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Research Article

PREPARATION AND EVALUATION OF TMC LOADED VORICONAZOLE NANOPARTICLES

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ABSTRACT

Objective: Ocular diseases affect a growing number of people across the globe. Hence, the present research work focused to prepare and evaluate voriconazole (VCZ) nanoparticles containing trimethyl chitosan (TMC) for ophthalmic drug delivery with primary goal is to develop topical ocular delivery systems with improved ocular bioavailability and reduced systemic side effects while maintaining the dosage form's simplicity and convenience.

Methods: In the present study, the nanoparticles are prepared using ionotropic gelation method. The physiochemical interactions between drugs and selected excipients were studied using various techniques such as FTIR, DSC, XRD, and H-NMR. The physiochemical properties of the nanoparticles such as size, PDI, pH, and drug content/entrapment efficiency were determined. The *in-vitro* drug release properties were characteristics and examined for the formulations. The synthesize form of chitosan, that is, tri-methyl chitosan is used due to solubility issue.

Result: The comparative study was done using TMC and cyclodextrin as a polymer out of which TMC polymer gives better results. The optimization is done using 3^2 factorial design using design expert software. The optimized batch follows the zero order release kinetics.

Conclusion: TMC loaded VCZ nanoparticles show better result with improved solubility and permeability.

Keywords: Voriconazole, Tri-methyl chitosan, Nanoparticles, Opthalmic drug delivery system.

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INTRODUCTION

Nanotechnology has been developed to overcome eye barriers and protect active substances. Mucoadhesive Chitosan nanocarriers increase eye contact time and act as permeability enhancers. Chitosan, on the other hand, does not get dissolve in both neutral and basic aqueous environments so its use is limited in drug delivery applications [1,2]. The use of Chitosan in ophthalmic drug delivery is restricted due to its insolubility at neutral and basic pH [3]. In presence of diseased conditions such as glaucoma, fungal infection, age-related macular degeneration, diabetic retinopathy, and dry eye syndrome that requires a novel drug delivery system for a prolonged period [4-6]. Hence, it has become necessary to create pharmaceutical formulations that provide sustained release and increased bioavailability with decreased frequency of administration. A significant challenge in achieving this goal is to overcome ocular barriers without causing permanent tissue damage. Hence, considering all these discussed potential drawbacks of the pure Chitosan molecule and conventional ophthalmic formulations, an innovative drug delivery system using modified Chitosan derivatives is need of the hours. The use of Chitosan in ophthalmic drug delivery is restricted due to its insolubility at neutral and basic pH. However, Chitosan is chemically modified to produce derivatives like Trimethyl Chitosan (TMC) that are soluble at both neutral and basic pH. Chemical changes can also be utilized to regulate hydrophobic, cationic, and anionic characteristics as well as attach various functional groups. Progress is being made quickly in this area, and the produced TMC has almost limitless application potential in a variety of fields. Furthermore, poor patient compliance, lesser corneal permeation, frequent dosage administration, and very less bioavailability are some of the major challenges associated with the ophthalmic drug delivery system. In the ophthalmic drug delivery system, most of the drug is drained away from the precorneal area in few minutes. As a result, frequent instillation of concentrated solutions is needed to achieve the desired therapeutic effects. However, by the tear drainage, the main part of the administered drug is transported through the nasolacrimal duct to the gastric intestinal tract where it may be absorbed, sometimes causing side effects. To increase the effectiveness of the drug, a dosage form should be chosen which increases the contact time of the drug in the eye. This may then increase the bioavailability, reduce systemic absorption, and reduce the need for frequent administration leading to improved patient compliance [7-10].

Voriconazole (VCZ) is the first second-generation triazole antifungal to hit the market. VCZ is in a class of antifungal medications called triazoles. It works by slowing the growth of the fungi that cause infection. VCZ is available in both oral and intravenous dosage forms. The systemic injection of VCZ, on the other hand, is associated with severe side effects and drug interactions. However, because of VCZ's low aqueous solubility, no commercial ocular product is currently available. Hence, considering the drawback of the current drug delivery system, a novel drug delivery system in the form of VCZ-TMC nanoparticles would be the better approach to overcome the serious issues associated with the current drug delivery system [11,12].

METHODS

Material

VCZ is obtained as a gift sample from Apollo Pharmaceutical, Mumbai, India. All the other ingredient's used are of analytical grade.

Method

Preparation of TMC

TMC was synthesized by reductive methylationaccording to a previous report with some modifications. Briefly, 2 g chitosan, 1.3 g sodium azide, 4 mL sodium hydroxide (10%w/v), and 3.5 mL methyl iodide were added and stirred at 50°C for 2 h. Next, 150 mL of distilled water (DW) was added and stirring was continued for 1 h before being dialyzed against DW overnight. A precipitate was formed by adding a large volume of acetone; it was washed with sodium hydroxide. The

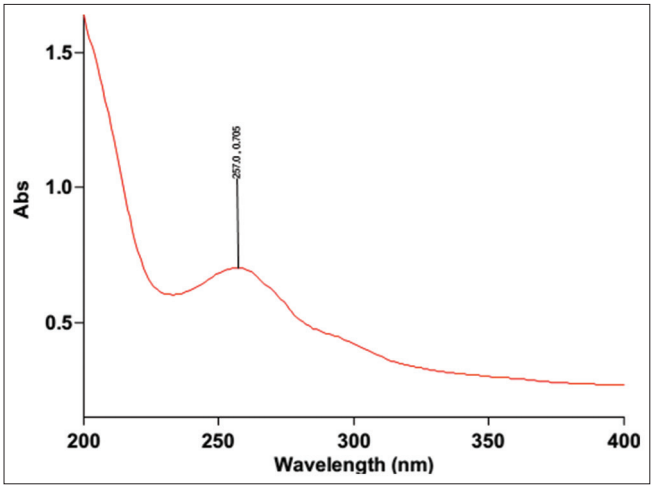

Fig. 1: UV- Spectra of pure voriconazole in simulated tear fluid

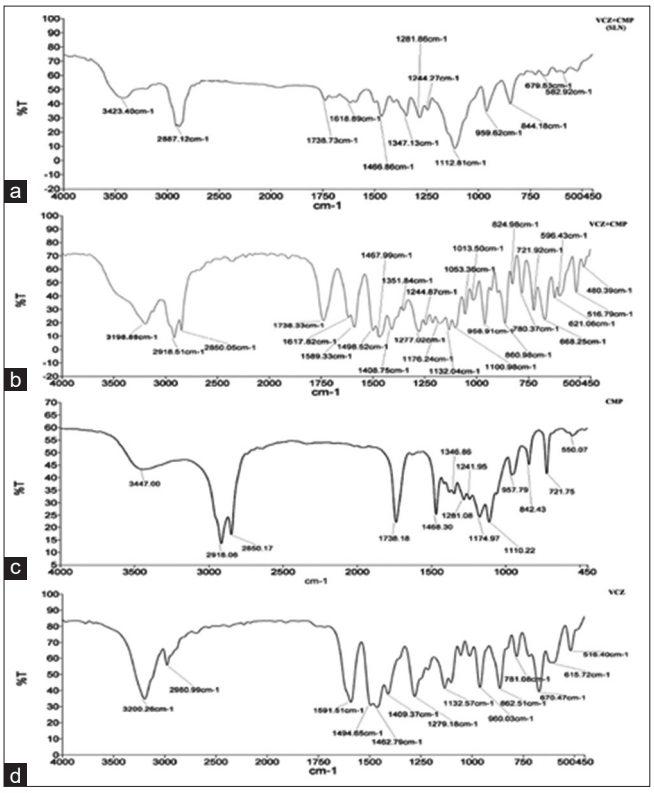

Fig. 2: FTIR spectra of (a) Drug, (b) TMC, (c) Physical mixture of drug and TMC, and (d) Nanoparticles

precipitate was suspended in water and dialyzed for 24 h, followed by freeze drying [13,14].

Preparation of nanoparticles by ionotropic gelation method

The drug-loaded (VCZ) nanoparticles will be prepared using the ionotropic gelation method as per the reported method. Briefly, 10 mg of TMC 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 TMC 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 TMC or cyclodextrin-drug solution drop by drop to induce ionotropic complexation. An opalescent dispersion formed after TPP

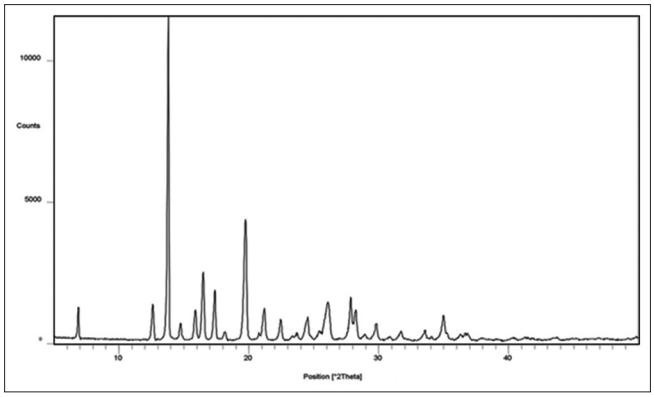

Fig. 3: XRD spectra of VCZ

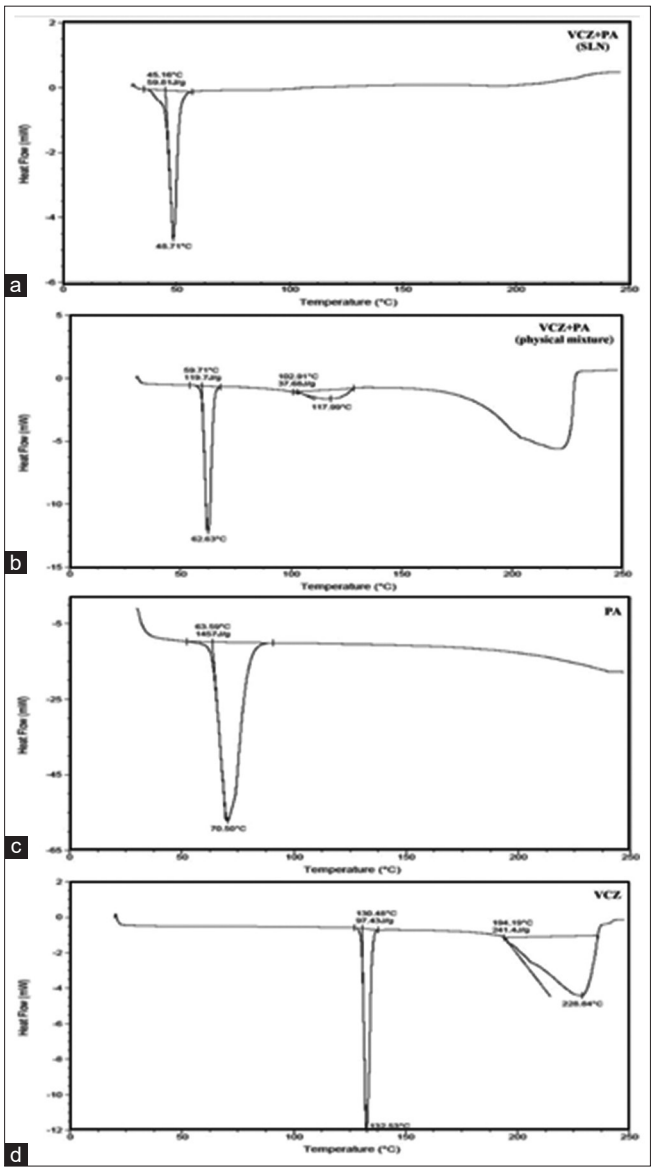

Fig. 4: DSC thermogram of (a) Drug, (b) TMC, (c) Physical mixture of drug and TMC, and (d) Nanoparticles

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 [15,16].

Table 1 : Dissolution test parameters for powder dissolution test of nanoparticles

Batches	Dissolution media (900 ml)	Paddle speed (RPM)	Bath temperature (°C)	UV Analysis (λmax) (nm)	Time (hours)
F1 to F9	Simulated tear fluid	100	37±0.5	257	12

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 aesthetic attributes. Particle size analysis of loaded and unloaded nanoparticles performed by nanoparticle size analyzer. 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 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 VCZ was determined spectrometrically [18].

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

In vitro release studies

In vitro release studies were performed in triplicate using USP Paddle method at 100 rpm and 37±0.2°C in 900 ml of simulated tear fluid. Samples were taken at appropriate time intervals. The samples were measured spectrophotometrically at 257 nm. Fresh dissolution medium was replenished each time when sample is withdrawn to compensate the volume [19]. The dissolution parameters are given in Table 1.

Surface morphology

Scanning electron microscopy (JSM-5200, Tokyo Japan) was used to analyze particle size and surface topography was operated at 30 kV acceleration voltage. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer 20 nm thick. Photographs were elaborated by an image processing program and individual NP diameters were measured to obtain mean particle size [20].

Encapsulation efficiency and drug loading capacity

For the determination of encapsulation, efficiency accurately weighed NPs (10 mg) were added to 10 mL of DW 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 257 nm.

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). VCZ concentration in the sample was determined using UV visible spectrophotometer at 257 nm [21]. The percentage drug loading capacity was determined using the following formula:

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

Formulation of VCZ loaded-TMC OR CD Nanoparticles

The formulation of voriconazole loaded – TMC OR CD Nanoparticles are given in Table 2

Table 2: Formulation of Voriconazole loaded TMC and CD nanoparticles

Batch	Drug solution	TMC	TMC Solution	TPP solution	CD	CD Solution
TMCF1	10	0.50	5	1.70	-	-
CDF1	10	-	-	1.70	0.50	5
TMCF2	10	0.50	5	1.85	-	-
CDF2	10	-	-	1.85	0.50	5
TMCF3	10	0.50	5	2.00	-	-
CDF3	10	-	-	2.00	0.50	5
TMCF4	10	1.25	5	1.70	-	-
CDF4	10	-	-	1.70	1.25	5
TMCF5	10	1.25	5	1.85	-	-
CDF5	10	-	-	1.85	1.25	5
TMCF6	10	1.25	5	2.00	-	-
CDF6	10	-	-	2.00	1.25	5
TMCF7	10	2.00	5	1.70	-	-
CDF7	10	-	-	1.70	2.00	5
TMCF8	10	2.00	5	1.85	-	-
CDF8	10	-	-	1.85	2.00	5
TMCF9	10	2.00	5	2.00	-	-
CDF9	10	-	-	2.00	2.00	5

TMC: Trimethyl chitosan

Table 3: Evaluation of different batches of nanoparticle

Batch	Yield (%)		Particle size		Zeta potential	
	TMC	CD	TMC	CD	TMC	CD
F1	84.56	75.65	123.32	132.52	−19	−20
F2	93.68	84.62	75.62	142.32	−11	−18
F3	89.65	89.65	54.52	110.23	−14	−17
F4	88.65	79.85	89.92	98.65	−15	−16
F5	75.98	86.65	94.62	101.32	−17	−15
F6	94.56	94.65	79.65	84.25	−13	−14
F7	84.54	74.65	82.54	99.65	−18	−20
F8	68.75	88.62	68.65	123.65	−16	−19
F9	80.21	72.32	58.65	104.65	−20	−17

TMC: Trimethyl chitosan

Table 4: Drug content values of different batches of nanoparticle

Batch	Drug content (%)		Drug loading (%)	
	TMC	CD	TMC	CD
F1	75.23	54.65	81.25	79.65
F2	88.32	74.65	87.65	68.95
F3	94.87	84.62	95.54	84.65
F4	85.65	68.65	86.65	79.86
F5	91.23	78.65	89.39	89.65
F6	86.45	91.32	95.99	90.33
F7	89.54	88.56	91.23	88.98
F8	94.65	81.32	86.22	93.65
F9	90.21	89.32	90.68	90.32

TMC: Trimethyl chitosan

Table 5: Results of comparative study

Batch	Particle size	Zeta potential	Drug content
F3 (using TMC)	54.52	−14	94.87
F6 (using cyclodextrin)	84.25	−14	91.32

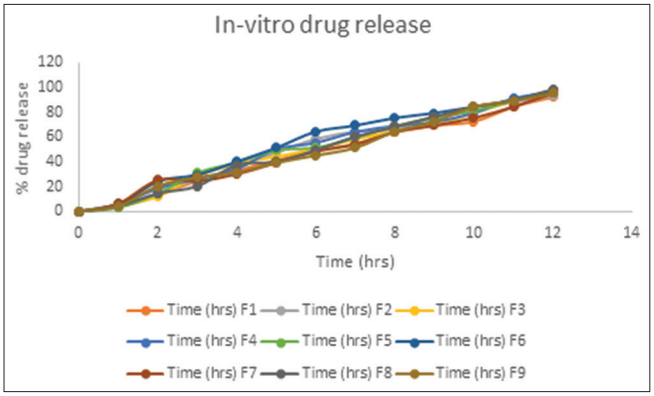

Fig. 5: Graphical presentation of comparative drug release profile for F1 to F9 formulations

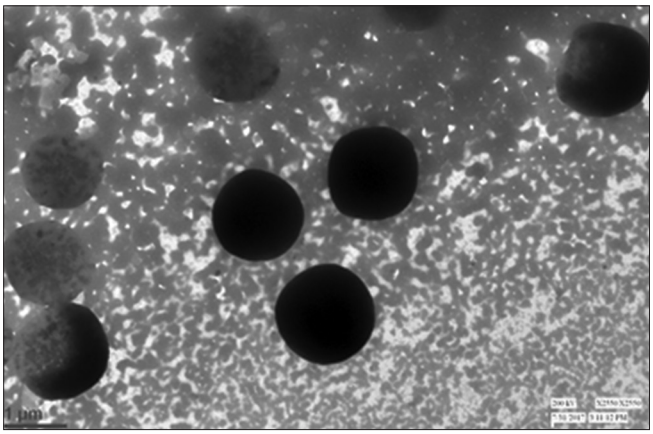

Fig. 6: SEM image of optimized batch

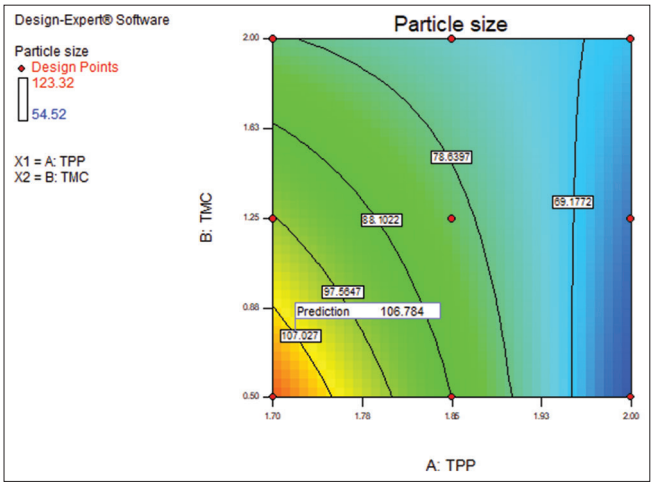

Fig. 7: Counter plot the effect of TPP solution and TMC on particle size

RESULT AND DISCUSSION

UV-spectroscopic analysis

Determination of λmax of VCZ in dissolution media

The standard solution (100 µg/ml) of pure drug (VCZ) was prepared in freshly prepared simulated tear fluid. The prepared solution was scanned between 200 and 400 nm by UV-visible spectrophotometer (Fig. 1).

Fourier trans form infra-red (FTIR) Spectroscopy

The FTIR spectra were taken on IR spectrophotometer using KBr pellet technique. The scanning range was 4000–400 cm^{-1}. The peaks

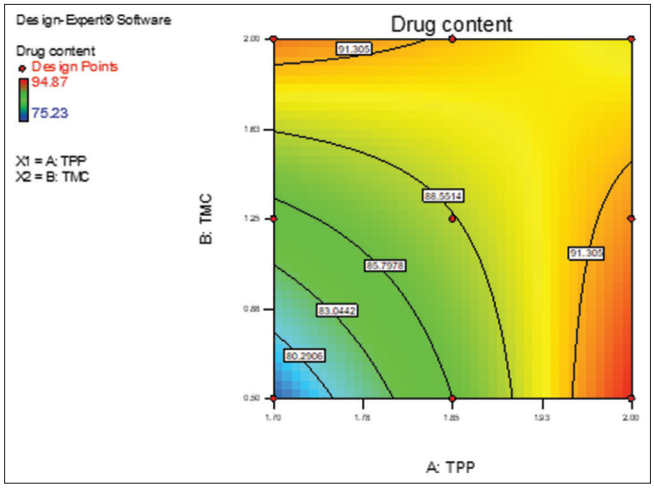

Fig. 8: Counter plot the effect of TPP solution and TMC on drug content

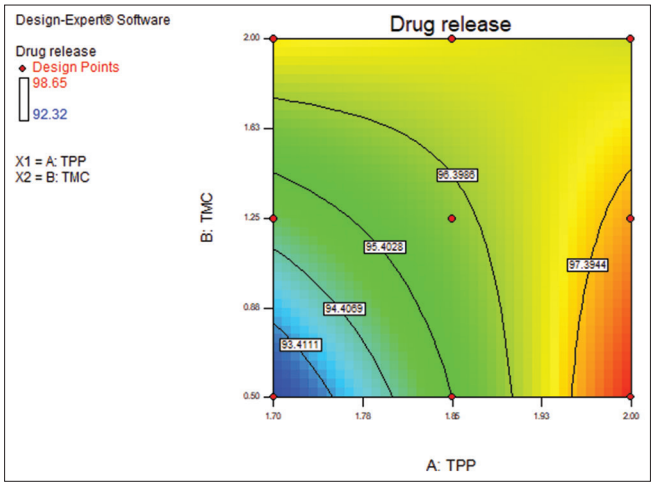

Fig. 9: Counter plot the effect of TPP solution and TMC on drug release

were interpreted for the confirmation of various functional groups (Fig. 2).

Nuclear magnetic resonance (NMR)

H-NMR is the technique used to identify hydrogen content and their positioning. A small amount of the sample was dissolved in $CDCl_3$ in a narrow glass vial and positioned in the sample holder of the instrument. NMR was recorded and interpreted for the validation of the compound.

X-ray diffraction (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 2θ 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 (BrukerLynxEyedetector). 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 2θ-scattered angles, which indicates the crystalline nature of the drug.

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 (Fig. 3).

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

Time (hours)	% Drug release of Nanoparticle								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	5.45	5.98	4.32	6.01	3.25	5.46	6.45	4.51	4.56
2	14.32	19.65	12.35	18.65	14.65	24.32	25.36	14.65	20.6
3	24.65	24.65	28.65	26.65	31.23	29.68	24.65	20.65	27.65
4	33.25	36.65	38.95	34.56	39.65	40.35	30.32	37.65	31.65
5	41.42	47.65	44.35	50.32	49.56	51.56	39.56	40.23	39.45
6	49.56	58.65	50.65	55.62	51.65	64.56	48.65	49.62	45.35
7	54.62	64.62	58.65	64.32	59.56	69.56	54.35	60.56	51.62
8	64.65	66.52	64.62	69.25	69.01	75.65	64.23	68.56	64.65
9	69.51	76.56	70.32	72.36	75.65	79.56	69.56	76.65	72.65
10	72.65	81.26	79.65	79.65	81.56	84.65	75.62	84.65	84.65
11	84.65	88.62	89.65	91.32	89.65	90.24	84.65	89.62	89.45
12	92.32	93.32	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
F3	54.52	−14	94.87	98.65

Synthesis of TMC

Quaternization (methylation) of amino groups in chitosan can be achieved with methyl iodide at elevated temperature in strong alkaline environment to bind the acid being generated during the reaction taking place and to avoid protonation of the unreacted primary amino groups. The degree of quaternization (DQ) can be altered by increasing the number of reaction steps or by increasing the reaction time or by controlling the reaction steps or by using different deacetylation grades of chitosan. At higher degrees of quaternization, however, evidence of O-methylation on the 3 and 6 hydroxyl groups of chitosan is found. In general, O-methylation led to less soluble products. It is desirable hence to prepare TMC polymers with a high DQ but with a low degree of O-methylation.

Evaluation of nanoparticles

Percentage yield

The percentage yield of different batches was determined by weighing the nanoparticle after drying and was found to be in range as shown in below Table 3.

Zeta potential

The zeta potentials of VCZ formulation are sufficient enough (−10−−20 mV) to stabilize the formulation.

Particle size analysis

The change in the concentration of polymer results in variation of particle size of nanoparticles. The results of particle size of various batches are discussed in the Table 3.

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 255 nm. The results are given in below Table 4

Comparative study of TMC and cyclodextrin polymer

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

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

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

***In vitro* drug release study**

In vitro drug release for drug-loaded nanoparticles for a period of 12 h was carried out by using simulated tear fluid at 37±5°C. From the dissolution profile of formulations F1 to F9, it is concluded that formulation batch F3 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 6.

Optimized batch

From the above results, F3 batch was found to be optimized.

Surface morphology

The surface morphology is done using scanning electron microscopy.

Optimization

Particle size

Counter plot

Figure shows that the counter plot of TPP solution and TMC is actual factor. It shows as TPP solution concentration increases the particle size decreases. TMC concentration increases, particle size was found to be decreased.

Three dimensional graphical presentations 3D surface

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

Drug content

Counter plot

Figure shows that the counter plot of TPP solution and TMC is actual factor. It shows as TPP solution concentration increases the drug content increases. Furthermore, as TMC concentration increases, drug content was found to be increased.

Three dimensional graphical presentations 3D surface

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

Drug release

Counter plot

Figure shows the counter plot of TPP Solution and TMC is actual factor. It shows as TPP solution concentration increases, the drug release

Table 8: The release data obtained

Zero order Equation line Y=	R2	First order Equation line Y=	R2	Higuchi equation line Y=	R2	Korsmeyerspeppas Equation line Y=	R2
8.4328×+0.5229	0.993	0.0927×+1.0379	0.8214	38.826×–39.297	0.9875	1.1163×+0.8333	0.9866

Table 9: Model fitting of optimize batch of voriconazole formulation

Run	Zero order	1st order	Higuchi	Korsmeryspeppas
(R2)	0.993	0.8214	0.9875	0.9866

Table 10: Stability study of optimized formulation

Time(day)	Drug content (%)	In vitro drug release (%)
0	99.13	99.884
30	97.52	96.522
60	95.61	93.548
90	88.32	91.365

increases. Furthermore, as TMC concentration increases, drug release was found to be increased.

Three dimensional graphical presentations 3D surface
The 3D in figure shows that as increase in concentration of TPP solution and TMC concentration, it shows increase in drug release. It was concluded from the graph that the factor A has significance effect on the particle size.

Kinetic treatment to dissolution data
From result shown in table, we can conclude that overall order of release data of drug after fitting in different mathematical model shows following results.

F3 batch
Zero order >Higuchi>Korsmeyerspeppas> first order.

DISCUSSION

From the R^2 value, it was concluded that the drug releases profile of optimize batch followed zero order kinetics and release pattern, respectively.

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 following table. Results of stability studies showed that there is no significant change in above mentioned parameter after elevated temperature and humidity condition during stability studies. Thus, it can be proved from the stability studies that the prepared formulation is stable and not much affected by elevated humidity and temperature conditions.

CONCLUSION

Eye drops (solutions) are one of the widely preferred topical formulations; however, their frequent administration and poor bioavailability have been the major issue of concern. The exploitation of nanotechnology in ophthalmic drug delivery could be an effective strategy in resolving these drawbacks. The point of major concern for nanoparticles is stability issues (particle aggregation and poor drug entrapment) on long-term storage, which needed further attention.

The objective of the present study was to develop optimum topical delivery systems of VCZ containing tri-methyl chitosan, which ensures the effective delivery of the drug with lesser side effects. The present study is aimed to synthesize N-TMC, a water-soluble chitosan derivative, which is used in the ophthalmic drug delivery. Chitosan is a natural polymer which is insoluble in water while TMC is soluble at neutral and basic pH. VCZ is a BCS Class II drug having low solubility and high permeability. Hence, VCZ nanoparticles are prepared to increase the solubility of VCZ and also to maintain the pH.

AUTHOR'S CONTRIBUTION

All author's contributed equally.

CONFLICT OF INTEREST

No.

AUTHOR'S FUNDING

No.

REFERENCES

1. Asasutjarit R, Theerachayanan T, Kewsuwan P, Veeranodha S, Fuongfuchat A, Ritthidej GC. Development and evaluation of diclofenac sodium loaded-N-trimethyl chitosan nanoparticles for ophthalmic use. AAPS PharmSciTech 2015;16:1013-24. doi: 10.1208/s12249-015-0290-4, PMID 25609376
2. Bunjes H, Unruh T. Characterization of lipid nanoparticles by differential scanning calorimetry, X-ray and neutron scattering. Adv Drug Deliv Rev 2007;59:379-402. doi: 10.1016/j.addr.2007.04.013, PMID 17658653
3. Donohue EK, Wilensky JT. Dorzolamide: A review. In: Seminars in Ophthalmology. Vol. 12. United Kingdom: Taylor and Francis; 1997. p. 119-26.
4. Felt O, Furrer P, Mayer JM, Plazonnet B, Buri P, Gurny RJ. Topical use of chitosan in ophthalmology: Tolerance assessment and evaluation of precorneal retention. Int J Pharm 1999;180:185-93. doi: 10.1016/s0378-5173(99)00003-4, PMID 10370189
5. Fouda NH, Abdelrehim RT, Hegazy DA, Habib BA. Sustained ocular delivery of dorzolamide-HCl via proniosomal gel formulation: *In-vitro* characterization, statistical optimization, and *in-vivo* pharmacodynamic evaluation in rabbits. Drug Deliv 2018;25:1340-9. doi: 10.1080/10717544.2018.1477861, PMID 29869516
6. Gupta KC, Ravi Kumar MN. An overview on chitin and chitosan applications with an emphasis on controlled drug release formulations. J Macromol Sci C 2000;40:273-308. doi: 10.1081/MC-100102399
7. Harikumar SL, Sonia A. Nanotechnological approaches in ophthalmic delivery systems. Int J Drug Dev Res 2011;3:9-19.
8. Kashin AS, Ananikov VP. A SEM study of nanosized metal films and metal nanoparticles obtained by magnetron sputtering. Russ Chem Bull 2011;60:2602-7. doi: 10.1007/s11172-011-0399-x
9. Kesarla R, Tank T, Vora PA, Shah T, Parmar S, Omri A. Preparation and evaluation of nanoparticles loaded ophthalmic *in situ* gel. Drug Deliv 2016;23:2363-70. doi: 10.3109/10717544.2014.987333, PMID 25579467
10. Li J, Jin X, Yang Y, Zhang L, Liu R, Li Z. Trimethyl chitosan nanoparticles for ocular baicalein delivery: Preparation, optimization, *in vitro* evaluation, *in vivo* pharmacokinetic study and molecular dynamics simulation. Int J Biol Macromol 2020;156:749-61. doi: 10.1016/j.ijbiomac.2020.04.115, PMID 32320806
11. Malik A, Gupta M, Mani R, Gogoi H, Bhatnagar R. Trimethyl chitosan nanoparticles encapsulated protective antigen protects the mice against anthrax. Front Immunol 2018;9:562. doi: 10.3389/fimmu.2018.00562, PMID 29616046
12. Marangon FB, Miller D, Giaconi JA, Alfonso EC. *In vitro* investigation of voriconazole susceptibility for keratitis and endophthalmitis fungal pathogens. Am J Ophthalmol 2004;137:820-5. doi: 10.1016/j.

ajo.2003.11.078, PMID 15126145

13. Min JB, Kim ES, Lee JS, Lee HG. Preparation, characterization, and cellular uptake of resveratrol-loaded trimethyl chitosan nanoparticles. Food Sci Biotechnol 2018;27:441-50. doi: 10.1007/s10068-017-0272-2, PMID 30263768
14. Mourya VK, Inamdar NN. Chitosan-modifications and applications: Opportunities galore. React Funct Polym 2008;68:1013-51. doi: 10.1016/j.reactfunctpolym.2008.03.002
15. Munier A, Gunning T, Kenny D, O'Keefe M. Causes of blindness in the adult population of the republic of Ireland. Br J Ophthalmol 1998;82:630-3. doi: 10.1136/bjo.82.6.630, PMID 9797662
16. Naik JB, Pardeshi SR, Patil RP, Patil PB, Mujumdar A. Mucoadhesive micro-/Nano carriers in ophthalmic drug delivery: An overview. BioNanoScience 2020;10:564-82. doi: 10.1007/s12668-020-00752-y
17. Üstündağ Okur NÜ, Yozgatlı V, Okur ME, Yoltaş A, Siafaka PI. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive *in situ* gels as ophthalmic drug carriers. J Drug Deliv Sci Technol 2019;49:323-33. doi: 10.1016/j.jddst.2018.12.005
18. Pandit J, Sultana Y, Aqil M. Chitosan-coated PLGA nanoparticles of bevacizumab as novel drug delivery to target retina: Optimization, characterization, and *in vitro* toxicity evaluation. Artif Cells Nanomed Biotechnol 2017;45:1397-407. doi: 10.1080/21691401.2016.1243545, PMID 27855494
19. Shastri D, Shelat P, Shukla A, Patel P. Ophthalmic drug delivery system: Challenges and approaches. Syst Rev Pharm 2010;1:113-20. doi: 10.4103/0975-8453.75042
20. Popa L, Ghica MV, Dinu-Pîrvu CE, Irimia T. Chitosan: A good candidate for sustained release ocular drug delivery systems. In: Chitin-Chitosan-Myriad Functionalities in Science and Technology. London, UK: InTech; 2018. p. 283-310.
21. Shinde UA, Joshi PN, Jain DD, Singh K. Preparation and evaluation of N-trimethyl chitosan nanoparticles of flurbiprofen for ocular delivery. Curr Eye Res 2019;44:575-82. doi: 10.1080/02713683.2019.1567793, PMID 30632402