Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

Design and development of itraconazole loaded nanosponges for topical drug delivery

Swalin Parija* and V. Sai Kishore

[1]Institute of Pharmacy & Technology, Salipur, Cuttack, Odisha-754202
[2]Department of Pharmaceutics, Bapatla college of Pharmacy, Bapatla-522101
*Corresponding Author: E-mail Id: Swalinpharma@gmail.com

Abstract

The aim of present research work is to develop a topical gel formulation of Itraconazole loaded nanosponges to increase the solubility, permeability, stability and to control the Itraconazole release for a prolonged period. Itraconazole loaded nanosponges was prepared by cross-linking different concentrations of β- Cyclodextrin with carbonate bonds of di phenyl carbonate in different proportions, which are porous as well as nanosized. Drug was incorporated by solvent evaporation method by dissolving the drug in various solvents like ethanol, acetone and chloroform. The prepared nanosponges were incorporated into carbopol gel. From the encapsulation efficiency of the drug loaded nanosponges formulations, it was observed that as the crosslinking ratio increased the encapsulation efficiency was found to be enhanced. It is also found that the encapsulation efficiency of drug loaded nanosponges were influenced by the solvent used for drug loading by solvent evaporation technique. Based on the drug encapsulation efficiency , drug content and extent of sustained nature , the gel prepared with β-Cyclodextrin and crosslinking agent in 1:1 ratio, chloroform as a solvent and carbopol as a gellling agent (IF12 formulation) was concluded to be the best formulation. All the formulations followed zero order release kinetics and mechanism of drug release was governed by Peppas model.The diffusion exponential coefficient(n) values were found to be in between 0.9402 to 1.1864 indicating non fickian diffusion mechanisam.

Keywords: Itraconazole, β-cyclodextrin, nanosponges, diffusion rate

Introduction

Nanosponges are the progression in nano technology, which are the prominent answers for the various formulation challenges like low aqueous solubility, controlled release and targeted release. As compared to nano paricles these are less prone to bursting and releases the drug in a controlled and predictable manner throughout the intended period of application or administration (1). Nanosponges are beneficial for the passive targeting of drugs to skin, there by accomplishing major benefits such as reduction of total dose, retention of dosage form on the skin for prolonged period. Nanosponge loaded topical dosage forms can act as local depot for sustained drug release as well as rate- limiting membrane barrier for inflection of systemic absorption and thus overcoming the limitations of topical formulations. They are non- irritating, non-mutagenic, non- allergenic and non- toxic. Itraconazole is an imidazole derivative and used for the treatment of local and systemic fungal infections. It is a BCS Class II drug having very low solubility and high permeability. The oral use of Itraconazole is not much recommended as it has many side effects.

Most of these infections spread only in the skin layers but upon prolonged time they may be converted to systemic infections which may be mortal. Oral administration of Itraconazole is not convenient due to its severe side effects and its short half-life (3–6 h) that requires frequent dosing(2). Itraconazole is a BCS Class II drug that has a dissolution rate limited poor bioavailability so it needs to be incorporated into a proper vehicle to have right

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

levels of topical absorption. The conventional topical Itraconazole formulations release the drug for a shorter period at high quantities which causes the adverse effects like stining, zerythema, edema, vesicat, edema, vesication, desquamation, pruritus and urticaria due to the toxicity on the epithelial cells of the skin . The conventional topical dosage cannot reside at the site of application for longer times and does not release the drug in sustained manner.

Various methods are available to sustain the release of the drug. Among them the nanosponges have some unique advantages, which are three dimensional sponges like nanostructure encapsulating the drug. The nanostructure have potential for decreased skin irritation and stabilization of sensitive activities. Moreover nanosponges have good penetration into stratum corneum by overcoming the skin barrier effect and maintaining the good physical and chemical stability(3).

Materials and Methods

Itraconazole was the generous gift from Aurobindo Pharma Ltd, Hyderabad. Carbapol 934 P was procured from SD Fine chemicals Ltd, Mumbai.β- cyclodextrin and Di phenyl carbonate were purchased from Sigma Aldrich (Milan, Italy). All other ingredients used were of analytical grade shows Table 1.

***Synthesis of β- cyclodextrin nanosponges*:** β- cyclodextrin based nanosponges was prepared using Di phenyl carbonate as a cross-linker. Nanosponges were prepared using different ratios of β-cyclodextrin and Di phenyl carbonate [1:0.25, 1:0.5.1:0.75 and 1:1]. Finely homogenized anhydrous β- cyclodextrin and Di phenyl carbonate were placed in a 100 ml conical flask. The system was gradually heated to 100 ^{0}C under magnetic stirring, and left to react for 5 h. During the reaction crystals of phenol appeared at the neck of the flask. The reaction mixture was left to cool and product obtained was broken up roughly. The solid was repeatedly washed with distilled water to remove unreacted β- cyclodextrin and then with acetone, to remove the unreacted Di phenyl carbonate and the phenol present as by-product of the reaction. After purification, nanosponges were stored at 25 ^{0}C until further use(4).

Table1. Composition of Itraconazole loaded nanosponges using different solvents

S. No	Batch code	Polymer : cross linking agent (mg)	Drug (mg)	Solvent
1	ILNS1	4000:1000	4000	Ethanol
2	ILNS2	4000:2000	4000	Ethanol
3	ILNS3	4000:3000	4000	Ethanol
4	ILNS4	4000:4000	4000	Ethanol
5	ILNS5	4000:1000	4000	Acetone
6	ILNS6	4000:2000	4000	Acetone
7	ILNS7	4000:3000	4000	Acetone
8	ILNS8	4000:4000	4000	Acetone
9	ILNS9	4000:1000	4000	Chloroform
10	ILNS10	4000:2000	4000	Chloroform
11	ILNS11	4000:3000	4000	Chloroform
12	ILNS12	4000:4000	4000	Chloroform

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

***Preparation of Itraconazole loaded nanosponges*:** The Itraconazole loading into β-cyclodextrin nanosponges was carried out by solvent evaporation technique. In this various solvents like chloroform, acetone and ethanol were used. In 100 ml of each solvent 4000 mg of Itraconazole was dissolved separately to form solutions. To the each solution, prepared nanosponges were added and triturated until the solvent evaporated. While triturating the clumps of nanosponges were segregated and absorbs the drug solubilised solvent. The solid dispersions were dried in an oven overnight (at 50 ^{0}C at atmospheric pressure) to remove any traces of solvents and were sieved through 60 # and used for further work(5).

***Preparation of Itraconazole nanosuspension*:** The dried drug encapsulated nanosponges were collected and required quantities of drug equivalent nanosponges were transferred into 250ml volumetric flask containing 100ml methanol in order to remove the free unencapsulated drug by solubilising in the methanol. The drug encapsulated nanosponges were separated from the free drug by membrane filtration by using 0.22µ membrane filter. The residual drug loaded nanosponges were collected and dispersed in distilled water by using ultra sonication to form a nanosuspension(6).

***Formulation of carbopol gel containing Itraconazole loaded nanosponges*:** 500 mg of carbopol 934 was dispersed in 5 ml of distilled water and allowed for swelling over night. The swelled carbopol was stirred for 60 minutes at 800 rpm. The previously prepared required Itraconazole equivalent nanosuspensions , methylparaben and propylparaben were incorporated into the polymer dispersion with stirring at 500 rpm, by a magnetic stirrer for 1 h. The P^{H} of above mixture was adjusted to 4.5 with tri ethanolamine (0.5%). The gel was transferred in to a measuring cylinder and the volume was made up to 10ml with distilled water (7).

Evaluation studies

***Fourier Transform Infrared (FTIR) spectroscopy*:** To confirm the formation of nanosponges, Fourier Transform Infrared (FTIR) spectroscopy studies was used. Potassium Bromide pellet method was used in the study. The spectra was studied for the conformational changes of optimized drug when compared with the pure drug and pure excipients spectrums(8). The spectra were recorded in the wave number region of 4000-500cm^{-1}.

Encapsulation efficiency: The encapsulation efficiency of nanosponges was determined spectrophotometrically (λmax = 261 nm). A sample of Itraconazole nanosponges (100 mg) was dissolved in 100 ml of methanol and kept it for overnight. 1 ml of the supernatant was taken and diluted to 10 ml with a solution containing 4.5 P^{H} phosphate buffer and was analysed at 260 nm using UV-visible spectrophotometer. From the absorbance the free drug content was calculated in Table 2. The methanol dispersion containing Itraconazole nanosponges was then ultra sonicated to release the encapsulated drug from the nanosponges structure(9). Then the solution was filtered by using 0.22µ filter

Table 2. Encapsulation efficiency of Itraconazole loaded nanosponges

S. No	Batch code	%Encapsulation efficiency (n=3)
1	ILNS1	98.21 ± 0.4
2	ILNS2	98.34 ± 0.7
3	ILNS3	98.40 ± 1.1
4	ILNS4	98.56 ± 0.9
5	ILNS5	98.36 ± 0.3
6	ILNS6	98.44 ± 0.6
7	ILNS7	98.54 ± 0.6
8	ILNS8	98.67 ± 1.2
9	ILNS9	99.29 ± 0.8
10	ILNS10	99.37 ± 1.6
11	ILNS11	99.43 ± 1.4
12	ILNS12	99.57 ± 0.9

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

paper and the filtrate was analysed at 260 nm using UV visible spectrophotometer for the total drug content. The encapsulation efficiency (%) of the nanosponges will be calculated according to the following equation:

Encapsulated drug content in nanosponges

Encapsulation efficiency = ---------- X 100

Total drug content

All measurements were performed in triplicate. The results of the best polymer and crosslinking agent ratio were analysed statistically for their significance of difference.

Determination of particle size distribution: The particle size distribution was determined by using Dynamic Light Scattering (DLS) technique. The equipment used for the particle size distribution is HORIBA particle size analyzer. In this technique the particle sizes of a batch of the nanosponges were observed and from the standard deviation and mean particle size of nanosponges, the Poly Dispersity Index (PDI) was calculated. The poly dispersity index is the indication for the nature of dispersity[10].

Determination of zeta potential: Zeta potential is a measure of surface charge of dispersed particles in relation to dispersion medium. It was determined by using HORIBA zeta sizer having the capability of determination of zeta potential. The zeta potential value is the indication of physical stability of the nanosponges(11).

Evaluation of drug loaded Nano sponges containing gels: The drug loaded Nano sponges containing gels were evaluated for P^H,Viscosity Spreadability, Extrudability and mucoadhesive time(12).

Drug content in the DLNS containing gel formulations: The sample of 1 gram of gel formulation containing 10 mg of Itraconazole was dissolved in methanol, filtered and the volume will be made to 20 ml with methanol in Table 3. The drug content

Table 3. Percentage of drug content in the Itraconazole loaded nanosponges containing gel formulations

S. No	Batch code	% Drug content
1	IF1	95.15
2	IF2	96.23
3	IF3	96.46
4	IF4	97.41
5	IF5	94.30
6	IF6	95.30
7	IF7	96.61
8	IF8	97.42
9	IF9	96.57
10	IF10	97.49
11	IF11	98.33
12	IF12	99.28

will be determined by diluting the resulting solution for 10 times with a solution containing7.4 P^H phosphate buffer and the absorbance was measured at 260 nm using UV Visible spectrophotometer (13).

In-vitro drug diffusion study: Modified Franz diffusion cell was used for these studies. Cellophane membrane was used as the simulation for the skin. Cellophane membrane was mounted in a modified Franz diffusion cell. The known quantity (1g of gel containing 100 mg of the drug equivalent DLNS) was spread uniformly on the cellophane membrane on donor side. The solution containing 7.4 P^H phosphate buffer solution was used as the acceptor medium, from which 3ml of samples were collected for every hour and the same amount of fresh medium was replaced to maintain sink conditions for 12 hrs. While taking the samples from the acceptor medium, precautions were taken that no air bubbles were formed in the acceptor medium(14). The fresh samples were analyzed at 260 nm by UV-spectrophotometer and the

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

amount of drug diffused for each hour was calculated. All the samples were analysed in triplicate.

Results and Discussion

The FTIR structure of formed nano sponges were studied by comparing with unreacted β-cyclo dextrin and diphenyl carbonate FTIR spectra. In all the ratio of nanosponges, the major peaks were observed at 940 cm^{-1} which represents the α-1,4 glycoside bond which is the indication that there was no change in the cyclodextrin linkages. The absence of peaks responsible for carbonyl group of the diphenyl carbonate at 1768 cm^{-1} in the nanosponges is the indication of the removal of C=O from diphenyl carbonate. The absence of peaks responsible for –C=C- at 1591 and 1497 cm^{-1} in the IR spectra of nanosponges is indication of absence of phenol rings which were present in the unreacted diphenyl carbonate. Similarly absence of an intense peak responsible for –C=O group at 1157 cm^{-1} in the IR spectra of nanosponges is the indication of removal of C=O group from the diphenyl carbonate which might be attached to the primary of secondary hydroxyl groups of β- cyclodextrin by leaving phenol as by product. All these changes infers that the formation of nanosponges by reacting of primary/secondary hydroxyl groups of betacyclo dextrin with the carbonyl groups of diphenyl carbonate. From the encapsulation efficiency of the drug loaded nanosponges formulations it was inferred that, as the crosslinking ratio increased the encapsulation efficiency was found to be enhanced. The order of encapsulation efficiency in the nanosponges is 1:1>1:0.75>1:0.5>1:0.25. It is also found that the encapsulation efficiency of drug loaded nanosponges are influenced by the solvent used for drug loading by solvent evaporation technique. Chloroform >Acetone >Ethanol.

The change in the encapsulation efficiency with respect to solvent might be due to the solubility of Itraconazole in the particular solvent. The extended sustained release was observed in all the 12 formulations. But the extent of sustained nature was varied from one ratio to other. The order of sustained action was as follows 1:1>1:0.75>1:0.5>1:0.25.

Based on the drug encapsulation efficiency, drug content and extent of sustained nature formulation 12 was concluded to be the best formulation. The results of the present investigation overlay the path and provide substantial information for the utilization of Beta cyclodextrin in the development of drug delivery systems. The optimized formulation (IF12) were evaluated for their particle size and zeta potential. The particle size (334 nm) and zeta potential (-26.7 mV) was found to be good enough to maintain the physical stability of the nanosponges.

Carbopol gels containing nanosponges prepared with β -cyclodextrin and Di phenyl carbonate in different ratios and by using ethanol as a solvent shown drug release for a period of 7 hours, 7.5 hours, 8 hours and 9.5 hours respectively. Carbopol gels containing nanosponges prepared with β -cyclodextrin and Di phenyl carbonate in different ratios and by using acetone as a solvent shown drug release for a period of 8 hours, 8.5 hours, 9 hours and 10 hours respectively. Carbopol gels containing nanosponges prepared with β -cyclodextrin and Di phenyl carbonate in different ratios and by using chloroform as a solvent shown drug release for a period of 8.5 hours, 9 hours, 9.5 hours and 11 hours respectively. Based on the drug encapsulation efficiency , drug content and extent of sustained nature , the gel prepared with polymer and crosslinking agent in 1:1 ratio, chloroform as a solvent and carbopol as a gellling agent (IF12formulation) was concluded to be the best formulation.The initial burst release decrease with increase in concentration of crosslinking agent. To ascertain the mechanism of drug release, the dissolution data was analyzed by zero order, first order, and Higuchi and Peppas equations. The correlation coefficient values (r) and diffusion kinetics values were shown in Table 4. Amount of drug diffused versus time

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

Table 4. *In vitro* drug diffusion kinetic data of Itraconazole loaded nanosponges containing gel formulations

Formulation	Correlation coefficient				Diffusion Rate Constant (mg/hr) Ko	T_{50} (hr)	T_{90} (hr)	Diffusion Exponent (n)
	Zero order	First order	Higuchi	Peppas				
IF1	0.9996	0.8771	0.9312	0.9977	13.62	3.63	6.54	0.9841
IF2	0.9996	0.8608	0.9267	0.9992	13.02	3.84	6.92	0.9919
IF3	0.9997	0.8425	0.9228	0.9995	12.25	4.08	7.34	0.9963
IF4	0.9998	0.7833	0.9221	0.9996	10.26	4.87	8.78	0. 9951
IF5	0.9932	0.8964	0.9058	0.9941	12.95	3.86	6.94	0. 9957
IF6	0.9973	0.8474	0.9043	0.9978	11.52	4.34	7.82	0. 9968
IF7	0.9961	0.8238	0.8979	0.9983	10.50	4.76	8.57	0.9957
IF8	0.9946	0.7977	0.8909	0.9993	9.76	5.12	9.23	0.9864
IF9	0.9997	0.8721	0.9296	0.9998	12.25	4.08	7.34	0.9817
IF10	0.9987	0.8535	0.9276	0.9990	11.76	4.25	7.65	0.9923
IF11	0.9998	0.8160	0.9223	0.9990	10.50	4.76	8.57	0.9942
IF12	0.9997	0.7916	0.9214	0.9942	8.99	5.56	10.01	0.9937

curves exhibited straight line for the formulations and confirmed that the diffusion rate followed zero order release kinetics. Percentage of drug release versus square root of time curves shows linearity and proves that all the formulations followed Peppas model.

The diffusion exponential coefficient(n) values were found to be in between 0.9841 to 0.9968 indicating non fickian diffusion mechanisam. These results indicated that the diffusion rate was found to be decrease with increase in concentration of crosslinking agent. The optimized formulation has good spreadability, extrudability and mucoadhesive nature in Table 5 and Fig 1. The P^H and viscosity of the formulation were appropriate for the topical drug delivery and nanosponges technique was a better choice for sustained release.

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

Table 5. Physical properties of optimized gel

Formulation	Viscosity (cps)	Extrudability (N)	Spreadability (g.cm/sec.)	pH	Muco adhesive Time
IF12	3985±72	92.41± 0.05	34.61±2.11	4.46±0.02	>12 hrs

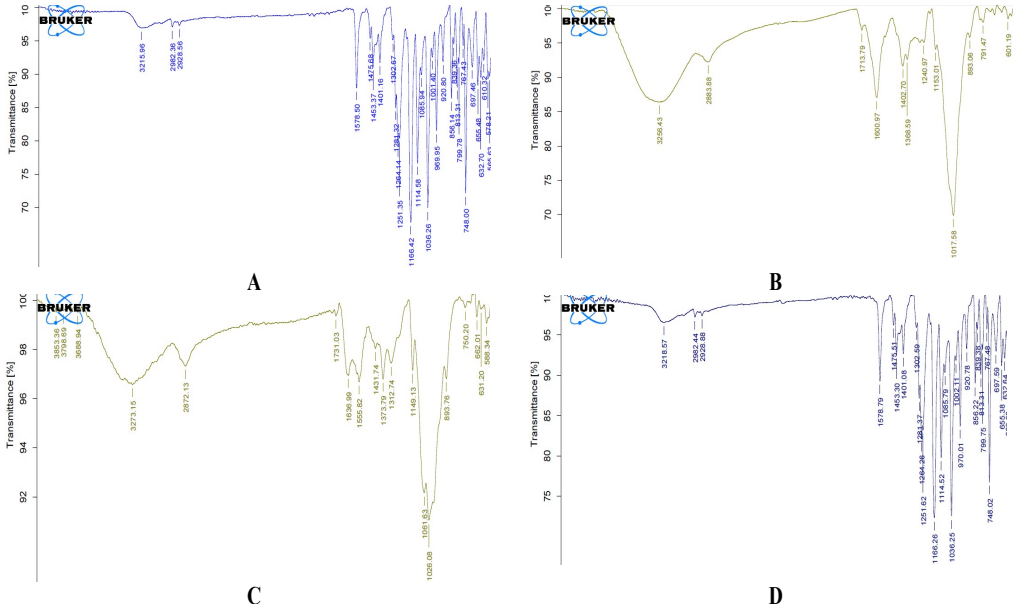

Fig 1. FT-IR spectra of Itraconazole(A) , β-Cyclodextrin (B), diphenyl carbonate (C)and optimized formulation(D)

References

1. Singh A, Development and Evaluation of Cyclodextrin Based Nanosponges for Bioavailability Enhancement of Poorly Bioavailable Drug. *World Journal of Pharmaceutical Sciences*. 2017; 6(2): 805-836.

2. Tripati K D. Antifungal drugs. Tripati K D(Ed). Essentials of Pharmacology, 6th edition, New Delhi: Jaypee Publicayion; 2008.

3. Jilsha G and Viswanad V, Nanosponge Loaded Hydrogel of Cephalexin for Topical Delivery. International Journal of Pharmaceutical Sciences and Research, 2015; 6(7): 2781-89.

4. Arvapally S, Harini M, Harshitha G, Kumar A,Formulation and *In-Vitro* Evaluation of Glipizide Nanosponges, American Journal of PharmTech Research, 2017; 7(3): 341-361.

5. Kesar PM, Kale K, Phadtare DG, Formulation And Evaluation of Topical Antifungal Gel Containing Itraconazole, International Journal of Current Pharmaceutical Research,2018; 10(4): 71-74.

6. Penjuri S, Nagaraju R, Saritha D, Sailakshmi B, Srikanth R, Formulation and

Vol. 16 (Supplementry Issue 2) 179 - 186, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.76

Evaluation of Lansoprazole Loaded Nanosponges, Turkish Journal of Pharmaceutical Sciences, 2016; 13(3): 304-310.

7. Priyanka D, Sindhu S, Saba M. Design Development and Evaluation of Ibuprofen Loaded Nanosponges for Topical Application,International Journal of ChemTech Research, . 2018; 11(2): 218-227.

8. Manyam N, Kumar K, Budideti R, Mogili S, Formulation and *In vitro* evaluation of Nanosponge loaded Extended Release Tablets of Trimethoprim, UPI Journal of Pharmaceutical, Medical and Health Sciences, 2018; 1(1): 78-86.

9. Kamble M., Zaheer Z., Mokale S., Zainuddin R, Formulation Optimization and Biopharmaceutical Evaluation of Imatinib Mesylate Loaded β-Cyclodextrin Nanosponges, *Pharmaceutical Nanotechnology*, 2019;7:343–361.

10. Patil BS, Dr. Mohite SK,Formulation Design & Development of Artesunate Nanosponge. *European Journal* of *Pharmaceutical* and Medical *Research,* 2016; 3(5): 206-211.

11. Swaminathan S, Vavia PR, Trotta F, Torne S, Formulation of betacyclodextrin based nanosponges of itraconazole, The Journal of *Inclusion Phenomena and Macrocyclic Chemistry*, 2017; 57:89-94.

12. Shrishail MG, Kamalapurkar Ka, Thorat Ys, Pathan Ma, Preparation and in-Vitro Evaluation of Itraconazole Loaded Nanosponges for Topical Drug Delivery. Indo American Journal of Pharmaceutical Research,2019;9(04):1999-2013.

13. Caldera F., Tannous M., Cavalli R., Zanetti M., Trotta F, Evolution of Cyclodextrin Nanosponges, *International Journal of Pharmaceutics,* 017; 531:470–479.

14. Pushpalatha R., Selvamuthukumar S., Kilimozhi D. Cyclodextrin nanosponge based hydrogel for the transdermal co-delivery of curcumin and resveratrol: Development, optimization, in vitro and ex vivo evaluation, Journal of *Drug Delivery* Science and *Technology,* 2019;52:55–64.