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FORMULATION AND EVALUATION OF POLYMERIC NANOPARTICLES LOADED IN HYDROGEL FOR OPTIMUM ANTIFUNGAL PURPOSE

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ABSTRACT

Objective: To develop miconazole nitrate -loaded chitosan nanoparticles by modified ionic gelation method for optimum antifungal purpose. **Methods:** Miconazole nitrate -loaded chitosan nanoparticles were prepared by ionic gelation method with the aid of sonication. The prepared hydrogel formulations were evaluated for physico chemical parameters like physical appearance, pH, drug release, drug content and rheological parameters like spredability and extrudability. Parameters such as the zeta potential, particle size, entrapment efficiency and *in vitro* drug release of the polymeric nanoparticles loaded in hydrogel were assessed for optimization**. Results:** All hydrogel formulations showed acceptable physico chemical and rheological properties and results were found to be within the limits. The drug release was found to decrease with increase in polymer concentration. The mean particle size of optimized formulation F2 nanoparticles was found to be 85.6 nm. and zeta potential of optimized formulation F2 nanoparticles was found to be -36.2 mV. There was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulations. The *in vitro* release behaviour of optimized formulation GF2 was found to follow Korsmeyer-Peppas model and provided sustained release over a period of 24 h. **Conclusion:** Polymer and crosslinking agent concentrations and sonication time are rate-limiting factors for the development of the optimized formulation. The chitosan nanoparticles developed would be capable of sustained delivery of a miconazole nitrate for optimum antifungal purpose.

KEYWORDS: Nanoparticles, Hydrogel, Antifungal, Chitosan, Sodium tripolyphosphate, Miconazole Nitrate.

INTRODUCTION

Hydrogels are widely used in topical drug delivery systems due to their physical and chemical properties such as controllable and prolonged release of drug.[1,2] These formulations on contact with the skin forms a semi occlusive film over the skin and release the drug in controlled manner.[3] Lipophilic drug can cross the Stratum corneum, but rate of diffusion decreases as it enters the more aqueous lower regions of the epidermis.[4] Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides have been developed and tested over the past decades. Both synthetic and natural polymers were studied with the aim of forming nanoparticles. However, among the variety of polymers that were used for drug-loaded nanoparticles, chitosan has received great attention in both the medical and pharmaceutical fields. Chitosan can be synthesized by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is very promising and have been widely exploited in pharmaceutical industry for controlling drug release.[5,6] Chitosan, a linear polysaccharide consisting of glucosamine and N-acetylglucosamine units, is biocompatible, biodegradable, and nontoxic in the application of peroral delivery of drugs.[6] The addition of chitosan can not only endow nanoparticles positive surface charge, but also prolong the time that the active ingredients contact with the epithelium and enhance absorption via the para-cellular transport pathway through the tight junctions.[7,8] Chitosan nanoparticles can easily be prepared by the ionic gelation method using tripolyphosphate as a crosslinking agent. The advantage of this method was attributed to its mild conditions achieved without applying harmful organic solvent, heat or vigorous agitation that are damaging to sensitive proteins. Its association with a conventional antifungal, such as miconazole, could provide an effective therapy. Miconazole is a fungistatic synthetic antifungal of the azole group which can cause a selective pressure and favor drug resistant isolates.[9] Nanostructured drug delivery systems provide combined therapy allowing a long-term therapeutic concentration at low doses. In addition, the drug released from these systems at the wanted site presents favorable therapeutic efficacy when compared to conventional drugs.[10-12]

Potent bactericidal activity against gram positive and negative bacteria has been seen in nanoparticles encompassing antibiotics, which can be extrapolated for possible antifungal efficacy too. Therefore the goal of our research to formulate and evaluate polymeric nanoparticles loaded in hydrogel for optimum antifungal purpose.

MATERIALS AND METHODS

Materials

Chitosan was used as carrier and sodium tripolyphosphate was used as crosslinking agent, both were purchased from Sigma-Aldrich. Miconazole Nitrate was received was received as gift sample from Modern science Pvt. Ltd., Nashik Mumbai. All other chemicals were of analytical grade and were obtained commercially.

Methods

Preparation of chitosan nanoparticles of miconazole

The chitosan nanoparticles associated to the drugs were produced according to the technique described by by ionotropic gelation method.[13] A chitosan solution (2 mg/ml) was prepared through the polymer dissolution by sonication in 0.1 M of acetic acid until the solution was clear (about 10 min). Next, 250 µl was added of a concentrated solution of miconazole (10 mg/l) and was dissolved in chitosan solution. The pH of the solution was adjusted to 4.4 with 0.1 M NaOH. A tripolyphosphate (TPP) solution (1 mg/ml) was prepared in miliQ water. Tripolyphosphate solution was added drop wise with a syringe to chitosan solution while stirring. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Nanoparticles were obtained which was air dried for twenty four hours followed by oven drying for six hours at 40˚C.

Table 1: Formulations of chitosan nanoparticles.

Sr. No	Formulation Code	Miconazole (mg)	Chitosan (mg)	STPP (mg)
1.	F1	10	250	100
2.	F2	10	250	100
3.	F3	10	250	100
4.	F4	10	500	100
5.	F5	10	500	100
6.	F6	10	500	100
7	F7	10	750	100
8	F8	10	750	100
9	F9	10	750	100

Evaluation of nanoparticles

Percentage yield

The prepared nanoparticles F1-F9 were collected and weighed from each formulation. The percentage yield (%) was calculated using formula given below:

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment efficiency

Amount of miconazole in each formulation was calculated by weighing 10 mg of chitosan nanoparticles from each batch. The powder of chitosan nanoparticles were dissolved in 10 ml 7.2 pH Phosphate buffer and centrifuged at 1000 rpm. This supernatant solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.4 pH Phosphate buffer. The supernant was analyzed for drug content by measuring the absorbance at 278nm. The entrapment efficiency (EE) was calculated using the equation:

EE (%) = [(Total drug added – amount of free drug)/total drug added] x 100

Measurement of mean particle size

The mean particle size of the nanoparticle was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern particle size analyser) at a scattering angle of 90°. A sample (0.5mg) of the nanoparticle suspended in 5 ml of distilled water was used for the measurement.[14]

Determination of zeta potential

The zeta potential of the drug-loaded nanoparticles was measured on a zeta sizer (Malvern particle size analyser) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.[15,16]

Formulation development of miconazole-loaded nanoparticles gel

Preparation of gel base

Gel base was prepared by dissolving Carbopol 934 (1-3% w/v) in double distilled water (80 ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10 ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.8. Gel preparation corresponding to 2.0 % w/w (2.0 mg of drug in 100 mg of gel) of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Table 2: Formulation of miconazole-loaded nanoparticles gel.

S. No.	Formulation code	Drug % (Equivalent to nanaoparticles)	Carbopol 934 (gm)	Water
1.	GF1	2	1	100
2.	GF2	2	2	100
3.	GF3	2	3	100

Characterization of prepared gel

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9. After calibration, the electrode was dipped into the prepared gel formulations. Then pH of selected formulation was measured and readings shown on display were noted.[17,18]

Measurement of viscosity

Viscosity measurements of prepared topical nanoparticles based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10 rpm.[17,18]

Drug content

Accurately weighed amount of gel formulation equivalent 5mg (100 mg of gel) was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using U.V. method at 278 nm.[17,18]

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube. Extrudability of gel required weight to extrude a 0.6cm ribbon of gel in 6 seconds.[17,18]

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied

with weight pan. To determine spreadibility, placing 0.25 to 0.50 g of gel between two slide and fix weight of 20 gm. was applied and time required by the top plate to cover a distance of 6 cm was noted. Good spreadibility show lesser time to spread.[17,18] Measurement of spreadability was done in triplicate and calculated by using the following formula:

Spreadability= (Weight×Length)/Time Where, S=Spreadability m=Weight tied to the upper slide (20 g) l=Length of the glass (6.0 cm) t=Time taken in seconds

In vitro drug diffusion study

The *in-vitro* diffusion study is carried by using franz diffusion cell with semipermeable membrane. The semipermeable membrane soaked in a buffer for 6-8 hours. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq.cms. The semipermeable is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 278 nm.[19]

Release kinetics study

To study the release kinetics of drug from the polymeric nanoparticles loaded in hydrogel, the data obtained from the *in vitro* release study were analysed using various kinetic models to describe the mechanism of drug release from the hydrogels.[20]

In order to investigate the mode of release from the polymeric nanoparticles loaded in hydrogel, the release data were analyzed with the following mathematical models.

$Qt = K_0 t$ (Zero Order Kinetics)

$\text{Log}\ (Q_t / Q_0) = - K_1 t / 2.303$ (First order Kinetics)

$Qt = K_{KP} t^n$ (Korsmeyer and Peppas equation)

$Qt = K_H t^{1/2}$ (Higuchi's equation)

Where, Qt is the percent of drug released at time't', K_0, K_1, K_{HC}, K_{KP} and K_H are the coefficients of Zero order, First order, Korsmeyer-Peppas and Higuchi's equations.

RESULTS AND DISCUSSION

The percentage yield of prepared formulations was found in the range of 63.12±0.14 to 75.65±0.32 percentage, which was within the acceptable limits. The outcomes of the results are discussed in the table 3.

Table 3: Percentage yield for different formulation.

Formulation	Percentage Yield (%)
F1	65.56±0.45
F2	75.65±0.32
F3	69.98±0.65
F4	65.45±0.85
F5	63.12±0.14
F6	65.78±0.25
F7	63.56±0.36
F8	68.89±0.78
F9	71.23±0.54

Percentage entrapment efficiency of prepared nanoparticles was found in range of 63.32±0.45 to 73.23±0.45 percentage. The outcomes of the results are discussed in the table 4. The maximum percentage yield and entrapment efficiency was found formulation F2. The optimized formulation among other batches subjected to further studies.

Table 4: Entrapment efficiency for different formulation.

Formulation	% Entrapment Efficiency of prepared nanoparticles
F1	65.56±0.32
F2	73.23±0.45
F3	70.12±0.45
F4	68.85±0.65
F5	65.45±0.56
F6	65.56±0.41
F7	63.32±0.45
F8	65.85±0.36
F9	68.89±0.25

The mean size of the nanoparticles was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Particle Size Analyzer from Malvern) at a scattering angle of 90°C. A sample (0.5mg) of the nanoparticles suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F2 nanoparticles were found 85.6 nm.

The zeta potential of the drug-loaded nanoparticles was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. A result of zeta potential of optimized formulation F2 nanoparticles was found to be -36.2 mV.

pH was found in range of 6.85±0.02 to 7.02±0.04for gel prepared by carbopol and sodium tripolyphosphate as gel base which is near to the pH of the skin and hence is found to be compatible with skin. The outcomes of the results are discussed in the table 5.

Bioavailability and therapeutic property of the formulation depends upon the spreadability. The spreadability is expressed of time in seconds based on the slip off from the gel by upper slide under certain load. Time taken for the separation of the two slides is less which indicates the topical formulation has better spreadability. The spreadability value was found to be 6.58±0.45 (g.cm/sec) and 8.85±0.25 (g.cm/ csec) for gel prepared by sodium tripolyphosphate and Carbopol. The observed results were comparable with the earlier literature.[17]

The viscosity was performed to assess the effect of the type and concentration of the gelling agent on the physical properties of the final polymeric nanoparticles loaded in hydrogel products and their viscosity.[21] Table 5 shows the viscosities of gel formulations at low and high shear rates. It can be seen in the figure that the effect of the types of gelling agents on the viscosity. Generally, the formulations of carbopol have higher viscosity than other formulations, because carbopol is a cross-linked polymer of acrylic acid with high molecular weight that has the ability to absorb and retain water upon neutralization, resulting in a viscous gel.

The drug content of formulations is shown in table 5. The percentage drug content of all prepared dermal formulations was found to be in the range of 97.25±0.25-99.25±0.12%. Therefore uniformity of content was maintained in all formulations. Extrudability was found in range of 125±3 to 165±2 gm for gel prepared by carbopol and sodium tripolyphosphate and the outcomes of the results are discussed in the table 5.

Table 5: Results of gel formulations.

Code	pH	Spreadability (gm.cm /sec.)	Viscosity (cps)	Drug Content (%)	Extrudability (gm)
GF1	6.85±0.02	12.23±0.45	4568±13	98.45±0.25	145±4
GF2	7.02±0.04	11.56±0.98	4251±11	99.25±0.12	165±2
GF3	6.95±0.02	10.32±0.65	3978±14	97.25±0.25	125±3

The *in-vitro* drug release profile of polymeric nanoparticles loaded in hydrogel was represented in table 6 and figure 1. It was found that the amount of drug diffused from all the formulation ranged from 11.32 to 19.98% during first hour. Formulation GF2 containing polymeric nanoparticles loaded in hydrogel showed complete drug release in 12hrs. Highest drug release was observed in formulation GF2 and lowest in formulation GF3. It was observed that the drug release was influenced by the polymer type and the concentration of the polymer used. The optimized formulation GF2 was subjected to further studies.

Table 6: *In vitro* drug release study of prepared gel formulation.

S. No.	Time (hr)	% Cumulative Drug Release		
		GF1	GF2	GF3
1	0.5	12.32	11.32	9.85
2	1	18.89	19.98	11.32
3	2	23.32	35.65	15.56
4	3	26.65	40.23	18.89
5	4	43.32	45.65	22.32
6	5	53.45	65.58	26.69
7	6	59.98	73.32	38.89
8	8	63.32	81.14	45.56
9	10	65.45	91.45	55.56
10	12	66.68	98.85	63.32

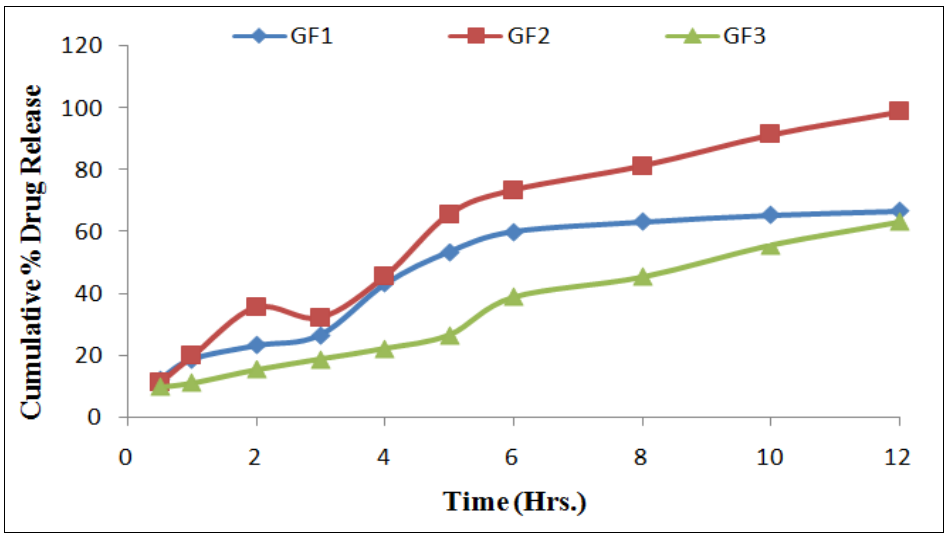

Figure 1: *In-vitro* drug release study of GF1, GF2 and GF2.

Table 7: *In-vitro* drug release data for gel GF2.

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	-0.301	11.32	1.054	88.68	1.948
2	1	0.000	19.98	1.301	80.02	1.903
3	2	0.301	35.65	1.552	64.35	1.809
4	3	0.477	40.23	1.605	59.77	1.776
5	4	0.602	45.65	1.659	54.35	1.735
6	5	0.699	65.58	1.817	34.42	1.537
7	6	0.778	73.32	1.865	26.68	1.426
8	8	0.903	81.14	1.909	18.86	1.276
9	10	1.000	91.45	1.961	8.55	0.932
10	12	1.079	98.85	1.995	1.15	0.061

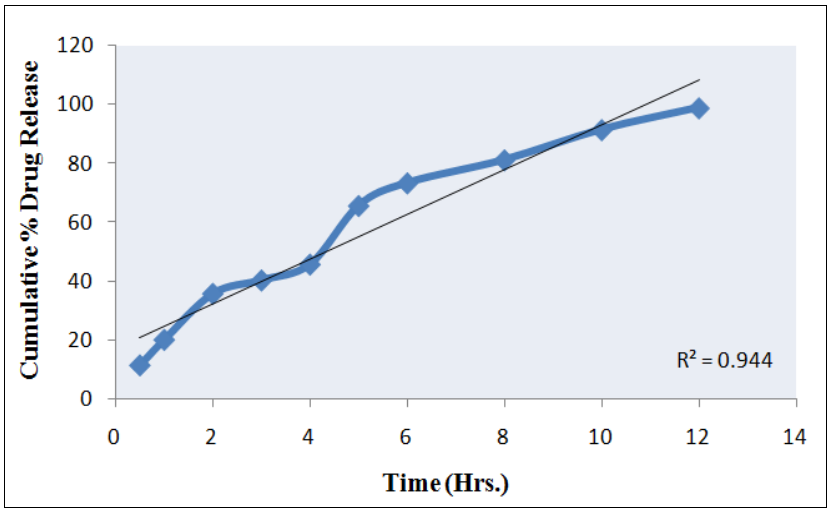

Figure 2: Graph of Zero order Release Kinetics of optimized gel GF2 (Cumulative % drug released Vs Time).

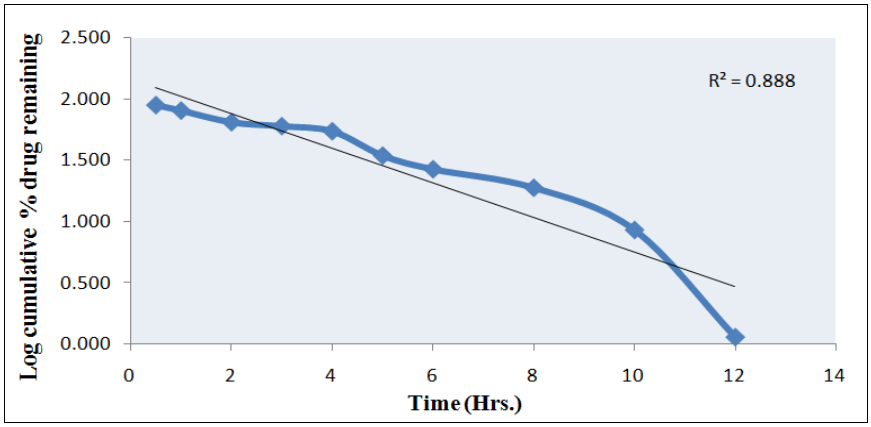

Figure 3: Graph of first order Release Kinetics of optimized gel GF2 (Log cumulative % drug remaining Vs Time).

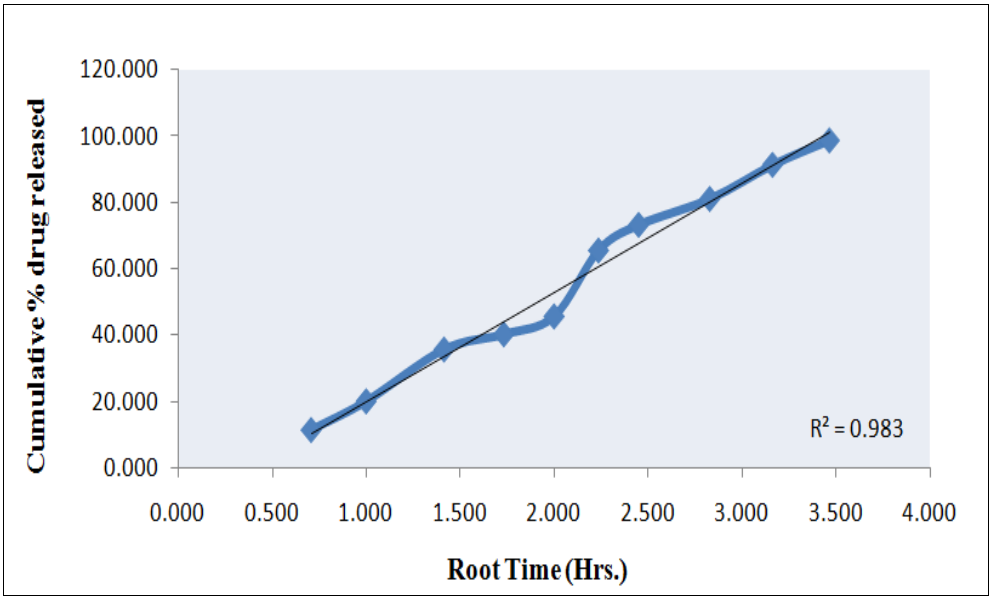

Figure 4: Higuchi release Kinetics (Cumulative % drug released Vs Root Time).

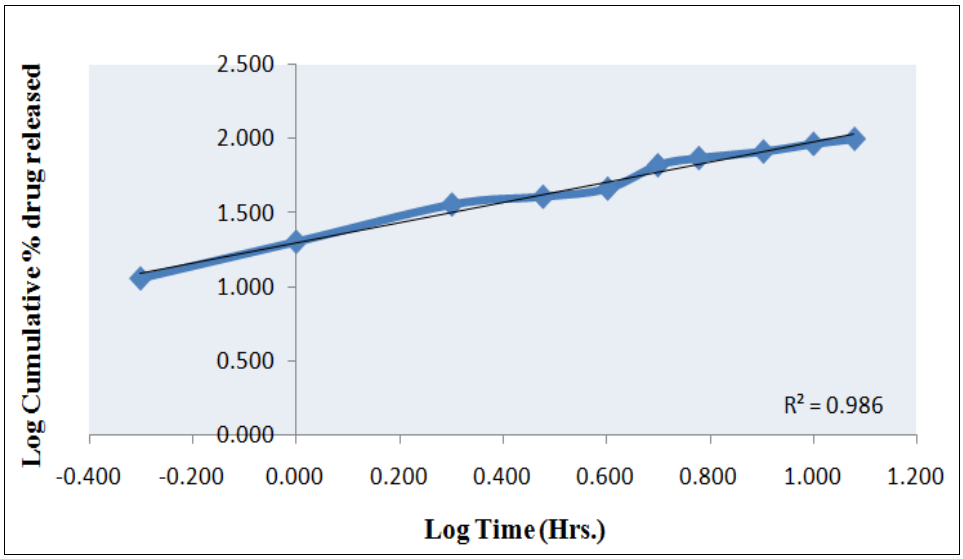

Figure 5: Korsmeyer-Peppas release Kinetics (Log Cumulative % drug release Vs Log Time).

Table 8: Release Kinetics Regression values of formulation GF2.

Formulation code	Zero order	First order	Higuchi	Korsmeyer-Peppas
GF2	0.944	0.888	0.983	0.986

In order to determine the release model, the *in vitro* drug release data were analyzed according to zero order, first order, and diffusion controlled mechanism according to Higuchi model. The results revealed that the release of miconazole from the optimized formulae GF2 obeyed the Higuchi model of matrix diffusion with highest R^2 values, as shown in table 8. This is in agreement with Gardouh *et al.* who stated that drug release by

diffusion involves three steps which include penetration of water into system causing welling of matrix followed by the conversion of solid lipid into rubbery matrix, and then the diffusion of drug from the swollen rubbery matrix takes place.[22] To confirm the release mechanism, the data were fitted to the Korsmeyer-Peppas equation to describe the drug release mechanism from matrix. The drug release kinetic study revealed that drug release followed krossmeyer peppas model in the formulations GF2 indicates that drug is diffused through the membrane. The Krosmeyer peppas graphs were plotted and the release rate constant, k, and the slope n, determined. The results showed that most of the 'n' values were between 0.888 and 0.986. Therefore it can be inferred that the drug may have followed case II transport mechanism.

CONCLUSION

In this study, chitosan nanoparticles loaded with miconazole were prepared based on our recently optimized ionotropic gelation method, which employed TPP as the crosslinker to investigate the physicochemical properties of nanoparticles. The *in vitro* release profile observed for these nanoparticles was characterized by an initial fast release followed by a controlled release phase. The optimized formulation with better bioadhesive property may improve the bioavaibility of topical administration of miconazole nitrate in polymeric nanoparticles loaded in hydrogel form and can be alternative to the conventionally administered topical formulations. To conclude, the present study confirms that polymeric nanoparticles loaded in hydrogel of miconazole may be the best one for delivery, which are intended for topical antifungal application.

REFERENCES

1. Emma R, Stepan G, Ashot M, e al. New Drug Delivery System for Cancer Therapy. International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering, 2013; 7(12): 899–904.
2. Enas MA. Hydrogel: Preparation, Characterization and Applications: A Review. Journal of Advanced Research, 2013; 6(2): 106–121.
3. Lackner TE, Clissold SP. Bifonazole. A Review of Its Antimicrobial Activity and Therapeutic Use In Superficial Mycoses. Drugs, 1989; 38(2): 204–225.
4. Chandra A, Sharma PK. Microemulsion: An Overview. Pharmacological Reviews, 2008; 6: 2–4.

5. Shi J, Alves NM, Mano JF. Chitosan coated alginate beads containing poly (N-isopropylacrylamide) for dual-stimuli-responsive drug release. J. Biomed. Mater. Res. B Appl. Biomater, 2008; 84(2): 595-603.
6. Murata Y, Jinno D, Liu D, Isobe T, et al. The drug release profile from calcium-induced alginate gel beads coated with an alginate hydrolysate. Molecules, 2007; 12(11): 2559-2566.
7. Kotze AF, Thanou MM, Luebetaen HL, de Boer AG, et al. Enhancement of paracellular drug transport with highly quaternized N-trimethyl chitosan chloride in neutral environments: In vitro evaluation in intestinal epithelial cells (Caco-2). J. Pharm. Sci., 1999; 88: 253-257.
8. Sarmento B, Ribeiro A, Veiga F, Sampaio P, et al. Alginate/Chitosan Nanoparticles are Effective for Oral Insulin Delivery. Pharm. Res., 2007; 24(12): 2198-2206.
9. Fothergill AW. Miconazole: A historical perspective. Expert Rev Anti Infect Ther, 2006; 4: 171-175.
10. Valenta C. The use of mucoadhesive polymers in vaginal delivery. Adv Drug Deliv Rev., 2005; 57: 1692-1712.
11. Fakruddin MD, Hossain Z, Afroz H. Prospects and applications of nanobiotechnology: a medical perspective. J Nanobiotechnology, 2012; 10: 31.
12. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater, 2002; 6: 319-327.
13. Calvo P, Remunan-Lopez C, Vila-Jato J et al.Novel hydrophilicchitosan-polyethylene oxide nanoparticles as protein carriers, J Appl Polym Sci., 1997; 63: 125-132.
14. Zhang X, Xia Q, Gu N. Preparation of all- trans retinoic acid nanosuspension using a modified precipitation method. Drug Dev Ind Pharm, 2006; 32: 857-863.
15. Barratt G. Characterization of colloidal drug carrier systems with zeta potential measurements. Pharm Technol Eur, 1999; 11: 25-32.
16. Lochmann D, Weyermann J, Georgens C, Prassl R, Zimmer A. Albumin-protamine-oligonucleotide nanoparticles as a new antisense delivery system. Part 1: Physicochemical characterization. Eur J Pharm Biopharm, 2005; 59: 419-429.
17. Yamini K, Onesimus T. Preparation and evaluation of herbal anti-acne gel. Inter J Pharma Bio Sci., 2013; 4(2): 956-60.
18. Singh K, Panghal M, Kadyan S, et al. Evaluation of antimicrobial activity of synthesized silver nanoparticles using *Phyllanthus amarus* and *Tinospora cordifolia* medicinal plants. J Nanomed Nanotech, 2014; 5(6): 250-255.

19. Doijad RC, Manvi FV, Rao SNM, e al. Sustained ophthalmic delivery of gatifloxacin from *in situ* gelling system. Indian J Pharma Sci., 2006; 68(6): 814–818.
20. Sabri, H. S., Alia, W. K., Abdullahb, B. H., Al-Anic, W. M. Formulation design and evaluation of anti-microbial activity of emulgel containing essential oil of *Myrtus communis* L. Inter J Pharm Sci Rev Res., 40: 271–277.
21. Kostyuk VA, Potapovich AI, Vladykovskaya EN, Korkina LG, Afanas'ev IB. Influence of metal ions on flavonoid protection against asbestos-induced cell injury. Arch Biochem Biophys, 2001; 385(1): 129-37.
22. Gardouh R, Gad Shadeed, Ghnaim HM, Ghorab MM. Design and characterization of glyceryl monostearate solid lipid nanoparticles prepared by high shear homogenization. British J Pharm Res., 2013; 3: 326-46.