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DEVELOPMENT AND EVALUATION OF COLLOIDOSOMES OF GLIBENCLAMIDE

Ramchandra N. Chilkawar*, S. M. Patil, Dr. B. K. Nanjwade, V. S. Panchal

Department of Pharmaceutics, KLE'S College of Pharmacy, Nippani, Karnataka, India.

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***Author for Correspondence**
Ramchandra N. Chilkawar
Department of Pharmaceutics, KLE'S College of Pharmacy, Nippani, Karnataka, India.

ABSTRACT

Glibenclamide (Glyburide) is a potent oral antidiabetic agent and orally active second generation sulphonyl urea used in lowering blood-glucose in patients with type II diabetes mellitus (NIDDM). Glibenclamide has short half life of 4-6 hours. The objective of this study is Development and evaluation of colloidosomes of Glibenclamide for controlled/sustained drug release. An attempt was made to formulate and evaluate colloidosomes of Glibenclamide as a model drug using oil in water emulsion based method by using CaCO3 as a colloidal particle with a view to deliver drug at controlled/ sustained manner in GIT and consequently into systemic circulation. The prepared colloidosomes were evaluated for particle size, shape and surface morphology, FTIR study, DSC, XRD, % yield, % drug entrapment efficiency, Zeta potential, *In-vitro* drug release studies. The release rates were studied using GRAPHPAD PRISM software.

The obtained Colloidosomes were found to be discrete and spherical in shape and had mean particle size range of 45 µm -115.14µm, drug entrapment efficiency was found to be 65.88 % to 76.28% All the colloidosomes formulations were stable and good sustained release of the drug for a period of 8 hours. The release profile was very promising as compared with marketed formulation and followed Zero order kinetic Model. This implies that developed formulations have a potential to deliver the drug in controlled release manner. This outcome from release profiling strongly recommends that developed Glibenclamide loaded colloidosomes can be useful delivery carrier to deliver drug in controlled release manner which is a prime requirement for the treatment of type II Diabetes mellitus (NIDDM).

KEYWORDS: Glibenclamide, Colloidosomes, antidiabetic agent, Colloidal particle, *In-vitro* drug release.

INTRODUCTION

Diabetes is a major problem worldwide and one of the most common cause for seeking medical consultation. The management of diabetes is a lifelong process, which involves proper planning and control of blood sugar, which should be both uniform and sustained. GLIBENCLAMIDE also known as GLYBURIDE is an orally active second generation sulphonyl urea used in lowering blood-glucose in patients with type II diabetes mellitus (NIDDM). Glibenclamide is potent, having marked insulinaemic action and may work when other antidiabetic agents fail. It is does not cross placenta and has been safely used in pregnancy i.e., gestational diabetes mellitus (GDM) without any adverse effect to the foetus. In contrast, other antidiabetic agents like Metformin, Rosiglitazone and Pioglitazone freely cross placenta.

Glibenclamide having half life of 4-6 hours. The usual initial dose is 2.5 to 5 mg. The usual maintenance dose is 1.25 to 20 mg daily. Because of this high dosing frequency it is necessary to develop sustained /controlled release preparations. The treatment will be effective if the drug substances are delivered directly to the site of absorption or action. Better treatment may be possible with a lower dose of the drug. Therefore it would be beneficial to develop a sustained release formulation which remains at the absorption site for an extended period of time.[1,2,3,4]

For delivery of drug directly to the site of infection, vesicular delivery system is an efficient method which leads to reduction of drug toxicity with least side effects. Vesicular drug delivery is a delivery system which reduces the cost of therapy by improving bioavailability of medication, in case of poor soluble drugs. They can incorporate both lipophilic as well as hydrophilic drugs. In the last few decades, accordingly a number of lipid based systems like lipospheres[5,6,7], liposomes[8], niosomes, ethosomes, transferosomes were developed.[9] Such delivery systems are used to delay drug elimination of rapidly metabolizable drugs and function as sustained release systems. This system also solves the problem of instability, insolubility, and rapid degradation and widely used in specialized areas like gene delivery, protein delivery, tumour targeting, targeting to brain.[10] Novel technology has shown great potential for improving the effectiveness and efficiency of delivery of bioactive compounds and nutraceuticals. Some recent advances in nanotechnology show their promise for poorly soluble, poorly absorbed and labile herbal extracts and phytochemicals as potential cosmetics.[8] Other than vesicular systems nanoparticles[11, 12] and microspheres have also

gained importance in colloidal systems. Among the vesicular series of system, colloidosomes are used as advanced tool for encapsulation of various materials such as drugs, dyes, cosmetics, biomaterials as filler in catalysis and waste removal.[13]

Colloidosomes (a term coined by Dinsmore et al. in 2002) are microcapsules characterized by a coating, or shell composed of self-assembled colloidal particle (see Fig. 1) that can range in size from nanometers to microns.[14] Colloidosomes constitue important part of vesicular systems, colloidosomes is the advanced tool in drug delivery. Colloidosomes are the hollow shell microcapsules consisting of coagulated or fused particles at interface of emulsion droplets. Colloidosomes have exciting potential applications in controlled release of drugs, proteins, vitamins as well as in cosmetics and food supplements. Colloidosomes have a great encapsulation efficacy with a wide control over size, permeability, mechanical strength and compatibility.[15]

The objective of present investigation is to formulate a colloidosomes using Glibenclamide as a model antidiabetic drug and subject the formulation for characterization and evaluation.

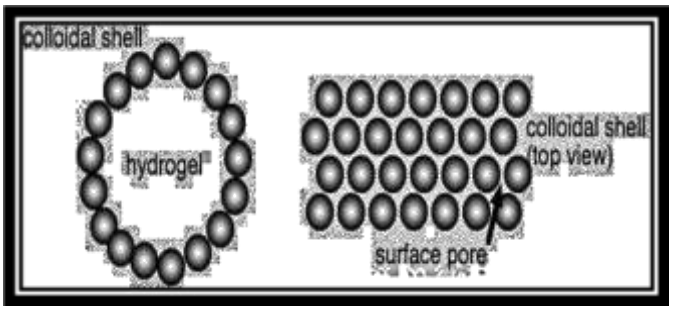

Fig. 1: Colloidosomes Structure

Fig.1: It is a sketch of colloidsomes, namely, cores of hydrogel coated with a shell composed of colloidal particles (left), and the surface pores formed in the shell by the packing of the particles on the hydrogel surface (right).

MATERIALS AND METHODS

Materials

Glibenclamide was kindly supplied by Micro labs Ltd Bangalore, India; Colloidal particle-Calcium carbonate was purchased from Molychem Chemicals Mumbai. Sodium alginate was procured from Himedia Laboratories Pvt. Ltd Mumbai. Sunflower oil was purchased from Germin seeds Pvt. Ltd. Bangalore. Distilled water, Ethanol and other solvents were purchased from local suppliers. All the chemicals were used as supplied, without further purification.

Formulation Of Colloidosomes [16, 17]

Selection of concentration of ingredients by preparing blank colloidosomes

The blank colloidosomes were developed so as to optimize the particle size, particle shape. Based on the results of above defined characteristics the selected concentrations of excipients were optimized and are shown below in table no.1

Preparation of Blank Colloidosomes

Blank colloidosomes were prepared by using oil-in-water emulsion based method. In a typical fabrication CaCO3 microparticles were dispersed in sunflower oil through stirring for 1 hour. Aqueous solution of sodium alginate was added into oil and oil-in-water emulsion was formed by stirring for several minutes. The emulsion was shaken for two hours and then left for 48 h. The obtained colloidosomal dispersion added to non aqueous phase (ethanol) and allowed to centrifuge to separate them from the supernatant. The obtained oil core colloidosomes are washed with ethanol and finally redispersed in water.

Preparation of Glibenclamide loaded Colloidosomes

The formulation chart for formulating Glibenclamide loaded colloidosomes is shown in following table no. 2. Colloidosomes of Glibenclamide were prepared by oil-in-water emulsion based method.

Table 1: Formulation table for Blank Colloidosomes

Blank Formulation	Oil and water ratio	Sodium alginate	$CaCO_3$ (mg)	Particle size in (µm)	Particle shape
B1	1:4	1%	40	102.11	Spherical
B2	1:5	1%	40	98.23	Spherical
B3	1:6	1%	40	95.45	Spherical
B4	2:4	1%	40	89.23	Spherical
B5	2:5	1%	40	85.42	Spherical
B6	2:6	1%	40	71.24	Spherical
B7	3:4	1%	40	65.34	Spherical
B8	3:5	1%	40	47.32	Spherical
B9	3:6	1%	40	42.24	Spherical

Drug loaded colloidosomes were developed using above excipients concentrations.

Table 2: Formulation Table of Glibenclamide loaded Colloidosomes

Formulation	Drug (mg)	oil and water ratio	Sodium alginate	$CaCO_3$ (mg)
F1	50	1:4	1%	40
F2	50	1:5	1%	40
F3	50	1:6	1%	40

F4	50	2:4	1%	40
F5	50	2:5	1%	40
F6	50	2:6	1%	40
F7	50	3:4	1%	40
F8	50	3:5	1%	40
F9	50	3:6	1%	40

Evaluation Of Glibenclamide Loaded Colloidosomes[18, 19, 20]

Particle size Analysis

Particle size of the prepared Colloidosomes was determined by optical microscopy. The Optical microscope was fitted with an ocular micrometer and a stage micrometer. The eyepiece micrometer was calibrated. The particle diameters of 50 Colloidosomes were measured randomly by optical microscope.

The average particle size was determined by using the Edmondson's equation:

$$= \frac{\Sigma\ nd}{\Sigma\ n}$$

Where, n – Number of colloidosomes observed

d – Mean size range

Percentage yield

The practical percentage yield was calculated from the weight of colloidosomes recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Practical Mass (Colloidosomes)}}{\text{Theoretical Mass (Polymer + Drug).}} \times 100$$

Determination of Entrapment Efficiency Percentage

Entrapment efficiency of Glibenclamide loaded Colloidosomes was estimated by centrifugation method. The prepared Colloidosomes were placed in centrifugation tube and centrifuged at 15000 rpm for 30 min. The supernatant (1ml) was withdrawn and diluted with ethanol. The unentrapped Glibenclamide was determined by UV spectrophotometer at 230 nm. The samples from the supernatant were diluted suitably before going for absorbance measurement. The free Glibenclamide in the supernatant gives the total amount of unentrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped and

was calculated using below equation no. Concentration of drug was calculated from equation of straight line obtained for standard curve for Glibenclamide.

$$\% \text{ E.E} = \frac{\text{Total amount of drug} - \text{Free dissolved drug}}{\text{Total amount of drug}} \times 100$$

Shape and surface morphology

Scanning electron microscopy was done to study the particle surface morphology and shape. The sample for the SEM analysis was prepared by sprinkling the colloidosomes on to one side of double-adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater (Jeol Ltd, Tokyo, Japan). The SEM analysis of the colloidosomes was carried out by using Jeol JSM 5300 (Jeol Ltd). The colloidosomes were viewed at an accelerating voltage of 15–20 kV.

Zeta Potential Determination[21]

Zeta potential was measured by using Zetatrac after appropriate dilution with distilled deionised water.

In Vitro Drug Release Studies

Colloidosomes equivalent to 5 mg of Glibenclamide was taken in to tube with both ends open. One end of the tube is closed with dialysis membrane. Now the tube containing drug loaded Colloidosomes is kept in a beaker containing buffer pH 1.2 (for initial 2 hours later then the medium was changed to pH 7.4 phosphate buffer solutions and drug release was studied for further remaining hours.). The tube is arranged in such a way that, it just touches the surface of the buffer solution. The whole set up is placed on a magnetic stirrer and rotated at 75 rpm. The temperature of buffer is maintained at 37 ± 0.5^{0}C.

At prefixed time (every 1 hour); 1 ml of solution were withdrawn. After suitable dilution, samples were assayed spectrophotometrically for the drug content at 230 nm using UV-Visible spectrophotometer.

Stability studies

From the nine batches of Glibenclamide loaded colloidosomes, formulation F7 was tested for stability studies. Formulation F7 was divided into 3 sample sets and stored at:

- $4^{o} \pm 1^{o}$C
- $25^{o}\pm 2^{o}$C and 60% RH ± 5% RH.

- $37^{o}\pm 2^{o}C$ and 65% RH ± 5% RH.

After one-month, the drug release of selected formulation (F7) was determined by the method discussed previously in vitro drug release studies, percentage Loading efficiency and SEM was also carried out for the same formulation.

RESULTS AND DISCUSSION

Particle size Analysis

The mean particle size ranged from 45 µm -115.14µm .The mean size was influenced by the concentration of water volume used in the formulation. As the volume fraction of water varies, some colloidosome deformed to nonspheral shape and even broken, also significant effect of stirring speed was observed for all formulations.

This may be due to the less availability of amphiphiles during emulsion formation and may be partly due to more partitioning of surfactant in to oil phase as the concentrations of aqueous phase was increased. Further as the stirring speed was raised there is decrease in average particle size of colloidosomes. This is due to high stress generated at the interface causing more creation of new surfaces which were appropriately stabilized by amphiphiles resulting in smaller particle size distribution. Mean particle size of all formulations are given in the Table no.3**.**

Table 3: % Yield, % Drug entrapment efficiency, Particle size of Colloidosomes of Glibenclamide

Formulation code	% Yield*	% Drug entrapment efficiency*	Particle size* (µm)
F1	81.33±2.1	74.48±2.1	115.14± 2.2
F2	82.74±1.4	71.15±2.4	113.34±3.2
F3	79.88±2.1	69.44±3.2	107.88± 4.3
F4	78.78±3.2	71.39±3.2	91.8±3.1
F5	81.95±2.3	65.88±0.98	87.96±1.1
F6	82.11±2.1	76.28±4.3	72.84±2.3
F7	81.32±3.1	74.46±2.1	70.32±1.4
F8	82.14±2.2	73.39±1.3	49.5±3.2
F9	81.67±1.4	73.18±1.2	45.0±2.1

*Each value represented as mean ± Standard Deviation of 3 observations

Percentage Yield

The % yields of all 9 formulations were very high for all colloidosomes obtained and were not affected by the type of polymer, the polymer**:** drug ratio, the stirring speed of the system and the ratio of the mixture of polymers. The yields of all formulations are shown in Table no. 3.

Percentage Drug entrapment efficiency

The % drug entrapment efficiency of Glibenclamide ranged from 65.88 to 76.28 % for colloidosomes of Glibenclamide using sodium alginate. It was seen that highest entrapment efficiency; when oil and water ratio maintained 2:6. Beyond this ratio when concentration increased the entrapment efficiency was found to be decreased. The reason for this is, during the cross linking process, the Colloidosomes will shrink and expel the drug molecules along with the water into the oil phase. This could be the reason for the loss of 10–20% of the drug during the encapsulation process. Moreover, higher drug loading lowered the percentage of entrapment and encapsulation, which indicates the wastage of drug during the microencapsulation process.

The % drug entrapment efficiency of the prepared Colloidosomes formulations are shown in Table no.3.

Shape and Surface Morphology

Morphology of the colloidosomes was investigated by scanning electron microscopy. Colloidosomes of Glibenclamide were spherical and their surface was smooth and devoid of cracks giving them good appearance. The SEM data obtained on the drug-loaded colloidosomes are shown in Figure no.2.

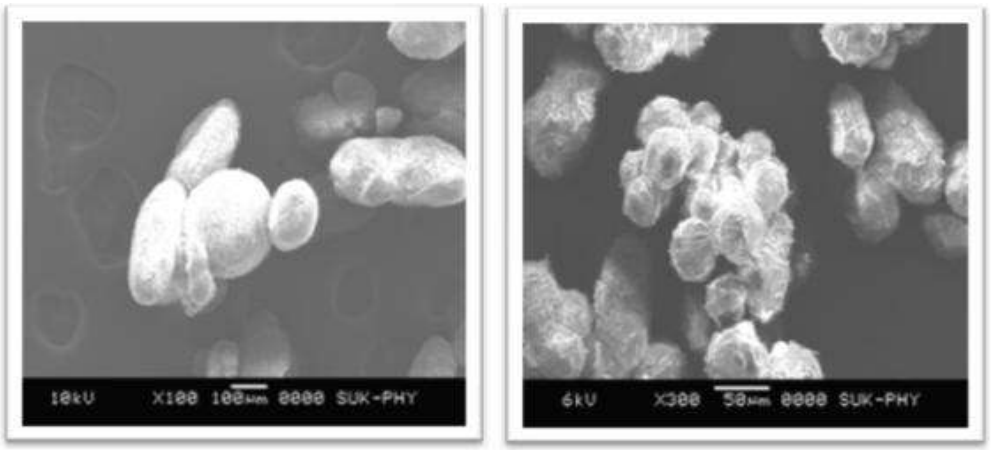

Figure 2: SEM images of Glibenclamide loaded Collidosomes formulation F7

In general; the colloidosomes were well formed and spherical in shape.

Zeta Potential

The stability of the drug delivery system was assessed by measuring the zeta potential of the particles by Zetatrac. If all the particles in a suspension have a large positive or negative zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. Zeta potential values of 9 formulations are presented in table no.4

Table 4: Zeta potential values

Formulation Code	Zeta potential* (meV)
F1	-18±1.1
F2	-15±0.9
F3	-19±13
F4	-21±1.2
F5	-23±1.6
F6	-28±1.5
F7	-26±1.5
F8	-25±1.4
F9	-22±1.3

*Each value represented as mean ± Standard Deviation of 3 observations

The zeta potential values of Glibenclamide loaded Colloidosomes were found in the range of -15 to -28. The formulation F6 shows highest Zeta potential values in comparison to other formulations. The high zeta potential value indicates high stability. This study confirms the stability characteristics of developed formulations.

In Vitro Drug Release Studies

In Vitro drug release studies of all the Colloidosomes formulations were carried out in a two different buffers, pH 1.2 and pH 7.4 using dialysis membranes. The study was performed for 8 hrs, and cumulative drug release was calculated at different time intervals. It was observed that the drug release from the formulations slightly increases as the particle size of the formulation decreases. All the formulation released the drug up to 8 hours which was very significant as compared with the marketed formulation Daonil which released nearly 100% drug within 6 hours.

The release profile depicted in the figure no.3 shows that the developed colloidosomes formulations were found to retard the drug release in comparison to marketed formulation Daonil. To ascertain the release mechanism the release data was fitted in four different kinetic models, namely

1. Zero order kinetic model
2. First order kinetic model
3. Higuchi's classical diffusion model
4. Peppa's Model

The best fit model was opted on the basis of linear regression analysis corresponding to high value of regression coefficient. The statistical analysis was performed by using GRAPHPAD PRISM software.

The data shows that all the formulations followed Zero order kinetic Model. This implies that developed formulations have a potential to deliver the drug in controlled release manner.

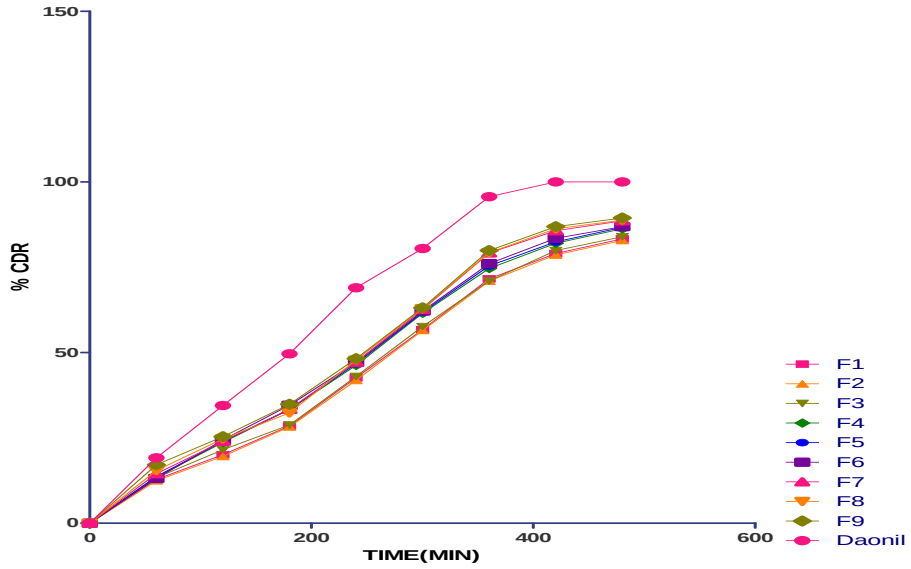

Figure 3. In vitro dissolution profile of formulation F1-F9 and marketed formulation Daonil®

Stability studies

Stability studies of the prepared Glibenclamide loaded colloidosomes were carried out by storing the formulation F7 at $4^{o} \pm 1^{o}C$, $25^{o} \pm 2^{o}C$ 60% ± 5% RH and $37^{o} \pm 2^{o}C$ 65% ± 5% RH for one month. Two parameters namely percentage drug entrapment efficiency and *in-vitro* release studies were carried out. The results of % drug entrapment efficiency and *in vitro* drug release after one month of storage are shown in Table no. 5 and 6 respectively.

Table 5: Stability Studies – % drug entrapment Efficiency after 30 Days Storage of Selected Formulation F7

Formulation	Percent Entrapment Efficiency* at 4° ± 1°C	Percent Entrapment Efficiency* at 25° ± 2°C and 60% RH ±5% RH	Percent Entrapment Efficiency*at 37° ± 2°C and 65% RH ±5% RH
F7	73.98±2.3	73.56±1.6	73.02±1.7

*Each value represented as mean ± Standard Deviation of 3 observations

Table 6: Stability Studies – *In Vitro* Release Studies of Selected Formulation F7

Time (min)	Cum % Drug Release* at 4° ± 1°C After 30 Days	Cum % Drug Release* at 25° ± 2°C and 60% RH±5% RH After 30 Days	Cum % Drug Release* at 37° ± 2°C and 65% RH±5% RH After 30 Days
0	0	0	0
60	14.59±058	14.78±0.68	14.99±0.76
120	23.89±1.1	23.77±1.3	23.97±1.3
180	33.11±1.5	33.32±1.4	33.58±1.3
240	47.28±1.6	48.32±1.7	48.75±1.5
300	63.02±1.5	63.98±2.1	64.05±1.7
360	78.25±2.3	77.62±1.8	77.42±2.2
420	85.74±2.7	85.84±2.3	85.94±1.3
480	88.72±2.7	88.78±2.5	88.93±1.6

*Each value represented as mean ± Standard Deviation of 3 observations

The SEM of the selected formulation F7 after one month of stability study is shown in the Figure no.4

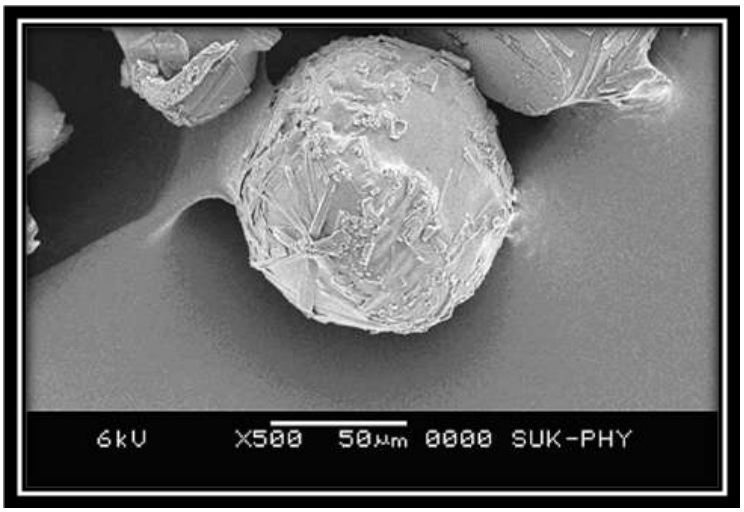

Figure 4: SEM of Formulation F7 after stability studies

These studies revealed that, there is a reduction in drug entrapment after storage for one month at $4^{o}\pm1^{o}C$, $25^{o}\pm2^{o}C$; 60% ±5% RH and $37^{o}\pm2^{o}C$; 65% ±5% RH. It was also revealed that formulation F7 stored at $4^{o}\pm1^{o}C$ showed maximum drug entrapment efficiency followed by the storage at $25^{o}\pm2^{o}C$; 60% ±5% RH and $37^{o}\pm2^{o}C$; 65% ±5% RH conditions.

CONCLUSION

1. The study showed that the Colloidosomes are having promising performance for loading of antidiabetic drug Glibenclamide. As the study showed that prepared formulation are having good surface morphology and loading efficiency. SEM analysis of the Colloidosomes revealed that all prepared colloidosomes was discrete, spherical in shape and had satisfactory surface morphology.
2. The high zeta potential value indicates high stability. This study confirms the stability characteristics of developed colloidosomes formulations
3. By varying the oil water ratio, it was found that Increase in water concentration in formulation leads to increase in particle size, % entrapment efficiency and slower release rate.
4. By performing in vitro drug release study it was observed that the drug release from the formulations slightly increases as the particle size of the formulation decreases.
5. Kinetic models were used to confirm release mechanism of the formulations. Glibenclamide release from all formulations followed zero order kinetics.
6. Stability studies for one month revealed that the formulations were stable up to at $40^{0}C$ at relative humidity of 75%. It should be stored in a cool and dry place.
7. The release profile was very promising as compared with the marketed formulation. The retardation in release is attributed to significant barrier provided by the partitioning of drug in oil phase to that water phase. The colloidal particle, CaCo3 adsorbed at the interface has given more rigid characteristics to the formulation and potentiated the retarded release of drug from formulation.

This implies that developed formulations have a potential to deliver the drug in controlled release manner. This outcome from release profiling strongly recommends that developed Glibenclamide loaded colloidosomes can be useful delivery carrier to deliver drug in controlled release manner which is a prime requirement for the treatment of Diabetes mellitus.

The future studies should be performed to asses the pharmacokinetic parameters of the formulations and to study scale up on large scale so as to give best alternative to existing formulation.

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