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Research Article

Development of Anti-diabetic Niosomes Formulation Containing Metformin and Gliclazide

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ABSTRACT

Metformin/Gliclazide niosomes were formulated with span 60 by ether injection method. Three batches MG1-MG3 were prepared in order to study influence of drug polymer ratio on the niosomes formation and *in vitro* drug release. The formulated niosomes were characterized by drug entrapment, vesicle size determination, and *in vitro* drug release. Optimized concentration of span 60 and cholesterol was found to be 1:1. In the *in-vitro* study, niosomes formulation of MG1 showed high percentage of drug release, 40.18 to 45.75% for about 8 hrs. This indicated that this batch of niosomes formulation exhibit sustained drug release pattern as the niosomes act as reservoir system for continuous delivery of drug. The quantity of Metformin/Gliclazide present in the niosomes and the release medium were estimated by a validated HPLC method. The formulated niosomes had acceptable physicochemical characters and released the drug over 6-8 h. The data obtained from *in vitro* release studies were fitted with various kinetic models and was found to follow Higuchi kinetics.

Introduction

Diabetes is a disease with skyrocketing prevalence. The number of ~280 million patients today will increase to ~450 million in 2030. Therefore, new approaches to research and development (R&D) in the pharmaceutical industry are needed in how we work and what we work upon [1]. Metformin hydrochloride and Gliclazide are oral hypoglycemic agents belonging to biguanide group and second generation sulphonyl urea, respectively (Fig. 1). Generally, they are individually used in the treatment of type II non-insulin dependent diabetes mellitus [2]. The combinations of both the drugs are more effective than individual therapy. Metformin acts by decreasing hepatic glucose production and improves insulin sensitivity by increasing peripheral glucose uptake. Because of its shorter and variable biological half-life of 1.5-4.5 h, it should be repeatedly administered (500 mg thrice a day) to maintain effective plasma concentration. Gliclazide reduces the glucose level by direct stimulation of insulin release from beta cells of pancreatic islet. Relatively Gliclazide is having longer biological half-life (6-15 h) depending upon individual and the dose is 80 mg two-three times a day with meals. Combination of Metformin-500 mg and Gliclazide-80 mg per tablet is available in India and it is to be taken two to three times a day to get required effect [3, 4]. Recently, lipid and nonionic surfactant based drug delivery systems specifically niosomes have drawn much attention from researchers as potential carriers of various bioactive molecules that could be used for therapeutic applications. Several commercial niosome-based drugs have already been marketed with a great success [5,6].In the present study, we attempted to formulate Metformin/Gliclazide dual drug loaded niosomes by using span 60 and cholesterol, with an intention to extend the drug release over a period of 8-12 h, hence it can be taken once a day. The formulations were analysed for drug content by a validated HPLC technique developed in our laboratory and characterized by various physicochemical parameters such as drug entrapment, vesicle size, and *in vitro* drug release.

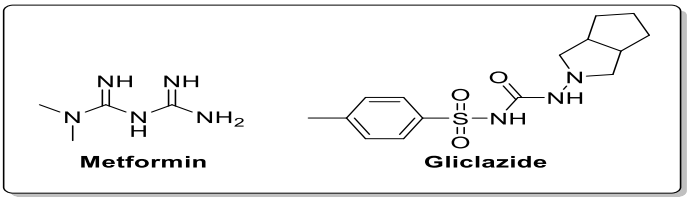

Fig 1: Representative structure of anti-diabetic drugs

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Materials and Methods

Chemicals

Span 60 (sorbitanemonoesterate, CDH lab reagent), Cholesterol (LOBA CHEME), Amoxycillin drug (Zydus Cadila), Chloroform (Ramkem), Phosphate Buffer Saline (pH 7.4), 0.1 N Hydrochloric acid, 0.1 N Sodium Hydroxide, Sodium Sulfate, Sulfuric acid, 0.1 N Glacial Acetic acid, Ammonium Hydroxide, Sodium Chloride, Ethanol, Methanol, Acetone, Dialysis membrane (LA390-60), Nutrient Agar and Dialysis membrane were from Hi Media.

Instruments

Hot air oven (Scientech,325 L), Incubator (Scientech), Digital Balance (Denver, Germany), Autoclave vertical, Laminar air flow chamber horizontal (Scientech), Shimadzu 1800 Double Beam UV-VIS Spectrophotometer (Japan), Centrifuge (Remi C-24 India), Vortex Mixer (Remi), Magnetic stirrer with hot plate (Remi), Digital pH meter (Eutech, Singapore), Vacuum Rotary Evaporator (Buchi Type), Trinocular Microscope (Olympus CH 20 I) with Ocular Micrometre.

Preparation of niosomes

Ether injection method: A mixture of Span 60 and cholesterol in different ratio was added to a beaker containing 10 ml diethyl ether organic solvent. To this mixture, 250 mg of amoxycillin was added portion wise with continuous stirring and maintaining a temperature of 30 ºC. This heated solution was finally filled in a 20 mL syringe. A solution 125 mg clavulanic acid dissolved in 10 mL phosphate-buffered saline was prepared separately. The contents of the syringe were injected drop wise into a mixture with continuous stirring [7].

IR Spectroscopy

Fourier transform infrared spectroscopy has been used to study the purity of API. The standard amoxicillin and API sample were mixed separately with IR grade KBr and were scanned over a range of 400-4000 cm^{-1} using FTIR instrument (FTIR-1700, Shimadzu, Kyoto, Japan).

HPLC

Stock solution of Amoxicillin was prepared by dissolving 20 mg of Amoxicillin in 200 ml standard volumetric flask containing approximately 200 ml of buffer solution and the solution was sonicated for 20 min and then the volume was made upto the mark with mobile phase. Subsequent dilutions of 2 ml to 20 ml this solution were made with mobile phase. The standard solutions prepared as above were injected into the 10 µL loop and the chromatogram was recorded.

Chromatographic condition:

Mobile phase: [ACN]: [Buffer]:: 4:96
Flow Rate: 1.5 ml/min
Spectrophotometer: 230 nm
Injection volume: 10 µL

Results and Discussion

Niosomal formulations of metformin- gliclazide were prepared with Span 60 as surfactants. Span 60 yields very stable niosomes with a narrow particle size distribution but they exhibit rather low entrapment efficiency. By adding cholesterol to surfactant in a 1:1 weight ratio both stability and entrapment efficiency increased. In addition, surface of Span 60 niosomes is flexible due to their head group structure.

Table 1: Formulation of Niosomes

S. No.	Formulation Code	Span 60	Cholesterol	Solvent	Drugs
1.	MG1	50 mg	50 mg	10 mL DEA	500 mg metformin + 60 mg gliclazide
2	MG2	50 mg	100 mg	10 mL DEA	500 mg metformin + 60 mg gliclazide
3	MG3	100 mg	50 mg	10 mL DEA	500 mg metformin + 60 mg gliclazide

Dynamic laser scattering was performed to characterize the size of the MG1, MG2 and MG3 niosomal formula. The formula showed small micro-vesicular size (8.15-10.50 µm) (Fig. 2). It was also really worth to mention that such micro-size ranged vesicles represent a good pharmaceutical carrier for passive targeting to infected cells.

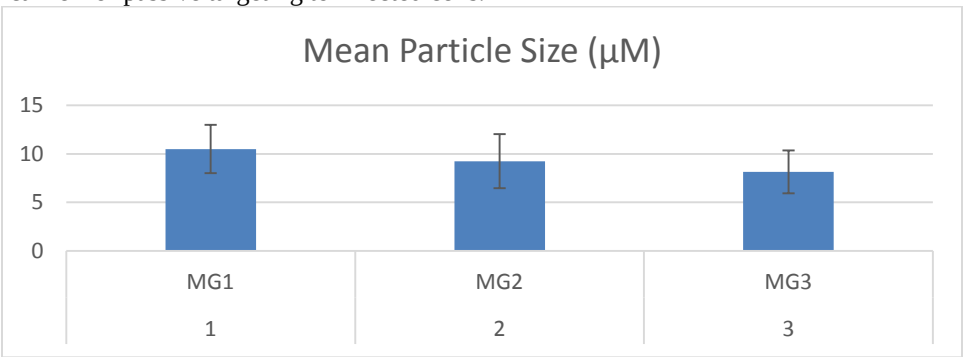


Fig 2: Mean particle size of niosomal formulations

In-vitro release

Release data expressed as percent drug released over 8 hours determined for three formulations are shown in Fig. 3. The release profile of the free unentrapped metformin and gliclazide showed that 75% of the drug was released within one hour. In comparison, the release profile of metformin from the niosomal formulations showed that 40.18% of the drug was released in 8 hours, reflecting the sustained release of metformin from the niosomal formulations. Similarly, the release profile of gliclazide from the niosomal formulations showed that 45.75% of the drug was released in 8 hours. The results of release profiles of the binary drugs indicate the effect on release of the changes in proportion of free to niosome-entrapped drug in the prepared formulations.

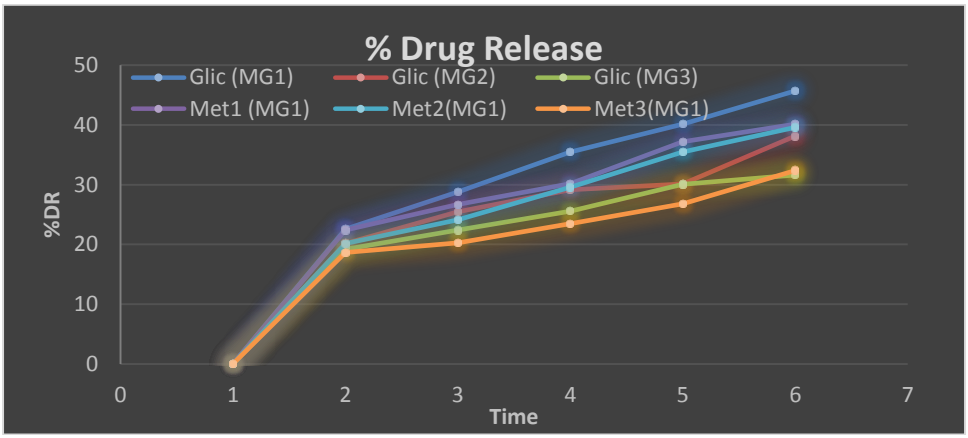


Fig 3: % Drug released of niosome formulations MG1, MG2 and MG3

Compatibility studies

Excipients compatibility studies the results depicts there was no change in color, no lump formation occurred in any of the mixture at different temperature & humidity conditions, when observed on different days (7th, 15, 30th days) interval in comparison to initial observation on 0th day. This confirmed that both the drugs were compatible with each other as well as with excipients. R_f values obtained from TLC studies on (7th, 15, 30th days) were approximately similar to R_f values of pure drugs and niosomes obtained on 0th day, predicting the compatibility of both drugs with gel excipients.

FT IR

Drug excipients interaction was also checked out by comparing the FT IR spectra of pure drug metformin, gliclazide and FTIR spectra of the physical mixture of drugs with excipients; span 60 and cholesterol. The FT IR spectra in this region of metformin-gliclazide binary system, of molar ratio 1:1, are shown in Fig 4. Frequencies assigned to primary amine N-H stretching, secondary amine N-H and imino C=N group of metformin whereas C=O and N-H stretching of gliclazide, all the bands were identified in free metformin-gliclazide and are reported in Table. IR spectra indicate no significant difference in characteristic peak at wave numbers of the drug in presence of the excipient. Thus, IR spectra indicated no drug-excipient interaction.

Table 2: Assignment of relevant IR absorption bands of metformin-gliclazide and niosome formulation

Assignment	Metformin(cm^{-1})	Gliclazide(cm^{-1})	Formulation MG1 (cm^{-1})
1º amine N-H	3370	-	3410
2º amine N-H	3216	-	3200
C=N	1678	-	1710
N-H (amide)	-	3300	3400
C=O	-	1590	1580

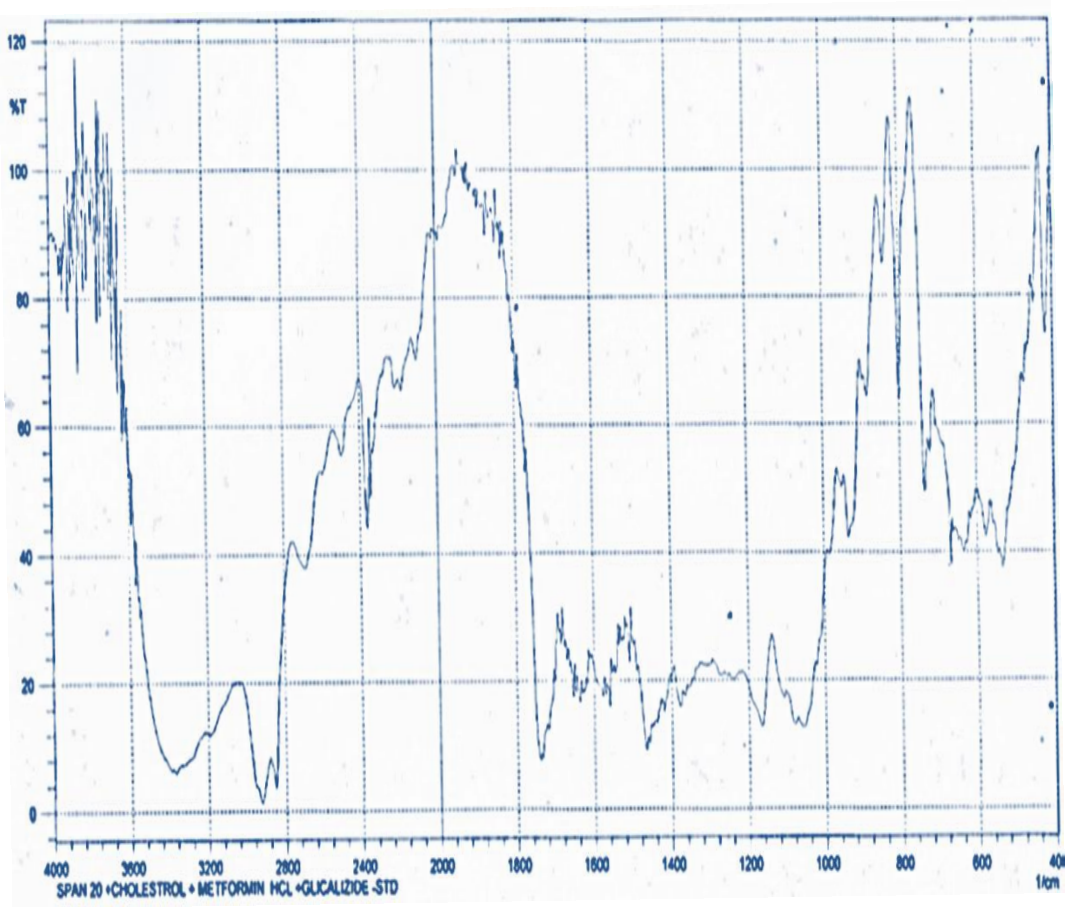
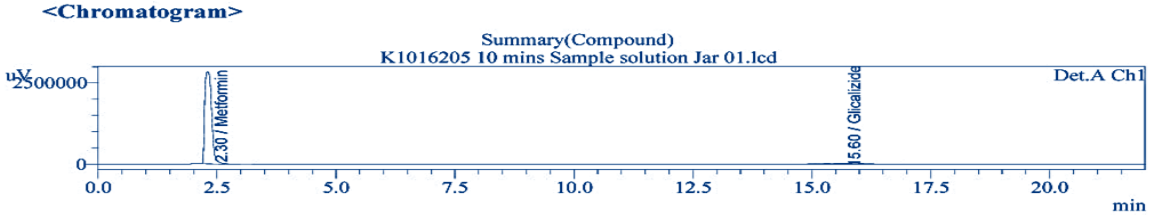


Fig 4: IR spectrum of formulation MG1

HPLC

The evaluation of the stability and compatibility of metformin and gliclazide binary mixtures with the span 60 and cholesterol after one month at 40°C and 75% RH was conducted using the HPLC-UV method described in the relevant USP monograph. The response was linear within the range studied (R^2 = 0.9870) (Fig. 5). The HPLC-UV method for quantitation of metformin and gliclazide can detect thermal degradation and chemical interactions with excepients, which decrease the concentration of drugs. The chromatograms of binary mixtures are shown in Figure. No alteration in peak area was detected, indicating that these excepients have sufficient compatibility for use in noisome formulation of metformin and gliclazide.



<< Detector A >>

ID#1 Compound Name: Metformin

Title	Sample ID	Ret. Time	Area	heoretical Plat	Tailing Factor
K1016205 10 mins Sample solution Jar (	Dissolution	2.30	28380187	1462	1.49

ID#2 Compound Name: Glicalizide

Title	Sample ID	Ret. Time	Area	heoretical Plat	Tailing Factor
K1016205 10 mins Sample solution Jar (	Dissolution	15.60	2885769	723	1.30

Fig 5: HPLC chromatogram of noisome formulation

Conclusion

Niosomes prepared from Span 60-cholesterol were able to encapsulate metformin and gliclazide. Average size, stability and RSV entrapment efficiency of niosomes are linked not only to their composition but also to the protocol employed in preparing the niosomes. Optimized concentration of span 60 and cholesterol was found to be 1:1. In the *in-vitro* study, niosomes formulation of MG1 showed high percentage of drug release, 40.18 to 45.75% for about 8 hrs. This indicated that this batch of niosomes formulation exhibit sustained drug release pattern as the niosomes act as reservoir system for continuous delivery of drug. In stability studies, the optimized formulation, MG1 Stability started to deteriorate from 2nd week where the niosomes vesicles are seen in non-spherical shape. On 21st and 28th day the niosomes formulation were examined and seen in non-spherical shape and are clumped for both storage condition. This is mainly due to disruption or aggregation of vesicles since it is exposed to chemical degradation like hydrolysis and oxidation. As for the drug release, niosomes formulation stored in room temperature and refrigerated condition showed 90% which mainly due to membrane-stabilizing effect of cholesterol. Thus, from the prepared niosomes formulation, it can be concluded that the vesicular system was more stable at 2º C - 8º C. The released data of optimized niosomes formulation MG1 of amoxicillin were analysed mathematically according to zero order, first order, and Higuchi equations. As for the Higuchi's model (r^2=0.9447), First order (r^2=0.8227), and zero order (r^2=0.7577) regression co-efficient obtained. The metformin drug release from niosomes does not obey first order kinetics, which means that the release of amoxicillin from the niosomes vesicle is independent to concentration gradient. Similarly, for gliclazide regression co-efficient obtained as Higuchi's model (r^2=0.9688), First order (r^2=0.8682), and zero order (r^2=0.7971). Best fitted Higuchi's model indicates that the drug release by diffusion.

Acknowledgement

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