DEVELOPMENT & CHARACTERIZATION OF AQUASOME OF ITRACONAZOLE FOR IMPROVED ANTIFUNGAL EFFECT

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Abstract

Numerous fungi have been found to be involved in human infection. Infectious diseases rank second globally in terms of cause of death, after cardiovascular diseases. One innovative method and great option for drug delivery is the use of aquasomes, which are self-assembling nanoparticulate carriers. Thus this study aims at development & characterization of aquasome of itraconazole for improved antifungal effect. In total six formulations of itraconazole containing aquasome were prepared. The vesicle size ranged from 120.32nm to 165.20nm while the surface charge varied from -26.65 mv to -38.74mv. Further it was observed that highest entrapment efficiency was associated with F3 formulation which is 74.65±0.22%. The *In vitro* drug release study of prepared Aquasomes suggested that about 98.78% drug is released in 12 hours. According to drug release kinetics study the R^2 value for zero order, first order, Higuchi and Korsmeyer estimated to be 0.937, 0.812, 0.994, and 0.651 respectively. Thus from obtained R^2 value it is clear that the itraconazole containing aquasome follows Higuchi release kinetics. Beside this the antimicrobial activity of aquasome is checked by well diffusion method. The zone of inhibition for 2.5mg/ml, 5 mg/ml and 10mg/ml was noted to be 8±0.74mm, 10±0.57mm, and 12±0.86mm respectively. The results from the antimicrobial activity indicated that prepared aquasome have potent anti-fungal activity. Along with that stability studies were carried out. The formulation was found to be stable for three months when kept at 4.0 ±0. 2°C. Thus, the aquasome are attractive vehicles for the delivery of a variety of conformationally sensitive compounds that exhibit enhanced biological activity.

Keywords: Fungal infections, Aquasome, Itraconazole, Vesicular drug delivery, Nanoparticulate carriers

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Introduction

Numerous fungi have been found to be involved in human infection. Infectious diseases rank second globally in terms of cause of death, after cardiovascular diseases. Even though viruses or bacteria are the primary cause of these infections, opportunistic fungal infections in humans and animals are becoming more commonplace globally. There are several ways that infectious pathogens can spread from animals to people and vice versa: vector-borne routes, faecal-oral, direct or indirect contact, and airborne, such as respiratory droplets and aerosols (Arendrup *et al*., 2009; Gnat *et al*., 2021).

Topical antifungal medications are used to treat the majority of superficial infections; the choice of medication depends on the location and severity of the infection as well as the causative organism, which is typically easy to identify. Fluconazole and itraconazole have favorable oral bioavailability and safety profiles, which make them popular choices for chemoprophylaxis. Lipid-associated formulations of amphotericin-B and intravenous itraconazole are safer than conventional amphotericin-B (the previous gold standard) and at least as effective when used in empirical therapy. The use of amphotericin-B formulations associated with lipids has been restricted due to their expensive acquisition costs (Meis and Verweiji 2001: Richardson and Warnock, 2012).

Since then, developments in the field of vesicular drug delivery have produced systems that enable drug targeting, entrap large drug molecules, and release conventional medications in a sustained or controlled manner. Recent years have seen a great deal of innovative research into the formulation and creation of small-dosage forms that enhance drug efficacy. There are various kinds of carriers for pharmaceuticals. They are carriers of particles, polymers, macromolecules, and cells (Jain *et al*., 2014; Alenzi *et al*., 2023).

One innovative method and great option for drug delivery is the use of aquasomes, which are self-assembling nanoparticulate carriers. Aquasomes proved to be a significant drug delivery system in the development of ceramic nanoparticles. The three-layered structure known as an aquasome is made of a solid crystalline core that has been covered in carbohydrates and onto which biologically active drug molecules are adsorbed. While the polyhydroxy oligomer coating guards against dehydration and gives stability to active drug molecules, the solid core provides structural stability (Gupta *et al*., 2021). Aquasome formulations are typically administered parenterally, although recent research indicates that alternative routes of administration may be possible. Aquasomes use a combination of sustained release and targeted molecular shielding to deliver their bioactive molecules. Aquasomes based on hydroxyapatite cores are widely used in implant preparation. Because of their ability to maintain conformational integrity and a high degree of surface exposure, aquasomes are successfully used to target specific sites for the delivery of peptide molecules like insulin and hemoglobin as well as enzymes like serratiopeptidase and to help target genes and vaccines (Patil *et al*., 2018; Manikiran *et al*., 2010).

Compared to fluconazole, ittraconazole appears to induce less resistance and has a wider spectrum of activity. It is also well tolerated. Due in large part to the significant antifungal activity of its primary metabolite, hydroxy-itraconazole, this triazole antifungal agent is very effective. But there is a lot of variation in the absorption of itraconazole from the capsule formulation—which has been around for a while. This is partially due to the fact that sufficient absorption necessitates both the presence of food and an acidic gastric environment. Some patients have reduced absorption due to low oral food intake, frequent vomiting, inability to tolerate solid-dose formulations, or difficulty swallowing the capsules. The oral solution absorbs more readily. If osmotic diarrhea is a concern due to the cyclodextrin in the oral solution, half the dose can be administered as an oral solution and the other half as a capsule to lower the dosage of cyclodextrin (Willems *et al*., 2001; De Beule, 1996).

Thus, with current scenario there is need to develop topical form of itraconazole with better bioavailability. Thus this study aims at development & characterization of aquasome of itraconazole for improved antifungal effect.

Materials and Methods

Procurement of drug

Itraconazole was obtained as gift sample from phramceutical industry.

Chemicals and Reagents

Tween 80, Glutaraldehyde, Gelatin, ethanol, methanol, water etc. wereobtained from Loba chemie Pvt. Ltd. Mumbai.

Formulation of Itraconazole loaded Aquasomes

Preparation of drug solution

Dissolve the drug Itraconazole in a suitable solvent, such as water or a water-alcohol mixture, to obtain a drug solution. Ensure that the drug is fully dissolved to achieve a homogenous solution.

Preparation of stabilizer solution

Dissolve the stabilizer gelatin in water to form a stabilizer solution. Heat the solution gently, if required, to ensure complete dissolution of the stabilizer.

Preparation of surfactant solution

Dissolve the surfactant (Tween 80, 1 to 3%) in water to create a surfactant solution. Mix the solution thoroughly to ensure proper dissolution.

Preparation of cross-linking agent solution

Prepare the cross-linking agent solution separately. Glutaraldehyde as the cross-linking agent, prepare a 1% glutaraldehyde solution by diluting it in water.

Formation of Aquasomes

Slowly add the drug solution to the stabilizer solution while continuously stirring. This step forms the drug-stabilizer complex. Gradually add the surfactant solution to the drug-stabilizer complex while maintaining continuous stirring. The surfactant will stabilize the complex and prevent agglomeration (Rojas Oviedo *et al.*, 2007).

Cross-Linking of Aquasomes

After the formation of the Aquasome suspension, add the cross-linking agent solution drop wise to the suspension while stirring gently. The cross-linking agent will promote the cross-linking of stabilizer molecules, forming a rigid shell around the drug-stabilizer-surfactant complex, resulting in the formation of Aquasomes.

Table 1: Different formulation of Aquasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Drug	50	50	50	50	50	50
Tween 80	1	2	3	1	2	3
Glutaraldehyde	1	1	1	1	1	1
Gelatin	0.5	1	1.5	0.5	1	1.5
Water	qs	qs	qs	qs	qs	qs

Characterization and evaluation of Itraconazole loaded Aquasomes

Characterize the Aquasomes using various techniques, Surface charge and vesicle size, entrapment efficiency, transmission electron microscopy (TEM), and *in-vitro* diffusion study (Yadav *et al*., 2020).

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the Aquasomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a Zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm (Khopade *et al.*, 2002).

Entrapment efficiency

One milliliter of Aquasomes suspension was centrifuged at 15.000 rpm for 1 h to allow the separation the entrapped drug from the un-entrapped drug. After removal of the supernatant, the sediment was lysed using methanol and then analyzed spectrophotometrically at 264nm using a UV spectrophotometer (Labindia 3000+). The EE% of in the prepared Aquasomes was calculated applying the following equation (Kulkarni *et al*., 2022).

$$\%\ Entrapment\ Efficiency = \frac{Terotical\ drug\ content - Practical\ drug\ content}{\text{Therotical drug content}} \times 100$$

***In vitro* drug diffusion study**

The dialysis diffusion approach was used to perform in vitro drug release of prepared aquasomes utilizing the dissolution test apparatus. The dissolving media was phosphate buffer pH 7.4. The dialysis technique was carried out utilizing a cellulose acetate dialysis membrane with a molecular weight cutoff of 12,000–14,000 moles. This membrane ensures drug penetration while retaining aquasomal vesicles. Before usage,

the membrane was soaked in fake tears for 12 hours. A glass cylinder with a length of 8 cm and a diameter of 1 cm was filled with four ml of aquasomal dispersion, and a dialysis membrane was threaded to the mouth of the cylinder. Each glass cylinder was attached to the shaft of the dissolution apparatus (USP Dissolution tester, Labindia DS 8000) and descended down into a 100 ml beaker containing 50 ml of as dissolution medium without touching the bottom surface of the beaker. The beaker was then placed into vessels of dissolution apparatus that contained about 100 ml of water to keep temperature at 34 ± 0.5°C. The glass cylinders were adjusted to rotate at a constant speed of 20 rpm. One ml of dissolution medium was withdrawn at predetermined time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 h). To maintain a consistent volume, the samples were changed with new dissolving media. The concentrations of drugs in samples were measured spectrophotometrically at 264nm, the wavelength of the drug (Jagdale and Karekar, 2020).

Anti-fungal studies

After preparation and sterilization of potato dextrose agar medium at room temperature, then the medium was poured into the Petri dishes and allowed to cool for some time at room temperature until it solidifies the it was inoculated with *Candida albicans* (fungal strain) and then well were bored in Petri dish with the help of sterile bore of 6mm diameter and calculated concentration of the drug loaded Aquasomes were placed in the bores and the Petri-plates were incubated at 37ºC for 48 hrs in incubator. The zone of inhibition was observed and the radius of the zone of inhibition was calculated (Magaldi *et al*., 2004).

Stability studies

Stability study was carried out for drug loaded Aquasomes at two different temperatures i.e. refrigeration temperature (4.0 ±0.2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content (Bajaj *et al*., 2012).

Results and discussion

In total six formulations of itraconazole containing aquasome were prepared. The vesicle size ranged from 120.32nm to 165.20nm while the surface charge varied from -26.65 mv to -38.74mv. Aquasomes should have particle sizes between 60 and 300 nm. Targeting the drug molecule is the main use of aquasomes in drug release. According to this perspective, particle size and particle size distribution (PSD) are crucial because they affect important colloidal characteristics like packing density, surface area, rheology, and film gloss.

Storage stability can be predicted using the zeta potential measurement. To prevent particle aggregation, a high zeta potential should be reached, either positively or negatively. Further it was observed that highest entrapment efficiency was associated with F3 formulation which is 74.65±0.22%. The *In vitro* drug release study of prepared Aquasomes suggested that about 98.78% drug is released in 12 hours.

The solubility, desorption, and diffusion of the drug through the matrix all affect the rate of drug release. Drugs added during formulation will cause a brief burst of effect in the system before a prolonged release. Drug release from a polymeric membrane happens through desorption or diffusion if the particle is coated with a polymer. Particle size also has an impact on drug release. The surface area to volume ratio will be higher for smaller particle sizes. Therefore, the medication is at or close to the particle's surface, resulting in a quicker release from aquasome.

According to drug release kinetics study the R^2 value for zero order, first order, Higuchi and Korsmeyer estimated to be 0.937, 0.812, 0.994, and 0.651 respectively. Thus from obtained R^2 value it is clear that the itraconazole containing aquasome follows Higuchi release kinetics.

Beside this the antimicrobial activity of aquasome is checked by well diffusion method. The zone of inhibition for 2.5mg/ml, 5 mg/ml and 10mg/ml was noted to be 8±0.74mm, 10±0.57mm, and 12±0.86mm respectively. The results from the antimicrobial activity indicated that prepared aquasome have potent anti-fungal activity. Along with that stability studies were carried out. The formulation was found to be stable for three months when kept at 4.0 ±0. 2°C.

Thus, the aquasome are attractive vehicles for the delivery of a variety of conformationally sensitive compounds that exhibit enhanced biological activity because of the special carbohydrate coating that covers the ceramic core. Thus, this method offers pharmaceutical scientists fresh hope for the delivery of a variety of molecules, such as bioactive molecules and viral antigens. Aquasomes require additional research to validate their efficacy and safety, determine their clinical utility, and initiate commercialization.

Table 2: Results of Vesicle size and Surface Charge of Aquasomes formulation

S. No.	F. Code	Vesicle size (nm)	Surface Charge (mv)
1	F1	165.25	-26.65
2	F2	136.85	-29.98
3	F3	120.32	-32.14
4	F4	132.25	-30.45
5	F5	146.65	-36.69
6	F6	140.32	-38.74

Table 3: Results of Entrapment efficiency

S. No.	F. Code	Entrapment efficiency (%)
1	F1	64.45±0.15
2	F2	69.95±0.23
3	F3	74.65±0.22
4	F4	68.85±0.18
5	F5	67.74±0.32
6	F6	64.41±0.18

Table 4: *In vitro* drug release study of prepared Aquasomes optimized formulation F3

S. No.	Time (hr)	Root T	Log T	% Cumulative Drug Release	% Cumulative Drug Release Remain	Log % Cumulative Drug Remain to be Release	Log % Cumulative Drug Release
1	0.5	0.707	-0.301	18.85	81.15	1.275	1.909
2	1	1	0	26.65	73.35	1.426	1.865
3	2	1.414	0.301	42.23	57.77	1.626	1.762
4	4	2	0.602	58.98	41.02	1.771	1.613
5	6	2.449	0.778	73.32	26.68	1.865	1.426
6	8	2.828	0.903	86.65	13.35	1.938	1.125
7	12	3.464	1.079	98.78	1.22	1.995	0.086

Table 5: Release Kinetics of aquasomes optimized formulation F3

Formulation	Zero order	First order	Higuchi	*Korsmeyer*
F3	0.937	0.812	0.994	*0.651*

Table 6: Antifungal activity of Aquasomes against *Candida albicans*

S. No.	Name of microbes	Zone of inhibition		
		2.5mg/ml	5 mg/ml	10mg/ml
1	*Candida albicans*	8±0.74	10±0.57	12±0.86

Table 7: Stability study of optimized formulation of Aquasomes

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temperature	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Average vesicles size (nm)	120.32	145.65	122.36	145.65	125.45	162.32
% EE	73.32	68.78	72.32	65.78	73.32	65.50
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid

Conclusion

In conclusion this study successfully created and evaluated the itraconazole containing aquasome for antifungal effect. The F3 formulation found to have all ideal parameters. In order to validate its clinical applications, research should also concentrate on assessing the factors linked to biocompatibility, safety, efficacy, and toxicity. Aquasomes may eventually become a viable alternative vesicular carrier for the effective delivery of a range of macromolecules, biologicals, and medications, despite these development-related problems.

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