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FORMULATION AND EVALUATION OF COLON TARGETED MICROSPHERES OF GLIPIZIDE

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ABSTRACT: The objective of the present study was to formulate and evaluate microspheres of Glipizide using guar gum and HPMC K4M as polymers for colon-specific delivery for better treatment of Type 2 diabetes. The glipizide microspheres were prepared by ionotropic gelation method by the use of various cross-linking agents. The microspheres were then studied for physical appearance, particle size, surface morphology, drug-polymer compatibility, and drug entrapment efficiency. The *in-vitro* drug release profile was studied in three different buffer medium using USP type I apparatus. Further, kinetic modeling was employed to find out the release mechanism. Glipizide microspheres showed high entrapment efficiency (89.80%) and the microspheres were free-flowing, non aggregated, and almost spherical between 500-700 μm in diameter. The FTIR spectrum showed that there is no interaction between the polymers and drug. The *in-vitro* release study found to be affected by using various cross-linking agents. The microspheres with barium chloride as cross-linking agents showing a small amount of drug release in acidic pH but show maximum drug release at the end of 12 h. It was found that the % cumulative release in microspheres encapsulated with guar gum was maximum for F2 (64.29%) and minimum for F6 (42.85%). The rate of drug release follows the Korsmeyer-Peppas model and zero-order kinetics. The colon targeted microsphere of Glipizide showed no change either in physical appearance, drug entrapment efficiency and dissolution pattern after performing stability study for 6 months. The study reveals that glipizide loaded microspheres can be used effectively for colon targeting.

INTRODUCTION: Colon targeted drug delivery (CTDD) refers to the targeted delivery of drugs into the lower GI tract, which occurs primarily in the large intestine (*i.e.*, colon).

Targeted delivery of drugs to the colon has attracted much interest recently for local treatment of a variety of colonic diseases such as irritable bowel syndrome (IBS), colorectal cancer, and inflammatory bowel diseases (IBD), which includes both ulcerative colitis and Chron's disease.

Apart from these local treatments, the colon is used for the systemic absorption of proteins and peptides and also for those drugs where a delay in drug absorption is required from a therapeutic point of view. Specific targeting of drugs to the colon is

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recognized to have several therapeutic advantages. Drugs, which are destroyed by the stomach acid and / or metabolized by pancreatic enzymes, are slightly affected in the colon, and sustained colonic release of drugs can be useful in the treatment of nocturnal asthma, angina, and arthritis. Treatment of colonic diseases such as ulcerative colitis, colorectal cancer, and Crohn's disease is more effective with direct delivery of drugs to the affected area. Likewise, colonic delivery of vermicides and colonic diagnostic agents require smaller doses¹. Glipizide is an anti-diabetic medication of the sulfonylurea class used to treat Type 2 diabetes. It is used together with a diabetic diet. It is active at very low doses, a characteristic feature of second-generation sulfonylureas.

It is practically insoluble in water and acid solution but as per the Biopharmaceutical Classification System (BCS), it is highly permeable. Glipizide having a short biological half-life (2-5h) requiring it to be administered in 2 to 3 doses of 2.5 to 10 mg per day. SR formulations that would maintain plasma levels of drug for 8 to 12 h might be sufficient for once a day dosing for Glipizide. Sustain release products are needed for Glipizide to prolong its duration of action and to improve patient compliance².

The objective of the study is to formulate and evaluate colonic microspheres of Glipizide using guar gum and HPMC K4M polymers. By directly targeting the drug to the colon, the maximum concentration of drug reaches and increases the residence time of the drug in the colon with improved patient compliance, lesser side effects, and an ideal drug delivery system. Different batches of Glipizide microspheres were prepared using a different cross-linking agent. The microspheres were then studied for physical appearance, entrapment efficiency, drug-polymer compatibility, surface morphology, *in-vitro* drug release studies.

MATERIALS AND METHODS:

Materials: Glipizide, guar gum and HPMC K4M were obtained from Yarrow chem. Products, Dombivali (E), Mumbai, sodium alginate from Chemvin Industries, Thrissur. All other chemicals and reagents used in this study were of analytical grade.

Methods:

Preparation of Calibration Curve of Glipizide:³

The stock solution was diluted accordingly to get a concentration of 100 µg/ml using phosphate buffer pH 7.4, 6.8, and 0.1N HCl (Solution B). From this solution 0.5, 1, 1.5, 2 and 2.5 ml solution were transferred into 10 ml volumetric flask to get 5, 10, 15, 20 and 25 µg/ml solutions respectively. The working standard solutions of glipizide were scanned in the UV region, and the absorbances were observed against phosphate buffer (pH 7.4, 6.8) and 0.1N HCl solution as blank at 276 nm. Finally, the calibration curve was plotted with a concentration on X-axis and respective absorbances on Y-axis.

Drug-Excipient Compatibility Study:

Fourier Transform Infra-Red Spectroscopy (FTIR):⁴

Preformulation studies regarding the drug-polymer interaction is very important in the selection of appropriate polymers. So the integrity and compatibility of pure drug glipizide with the polymers were studied using Fourier Transformed Infrared Spectroscopy (FTIR). The FTIR spectra of pure drug, polymers, and the physical mixture of drug and polymers were taken. The spectra are run between 4000 – 400 cm⁻¹.

Preparation of Colon Targeted Microspheres of Glipizide:

Required quantity of Guar gum and HPMC K4M individually were dissolved in distilled water (I). The same quantity of sodium alginate was dissolved in distilled water and 10mg of drug dispersed into this solution (II). I and II solution were mixed and dropped into a different cross-linking agent solution with 21 gauze needle. The microspheres allowed hardening in the solution for 15 min. Then microspheres were filtered and dried primarily at room temperature and after it, in a hot air oven at 35 °C for about 30 min. Microspheres were then stored in glass bottles, capped tightly. After that, microspheres were weight equivalent to the dose of the drug and filled manually into hard gelatin capsule shells².

Evaluation of Colon Targeted Microspheres:

Particle Size and Surface Morphology: Particle size was determined by optical microscopy. The most widely used procedures to visualize microparticles by using scanning electron microscopy (SEM). Morphological examination of

the surface and internal structure of the dried microspheres was performed by using a Scanning electron microscope (model-JEOL JSM-6390

England) using platinum sputter technique. The working distance is 50 micrometer. Photographs were taken with $100 \times$ magnifications ².

TABLE 1: FORMULATION COMPONENTS OF GLIPIZIDE MICROSPHERES

Formulation Code	Drug (mg)	Polymer	Drug-polymer ratio	Cross-linking agent	Conc. of cross-linking agent (% w/v)
F1	10	Guar gum	1:7	Calcium chloride	9
F2	10	Guar gm	1:7	Barium chloride	9
F3	10	Guar gum	1:7	Zinc sulphate	9
F4	10	HPMC K4M	1:7	Calcium chloride	9
F5	10	HPMC K4M	1:7	Barium chloride	9
F6	10	HPMC K4M	1:7	Zinc sulphate	9

Micromeritic Properties: Micromeritic properties including the angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio were determined to check the flow properties of microspheres ⁵.

Drug Entrapment Efficiency (DEE): ⁶ The amount of drug entrapped was estimated by crushing 100 mg of microspheres in 100 ml of phosphate buffer pH 7.4 for 24 h, the solution was then filtered, and the absorbance was measured by UV spectrophotometer against appropriate blank. The amount of drug entrapped in the microspheres was calculated by,

$$\text{DEE} = (\text{Amount of drug actually present}) / (\text{Theoretical drug load expected}) \times 100$$

In-vitro Drug Release Studies: A USP dissolution rate testing apparatus I basket type dissolution apparatus was used to study the *in-vitro* drug release from microspheres. A weighed amount of microspheres (equivalent to 10mg drug) was placed in the basket (basket covered with muslin cloth). Using 0.1 N HCl for first 2 h, phosphate buffer of pH 6.8 for next 2 h and phosphate buffer of pH 7.4 for last 6 h as dissolution medium. Distilled water and dissolution fluid were maintained at $37 \pm 0.5^\circ\text{C}$. An aliquot (5 ml) of the sample solution was withdrawn at predetermined time intervals, filtered through Whatman filter paper and analyzed spectrophotometrically at 276 nm for 0.1N HCl buffer, phosphate buffer of pH 6.8 and phosphate buffer of pH 7.4.

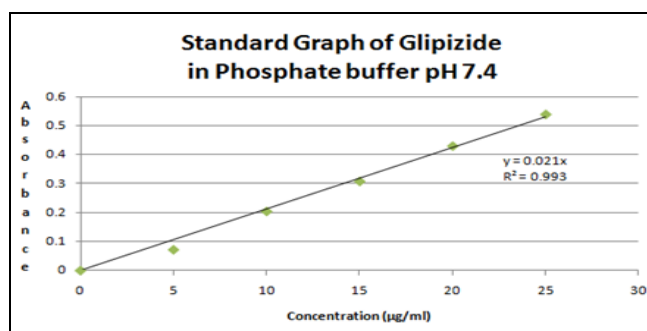


FIG. 1: STANDARD GRAPH OF GLIPIZIDE IN PHOSPHATE BUFFER pH 7.4

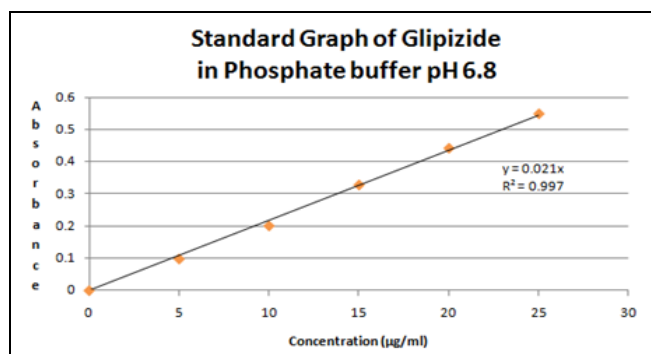


FIG. 2: STANDARD GRAPH OF GLIPIZIDE IN PHOSPHATE BUFFER pH 6.8

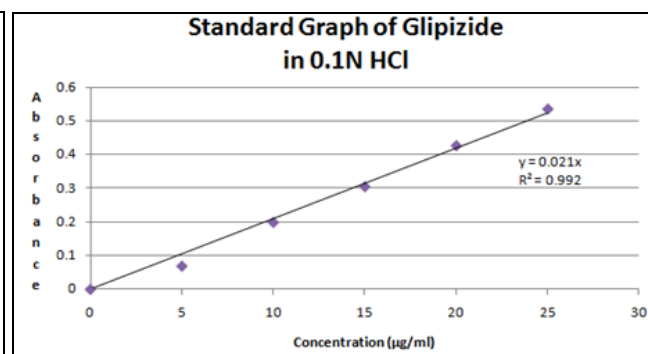


FIG. 3: STANDARD GRAPH OF GLIPIZIDE IN 0.1N HCl

Drug Release Kinetics of Best Formulation: To examine the drug release kinetics and mechanism, the cumulative drug release data were fitted to models representing zero order, first order, Higuchi's model and Korsmeyer-Peppas model⁸.

Stability Studies: The principles of accelerated stability studies are adopted. ICH specifies the length of study and storage conditions. Best formulation was stored at a different temperature at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ at $65\% \pm 5\%$ RH for 6 months and $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ at $75\% \pm 5\%$ RH for 6 months⁹.

RESULTS AND DISCUSSION:

Calibration Curve of Glipizide: The absorbance of the solution was measured at 276 nm (λ_{max} of glipizide) and plotted calibration curve by taking concentration and absorbance on X and Y-axis

respectively. The calibration curve was found to be linear in concentration range 0-25 $\mu\text{g/ml}$. The curve obeyed Beer Lambert's law is above concentration range.

Fourier Transform Infrared Spectroscopy: Drug- polymer compatibility studies were carried out using Fourier Transformed Infrared Spectrophotometer. The compatibility of pure drug glipizide with polymers used in the formulation, guar gum, and HPMC K4M was studied.

The individual spectra are compared with the physical mixture of drug and polymer, and no major differences were found in the absorption position of the peaks. Hence there are no incompatibility problems with the drug and polymers.

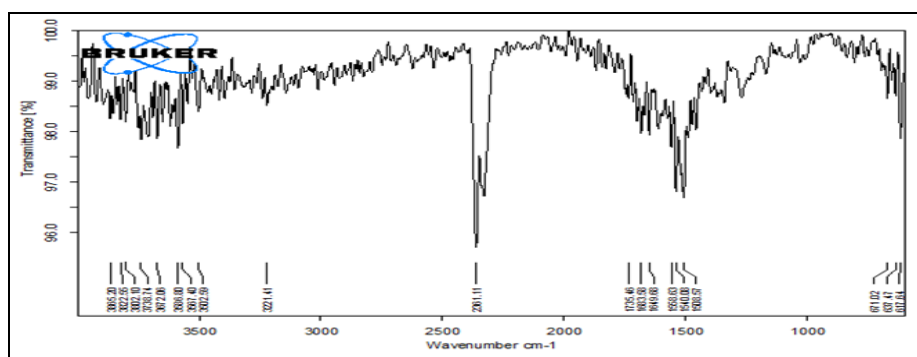


FIG. 4: FTIR SPECTRA OF GLIPIZIDE

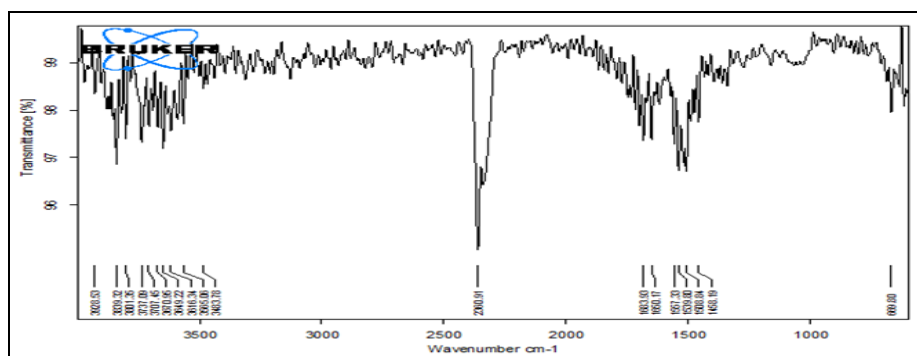


FIG. 5: FTIR SPECTRS OF GLIPIZIDE AND GUAR GUM MIXTURE

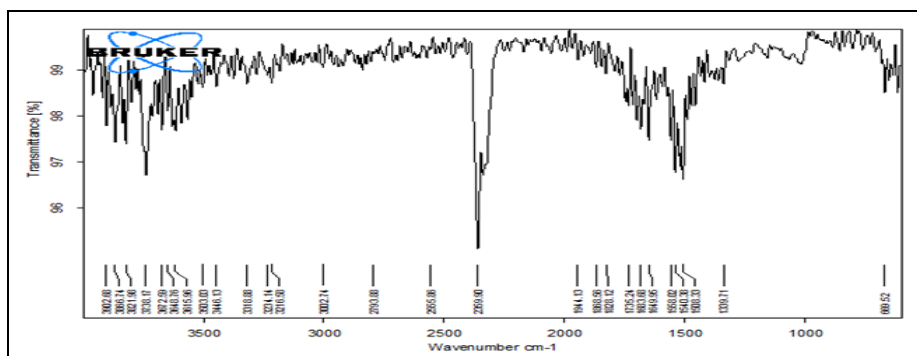


FIG. 6: FTIR SPECTRS OF GLIPIZIDE AND HPMC K4M MIXTURE

Particle Size and Surface Morphology: The particle size of all formulations was found within the range of $583.44 \pm 0.28 - 642.60 \pm 0.91 \mu\text{m}$.

From the results it was found that the maximum particle size range was found to be $630.36 \pm 0.68 - 642.60 \pm 0.91 \mu\text{m}$ in Glipizide microspheres with sodium alginate and HPMC K4M for Batch F4 to F6. The surface morphology of Glipizide microspheres was seen by Scanning Electron Microscope. The surface morphology was done for formulation F2. Samples were stuck on double-sided carbon tape on electron microscope brass tab

and coated with gold in ion sputter. Picture of microspheres was taken by random scanning of the stub. Glipizide microspheres were found almost spherical, free-flowing and non-aggregated.

Micromeritic Properties: From the results the bulk density was found to be from $0.546 \pm 0.0047 - 0.622 \pm 0.003 \text{ g/cc}$, tapped density from $0.612 \pm 0.017 - 0.765 \pm 0.005 \text{ g/cc}$, angle of repose was from $31.82^\circ \pm 0.098 - 40.2^\circ \pm 0.28$, Carr's index was from, $10.7 \pm 1.83 - 18.64 \pm 0.108$ and Hausner's ratio was from $1.11 \pm 0.023 - 1.226 \pm 0.0047$. All formulations were showed good flow property.

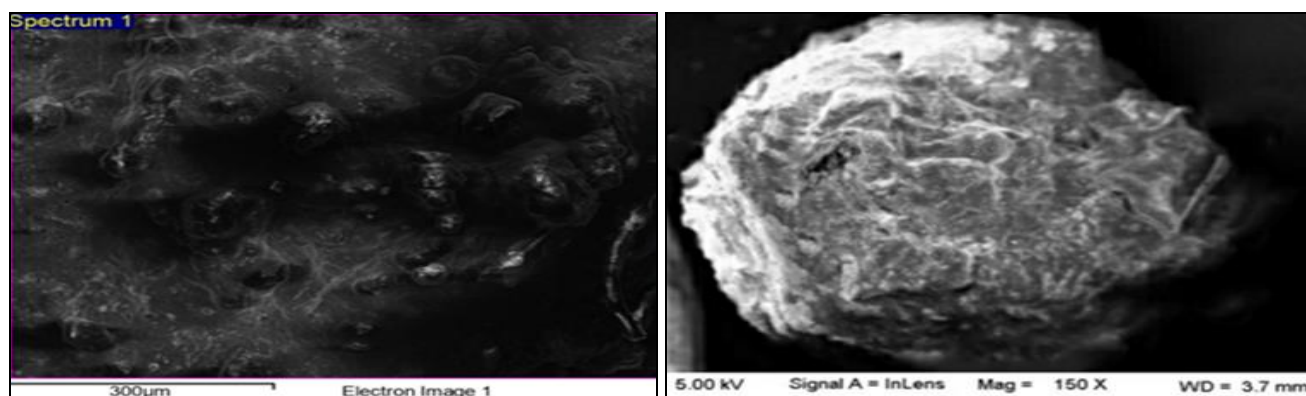


FIG. 7: SCANNING ELECTRON MICROGRAPH OF BEST FORMULATION F2

TABLE 2: MICROMERTITIC PROPERTIES OF MICROSPHERES

Formulation Code	Angle of Repose($^\circ$) \pm SD	Bulk Density (g/cc) \pm SD	Tapped Density (g/cc) \pm SD	Carr's Index (%) \pm SD	Hausner's Ratio \pm SD
F1	33.8 \pm 0.17	0.546 \pm 0.0047	0.612 \pm 0.017	10.7 \pm 1.83	1.11 \pm 0.023
F2	31.82 \pm 0.098	0.553 \pm 0.0047	0.626 \pm 0.002	12.2 \pm 0.28	1.133 \pm 0.0047
F3	37.56 \pm 0.2	0.622 \pm 0.003	0.765 \pm 0.005	18.64 \pm 0.108	1.226 \pm 0.0047
F4	34.65 \pm 0.17	0.575 \pm 0.017	0.674 \pm 0.03	14.54 \pm 0.64	1.16 \pm 0.009
F5	32.28 \pm 0.094	0.546 \pm 0.0047	0.635 \pm 0.007	13.9 \pm 0.21	1.156 \pm 0.0047
F6	40.2 \pm 0.28	0.62 \pm 0.007	0.746 \pm 0.023	16.85 \pm 3.37	1.20 \pm 0.049

Drug Entrapment Efficiency: The percentage drug entrapment efficiency of microspheres varied from 69.95-89.80%. The microspheres of batch F2 cross-linked with barium chloride were showed highest entrapment efficiency. Drug entrapment efficiency was found to decrease with cross-linking agent zinc sulfate when compared to other cross-linking agents.

In-vitro Drug Release Study: It was seen from the observation data that the cumulative percentage release from all the drug-loaded batches of microspheres fell within the range of 42.85% to 64.29% in 12 h study, and it was found that the % cumulative release in microspheres encapsulated with guar gum was maximum for F2 (64.29%) and

minimum for F6 (42.85%). The results of *in-vitro* release study indicated that the amount of drug release decreased significantly in the microspheres cross-linked with zinc sulfate.

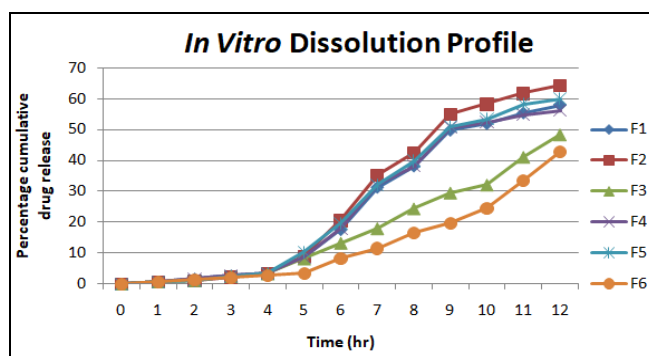


FIG. 8: IN-VITRO DISSOLUTION PROFILE OF MICROSPHERES

It was found that the release of drug from all drug-loaded microspheres was found to follow a slow or sustained manner for a period of 12 h. Also, the percentage cumulative drug release in 0.1N HCl is very less (at first 2 h), which indicate that polymers will prevent the release of drug in the stomach, and proper drug release is occur only when the drug reaches in the colonic region. This further confirms the targeted delivery of the drug to the colon.

Drug Release Kinetics: The data obtained from the *in-vitro* drug release studies of formulation F2 was fitted into the various kinetic models, and it was found that the drug release seems to follow zero-order kinetics as it is evidenced by correlation coefficient ($R^2=0.944$) and Korsmeyer-Peppas was found with regression coefficient ($R^2=0.946$).

TABLE 3: REGRESSION COEFFICIENTS OF DIFFERENT KINETIC MODELS

Formulation	Zero-order (R^2)	First-order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)
F2	0.944	0.904	0.884	0.946

TABLE 4: STABILITY STUDIES AT $40 \pm 2 \text{ }^\circ\text{C} / 75 \pm 5 \text{ \% RH}$

Parameters studied	Initial	After 3 rd month	After 6 th month
Physical appearance	Off white color, odorless	No change	No change
Particle size (μm)	583.44	582.95	582.31
Drug entrapment efficiency	89.80%	89.31%	88.95%
<i>In-vitro</i> drug release	64.29%	64.02%	63.87%

CONCLUSION: Formulation F2 was selected as the optimized formulation which has a particle size of $583.44 \pm 0.28 \mu\text{m}$ with drug entrapment efficiency of $89.80 \pm 0.167\%$ and showed *in vitro* drug release of 64.28% at the end of 12 h. The rate of drug release follows the Korsmeyer-Peppas model and zero-order case II transport mechanism. The selected formulation was evaluated for its stability and was stable at room temperature and accelerated temperature over six months.

The result of this investigation reveals that the ionotropic gelation method can be successfully employed to fabricate Glipizide loaded microspheres. The entrapment efficiency and the *in-vitro* drug release were found to be greatly influenced by the cross-linking agent.

It is concluded that Glipizide microspheres of guar gum cross-linked with barium chloride is free-flowing, almost spherical and maximum entrapment efficiency are prepared and analyzed for *in-vitro* drug release and found to be maximum in colonic simulated conditions.

Therefore, it was ascertained that the drug permeation from the microsphere of optimized formulation F2 should follow zero-order kinetics and Korsmeyer-Peppas equation explains the diffusion-controlled release mechanism. In this study the n value of F2 was found to be between 0.85-1.0 (0.946) then it shows non-fickian case II transport.

Stability Studies: Accelerated stability studies were carried out as per ICH guidelines. From the stability studies data which was carried out for formulation F2 for a period of 6 months showed that the prepared microsphere passes stability studies with no much significant change in physical appearance, particle size, drug entrapment efficiency, and *in-vitro* drug release.

So, our objective of delivering Glipizide microspheres to the colon for type 2 diabetes to enhance its duration of action, decrease frequent dosing, and to minimize the side effects were achieved through optimized microspheres parameters.

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CONFLICTS OF INTEREST: None of the authors has any conflict of interest in the context of this work.

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