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SELF-EMULSIFYING SOLID LIPID MICROPARTICLES OF GLIBENCLAMIDE BASED ON AMPHIPHILIC NON-IONIC CAPRYLOCAPROYL MACROGOL-8-GLYCERIDE

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ABSTRACT

Solid lipid microparticles are drug carriers that can be used to improve the efficacy, bioavailability and stability of drugs. The drug delivery system can also be used to control or modify the release of active pharmaceutical ingredients. The aim of this study is to prepare and characterize self-emulsifying solid lipid microparticles of glibenclamide, a hypoglycaemic anti-diabetic agent, using caprylocaproyl macrogol-8-glyceride (LABRASOL®). The microparticles were prepared by hot homogenization technique and batches were prepared with glibenclamide (0.05%w/v), stearic acid (1.5-2.5%w/v), MAISINE® oil (0.5-1.5%w/v), LABRASOL® (1-2%w/v), sodium benzoate (0.5%w/v) and distilled water. The dispersion of self-emulsifying solid lipid microparticles were evaluated for particle size, viscosity, pH, self-emulsification time, effect of aqueous dilution and centrifugation on stability, encapsulation efficiency and *in vitro* drug release and diffusion study. The results showed relatively stable formulations with self-emulsification time of 1.75-4 min, particle size range of 6.0 – 47.6µm, pH of around 6, viscosity of 8-78 mPas, encapsulation efficiency of mostly around 30%. Optimized formulations showed almost 100% drug diffusion through dialysis membrane in 30 min. In conclusion, the self-emulsifying solid lipid microparticles showed satisfactory physicochemical properties and diffusion capabilities, and are valuable carriers for glibenclamide in the management of diabetes mellitus.

KEYWORDS: Particle size; self-emulsification; diffusion.

INTRODUCTION

Solid lipid microparticles and self-emulsifying drug delivery systems are carrier systems that can effectively deliver active pharmaceutical ingredients.

Solid lipid microparticles (SLM) are micro-scale drug carriers possessing matrix made from fatty acids, glyceride, fatty alcohol and solid wax with high melting points.[1] The entrapment of biologically active substances in fabricated micro-containers may provide the benefit of controlled drug release, protection against degradation, reduction of irritations and side effects and improved drug bioavailability amongst other advantages.

Self-emulsifying drug delivery systems are isotropic mixtures of oils, surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/ co-surfactants. These self-emulsifying formulations also contain solubilized drugs. Upon mild agitation after dilution in aqueous media such as gastro-intestinal fluids, these systems can form fine oil-in-water (o/w) microemulsions that provide effective dispersion of drugs.[2] Emulsification process may be associated with the ease with which water penetrates the oil-water interface with formation of liquid crystalline phase resulting in swelling at the interface, thereby causing increased ease of emulsification.[3] Solubilization of drugs, especially those with poor or low aqueous solubility, will facilitate their absorption and efficacy.

Glibenclamide is a second-generation sulfonylurea antidiabetic agent which lowers blood glucose acutely by stimulating the release of insulin from the pancreas, although extrapancreatic mechanisms may also be involved. Glibenclamide is a drug administered in the management of Diabetes Mellitus. The drug is practically insoluble in water and formulation in lipid-based carriers is a viable channel to improve its

bioavailability and therapeutic efficacy. The aim of this study is to formulate and evaluate self-emulsifying solid lipid microparticles of glibenclamide using amphiphilic non-ionic surface active agents.

MATERIALS AND METHODS

Materials

Stearic acid, LABRASOL®, MAISINE® oil (Gattefosse, France), glibenclamide powder (Juhel Pharmaceuticals, Nigeria).

Method of preparation

Hot homogenization method [4] was used in the preparation of the glibenclamide solid lipid microparticles. Eight batches of the formulations were prepared with different concentrations of excipients as expressed in Table 1. Glibenclamide was dispersed in molten stearic acid and MAISINE oil according to Table 1 at 75 °C in a water bath. Also LABRASOL and sodium benzoate were dispersed in distilled water at same temperature. The lipid mixtures and aqueous dispersions were mixed to form a pre-emulsion. These were further homogenized using a homogenizer (Ultra-Turrax® T25 basic digital, Ika Staufen, Germany) at 5000 rpm for 5 min, to produce solid lipid microparticles dispersions. The relative amounts of the LABRASOL, stearic acid and MAISINE oil allows for self-emulsifying qualities. This was the procedure used for all the batches which contained the same concentration of the drug (glibenclamide) but different concentration of excipients.

Table 1: Quantities of constituents used in glibenclamide self-emulsifying microparticles formulations.

Ingredient	Batch A	Batch B	Batch C	Batch D	Batch E	Batch F	Batch G	Batch H
Glibenclamide	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g
Stearic acid	2.5g	1.5g	1.5g	2.0g	2.0g	1.5g	1.83g	1.75g
Maisine oil	0.5g	1.5g	0.5g	1.0g	0.5g	1.0g	0.83g	0.9g
Labrasol	1.0g	1.0g	2.0g	1.0g	1.5g	1.5g	1.33g	1.35g
Sodium benzoate	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g
Water Qs	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

Particle morphology and size determination

Each batch bottle was shaken after which a drop of the formulation was collected and placed on a clean glass slide along with a drop of distilled water to obtain a dilute concentration of the sample on the slide and thus an appropriate distribution of the particles. The slide was then viewed using a light microscope with an attached camera (Motic, China) and the size and morphology of the solid lipid particles examined.

Viscosity of the formulation

The viscosity of the dispersions of solid lipid microparticles was determined using a viscometer with rotor no 2# at a speed of 60 rpm and values were recorded in milli Pascal seconds, (mPa.s). An optimization analysis was done based on a simplex centroid design using results of viscosity and design expert® 12 software.

Determination of self-emulsification time

A 0.1 ml quantity of each of the eight samples of different batches was added to 100 ml of distilled water (in eight different beakers, respectively) maintained at 37 ^{0}C with gentle agitation of the content in each beaker using a magnetic stirrer and timer started. The samples were examined and the time taken for each batch of sample to develop homogenous emulsion was recorded. An optimization analysis was done based on a simplex centroid design using results of self-emulsification time and design expert® 12 software.

Determination of pH of the formulations

Using a pH meter, the pH of dispersion of each batch of the solid lipid microparticles was obtained and recorded as well. The pH of the dispersions was evaluated using a validated pH meter (HANNA Instruments, Padova, Italy). The electrode part was immersed into 50 ml of the dispersions and the reading recorded.

Physical stability testing

The batches were left to stand without agitation, immediately after preparation, for about 30 min and their resulting appearances was noted. Also after three weeks, their results were all observed and recorded.

Centrifugation test

A 2 ml quantity of each fresh sample was transferred into a bottle and 2 ml of phosphate buffer was added. The resulting solution was centrifuged at 3000 rpm for 30 min. The samples were then observed carefully for drug precipitation, as well as phase separation.

Aqueous dilution test

The test was done for all the batches of the formulation to check for instability. One milliliters of each batch of formulation was transferred into a 100 ml beaker then the volume was made up to 10 ml with fresh phosphate buffer and stirred with a glass stirrer. The solution was thereafter observed after 20 min to know if aggregation occurred.

Encapsulation efficiency

A 2 ml quantity of each fresh sample was transferred into a bottle and then 2 ml of phosphate buffer was added. The resulting solution was spun at 3000 rpm for 30 min. The supernatant from the centrifugation was collected and filtered. From the filtrate, 1ml was collected and diluted with 4 ml of phosphate buffer pH 7.4. The solution obtained thereafter was analyzed for absorbance readings using a UV-VIS spectrophotometer (Jenway,

India). The encapsulation efficiency was calculated using the formula in Equation 1,

Encapsulation efficiency % = $\frac{W_{total} - W_{free}}{W_{total}} \times 100 \ldots\ldots\ldots\ldots\ldots 1$

Where W_{total} is the weight of the drug added to the system

W_{free} is the weight of free drug added to the medium found in the supernatant

In vitro drug release and diffusion study

The *in vitro* release was studied using dialysis method with modifications.[4] Here the dissolution medium was 200 ml of phosphate buffer, pH 7.4, maintained at a temperature of 37 ^{0}C. For each batch, 2ml of the formulation was placed in 6 cm of the dialysis membrane which was tied at one end before the formulation was introduced and the other end tied after placing the formulation. The dialysis membrane and content were placed in a mini basket cage which was suspended into a beaker containing the dissolution medium. The beaker was standing on a magnetic stirrer set-up and content stirred at speed of 50 rpm.

At intervals of 30 min, aliquots of 5 ml were withdrawn from the solution in the beaker and replaced with 5 ml of fresh phosphate buffer. The 5 ml test solutions removed at every 30 min was analyzed using the UV-VIS spectrophotometer to obtain the drug content. This method was used for all the batches of the formulations.

RESULTS AND DISCUSSION

Particle morphology and size

The particles dispersed in the formulations were largely spherical in shape although some are not perfect spheres. This is expected since the self-emulsifying capacity of the system may lead to gradual erosion of the periphery of the suspended particles into the continuous phase. The images of the particles in each batch are presented in Fig.1. The particles in the formulations showed sizes within the range of 11-49 µm. The dispersed particles are within the lower micro range. This implies that the formulation can be administered without discomfort and the product can provide intermittent emulsified delivery of active constituent.

Viscosity

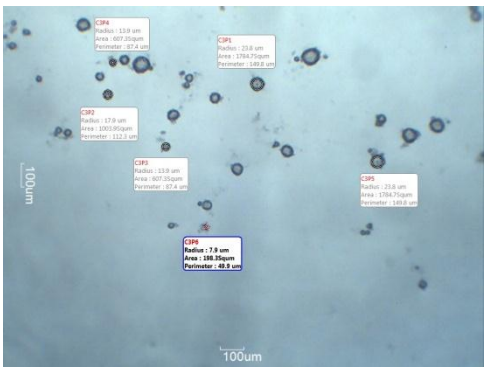
The formulations were of relatively low viscosity (Table 2) and this can be said to be due to the low concentrations of the stearic acid employed for the study and this is desirable to ease the process of self-emulsification, facilitate drug release and diffusion. Batch A (Stearic acid 2.5%w/v, MAISINE oil 0.5%w/v, and LABRASOL 1%w/v) showed the lowest viscosity of 8 mPas. Batches with the minimum amounts of LABRASOL® surfactant (caprylocaproyl macrogol-8-glyceride) showed the lowest viscosity. Although batch C contained higher quantity of LABRASOL, it still showed low resistance to flow (viscosity of 11 mPas) since stearic acid and MAISINE oil were both present at minimal amounts. The result showed different combinations of LABRASOL®, MAISINE® and stearic acid resulted in interactions that caused synergisms and antagonisms with respect to viscosity.

Based on optimization results obtained using Design expert® 12 software, Equation 2 can be used to predict viscosity from any combination of the variables. The equation is a special cubic model.

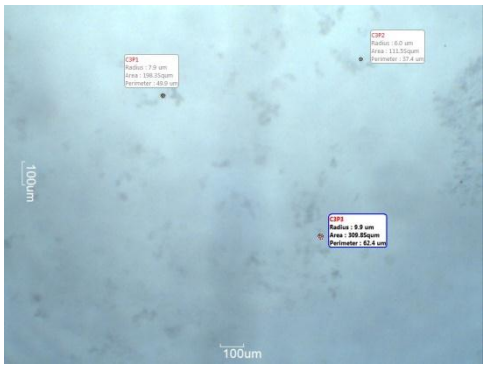
Viscosity = 8.28A + 18.15B + 11C – 3.73AB + 238.17 AC + 247.8 BC – 765.32 ABC …2

Where A, B, C represent Stearic acid, MAISINE and LABRASOL levels, respectively.

The equation further confirmed that Labrasol has a significant effect on the viscosity. At minimum Labrasol and mid-level stearic acid and Maisine (AB), a slightly negative or reductive effect was observed on viscosity. Furthermore, mid-level Labrasol and stearic acid or Maisine caused a high positive or incremental impact on viscosity. At centroid levels of the three (ABC), an even greater reductive effect was observed. The 3D response surface plot and contour plot are presented in Figs.2 and 3.



A



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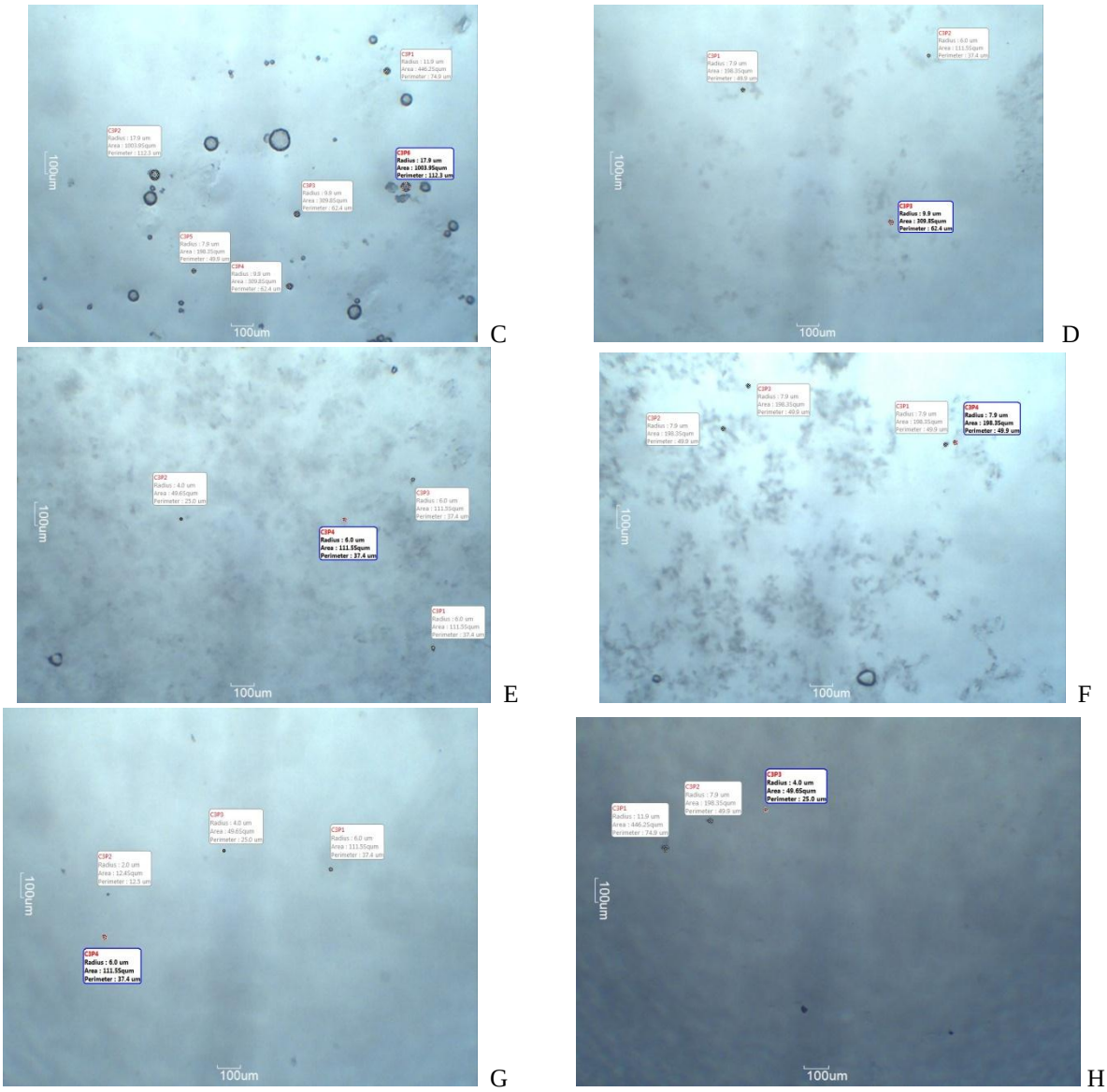

Fig. 1: Photomicrograph of batches A,B,C,D,E,F.G and H prepared with different mixtures of stearic acid, MAISINE oil, LABRASOL.

Table 2: Particle size, Viscosity and Self-emulsification time of the formulations.

Batch	Particle size (µm)	Viscosity (mPas)	Self-emulsification time (Min)
A	34.75	8.0	3.0
B	48.6	18.5	2.0
C	28.8	11.0	2.08
D	17.8	12.0	1.75
E	11.0	68.2	2.00
F	15.8	78.5	3.03
G	12.95	51.0	4.00
H	15.9	27.5	3.00

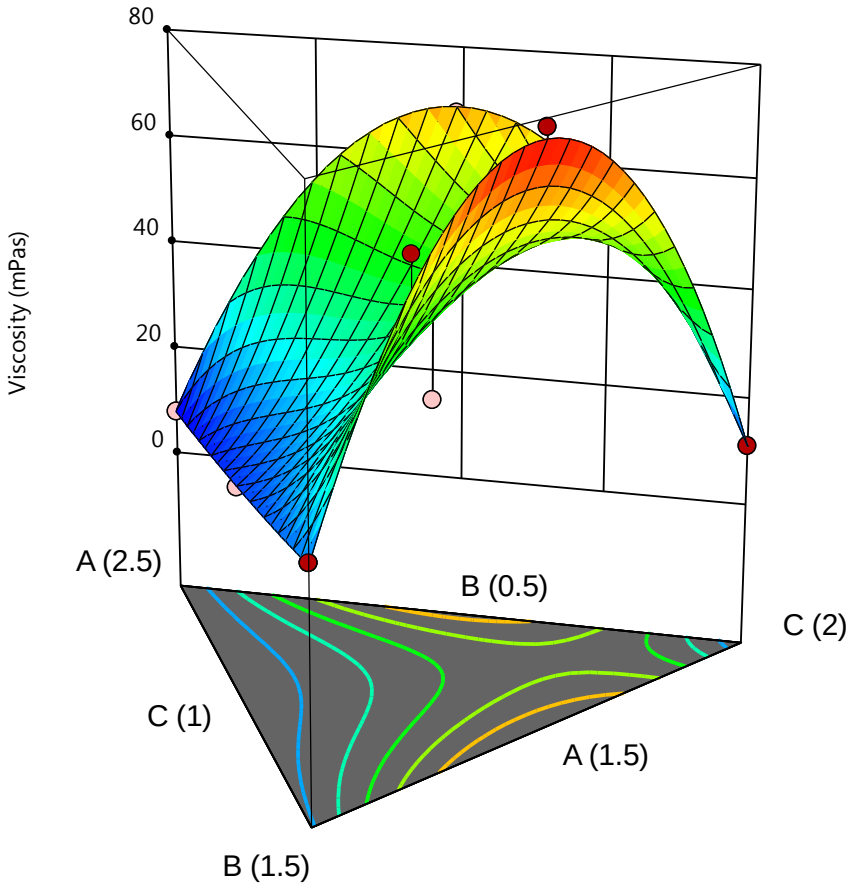

Fig. 2: 3D Response surface plot of viscosity based on mixtures of Stearic acid (A), MAISINE (B) and LABRASOL (C).

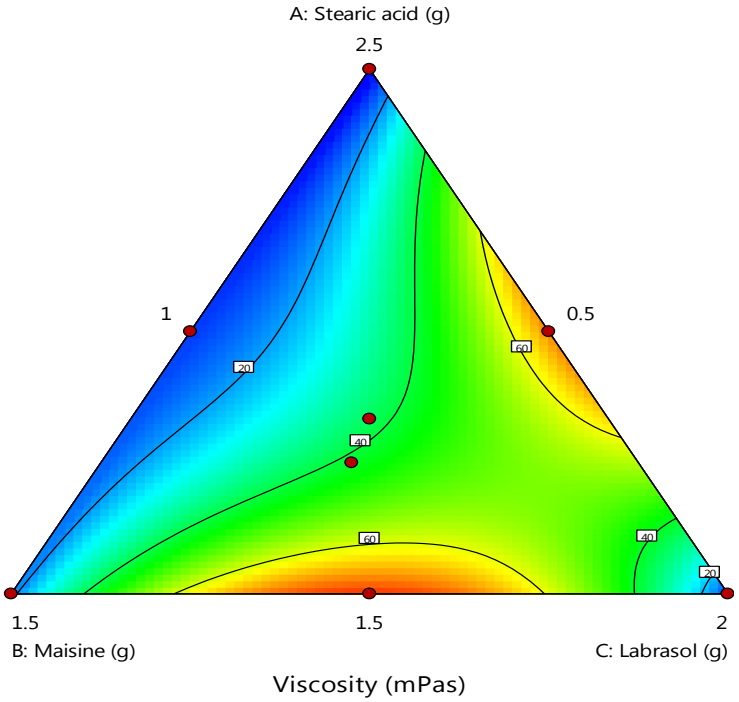

Fig. 3: Response surface contour plot of viscosity based on mixtures of Stearic acid (A), MAISINE (B) and LABRASOL (C).

Self-emulsification time

The time taken for the various batches of formulations to form homogenous oil-in-water emulsions was observed and results presented in Table 2. The results showed that all the batches were able to form homogeneous whitish dispersions within the time recorded and batch D was the fastest at 105 sec (1 min 45 sec). Batch D contains stearic acid, Maisine oil and Labrasol at concentrations of 2 %w/v, 1%w/v and 1%w/v, respectively. During the self-emulsification process, the lipid formulation becomes exposed to more volume of water which causes further emulsification especially with peripheral surfactant having access to the water.

Based on optimization results obtained using Design expert® 12 software, Equation 3 can be used to predict self-emulsification time from any combination of the variables. The equation is a special cubic model.

$SET = 3.01A + 1.99B + 2.08C - 2.95AB - 2.03AC + 3.69BC + 34.64ABC$ …….3

*SET is self-emulsification time

Where A, B, C represents Stearic acid, MAISINE and LABRASOL levels, respectively.

The optimization data (Equation 3, Figs. 4 and 5) showed that increase in each of the components of the mixture causes a slight increase in self-emulsification time, although more effects were observed with stearic acid. The combined effect of the three at centroid levels showed more increase in self emulsification time. However a reductive effect can be obtained when stearic acid and MAISINE or stearic acid and LABRASOL are maintained at moderate level while the third component is maintained at low levels, respectively. There is an increasing interest in the use non-ionic tensides both as a surfactant and as a co-surfactant because of high stability, low toxicity, low irritancy and biodegradability of many non-ionic surfactants.[5] However, it has been reported that compositional variables including oil, presence of other amphiphiles, hydrophilic molecules or electrolytes and temperature may have an influence on hydrophilic and hydrophobic properties, the geometry of the surfactant molecule and the capacity of a surfactant to generate microemulsion.[6]

pH of formulations

The pH of the formulations was around 6. Despite changes in the concentrations of LABRASOL, MAISINE and stearic acid from their maximum to minimum values based on design model, the pH values of the batches were similar ($p > 0.05$). The mixtures of the lipids and amphiphilic surfactant did not significantly affect the pH values.

Physical stability

After 30 min of storage, batches A and D showed two slightly different layers of different viscosity. The formulations were redispersed by thorough agitation and restored their initial appearance after few minutes. After three weeks of storage, there was a thickening of a layer in the formulations. However, the system became homogenous after agitation.

Centrifugation

After 30 min of centrifugation, it was observed that there was minimal drug precipitation or particle aggregation with a small volume of clear supernatant for batches A, D, and H. These preparations have relatively higher amounts of stearic acid with less quantities of LABRASOL that should maintain balance and stability. For the other batches, the clear supernatant was of larger volume and with little precipitation or aggregation. No phase inversion was observed in any of the bottles containing the batches of the formulation. Also no creaming or cracking of the formulations were observed after centrifugation. Centrifugation is explored as a mechanical process that employs applied centrifugal force fields to facilitate separation of components of a mixture according to density and/or particle size.[7]

Dilution

The formulations behaved differently upon dilution in the presence of water. Formulations with relatively higher concentrations of MAISINE oil (Glycerol monolinoleate) were most stable upon dilution. This shows the importance of liquid oil in emulsification and dilution stability. Stearic acid tends to resolidify after initial heat-based liquefaction and nanostructuring with Maisine oil was designed to improve accommodation of more guest drug molecules. Dilution generally leads to globule divisions and spontaneous curvature of surfactant films in liquid disperse phases but since the prepared formulations have solid or semisolid disperse phase, the break-up is relatively slow and partial with MAISINE oil probably providing the internal structure for solid lipid disruption.

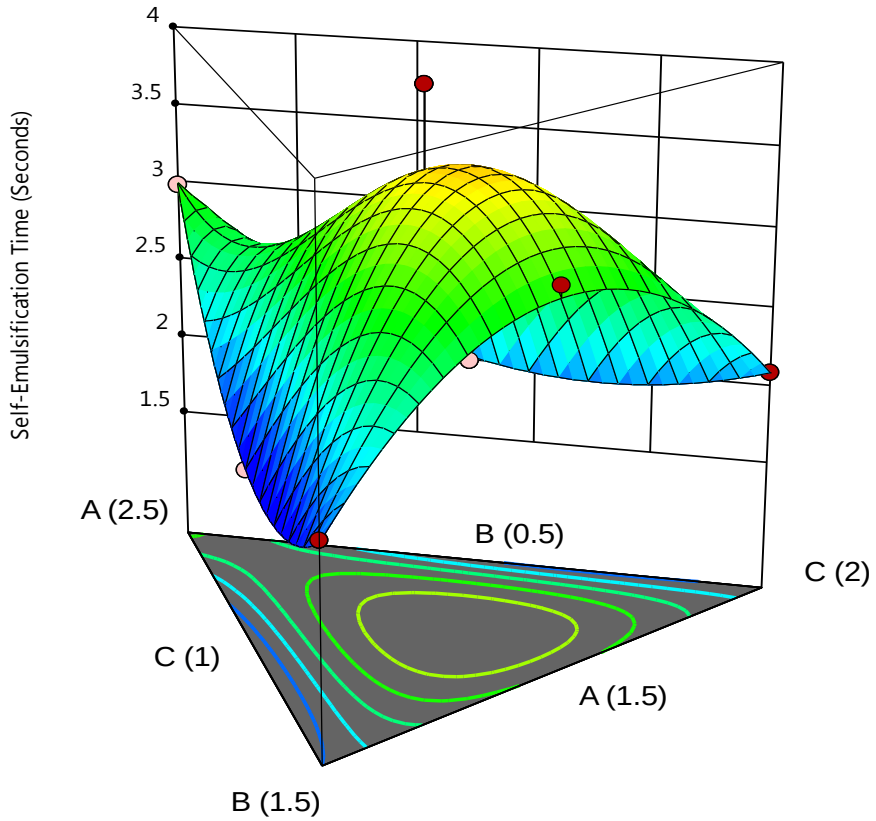

Fig. 4: 3D Response surface plot of self-emulsification time based on mixtures of Stearic acid (A), MAISINE (B) and LABRASOL (C).

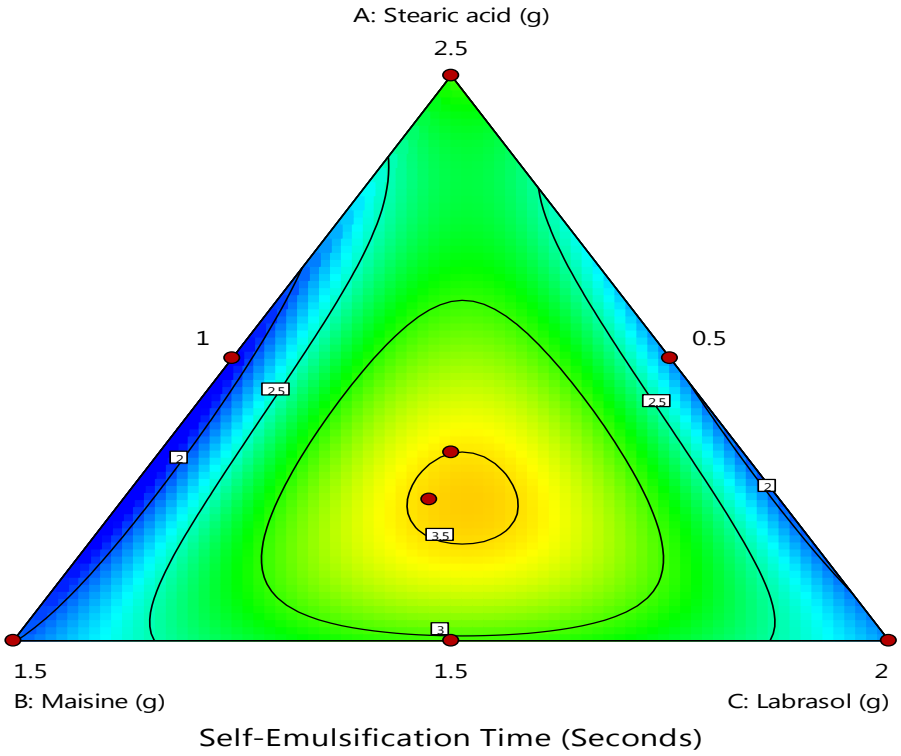

Fig. 5: Response surface contour plot of self-emulsification time based on mixtures of Stearic acid (A), MAISINE (B) and LABRASOL (C).

Table 3: Percentage drug encapsulation efficiency of the formulations.

Batches	% Drug Encapsulation efficiency
A	28
B	30
C	36
D	38
E	12
F	10
G	24
H	20

Drug release and permeation through dialysis membrane

The passage of glibenclamide from the formulations through dialysis membrane was assessed and the result (Fig. 6) showed that batches A and D containing minimum amounts of LABRASOL® and high or mid-level Stearic acid/MAISINE®, respectively, had the highest percentage drug release. These two formulations showed relatively low viscosity and self-emulsification time. The combined effect of these attributes may have facilitated high rate of drug release, permeation and diffusion shown by the two batches. Batches A and D showed 96% and 98% drug released and permeated through the membrane, respectively, after 30 min while both showed 100% release and permeation after 1 h. For almost all the batches, the drug release became steady after an initial burst release. This shows that the optimal formulations can effectively deliver glibenclamide in the management of diabetes mellitus. The low viscosity of the formulations may have aided the drug release and diffusion. From Fick's law of diffusion, it is obvious that the amount of drug in solution that passes through a unit area of a dialysis membrane is directly proportional to the concentration difference across the membrane. Lower viscosity implies that already permeated drug can move away from the membrane, thereby allowing more drug molecules to pass through. A sink condition is favoured with reduced viscosity. A reduced self-emulsification time would also imply effective solubilization of drug within the formulation and this may aid drug delivery, permeation and diffusion. After human ingestion, digestion of formulations will surely have a profound effect on the state of dispersion of the lipid formulation and the biological fate of the drug.[8] Digestion of dietary triglycerides in the small intestine is usually very rapid and many non-ionic esters such as mixed glycerides and surfactants will be substrates of pancreatic lipase enzymatic activity.[9]

The droplet size of an emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release, and consequently, absorption.[10] The particle size of the emulsifying particles and the droplet size of the resulting emulsion from free and molten lipid have effect on drug release. In the GIT, the body temperature of 37 °C would facilitate liquification of the solid particles with formation of oil droplets dispersed in aqueous medium and subsequent decrease in the size of the droplets in vivo.

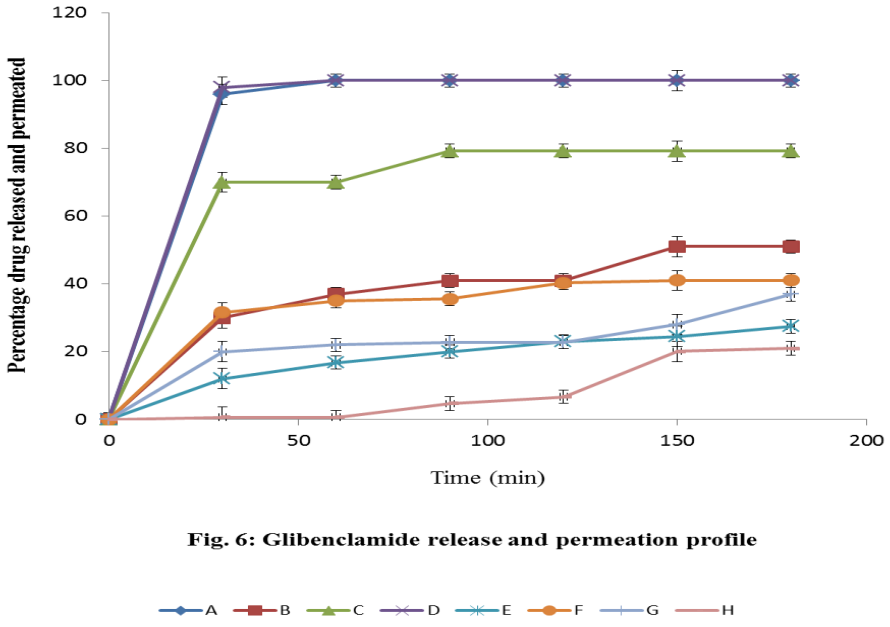

Fig. 6: Glibenclamide release and permeation profile

CONCLUSION

The self-emulsifying solid lipid microparticles showed satisfactory physicochemical properties and diffusion capabilities, and are valuable carriers for glibenclamide in the management of diabetes mellitus. The formulations contained both lipid-entrapped drug and free drugs solubilized by LABRASOL, a caprylocaproyl macrogol-8-glyceride. This arrangement would allow for immediate release of glibenclamide from a self-emulsifying entity, followed by prolonged release of the lipid-entrapped portion of the drug. The study clearly showed that batches or combinations that resulted in low viscosity and self-emulsification time generally caused improved drug release, diffusion and permeation.

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