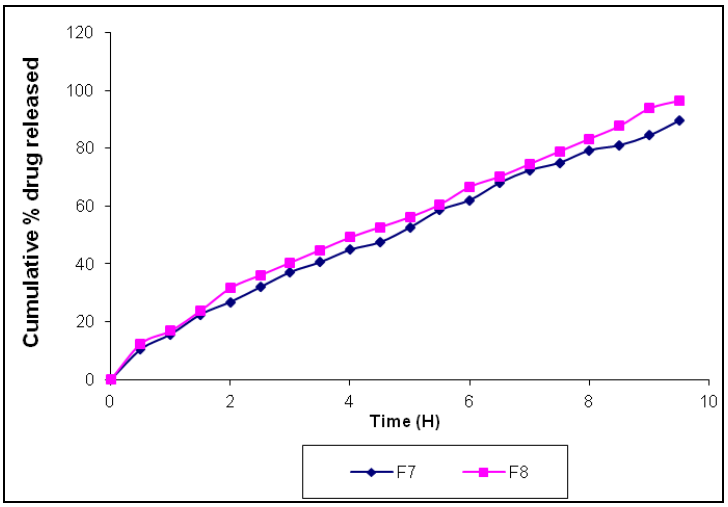

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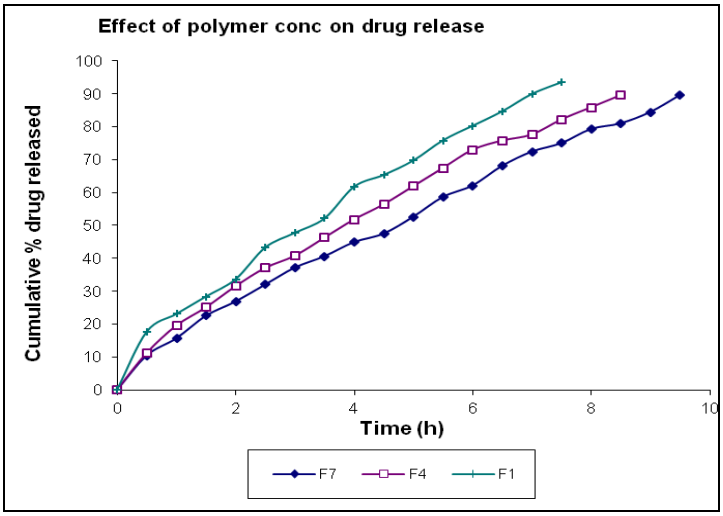
FIG. 6: EFFECT OF HYDROPHOBIC DISPERSANT CONC. ON DRUG RELEASE



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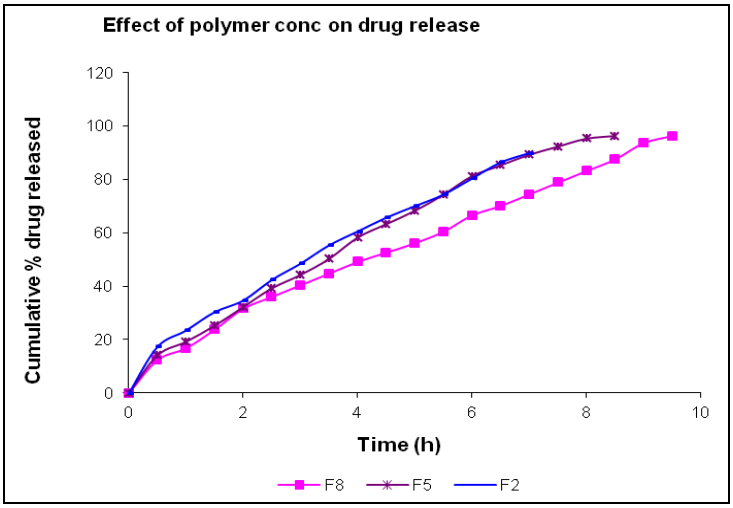

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FIG. 7: EFFECT OF DRUG POLYMER RATIO ON DRUG RELEASE

***In vitro* Release Kinetics:** *In vitro* release profile was analyzed by various kinetic models in order to find out the mechanism of drug release from the microspheres [27, 28]. The release kinetics determination by conventional way i.e., by comparing the correlation co-efficient was method found to be more complex. Therefore, to avoid any ambiguity the data obtain was fitted to Korsemeyer-Peppas Model in order to find out 'n' value which describe the drug release mechanism [30]. The 'n' value of all formulation lies between 0.5 - 1, indicating the drug release to be non-fickian diffusion controlled which shown in **Table 9**.

TABLE: 9 VARIOUS PARAMETERS OF THE MODEL EQUATIONS OF THE GLIBENCLAMIDE *IN VITRO* RELEASE KINETICS

Formulation	Zero order	First order	Higuchi	Hixon Crowell	Korsemeyer-Peppas	
					r^2	n
F1	0.9971	0.964	0.992	0.9886	0.9922	0.6612
F2	0.9980	0.972	0.993	0.9907	0.9951	0.6122
F3	0.9950	0.982	0.995	0.9955	0.9993	0.7275
F4	0.9935	0.963	0.993	0.9900	0.9951	0.7443
F5	0.9974	0.975	0.992	0.9918	0.9987	0.7228
F6	0.9979	0.925	0.991	0.972	0.9972	0.6901

(r^2 = CORRELATION CO–EFFICIENT), (n = RELEASE EXPONENT)

TABLE 2: PARTICLE SIZE DISTRIBUTIONS OF GLIBENCLAMIDE MICROSPHERES

Sieve opening (µm)	Formulation code					
	% Weight retained on each sieve					
	F1	F2	F3	F4	F5	F6
710	32.51	0	15.32	10.20	57.98	27.80
500	45.64	95.18	75.09	53.09	29.88	40.81
355	21.83	4.81	9.57	26.66	12.12	31.37
250	0	0	0	10.04	0	0

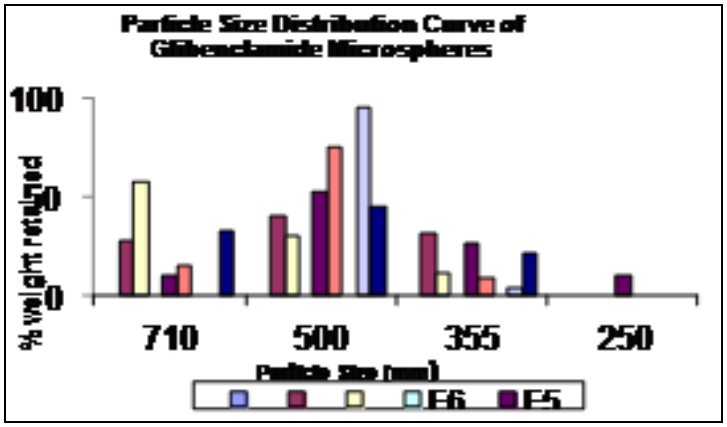

FIG. 1: PARTICLE SIZE DISTRIBUTION CURVE OF VARIOUS FORMULATIONS

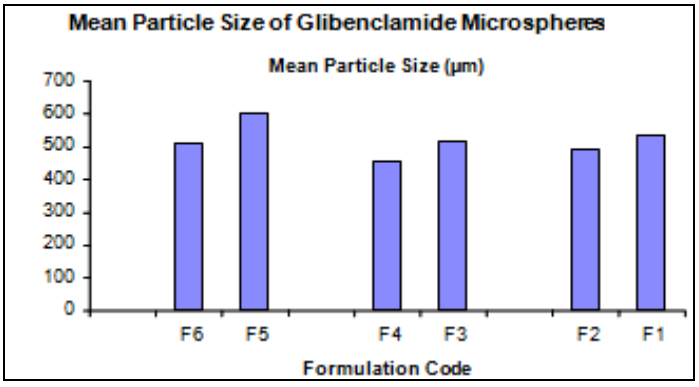

FIG. 2: MEAN PARTICLE SIZE OF VARIOUS FORMULATIONS

TABLE 7: DISSOLUTION PROFILE OF VARIOUS FORMULATIONS OF GLIBENCLAMIDE MICROSPHERES

Time (h)	FORMULATION CODE					
	Cumulative % drug released					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	17.67	17.33	11.20	14.18	10.46	12.38
1	23.07	23.48	19.51	19.28	15.67	16.83
1.5	28.34	30.37	25.064	25.27	22.53	23.8
2	33.62	34.7	31.495	32.27	26.85	31.66
2.5	43.24	42.43	37.025	39.28	32	36.07
3	47.69	48.48	40.723	44.31	37.15	40.44
3.5	52.09	55.37	46.225	50.31	40.6	44.81
4	61.7	60.56	51.746	58.3	44.89	49.19
4.5	65.28	65.73	56.355	63.34	47.49	52.69
5	69.67	70.05	61.867	68.34	52.62	56.19
5.5	75.8	74.36	67.387	74.34	58.62	60.55
6	80.22	80.38	72.908	81.34	62.08	66.66
6.5	84.61	86.42	75.695	85.38	68.07	70.18
7	88.88	89.89	77.545	89.38	72.39	74.54
7.5	92.41	93.43	82.118	92.39	74.98	78.92
8	95.76	97.02	85.807	95.4	79.26	83.29
8.5	97.05		89.488	96.42	81	87.66
9			92.563	98.12	84.42	93.76
9.5			95.375		89.56	96.42
10			97.574		93.78	98.34
10.5					95.45	
11					97.31	
11.5					98.97	

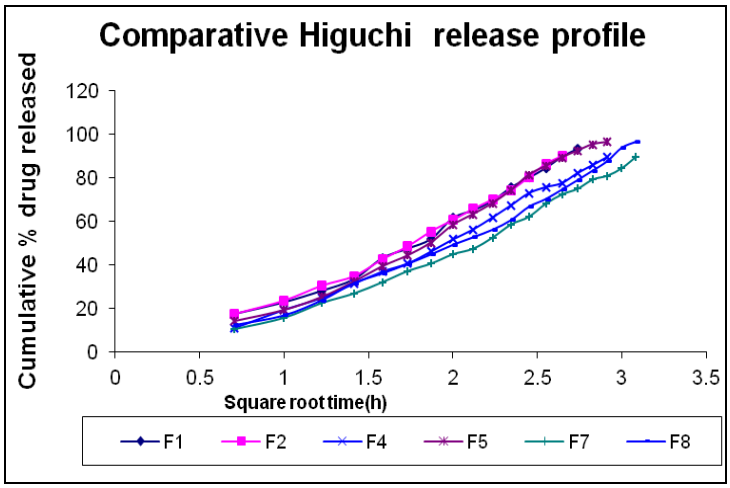

FIG. 5: COMPARATIVE HIGUCHI ORDER RELEASE OF DIFFERENT GLIBENCLAMIDE MICROSPHERES

CONCLUSION: Microspheres were prepared successfully by non-aqueous emulsion solvent evaporation method. The drug: polymer ratio, amount of dispersant and stirring speed influences the mean particle size of the microspheres. The drug entrapment efficiency was 45-92%, from *in vitro* dissolution study; it is evident that when the drug is encapsulated in the polymeric system the release is sustained for several hours. In the present investigation, the t_{90}% of pure glibenclamide was about 37 min. whereas in case of formulated glibenclamide loaded microspheres t_{90}% was 4.1-9.4 hrs. From this finding, the formulation no. F5 is the optimized batch, which may be the ideal batch for the investigation of *in vivo* study.

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