DESIGN AND DEVELOPMENT OF POLYMERIC NANOPARTICLES LOADED IN HYDROGEL OF MICONAZOLE FOR FUNGAL TREATMENT

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ABSTRACT: Miconazole is one of the broad spectrum antifungal compounds of the imidazole group. In Present investigation chiosan nanoparticles of Miconazole was prepared and evaluated for Percentage yield, Drug entrapment, Zeta potential and particle size and further prepared nanoparticles incorporated in gel base and evaluated. Percentage yield of different formulation was determined by weighing the nanoparticles after drying. The optimized formulation among other batches subjected to further studies.The results of measurement of mean particle size of optimized formulation F2 nanoparticles were found 85.6 nm. Gel preparation corresponding to 2.0% w/w (2.0 mgof drug in 100 mg of gel) of drug was incorporated into the gel base to get the desired concentration of drug in gel base.Gels were evaluated by means of pH, spreadability, viscosity and drug content and extrudability. pH was determined using digital pH meter.The viscosity of gels was determined by using a Brookfield viscometer DV-II model. It was concluded that prepared gel containing Miconazole nanoparticles was optimized and successfully formulated in the form gel can be use for topical preparation for antifungal effect.

KEYWORDS: Viscosity, Evaluation, Drug, Gel, Spreadability.

INTRODUCTION: Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Da can pass through it . To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. At the skin, molecules contact cellular debris, microorganisms, sebum and other materials, which negligibly affect permeation. The penetrant has three possible pathways to the viable tissue-through hair follicles with connected sebaceous glands, via sweat ducts, or across continuous stratum corneum between these appendages. The incidence of superficial fungal infections of skin, hair and nails has been increased in worldwide. It has been estimated that about 40 million people have suffered from fungal infections in developing and under developed nations. The progression of fungal infections can be rapid and serious due to compromising with immune function. Dermatophytes are one of the most frequent causes of tinea and onchomycosis. Candidal infections are also among the most widespread superficial cutaneous fungal infections even, candida can invade deeper tissues as well as the blood which leads to life- threating systemic candidiasis. Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections. The main classes of topical antifungals are polyenes, azoles, and allylamine/benzylamines. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays. The efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Hence, the effective drug concentration levels should be achieved in the skin. In topical administration of antifungals, the drug substances should pass the stratum corneum, which is the outer most layer of the skin, to reach lower layers of the skin, particularly into viable epidermis. In this context, the formulation may play a major role for penetration of drugs into skin. Development of alternative approaches for topical treatment of fungal infections of skin

encompasses new carrier systems for approved and investigational compounds. Delivery of antifungal compounds into skin can be enhanced with the carriers including colloidal systems, vesicular carriers, and nanoparticles. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of absorbing large amounts of water or biological fluids. Due to their high watercontent, porosity and soft consistency, they closely simulate natural living tissue, more than any other class of synthetic biomaterials. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are prepared from materials such as gelatin, polysaccharides, cross-linked polyacrylamide polymers, polyelectrolyte complexes, and polymers or copolymers derived from methacrylate esters. They are insoluble in water and are available in dry or hydrated sheets or as a hydrated gel in drug delivery systems designed for single use. Furthermore, hydrogels can be formulated in a variety of physical forms, including labs, microparticles, nanoparticles, coatings, and films. As a result, hydrogels are commonly used in clinical practice and medicine with a wide range of applications, including Tissue Engineering and Regenerative Medicine, Diagnostics, Cellular immobilization, Separation of biomolecules or cells, and barrier materials to regulate biological adhesions. These unique physical properties of hydrogels have stimulated particular interest in their use in drug delivery applications. Their highly porous structure can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of a small molecule or a macromolecule through the gel network [9]. Since the polymer cannot dissolve due to the covalent crosslinks, water uptakes farTopical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections. The main classes of topical antifungals are polyenes, azoles, and allylamine/benzylamines. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays.

The efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Hence, the effective drug concentration levels should be achieved in the skin. In topical administration of antifungals, the drug substances should pass the stratum corneum, which is the outer most layer of the skin,to reach lower layers of the skin, particularly into viable epidermis. In this context, the formulation may play a major role for penetration of drugs into skin. Development of alternative approaches for topical treatment of fungal infections of skin encompasses new carrier systems for approved and investigational compounds. Delivery of antifungal compounds into skin can be enhanced with the carriers including colloidal systems, vesicular carriers, and nanoparticles. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of absorbing large amounts of water or biological fluids. Due to their high watercontent, porosity and soft consistency, they closely simulate natural living tissue, more than any other class of synthetic biomaterials. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are prepared from materials such as gelatin, polysaccharides, cross-linked polyacrylamide polymers, polyelectrolyte complexes, and polymers or copolymers derived from methacrylate esters. They are insoluble in water and are available in dry or hydrated sheets or as a hydrated gel in drug delivery systems designed for single use. Furthermore, hydrogels can be formulated in a variety of physical forms, including labs, microparticles, nanoparticles, coatings, and films. As a result, hydrogels are commonly used in clinical practice and medicine with a wide range of applications, including Tissue Engineering and Regenerative Medicine, Diagnostics, Cellular immobilization, Separation of biomolecules or cells, and barrier materials to regulate biological adhesions. These unique physical properties of hydrogels have stimulated particular interest in their use in drug delivery applications. Their highly porous structure can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity

also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of a small molecule or a macromolecule through the gel network. Since the polymer cannot dissolve due to the covalent crosslinks, water uptakes far in excess of those achievable with hydrophilic linear polymers can be obtained. Hydrogels are also generally highly biocompatible, which may be attributed to the high water content of hydrogels. Biodegradability or dissolution in case of hydrogels may be brought about by enzymatic, hydrolytic, or environmental (e.g. pH, temperature, or electric field) pathways; however, degradation is not always desirable depending on the time frame and location of the drug delivery device. Hydrogels are relatively deformable and can conform to the shape of the surface onto which they are applied. In the latter context, the mucoadhesive or bioadhesive properties of some hydrogels can be advantageous by keeping them immobilized at the site of application or in applying them on surfaces that are not horizontal. However the amount and homogeneity of drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs. The high watercontent and large pore sizes of most hydrogels often result in relatively rapid drug release, over a period of few hours to a few days. Ease of application can also be problematic; although some hydrogels are sufficiently deformable to be injectable, many are not, necessitating surgical implantation.

Properties of hydrogels

Mechanical properties: Mechanical properties of hydrogels are very important from the pharmaceutical and biomedical point of view. The evaluation of mechanical property is essential in various biomedical applications viz. ligament and tendon repair, wound dressing material, matrix for drug delivery, tissue engineering and as cartilage replacement material. The mechanical properties of hydrogels should be such that it can maintain its physical texture during the delivery of therapeutic moieties for the predetermined period of time. By changing the degree of crosslinking the desired mechanical property of the hydrogel can be achieved.

Biocompatible properties: It is important for the hydrogels to be biocompatible and nontoxic in order to make it applicable in biomedical field. Most polymers used for this purpose must pass cytotoxicity and in-vivotoxicity tests.

Desired features of hydrogel material: The functional features of an ideal hydrogel material can be listed as follows:

- Must have highest absorption capacity (maximum equilibrium swelling) in saline. Must show desired rate of absorption (preferred particle size and porosity) depending on the application requirement.
- Must exhibit the highest absorbency under load. Should show lowest soluble content and residual monomer.
- Have lowest price. Must have highest durability and stability in the swelling environment and during the storage.
- Must have highest biodegradability without formation of toxic species following the degradation. pH-neutrality after swelling in water.
- Colorless, odorless, and absolutely non-toxic. Must have good photostability.
- Re-wetting capability (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it; depending on the application requirement.

Common uses for hydrogels:

- As control-release delivery systems.
- Provide absorption, desloughing and debriding capacities of necrotics and fibrotic tissue.
- Hydrogels that are responsive to specific molecules, such as glucose or antigens can be used as biosensors as well as in DDS.

- Used in disposable diapers where they "capture" urine, or in sanitary napkins.
- Contact lenses (silicone hydrogels, polyacrylamides).
- Common ingredients aree.g. polyvinyl alcohol, sodium polyacrylate, acrylate polymers and copolymers with an abundance of hydrophilic groups. Natural hydrogel materials are being investigated for tissue engineering, these materials include agarose, methylcellulose, hylaronan, and other naturally derived polymers.

MATERIALS AND METHODS

Preparation of chitosan nanoparticles of Miconazole: Chitosan nanoparticles were prepared by ionotropic gelation method.
PreparationI: Chitosan stock solution (0.25 to 0.75 %w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature.
Preparation II: The drug (10 mg) was dissolved in chitosan solution.
Preparation III: 1% Sodium tripolyphosphate solution was prepared in water.

Preparation IV: Sodium tripolyphosphate solution was added dropwise 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 prepared

S. 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

RESULTS AND DISCUSSION

Evaluation of nanoparticles of Miconazole

PercentageYield

Percentage yield of different formulation was determined by weighing the nanoparticles after drying. The percentage yield of different formulation was in range of 63.12±0.36 – 75.65±0.32%.

Table 2: 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

***Average of three determinations (n=3)**

Entrapment Efficiency

The drug entrapment of different formulations was in range of 63.32±0.45-73.23% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Miconazole nanoparticles.

Table 3: 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

***Average of three determinations (n=3)**

The maximum percentage yield and entrapment efficiency was found formulation F2. The optimized formulation among other batches subjected to further studies.

Results of Characterization of Transfersomes Gel: Total three formulations (GF1, GF2 and GF3) were prepared by using different concentration of Carbopol 934. 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. Gels were evaluated by means of pH, spreadability, viscosity and drug content and extrudability. pH was determined using digital pH meter. The pH values of the prepared transfersomes gel were within acceptable limits of 6.85-7.02 for GF1 (6.85±0.02) GF2 (7.02±0.04) GF3 (6.95±0.02). The Spreadability was measured on the basis of slip and drag characteristics of the gels and was in the range of10.32±0.65 to 12.23±0.45gms/cm sec. The Spreadability of GF1, GF2, and GF3 was found to be 12.23±0.45, 11.56±0.98 and 10.32±0.65 respectively. The viscosity of gels was determined by using a Brookfield viscometer DV-II model. The temperature which alters the viscosity was maintained at 25 because the increase of temperature decreases the viscosity of gels and vice versa. Viscosity for GF1, GF2 and GF3 found to be 4568±13cps, 4251±11 cps and 3978±14cps respectively.

Table 4: 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

***Average of three determinations (n=3)**

In transdermal drug delivery system pH plays an important role, the result of pH study of formulation shows that all the formulations are suitable for skin delivery. The pH values of the prepared gels were within acceptable limits of 6.99-7.02. Viscosity of the gels increases with an increase in concentration polymer. The increase in viscosity with the polymer concentration is because of increase in bonds between the polymer molecules which lead to formation of a hard and dense compact mass. This hardness is due to less amount of liquid in gels with high polymer concentration as compared to gels of low polymer concentration or in other words it can be said the higher the polymer concentration more shear stress is required to produce a specified rate ofshear.

The viscosity and Spreadability values indicate that the formulations are efficient to hold up the Transfersomal formulation. Moreover the GF2 formulation has a optimum viscosity which can be spread evenly over the skin and will maintain contact for longer period of time with the skin and thereby leading to maximum therapeutic effect. Also the pH of the formulations indicates to be in the range of 6.99±0.02 to 7.02±0.04 and hence they are suitable for the application to the skin and will not lead to any irritation and adverse effects.

In vitro drug release study of prepared gel formulation

Table 5 : In vitro drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release		
		GF1	GF2	GF3
1	0.5	12.32±0.25	11.32±0.32	9.85±0.36
2	1	18.89±0.32	19.98±0.45	11.32±0.25
3	2	23.32±0.14	35.65±0.65	15.56±0.14
4	3	26.65±0.65	40.23±0.74	18.89±023
5	4	43.32±0.47	45.65±0.65	22.32±0.14
6	5	53.45±0.32	65.58±0.32	26.69±0.23
7	6	59.98±0.74	73.32±0.14	38.89±0.14
8	8	63.32±0.23	81.14±0.65	45.56±0.74
9	10	65.45±0.25	91.45±0.32	55.56±0.36
10	12	66.68±0.21	98.85±0.74	63.32±0.25

***Average of six determinations (n=6)**

SUMMARY AND CONCLUSION

Miconazole is one of the broad spectrum antifungal compounds of the imidazole group. This antifungal agent is a fungicide used in the treatment of fungal infections in a topical and transdermal. This drug works by inhibiting ergosterol biosynthesis on the fungal cell membranes that cause damage to the cell wall of the fungus, resulting in increased membrane permeability, and ultimately, causing the fungal cell to lose its cellular nutrients. The drug is mainly used for the treatment of mycosis skin diseases. The bioavailability of miconazole nitrate is very low when taken orally because it is very difficult to dissolve and has a small absorption, and therefore, the use of miconazole nitrate as an antifungal agent is given topically, but the main problem of this drug in topical treatment is poor skin penetration. In Present investigation chiosan nanoparticles of Miconazole was prepared and evaluated for Percentage yield, Drug entrapment, Zeta potential and particle size and further prepared nanoparticles incorporated in gel base and evaluated. Percentage yield of different formulation was determined by weighing the nanoparticles after drying. The percentage yield of different formulation was in range of63.12±0.14– 75.65±0.32%. The drug entrapment of different formulations was in range of 63.32-73.23% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Miconazole nanoparticles. The maximum percentage yield and entrapment efficiency was found formulation F2. The optimized formulation among other batches subjected to further studies. The results of measurement of mean particle size of optimized formulation F2 nanoparticles were found 85.6 nm. Results of zeta potential of optimized formulation F2 nanoparticles was found to be-36.2 mV.

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