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Original Article

Preparation and Evaluation of Nanogel Attached Through Methotrexate for Managing Psoriasis

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ABSTRACT

There is novel preparation of nanoparticle for targeted delivery of methotrexate. The drug of choice for the treatment of Psoriasis. We find very enchanted result of the prepared formulation that is very high stable and very less toxic as earlier pure methotrexate and our study reveals that the dose reduction and targeted therapy have very great importance in managing psoriasis. The stability was good as we find from the study and nanogel was followed sustained release pattern of the nanogel with good spreadability and intrinsic homogeneity. Moreover, if we discuss about the various parameters performed in the research such as particle size and zeta potential then these both of the tests give the information about better ADME and confirm the delivery potential respectively. Entrapment efficiency is needed to know the exact amount of drug contained or engulfed by the nanoparticle. The current research focused on the nano formulation to treat the Psoriasis for better drug release with less toxicity.

Keywords: Psoriasis, Methotrexate, chitosan, Optimization, Nanoparticles.

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INTRODUCTION

Psoriasis is a hyper proliferative, autoimmune skin disorder characterized by patches of abnormal skin. It is a common chronic inflammatory disease of the skin that is increasingly being recognized as a systemic inflammatory disorder. Psoriatic arthritis is a well-known comorbidity of psoriasis [1]. A rapidly expanding body of literature in various populations and settings supports additional associations between psoriasis and cardiovascular\diseases, gastrointestinal diseases, kidney disease, malignancy, infection, and mood disorders. The pathogenesis of comorbid disease in patients with psoriasis remains unknown; however, shared inflammatory pathways, cellular mediators, genetic susceptibility, and common risk factors are hypothesized to be contributing elements. As additional psoriasis comorbidities continue to emerge, education of health care providers is essential to ensuring comprehensive medical care for patients with psoriasis [2].

Aim of Research

- Preparation of Nanogel of optimized nano size to get better bioavailability.
- Characterization of the prepared formulation to evaluate its parameters.
- Invitro release of the drug from nanogel to confirm the pattern of treatment.
- Evaluate the toxicity of the formulation for its safety issue.

METHODOLOGY

Ion gelation technique was employed for the fabrication of blank nanogel and BCNL with the help of Pluronic 127.The blank Chitosan nanogel (CNGL) 0.4% wt/vol of chitosan was dissolved in 1% v/v aqueous acetic acid solution followed by the drop wise addition of 0.4% Sodium tripolyphosphate solution (TPP) at the rate of 2 ml/min.17 The resulting particle dispersion so obtained were processed using probe sonicator (S-4000; Misonix, Farmingdale, NY) at medium amplitude (50%) for 5 min to obtain CNGL of size less than 100 nm. To the above aqueous chitosan particles dispersed in 22% wt/vol Pluronic 127 was added and stirred by using magnetic stirrer (REMI) for about 2 h at 1000 rpm for the final synthesis of biodegradable CNGL[3].

Preparation of blank chitosan nanoparticle: Chitosan nanoparticles were prepared by using ion gelation technique. A requisite quantity of 0.4%(wt/vol) of chitosan (5mg, 10mg and 15mg) was dissolved in 1% aqueous acetic acid (50ml) under magnetic stirring followed by drop wise addition of 0.4%(wt/vol) Sodium tripolyphosphate solution(TPP) at the rate of 2ml/min under magnetic stirrer (REMI) for about 2h at 1000 rpm. The particle dispersion proceeds using probe sonicator (S-4000; sonicator name) and obtain chitosan nanogel particles size less than 100nm[4,5].

Optimization of chitosan Nanoparticles: There are various parameters which affect the preparation, properties and stability of nanoparticles. These formulation parameters were identified and optimized to get uniform preparation and highest encapsulation efficiency. Different types of variables studies include concentration of polymer, amount of drug, surfactant concentration, and process variables include stirring speed, stirring time and sonication time [6]. All these parameters were optimized by taking effect on particle size, entrapment efficiency and zeta potential.

Characterization of nanoparticle: The optimized formulation chitosan nanoparticles (CNP) were selected and characterized for various attributes viz, size, shape, zeta potential, entrapment efficiency, hemolytic toxicity and stability study.

Particle size and zeta potential determination: The average particle size, zeta potential and poly dispersity index (PDI) of chitosan nanoparticles were determined by a Zetasizer (DTS ver.4.10, Malvern Instrument, England).Briefly nanoparticle sample dispersion was added in polystyrene cuvettes diluted with ultra-pure deionized waterand analyzed at a 90^{o} fixed angle.The Zeta potential of NPs formulation was measured by determining electrophoresis mobility with a laser based multiples angle particle electrophoresis analyzers, Malvern Zetasizer (DTS Ver.4.10,Malvern Instruments, England).

The nanoparticles were suspended in ultra-pure de-ionized water kept in an electrophoresis cell with an electric field of 15.24V/cm and the zeta potential were measured.

Estimation of drug entrapment efficiency: The drug entrapment efficiency EE of MTX in NLC was determined by an indirect method. Briefly, the NLC suspension was centrifuged at 10000 rpm for 10 min at 4 C. The obtained supernatant was diluted with methanol and the amount of the free MTX present in the supernatant was quantified using UV method.
The EE was calculated using the following formula:
EE = Amount of MTX added in formulation - Amount present in supernatant/Amount of MTX added in formulation 100

In-vitro release study: In vitro release of methotrexate from chitosan particles the release

characteristics of methotrexate from chitosan particles were studied by using dialysis tube. 5ml methotrexate loaded chitosan nanoparticles was placed into dialysis bag of MWCO 1200KD_a (Himedia, Mumbai India), tied at both the ends are placed in a beaker containing (100ml) of phosphate buffer (pH ~ 7.4). The beaker was placed over a magnetic stirrer at 1000rpm and the temperature maintained at 37±1^oC throughout the procedure. At specific time intervals, the dissolved medium (5ml) was taken and was replaced with an equivalent volume of fresh buffer solution and sink condition was maintained. The amount of released methotrexate in the sample was analyzed by a spectrophotometer over wavelengths ranging from 280- 300 nm.

Evaluation parameter of Methotrexate Nanogel

Homogeneity: The prepared nanogels were visually inspected for clarity; color and transparency. The prepared nanogels were evaluated for the presence of any particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

Drug content: Methotrexate loaded nanoparticle were mixed with Phosphate buffer (7.4) and sonicated for 10 min to obtain a clear solution and filtered. Concentrations of Methotrexate were determined by UV at λmax 300nm. Calibration curve of chitosan nanoparticle in nanogel was performed in phosphate buffer (7.4). The calibration curve was found to be linear in the concentration range of 5-30 µg/ml having coefficient of regression value R^2 = 0.899 and line equation, y = 0.052x + 0.356.

Spreadability: Spreadibility of the chitosan nanogel determined by using two glass slides of known standard dimensions. Formulation whose spreadability to be determined was place on one slide and then other slide was kept over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present and the adhering gel was whipped off. The two slides were placed onto a stand such that only the lower slide is held firm by the opposite fangs of the clamp clips and allow the upper slide to slip freely over it by the force of 20gm weight to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using the formula. Result shown on Table 3.11 and fig 3.15.

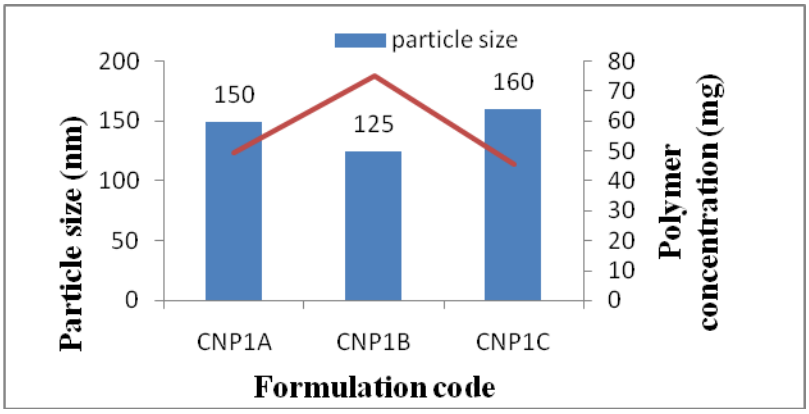
Spreadability (S) = M X L/T
Where,
S= is the spreadability,
M= is the weight in the pan (tied to the upper slide),
L= is length moved by the glass slide,
T= represents the time taken to separate the slide completely from each other.

Stability studies: The stability study confirms and gives evidence that how the quality of the formulation varies with the change in the external environment and how the environmental factors affect the formulation such as temperature, humidity and effect of light in most of the cases. There is main factor of stability study is of aged product because of its more pharmaceutical significance. Therefore, accelerated stability study is preferred and it was performed for pure and prepared formulations. These formulations were taken in the borosilicate glass vials and vials were stored in different temperature conditions such as in refrigerator at 4 °C, room temperature and in dark room at 37 °C for 90 days and the samples were evaluated by the ultraviolet spectrophotometry and the effect of change due to storage was noted as percent drug residual content in the nanoparticle formulation. While the initial drug amount considered as 100 percent.

RESULTS

Table 1. Effect of drug concentration

S.No.	Drug:Polymer concentration(mg)	Aqueous acetic acid(ml)	Surfactant (mg)	Particlesize(nm)	Entrapment efficiency (%)
CNP1A	5:10	1%	0.4mg	150±1.08	49.12±0.30
CNP1B	10:10	1%	0.8mg	125±1.10	75.40±0.20
CNP1C	**15:10**	**1%**	**1mg**	**160±1.20**	**45.28±0.21**

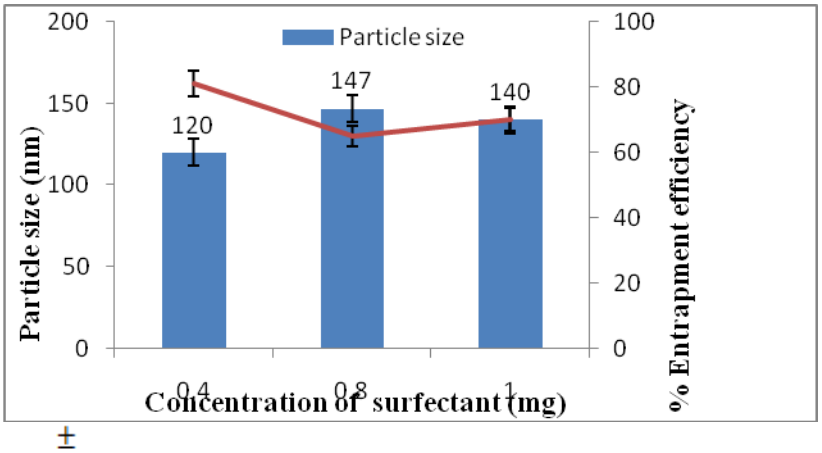


S.D. ± Mean (n=3)

Fig 1. Optimization of concentration of drug with respect to entrapment efficiency and particle size

Table 2. Effect of surfactant concentration

S. No.	Drug:Polymer concentration(mg)	Aqueousacetic acid(ml)	Surfactant (mg)	Particle size (nm)	Entrapmente efficiency (%)
CNP1SA	10:10	1%	0.4mg	120±1.30	81.01±0.30
CNP1SB	10:10	1%	0.8mg	147±1.40	65.03±1.35
CNP1SC	**10:10**	**1%**	**1 mg**	**140±1.10**	**70.20±1.25**



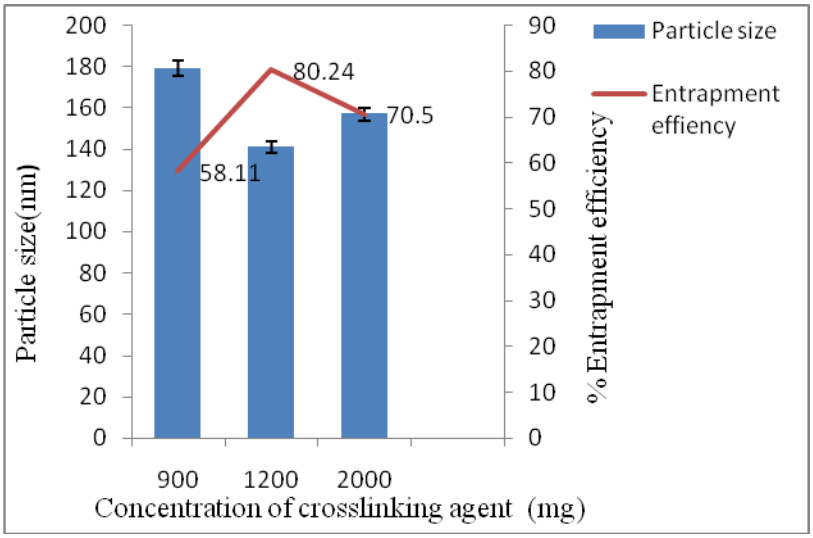
±

S.D. Mean (n=3)

Fig 2. Optimization of surfactant concentration with respect to particle size.

Table 3. Effect of crosslinking agent concentration

S. No.	Drug:Polymer concentration (mg)	Tripolyphosphate (mg)	Aqueous acetic acid(ml)	Surfactant (mg)	Particle size (nm)	Entrapment efficiency (%)
CNPCL1	10:10	0.4	1%	0.4mg	140±1.50	60.05±1.60
CNPCL2	10:10	0.6	1%	0.8mg	129±1.30	70.09±1.45
CNPCL3	**10:10**	**0.8**	**1%**	**1 mg**	**120±1.19**	**80.12±1.20**

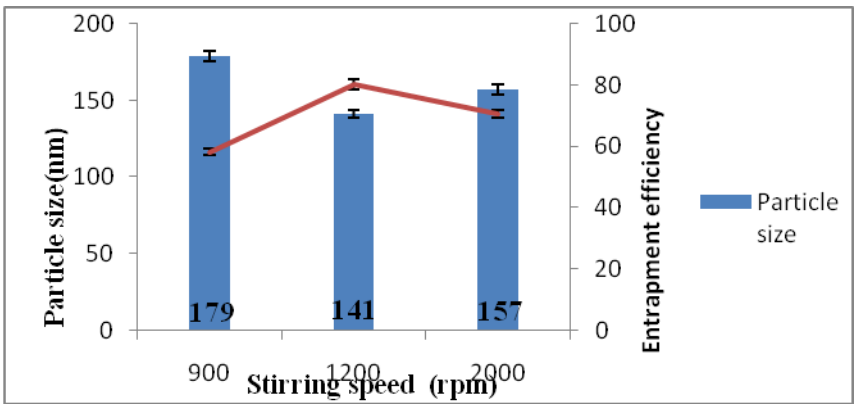


S.D. ± Mean (n=3)

Fig 3 Optimization of crosslinking agent with respect to particle size and entrapment efficiency

Table 4. Optimization of stirring speed

S.No.	Stirring speed (rpm)	Particle size (nm)	Entrapment efficiency (%)
CNPSS1	900	179±1.40	58.11±2.09
CNPSS2	1200	141±1.25	80.09±2.46
CNPSS3	2000	157±1.30	70.12±1.32



S.D. ± Mean (n=3)

Fig 4. Optimization of stirring speed with respect to particle size and entrapment efficiency

Spreadibility:

Table 5: Spreadability of nanogel

S.No	Weight of the pan (M)	Length moved by glass slide (L)	Time taken to separate the slide (T)	Spreadibility (S)
1.	14g	1cm	1 min	14
2.	30g	1-3cm	2 min	45
3.	40g	3-5cm	3.5 min	57.14

***In-vitro* release study**

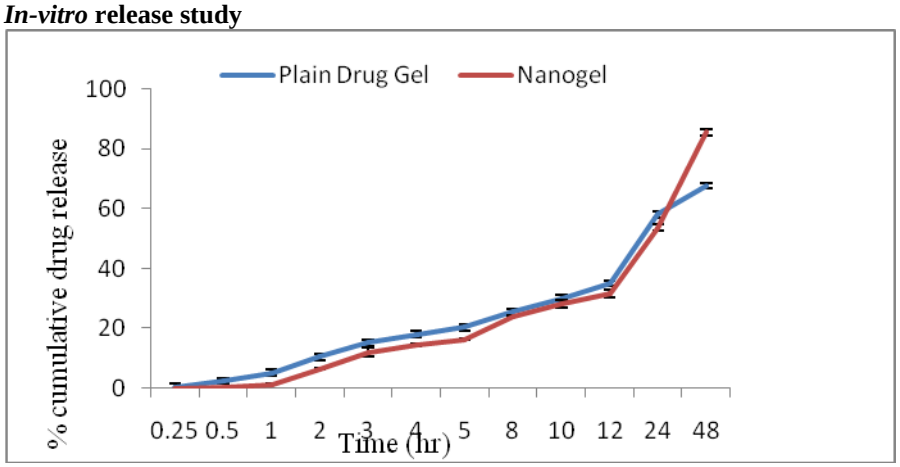


Fig 6. Release pattern of drug and drug from nanogel.

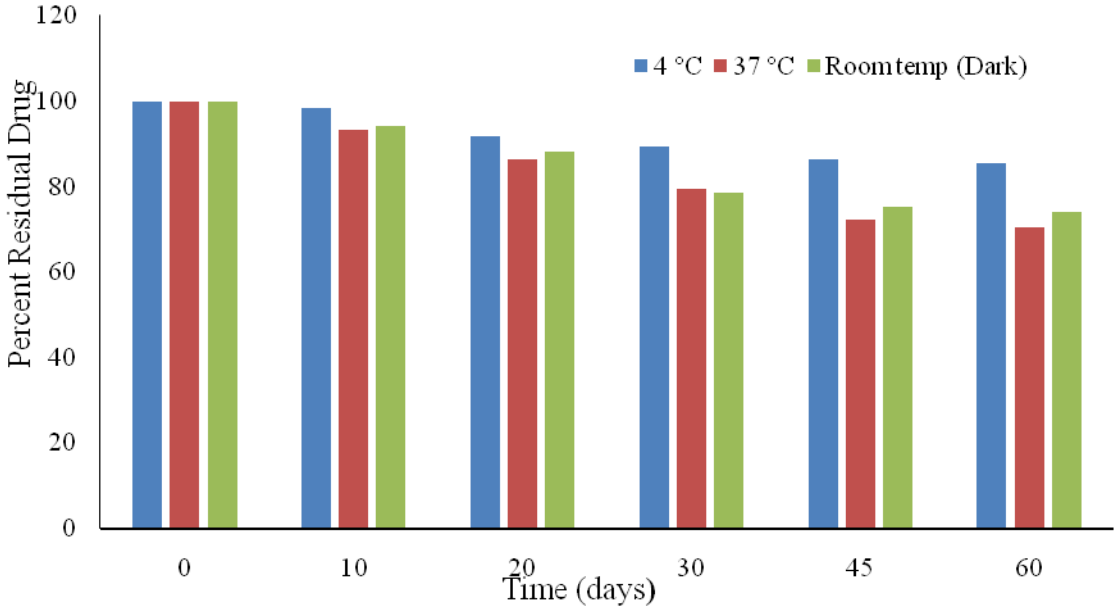


Fig.7 Stability study of the formulations with respect to percent drug remained and the time taken.

DISCUSSION

Particle size and zeta potential: Particle size is one of the major factors to include in the characterization of the prepared formulation for delivery. It gives the exact idea of the particle which should be convenient to the patient and easily transported through the systemic circulation and also with good absorption and penetration through the membrane. Whereas zeta potential of the particle was measure to confirm the delivery potential and attachment of drug to the receptor site and it was finding that both are zeta and size is within range as per need.

Entrapment efficiency: Entrapment efficiency is needed to know the exact amount of drug contained or engulfed by the nanoparticle and it was analyzed to decide the dose of the nanoparticle containing drug so that equivalent amount of drug can be given to the patient and also there concentration was measured.

Spreadability and Homoginity: The drug formulation nanogel was prepared for the topical application and for that rate and flexibility to spred through skin is to be measured. It also confirms the effective area of the formulation on the topical skin to be applied. Spreadability of the nanogel was good and up to the standard parameter suggestive for topical application. To spread equal amount of drug throughout the surface area of skin homogeneousness is measured and it was finding that the nanogel containing drug was equally distributed through the surface with average size range within formulation.

***In -vitro* release and stability:** The release pattern of the drug was measured to confirm that which type of release of the drug is containing the

formulation so that the dose interval may be decided by this study the release of nanogel was sustained and it was decided to study out stability study of the drug which confirm the how long drug formulation can be used what will be the temperature and environmental condition to store the drug and which type of container need for storing the formulation.

CONCLUSION

In the current work there is novel preparation of nanoparticle for targeted delivery of methotrexate. The drug of choice for the treatment of Psoriasis. Ion gelation technique was employed for the fabrication of blank nanogel. Pluronic and Chitosan was taken in to produce this nanogel.nano formulation was esteemed to be good release pattern and it was suggesting that the prepared formulation can be explored for the further study for management of targeted delivery of psoriasis and also be a game changer for this disease management. Further the formulation it can also use self-nano-emulsifying drug delivery system SNEDDS to achieve the higher bioavailability of the drug for various skin diseases.

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