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Synergistic Effect of Diclofenac Sodium and Sulfamethoxazole in Pure form, Microparticle Formulation and in Carbopol Incorporated Gel Containing Microparticle Formulation

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Abstract: As infectious diseases are connected with pain and infections, treating pain and infection should be prioritized. Patients suffering from bacterial infection may need diclofenac sodium (DS) during the regular use of sulfamethoxazole (SM) treatment. So, simultaneous or following administration of DS and SM may be needed. The goal of this study was to prepare six microparticle formulations (MF-1-MF-6) made up of Polyethylene glycol-6000 (PEG-6000) with and without chitosan and that was loaded with two drugs (DS and SM) by cold/hot (melt) dispersion method. The formulated microparticle was then mixed with carbopol gel (0.75 and 1.00% w/v) and evaluated. The percentage yield for all the formulated microparticles was found to be between 94.13% and 97.18%. The drug content for both DS and SM is within the prescribed limits. The microparticle size studies revealed that microparticles prepared without chitosan had smaller particles than those prepared with chitosan. The *in vitro* drug release results illustrate that the release of DS and SM from prepared microparticles is increased for the preparation containing chitosan compared to without chitosan preparation. When compared to microparticles alone, the microparticles in gel formulations demonstrated slow drug release. Based on the obtained results of the work, it may be concluded that chitosan increases particle size, and subsequently, drugs are released more rapidly. The research also suggests that DS and SM produce synergistic drug release when administrated together in pure, microparticle, and gel form.

Keywords: carbopol 940; chitosan; diclofenac sodium; microparticle; PEG-6000; sulfamethoxazole.
Abbreviations: DS: Diclofenac sodium; SM: Sulfamethoxazole; PEG: Polyethylene glycol; MF: Microparticle formulation; GF: Gel formulation.

1. Introduction

DS is a classic nonsteroidal anti-inflammatory drug used to treat chronic forms of arthritis and mild-to-moderate acute pain to reduce increased body temperature and inflammation is also available without a prescription. Regarding administration routes,

diclofenac is available orally and topically [1]. DS topical formulations are often preferred over oral DS since it has better tolerability on patients experiencing serious gastrointestinal problems and prevents first-pass metabolism [2]. For treating musculoskeletal pain, the physician may feel the topical route is reasonable for patients with a history of risk or adverse side effects. Most studies found comparable efficacy in comparing oral versus topical applications of DS, with minimal side effects utilizing the topical route [3]. DS is an official quality standard in Indian, British, United States (US), and European Pharmacopeias. The structure of DS is shown in Figure 1.

Antibiotic therapy has been critical in treating bacterial infectious illnesses [4] for the past 94 years [5]. Sulfonamides (sulfa medicines) are a type of synthetic bacteriostatic antibiotic used to treat bacterial infections. Bacterial resistance to a class of antibiotics, which includes sulfonamides, has changed over the years [6]. Before the discovery of penicillin in 1928, sulfonamides (sulfa drugs) 1934 were the main source of antibacterial treatment [7]. SM belongs to the synthetic broad-spectrum sulfonamide antibiotic of the biopharmaceutical classification system class IV, and it is characterized by low solubility and poor permeability [8]. SM is used orally to treat bacterial infections like urinary tract infections [9], bronchitis, and prostatitis. SM is effective against gram-negative and gram-positive bacteria like *Listeria monocytogenes* and *Escherichia coli*. As well, SM is used to treat protozoan infections [10].

Infectious diseases associated with bacteria can cause pain and infections, and that pain and infection should be treated with equal priorities. SM is often used in combination with trimethoprim (antibacterial drug) as cotrimoxazole. Cotrimoxazole is used to treat infections that can cause pain. [11]. The structure of SM is shown in Figure 1.

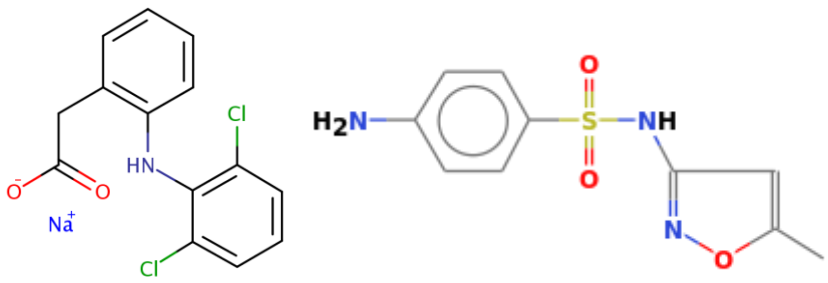

Figure 1. Structure of Diclofenac sodium and Sulfamethoxazole**.**

Trimethoprim/sulfamethoxazole was previously used as the first-line antimicrobial for many infections. Moreover, widespread resistance has lost its effectiveness for outpatient evidence-based coverage of infectious diseases. Trimethoprim/sulfamethoxazole reports currently include the medical assistance of simplified cystitis in patients without the latest antimicrobial use, hospital stays, or reoccurring UTI in the previous year, as well as the treatment of non-bloody diarrheal disease on an outpatient basis. It is no longer supported in respiratory or skin/soft tissue infectious diseases due to high resistance in swollen tonsils and staph species [12].

Patients suffering from bacterial infection may require the administration of diclofenac during the normal use of SM therapy. As a result, simultaneous or successive administration of DS and SM may be necessary. It was reported that successful administration of DS and SM resulted in significant improvements in Whipple's disease with normal duodenal histology and ankylosing spondylitis. SM or DS cannot be avoided entirely, so the administration must be staggered over time if there is no other alternative [11].

Topical administration is widely used to release drug molecules locally owing to its simplicity of use and low cost. Compared to oral administration, topical administration prevents first-pass metabolism in the hepatic, gastric pH fluctuations in the stomach, and variations in plasma concentrations. It is more effective to deliver a drug topically because it penetrates deeper into the skin and is more readily absorbed [13].

This present work aimed to determine the effect of dual drug DS and SM microparticles with and without chitosan. Then the prepared microparticles were evaluated for various studies, and then it was incorporated in carbopol gels, and the gels were evaluated. The objectives of the current work were, therefore, (i) to prepare the microparticles with and without chitosan by hot (melt) dispersion method, (ii) to evaluate the prepared microparticles for various studies, (iii) to compare the drug release profile between microparticles prepared with and without chitosan and also alone, (iv) incorporated the prepared microparticle to different concentration of carbopol gel, (v) evaluation of gel containing microparticles, and (vi) compared the drug release profile between different gel concentration containing microparticles prepared with and without chitosan.

2. Materials and Methods

2.1. Materials.

DS and SM were obtained from Aarti Drugs Limited, (Ltd.,) Chennai. Chitosan was purchased from Yarrow Chem Products, Mumbai. PEG-6000 was procured from Loba Chemie Private (Pvt.,) Ltd., Mumbai. Coconut oil was procured from the local market. Propylene glycol and Span-80 were procured from Reachem laboratory chemicals Pvt., Ltd., Chennai. *n*-hexane was obtained from Avantor performance India Ltd., Maharashtra. Carbopol-940 was obtained from Merck specialties Pvt., Ltd., Mumbai. All other compounds utilized were of analytical grade.

2.2. Methods.

2.2.1. Preparation of DS and SM microparticles.

2.2.1.1. DS and SM Microparticle prepared without chitosan.

Microparticles containing DS (MF-1), SM (MF-2), and a combination of DS and SM (MF-3) without chitosan were prepared with slight modification, which was already done by Tamilvanan *et al.* 2014 [14]. The list of ingredients used for preparing the microparticles is shown in Table 1. In a china dish, 6 gm of PEG-6000 was taken and melted by placing it in a hot water bath. For MF-1 formulation, 50 mg of DS, MF-2 formulation 100 mg of SM, and for MF-3 formulation, 50 mg of DS and 100 mg of SM were added to the molten PEG-6000 and thoroughly mixed until DS and SM were melted completely. The oil phase was made by thoroughly mixing coconut oil and Span-80 (surfactant) with a mechanical stirrer for 5 min. The molten mixture (PEG-6000+drug) was added to the oil phase as a thin film. The preparations were mixed in a mechanical stirrer at 800 rpm for 60 min.

After 60 min, *n*-hexane (non-solvent) was added dropwise (with the help of a burette) to the above preparation with continuous stirring. It took about 45-60 min to complete the addition of *n*-hexane. The role of *n*-hexane in this preparation is to harden the microspheres. Stirring was continued for an additional 30 min. With the help of filtration, the microspheres

were collected and washed three to four times with *n*-hexane and dried for 12 hrs. Until further use, the dried microparticles were placed in a desiccator.

2.2.1.2. DS and SM Microparticle prepared with chitosan.

Microparticles containing DS (MF-4), SM (MF-5), and a combination of DS and SM (MF-6) with chitosan were prepared with slight modification, which was already done by Tamilvanan *et al*. 2017 [15]; the list of ingredients used for preparing the microparticles was shown in Table 1. In a china dish, 6 gm of PEG-6000 was taken and melted by placing it in a hot water bath. For MF-4 formulation, 50 mg of DS, MF-5 formulation, and 100 mg of SM; for MF6 formulation, 50 mg of DS and 100 mg of SM were added to the molten PEG-6000 and thoroughly mixed until DS and SM were melted completely. Propylene glycol and chitosan were added to the above and thoroughly mixed. The oil phase was made by thoroughly mixing coconut oil and Span-80 (surfactant) with a mechanical stirrer for 5 min. The molten mixture (PEG-6000+propylene glycol+chitosan+drug) was added to the oil phase as a thin film. The preparations were mixed in a mechanical stirrer at 800 rpm for 60 min.

After 60 min, *n*-hexane (non-solvent) was added dropwise (with the help of a burette) to the above preparation with continuous stirring. It took about 45-60 min to complete the addition of *n*-hexane. The role of *n*-hexane in this preparation is to harden the microspheres. Stirring was continued for an additional 30 min. With the help of filtration, the microspheres were collected and washed three to four times with *n*-hexane and dried for 12 hrs. Until further use, the dried microparticles were placed in a desiccator.

Table 1. DS and SM microparticle formulations.

Ingredients	**Microparticle formulation code**					
	MF-1	**MF-2**	**MF-3**	**MF-4**	**MF-5**	**MF-6**
PEG-6000 (gm)	6	6	6	6	6	6
DS (mg)	50	-	50	50	-	50
SM (mg)	-	100	100	-	100	100
Coconut oil (mL)	50	50	50	50	50	50
Span-80 (mg)	100	100	100	100	100	100
Chitosan (mg)	-	-	-	75	75	75
Propylene glycol (mL)	-	-	-	0.5	0.5	0.5
n-hexane (mL)	50	50	50	50	50	50

2.2.2. Fourier-transform infrared spectroscopy (FT-IR studies).

Drugs and polymer interactions were studied by using FT-IR [16]. This method is useful in finding the interactions between the drugs and the polymer. By this method, analysis of pure drug, combination, and prepared microparticles were carried out. The peak and patterns obtained by the unadulterated drug were compared with the combination and different formulations.

2.2.3. Determination of percentage yield.

All the prepared microparticles were dried in a hot air oven, and the percentage yield for the dried microparticles can be determined with the help of formula (1) [17].

$$\text{Percentage yield} = \frac{\text{Quantity of microparticles obtained}}{\text{Total quantity of drug and other excipients used}} \times 100 \quad (1)$$

2.2.4. Determination of drug entrapment efficiency.

The amount equivalent to 5 mg of DS and 10 mg SM of microparticle was weighed, and it was initially dissolved in methanol. The resulting solution was further diluted with phosphate buffer pH 7.4, and absorbance was measured at 276 nm for DS and 257 nm for SM in an Ultraviolet (UV) spectrophotometer against pH 7.4 as blank. The drug entrapment efficiency can be calculated using the below formula [18].

$$\% EE = \frac{\text{Total amount of drug added to the formulation} - \text{Unencapsulated drug}}{\text{Total amount of drug added to the formulation}} \times 100$$

2.2.5. Particle size determination by sieving method.

Particle size was determined according to the procedure given by Subrahmanyam [19]. Standard sieves set consists of a range of sizes chosen (sieve numbers 10, 22, 36, 44, 65, 80, 100, and 120). The coarsest sieve (sieve number 10) was placed on top, followed by the finest sieve (sieve number 120). The pan was kept below the sieve number 120. 10 gm of dried microparticles was placed on the coarsest sieve, and the mechanical sieve shaker was shaken for 15 min. After 15 min, the microparticle retained on every sieve was collected separately and weighed. The percentage of granules retained on top of every sieve can be calculated with the following formula (3).

$$\% \text{ weight of granules retained (n)} = \frac{\text{Weight of granules retained}}{\text{Total weight of granules}} \times 100 \quad (3)$$

Percentage undersize is calculated by adding the values of % weight of granules retained. Percentage oversize is calculated by subtracting the values of % weight of granules retained from 100. The graph can be plotted by the arithmetic mean size of the opening (µm or mm) on the x-axis and percentage under-size and over-size on the y-axis.

2.2.6. *In-vitro* drug release.

The release of pure DS, SM individually, as well as a combination of both drugs and the prepared microparticle formulations (MF-1 to MF-6), were determined in USP II apparatus type 1 (basket), with 900 mL of phosphate buffer of pH 7.4 maintained at 37±2 °C the stirring speed of 50 rpm. About 1 mL of the sample was taken every five minutes, and the same 1 mL pH 7.4 was replaced to maintain the sink state. The obtained solution was made up to 10 mL with pH 7.4 and analyzed by UV method at 276 nm for DS and 257 nm for SM, using pH 7.4 as a blank. DS and SM release was determined from their respective absorbance reading [20].

2.2.7. Gel preparation.

The required amount of distilled water was taken in a beaker, and the weighed amount of carbopol (0.5, 0.75, 1.00, 1.25, and 1.5 % w/w) was slowly added (to prevent the agglomeration of the particle). It was thoroughly mixed with the help of a magnetic stirrer. Care must be taken at the time of mixing to avoid the formation of air bubbles in the gel. The mixing process was continued until a clear gel was obtained (Table 2).

Table 2. Gel preparation with carbopol-940.

Carbopol	Water	Equipment	Mixing time
0.5 gm	Q.S to make up to 100 gm	Magnetic stirrer	Until a homogeneous solution/gel formation
0.75 gm	Q.S to make up to 100 gm	Magnetic stirrer	Until a homogeneous solution/gel formation
1.0 gm	Q.S to make up to 100 gm	Magnetic stirrer	Until a homogeneous solution/gel formation
1.25 gm	Q.S to make up to 100 gm	Magnetic stirrer	Until a homogeneous solution/gel formation
1.5 gm	Q.S to make up to 100 gm	Magnetic stirrer	Until a homogeneous solution/gel formation

Q.S-Quantity Sufficient

2.2.8. Gel evaluation studies.

2.2.8.1. Spreadability test.

The spreadability test was performed with slight modifications previously done by Alshehri and Imam [21]. 1 gm of gel was placed in a glass slide, which was then covered with another glass slide. For uniform gel spreading, the two glass slides were compressed by placing a 100 gm weight over them and leaving them for 1 min. After 1 min, the excess gel on the slide's side was wiped away with a clean cloth. One glass slide was fixed, while the other was movable. To the movable slide, 50 gm of weight was tied. The time it took the movable glass slide to pass the 7.5 cm distance was recorded. The test was carried out in triplicate. The following formula was used to calculate spreadability (4).

$$S = \frac{M \times L}{T} \quad (4)$$

where,

S is the spreadability
M is the weight (gm) of the upper glass slide
L is the length (cm) moved on the glass slide, and
t is the time (sec) taken to cross 7.5 cm.

2.2.9. Microparticles mixed with gel.

Accurately equivalent to 50 mg of DS and 100 mg of SM microparticle from various formulations (MF-1, MF-2, MF-3, MF-4, MF-5, and MF-6) were weighed and mixed with 5 gm of gel (0.75, and 1.00 % w/w). The mixing was done in a magnetic stirrer for 15 min. The microparticle formulations mixed with gels were mentioned as GF (Gel Formulation). The details are shown in Table 3.

Table 3. Gel formulation containing microparticles.

Microparticle formulation	Microparticle taken	Gel concentration (% w/v)	Code for gel formulation containing microparticle
MF-1	Equivalent to 50 mg of DS	0.75	GF-1
		1.00	GF-2
MF-2	Equivalent to 100 mg of SM microparticle	0.75	GF-3
		1.00	GF-4
MF-3	Equivalent to 50 mg of DS and 100 mg of SM microparticle	0.75	GF-5
		1.00	GF-6

Microparticle formulation	Microparticle taken	Gel concentration (% w/v)	Code for gel formulation containing microparticle
MF-4	Equivalent to 50 mg of DS	0.75	GF-7
		1.00	GF-8
MF-5	Equivalent to 100 mg of SM microparticle	0.75	GF-9
		1.00	GF-10
MF-6	Equivalent to 50 mg of DS and 100 mg of SM microparticle	0.75	GF-11
		1.00	GF-12

2.2.9.1. Centrifugation test.

The centrifugation test was performed with a slight modification previously performed by Kupiec et al. [22]; about 10 gm of formulation (GF-1 to GF-12) was taken in a centrifugation tube and centrifuged at 3000 rpm for 30 min at room temperature.

2.2.9.2. Drug content studies.

The drug content for GF-1 to GF-12 was determined by dissolving an accurately weighed amount of gel (about 0.1 gm) in 50 mL of methanol. These solutions were appropriately diluted and made with pH 7.4. The final solutions were filtered with 0.45 µm membrane filters, and the final solutions were analyzed at 276 nm for DS and 257 nm for SM by UV method. The linear regression equation derived from the calibration data was used to calculate drug content [23,24].

2.2.9.3. Permeation studies.

A cellophane membrane (placed in glycerin for 12 hrs before use) was fixed to the Franz-diffusion cell apparatus. The gel sample (equivalent to 5 mg of DS and 10 mg of SM) was spread over the cellophane membrane and placed between the donor and receptor compartment. The receptor compartment contains phosphate buffer pH 7.4. A magnetic stirrer at 50 rpm was used to agitate the receptor compartment, and a temperature of 37±2 °C was maintained. Every 5 min for 30 min, 45, and 60 min, and then every 30 min for up to 6 hrs, 0.5 mL of samples from the receptor compartment were pipette out and replaced with the same 0.5 mL of pH 7.4 to maintain the sink condition. The samples were appropriately diluted to a pH of 7.4. The absorbance of the resulting solution was then measured using a UV spectrophotometer at 276 nm for DS and 257 nm for SM against a phosphate buffer solution pH 7.4 as a blank [23,24].

2.2.10. Kinetics study for the gel formulation contains microparticles.

Based on Sekharan *et al.* [25,26], five different kinetic models were used to calculate the release kinetics of drug samples from in vitro studies, such as the zero-order, first-order, Higuchi model, Hixson-Crowell, and Korsmeyer-Peppas model.

3. Results and Discussion

3.1. Screening of excipients.

3.1.1. Chitosan.

In nature, chitosan is a naturally occurring cationic polysaccharide that forms when alkaline deacetylation degrades chitin, a copolymer of glucosamine and N-acetylglucosamine

units. It has been receiving great attention in the medical and pharmaceutical fields as a carrier for drugs due to its environment-friendly and safe [27,28].

3.1.2. PEG-6000.

PEG-6000 is a hydrophilic polymer produced by the polymerization of ethylene oxide. It is commonly used in the cosmetic, food, and pharmaceutical industries as a solvent, plasticizer, surface active agent, ointment and suppository base, and lubricating agent in tablet and capsule preparations. In the manufacturing process, as a carrier, it increases the dissolution properties of drugs [29].

3.1.3. Coconut oil.

Coconut oil is selected based on the experiments done by Jelvehgari and Montazam [30] suggested that drugs in the oil phase are more likely to be incorporated into microspheres and do not dissolve into the external organic solution as often as in the water phase.

3.1.4. Span-80.

Because of the presence of Span 80, the oil phase could absorb water from the aqueous phase. The concentration of Span 80 had a massive effect on the microsphere structure. The morphologies of microspheres were related to the concentration of Span 80 [31].

3.1.5. n-hexane.

Based on Jelvehgari *et al.* [32], n-hexane is a non-solvent that is used in microparticle preparation to make the microparticle harder and leave their surfaces porous. Cold/hot dispersion is a simple aqueous-free procedure for preparing microparticles because it affords an easy preparation procedure. According to Tamilvanan *et al.* [14], microparticles prepared using PEG-6000 alone, irregularly shaped microparticles are produced without adding chitosan and propylene glycol. Microparticles with spherical shapes are due to the combination of PEG-6000 with chitosan and propylene glycol.

3.2. FT-IR Studies.

The FT-IR spectrums of DS, SM, a combination of DS and SM, and MF-1 to MF-6 formulations are shown in Figures 2 and 3.

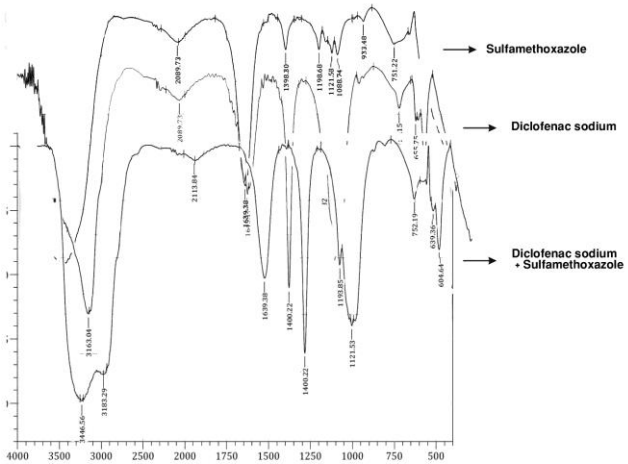

Figure 2. FT-IR spectrum of pure SM, DS and combination of DS and SM.

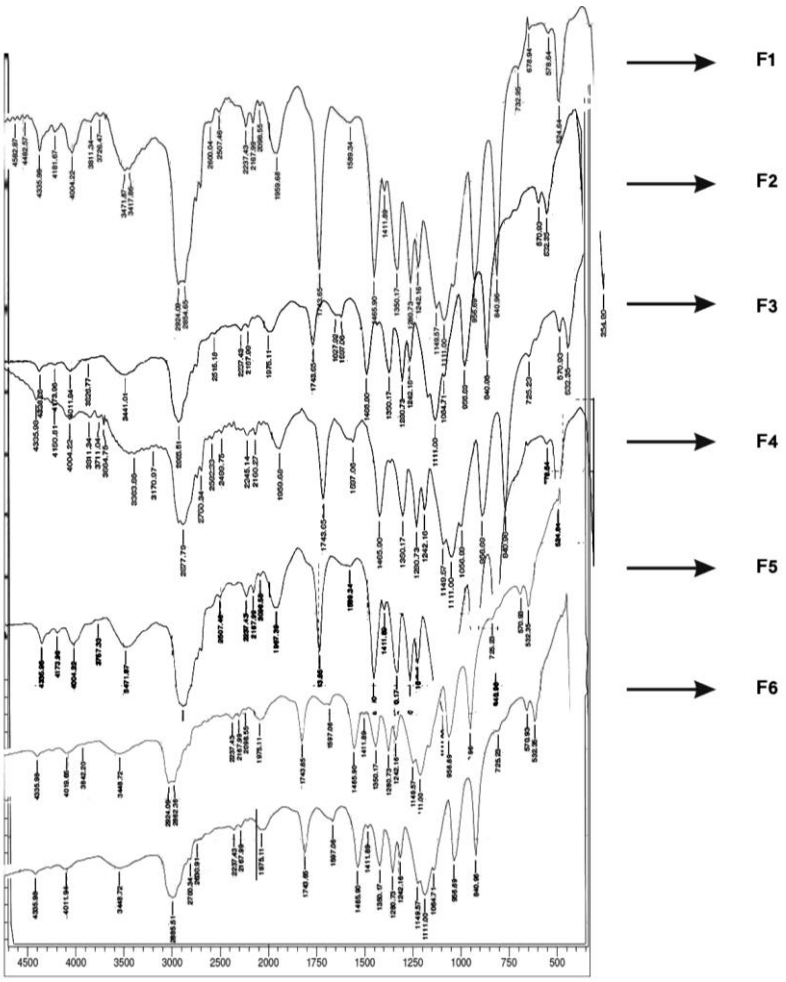

Figure 3. FT-IR Spectrum of MF-1 to MF-6 formulation.

For DS, the peak observed (cm^{-1}) and the group was found to be 3163.4-N-H stretching; 1645.17-C=O stretching; 1400.22-COO stretching; 1194.82-C-N (amines) stretching; 1120.56, 1095.49-C-O stretching; 753.15-ortho disubstituted aromatic and 655.75; 603.68-C-Cl stretching.

For SM, the peak observed (cm^{-1}) and the group was found to be 3476.45-N-H stretching; 1639.38-isoxazole ring stretching; 1398.3-asymmetric SO_2 stretching; 1198.68-C-N (amines) stretching; 1121.53, 1088.74-C-O stretching and 933.48-N-O stretching vibration mode, and 751.22-C-H bending.

For the combination of DS and SM, the peak observed (cm^{-1}), group, and the peak due to was found to be 3183.29, 3446.56-N-H stretching (DS and SM); 1639.38-isoxazole ring stretching (SM); 1400.22-COO stretching (DS); 1193.85-C-N (amines) stretching; 1121.53-C-O stretching (DS and SM); 752.19-ortho disubstituted aromatic and 639.36, 604.64-C-Cl stretching (DS).

For PEG-6000, the peak observed (cm^{-1}) and the group were found to be 2889.17-aliphatic C-H stretching; 1467.73-C-H bending; 1343.33-C-H bending; 1280.65-C-O-H stretching and 1111.89-C-O stretching.

For the combination of DS, SM, and PEG-6000, the peak observed (cm^{-1}), group and the presence of peak due to was found to be 3443.66, 3359.77, 3048.28-N-H stretching (SM and DS); 2923.88-aliphatic C-H stretching (PEG-6000); 1746.42-C=O stretching (DS); 1595.02-C=N stretching; 1504.37-N-O stretching (SM); 1453.26-C-H bending (PEG-6000); 1354.9-asymmetric SO_2 stretching (SM); 1314.4-C-H bending (PEG-6000); 1163.96-C-N

(amines) stretching (DS; SM); 1068.49-C-O stretching (DS; SM; PEG-6000); 745.44-ortho disubstituted aromatic and 675.04-C-Cl stretching (DS).

For MF-1 formulation, the peak observed (cm^{-1}), group, and the peak due to was found to be 3471.87, 3417.86-N-H stretching; 1589.34-C=O stretching; 1411.89-COO stretching; 1149.57-C-N (amines) stretching; 732.95-ortho disubstituted aromatic; 678.94, 578.64-C-Cl stretching the above-shown peaks values were due to the presence of DS. 2924.09, 2854.65-aliphatic C-H stretching; 1465.9-C-H bending; 1350.17-C-H bending; 1280.73-C-O-H stretching, and 1111-C-O stretching, the above-shown peaks values were due to the presence of PEG-6000.

For MF-2 formulation, the peak observed (cm^{-1}), group, and the peak due to was found to be 3441.01-N-H stretching; 1627.92-isoxazole ring stretching; 1350.97-asymmetric SO_2 stretching; 1149.57-C-N (amines) stretching; 1111; 1064.71-C-O stretching; 956.69-N-O stretching vibration mode, and 732.95-C-H bending, the above-shown peaks values were due to the presence of SM. 2885.51-aliphatic C-H stretching; 1465.9-C-H bending; 1350.17-C-H bending; 1280.73-C-O-H stretching and 1111-C-O stretching, the above-shown peaks values were due to the presence of PEG-6000.

For MF-3 formulation, the peak observed (cm^{-1}), group, and the peak due to was found to be 3170.97-N-H stretching; 1465.9-COO stretching; 752.23-ortho disubstituted aromatic; and 725.23, 570.93-C-Cl stretching, the above-shown peaks values were due to the presence of DS. 3363.86-N-H stretching; 1597.06-isoxazole ring stretching; the above-shown peak values were due to the presence of SM. 1280.73, 1242.16-C-N (amines) stretching, and 1149.57-C-O stretching, the above-shown peak values were due to the presence of DS and SM. 2877.79-aliphatic C-H stretching; 1465.9-C-H bending; 1350.17-C-H bending, 1280.73-C-O-H stretching, and 1111-C-O stretching; the above-shown peaks values were due to the presence of PEG-6000.

For MF-4 formulation, the peak observed (cm^{-1}), group, and the peak due to was found to be 3471.87-N-H stretching; 1743.65-C=O stretching; 1411.89-COO stretching; 1242.16-C-N (amines) stretching; 1149.57-C-O stretching and 578.64-C-Cl stretching the above-shown peaks values were due to the presence of DS. 2885.51-aliphatic C-H stretching; 1465.9-C-H bending; 1350.17-C-H bending; 1280.73-C-O-H stretching, and 1111-C-O stretching; the above-shown peaks values were due to the presence of PEG-6000.

For MF-5 formulation, the peak observed (cm^{-1}), group, and the peak due to was found to be 3448.72-N-H stretching; 1597.06-isoxazole ring stretching; 1411.89-asymmetric SO_2 stretching; 1242.16-C-N (amines) stretching; 1149.57-C-O stretching; 956.69-N-O stretching vibration mode and 725.23-C-H bending, the above-shown peaks values were due to the presence of SM. 2862.36-aliphatic C-H stretching; 1465.9-C-H bending; 1350.17-C-H bending; 1280.73-C-O-H stretching and 1111-C-O stretching, the above-shown peaks values were due to the presence of PEG-6000.

For MF-6 formulation, the peak observed (cm^{-1}), group, and the peak due to was found to be 1743.65-C=O stretching; 1411.89-COO stretching; 1242.16-C-N (amines) stretching; 1149.57-C-O stretching, and 725.23, 570.93-C-Cl stretching, the above-shown peaks values are due to the presence of DS. 3448.72-N-H stretching, 1597.06-isoxazole ring stretching, 1149.57-C-O stretching, and 968.69-N-O stretching vibration mode, the above-shown peaks values were due to the presence of SM. 2885.51-aliphatic C-H stretching; 1465.9-C-H bending; 1350.17-C-H bending; 1280.73-C-O-H stretching, and 1111-C-O stretching; the above-shown peaks values were due to the presence of PEG-6000.

The combination of DS and SM peaks showed that there were no interactions between the two drugs. The combination of DS, SM, and PEG-6000 peak showed that there were no interactions between the two drugs and PEG-6000. The MF-1 to MF-6 formulation peak showed no interactions between two drugs, PEG-6000 and other excipients used in the microparticle preparation.

3.3. Determination of percentage yield.

The percentage/calculated yield (%) for the microparticles prepared by cold/hot-dispersion method, without chitosan, and with chitosan were given in Table 4. The percentage yield for the prepared microparticle for MF-1 to MF-6 formulation was found to be in the range of 94.13 to 97.18 %. The drug entrapment efficiency of DS was found to be between 95.04% and 96.76 %; for SM, it was between 95.52% and 96.95%.

3.4. Determination of drug entrapment efficiency.

The drug entrapment efficiency for all the prepared microparticle formulations (MF-1 to MF-6) was shown in Table 4.

3.5. Particle size determination by sieving method.

Using a sieving method, the size of prepared microparticle formulations was determined (MF-1 to MF-6). The particle size for formulations without chitosan was 584.34 µm for MF-1, 583.95 µm for MF-2, and 591.19 µm for MF-3. Using chitosan as an excipient, the MF-4 formulations showed that the particle size was 727.22 µm, MF-5 formulations showed 723.07 µm, and MF-6 formulations showed 737.37 µm. The results are shown as a value in Table 4 and Figure 4.

In this study, results for the particle size showed that when compared to microparticle formulations prepared with chitosan, microparticle formulations prepared without chitosan had smaller particles. Phromsopha and Baimark's [33] results also suggested that the concentration of chitosan had the strongest influence on the increase in particle size. Due to a decrease in solution viscosity, the particle size may have been small. The same finding was reported by others [34-37]. Formulations containing chitosan tend to form large particles as a result of the particles aggregating.

Table 4. Calculated yield (%), drug entrapment efficiency (%), and particle size for the prepared different microparticle formulations.

Formulations		% yield	Drug entrapment efficiency (%)	Particle Size (µm)
MF-1	Without chitosan	95.76	95.04	584.34
MF-2		94.13	95.52	583.95
MF-3		95.12	DS-96.76 SM-96.95	591.19
MF-4	With chitosan	96.87	95.23	727.22
MF-5		96.20	95.55	723.07
MF-6		97.18	DS-96.61 SM-96.88	737.37

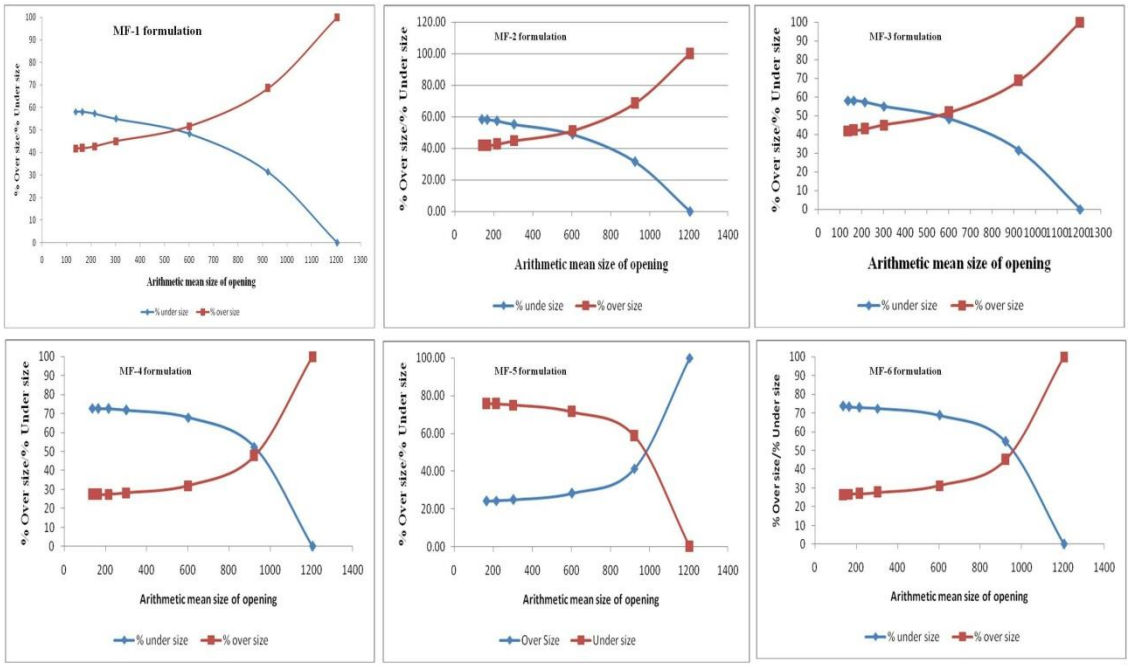

Figure 4. MF-1 to MF-6 formulation particle size determined by the sieving method.

3.6. In-vitro drug release studies for microparticles.

The percentage of drugs released at different time intervals was noted; the results are shown in Figure 5.

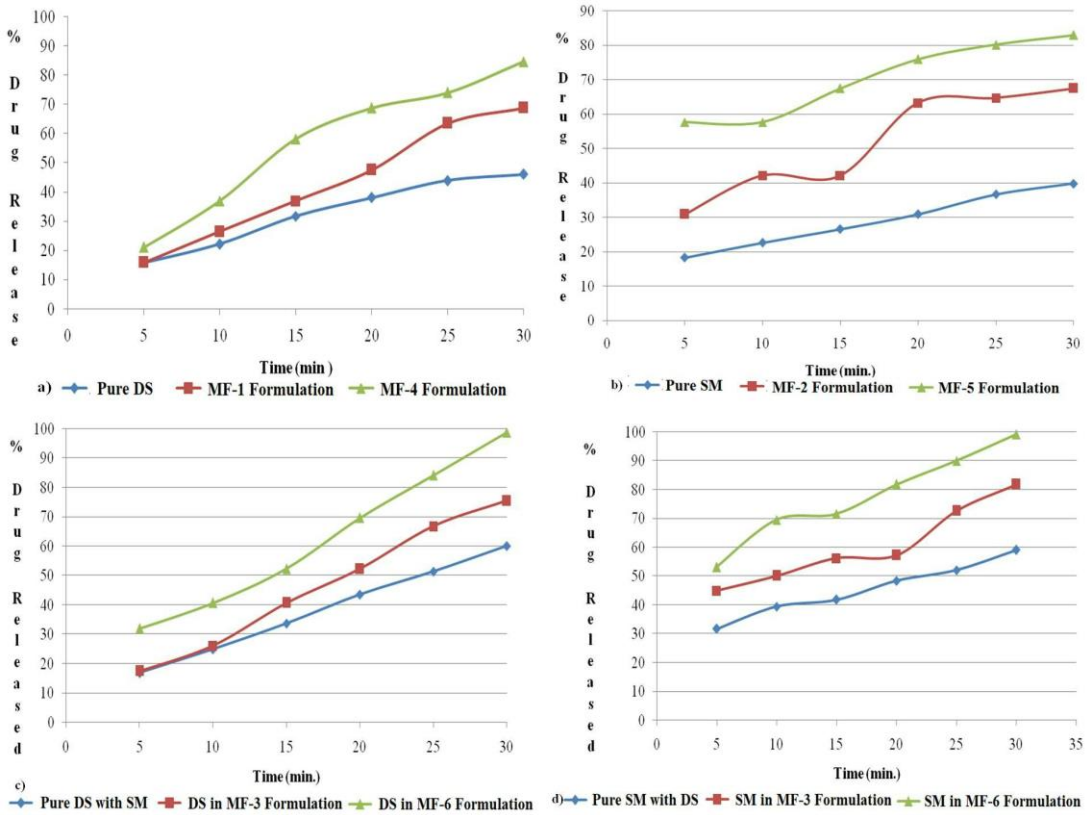

Figure 5. *In vitro* drug release study comparing the release of the (**a)** DS, MF-1, and MF-4 formulations; **(b)** SM, MF-2, and MF-5 formulations; (**c)** DS combined with SM, DS release in MF-3, and DS release in MF-6 formulations; **(d)** SM combined with DS, SM release in MF-3, and SM release in MF-6 formulations.

The *in vitro* release study showed that at 30 min 46.06% of pure DS, and 39.8% of pure SM were released. During concurrent administration, the SM increases the DS to 60.10% (from

46.06%). Similarly, DS increases SM to 59.11% (from 39.8%). It shows the synergistic effect of the two drugs. The same result was concluded by Hossain et.al. The release of DS in MF-1 showed 68.82%, and MF-4 showed 84.71% at 30 min. The release of SM in MF-2 showed 67.50%, and MF-5 showed 82.97% at 30 min. At 30 min, the combined DS and SM, SM increased the DS release from 68.82% to 75.48% in the absence of chitosan and 84.71% to 98.71% in the presence of chitosan. DS also increased the concentration of SM from 67.5% to 81.81% without chitosan and from 82.97% to 99.21% with chitosan.

As a result of the drug release study, when both drugs (DS and SM) are combined in pure form, the amount of drug released in pure form is increased compared to each drug released separately. The *in vitro* results showed that the release rate of DS and SM in microparticles was faster when compared to the pure form because of a higher amount of PEG-6000 used in the microparticle preparation. This PEG-6000 is a highly water-soluble polymer that makes the matrix more permeable, allowing more drugs to exit from the matrix. As compared to pure drug (DS and SM), the microparticles released the drug more rapidly. Chitosan-based microparticles demonstrated higher drug release levels than the formulations prepared without chitosan.

The *in vitro* study of a combination of DS and SM gives an increasing release rate when compared to the DS and SM alone. The increase may be because the structural activity relationship (SAR) of both the DS and SM is different; in combination, it may alter the structural activity relationship of both drugs.

Generally, diclofenac is a monocarboxylic acid with phenylacetic acid containing (2,6-dichlorophenyl) amino group at the second position. DS has a PKa value of 4.15. The twist angle between phenyl rings determines the drug's lipophilicity. The carboxylic acid group determines the hydrophobic action. The carboxylic group is replaced with an uncharged polar group. It made a high affinity. The methylene group present in the structure increases the action [38].

SM is an isoxazole (1,2-oxazole) substance with a 4-amino benzene sulfonamide group at position three and a methyl group at position five. It has the role of an antibacterial agent. SM has a pKa value of 6.6-7.4. It has a free aromatic amino group. The substitution in the aromatic ring is devoid of the action. The amino group at the iso-oxazole ring has more acidic and ionizable [39].

While combining both DS and SM, there is an increase in its *in vitro* properties. This could be due to the amino groups found in both drugs. It decides the ionizing property of the drug. And the pKa value of the two drugs is different. These two criteria may result in a change in the *in-vitro* property.

3.7. Gel evaluation studies.

The different concentrations of the prepared gels were tested for the spreadability test.

3.7.1. Spreadability test.

The gel base was prepared with four different concentrations of carbopol-940 (0.75%, 1%, 1.25% and 1.5% w/v). Table 5 displays the spreading tendency of the gel at four different concentrations.

Table 5. Spreadability value for carbopol gel in different concentrations.

Concentrations (% w/w)	Spreadability value
0.75	6.59 ±0.1463
1	3.77±0.0481
1.25	2.63±0.0537
1.5	1.75±0.0442

The spreadability value for 0.5 percent concentration is greater, and the consistent value is less when compared to other concentrations, but it was not chosen for the formulation because it has less viscosity in nature and is more free-flowing than other concentrations. Similarly, 1.25 and 1.50% were very viscous compared to the previous two concentrations (0.75 and 1%), so they were not chosen for further studies. The spreadability was found to be satisfactory for gel concentrations of 0.75 and 1%. As a result, 0.75 % and 1% carbopol gel were chosen for further studies.

3.7.2. Gel permeation studies.

Permeation studies were conducted for the gel preparation (0.75 and 1% w/w) containing microparticles. Figure 6 shows the results of permeation studies for 6 hrs. At 6 hrs, the DS released from GF-1 formulation was 25.88%, and GF-2 formulation was 20.59%, the SM released from GF-3 formulation was 18.75%, and from GF-4 formulation was 14.69%, the DS released from GF-5 formulation was 29.68% while the SM released from GF-5 formulation was found to be 22.25%. The release of DS from the GF-6 formulation was found to be 25.81%, while the release of SM from the GF-6 formulation was 17.61%. The DS released from the GF-7 formulation was 30.59%, and the GF-8 formulation was found to be 26.47%. SM released from GF-9 formulation showed 19.84%, and GF-10 formulation showed 16.56%. The release of DS from GF-11 showed 33.87%, while the release of SM from GF-11 showed 24.77%. The release of DS from the GF-12 formulation showed 29.68%, while the release of SM from the GF-12 formulation showed 21.13%.

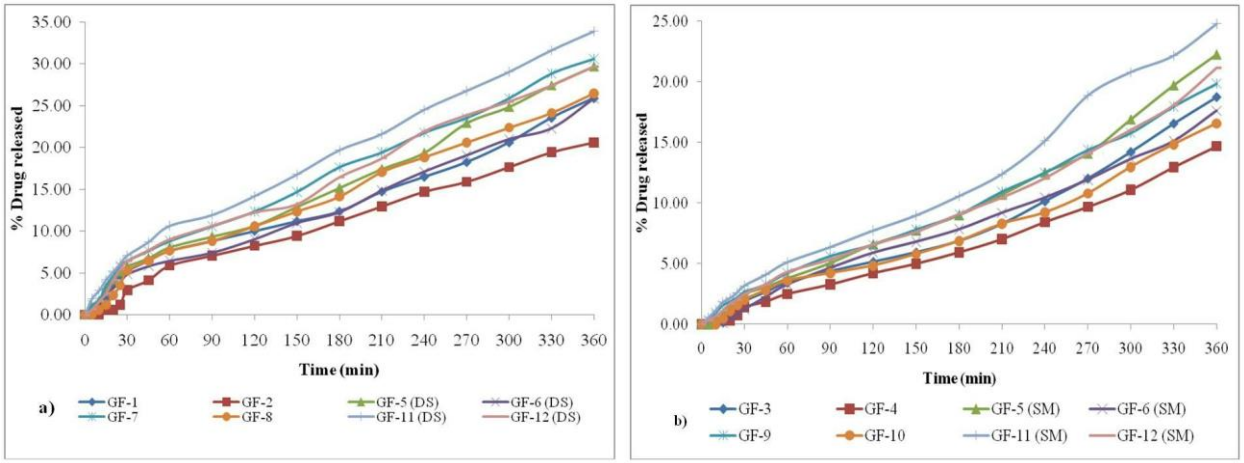

Figure 6. *In vitro* release **c**omparison of **(a)** DS released from different gel formulations [GF-1, GF-2, GF-5 (DS), GF-6 (DS), GF-7, GF-8, GF-11 (DS) and GF-12 (DS)] and **(b)** SM released from different gel formulations [GF-3, GF-4, GF-5 (SM), GF-6 (SM), GF-9, GF-10, GF-11 (SM) and GF-12 (SM)].

When compared to microparticles alone, drug release from the gel was slow, whereas, in microparticles alone, the drug release was rapid. The drug released from the gel is also affected by the gel concentration. When compared to the formulations prepared without chitosan (GF-1 to GF-6), the formulations prepared with chitosan (GF-7 to GF-12)

demonstrated greater drug release in the specified time (6 hours). In the gel formulation, also chitosan increases the drug release. The release of the drug from the gel formulation is based on the gel concentration and the polymer chitosan. When compared to high viscous gel concentration (1 % w/v), low viscous gel concentration (0.75 % w/v) showed more and, to some extent, faster drug release. We can also see synergistic drug release for both drugs (DS and SM) in both concentrations (0.75 and 1% w/v), prepared with and without chitosan.

3.8. Release kinetics study of the microparticulate drugs from the gel.

Several release kinetic models have been fit to the data, including zero order, first order, Higuchi, Hixson, and Korsmeyer-Peppas models. The results are shown in Table 6.

Table 6. Drug release kinetics of carbopol gel formulation containing microparticles.

Formulations	Zero-order	First-order	Higuchi	Hixson Crowell	Korsmeyer-Peppas	n value
GF-1	0.9783	0.9788	0.9604	0.9789	0.9544	0.7780
GF-2	0.9851	0.9898	0.9849	0.9885	0.9021	0.8984
GF-3	0.9767	0.9708	0.9272	0.9729	0.8287	0.8779
GF-4	0.9864	0.9842	0.9513	0.9851	0.8473	0.7808
GF-5 (DS)	0.9905	0.9887	0.9616	0.9898	0.9751	0.7224
GF-5 (SM)	0.9851	0.9773	0.9298	0.9802	0.9543	0.8432
GF-6 (DS)	0.9889	0.9879	0.9623	0.9886	0.9377	0.8390
GF-6 (SM)	0.9933	0.9919	0.9649	0.9926	0.8050	0.9536
GF-7	0.9899	0.9930	0.9796	0.9925	0.9775	0.7050
GF-8	0.9837	0.9892	0.9840	0.9878	0.9408	0.8548
GF-9	0.9948	0.9919	0.9540	0.9931	0.9825	0.8484
GF-10	0.9812	0.9774	0.9386	0.9789	0.9179	0.7672
GF-11 (DS)	0.9919	0.9955	0.9826	0.9949	0.9948	0.6454
GF-11 (SM)	0.9880	0.9817	0.9345	0.9841	0.9905	0.8210
GF-12 (DS)	0.9845	0.9891	0.9787	0.9880	0.9593	0.7785
GF-12 (SM)	0.9884	0.9826	0.9402	0.9848	0.9898	0.8863

Based on the release kinetics, it was determined that most drugs released were by zero-order release, meaning the same quantity of the drug was released per unit time. The "n" value for all the formulations (GF-1 to GF-12) was found to be between 0.6454 and 0.9536, which falls between 0.5 and 1.00, which demonstrates that the drug release was non-Fickian diffusion (anomalous). Thus from the results, it can be suggested that the drug from the carbopol 940 formulations was released by more than one mechanism, which may be a combination of diffusion and erosion mechanisms.

4. Conclusions

From this work, it was concluded that when DS and SM are taken together, they exhibit synergistic release compared to an individual drug in pure form, in the microparticles formulation, and as well as in the gel formulation. The particle size of the microparticles prepared without chitosan was smaller than those prepared with chitosan. Compared to the microparticles prepared without chitosan and the pure drug, the microparticles prepared with chitosan showed faster release. Among the two gel concentrations (0.75 and 1% w/v), 1 % w/v showed less drug release.

Having more than one active pharmaceutical ingredient on a single dosage form, such as a tablet, is common practice and has even been successful on the market. However, microparticles containing more than one active pharmaceutical ingredient are unusual. None of the microparticles that have been introduced into the market use the concept of dual drug

loading. In this work, by cold/hot-dispersion method, the two drugs, DS and SM, were encapsulated into microparticle formulation with and without chitosan.

According to our current pilot experiment, the microparticles formed after the particle formation were observed through IR and UV spectroscopy and particle size analysis, indicating that DS and SM showed an increase in drug release in pure form, prepared microparticle and microparticle encapsulated gel. Therefore additional experimental studies are required to confirm the predicted increase in release between these two drugs (DS and SM) occurs at the molecular (solid solution) level surrounded by the hydrophilic polymer-based microparticles.

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Conflicts of Interest

The authors declare no conflict of interest.

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