Pharmaceutıcal Modulatıon Of Conventıonal Drugs; The Case Of Fluconazole

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Abstract

Fluconazole is a third-generation triazole class antifungal drug used to treat systemic or superficial fungal infections, a potent and specific inhibitor of sterol synthesis. Fluconazole (FCZ) acts by preventing ergosterol production. It has been used since FDA approval. It is used in vaginal and oral infections of Candida and systemic candidiasis. It can cause stomach upset, diarrhoea, malaise, vomiting, rash, red blood cell reduction and hepatotoxicity as side effects. Therefore, it is important to develop different pharmaceutical forms that will reduce the side effects of FCZ. The change of the conventional FCZ form with different methods and different carriers is a matter of curiosity. Modulations using solid lipid nanoparticle (SLN) carrier systems provide tissue penetration, ease of use, and bioavailability efficiency. This form is enjoyable for both local and systemic treatment. It has become inevitable to develop new modulations in the treatment of mycosis infections such as C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, Cryptococcus neoformans, which also cause oropharyngeal, oesophagal, vaginal and urinary system candidiasis, cryptococcal meningitis, and candida-induced peritonitis.

It aims to produce a 300nm SLN form (FCZ-SLN) to optimize the oropharyngeal, vaginal and inhaled systemic absorption of FCZ. The main goal of FCZ is the characterization, and the ultimate goal is the nanopharmaceutical modulation of a conventional agent with experimental animals and phase studies.

In this study, the hot homogenization technique described by Müller was used for SLN-FCZ. FCZ was prepared as a hot lipid solution at 80ºC using 5% lipid fluid (Compritol 888) and 3% Tween 80 as surfactant. FCZ was added to the lipid solution, and Tween 80 was slowly added while slowly mixing with Ultra-Turrax (T25, Janke & Kunkel IKA®, Germany) for 10 minutes. Then, the FCZ-SLN complex was obtained by mixing at 20,500 rpm for 1 minute. Then, it was filtered through a 0.2µm filter and cooled to room temperature. It was stored in dark-coloured, sterile 5 mL glass tubes with screw caps at 6-8ºC. SLN-FCZ characterization The average diameter, particle size, and polydispersity index (PI) of SLNs were measured by photon correlation spectroscopy (PCS) using Nano Zetasizer (ZS, Malvern, UK) at a fixed angle of 90° at room temperature. SLNs were spread on a Cu grid, stained with uranyl

acetate, and observed under a transmission electron microscope (TEM). FCZ-loaded SLNs were confirmed by TEM (TEM FEI Tecnai™ Bio Twin).

Obtaining FCZ-SLN nanoparticles designed to be administered via inhalation is a scientific infrastructure research. It can be nebulized directly into the lungs as an aerosol in experimental animals, and its pharmacokinetics can be evaluated. With this research, FCZ has been added to the pharmaceutical modulation of conventional drugs for inhalation. In addition to conventional molecules used in treatment, some phytotherapeutic agents can also be adapted.

Keywords: Fluconazole, inhaler, pharmaceutical nano-design, SLN drug

Konvansiyonel İlaçların Farmasötik Modülasyonu; Flukonazol Örneği

Özet

Flukonazol sistemik veya yüzeysel mantar enfeksiyonlarının tedavisinde kullanılan üçüncü nesil triazol sınıfı, sterol sentezini güçlü ve özgün inhibe eden antifungal bir ilaçtır. Flukonazol (FCZ) ergosterol üretimini önleyerek etki eder. FDA onayından beri (1990) kullanılmaktadır. Kandidanın vajinal ve oral enfeksiyonları ile sistemik kandidiyaziste kullanılır. Yan etki olarak mide rahatsızlığı, ishal, kırgınlık, kusma, kızarıklık, kırmızı kan hücrelerinde azalma ve hepatotoksisiteye neden olabilir. Bu nedenle, FCZ'nin yan etkilerini azaltacak farklı farmasötik formlarının geliştirilmesi önem arz eder. Farklı yöntemler ve değişik taşıyıcılar ile konvansiyonel FCZ formun değişimi merak konusudur. Katı lipid nanopartikül (SLN) taşıyıcı sistemler kullanılarak yapılan modülasyonlar, doku penetrasyonu, kullanım kolaylığı ve biyo-yararlanımda verimilik sağlarlar. Bu form hem lokal hem de sistemik tedavi için ilgi çekicidir. Orofaringeal, özofagial, vaginal ve üriner sistem kandidiyazisi ile kriptokokkal menenjit, kandida kaynaklı peritonite de neden olan C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, Cryptococcus neoformans gibi mikoz enfeksiyonların sağaltımında yeni, yeni modülasyonların geliştirilmesi kaçınılmaz olmuştur (Choudhury and Roy (2016); Gupta & Vyas, 2012; Silvestre et al., 2020).

FCZ'un Orofaringeal, vaginal ve inhaler sistemik emilimini optimize etmek için 300nm SLN formunun (FCZ-SLN) üretilmesini amaçlar. FCZ'ün karakterize edilmesi temel olup nihai hedef deney hayvanları ve faz çalışmaları ile konvansiyonel bir ajanın nano-farmasötik modülasyonudur.

Bu çalışmada SLN-FCZ için Müller'in(Müller, Mäder, & Gohla, 2000) tarif ettiği sıcak homojenizasyon tekniği kullanıldı. FCZ, %5 lipit akışkan %5 (Compritol 888) ile %3 yüzey aktif madde olarak Tween 80 kullanılarak 80ºC sıcak lipit bir eriyik hazırlandı. Lipit eriyiğe FCZ eklenip Tween 80 yavaşça ilave edilirken Ultra-Turrax (T25, Janke & Kunkel IKA®, Germany) ile 10 dakika yavaşça karıştırıldı. Akabinde 20,500 rpm'de 1 dakika karıştırılarak FCZ-SLN kompleksi elde edildi. Sonra 0,2µm'lik

filtreden geçirilip oda sıcaklığına soğutuldu. Vidalı kapaklı, koyu renkli, steril 5 mL cam tüplerde 6-8ºC'de saklandı. SLN-FCZ karakterizasyonu SLN'lerin ortalama çapları, parçacık boyutu, polidispers indeksi (PI), 90° sabit bir açıyla, oda sıcaklığında Nano Zetasizer (ZS, Malvern, UK) kullanılarak foton korelasyon spektroskopisi (PCS) ile ölçüldü. SLN'ler bir Cu ızgarası üzerine yayılarak Uranil asetat ile boyandı ve transmisyon elektron mikroskobunda (TEM) gözlemlendi. FCZ yüklü SLN'ler TEM (TEM FEI Tecnai™ Bio Twin) ile doğrulandı.

İnhaler yolla verilmek üzere tasarlanan FCZ-SLN nano-partikül elde edilmesi bilimsel bir altyapı araştırmasıdır. Deney hayvanlarında aerosol olarak direkt akciğerlere nebulize edilip farmakokinetiği değerlendirilebilir. Bu araştırma ile konvansiyonel ilaçların inhaler verilmek üzere farmasötik modülasyonuna FCZ de eklendi. Tedavide kullanılan konvansiyonel moleküllerin yanı sıra bazı fitoterapötik ajanlar da uyarlanabileceği (Malamatari, Charisi, Malamataris, Kachrimanis, & Nikolakakis, 2020) düşünülebilir. Bu da sofistike mühendislik mahareti gerektirir. Bu araştırma ile SLN nano-partiküler ilaçların farmasötik modülasyonlarının mümkün olduğu gösterildi. Araştırmanın uygulanabilirliği tedavide daha verimli farmasötik ilaçların üretilmesine olanak sağlayacağı değerlendirilmektedir.

Anahtar kelimeler: Flukonazol, inhaler, farmasötik nano tasarım, SLN ilaç

Introduction

Fluconazole is a widely used antifungal agent of the triazole group. This agent is used to treat systemic Candida infections, including oropharyngeal, esophageal, vaginal candidiasis, candidemic peritonitis, pneumonia, and cryptococcal meningitis. It reduces the incidence of prophylactic candidiasis in patients with bone marrow transplantation and those receiving radiotherapy or cytotoxic chemotherapy. FCZ is more successful than other azole antifungal treatments in treating soft tissue and lung infections and infections caused by coccidioidomycosis. It is reasonably well tolerated. FCZ is used for off-label blastomycosis, histoplasmosis, and coccidioidomycosis. It is also effective in treating meningitis, a bone-joint infection caused by coccidioidomycosis. Fluconazole is also a good option for the treatment of pneumonia in immunocompromised patients and pneumonia in HIV-positive patients. Although antifungals reduce morbidity and mortality, their side effects, drug interactions, and their lack of desired concentration in the target tissue are serious problems. The risk of resistance development and toxicity in systemic use reduces the efficiency of the treatment. In this regard, FCZ is the least problematic of the azole antifungals.

Pharmaceutical modification of drugs increases drug efficiency and also improves treatment performance. Inhaled pharmaceutical drugs such as corticosteroids, tobramycin, ribavirin, and beta-agonists have many advantages in treatment. Rationally designed drug delivery systems can improve treatment acceleration and overcome many limitations. For this purpose, "pharmaceutical modulations" are the most efficient gains that can be made to reduce the side effects of existing conventional drugs, increase their effectiveness, and optimize their accumulation in the target tissue (Slavin, Asnis, Häfeli, & Bach, 2017). The physical dimensions and particle diameters of solid pharmacological agents are essential for their absorption as well as their alveolar penetration. In particular, the intercellular penetration efficiency of nanoparticles (NP) increases their bioavailability. It reduces the dose and limits their side effects. In short, it can make the safety range more efficient while optimizing its effectiveness. The ability of the drug to cross the membrane depends on the structure of the tissue and the molecule-membrane properties. The main thing is to deliver the appropriate pharmaceutical form to the target tissue through the ideal route. Therefore, the preparation, carriers and characterization of NPs are essential. In this way, the first commercialization of liposomal Amphotericin-B has given great excitement and hope. It has been a significant progress that has allowed the clinical use of an effective antifungal drug with minimum toxicity. Inspired by this, many other antifungal nanoparticle formulations have been studied. (Bobo, Robinson, Islam, Thurecht, & Corrie, 2016; Soliman, Mohamed, & Khatera, 2019). Adaptation of molecule lipophilicity, absorption efficiency, increased stability, targeting infected tissues, and improved antifungal activity have led to promising results regarding antifungal treatment. Thus, promising results regarding antifungal treatment have emerged on the horizon.

Pharmacokinetically, almost all FCZ (≥90%) is absorbed from the gastrointestinal tract. FCZ's oral and IV pharmacokinetics are similar and reach maximum plasma concentrations in 1-2 hours. Fluconazole absorption is not affected by gastric pH. Suspensions and tablets are bioequivalent. This is an important feature. A single daily dose reaches Steady-state concentration in 4-5 days. Fluconazole passes into all body fluids, including cerebrospinal fluid and milk. It can be found in milk in amounts close to plasma concentrations.

There are no adequate and well-controlled studies on the use of fluconazole in pregnant women. Available human data do not show an increased risk of congenital anomalies after treatment of pregnant women with standard doses (<200 mg/day) of fluconazole as a single dose or multiple doses during the first trimester. It crosses the placenta in rats. It is not known

whether this occurs in humans. Unlike some azoles such as ketoconazole, itraconazole, and miconazole, it is only slightly (11–12%) bound to plasma proteins. The drug is excreted primarily by the kidney, with a mean body clearance of approximately 0.23 mL/min/kg in adults. In regular volunteers, approximately 80% of the administered dose is excreted unchanged in the urine. Approximately 11% of the dose is also excreted in the urine as metabolites. 93.3% of 50 mg of radioisotope-labeled FCZ is excreted in the urine. FCZ clearance is proportional to creatinine clearance. Therefore, hemodialysis and peritoneal dialysis can remove it from the blood.

The half-life is inversely proportional to age. While it is 88 hours in newborns at the first dose, it is 19.5-25 hours between 9 months and 13 years of age and 15.2-17.6 hours between 5-15 years of age. Plasma concentrations increase, and the half-life is prolonged in patients with renal insufficiency. Pharmacokinetics in children are based on studies conducted for adults. The apparent volume of distribution is similar to the total body fluid volume of distribution.

Fluconazole crosses the blood-brain barrier. The meninges become increasingly permeable to fluconazole in inflammatory states. (Madu et al., 1994). This facilitates the treatment of meningitis. FCZ is metabolized very little in the liver. Fluconazole is a CYP2C9, CYP3A4 and CYP2C19 inhibitor. It is likely to have a different metabolism pathway than its counterparts. FCZ penetrates well into all body fluids examined. This feature makes it ideal for the treatment of systemic fungal infections. Fluconazole levels in saliva and sputum are similar to plasma levels. In patients with fungal meningitis, fluconazole levels in cerebrospinal fluid (CSF) are up to 80% of plasma levels. Fluconazole accumulates in the stratum corneum, epidermis-dermis and sweat glands at higher concentrations than in serum (Koks, Meenhorst, Hillebrand, Bult, & Beijnen, 1996) No evidence of carcinogenic risk was demonstrated in mice and rats treated orally for 24 months at doses approximately 2-7 times the human recommended dose of fluconazole (Aoyama et al., 2012; Bury, Tissing, Muilwijk, Wolfs, & Brüggemann, 2021; Debruyne & Ryckelynck, 1993; Lee et al., 1992; Watt et al., 2018). There are insufficient and controlled studies on the use of fluconazole in pregnant women. Although it is said to affect steroid hormones, this has not been proven (Rohman & Lias, 2021; Yang, Wang, & Elmquist, 1996).

The most optimal thing to do is to increase its efficiency by editing the molecule. For this purpose, as with all drugs, FCZ requires trying different delivery methods in the form of emulsions by bringing them to micro and nano sizes. Nanoemulsions are thermodynamically and kinetically more stable than emulsions. They are evaluated as colloidal carriers to improve

the effectiveness and tolerability of antifungal drugs. Nanoemulsions have better dissolving capacity for large amounts of drugs with low solubility, compatibility, and ability to protect drugs from enzymatic degradation and hydrolysis. Their capacity to penetrate deeper layers makes them ideal drug delivery vectors. For topical mycosis treatments, FCZ-loaded SLN-based gels, liquids, and pharmaceutical forms that can melt and disperse at body temperature can be a good example.

Liposomes, solid lipid nanoparticles (SLN), nanocarriers and nano-biomaterials are promising for the conversion of antifungals into NPs. (Soliman et al., 2019). In addition to being versatile and functional, thanks to their diverse range of properties, NPs can overcome the negative characteristics of many drugs. When health authorities saw the effectiveness of NP antifungals in invasive mycosis treatments, the transformation and adaptation of other antifungal agents to the clinic became the focus of studies. Increasing the effectiveness, reducing adverse effects, providing easy access to the tissue, and significantly minimizing nephrotoxicity and hepatotoxicity depend on the sophisticated preparation of the molecule. This preparation requires overcoming the passage mechanism in the organism and the NP molecule (Beloqui, Solinís, Rodríguez-Gascón, Almeida, & Préat, 2016). Therefore, it is not easy to adapt it to the clinic.

The most critical shortcomings of conventional drugs are limited penetration into the tissue, low absorption, limited distribution and low pharmacokinetics (PK). This situation causes the drug's effectiveness to decrease in practice, its side effects to increase, and its stability to be lost to the tissue. While these situations are undesirable features, they all seem correctable in NP drugs. On the other hand, topical drug delivery reaches high drug concentrations in a limited area, which allows for small drug doses, fewer side effects and low treatment costs. This is also very attractive in terms of efficiency (Verma & Utreja, 2019).

Patient compliance can also be ensured by rearranging the application routes. Conventional drug application methods for superficial mycosis treatment are simpler and more efficient. Topical antifungal drug administration using liposomes has improved penetration and increased therapeutic efficacy (Paralıkar, 2015). For this purpose, various carrier systems have been tested using liposomal miconazole, terbinafine, ketoconazole, FCZ, itraconazole, and clotrimazole (Chen et al., 2012; Sousa, Ferreira, Reis, & Costa, 2020). When oral, dermal, sub-dermal and vaginal activity capabilities of NP carriers were tested in different experimental animals, the efficiency of drugs in NP systems was found to be much better and more compatible than conventional pharmaceutical preparations (Mehnert & Mäder, 2012; Wolverton & Wu, 2019). Of course, the authorities must design the legal legislation in line with

these developments and this must be carried out in parallel (Guidance, 2011). For example, ex vivo penetration experiments performed on pregnant sheep vaginal tissue showed that chitosan-coated liposomes had higher clotrimazole tissue retention and good penetration compared to the control group (Arikan et al., 2002; Jøraholmen, Vanić, Tho, & Škalko-Basnet, 2014; Souto, Wissing, Barbosa, & Müller, 2004).

The NP field has also paved the way for administering phytotherapeutic agents as NPs or their use as carriers. When some agents, especially curcumin, from the antifungal phytotherapeutic agents, were turned into NP, this field became more attractive and interesting (Berginc, Suljaković, Škalko-Basnet, & Kristl, 2014; Boonme et al., 2016; Iadnut et al., 2019; Iwalokun, Ogunledun, Ogbolu, Bamiro, & Jimi-Omojola, 2004; Krishna, 2021; Sousa et al., 2020). SLN carrier systems have attracted interest in the local treatment of skin fungal infections because they facilitate the penetration of loaded active materials through the stratum corneum layer (Gupta ve Vyas, 2012). SLNs are more effective colloidal carriers than microemulsions and liposomes because they are replaced by solid lipids dispersed in the appropriate liquid. Therefore, many unique properties such as small size, large surface area, high drug loading and interaction of phases at the interfaces have been attributed to SLNs. This has improved the performance of pharmaceuticals. For example, subconjunctival injection of drug-loaded liposomes for fungal keratitis provided sustained drug release for up to 120 days. Or, rabbits treated with FCZ liposomes showed 86.4% recovery after 3 weeks (Habib, Fouad, Abdel-Rhaman, & Fathalla, 2010).

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLCs) are colloidal nano-drug carriers of physiological lipids dispersed in an aqueous surfactant solution. They were first introduced in the early 1990s as an alternative to polymeric NPs. SLNs are solid at room and body temperature. This physical state allows sustained long-term drug release via lipids. SLNs provide significant advantages for drug delivery, including the ability to overcome biological barriers, the ability to modify their surfaces, the possibility of co-administration of several drugs, and the preservation of the drug from degradation. However, SLNs also have some disadvantages, such as low drug loading capacity and inability to retain the drug for long periods during storage. The aspects of this technology that need to be developed include the confinement of the drug in the SLN crystal cage, storage time, and the ability to keep lipid molecules functional. Nanostructured lipid carriers (NLCs) are second-generation SLNs with a solid lipid matrix in specific proportions at room and body temperature. Therefore, they can be better loaded with drugs and adapt to different modulations of drug release. Since they have long stability, they also eliminate some disadvantages of SLNs. For example, itraconazole can

gain 99.98% encapsulation efficiency and the potential to maintain its stability for more than 6 months in this form (Pardeike, Weber, Zarfl, Pagitz, & Zimmer, 2016). This is less the case with SLNs. Amphotericin-B, voriconazole, fluconazole, ketoconazole, terbinafine, and griseofulvin, which are encapsulated in SLNs and whose physicochemical properties, stability and pharmacokinetics have been evaluated, have been shown to have better absorption and penetration properties than conventional drugs and have been proven to be ideal between 50-450 nm (Moazeni et al., 2016; Pardeike et al., 2016).

There is no study on targeting the lung tissue or the entire system via this tissue with liquid pharmaceutical aerosol (Cheng et al., 2020). Inhaled steroids before long-term oral or parenteral asthma treatments with FCZ result in a dose reduction (Ward Jr, Woodfolk, Hayden, Jackson, & Platts-Mills, 1999). With local targeting, the continuity of the drug delivery to the region, the increase in bioavailability and the effectiveness of the treatment are increased. In addition, the decrease in systemic toxicity and increased patient compliance emerge as an advantage (Monforte et al., 2010).

Mycosis infections such as Candida are challenging to diagnose, persistent and chronic, and therefore difficult to treat. Pulmonary mycoses such as invasive aspergillosis pose a significant threat to immunosuppressed patients. The risk of pulmonary aspergillosis, cryptococcosis, blastomycosis, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis is always higher in the hospital setting. Immunosuppressed individuals such as HIV and AIDS patients and organ transplant recipients typically have a high risk of opportunistic, asymptomatic, and disseminated mortality. Flucytosine, amphotericin-B combination followed by FCZ is the primary treatment for severe symptomatic pulmonary cryptococcosis. Triazole antifungals and echinocandins are recommended for cryptococcal and Candida patients, regardless of immunosuppression status, as they exhibit excellent lung penetration. Fluconazole has an important place here.

Pulmonary mycosis treatment is complex due to cancers, increased autoimmune disorders, and immunosuppressive drug use. Treatment of respiratory tract fungal diseases requires long-term hospitalization. The risk of other nosocomial infections may increase accordingly. FCZ is a broad-spectrum antifungal agent and is still successfully used in combination with other antifungal agents to treat opportunistic infections. Considering drug resistance and adverse effects of systemic administration, specific inhaled antifungal therapy may be a suitable alternative. Different pharmaceutical forms of FCZ have been tried for this purpose. Powder, liposomal forms, polyelectrolyte complex, and chitosan/alginate SLN forms

have been studied. Studies have focused more on this direction since mucosal infections are more prevalent (Darwesh, Aldawsari, & Badr-Eldin, 2018; Hamishehkar, Pourtahmaseb, Babazadeh, & Alipour, 2018). FCZ-encapsulated liposomal structures have been successfully used in candida keratitis. The stability and drug release of these formulations were found to be satisfactory (El-Housiny et al., 2018; Sousa et al., 2020).

This study aims to enable FCZ nanoparticles, which have been successfully used topically in different pharmaceutical forms, to be used as inhalers. The FCZ used consists of 300nm SLN particles. The aim is to convert fluconazole from systemic use to an inhaled treatment tool. The research is infrastructure research designed to use pharmaceutical nanoparticles FCZ as inhalers.

Materials and Methods

Fluconazole powder form (USP/Ph.Eur.) was obtained from İnd-Swift® laboratories limited through Sanovel® Turkey. Compritol and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Merck® and Schuchardt (Darmstadt, Germany). Other chemical analytes were obtained locally.

Obtaining Solid Lipid Nanoparticle Fluconazole (SLN-FCZ)

SLNs are obtained from solid lipid carriers in a similar way to emulsions. Two methods are used for receiving. Hot or cold homogenization under high pressure. They can also be obtained by solvent evaporation, solvent emulsification-diffusion, ultrasonication, shear (high-speed) homogenization, microemulsion and supercritical fluid methods. The high-speed hot homogenization technique is more efficient in obtaining SLN. The equipment used in this method is widespread and economical. Its reproducibility is a significant advantage (Mehnert & Mäder, 2012). In this method, the drug homogenized in lipid is dissolved homogeneously in the desired diameter by adjusting the temperature. In this study, the hot homogenization technique described by Müller was used to prepare SLN-FCZ (Müller et al., 2000). First, the drug solution was dispersed in a hot surfactant solution. The maximum solubility was determined by checking the solubility of FCZ in molten lipids under light with the naked eye. Then, the pre-emulsion was homogenized. Then, the hot nanoemulsion was cooled to 24ºC (room temperature) to obtain solid lipid NP (Shrimal, Jadeja, & Patel, 2020; Sparrelid et al., 1997).

A hot lipid solution was prepared at 80ºC using FCZ 5%, lipid fluid 5% (Compritol 888) and 3% surfactant, Tween 80. FCZ was added to the lipid solution, and Tween 80 was slowly added while slowly mixing with Ultra-Turrax (T25, Janke & Kunkel IKA®, Germany) for 10

minutes. The FCZ-SLN complex was obtained by rapid mixing at high speed (20,500 rpm) for 1 minute. This complex structure was passed through a 0.2 µm filter, and the resulting solution containing the nanoparticle system was cooled to normal room temperature. It was stored at room temperature by transferring to 5 mL, screw-capped, sterile, dark-coloured glass tubes. The same procedure was repeated without FCZ-SLN for control purposes.

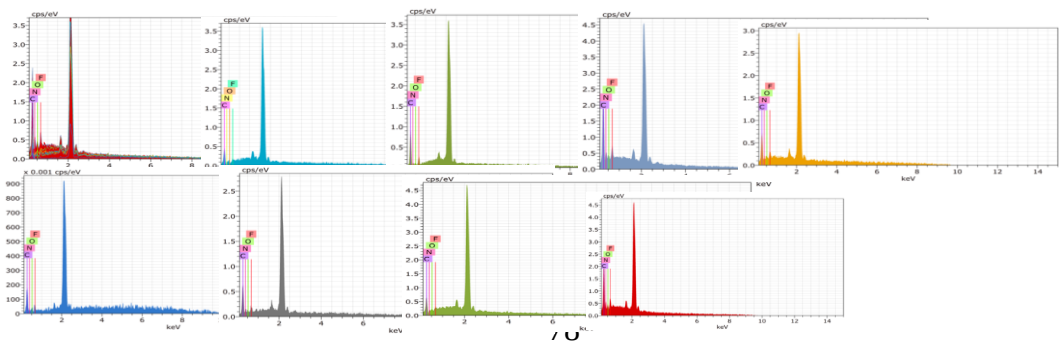
Characterization of SLN-FCZ

The diameters (average particle size) and polydispersity index (PI) of SLNs were determined by photon correlation spectroscopy (PCS) using a Nano Zetasizer (ZS, Malvern, UK) at a fixed angle of 90° and 25°C. PI is known as the measure of the size difference of nanoparticle clusters. To evaluate the stability of colloidal dispersity, zeta potential was measured by Zetasizer at 25°C. SLNs were suspended in distilled water (pH 7). Samples were analyzed in triplicate. Subsequently, SLNs were spread on a copper grid and stained with Uranyl acetate. Samples were observed by transmission electron microscope (TEM). The elemental structure was confirmed by TEM (TEM FEI Tecnai™ Bio Twin) (Table 2, Graph 3).

Mass percent (%) Spectrum

	C	N	O	F
1	37.26	26.61	16.59	19.55
2	47.78	26.35	8.86	17.00
3	37.27.	29.23	16.41	17.09
4	34.05	25.48	19.21	21.27
5	42.00	22.33	14.40	21.28
6	39.88	30.73	13.10	16.30
7	32.02	24.09	21.51	22.39
8	35.39	29.67	17.28	17.66
Mean value:	38.20	26.81	15.92	19.07
Sigma:	4.99	2.90	3.87	2.36
Sigma mean:	1.76	1.03	1.37	0.83

Table 2. Percentage composition data of elements (C, N, O, F) in different spectra of fluconazole.



Graph 3. Percentage composition data of elements (C, N, O, F) in different spectra of fluconazole.

Zeta potential and FCZ-SLN size

Zeta potential is the sliding motion of particles moving in a liquid between the moving Stern layer and the diffusion surface. The potential for this sliding plane is an indicator of electrokinetic effects. The magnitude of the zeta potential is directly proportional to the surface potential, the charge and the concentration of counter ions. The zeta potential of the particles was determined using Nano Malvern Zetasizer (Malvern Instruments). To determine the Stern potential, measurements were made in bidistilled water adjusted to 50 µS/cm conductivity with 0.9% (w/v) NaCl solution. SLN-FCZ size, zeta potential measurements, PI and electrical conductivity measurements were made using Zetasizer Nano Series (Nano-ZS) (Malvern Instruments, UK) (Table 1). The measurements were recorded by diluting with bidistilled water. Distilled water was adjusted to 50 µS conductivity with NaCl before the measurement.

In repeated measurements, the device temperature was set to 25ºC, and the light scattering was set to 90°. Measurements were made at different times during the day to verify the process (Graphs 1 and 2). Dynamic Light Scattering measures Brownian motion, the free motion of solid particles in a liquid, and relates it to particle size. Intensity waves are tested in the light scattered in all directions by illuminating the particles with a laser. When a screen is held close to the particle, it can be observed that bright and dark areas are formed. Brownian motion in a liquid is a random collision motion with the molecules of the liquid. The relationship between Dynamic Light Scattering and Brownian motion is essential because small particles behave quickly, and large particles behave slowly. The bright light scattered by the movement of the particles is constructive, and the dark light is destructive. This phase causes an increase and decrease in the intensity of the areas. This intensity fluctuation can be measured by the degree of similarity between two unique signals simultaneously with a correlator. As the instantaneous time intervals become smaller, a perfect correlation is achieved since the signals are the same. The size can be algorithmically determined by starting from the function of this correlation. Here, Zeta potential measures this fluctuation rate in intensity. It uses this to calculate the size of the particles. An important factor affecting the zeta potential is pH. As alkali is added to the suspension, the particles tend to become more negative, and as acid is added, they tend to become more positive. The isoelectric point is the point at which the colloidal system is least stable.

Nanoparticle stability

For colloidal system stability, the FCZ-SLN system was suspended in 5 ml buffer solution at pH = 5.5 with 20 min sonication and three solutions were prepared. After 30 days of storage at 4 °C, the suspensions were checked with light under a white background to see if there was any agglomeration or precipitation. The average diameters and Zeta potential of the nanoparticles were measured.

Result

SLNs are prepared for different purposes by different methods depending on their particle size, shape, controlled drug release, entrapment efficiency and stability (Cipolla, Blanchard, & Gonda, 2016; Debnath, Saisivam, & Omri, 2017; Dimer, de Souza Carvalho-Wodarz, Haupenthal, Hartmann, & Lehr, 2015; Gavaldà et al., 2005; Gontijo et al., 2014; Shilakari Asthana, Sharma, & Asthana, 2016; Togami, Chono, & Morimoto, 2012; Xu et al., 2016) Particle size is the most important physical property of colloidal carrier systems in determining the physical stability and activity of SLNs. In this study, the hydrophobic FCZ powder form SLN-FCZ was practically brought to 300 nm particle size. The stability of SLN-FCZ was maintained with Tween 80. These results prove that the SLN formulation brought the SLN-FCZ particle to the desired size, as shown in Table 1.

Table 1. SLN-FCZ PI value*

Formülasyon	Particle Size (nm)		Polydisperse Index (PI)	
	Mean (n=3)	S.D.	Mean (n=3)	S.D.
Fluconazole-SLN	3281	±2,99	0,422	±0,04

** Continuous quantitative variables are expressed as mean and standard deviation and qualitative variables are expressed as n, median value, and (Q1) and (Q3) percentage values. Kolmogorov-Smirnov and Shapiro-Wilk tests were used in normality tests of variables. Kruskal-Wallis One Way Analysis of Variance on Ranks test was applied to independent variables that did not show normal distribution. Probability values of $p<0.05$ were accepted as significant. All data analyses were performed with IBM SPSS Statistics 22 package programs.*

Surface Morphological Investigation on FCZ-SLN Particles

The surface character and shape structures of FCZ-SLN obtained by the hot homogenization technique were examined in TEM. As an examination method in TEM, the nanoparticles were separated from the supernatant by centrifugation, suspended in 100 µL of MiliQ water and impregnated by dropping onto carbon-coated copper grids. They were dyed by treating with 2% Uranium acetate salt (Uranyl acetate) for 20 seconds. Then, the excess dye was removed with water and dried. NPs were examined in TEM after 24 hours of drying. The elemental structure was extracted (Figure 1).

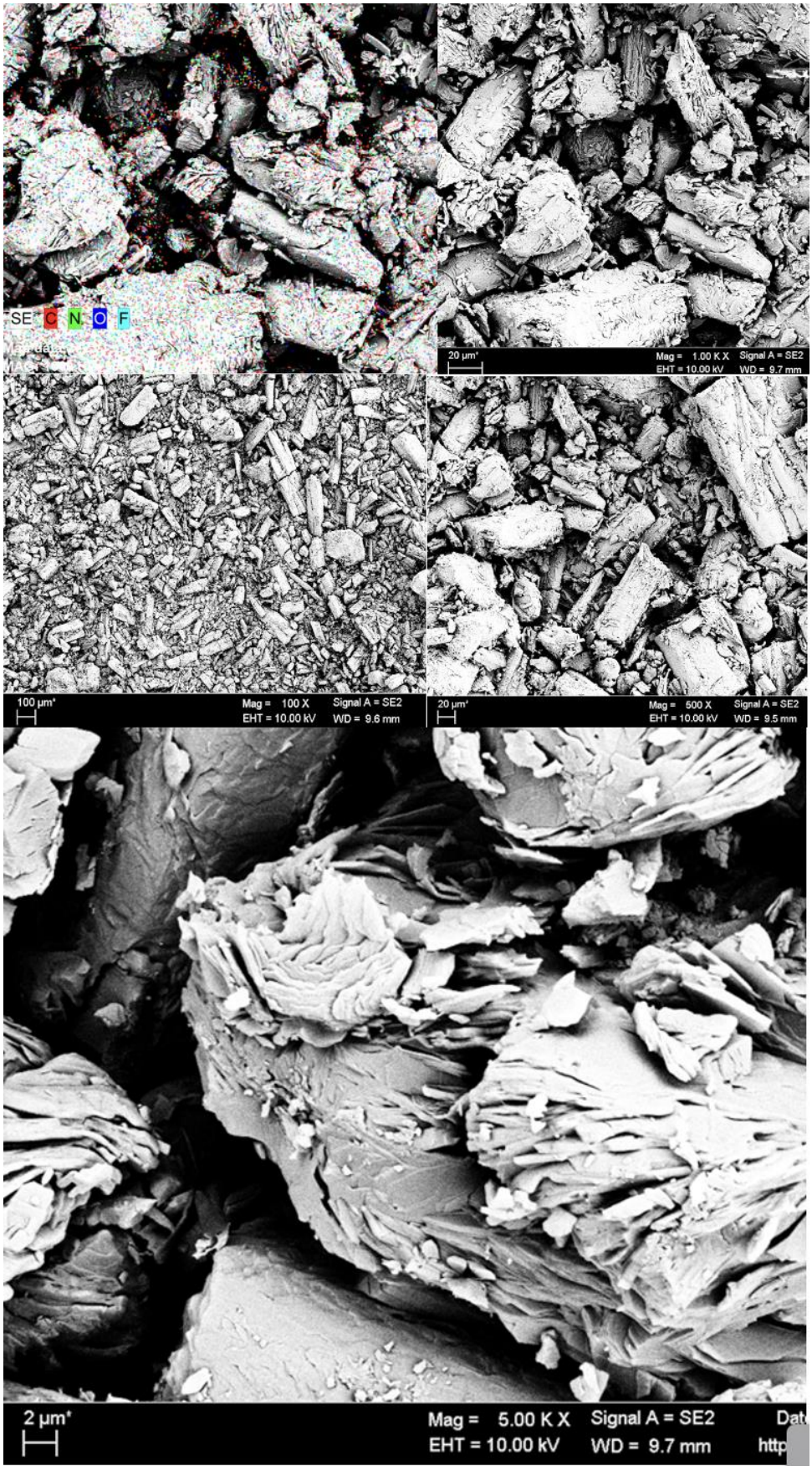
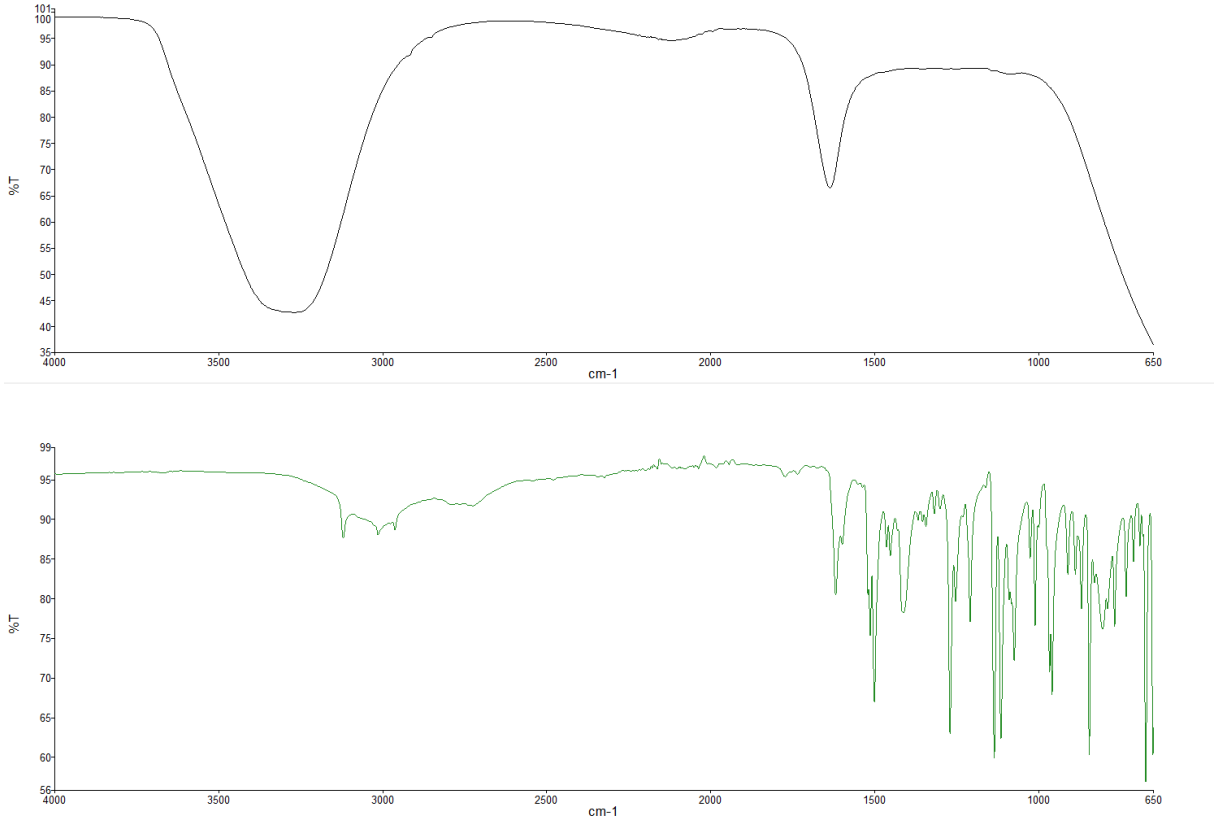

SE C N O F
20 µm
Mag = 1.00 K X
EHT = 10.00 kV
Signal A = SE2
WD = 9.7 mm
100 µm
Mag = 100 X
EHT = 10.00 kV
Signal A = SE2
WD = 9.6 mm
20 µm
Mag = 500 X
EHT = 10.00 kV
Signal A = SE2
WD = 9.5 mm
2 µm
Mag = 5.00 K X
EHT = 10.00 kV
Signal A = SE2
WD = 9.7 mm

Figure 1. Electron microscopy data of elemental (C, N, O, F) percentage composition of fluconazole in different spectra.

Graphs 1 and 2. FCZ-SLN measurements



Fluconazole is a member of the triazole class of antifungal agents (used against fungal infections) and is a potent and specific inhibitor of fungal sterol synthesis. It can be administered orally or intravenously. Fluconazole is active in various fungal infection models in experimental animals. Its activity has been demonstrated in opportunistic mycoses. It is effective in systemic candidiasis, even in immunocompromised animals. It is effective in infections caused by Cryptococcus neoformans, including intracranial infections, infections caused by Microsporum and Trichophyton species (Cheng et al., 2020; Garg et al., 2020).

Fluconazole has also been shown to be active in animal models of endemic mycoses, including Blastomyces dermatitides and Coccidioides immitis. It is also successful in Histoplasma capsulatum infections in normal and immunosuppressed animals, including intracranial infections (Watt et al., 2018; Yang et al., 1996).

The most commonly used triazoles are fluconazole, itraconazole, terconazole, voriconazole, isavuconazole, posaconazole, iodiconazole and fosfluconazole. Imidazoles include clotrimazole, (Souto et al., 2004) oxiconazole, miconazole, econazole, tioconazole and ketoconazole in treatment protocols. Fluconazole and other azoles are less toxic to the human

body than imidazoles. They cause fewer drug interactions (Madu et al., 1994; Trombino, Mellace, & Cassano, 2016).

Fluconazole accumulates in the stratum corneum, epidermis-dermis, and sweat glands at higher concentrations than in serum. Fluconazole tends to precipitate in the stratum corneum. The long plasma elimination half-life allows the treatment of vaginal candidiasis with lower doses. Studies have compared the salivary and plasma concentrations of a single 100 mg capsule and an oral suspension administered by holding it in the mouth for 2 minutes and shaking. The maximum salivary concentration of fluconazole for the suspension 5 minutes after ingestion of the dose was 182 times the maximum salivary concentration seen 4 hours after ingestion of the capsule (Koks et al., 1996).

Conclusion

Several topical and oral antifungal treatments have been tried to induce healing in fungal infections. Topical therapy is preferred because it is fast, effective and well-tolerated (Yetisgin, Cetinel, Zuvin, Kosar, & Kutlu, 2020) Nanopharmaceuticals are likely to be used for dermatological and cosmeceutical purposes. Apart from Amphotericin-B, no antifungal drugs are used in inhaled form (Cheng et al., 2020).

Nanopharmaceuticals modulations of triazoles have been tested in different forms in dermatology, and good results have been obtained (Chen et al., 2012). When FCZ-SLN was prepared for Pityriasis versicolor, a dermatological case, and its gel pharmaceutical form was tested, excellent results were obtained. The ability of the nanostructure to be well stored in lipophilic character, to optimize (El-Housiny et al., 2018) the liberation of the vehicle substance and, more importantly, to have good penetration are superior to conventional drugs. Such a change in traditional drugs is worth working on in terms of preventing the development of resistance and not exposing the entire organism to the drug.

With a sophisticated engineering design, an artificial intelligence and nano-bio pharmaceutical treatment can change its structure completely. The 300nm FCZ-SLN particle structures obtained in this study seem suitable for spray, vaginal gel and inhaler nebulization. This research is valuable for building on pharmacokinetic studies. Less side effects and more accumulation of drugs used for treatment in the target organ are desired situations. The main

problem solved in this scientific research is to provide drug density in the target tissue by optimizing the molecule size and changing the use route.

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