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PREPARATION AND EVALUATION OF NANOPARTICLE GEL USED IN PSORIASIS TREATMENT

Siddiqua Habeeb*[1], Md. Hamza Habeeb[2] and Bhargav Bhongiri[3]

[1]Lecturer in Pharmacy, Government Polytechnic for Women Nizamabad.
[2]Assistant Professor, MESCO College of Pharmacy, Karwan, Hyderabad, Telangana.
[3]Associate Director, Synpharma Research Lab, Dilsukhnagar, Hyderabad, Telangana.

***Corresponding Author: Siddiqua Habeeb**
Lecturer in Pharmacy, Government Polytechnic for Women Nizamabad.

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ABSTRACT
The objective of the present study is to formulate and evaluate Nano particulate gel containing methotrexate. It is used in the treatment of psoriasis. Methotrexate nanoparticles were formulated by the nanoprecipitation technique and evaluated for particle size, drug entrapment efficiency, SEM analysis, and FTIR studies. Optimized formulation of methotrexate nanoparticles further converted into the gel using Carbopol 934. Methotrexate loaded nanoparticles showed a spherical shape with smooth surface. In vitro anti-psoriatic study suggested that the developed methotrexate Nano particulate gel formulation exhibits improved dermal delivery of methotrexate and also nanoparticles had a superior effect on cell growth as compared to the free methotrexate. Pharmacokinetic study established that the amount of methotrexate reaching the viable layer is slightly high for Nano particulate gel as compared to free methotrexate. Thus, methotrexate loaded Nano particulate gel formulation might be the potential delivery system for the treatment of psoriasis.

KEYWORDS: Methotrexate, FTIR Studies, Precipitation method, Nanoparticles, Carbopol 934, In vitro drug release studies.

INTRODUCTION
Nanoparticle technology offers a series of advantages for drug delivery, including high loading yield, combination therapy, controlled release, prolonged circulation, and targeted delivery.[1] As a result, a myriad of nanoparticle-based drug delivery systems have been developed to improve therapeutic index of drugs by altering their pharmacokinetics and biodistribution profiles, resulting in nanomedicines for clinical treatment of various diseases.[2] NP-gel formulation has also been used to directly modulate nanoparticle release with varying matrix porosity (i.e. the fraction of void space in the polymer network)[3] Nanoparticles can be embedded into hydrogel network by mixing with monomer solution, followed by gelation (i.e., nanocomposite hydrogel, Figure 1A). Alternatively, they can be incorporated into gel matrix after gel formation by allowing the gel network to swell and 'breath in' nanoparticles for entrapment, a method especially useful when nanoparticles interfere with the gelation process. Furthermore, inorganic nanoparticles are often grown in situ within gel matrix by loading nanoparticle precursors into a gel first, followed by reduction reactions for nanoparticle formation.[4] Psoriasis confers significant physical and psychological distress and impairment usually resulting in a detrimental impact on patient quality of life. The exact cause of the origin of the disease is unknown, but it is considered as a multifactorial disorder associated with an overexpression of proinflammatory chemokines and cytokines produced by Th1 cells.[5] Methotrexate is one of the oldest and highly efficacious antineoplastic drugs; inhibit dihydrofolate reductase, blocking the conversion of dihydrofolic acid to tetrahydrofolic acid which is an essential coenzyme required for one carbon transfer reactions in denovo purine synthesis and amino acid interconversion.[6] Methotrexate is used for various certain malignancies, autoimmune diseases such as rheumatoid arthritis and psoriasis.[7]

MATERIALS
Methotrexate was obtained from Hetero lab, HYD. Eudragit and Carbopol 934 were procured from Synpharma Research Labs, Hyderabad, and other chemicals, and the reagents used were of analytical grade.

METHODOLOGY

Fourier-transform infrared spectroscopy analysis

The Fourier-transform infrared spectroscopy (FTIR) spectra of pure and Methotrexate loaded nanoparticles were obtained by a computerized FTIR spectroscopy (Shimadzu 8400s FTIR spectrometer, Japan) operating in the scanning wave number range of 4000 and 700 cm-1.[8]

Preparation of Methotrexate nanoparticles

Methotrexate nanoparticles were prepared through a nanoprecipitation method using the various polymer concentrations. Eudragit L100 and methotrexate were dissolved in methanol. The mixture formed was added drop-wise into the aqueous solution of polyvinyl alcohol (PVA) as a surfactant and was magnetically stirred at 500 rpm for 180 min for complete removal of organic solvent. The hardened methotrexate nanoparticles were recovered by centrifugation at 15000 rpm, washed repeatedly with deionized water, collected, and dried at 40°C for 1 h.[9]

Prepared methotrexate nanoparticles incorporated gels

For the preparation of methotrexate nanoparticles containing gel, the required quantity of Carbopol 934 was taken in enough quantity of water for 24 h. After that 0.5 mL of methyl paraben (0.5%w/v) added to the hydrated gel and stirred for about 4 h. The gel was neutralized by the addition of triethanolamine drop by drop until a clear transparent gel formed. Then Methotrexate loaded nanoparticles (F6) were incorporated in to gel with mechanical mixing.[10]

Table 1: Formulation development.

F. Code	Drug	Eudragit(mg)	Poly vinyl alcohol (%)
F1	25	100	0.25
F2	25	200	0.25
F3	25	300	0.25
F4	25	400	0.25
F5	25	100	0.5
F6	25	200	0.5
F7	25	300	0.5
F8	25	400	0.5

CHARACTERIZATION

Particle Size and Zeta Potential

The particle size and particle size distribution of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with distilled water. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern Instruments, UK). Samples were prepared by diluting with distilled water.[11]

Scanning electron microscope (SEM) Analysis

The surface morphological study of Methotrexate loaded nanoparticles were done with scanning electron microscopy (Model JSM 5610 LV SEM, Japan). The Scanning electron microscopy of methotrexate loaded nanoparticles gel showed relatively smooth surface nanoparticles distributed uniformly and have spherical shapes.[12]

Encapsulation efficiency and drug loading

The encapsulation efficiency (% EE) and drug loading of prepared methotrexate loaded nanoparticles gel were determined by dissolving a known quantity of nanoparticles in 10 mL of methanol under sonication for 1 hr. The samples were then filtered through a membrane filter and analysed at 249 nm using high-performance liquid chromatography (HPLC) (Shimadzu LC10 AD). The % EE and the loading of methotrexate into nanoparticles gel were determined according to the following equations.[13]

DEE = (Total Drug conc. – Supernatant Drug conc.)/ (Total Drug conc.) x 100%

In-vitro diffusion study

The diffusion studies were performed by applying 1 g of the gel uniformly to the dialysis membrane. The membrane was mounted between the compartments of Franz diffusion cell. Reservoir compartment was filled with 15 mL of 6.8 pH phosphate buffer. The study was carried out at (37±2) °C and was carried out for 24 h. The sample (1 mL) was withdrawn from reservoir compartment in successive intervals. Each time reservoir compartment was replenished with 1 mL of 6.8 pH phosphate buffer solution to maintain sink condition.[14]

Release kinetic study

Data obtained from in-vitro release studies of the nanoparticles from various gel formulations were fitted to various kinetic equations such as zero order, First order, Higuchi model and Korsmeyer-Peppas model.[15]

RESULTS AND DISCUSSION

FT-IR Spectrum of Methotrexate

FT-IR Spectra of drug and polymers were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between drug and polymers. It also confirmed that the stability of drug during process.

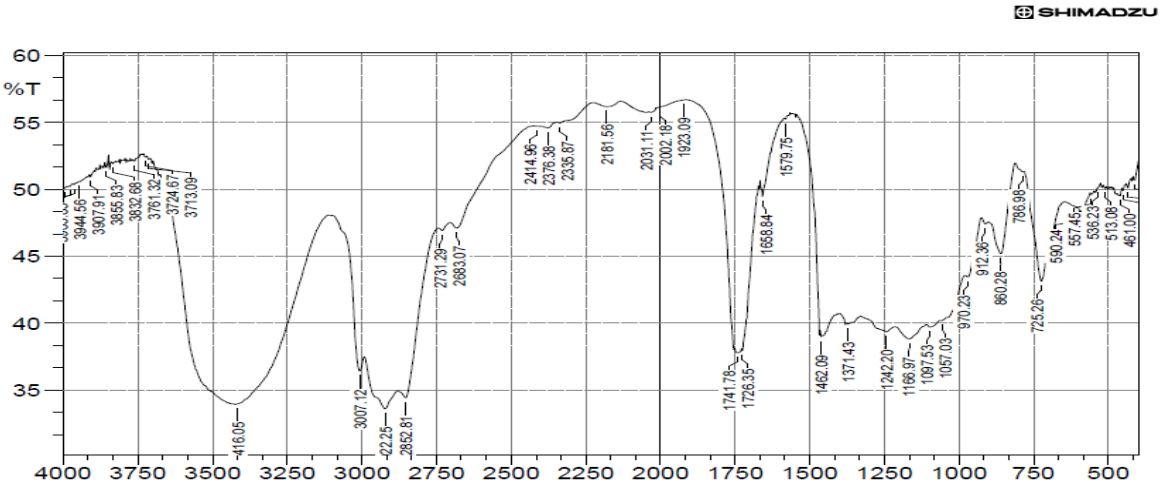

Fig-1: FT-IR Sample for Methotrexate.

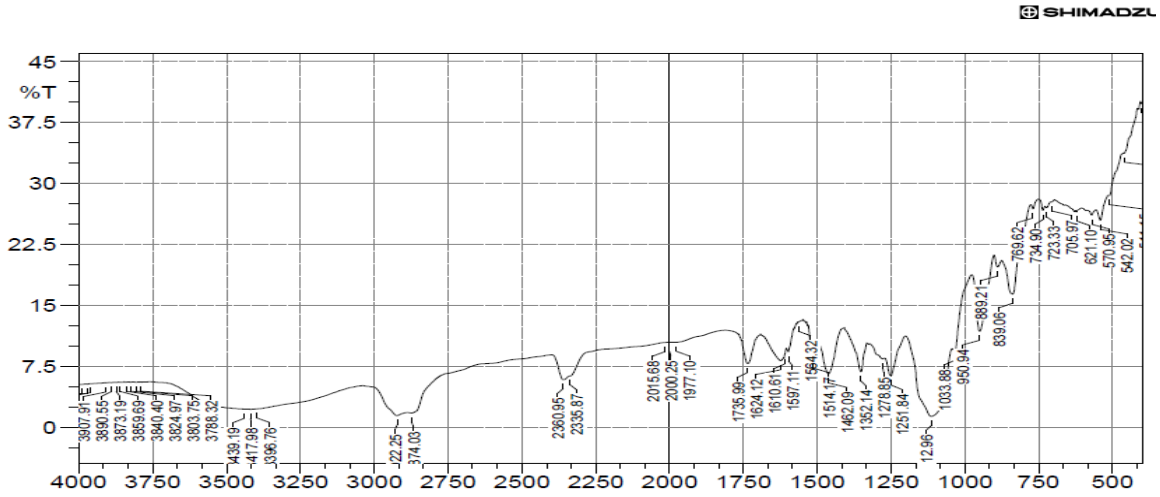

Fig-2: FT-IR Sample for physical mixture of drug and excipients.

Zeta potential

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized nanoparticle gel formulation was measured and found to -38.01 mv. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable.

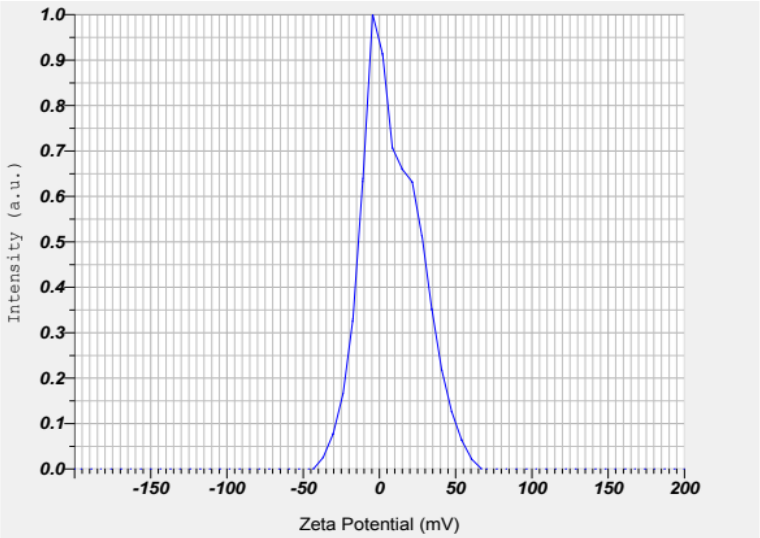

Fig-3: Zeta potential of Optimized formulation.

Table 2: Evaluation Studies of Zeta potential Nano sponges.

F. no	Zeta Potential(mV)
F1	-32.54
F2	-31.58
F3	-35.49
F4	-35.84
F5	-37.89
F6	-38.01
F7	-34.90
F8	-38.72

Particle size

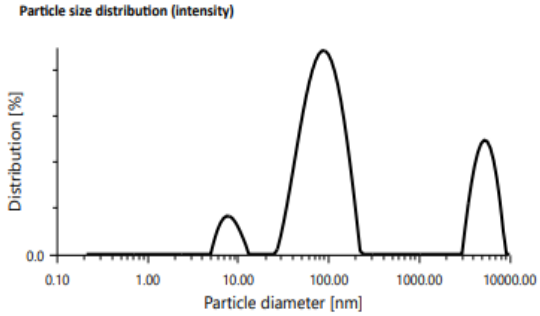

Fig-4: Particle size of optimized formulations.

In general, particle size was with a diameter of < 140 nm. The surfaces of the Nanoparticle were smooth.

Characterization of nanoparticle

Table 3: Evaluation Studies of particle size Nanoparticle.

F. no	Particle size (nm)
F1	144
F2	152
F3	163
F4	141
F5	143
F6	140
F7	156
F8	151

Entrapment efficiency

The drug entrapment efficiency of all 8 formulations was evaluated. From the F6 formulation showed maximum drug entrapment efficiency 83.69 compared to other formulations. The zeta potential or the change on the surface of colloidal particles in methotrexate nanoparticles was measured by electrophoretic light scattering mode using zetasizer Nano ZS. The particle charge of methotrexate nanoparticles were quantified at 25^{o} C. The samples were diluted approximately with the deionized water for the measurements of particle size.

Table 4: Evaluation Studies of Entrapment efficiency Nanoparticles.

F. no	Entrapment Efficiency (%)
F1	82.69
F2	81.98
F3	78.14
F4	82.47
F5	79.83
F6	83.69
F7	82.51
F8	80.93

SEM Analysis

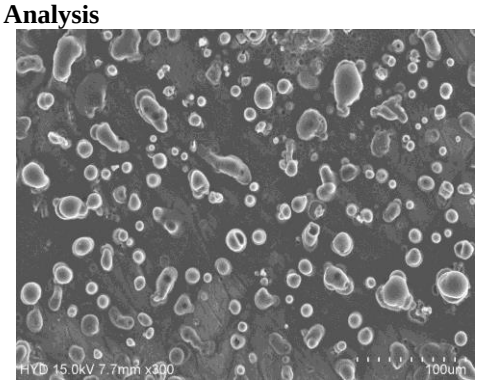

Fig-5: SEM Analysis of Optimized Formulation.

Characterization of Methotrexate Nano particulate gel

pH

The pH of Methotrexate loaded nanoparticulate gel was found to be 6.7.

Drug content

Drug content was found to be 96.82%. The spread ability of Methotrexate loaded nanoparticulate gel were 45.26 cm.

In vitro release study

Table 5: In vitro drug release studies of all formulations.

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	24.58	24.96	27.84	25.64	22.79	24.85	24.96	23.46
2	32.69	34.78	35.98	34.87	36.88	36.59	34.17	33.58
3	47.89	46.85	45.25	43.98	42.58	46.95	45.91	43.78
4	59.28	58.64	55.58	51.25	53.98	57.84	54.58	59.86
6	69.86	68.98	67.98	64.82	65.98	69.85	74.28	75.89
8	75.89	74.28	72.19	73.69	75.86	74.96	75.86	72.54
10	81.58	82.25	80.15	83.56	80.19	85.79	84.96	82.54
12	93.56	95.82	93.54	92.96	94.78	96.89	95.15	93.65

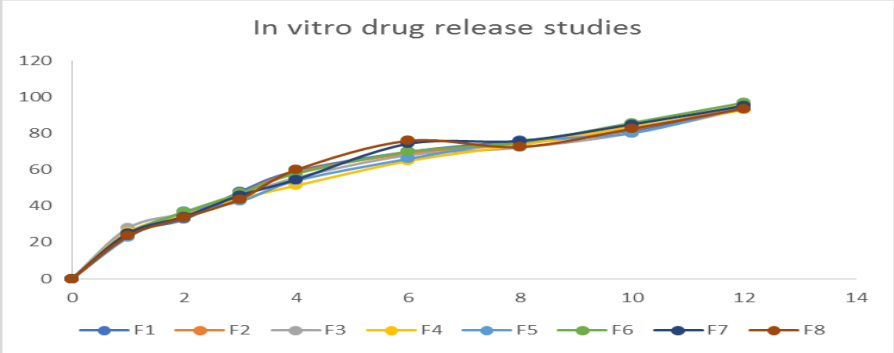

Fig-6: In vitro drug release studies of all formulations.

Drug release kinetics

Table 6: Drug release kinetics of optimized formulations.

Time (hrs)	%CDR	SQARE T	LOG T	LOG%CDR	ARA	LOG%ARA
0	0	0	0	0	0	0
1	24.85	1.000	0.000	1.395	75.150	1.876
2	36.59	1.414	0.301	1.563	63.410	1.802
3	46.95	1.732	0.477	1.672	53.050	1.725
4	57.84	2.000	0.602	1.762	42.160	1.625
6	69.85	2.449	0.778	1.844	30.150	1.479
8	74.96	2.828	0.903	1.875	25.040	1.399
10	85.79	3.162	1.000	1.933	14.210	1.153
12	96.89	3.464	1.079	1.986	3.110	0.493

Zero order kinetics

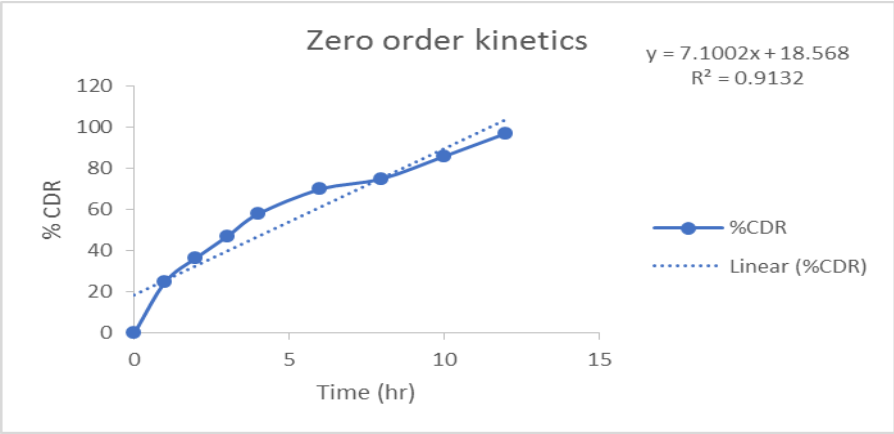

Fig-7: Zero order kinetics of optimized formulation.

First order kinetics

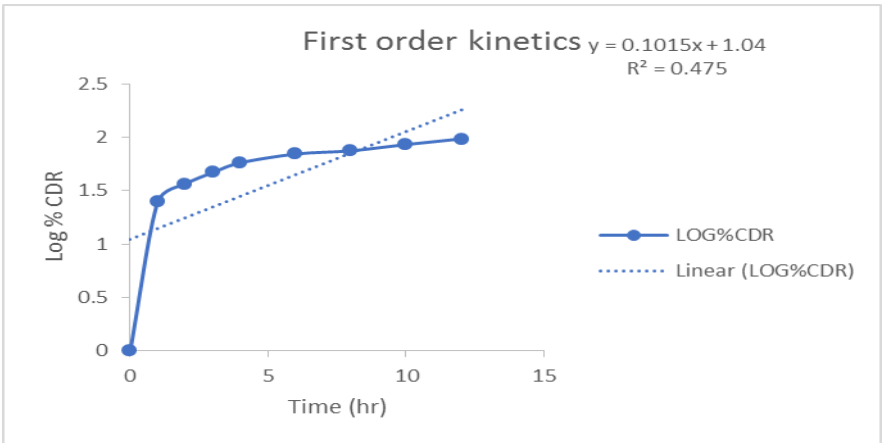

Fig-8: First order kinetics of optimized formulation.

Higuchi model

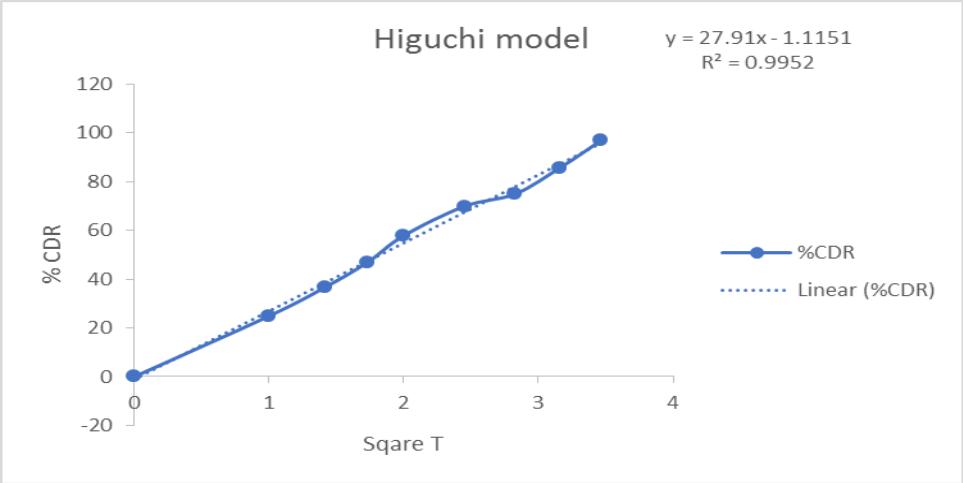

Fig-9: Higuchi model of optimized formulation.

Korsmeyer peppas

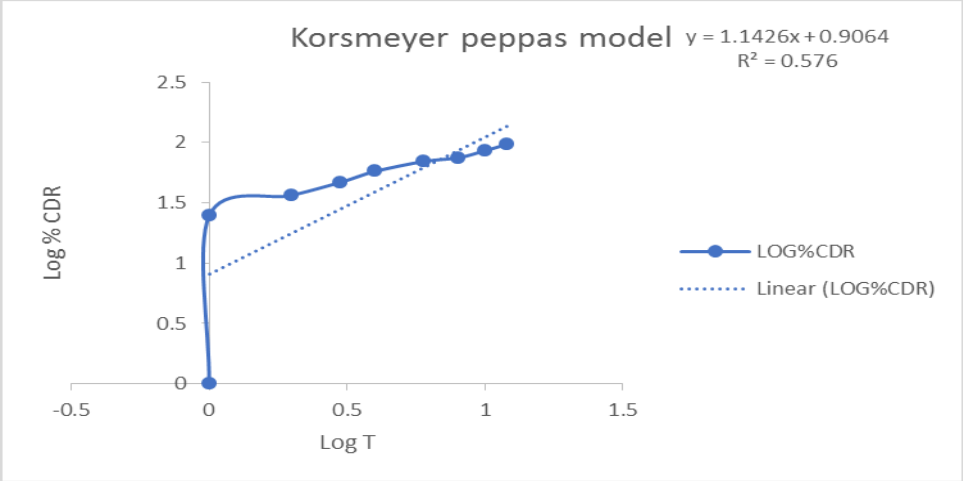

Fig-10: Korsmeyer peppas of optimized formulation.

CONCLUSION

Methotrexate nanoparticles were successfully formulated using a modified nanoprecipitation technique, and nanoparticles converted into the gel by using Carbopol 934 as a gelling agent. Physicochemical characterization including particle size, Zeta potential, scanning electron microscopy, and in-vitro release profile were carried out. In-vitro drug release pattern of nanoparticle gel showed fast and control release. Immediate releases as well as sustained release both are of interest for topical application. Immediate release can be useful to improve the penetration of drug & maintain the concentration work as loading dose, while sustained release supplied the drug over a prolonged period of time. The developed methotrexate nanoparticle gel formulation improved dermal delivery of methotrexate and exhibits enhanced in vitro anti-psoriatic efficacy.

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