ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 4, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

FORMULATION AND *IN VITRO* CHARACTERIZATION OF THIOCOLCHICOSIDE PRONIOSOMES FOR ORAL DELIVERY

DEEPA ANTIL[1]*, RAHUL SHARMA[1], BHARAT BHUSHAN[2]

[1]Department of Pharmaceutics, Hindu College of Pharmacy, Sonipat, Haryana, India. [2]Department of Pharmaceutical Chemistry, Hindu College of Pharmacy, Sonipat, Haryana, India. Email: geetaantil2309@gmail.com

Received: 27 September 2022, Revised and Accepted: 01 February 2023

ABSTRACT

Objective: The main objective of the present study was to develop a controlled release formulation of proniosomes of thiocolchicoside.

Methods: The formulations of proniosomes were prepared with thiocolchicoside varying the Span 60 and cholesterol ratio in the range of 4.5:1–1:4.5 using sucrose stearate as carrier by slurry method. The different proniosomal formulations prepared were characterized for micromeritic properties and further %Entrapment efficiency, %Drug content, and loading efficiency were also determined. The best optimized formulation F8 was characterized for particle size distribution, zeta potential, scanning electron microscopy, *in vitro* dissolution studies, and *in vitro* release kinetics to determine the release pattern of the drug from the formulation. Further, the formulated proniosomes were subjected to stability studies.

Results: The Fourier-transform infrared spectroscopy study showed no interaction between drugs and other excipients. The entrapment efficiency of proniosomes formulations found within the range of 49.71–83.62%. The formulation F8 was characterized for the *in vitro* dissolution studies which showed drug release 94.30% within 24 h when compared with pure drug. Kinetic analysis of drug release profiles showed that the drug release was followed by Korsmeyer–Peppas model (R^2=0.9413) resulted in controlled release. The mean particle size and zeta potential of proniosome derived niosomes were found to be 118.34 nm with polydispersity index 14.9% and −36.8, respectively, and has reasonably good stability characteristics as well.

Conclusion: Proniosomal formulation of thiocolchicoside may be used as controlled drug delivery system for oral administration. Hence, proniosomes could act as a promising alternative option for oral drug delivery.

Keywords: Thiocolchicoside, Proniosomes, Niosomes, Controlled release, Particle size, *In vitro* release.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i4.46456. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The conventional dosage forms have poor patient compliance due to variations in plasma drug levels, which necessitate high doses and frequent administration. Novel drug delivery systems are being developed rapidly to overcome the limitations of conventional dosage form; improving the patient compliance, safety and efficacy, effectiveness of an existing drug by optimizing the delivery and dosage, minimizing the side effect, and finding the therapeutic uses. Their objective is to encourage research to develop technologies for targeted, controlled, and sustained delivery of drug with reduced side effects that efficiently deliver drugs into cells [1]. Controlled drug delivery systems have therefore become a viable alternative to conventional drug delivery, since they prolong the duration of the drug release and maintain plasma levels within the therapeutic range while minimising side effects [2].

For a number of decades, oral route is preferred over other routes for drug administration. However, majority of the newly discovered and existing drugs administered by oral route frequently encounter bioavailability problems due to several reasons such as poor dissolution, unpredictable absorption, inter and intrasubject variability, and lack of dose proportionality [3]. Nowadays, vesicular delivery systems are preferred because they provide an efficient means of administration to the site of infection, reduce medication toxicity, and have no side effects. The encapsulation of a drug in vesicular structures is projected to prolong the drug presence in systemic circulation. Liposomes, niosomes, transferosomes, and pharmacosomes as vehicles for regulated delivery are among the novel ways employed for drug delivery through vesicular system [4].

Colloidal particulate drug delivery systems such as liposomes [5] or niosomes [6] are very distinct when compared to conventional dosage forms because the particles can act as the drug containing reservoirs and altered the particle composition or surface to adjust the rate of drug release or affinity toward the target site. Niosomes are non-ionic surfactant vesicles and can entrap both amphiphilic and hydrophobic solutes [7]. Niosomes considered to be an alternate to liposomes because they possess less chemical stability problems and low cost; however, they are associated with problems related to physical stability, such as fusion, aggregation, sedimentation, and leakage on storage.

The proniosome approach deprecates these problems using dry free-flowing product that is more stable during sterilization and storage. Proniosomes are the dry powder formulations containing water-soluble carrier particles coated with surfactant and hydrated by aqueous media on brief agitation to form niosomal dispersion. The niosomes formed after dispersion is more uniform in size and similar to conventional niosomes. Easy distribution, transfer, measuring, and storage make proniosome a versatile delivery system. The proniosomes preparation methods for oral delivery include slurry method and the spraying of surfactant on water-soluble carrier particles [8,9].

Thiocolchicoside is a potent muscle relaxant having analgesic and anti-inflammatory activity. It is a natural derivative of colchicine and a semisynthetic derivative of the naturally occurring colchicoside extracted from the seeds of *Gloriosa superba* (*Liliaceae*), used for relieving rheumatic and muscle pain. In addition, it also has anti-inflammatory and analgesic action [10]. It is the competitive antagonist of GABA receptor, glycine receptor, as well as nicotinic acetylcholine receptors [11]. Being less sedating than other centrally acting muscle

relaxants, thiocolchicoside is commonly used in the treatment of symptomatic spasms and contractures in muscular, rheumatic, and neurologic disorders [10] and in non-articular rheumatism, it inhibits the action of cyclooxygenase enzyme and resulting in lowering the pain and swelling of body cells. This drug can also be used for the treatment of acute and chronic lumbar and sciatic pain, post-traumatic and post-operative pain, cervicobrachial neuralgia, and also persistent torticollis. Thiocolchicoside also has some side effects such as drowsiness, impaired judgment, and impaired body movements [11]. The marketed preparations of thiocolchicoside in India are in the form of oral, parenteral, and topical formulations. The maximum recommended oral dose is 8 mg twice a day.

In the recent past, several strategies have been adopted to enhance the dissolution characteristics and bioavailability of thiocolchicoside by formulating it into microsphere [12], nanoemulsion [13], and ethosomes [14]. However, no relevant studies about thiocolchicoside proniosomes for oral administration have been reported yet. Furthermore, a limited number of studies considering the preparation and evaluation of proniosomes, to our knowledge, were published. The majority of the publications were focused on the utilization of proniosomes in the transdermal drug delivery. Henceforth, the present study encompasses the formulation and characterization of proniosomes of thiocolchicoside for oral delivery.

METHODS

Thiocolchicoside was a kind gift sample from Bio deal Pharmaceuticals Pvt. Ltd., Chandigarh, India. Span 60 was purchased from Sisco Research Laboratories Pvt. Ltd., New Delhi, India. Cholesterol was obtained from Research Lab Fine Chem Industries, Mumbai, India. Sucrose was procured from Qualikems fine chem Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade and solvents were of HPLC grade. Freshly collected double distilled water was used all throughout the experiments.

Analytical method development

Determination of absorption maxima of thiocolchicoside

Absorption maxima is the wavelength at which maximum absorption occurs. It is important to determine the absorption maxima of the substance under study for the accurate analytical work. For preparation of working standard, 10 mg of accurately weighed drug dissolved in 10 mL of methanol (1 mg/mL).

Dilution 1: Further, 1 mL of the stock solution was pipette out into a 100 mL volumetric flask, and the volume was made up of methanol.

Dilution 2: From this stock solution, pipette out 1 mL and dilute to 10 mL with methanol and subject for UV scanning in the range of 200–400 nm using double beam UV spectrophotometer [15].

Standard calibration curve in methanol procedure

Form this standard stock solution, a series of dilution (3, 6, 9, 12, 15, 18, 21, 24, and 27 μg/mL) were prepared by dilution with methanol. The absorbance of these solutions was measured spectrophotometrically against blank of methanol at respective λ_{max} for thiocolchicoside and repeated for 3 times. The average peak area was calculated. A calibration plot was drawn between concentration and peak area. The calibration equation and R^2 value were reported.

Preparation of proniosomes

Proniosomes formulations were prepared using slurry method. The composition of different proniosomal formulations of thiocolchicoside is given in Table 1. In brief, accurately weighed amounts of lipid mixture (250 μM) comprising of span 60 and cholesterol at various molar ratios (4.5:1, 4:1, 2.5:1, 1.5:1, 1:1, 1:1.5, 1:2.5, 1:4, and 1:4.5, respectively) and thiocolchicoside (8 mg) were dissolved in 20 mL of solvent mixture containing chloroform and methanol (2:1). The resultant solution was transferred into a 250 mL round bottomed flask containing a carrier, that is, sucrose stearate (300 mg) to form slurry. The flask was attached to a rotary flash evaporator (Perfit India, India) and the organic solvent was evaporated under reduced pressure at a temperature of 45±2°C and 600 mm of Hg pressure until the formation of a dry and free-flowing product. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature. The obtained proniosomes powder was stored in a tightly closed container at 4°C for further evaluation.

Table 1: Composition of proniosomes formulations F1–F11

Formulation	Drug (mg)	Span 60 (mg)	Cholesterol (mg)	Sucrose (mg)
F1	8	53.8	9.6	300
F2	8	53.8	19.3	300
F3	8	53.8	28.9	300
F4	8	53.8	38.6	300
F5	8	53.8	48.2	300
F6	8	53.8	57.9	300
F7	8	96.8	19.3	300
F8	8	86.0	19.3	300
F9	8	75.3	19.3	300
F10	8	64.5	19.3	300
F11	8	43.0	19.3	300

Preparation of niosomes from proniosomes

Proniosomes were transformed to niosomes by hydrating with phosphate buffer (pH 6.8) at 80°C using vortex mixture for 2–3 min. The niosomes were sonicated twice for 30 s using sonicator and then subjected for further studies [9,16].

Characterization/evaluation of proniosomes

Optical microscopy

The proniosome powder was placed on a cavity glass slide and observed. Then, few drops of water were added. The formation of vesicles was monitored through an optical microscope and photomicrograph was taken. For the morphological evaluation, proniosome powder was transformed to niosomes by hydrating with phosphate buffer (pH 6.8) at 80°C using vortex mixer for 2 min. The niosomes dispersion was placed over a glass slide and the vesicles formed were observed at a magnification of 450× through an optical microscope.

Fourier-transform infrared spectroscopy (FTIR) spectroscopy

Infrared spectroscopy was performed to confirm the interactions between drug and excipients. FTIR spectra were obtained by attenuated total reflectance technique using an FTIR spectrometer (Perkin Elmer, USA). After cleaning of crystal area, the solid material was placed, the pressure arm was positioned, and the spectrum was recorded [17]. Samples assessed encompass pure thiocolchicoside and physical mixture of thiocolchicoside with Span 60, cholesterol, and sucrose stearate.

Micromeritic properties of proniosomes

The flow properties of powder are necessary in handling and processing operations. The flow properties were studied by measuring the Angle of repose, Carr's compressibility index, and Hausner's ratio. The Carr's index and Hausner's ratio were calculated from the bulk and tapped density of the proniosomes.

Angle of repose

The angle of repose of the formulations was determined by employing fixed funnel method. All the prepared proniosomes formulations were weighed and passed through the funnel, which was fixed at a position so that the 13 mm outlet orifice of the funnel was 2 cm above the surface. The proniosomes formed a pile. From that, the height "h" and the radius "r" of the pile were measured, and the angle of repose (θ) was determined using the formula,

$\tan \theta = h/r$

Bulk and tapped density

Bulk and tapped densities were determined using the following equations:

Bulk density = Powder weight/bulk volume

Tapped density = Powder weight/tapped volume

Compressibility index

The compressibility of dry proniosomes was calculated by Carr's Index as follows:

Carr's Index (%) = [(tapped density-bulk density)/tapped density] × 100

Hausner's ratio

It is the ratio of tapped to bulk density. It provides an idea about the flow properties of the powder [18] and can be determined as follows:

Hausner's ratio = Tapped density/bulk density

Drug content

Thiocolchicoside content in all the prepared formulations of proniosomes was assayed by a UV-visible spectrophotometer. Proniosomes (100 mg) were dissolved in 10 mL methanol by shaking the mixture for 5 min. 1 mL of the resultant solution was taken and diluted to 10 mL with methanol. Then, aliquots were withdrawn, and absorbance was recorded at 258 nm using a UV visible spectrophotometer and drug content was calculated [19].

Drug entrapment efficiency and drug loading (DL) capacity

The entrapment efficiency was determined by calculating the amount of drug entrapped in the proniosomes. For this, an appropriate amount of dispersion was transferred in centrifuge tubes. The dispersion was centrifuged (Remi Scientific Instruments, India) for 15 min at 15000 rpm. After centrifugation, the supernatant was collected and %Drug Entrapment amount of free thiocolchicoside was determined spectrophotometrically (λ_{max} = 258 nm).

DL efficiency (DL) of the drug-loaded system was also calculated concerning the yield of the nanoparticles obtained after centrifugation [20].

The %EE and %DL were calculated as per the equation given below, with all the measurements being performed in triplicate.

$$\%\ \text{Entrapment Efficiency} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}} \times 100$$

$$\%DL = \frac{\text{Amount of total entrapped drug}}{\text{Total weight of formulation}} \times 100$$

Particle size, polydispersity index (PDI), and zeta potential

The mean particle size, size distribution as PDI, and zeta potential of niosomes formulations were determined by dynamic light scattering method using Malvern Zeta Sizer (Anton Paar India Pvt. Ltd., India), which analyses the fluctuations in light scattering due to the Brownian motion of the particles. Light scattering was monitored at 25°C at a scattering angle of 90°. The samples were diluted with distilled water in 1:10 ratio before the measurement. Zeta potential was determined to measure the stability of reconstituted niosomes [18].

Scanning electron microscopy (SEM)

Proniosomes were sprinkled on to the double-sided tape affixed on the aluminium stub, placed in the vacuum chamber. The samples were observed for morphological characterization using a gaseous secondary electron detector. SEM images were recorded at 15 KeV accelerating voltage [9].

In vitro drug release study of proniosomes

In vitro dissolution study of proniosomal powders and pure drug was performed using USP type II (paddle) apparatus in both 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). The volume of dissolution medium used was 900 mL and maintained at a temperature of 37±0.5°C with paddle speed 50 rpm. An aliquot of 5 mL was collected and replaced with fresh dissolution medium at predetermined time intervals up to 24 h to maintain constant volume and the sink conditions. The samples were filtered by passing through membrane filter and analyzed by UV spectrometer at 258 nm [21].

In vitro drug release kinetics

To describe the kinetics of the release process of the drug in the different formulations, zero order, first order, Higuchi, and Korsmeyer and Peppas models were fitted to the dissolution data of the optimized formulation using linear regression analysis.

Zero-order kinetics

It is the cumulative amount of %drug released versus time,

$C = K_0 t$

Where K_0 is zero-order rate constant (concentration/time) and t is time (hours).

First-order kinetics

It is the cumulative log percentage of log (%) cumulative drug remaining versus time,

$$\text{Log } C = \frac{\text{Log Co} - kt}{2.303}$$

Where Co is initial concentration of the drug, k is first-order constant, and t is time.

Higuchi model

It is the cumulative percentage of drug released versus square root of time,

$Q = K t_{1/2}$

Where K is constant reflecting the design variables of the system and t is time (hours).

Korsmeyer–Peppas equations

Determines the mechanism of drug release from the polymer matrix. Log cumulative %drug released versus Log time, and from the slope of the straight line, the n was calculated, Q =Kt n

Where, Q is % drug released at time t, K is a constant and n is an exponent characterizes the mechanism of release of tracers [22].

Stability studies

Stability studies are carried out by storing the proniosomes at refrigeration temperature and room temperature according to ICH guidelines. Drug content and %Entrapment efficiency was periodically monitored [23].

RESULTS AND DISCUSSION

Analytical method development: Determination of λ_{max} of thiocolchicoside

Fig. 1 shows that pure drug was scanned over a range of 200–400 nm, the peak was observed at the 258 nm which confirmed the identification of thiocolchicoside in methanol.

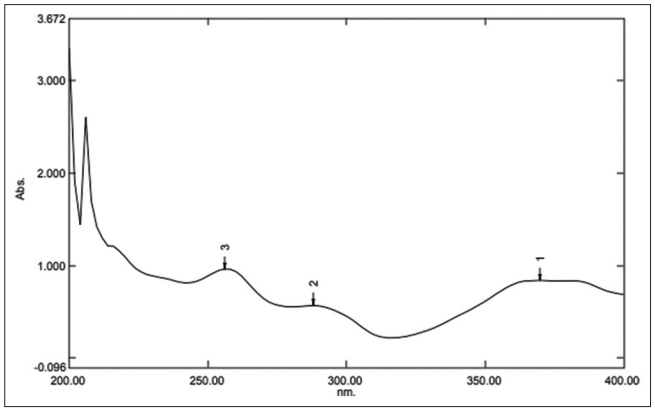

Fig. 1: Graph indicating λ_{max} of thiocolchicoside

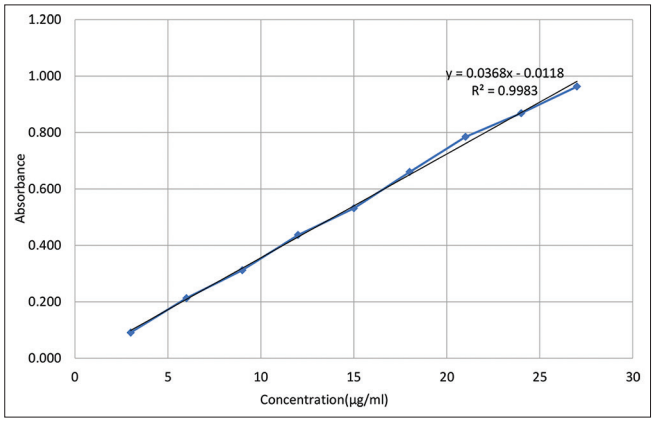

Fig. 2: Calibration curve of thiocolchicoside in methanol at λ_{max} =258 nm

Table 2: Standard calibration curve values of thiocolchicoside

Concentration (µg/mL)	Absorbance±SD*λmax=258 nm
3	0.091±0.001
6	0.213±0.001
9	0.312±0.001
12	0.437±0.001
15	0.532±0.001
18	0.660±0.002
21	0.785±0.001
24	0.869±0.001
27	0.963±0.001

*All the values are expressed in SD (n=3). SD: Standard deviation

Preparation of standard calibration curve of thiocolchicoside

From the Table 2 and Fig. 2, it has been resulted that standard graph of thiocolchicoside has showed good linearity with R^2 value 0.998 and the slope 0.0368 in methanol.

Drug-excipient compatibility studies by FTIR studies

IR spectral analysis was carried out using FT-IR, and the results showed that there were no/interactions between drugs and excipients. Figs. 3 and 4 show that the pure drug and its combination with excipients were subjected to FTIR studies. The characteristic peaks of thiocolchicoside showed IR absorption at 3292.68, 1602.76, 1427.81, 1074.15, and 857.94 cm^{-1}. All these peaks also have appeared in a physical mixture of the drug with Span 60, cholesterol, and sucrose stearate, which indicates compatibility and no chemical interaction between thiocolchicoside and the excipients confirms the stability of the drug during the formulation.

Characterization of proniosomes

Micromeritic properties of proniosomes

The angle of repose for all the formulations was found to be in the range of 26.76±0.09° to 34.29±0.60° and Carr's index values were found up to 15%, designates the excellent to good flow property according to IP limits. In addition, the dispersions showed a Hausner's ratio <1.25 is an indication of good flowability (Table 3).

%Drug content, %entrapment efficiency, and %DL

Higher entrapment efficiency of the vesicles of a formulation containing surfactant Span 60 is expected due to its higher alkyl chain length. F8 formulation showed highest entrapment efficiency of 83.62±0.34% which may have an optimum surfactant cholesterol ratio to provide a high entrapment of thiocolchicoside. The niosomal formulations having high surfactant concentration (F8, F9, and F10) showed the higher entrapment efficiency which might be due to the high fluidity of the vesicles. The formulation with very low cholesterol content (F1) was also found to cause low entrapment efficiency (55.59±0.20%), which might be due to leakage of the vesicles. It was also observed that formulation with very high cholesterol content (F6) had a low effect on drug entrapment (55.42±0.26%). This could be due to cholesterol beyond a certain level starts disrupting the regular bi-layered structure which leads to the loss of drug entrapment. %Drug content of all the formulations was found to be 87.34±0.85 to 98.66±0.85%. The % DL was found to be 16.97±0.010 to 20.33±0.011%. Entrapment efficiency, %drug content, and %DL obtained for all the formulations are given in Table 4.

SEM

SEM was carried out to determine the surface morphology of the proniosomes. Formulation F8 was selected as best formulation and, therefore, subjected to SEM to obtain the SEM images of proniosome powder (represented in Fig. 5). Most of the vesicles are well identified,

Table 3: Micromeritic properties of the prepared proniosomes formulations

Formulation code	Angle of repose (°)±SD*	Bulk density (g/mL)±SD*	Tapped density (g/mL)±SD*	Hausner's ratio±SD*	Carr's index (%)±SD*
F1	31.39±0.32	0.20±0.01	0.24±0.01	1.19±0.01	16.08±0.51
F2	28.63±0.10	0.20±0.01	0.22±0.00	1.10±0.03	08.87±2.87
F3	26.76±0.09	0.20±0.00	0.22±0.01	1.10±0.04	08.77±3.04
F4	31.49±0.30	0.20±0.01	0.24±0.01	1.17±0.08	14.42±5.74
F5	30.14±0.05	0.21±0.00	0.23±0.01	1.08±0.04	07.41±3.21
F6	32.35±0.38	0.20±0.01	0.23±0.01	1.16±0.04	13.77±2.84
F7	34.10±0.25	0.20±0.01	0.23±0.01	1.17±0.04	14.33±3.32
F8	28.42±0.45	0.20±0.00	0.22±0.01	1.08±0.04	07.41±3.21
F9	31.71±0.21	0.20±0.00	0.23±0.01	1.15±0.04	12.96±3.21
F10	31.59±0.13	0.21±0.00	0.24±0.01	1.16±0.05	13.73±3.40
F11	34.29±0.60	0.20±0.00	0.24±0.01	1.18±0.04	14.81±3.21

*Mean±SD (n=3). SD: Standard deviation

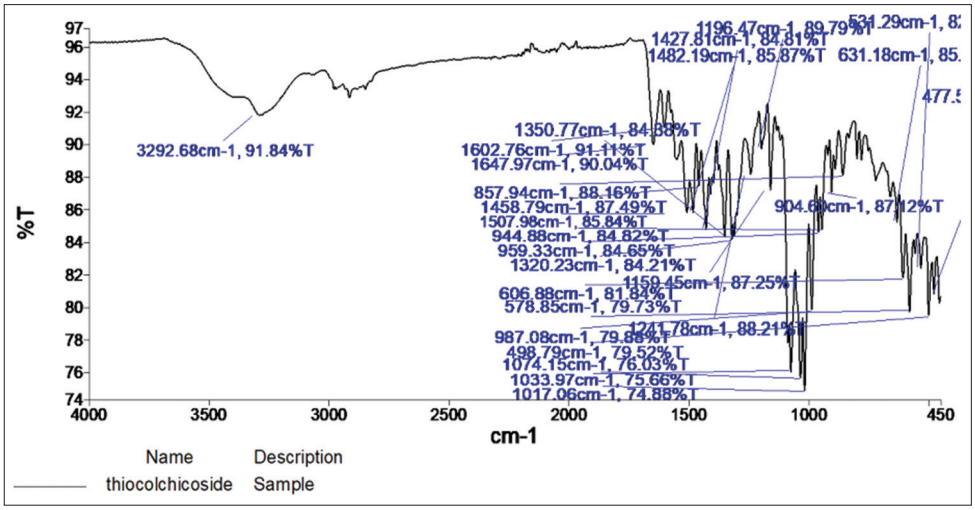

Fig. 3: Fourier-transform infrared spectroscopy spectrum of pure drug

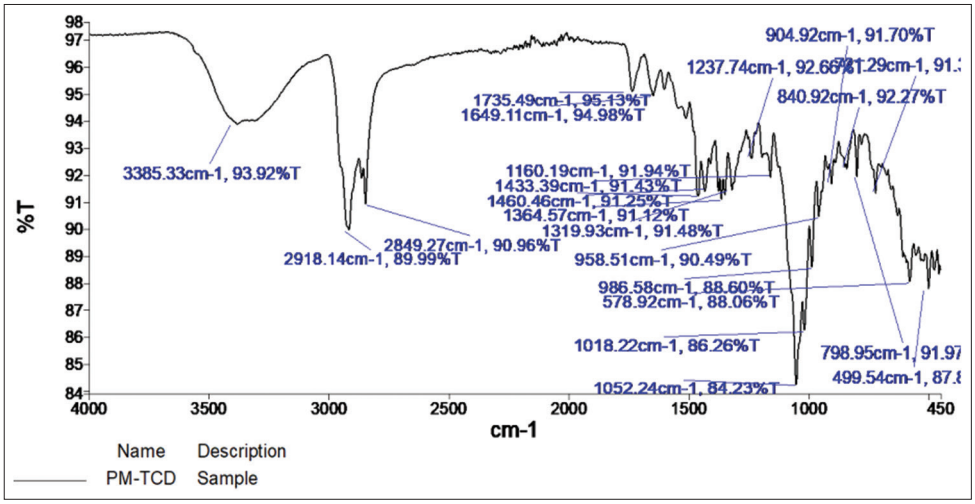

Fig. 4: Fourier-transform infrared spectroscopy spectra of physical mixture of drug with excipients

Table 4: Percentage drug content, entrapment efficiency, and % drug loading of all proniosomes formulations

Formulation code	Percentage drug content±SD*	Percentage EE±SD*	Percentage DL±SD*
F1	87.34±0.85	55.59±0.20	19.63±0.008
F2	97.76±0.68	75.80±0.26	19.98±0.011
F3	91.64±0.34	68.44±0.34	19.19±0.014
F4	96.74±0.68	65.05±0.17	18.58±0.007
F5	88.93±0.59	61.03±0.35	17.58±0.013
F6	91.76±0.20	55.42±0.26	16.97±0.010
F7	94.93±0.39	68.44±0.45	17.67±0.017
F8	98.32±0.52	83.62±0.34	18.72±0.010
F9	98.66±0.85	78.69±0.26	19.02±0.010
F10	92.21±0.71	77.45±0.34	19.49±0.014
F11	93.34±0.68	70.71±0.26	20.33±0.011

*Each value is average of three independent determinations. EE: Entrapment efficiency, DL: Drug loading, SD: Standard deviation

spherical and discreet with smooth boundaries having large internal aqueous space, and also, some of them were slightly spherical and or irregular in shape. The photomicrographs depicted the crystalline structure of the prepared Proniosomes at 230× and 650× magnification at 15 keV.

Table 5: Particle size, polydispersity index, and zeta potential of F8 formulation of proniosomes

Formulation	Particle size (nm)	PDI (%)	Zeta potential (mV)
F8	118.34	14.9	−36.8

PDI: Polydispersity index

Particle size, PDI, and zeta potential

Formulation F8 was selected and further characterized for particle size, zeta potential, and polydispersity index, as shown in Table 5. Formulation F8 showed a minimum particle size of 118.34 nm and PDI 14.9 as shown in Fig. 6 and Fig. 7. The low PDI indicates a narrow range of particle size distribution. As expected, all the formulations showed negative zeta potential which is due to the outer surfactant layers. F8 formulation showed an adequate zeta potential-36.8 mV indicates the good stability of thiocolchicoside niosomes from flocculation when seen in the context of its lower particle size.

In vitro dissolution studies

Drug release graph for pure drug and drug in proniosomes formulation (Fig. 8) was significantly different from the profile of drug

Table 6: *In vitro* dissolution study of formulation F8 versus pure drug

Dissolution medium	Time (h)	Percentage cumulative drug release of pure drug±SD*	Percentage cumulative drug release of formulation (F8)±SD*
0.1N HCl	0	0	0
	0.25	28.37±0.306	15.12±0.467
	0.5	33.16±0.467	21.14±0.636
	1.00	44.57±0.611	32.96±0.306
	2.00	63.63±0.636	43.66±0.529
Phosphate buffer, pH 7.4	3.00	85.54±0.917	58.43±0.176
	4.00	99.80±0.176	69.95±0.611
	6.00	99.80±0.176	85.94±0.353
	8.00	-	91.24±0.769
	10.00	-	94.10±0.809
	24.00	-	94.30±0.179

*Each value is average of three independent determinations. SD: Standard deviation

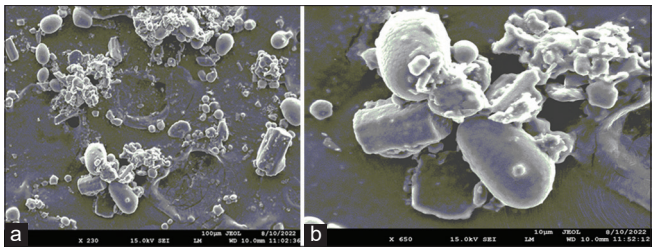

Fig. 5: Scanning electron microscopy images of proniosomes formulation F8 at (a) 230× and (b) 650× magnification

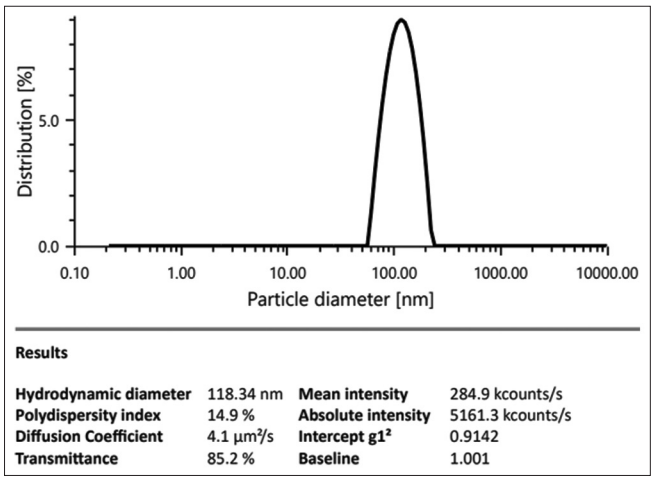

Fig. 6: Particle size distribution of F8

alone. In the pure drug solution, 33.16±0.467% thiocolchicoside was released within 30 min and followed by 99.80±0.176% in the 6th h, respectively. On the other hand, the release of drug in proniosomes formulation was considerably reduced with a maximum of 15.12±0.467–43.66±0.529% release in the acidic media within 2 h. After that, the percent cumulative drug release was found to be up to 94.30±0.179% within 24 h in phosphate buffer medium, pH 7.4. The *in vitro* drug release of formulation F8 and pure drug was given in a Table 6.

In vitro drug release kinetic studies

To predict the release mechanism and compare release profile, mathematical models are applied commonly. For the optimized formulation F8, the % drug release versus time (zero order), log percent drug remaining versus time (first order), log per cent drug release versus square root of time (Higuchi plot), and log of log

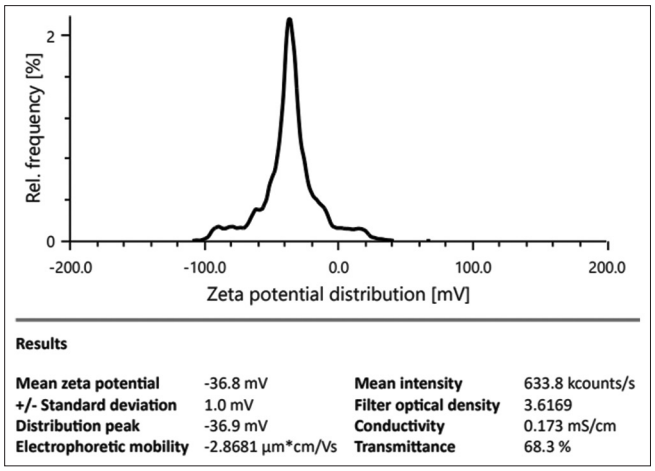

Fig. 7: Zeta potential of F8 formulation

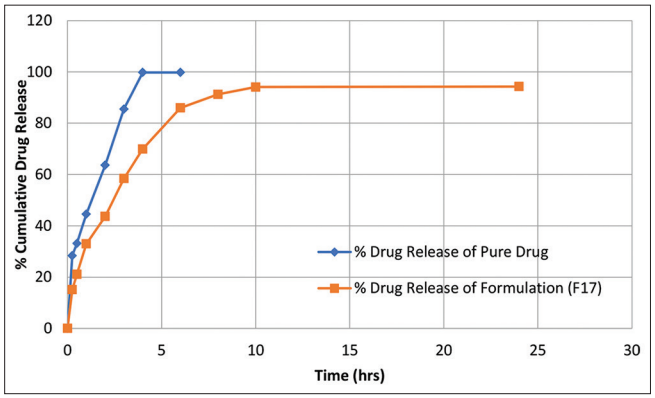

Fig. 8: %Cumulative drug release from formulation F8 and Pure drug

Table 7: R^2 values of F8 formulation for all the kinetic models

Formulation	Zero order	First order	Higuchi	Korsmeyer–Peppas
F8	0.550	0.700	0.798	0.941

% drug release versus log time (Korsmeyer–Peppas model) were plotted. In each case, R^2 value was calculated from the graph and reported in Table 7 and Figs. 9-12. Considering the determination coefficients, Korsmeyer–Peppas model was found linearity

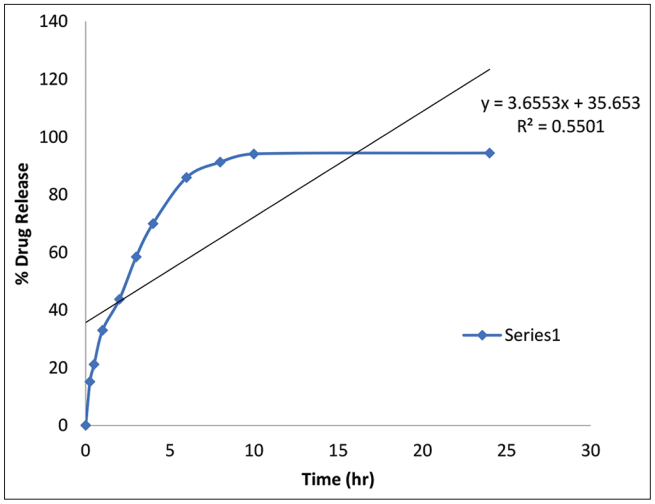

Fig. 9: Zero order plot for F8 formulation

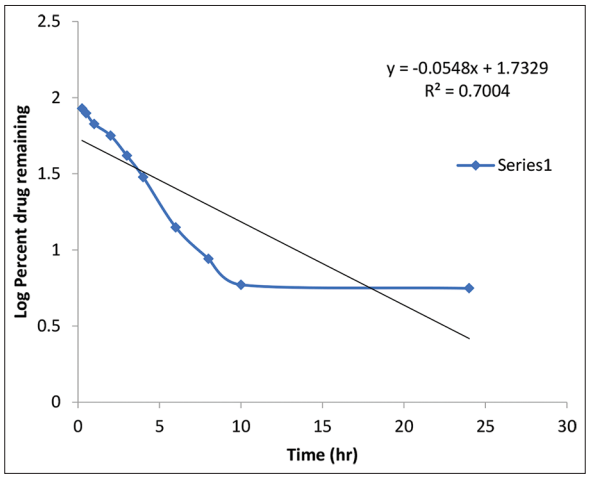

Fig. 10: First-order plot for F8 formulation

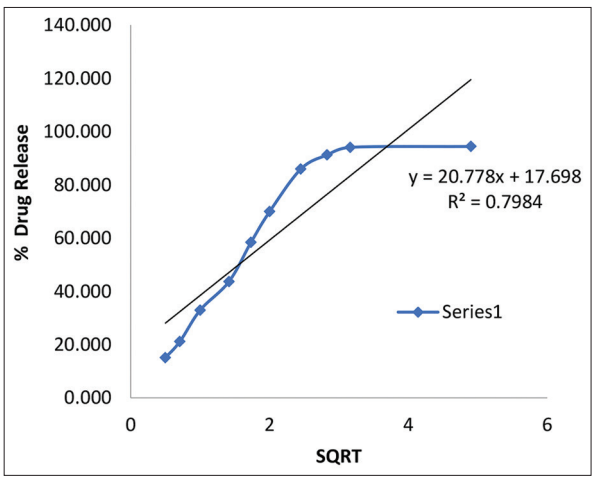

Fig. 11: Higuchi model plot for F8 formulation

R^2 = 0.9413, to fit the best release data. It could be concluded from the results that the drug was released from proniosomes by a controlled mechanism.

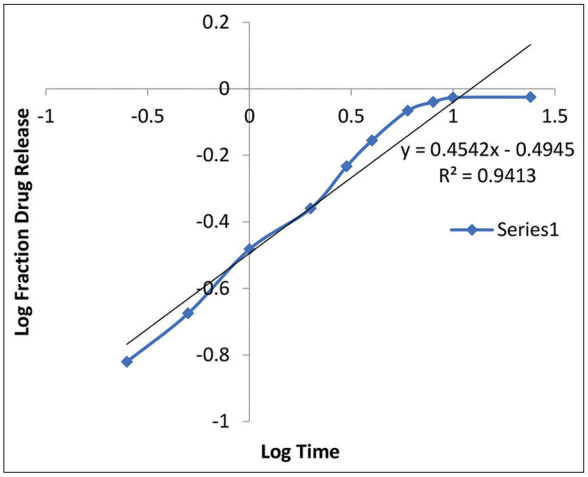

Fig. 12: Korsmeyer–Peppas model plot for F8 formulation

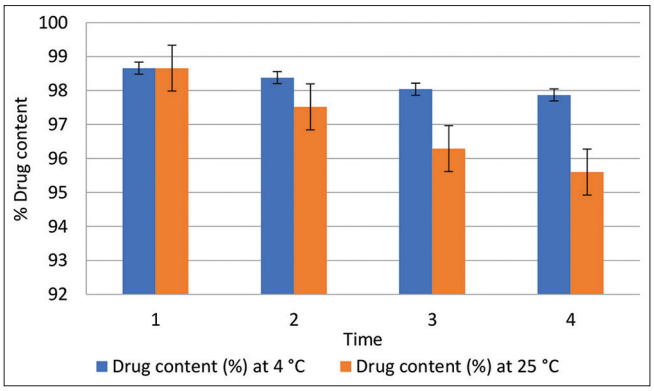

Fig. 13: Stability of F8 formulation indicating the %Drug content analysis

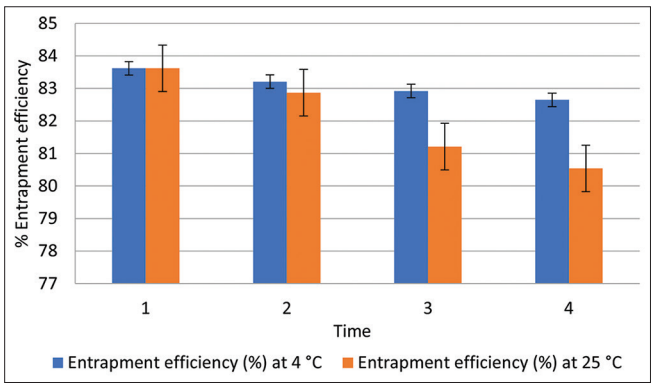

Fig. 14: Stability of F8 formulation indicating the %Entrapment efficiency

Stability studies of the best formulation

Selected formulation F8 was stored at 4°C±2°C and 25±2°C/65±5% RH for a period of 45 days. Table 8 shows that samples were analyzed after storage for 45 days and evaluated for %Entrapment efficiency and %Drug content in 0, 15, 30, and 45 days after storage. From the Table 8 and Figs. 13 and 14, it can be concluded that the drug leakage from the vesicles was least at 4°C. This may be attributed to phase transition of non-ionic surfactant and lipid causing leakage of vesicles at higher temperatures during storage.

Table 8: Stability studies for optimized formulation F8

Time of storage (days)	Temperature of storage			
	4±2°C (refrigerator temperature)		25±2°C (room temperature)	
	Drug content (%)	Entrapment efficiency (%)	Drug content (%)	Entrapment efficiency (%)
0	98.66	83.62	98.66	83.62
15	98.38	83.21	97.52	82.87
30	98.04	82.92	96.29	81.21
45	97.87	82.65	95.60	80.54

CONCLUSION

Proniosomes are promising drug carriers offers significant improvement in drug delivery by eliminating physical stability problems, such as aggregation or fusion of vesicles and leaking of entrapped drugs during storage. The slurry method was found to be simple, and hence, the slurry method was used to formulate proniosomes using sucrose stearate as the carrier. F1-F11 formulations of proniosomes were prepared. The optimized proniosomes formulation containing span 60 and cholesterol in equimolar ratio exhibited low size, high surface charge, and entrapment efficiency. The formulations possess good flow properties and the *in vitro* dissolution behavior was improved compared to control. The F8 formulation was optimized for its better particle size (151.76), PDI (0.12), and better zeta potential (−28.8). By this study, we concluded that thiocolchicoside could be successfully entrapped within the bilayer of the vesicles with high entrapment efficiency. Proniosomes based niosomes formed from Span 60 and cholesterol using sucrose stearate as a carrier is a promising approach to control the drug release for an extended period.

ACKNOWLEDGMENT

The authors would like to express gratitude to the Hindu College of Pharmacy, for their support and providing laboratory facilities about the research.

AUTHORS' CONTRIBUTION

All authors have contributed equally.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

AUTHORS FUNDING

None.

REFERENCES

1. Patil S, Mhaiskar A, Mundhada D. A review on novel drug delivery system: A recent trend. Int J Curr Pharm Res 2016;6:89-93.
2. Adepu S, Ramakrishna S. Controlled drug delivery systems: Current status and future directions. Molecules 2021;26:1-48. doi: 10.3390/molecules26195905, PMID 34641447
3. Löbenberg R, Amidon GL. Modern bioavailability, bioequivalence and biopharmaceutics classification system: New scientific approaches to international regulatory standards. Eur J Pharm Biopharm 2000;50:3-12. doi: 10.1016/s0939-6411(00)00091-6, PMID 10840189
4. Kumar R, Kumar S, Jha SS, Jha AK. Vesicular system-carrier for drug delivery. Der Pharmacia Sinica 2011;2:192-202.
5. Betageri G, Habib M. Liposomes as drug carriers. Pharm Eng 1994;14:76-7.
6. Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. J Control Release 1994;30:1-15. doi: 10.1016/0168-3659(94)90039-6
7. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes-non-ionic surfactant vesicles. J Pharm Pharmacol 1985;37:863-8. doi: 10.1111/j.2042-7158.1985.tb04990.x, PMID 2868092
8. Blazek-Welsh AI, Rhodes DG. Maltodextrin-based proniosomes. AAPS PharmSci 2001;3:E1. doi: 10.1208/ps030101, PMID 11741252
9. Gurrapu A, Jukanti R, Bobbala SR, Kanuganti S, Jeevana JB. Improved oral delivery of valsartan from maltodextrin based proniosome powders. Adv Powder Technol 2012;23:583-90. doi: 10.1016/j.apt.2011.06.005
10. Umarkar AR, Bavaskar SR, Yewale PN. Thiocolchicoside as muscle relaxant: A review. Int J Pharm Biol Sci 2011;1:364-71.
11. Shukla T, Pandey SP, Upmanyu N. Simultaneous estimation of thiocolchicoside and ketorolac tromethamine using UV spectroscopy. J Indian Chem Soc 2019;96:305-10.
12. Sachan AK, Kumar S, Singh S, Kumari K, Kumar K. Formulation and evaluation of floating microsphere of muscle relaxant drug thiocolchicoside. J Chem Pharm Res 2020;12:40-9.
13. Kumar D, Ali J, Baboota S. Omega 3 fatty acid-enriched nanoemulsion of thiocolchicoside for transdermal delivery: Formulation, characterization and absorption studies. Drug Deliv 2016;23:591-600. doi: 10.3109/10717544.2014.916764, PMID 24892633
14. Patel TB, Patel TR, Patel MN, Suhagia BN. Design and development of ethosomes for enhanced transdermal delivery of thiocolchicoside. Pharm Lett 2015;7:58-68.
15. Rele VR. UV-spectrophotometric estimation of thiocolchicoside by derivative method in pharmaceutical dosage form. Pharm Lett 2015;7:221-6.
16. Khatoon M, Shah KU, Din FU, Shah SU, Ur Rehman A, Dilawar N, *et al*. Proniosomes derived niosomes: Recent advancements in drug delivery and targeting. Drug Deliv 2017;2:56-69.
17. Ravi GS, Charyulu NR, Dubey A, Hebbar S, Mathias AC. Phytosomes: A novel molecular nanocomplex between Phyto molecule and phospholipid as a value-added herbal drug delivery system. Int J Pharm Sci Rev Res 2018;51:84-90.
18. Nasr A, Qushawy M, Swidan S. Spray dried lactose based proniosomes as stable provesicular drug delivery carriers: Screening, formulation, and physicochemical characterization. Int J Appl Pharm 2018;10:125-37. doi: 10.22159/ijap.2018v10i5.27732
19. Bhalerao A, Chaudhari PP. Formulation of Solid lipid nanoparticles of cilnidipine for the treatment of hypertension. J Drug Deliv Ther 2019;9:212-21. doi: 10.22270/jddt.v9i3.2849
20. Katrolia A, Chauhan SB, Shukla VK. Formulation and evaluation of metformin hydrochloride loaded curcumin-lycopene Niosomes. SN Appl Sci 2019;1:1-6. doi: 10.1007/s42452-019-1768-6
21. Annepogu H, Ahad HA, Nayakanti D. Determining the best poloxamer carrier for thiocolchicoside solid dispersions. Turk J Pharm Sci 2020;17:372-80. doi: 10.4274/tjps.galenos.2019.78800, PMID 32939132
22. Veerareddy PR, Bobbala SK. Enhanced oral bioavailability of isradipine via proniosomal systems. Drug Dev Ind Pharm 2013;39:909-17. doi: 10.3109/03639045.2012.717945, PMID 22998221
23. Cheriyan P, George BJ, Thomas N, Raj P, Samuel J, Carla SB. Formulation and characterization of maltodextrin based proniosomes of cephalosporins. World J Pharm Res 2015;3:62-74.