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Research Article

FORMULATION AND EVALUATION OF CLOBETASOL-17-PROPIONATE-LOADED CARBOXYMETHYL CHITOSAN NANOPARTICLE

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ABSTRACT

Objective: Formulation and evaluation of clobetasol-17-propionate-loaded carboxymethyl chitosan nanoparticle. Psoriasis is a chronic skin disorder caused due to the autoimmune factors. It has a detrimental psychological and physiological impact on patients due to the emergence of apparent skin. The systemic therapy with anti-psoriatic drugs such corticosteroids, immunosuppressant, and gene suppressors causes severe side effects. As a result, increasing the effectiveness and safety of the aforementioned medicines when applied topically would be extremely useful in avoiding the side effects associated with the systemic route of administration.

Methods: Chitosan (CS) has not been widely used in the clinic applications but due to its limited solubility and poor mechanical characteristics. CS, on the other hand, is chemically changed to form carboxymethyl (CMC), which is soluble at both neutral and basic pH. Chemical modifications can also be used to attach different functional groups and control hydrophobic, cationic, and anionic properties. CMC is a promising carrier that might possibly traverse the thick scales of psoriatic skin since it is a penetration enhancer that allows drug diffusion through either the transcellular or paracellular pathways. Comparative study is done using CMC as a polymer and CD as a polymer.

Results: CP-loaded CMC nanoparticles show better result results than CP-loaded CD polymer.

Conclusion: Clobetasol-17-propionate-loaded carboxymethyl chitosan nanoparticle shows better results with improved solubility.

Keywords: Psoriasis, Skin, Topical drug delivery, Carboxymethyl chitosan, Nanoparticles.

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INTRODUCTION

Psoriasis, an immune-mediated inflammatory dermatosis, has been widely viewed as an autoimmune disease in recent years, in light of psoriasis flare-ups being triggered by bacterial microbiota through molecular mimicry, such as between streptococcal and keratin proteins, the presence of homologous peptides between these proteins, and CD8+T cells' response to these homologous peptides. Psoriasis is a chronic skin disorder caused due to the autoimmune factors. It has a detrimental psychological and physiological impact on patients due to the emergence of apparent skin disfiguration expressed as erythematous plaques coated in silvery scales, as well as impairment of the skin's barrier function. As a result, it has a significant impact on patient's quality of life. Clobetasol propionate is a corticosteroid used to treat skin conditions such as eczema, contact dermatitis, seborrheic dermatitis, and psoriasis. It is applied to the skin as a cream, ointment, or shampoo. In the United States, clobetasol propionate is the most often used topical treatment for psoriasis. Ointment preparations, according to conventional dermatological wisdom, have the maximum efficacy (because to their occlusive nature and moisturizing capabilities) and are best suited for psoriasis. Patients, on the other hand, frequently find the administration of ointment to be unpleasant, creating concerns about both short- and long-term therapy adherences. Hence, to bypass the problems associated with current drug delivery technologies, newer drug delivery for clobetasol propionate is need of the hours [1-5].

CS has long been utilized in topical as well as transdermal preparations in the form of gel formulations to treat a variety of skin diseases, including burns, wounds, fungal, and microbial infections. However, its application has been restricted due to insolubility at neutral and basic pH. The use of CS alone in gelling system is having number of limitations due to the solubility issues associated with it. CS, on the other hand, is chemically changed to form carboxymethyl chitosan (CMC), which is soluble at both neutral and basic pH. Chemical modifications can also be used to attach different functional groups and control hydrophobic, cationic, and anionic properties. Progress is being made fast in this area, and the CMC generated has nearly infinite application possibilities in a wide range of industries [6-9].

CMC is a promising carrier that might possibly traverse the thick scales of psoriatic skin since it is a penetration enhancer that allows drug diffusion through either the transcellular or paracellular pathways. Because of the cationic character of the polymer and the polyanionic nature of skin cells' surface, CMC containing nano/ microparticles is recommended for dermal application because they provide adequate retention in the skin through bioadhesion. As a result, employing CMC as a carrier to bypass the stratum corneum (SC) and enhance drug delivery to the epidermal skin layer with low systemic absorption in the treatment of psoriasis seems promising, especially when produced as nanoparticles. The above results may be due to the fact that polymeric nanoparticles have been shown to have higher retention in the SC layer than lipidic systems, making them better candidates for topical treatment of skin diseases, especially when using lipophilic drugs, as they act as drug reservoirs to regulate skin permeation and allow for drug retention in the upstream. Small polymeric nanoparticles (100 nm or fewer) would selectively aggregate and have a longer retention period on inflamed skin than on healthy skin, especially when positively charged. The gelling system has already been widely utilized in the topical as well as transdermal drug delivery applications due to numerous advantages in comparison to other dosage forms. The use of various bioadhesive polymers in gelling system would increase the contact time with the skin leading to the better therapeutic effect in psoriatic treatment. As a result, it's thought that loading polymeric CMC nanoparticles with an efficient antipsoriatic drug in suitable gelling system developed with suitable bioadhesive polymers will increase their therapeutic efficacy in the treatment of psoriatic plaques [10-14].

METHODS

Materials

Clobetasol propionate is obtained as a gift sample from Dermocare Laboratories, India. All the other ingredients used are of analytical grade.

Methods

Preparation of carboxymethyl chitosan [15]

CS (0.640 g) was added to 10 M aqueous NaOH (10 mL) with stirring. The mixture was kept for several hours at room temperature and was stirred every 30 min to avoid aggregation of CS. After treatment with base, the CS mixture was transferred into an Erlenmeyer flask and 7 M aqueous chloroacetic acid was added dropwise over 5 min. Then, while stirring 1 M aqueous acetic acid was added dropwise into the mixture until the solution reached the correct pH. The reaction mixture flask was then placed into the water bath in the microwave oven and the temperature was adjusted by the recycling thermostated bath water. The reaction mixture was heated for some minutes under microwave irradiation. After cooling and filtering, the solution was concentrated and adjusted to the neutral pH by adding chloroacetic acid. Acetone was added to the solution, which resulted in the formation of a white precipitate, which was filtered and washed with a 3:1 solution of MeOH/water. White CMC was obtained after drying.

Preparation of nanoparticles by ionotropic gelation method [16]

The drug-loaded nanoparticles will be prepared using the ionic gelation method as per the reported method. Briefly, 10 mg of CMC or cyclodextrin will be dissolved in 5 ml 10 mM HEPES buffer (pH 7.4), and drug solutions will also be prepared in the same buffer at a definite concentration. The drug solution will be added to the CMC or cyclodextrin solution achieving a final concentration ranging from 0.1 to 0.5 mg/ml under constant stirring. While continuous stirring 1–2 ml of the crosslinking agent, TPP solution (1.7–2.0 mg/ml) will be added to the CMC or cyclodextrin drug solution drop by drop to induce ionic complexation. An opalescent dispersion formed after

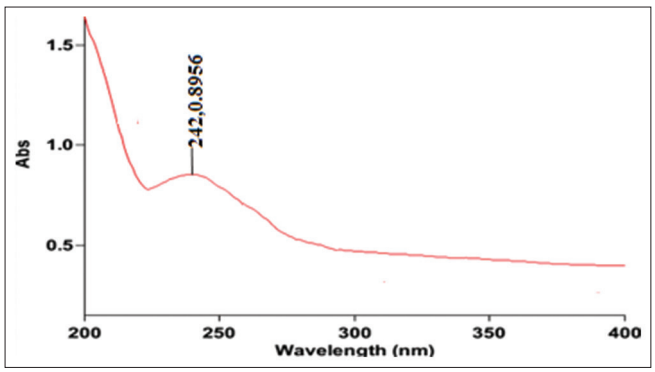

Fig. 1: UV spectra of pure CP in mixture of PBS pH 7.4:ethanol (70:30)

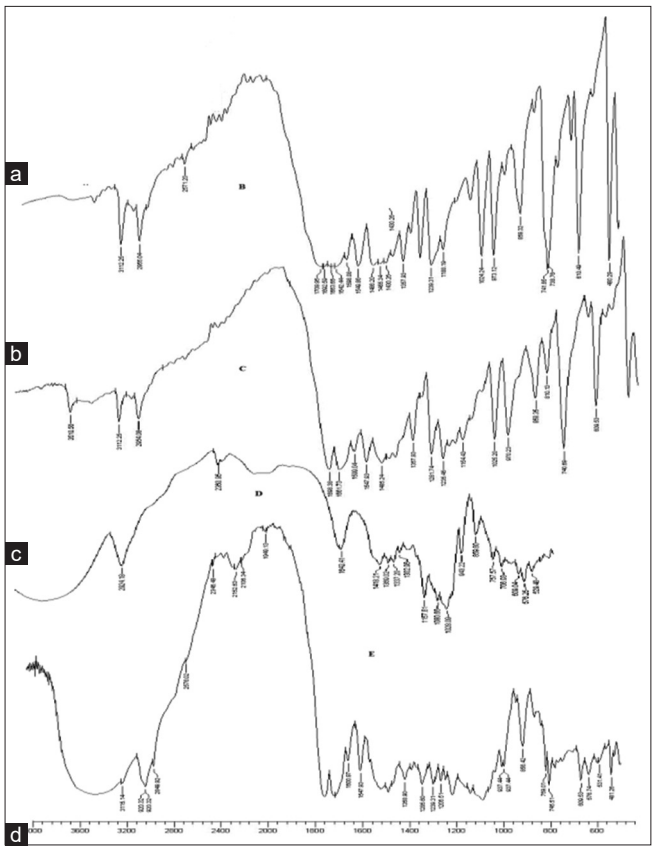

Fig. 2: FTIR spectra of (a) drug, (b) CMC, (C) physical mixture of drug and CMC, and (d) nanoparticles

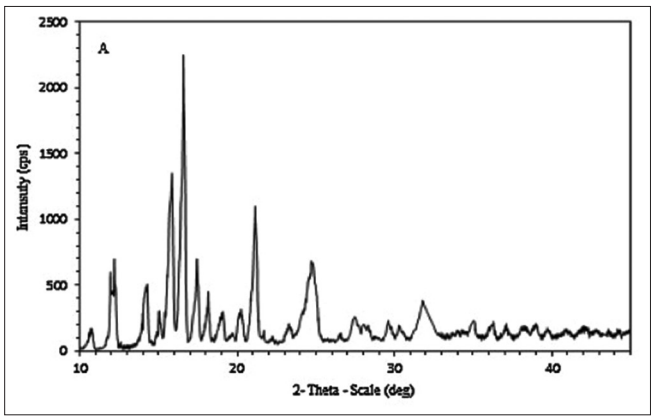

Fig. 3: XRD spectra of CP

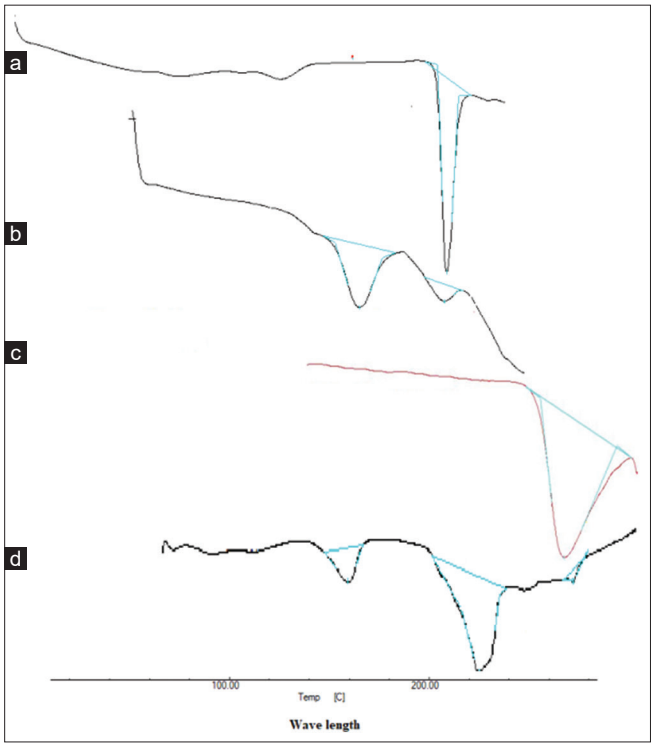

Fig. 4: DSC thermogram of (a) drug, (b) CMC, (C) physical mixture of drug and CMC, and (d) nanoparticles

Table 1: Formulation of CP-loaded CMC or cyclodextrin nanoparticles

Batch	Drug solution	CMC	CMC solution	TPP solution	Cyclodextrin	Cyclodextrin solution
CMCF1	10	0.50	5	1.70	-	-
CDF1	10	-	-	1.70	0.50	5
CMCF2	10	0.50	5	1.85	-	-
CDF2	10	-	-	1.85	0.50	5
CMCF3	10	0.50	5	2.00	-	-
CDF3	10	-	-	2.00	0.50	5
CMCF4	10	1.25	5	1.70	-	-
CDF4	10	-	-	1.70	1.25	5
CMCF5	10	1.25	5	1.85	-	-
CDF5	10	-	-	1.85	1.25	5
CMCF6	10	1.25	5	2.00	-	-
CDF6	10	-	-	2.00	1.25	5
CMCF7	10	2.00	5	1.70	-	-
CDF7	10	-	-	1.70	2.00	5
CMCF8	10	2.00	5	1.85	-	-
CDF8	10	-	-	1.85	2.00	5
CMCF9	10	2.00	5	2.00	-	-
CDF9	10	-	-	2.00	2.00	5

CMC: Carboxymethyl

Table 2: Solubility study of drugs in different solvent

S. No.	Media	Solubility (mg/ml)
1	Water	2 µg/ml
2	Ethanol	10 mg/ml
3	Ether	1 in 1000
4	Acetone	1 in 10
5	Dichloromethane	1 in 10

Table 3: Evaluation of different batches of nanoparticle

S. No.	Batch	Yield (%)	Particle size	Zeta potential
1	CMCF1	87.56	87.65	–18
2	CDF1	75.56	101.42	–25
3	CMCF2	95.68	59.87	–14
4	CDF2	81.53	132.35	–21
5	CMCF3	75.68	68.69	–20
6	CDF3	69.84	84.65	–19
7	CMCF4	84.89	75.65	–17
8	CDF4	80.45	84.56	–16
9	CMCF5	94.65	95.62	–16
10	CDF5	73.56	87.65	–18
11	CMCF6	79.68	105.36	–12
12	CDF6	65.32	98.65	–30
13	CMCF7	83.54	129.67	–19
14	CDF7	76.45	101.32	–24
15	CMCF8	92.65	118.65	–13
16	CDF8	78.95	145.65	–24
17	CMCF9	89.55	84.65	–15
18	CDF9	77.01	132.32	–17

TPP addition will indicate the formation of nanoparticles. The nanoparticles will be collected by centrifugation at 12,000 rpm for 15 min on a 10 µl glycerol bed. The particles will be stored at −20°C until further use.

Characterization of nanoparticles

For further characterization, nanoparticles was performed to access interaction if any between the drug and polymer and also to find out what properties of polymer make them an effective material for solubility and bioavailability enhancement.

Particle size measurement

The sizes of particles are maintained during polymerization for the formation of free following powders having fine esthetic attributes. Particle size analysis of loaded and unloaded nanoparticles performed.

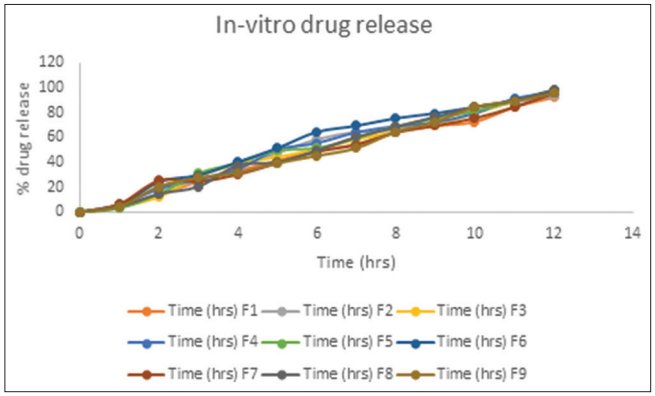

Fig. 5: Graphical presentation of comparative drug release profile for F1–F9 formulations

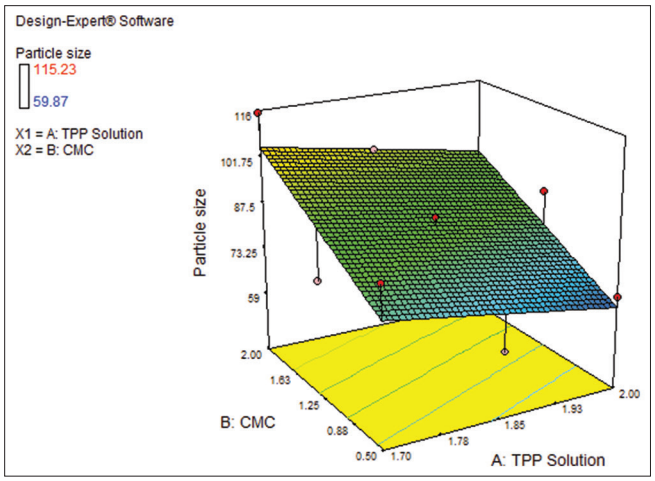

Fig. 6: 3D surface plot of particle size of CP with respect to TPP solution and CMC

Cumulative graph is maintained or plotted as particle size against time to study effect of particle size on drug release [17].

Drug content

To calculate the drug content, accurately weighed quantity of nanoparticles (10 mg) with 5 ml of methanol in a volumetric flask was

Table 4: Drug content values of different batches of nanoparticle

Batch	Drug content (%)	Drug loading (%)	Batch	Drug content (%)	Drug loading (%)
CMCF1	84.56	86.54	CDF1	75.65	84.65
CMCF2	98.85	97.65	CDF2	84.62	89.65
CMCF3	83.21	80.65	CDF3	70.32	78.95
CMCF4	79.65	82.65	CDF4	89.84	85.62
CMCF5	94.56	94.56	CDF5	77.86	88.56
CMCF6	86.65	90.54	CDF6	80.32	82.65
CMCF7	93.65	88.45	CDF7	81.23	84.65
CMCF8	96.65	97.54	CDF8	79.85	87.65
CMCF9	89.65	89.65	CDF9	73.65	92.32

Table 5: Results of comparative study

Batch	Particle size	Zeta potential	Drug content
F2 (using CMC)	59.87	–14	98.85
F4 (using CD)	84.56	–16	89.84

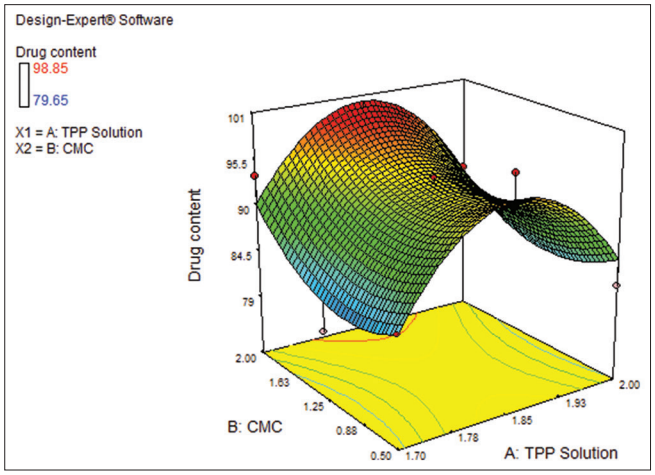

Fig. 7: 3D surface plot of drug content of CP with respect to TPP solution and CMC

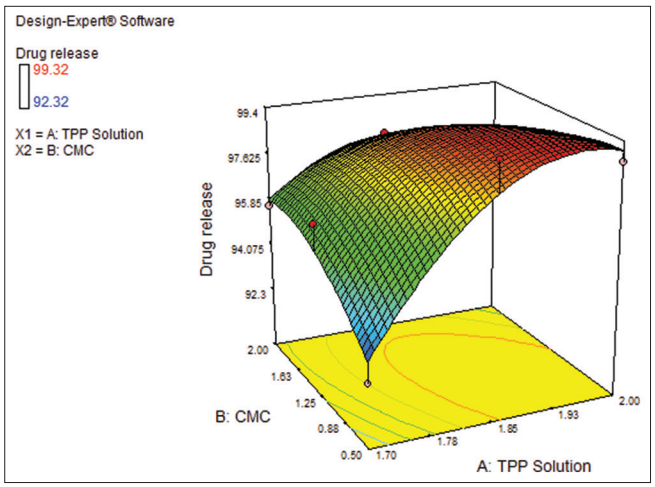

Fig. 8: 3D surface plot of drug release of CP with respect to TPP solution and CMC

shaken for 1 min using vortex mixer. The volume was made up to 10 ml. Then, the solution was filtered and diluted and the concentration of CP was determined spectrometrically [18].

$$\%\,Drug\,content = \frac{Actual\,drug\,content}{Theoretical\,drug\,content} \times 100$$

In vitro release studies

In vitro drug release studies of the nanoparticles were carried out using modified Franz diffusion cell during 24 h. Mixture of PBS pH 7.4:ethanol (70:30) was used as receptor medium and sink condition was determined. The receptor phase was kept at a constant temperature of 37°C and stirred by a magnetic stirrer. At appropriate time intervals, 0.5 ml of samples were collected and replaced by an equal volume of fresh receptor medium. The clobetasol propionate content was analyzed by UV at a wavelength of 242 nm [19].

Encapsulation efficiency and drug loading capacity

For the determination of encapsulation, efficiency accurately weighed NPs (10 mg) were added to 10 mL of distilled water and after the equilibrium solubility was attained, clear supernatant after centrifugation was filtered and 1 mL of the filtrate was mixed with 4 mL of methanolic HCl. Resulting sample was analyzed on UV–visible spectrophotometer at 242 nm [20].

For the determination of drug loading capacity, NPs (5 mg) were dissolved in 5 mL of methanolic HCl and the solution was filtered through 0.2 µm filter (Axiva Syringe filter) [21]. VCZ concentration in the sample was determined using UV–visible spectrophotometer at 300 nm. The percentage drug loading capacity was determined using the following formula:

% Drug loading = (Mass of drug in NP/Mass of NP recovered)×100

RESULTS AND DISCUSSION

UV spectroscopic analysis

Determination of λ max of CP in dissolution media

The standard solution (100 µg/ml) of pure drug (CP) was prepared in freshly prepared mixture of PBS pH 7.4:ethanol (70:30). The prepared solution was scanned between 200 and 400 nm by UV–visible spectrophotometer.

Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra were taken on IR spectrophotometer using KBr pellet technique. The scanning range was 4000–400 cm^{-1}. The peaks were interpreted for the confirmation of various functional groups.

XRD

The XRD measurements were carried out using Bruker D8 Advance X-ray diffractometer. The X-rays (Cu K-alpha) were produced using a sealed tube and the samples were scanned over a 2 θ range of 2–50° with a scanning rate of 5°/min. The X-rays were detected using a fast counting detector based on silicon strip technology (Bruker Lynx Eye detector). The XRD spectrum of VCZ exhibited sharp peaks at 6.9°, 12.6°, 13.8°, 15.9°, 16.5°, 17.4°, and 19.8° at 2 θ-scattered angles, which indicates the crystalline nature of the drug.

Table 6: *In- vitro* drug release studies of different batches of nanoparticle

Time (h)	% drug release of nanoparticle								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
2	5.45	5.98	4.32	6.01	3.25	5.46	6.45	4.51	4.56
4	14.32	19.65	12.35	18.65	14.65	24.32	25.36	14.65	20.6
6	24.65	24.65	28.65	26.65	31.23	29.68	24.65	20.65	27.65
8	33.25	36.65	38.95	34.56	39.65	40.35	30.32	37.65	31.65
10	41.42	47.65	44.35	50.32	49.56	51.56	39.56	40.23	39.45
12	49.56	58.65	50.65	55.62	51.65	64.56	48.65	49.62	45.35
14	54.62	64.62	58.65	64.32	59.56	69.56	54.35	60.56	51.62
16	64.65	66.52	64.62	69.25	69.01	75.65	64.23	68.56	64.65
18	69.51	76.56	70.32	72.36	75.65	79.56	69.56	76.65	72.65
20	72.65	81.26	79.65	79.65	81.56	84.65	75.62	84.65	84.65
22	84.65	88.62	89.65	91.32	89.65	90.24	84.65	89.62	89.45
24	92.32	99.32	98.65	96.35	97.65	98.45	95.62	97.86	95.62

Table 7: Results of optimized batch

Batch	Particle size	Zeta potential	Drug content	Drug release
F2	59.87	–14	98.85	99.32

Table 8: Stability study of optimized formulation

Time(day)	Drug content (%)	*In vitro* drug release (%)
0	98.658	99.884
30	96.52	96.522
60	95.61	94.548
90	89.32	92.365

Differential scanning calorimeter (DSC)
Small amount of sample (2 mg) was placed in the DSC aluminum pan and sealed. It was then heated under nitrogen flow at scanning rate of 10°C/min in the temperature range of 20–250°C. An empty aluminum pan was placed as a reference. Endothermic peaks were recorded. A sharp peak was observed at 132°C, indicative of its melting point this was near the reported melting point of the drug, that is, 128–130°C. The DSC thermogram confirmed the crystalline nature of the drug.

Solubility studies
Solubility studies were performed to analyze the solubility enhancing properties of nanoparticles. Solubility studies provided the basis for selection of the best ratio that was to be forwarded for formulation.

Evaluation of nanoparticles

Percentage yield

The percentage yield of different batches using CMC and cyclodextrin as a polymer was determined by weighing the nanoparticle after drying and as shown in below table.

Zeta potential

The zeta potentials of CP formulation is sufficient enough (–10–30 mV) to stabilize the formulation.

Particle size analysis

The particle size of CP-loaded CMC nanoparticles and CP-loaded CD nanoparticle is shown below. The change in the concentration of polymer results in variation of particle size of nanoparticles.

Drug content determination
The various batches of the nanoparticle were subjected for drug content analysis. The powdered nanoparticle (10 mg) was dissolved in adequate quantity (10 ml) of methanol. The UV absorbance was measured using a UV spectrometer at 251 nm.

Comparative study of CMC and cyclodextrin polymer
Nanoparticles are prepared using ionic gelation method. The comparative study was done between polymer CMC and polymer cyclodextrin.

Then, the prepared nanoparticles are evaluated. The nanoparticles prepared using CMC shows better results than the nanoparticles prepared using cyclodextrin. The results are as shown below:

From the above-mentioned results, the CP-loaded CMC nanoparticles show good drug content with minimum particle size than CP-loaded CD nanoparticles. Hence, CMC polymer is better than the cyclodextrin polymer.

***In vitro* drug release study**
In vitro drug release for drug-loaded nanoparticles for a period of 24 h was carried out by using mixture of PBS pH 7.4:ethanol (70:30) at 37±5°C. From the dissolution profile of formulations F1–F9, it is concluded that formulation batch F2 shows better drug release profile than other formulations. Cumulative % release has been shown for average of three preparations. Cumulative % drug release for all the formulations is depicted in table below.

Optimized batch
From the above results, F2 batch was found to be optimized.

Optimization

Effect analysis on particle size

Three-dimensional graphical presentations 3D surface
The 3D in figure shows that as increase in concentration of TPP solution, particle size decreases while as CMC concentration increases, particle size also increases. It was concluded from the graph that the factor A has significance effect on the particle size.

Effect analysis on drug content

Three-dimensional graphical presentations 3D surface

The 3D in figure shows that as increase in concentration of TPP solution and CMC concentration, it shows increase in drug content. It was concluded from the graph that the factor A has significance effect on the drug content.

Effect analysis on drug release

Three-dimensional graphical presentations 3D surface

The 3D in figure shows that as increase in concentration of TPP solution and CMC concentration, it shows increase in drug release at some point, then there is decrease in drug release. It was concluded from the graph that the factor A has significance effect on the drug release.

Stability studies
Optimized formulation was subjected to stability studies as per ICH guidelines. Various parameters such as drug content and *in vitro*

drug release were measured before and after 30, 60, and 90 days of stability. Results of stability studies are shown in the following table. Results of stability studies showed that there is no significant change in above-mentioned parameter after one from the stability studies that the prepared formulation is stable and not much affected by elevated humidity and temperature conditions.

CONCLUSION

Psoriasis often reoccurs and is rarely cured, and hence, patients may receive therapy periodically over many years. Hence, developing efficient vehicles for delivering clobetasol to treat psoriasis are especially important. CP-loaded CMC nanoparticles formulations were developed in this study to achieve this aim. Enhanced permeation and sustained release of clobetasol were obtained with nanoparticle. Enhanced permeation can be useful for improving the skin absorption of drugs, while sustained release is important for drugs with irritating effects at high concentrations or to supply the skin with drugs over a prolonged period of time. The comparative study using both polymers, that is, CMC and CD was done. From the obtained results, CP-loaded CMC nanoparticles show better results. However, optimized CP nanoparticles exhibited better stability.

AUTHORS' CONTRIBUTION

All authors contributed equally.

CONFLICTS OF INTEREST

No.

AUTHORS' FUNDING

No.

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