

FORMULATION AND IN-VITRO EVALUATION OF SITAFORMIN INVASOMAL SOLUTION

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ABSTRACT- PCOS is a common disorder with long term effects on women's overall health, and has been an important research topic over the past six decades. The clinical features of PCOS are chronic anovulation, polycystic ovary, and excessive levels of androgens. Increased risk of type 2 diabetes mellitus (T2DM), cardiovascular disease, non-alcoholic fatty liver disease (NAFLD), obesity, anxiety, and depression, are also associated with PCOS. In addition, immune function in PCOS patients is impaired and inflammatory markers are increased. Therefore, the optimal treatment for comorbid conditions in patients with PCOS is debated. Research in recent years has revealed that insulin resistance plays a major role in the pathogenesis of PCOS. Therefore, there is a growing interest in the use of insulin sensitizers and T2DM drugs in the treatment of PCOS. Invasomes are new vesicles that contain terpene and have more penetration than traditional liposomes. These are soft liposomal vesicles that aid in penetration thanks to their high membrane fluidity and terpene content. Terpene and ethanol are distinguishing characteristics of Invasome. These vesicles have demonstrated the benefits of liposomes, which operate as potential carriers and promote terpene penetration, potential carriers and terpene penetration augmentation, and the capacity to alter the SC packing order, so facilitating skin distribution. The aim of this study was to establish and evaluate a thermosensitive invasomal solution containing metformin HCL and sitagliptin phosphate (Sitaformin) to improve drug entrapment, sustain drug release, and increase drug bioavailability. Sitagliptin phosphate and metformin HCL invasomes were prepared using the conventional thin-film evaporation method, with a 1:2 molar ratio between Sitaformin and phospholipid. The positive interaction between sitagliptin phosphate, metformin HCL, and phospholipids was confirmed using Fourier transform infrared spectroscopy (FTIR). The results suggest that developing a thermosensitive invasomal solution of metformin hydrochloride and sitagliptin phosphate with improved bioavailability and physical stability could enhance its effectiveness.

Keywords- Invasomes, Metformin Hydrochloride, Sitagliptin phosphate, Phospholipid- Soya Lecithin

How to cite this article: Tanuja Sharma¹ Vijay Sharma² Shubham Thakur³ Meenakshi Verma⁴ Anchal Thakur⁵ Pushpender Sharma⁶ Prof. (Dr.) Atul Kumar Gupta^{7*}. FORMULATION AND IN-VITRO EVALUATION OF SITAFORMIN INVASOMAL SOLUTION. Int J Drug Deliv Technol. 2026;16(84s):136-142. DOI: 10.25258/ijddt.16.84s.16.

1 Introduction:

A. Novel Drug Delivery Systems (NDDS): NDDS, or innovative drug delivery methods, are commonly used to address pharmaceutical bioavailability challenges. This refers to how quickly and efficiently a medication reaches the target tissue after administration [1]. Most new treatments today have low bioavailability, requiring higher doses because only a small portion of the administered dose

enters systemic circulation and reaches the intended target. This results in substantial medication wastage. The main objective of pharmaceutical technology is to enhance the solubility and permeability of drugs with low bioavailability [2]. Over the past few decades, NDDS technology has evolved into various drug delivery methods. Modern equipment, computational power, and mathematical models

have revolutionized the formulation process, transforming drug delivery from simple tablets to programmable, time-controlled smart systems. NDDS, such as liposomes, niosomes, invasomes, and others, prevent pre-systemic metabolism, which reduces the negative effects of drug accumulation and increases targeted tissue delivery, particularly in pediatric and elderly patients [3]. This helps protect against physical and chemical degradation by **improving** solubility, permeability, stability, and long-term administration [4].

B. Benefits of Novel Drug Delivery Technologies:

- Enhances drug efficacy.
- Facilitates distribution to target sites.
- Reduces toxicity and adverse effects.
- Provides treatment for diseases previously considered incurable.
- Potential use as a preventive measure.
- Reduces short- and long-term healthcare costs [5].

C. Invasome:

Invasome are new vesicles that contain terpene and have more penetration than traditional liposomes. Invasome are soft liposomal vesicles that aid in penetration thanks to their high membrane fluidity and terpene content. Terpene and ethanol are distinguishing characteristics of Invasome [6]. These soft vesicles have manifested the advantages of liposomes, that operate as potential carriers and promote terpene penetration, potential carriers and terpene penetration augmentation, and the capacity to alter the SC packing order, so facilitating skin distribution [7].

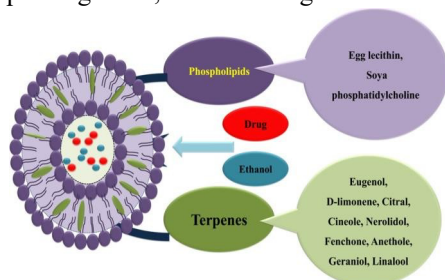


Fig.1 Composition of Invasome

D. PCOS (POLY-CYSTIC OVARY SYNDROME):

Over the past 60 years, PCOS, a frequent condition with long-term implications on women's general health has received significant research attention. Chronic anovulation, polycystic ovary, and high androgen levels are the clinical signs of PCOS. A higher risk of non-alcoholic fatty liver disease (NAFLD), obesity, anxiety, depression, and type 2 diabetes mellitus (T2DM) is also associated with PCOS [8]. Additionally, PCOS individuals have decreased immunological function and elevated inflammatory markers. As a result, there is disagreement on the best course of action for PCOS patients with comorbid illnesses. Recent studies have shown that a key factor in an etiology of PCOS is insulin resistance. As a result, there is increasing interest in using T2DM and insulin sensitizer medications to treat PCOS. [9]

E. Artificial Intelligence (AI) in Drug Formulation and Design: AI has become a potent instrument in many fields, and the discovery and formulation of drugs is no different. An overview of AI's uses in drug formulation and development is intended to be given in this review article, along with an examination of its potential future developments. The article starts off by outlining the basic ideas of artificial intelligence (AI), such as machine learning, deep learning, and artificial neural networks, as well as how they apply to the pharmaceutical sector. The article also covers the network and tools of artificial intelligence (AI) and its uses in the pharmaceutical development process, covering a number of topics such drug delivery, clinical trial management, quality control, manufacturing, and drug discovery [10].

F. AI in Drug Delivery Systems Based on Nanoparticles: These days, artificial intelligence (AI) has such a significant impact on the medical field that it is necessary to enhance AI, a drug based on nanotechnology, and its delivery method while working with nanomedicine, including the domains of cancer cell, biomedicine, and nanobiology [11]. In order to demonstrate what artificial intelligence is and how it functions, this chapter specifically focuses on its description. The field of nanotechnology and nanoscience is then explained. Lastly, it links them to the delivery of a medicine delivery system based on nano-technology [12]. The International Labor Organization (ILO) estimates that there are casual rag pickers globally around 15–20 million who get extremely low wages and frequently live below the poverty line. The UN Inter-Agency Task Force on Social and Solidarity Economy (2019) claims that. Rag picking occurs at every level of the waste management process, which involves source separation at the home or other location where garbage is produced [13]. Here, resources are recovered from garbage to meet demands and are either sold, given away, or reused. For example, discarded newspapers are utilized for packaging [14].

Table1. Literature Distribution of Across Major Continents (June 30th 2022).

Major Continents	Countries	Number of studies
South Africa	Brazil, Colombia, Nicaragua, Paraguay	Thirty (22.39%)
Asia	Bangladesh, India, Indonesia, Iran, Thailand, Sri Lanka, Nepal, Pakistan, Palestine, South Korea,	Sixty-Nine (51.5%)
Africa	Zimbabwe, South Africa, Cameroon, Ethiopia, Ghana, Malawi, Nigeria, and	Thirty - two (23.88%)

	Botswana	
Europe	Denmark, UK	Three (2.23%)

THE DETAILS HAVE BEEN COLLECTED IN STUDY DOMAIN INTO SUBGROUPS. THE 25 COUNTRIES HAVING STUDY AREA IN THE DATABASE OF LITERATURE ARE DEMONSTRATED BY THE PUBLISHED ARTICLE NUMBERS. 30% RESEARCH STUDIES HAVE BEEN DONE INDIA COMPARE TO THE ON RELATED TOPICS IN ANOTHER COUNTRIES THE INDIA HAS LEADING NATION [15].

II OBJECTIVES OF PRESENT STUDY

- To enhance drug entrapment
- To sustain the release of drug
- To improve drug solubility

III MATERIALS AND METHODS

A. Material: Metformin Hydrochloride and Sitagliptin Phosphate were obtained from Ipca Laboratories Ltd., Aurangabad and Lee Pharma Ltd., Andhra Pradesh. Phospholipid 90G and absolute methanol were bought from Lipidome Lifesciences, Ahmadabad and Fisher Scientific, India PVT. Ltd.

B. MTF-HCL and STF Invasomal Preparation

Conventional thin film evaporation method was utilized for the formulation and preparation of Sitaformin Invasome. In brief, MTF-STF and terpene were added to a methanol: chloroform 2:1(v/v) with combination of Soya phospholipid 90G and to obtain a clear solution, vortex for five minutes and sonicate for five minutes [16].

In the prepared mixture Using a syringe, a fine stream of distilled water (up to 10% V/V) was continuously vortexed. And continued vertexing for 5 minutes we obtained the final invasomal formulation (Table 2) [17].

Table2. Different Composition and Formulation of Invasomal Solutions

<i>S r . N o .</i>	<i>Form ulation n code</i>	<i>Metf ormi n HCL (mg)</i>	<i>Sita glipt in Pho spha te (mg)</i>	<i>Phosp holipi d 90G(% W/V)</i>	<i>Ter pen e (%V /V)</i>	<i>10 % Et ha nol (% V/ V) in SV F Bu ffe</i>
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						<i>r</i>
1	F1	500	50	5	1	10
2	F2	500	50	6	1	10
3	F3	500	50	7	1	10
4	F4	500	50	8	1	10
5	F5	500	50	9	1.5	15
6	F6	500	50	10	1.5	15
7	F7	500	50	11	0.5	15

C. Characterization of Loaded STF-MTF Invasomal Solution

a. Entrapment Efficiency

Determining the entrapment efficacy of the invasomal formulations the Ultracentrifugation method was used. The invasomal solution was ultra centrifuge at 15000 rpm for 15 minutes. Then, collected the sediment materials and diluted with ethanol [18]. for analyzing the sitaformin content the UV spectrophotometry was employed to analyze the 426 nm wavelength. The calculations were used to get the entrapment efficiency % [19].

% Efficiency of entrapment = Entrapped drug recovered amount/total amount of drug $\times$ 100 [20]

b. Determination of Size of Particles

The size of the vesicles was ascertained using the Zeta Sizer (Nano- ZS, Malvern, U.K.). Every determination was made three times [21].

c. Shape of the vesicles and morphology

Using a 100KV accelerating voltage, a Transmission Electron Microscope (TEM) was used to visualize the enhanced intracellular solution. Negative staining was applied to the samples. Samples were negatively stained with a 1% aqueous solution of phosphor-tungstic acid [22]. For staining, the invasomal mixture was dried on a tiny-coated grid. To eliminate the excess solution, blotting was employed. After the material had dried, it was inspected under a microscope. [23]

IV. RESULT AND DISCUSSION

A. Visual Appearance

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Appearance of invasome: The appearance of invasome were shown in formulation(F1) to (F7) were shown in table below

Table 3: Appearance of different invasome formulation contain Sitaformin

Sr.NO	Formulation Code	Appearance
1.	F1	Translucent Formed
2.	F2	Translucent Formed
3.	F3	Milky Formed
4.	F4	Translucent Formed
5.	F5	Milky Formed
6.	F6	Milky Formed



Fig 2: Sitaformin Invasome

Discussion: From the Table 3 and Fig 2 (F1 &F2) were selected for further evaluation due to their milky white.

B. Optical Microscopy: Optical microscopy of drug loaded invasome formulation was determined by optical microscopy at 100x magnification and result was in Fig 3.

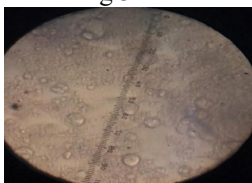


Fig 3. Optical microscopy of invasome

Discussion: Uniform, regular and spherical vesicles were observed in optical microscopic view.

C. pH : The pH of all formulation was given in a Table 4.

Table 4: pH of Different Invasome Formulation Containing MTF-STF

Sr.NO.	Formulation Code	pH (Mean $\pm$ SD) (N=7)
1.	F1	4.28 $\pm$ 0.02
2.	F2	4.34 $\pm$ 0.03
3.	F3	4.62 $\pm$ 0.01

4.	F4	4.66 $\pm$ 0.03
5.	F5	4.23 $\pm$ 0.02
6.	F6	4.20 $\pm$ 0.04
7.	F7	4.24 $\pm$ 0.02

Discussion: From the Table. 4 it was found that pH of all formulation was found to be in a range 4.20 $\pm$ 0.04 to 4.66 $\pm$ 0.03.

D. Entrapment efficiency: Percentage drug entrapment of all formulation was given in a Table 5:

Table 5: Percentage Entrapment Efficiency of Different Invasome Formulation Containing MTF-STF

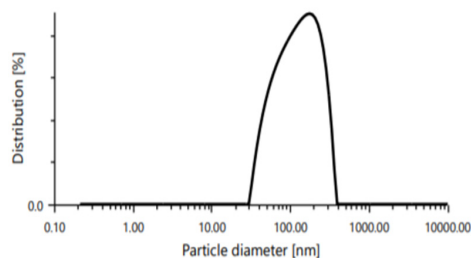
Sr. No.	Formulation code	%Entrapment Efficiency of Metformin Hydrochloride (Mean $\pm$ SD)	%Entrapment Efficiency of Sitagliptin Phosphate (Mean $\pm$ SD)
1	F1	47.59 $\pm$ 0.19	49.03 $\pm$ 0.31
2	F2	52.49 $\pm$ 0.16	47.52 $\pm$ 0.41
3	F3	58.18 $\pm$ 0.09	59.54 $\pm$ 0.15
4	F4	68.24 $\pm$ 0.09	68.70 $\pm$ 0.15
5	F5	80.86 $\pm$ 0.09	81.85 $\pm$ 0.21
6	F6	96.44 $\pm$ 0.01	96.59 $\pm$ 0.02
7	F7	31.62 $\pm$ 0.09	37.98 $\pm$ 0.15

Fig 4: Percentage Drug Entrapment of Invasomal Formulations Containing MTF-STF.

Discussion: From the Table 5 and Fig.4, it was found that Percentage drug entrapment of all formulation was found to be in a range 37.98 $\pm$ 0.15 to 96.59 $\pm$ 0.02. These outcomes explain significant result on percent entrapment efficiency of invasome was observed with lipid concentration. The percentage drug content was found maximum in F6 formulation compared to others as shown in Table 5 and Fig.5 On the basis of percent drug entrapment, F6 was selected as optimized formulation. Thus, it has been carried further for in vitro evaluation of release study.

E. Zeta Potential and Particle Size Determination: The particle size of invasomal solution was shown in figure no.6

Fig6: Particle size peak of invasomal formulation (F6)



Discussion: Fig 6: demonstrated particle size of invasome formulation was 129.57 nm with PDI 26.0%.

F. Transmission electronic microscopy (TEM): The TEM of invasome was shown below fig no.7

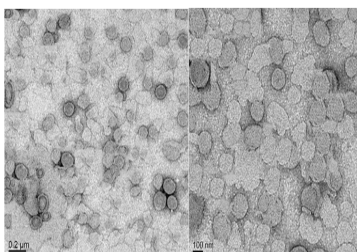


Fig 7: TEM Image of Formulation F6

Discussion: Morphological examination of the optimized Sitaformin loaded invasome was carried out using transmission electron microscope (TEM). As shown in Fig.7 the developed invasomal solution were showed the medication included within the lipid matrix in Figure 7. They display recognized spherical vesicles with distribution in uniform size.

G. FTIR Of Sitagliptin +Metformin: The FTIR study of combination of both drug were shown in fig no.8

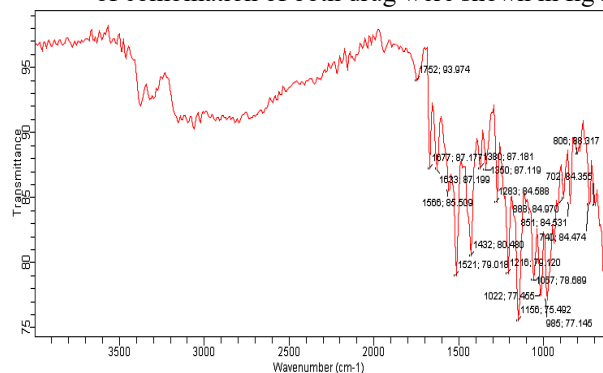


Fig 8. FTIR spectrum of STF-MTF

H. FTIR of MTF-STF-Phospholipid (soya lecithin): The FTIR study of MTF-STF and soya lecithin were shown in fig no.9

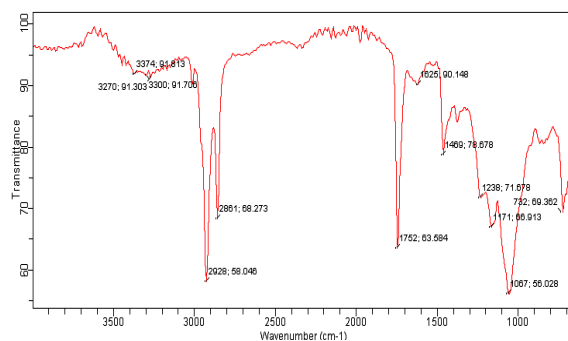


Fig. 9 FTIR spectrum of STF-MTF and Soya Lecithin

I. FTIR of MTF-STF- Carbopol 934: The FTIR study of MTF-STF and Carbopol 934 were shown in fig no. 10

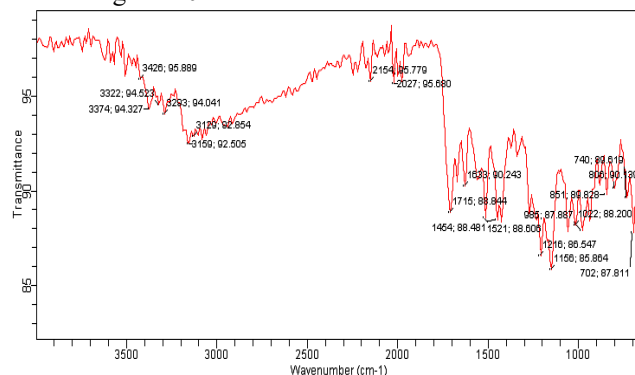


Fig. 10 FTIR spectrum of STF-MTF and Carbopol 934

Table 6: FTIR interpretation data

Sr.NO	Observed (cm-1)	Stretching
1.	1669.16	C-H Stretching
2.	1633.85	C-C Stretching
3.	1555.78	C=O Stretching
4.	1513.94	C=N Stretching
5.	1451.09	N-H Stretching
6.	1426.28	C-H Stretching
7.	1370.58	C-C Stretching
8.	1206.99	C=O Stretching
9.	1052.14	C=N Stretching
10.	977.6	N-H Stretching
11.	880.54	C-H Stretching
12.	780.49	C-C Stretching

V. Conclusion

PCOS, or polycystic ovarian syndrome, is a complex and multifaceted disease, therapy has been the subject of much study. A newer treatment strategy is the use of invasomal

solutions. Vesicular carriers called invasomes are intended to improve medication administration via the skin, possibly providing a non-invasive substitute for conventional therapeutic approaches. Increased medication administration, better patient adherence, focused treatment, fewer adverse effects, and synergistic outcomes. Nowadays, the medical profession is so affected by artificial intelligence (AI) that it is important to improve AI, a drug based on nanotechnology. An exciting development in PCOS therapy is the use of intrusive solutions, which provide a tailored, non-invasive, and patient-friendly substitute for conventional treatments. Their potential to lessen side effects, increase patient compliance, improve medication administration, and potentially have synergistic effects makes them an invaluable addition to the therapeutic toolbox for treating PCOS. To properly determine their effectiveness and safety profile in the therapy of PCOS, more clinical research and long-term assessments are necessary.

VI. Acknowledgement

I would like to sincerely thank everyone who helped to ensure the successful conclusion of this research project. First of all, we thank [Bahra University] for giving the required facilities to govern this work. Our deepest appreciation goes to [Ms. Vibha Sharma], whose guidance, support, and encouragement have been invaluable throughout this study. We are particularly thankful to Lab Technicians for their technical assistance in the laboratory, which was crucial for the in vitro evaluation of the invasomal solution.

And also, we thank our families and friends for their unwavering support, guidance and during the course of this research.

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