Research Article

Formulation and evaluation of Metformin Hydrochloride-loaded Curcumin–Lycopene Niosomes

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Abstract

Curcumin and lycopene, both are naturally occurring potent antioxidants which can be used in the treatment of oxidative stress. The biological sources to obtain curcumin and lycopene are *curcuma longa* and *Solanum lycopersicum*, respectively. The aim of this research study is to develop curcumin–lycopene conjugate-based biguanide niosomes. Span 60 as non-ionic surfactant and cholesterol as a stabilizer were used for the preparation of metformin hydrochloride-loaded niosomes by the ethanol injection method. This research dealt with the composition, formulation, evaluation and optimization of niosomes. The particle size and polydispersity index were found to be in the range from 139.6 to 509 and 0.152 to 0.541 nm, respectively. The entrapment efficiency and drug loading capacity were observed in the range of 40.43–79.02 and 26.954 to 78.17%, respectively. The in vitro drug release studies showed the sustained release of drug in all formulations up to the period of 24 h. Kinetic analysis of in vitro drug release studies showed that when the ratio of Span 60 and cholesterol is 1:1.5 and 1:2, the formulations followed first-order kinetics. When the ratio changed to 1:1, the formulation followed the Korsmeyer–Peppas model and finally at the ratio of 1.5:1 and 2:1, formulations followed the Higuchi kinetics and Hixson–Crowell kinetics, respectively. Formulation S2 is concluded as an optimized formulation on the basis of all evaluation parameters.

Keywords Curcumin–lycopene conjugate · Metformin hydrochloride · Niosomes · Ethanol injection method · Span 60

1 Introduction

Niosomes are defined as vesicular structures composed of non-ionic surfactant and cholesterol. They are known for the entrapment of hydrophobic as well as hydrophilic drugs [1] (Fig. 1).

Advantages of Niosomes:

1. Niosomes provide better patient compliance.
2. They are capable of entrapping hydrophilic, lipophilic as well as amphiphilic drugs.
3. They provide controlled and sustained release of drugs.
4. Oral bioavailability of the drug can be improved by using niosomes [1].
5. They are biodegradable, biocompatible and non-immunogenic to the body.
6. They provide targeting of drugs to various organs.
7. They provide enhanced stability of entrapped drugs [3].

Curcumin is an active constituent of *Curcuma longa* and is extracted from its rhizomes. Chemically, CUR (diferuloyl-methane) is 1,7-bis(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3,5-dione and it is represented as $C_{21}H_{20}O_6$ [4]. Oral route is the main route of administration of turmeric preparations. But due many barriers such as its
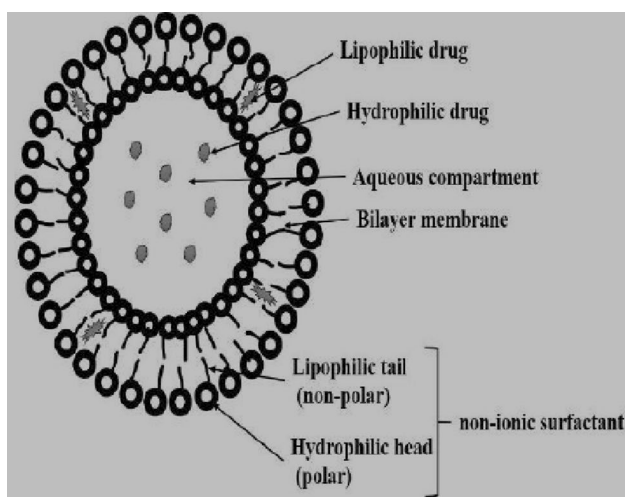
Fig. 1 Structure of niosome [2]

hydrophobicity at physiological pH, limited absorption, rapid metabolism and excretion, it has low bioavailability. To overcome the problems of poor solubility and low bio-availability, nanoparticle-based drug delivery system has been suggested [5].

Lycopene is a polyene, unsaturated hydrocarbon carotenoid. It has a molecular weight of 536.888 g/mol. It is an acyclic open-chain unsaturated carotenoid having 13 double bonds, 11 of them are conjugated double bonds arranged in a linear array. Antioxidant property of lycopene is associated with its extended system of conjugated double bonds.

In this research, curcumin–lycopene conjugate was prepared to get the synergistic effect of their antioxidant activity. Metformin hydrochloride that is the choice of drug was loaded in the conjugate in the form of niosomes by using ethanol injection method. This was done in order to obtain a targeted drug delivery system and to improve the oral bioavailability of the drug.

2 Materials and methods

2.1 Materials

Cholesterol, Span 60 (Central Drug House, New Delhi), ethanol (Changshu Hongsheng Fine Chemicals Co. Ltd.), curcumin (Central Drug House, New Delhi), lycopene (Medcure Pharma, Baddi), dichloromethane (S D Fine Chem Limited, Mumbai) and metformin hydrochloride (Accent Pharma, SIDCO, Jammu) were used in the study.

2.2 Preparation of niosomes

Ethanol injection method [6] was used for the preparation of niosomes. Firstly, curcumin and lycopene in specified amounts were dissolved in 10 mL of ethanol and dichloromethane, respectively. Both the solutions were mixed by using magnetic stirrer. Then, the cholesterol and Span 60 were added in the ratio as specified in Table 1 into above solution and stirred until a homogenous mixture was obtained. This will form the lipid phase (A) of the formulation. Aqueous phase (B) was prepared by dissolving 50 mg of drug in 100 mL distilled water. Finally, the solution A was added dropwise into solution B, maintained at 60 °C with continuous stirring at 1750 rpm. The mixture was stirred until the organic solvents get evaporated completely.

2.3 Characterizations of niosomes

2.3.1 Morphology

The prepared niosomal formulation was studied under the microscope for morphological evaluation. A drop of preparation was poured on a glass slide and placed under the pre-adjusted optical microscope. The morphology was then studied carefully [7].

Table 1 Formula for different batches of niosome

Ingredients → Formulations ↓	MH (mg)	CUR (mg)	Lycopene (mg)	Span 60 (mg)	Cholesterol (mg)	Stirring speed (rpm)
S1	50	25	25	50	50	1750
S2	50	25	25	75	50	1750
S3	50	25	25	50	75	2000
S4	50	25	25	50	100	1750
S5	50	25	25	100	50	1750

MH metformin hydrochloride, *CUR* curcumin

2.3.2 Particle Size and polydispersity index (PdI)

Dynamic light scattering technique was used to determine the particle size and PdI of formulated niosomes. The samples were firstly diluted with the help of distilled water and then were filled in the cuvettes. The cuvettes were placed in the instrument (Zetasizer Nano ZS-90; Malvern Instruments Ltd. UK), and finally, particle size and PdI were determined at the angle and temperature of 90° and 25°, respectively [7,8].

2.3.3 Entrapment efficiency (%EE)

10 mL of prepared formulation was taken and centrifuged at 7000 rpm at −4 °C for 1 h to separate the free drug from the niosomes. The supernatant was then collected and diluted properly with phosphate buffer saline (PBS) at pH 6.4. Finally, the dilutions were run at 232 nm using UV-spectrophotometer to determine the amount of free drug. The amount of entrapped metformin was calculated by subtracting the amount of free drug from the total drug present in the 10 mL of niosomal preparation [9, 10].

$$\%EE = \left(d_t - d_f / d_t\right) \times 100$$

Here, d_t = total amount of drug; d_f = amount of free drug

2.3.4 Drug loading capacity (%DL)

It can be calculated as per formula.

$$\%DL = \frac{Amount\ of\ total\ entrapped\ drug}{Total\ weight\ of\ nanoparticles} * 100$$

2.3.5 In vitro drug release studies

An amount of niosomal formulation, equivalent to 2.0 mg of drug, was calculated and filled in the dialysis bag. A beaker containing 100 mL of PBS at pH 6.4 was placed over a magnetic stirrer at 100 rpm, and the temperature was maintained at 37 ± 1 °C. Then, the bottom of the dialysis bag was dipped into the beaker. Finally, aliquots (5 mL) were withdrawn periodically from the beaker and the equal volume of PBS was added to maintain the sink conditions. The test was continued for 24 h. The withdrawn aliquots were then subjected to UV-spectrophotometry analysis at 232 nm to determine the concentration of drug [11, 12].

2.3.6 Kinetic analysis of in vitro release profiles

To investigate the mechanism of drug release from prepared niosomes, the release data were analysed by using different mathematical models as follows [13].

Zero order A graph of %CDR against time was plotted to study the release kinetics.

First order A graph of log %DR against time was plotted to study the release kinetics.

Higuchi model A graph of %CDR against SQRT was plotted to study the release kinetics.

Korsmeyer–Peppas model A graph log %CDR against log time was plotted to study the release kinetics.

Hixson–Crowell model A graph of CBRT %DR against time was plotted to study the release kinetics [14].

2.3.7 Scanning electron microscopy

The surface morphology of the formulation was studied by using SEM. The prepared niosomal formulation was deep frozen and lyophilized prior to SEM. A double-sided conducting tape was taken, and the lyophilized sample was spread over it. Then, the coating was done with gold by using gold sputter under vacuum condition but in the presence of argon gas at 50 mA for 100 s. Finally, the sample was analysed under the microscope and the images were taken (Fig. 2).

2.3.8 Physical stability testing

The physical stability of niosomes was studied by leakage of the drug from niosomal vesicles in the innate prepared form that is dispersion stored at room temperature as well as under refrigeration. The optimized formulation was taken in the dispersion form and stored in sealed vessels at 2–8 °°C and at room temperature for a period of 90 days. The samples were withdrawn at regular interval of time, i.e. 0, 15, 30, 60 and 90 days and then evaluated for percentage drug retained [15].

Fig. 2 Lyophilized formulation

Fig. 3 MH niosomes under microscope

Table 2 Particle size and polydispersity index of niosomes

Formulations	Particle size (nm)	PdI
S2	395.9	0.337
S4	440	0.454
S6	253.6	0.152
S8	139.6	0.223
S9	509	0.541

3 Results and discussions

3.1 Morphology

The microscopic view as depicted in Fig. 3 of the drug loaded niosomes shows the niosomes formed were spherical in shape.

3.2 Particle size and PdI

The particle size and PdI was found to be in the range of 139.6–509 nm and 0.152–0.541, respectively, as shown in Table 2.

3.3 Entrapment efficiency and drug loading capacity

The entrapment efficiency and drug loading capacity were observed in the range of 40.43–79.02 and 26.954–78.17%, respectively, as shown in Table 3.

3.4 In vitro drug release studies

Figure 4 shows the drug release profiles in PBS at pH 6.4. Formulation S4 showed the minimum release of 22.221%, whereas formulation S2 showed the maximum release of 70.607% over a period of 24 h. It is clear from the graph that the MH release in vitro has undergone rapid initial burst followed by slow sustained release. The rapid release

Table 3 Entrapment efficiency and drug loading capacity of niosomes

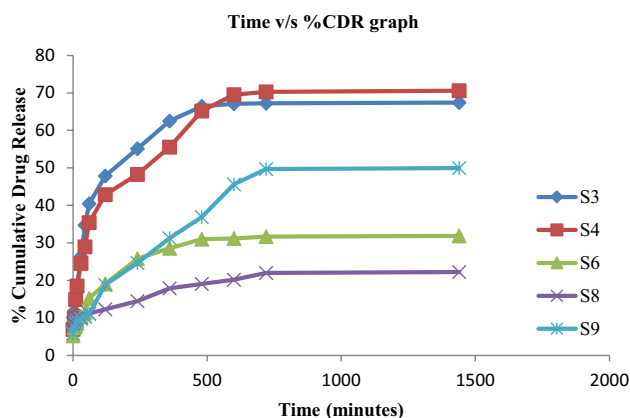| Formulations	Percentage entrapment efficiency (%EE)	Drug loading capacity (%DL)
S2	69.73	34.865
S4	79.02	78.17
S6	40.43	26.954
S8	62.77	31.385
S9	48.21	48.21

Fig. 4 Time vs % CDR graph

may be due to drug incorporated in fatty acid chains of lipid bilayers. This led to the rapid release of MH upon dispersing vesicles in buffer until reaching equilibrium [16].

3.5 Kinetic analysis of in vitro release studies

See Tables 4 and 5.

3.6 Scanning electron microscopy

See Figs. 5, 6, 7, 8 and 9.

3.7 Physical stability testing

The optimized formulation S2 was investigated for physical stability studies. The results of this investigation showed that after the period of 30 days at room temperature, the percentage drug entrapped in niosomes was found to be 99.93%, whereas at 2–8 °C, it was 99.98%. At the end of the study, i.e. after 90 days, the percentage drug entrapped was found to be 99.6% and 99.97% at room temperature and at 2–8 °C, respectively.

On the basis of derived particle size, polydispersity index, entrapment efficiency, drug loading capacity, in vitro drug release studies, kinetic analysis of drug

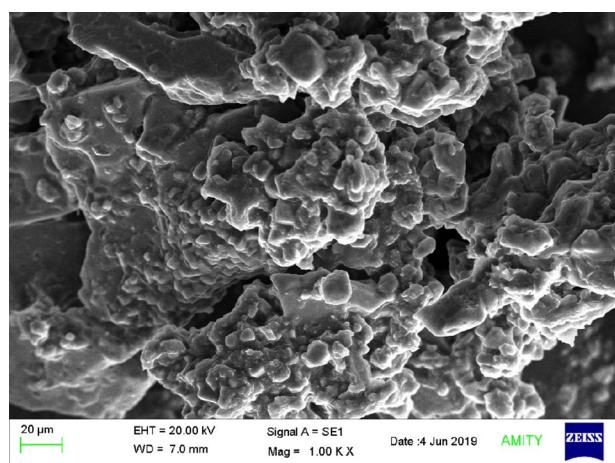**Table 4** Statistical kinetic values for different formulations

Batch	Zero-order model	First-order model	Higuchi model	Korsmeyer–Peppas model	Hixson–Crowell model
S1	0.8152	0.9137	0.9521	0.9849	0.8659
S2	0.9241	0.979	0.981	0.9351	0.968
S3	0.9783	0.9794	0.9472	0.8116	0.9791
S4	0.9824	0.9845	0.9543	0.7983	0.9839
S5	0.986	0.978	0.9431	0.843	0.99

Table 5 Kinetic model followed by different formulations

Batch	Span 60:CH	Kinetic model	Remarks
S1	1:1	Korsmeyer–Peppas model	Describes the drug release from a polymeric system [13]
S2	1.5:1	Higuchi model	Describes the release of drug from the insoluble matrix [13]
S3	1:1.5	First order	Describes the water soluble drug dissolution in porous matrices [14]
S4	1:2	First order	Describes the water soluble drug dissolution in porous matrices
S5	2:1	Hixson–Crowell model	Change in surface area and diameter of the particles describes the release from the system [14]
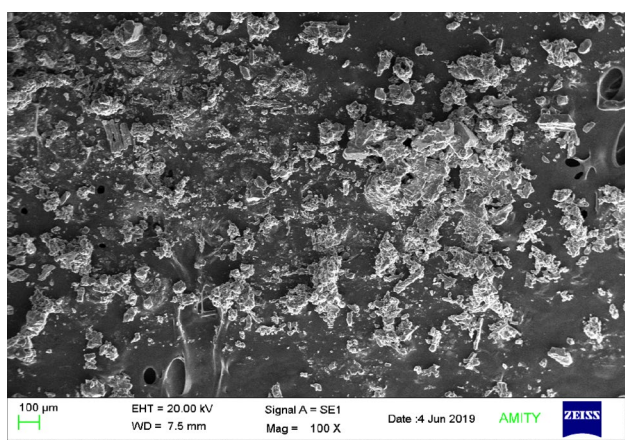
Fig. 5 Formulation at × 100 magnification

Fig. 6 Formulation at × 1000 magnification

release studies and scanning electron microscopy, formulation S2 are selected as an optimized formulation. This is due to the fact that S2 gives the desired particle size and entrapment efficiency. It also provides the sustained release of metformin hydrochloride over the period of 24 h with maximum drug release of 70.607%. Formulation S2 follows Higuchi model (plots show the highest linearity). Higuchi kinetics explains that the drug diffuses at a slower rate as the distance for diffusion increases, referred to the square root kinetics. In niosomes, liquid penetrates the matrix and dissolves the drug, which then diffuses into the exterior liquid. Higuchi tried to relate the drug release rate to the physical constants based on simple law of diffusion (Fick's first law). Also, the results showed that the niosomes were more stable under refrigeration, i.e. at 2–8 °C. The percentage drug retained in the niosomes after the period of 90 days under refrigeration was found to be 99.97%.

4 Conclusion

In this research work, five formulations were prepared at different ratios of Span 60 and cholesterol by using ethanol injection method. After that, various evaluation tests were performed to determine particle size, polydispersity index, percentage entrapment efficiency, drug loading capacity and in vitro drug release. Kinetic models were also studied for different formulations. From the obtained results, it was concluded that S2 is an optimized formulation with desired particle size and entrapment efficiency of
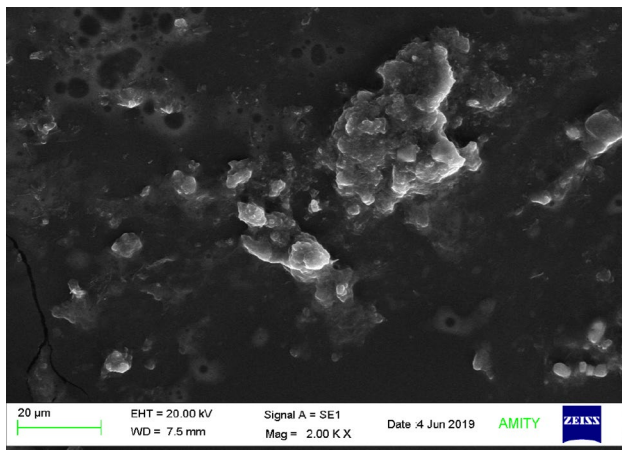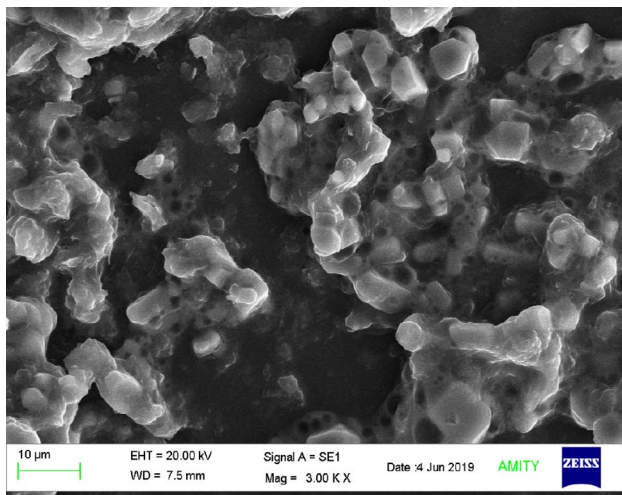
Fig. 7 Formulation at × 2000 magnification

Fig. 8 Formulation at × 3000 magnification

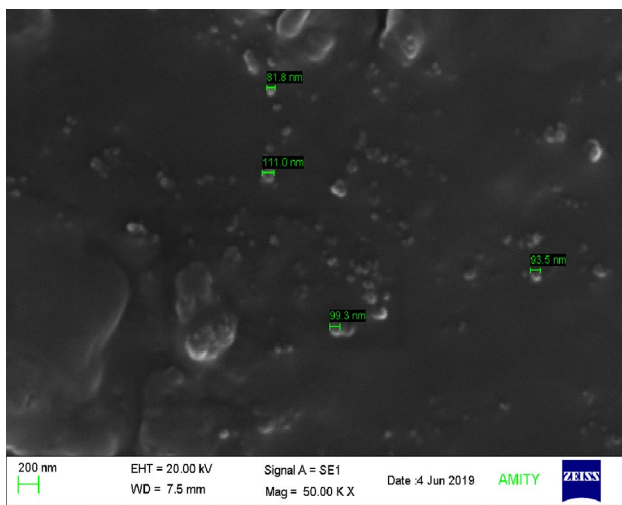**Fig. 9** Formulation at × 50,000 magnification

440 nm and 79.02%, respectively. It also showed the maximum release of 70.607% over a period of 24 h. Finally, formulation S2 followed Higuchi model kinetics, the reason being that plots shows the highest linearity and showed the high physical stability under refrigeration conditions by retaining 99.97% drug after a period of 90 days.

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