EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 12
242-249
Year: 2022

FORMULATION AND DEVELOPMENT OF METHOXSALEN LOADED NANOGEL FOR MANAGEMENT OF PSORIASIS

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Article Received on 01/10/2022 **Article Revised on 22/10/2022** **Article Accepted on 12/11/2022**

ABSTRACT
Nanomedicine, a novel concept, bears much hope in delivering drug candidates having low solubility and bioavailability. Nanogel, one of the emerging tools, is considered as ideal carriers for the topical delivery of lipophilic drugs to overcome these challenges in the management of psoriasis and related skin problems. Psoriasis is an auto-immune and chronic inflammatory disease affecting 2-3% population of the world. Current available treatment of psoriasis has limitations such as systemic side effects and low percutaneous permeation, which evokes a dire need to develop an alternative nanocarriers system. Methoxsalen is powerful anti-psoriatic agent, widely used in the treatment of psoriasis and also used in vitiligo. The pharmacokinetic parameters of methoxsalen make it a suitable candidate for development of nanogel. The present work is to formulate and evaluate the methoxsalen nanogel. The nanogel of methoxsalen is prepared by solvent diffusion method (high speed homogenization) using carbopol 940 and Poloxamer 407 as polymers and triethanolamine as a gelling agent and evaluated for homogeneity, pH, spreadability, extrudability, drug content studies, viscosity, in-vitro diffusion and stabilities studies. FTIR studies revealed that the drug and polymer are compatible with each other during preparation. Homogeneity and extrudability studies reveal that the nanogel was homogenous and easily extrudable. The pH data shows all the formulations are in the range of 4.9 to 6.9 and they are in compatible to skin pH. Viscosity studies show the results in the range of 23,486-51,486cps, and having good viscous property. The drug content studies of formulations were from 86 to 94 %. *In vitro* diffusion studies of prepared nano gel follow first order dissolution kinetics with controlled release mechanism. From the stability studies data, it was found that there was no such difference in drug content and *In-vitro* drug release. This indicates the prepared nano gel formulations are stable. Formulation F3 shows good results for the *invitro* diffusion studies for controlled release.

KEYWORDS: Methoxsalen, Nanomedicine, Nanogel, Psoriasis, Solvent diffusion method.

INTRODUCTION
Psoriasis is a chronic; T lymphocyte (T-cells) mediated auto-immune inflammatory condition identified by abnormal, rough and red-coloured blotch on skin due to epidermal hyper-proliferation and mostly affects knee, elbow, trunk and scalp.[1] The prevalence of the disease is about 2% worldwide but varies according to regions. The disease incidences are comparatively low in population of Asia and Africa and higher in Caucasian and Scandinavian population.[2]

Tumour necrosis factor- α (TNF-α) binds to receptor present on keratinocyte that activates hyper proliferation. Interlukin 23 (IL23) plays a crucial role in psoriasis and helps in differentiation of Th17cells and produce IL23, IL22. Psoriatic plaque shows high level of vascular growth factor which increases angiogenesis results bleeding point when peeled off.[3] Psoriasis is derived from Greek word "psora ", means itch and joints and tendons are affected along with itchy sensation on the body. In psoriasis both the environment and inherited factors play important role to cause the disease. Stress, damage to skin, alcohol, and exposure of sunlight may cause psoriasis. Also, some medications given in high blood pressure (BP), angina and malaria may worsen the psoriatic condition. In patients with smoking habit and obesity, the treatment of psoriasis is very difficult.[4] Topical drug delivery system (TDDS) is a type of dosage form which distributes an adequate amount of drug across the skin.[5] TDDS gives a greater chance of success of drug delivery over traditional methods like use of injectables and oral formulations. Nanogels are potential form of the delivery of large number of drugs to different organs of the body owing to their high biocompatibility, high drug loading capacity, high biodegradability (and hence low cytotoxicity), good permeation capabilities and tissue mimicking properties. Their high-water retention makes them ideal capable of incorporation of bulky drugs like proteins, peptides, oligonucleotides and other macromolecules.[6] Nanogel

have three-dimensional structure formed by chemically or physically cross-linked polymers with hydrophilic or amphiphilic macromolecular chains, able to swell, by holding a great amount of water, with no dissolving but maintaining the structure intact. The great water content correlates with the fluid-like transport properties for the biologically active molecules significantly smaller than the gel pore size.[7] Methoxsalen is an anti-psoriatic drug which is also known as xanthotoxin or 8-methoxypsoralen. It is extracted from the plant *Ammi majus* belonging to the family Apiaceae. The IUPAC nomenclature of this molecule is 9-methoxy-7H-furo [3,2- g] chromen-7-one. Tablets of 10 mg dose are widely prescribed by the physicians as a part of PUVA (Psoralen + UVA) therapy in the treatment of vitiligo and psoriasis.[8] Methoxsalen belongs to the class of furanocoumarins, a class of organic natural molecules which comprises of coumarin moiety annulated with furan.[9] In case of psoriasis, methoxsalen has been administered topically or systemically. Earliest topical formulation such as ointment, cream, and lotion produced a persistent hyperpigmentation.[10] Then it was replaced by oral therapy. But bioavailability of methoxsalen is highly variable because of its low water solubility and marked first pass effect. It also causes nausea, nervousness and mental depression after oral administration,[11] because of these short coming topical deliveries of methoxsalen by means of some topical formulation was attempted. The aim of the present study was to statistically optimize nanogel for enhanced skin delivery of a model drug of methoxsalen, which was effective candidate for the treatment of psoriasis.

MATERIALS AND METHODS

Materials

Methoxsalen was provided as a gift sample from Alembic Limited, Vadodara, Gujarat, India. Poloxamer 407 and triethanolamine were purchased from SD Fine Chemicals Mumbai, India. Carbopol 940 was purchased from CDH Laboratories New Delhi, India. Liquid paraffin, propylene glycol, methyl parabens and propyl parabens extra pure were purchased from Hi-Media laboratories Mumbai, India. All other chemicals used were of analytical grade and were used without any further chemical modification.

Preformulation studies

Physical characteristics

By visual examination, the drug was identified for physical characters like colour, texture, odour etc.

Solubility

Solubility of the drug was determined by taking some quantity of drug (about 10 mg) in the 10 ml volumetric flasks separately and added the 10 ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, chloroform and 7.4 pH buffer) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature).

Melting point

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

FTIR spectroscopy

The concentration of the sample in KBr should be in the range of 0.2% to 1%. The pellet is a lot thicker than a liquid film, consequently a decrease concentration in the sample is required (Beer's Law). For the die set that you'll be the usage of, about 80 mg of the mixture is wanted. Too excessive of an attention causes typically difficulties to obtain clean pellets. FTIR spectra of the samples were recorded over a spectral region from 4700 to 400 cm-1 using 20 scans with 4 cm-1 resolution.

Determination of λ_{max} of methoxsalen

Methoxsalen, 100 mg, was accurately weighted into a 100 ml volumetric flask, dissolved in methanol and the volume was made up with methanol. Pipette 1 ml of this solution into a 10 ml volumetric flask with methanol as the volume and marks it as stock. Prepare an appropriate dilution to bring the concentration down to 2-12µg/ml. The resulting solution is scanned with a UV spectrophotometer (UV-1700 Shimadzu corporation, Japan) in the range of (200-400 nm) to determine the absorption maximum (λ max). Concentration vs. absorbance was shown on a graph.

Preparation of nanogel

The nanogel is prepared from modified emulsion solvent diffusion method.[12] In the first step accurately weighed quantity of drug is dissolved in ethanol and propylene glycol with stirring (organic phase). In the second step aqueous phase is prepared by using Carbopol or Poloxamer dissolved in water with continuous stirring and heat for a 20min in a magnetic stirring. And the drug phase is sonicated under ultrasonic bath Sonicator for 10min. In third step, drug phase is added drop by drop into aqueous phase during high speed homogenization for 30 min at 6000 rpm to from emulsion. The emulsion is converted into nanodroplet by homogenizer results in o/w emulsion formed. In forth step, o/w emulsion is homogenized for 1 hour at 8000 rpm and triethanolamine is added with continues stirring to from nanogel Table 1.

Table 1: Composition of methoxsalen nanogel.

S. No.	Components	Formulations					
		F1	F2	F3	F4	F5	F6
	Methoxsalen (mg)	50	50	50	50	50	50
1	Carbapol (% W/V)	5	10	15	-	-	-
2	Poloxamer 407 (%W/V)	-	-	-	5	10	15
3	Propylene glycol (% W/V)	10	10	10	10	10	10
4	Ethanol (ml)	10	10	10	10	10	10
5	Triethanolamine	1- 2 drop	1- 2 drop	1- 2 drop	1- 2 drop	1- 2 drop	1- 2 drop
6	Purified water (ml)	10	10	10	10	10	10

Evaluation of methoxsalen nanogel

Physical characteristic

The prepared nanogel formulations were inspected visually for their color, appearance and consistency.

Determination of pH

The pH of nanogel formulations was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurement of pH of each formulation was done in triplicate and average values were calculated.[13]

Extrudability study

The nanogel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.[14] The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon gel in 10 seconds.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Homogeneity

The developed nanogels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Viscosity

The measurement of viscosity of the prepared nanogel was done using Brookfield digital viscometer. The viscosity was measured using spindle no S-96 at 10 rpm and 25^0C. The sufficient quantity of gel was filled in appropriate wide mouth container. The nanogel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the viscometer. Samples of the nanogel were allowed to settle over 30 min at the constant temperature (25 ±/1^0C) before the measurements.[15]

Spreadability

Two glass slides of standard dimensions (6×2) were selected. The nanogel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the nanogel formulation between the two slides was traced uniformly to form a thin layer. The weight was removed and the excess of the nanogel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each nanogel formulation.[16,17]

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)
l= length of glass slide (6cms).
t = time taken is seconds.

Drug content studies

A 0.5 gm of the prepared nanogel was diluted with 10 ml of ethyl acetate and filtered with a 0.45 µm filter. Total drug content was determined by UV spectrophotometry at 249 nm using the formula[18]:

$$DC = \frac{Total\ amount\ of\ nanogel \times Amount\ of\ drug\ in\ 0.5\ gm\ nanogel}{Amount\ of\ nanogel\ in\ gm\ W\ Initial\ drug - W\ free\ drug}$$

***In vitro* drug release**

The release of methoxsalen from optimized nanogel was determined by membrane diffusion technique using Franz diffusion cell. The nanogel equivalent to 5%w/w of methoxsalen was taken in donor compartment. The donor and receptor compartment were separated by synthetic cellophane membrane. The synthetic cellophane membrane was mounted between donor and receptor compartment of cell. The receptor medium was filled with phosphate buffer pH 7.4. The assembly was stirred at 200 rpm and receptor compartment was replenished with equal volume of phosphate buffer. Aliquots each of 1 ml was withdrawn periodically at an

interval of 2, 4, 6, 8, 10, 12 and 24 hrs and replaced by an equal volume of receptor medium.[19] The aliquots were suitably diluted with receptor medium and analyzed by UV visible spectrophotometer.

Drug release kinetics study

The results of in-vitro release profile obtained for all the formulations were plotted in kinetic models as follows,

1. Cumulative of drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining to be absorbed versus time (First order model)
3. Cumulative amount of drug release versus square root of time (Higuchi model)
4. Log cumulative drug released versus log time (Korsmeyer-Peppas model).[20]

RESULTS AND DISCUSSIONS

The melting point of methoxsalen (pure drug) was found to be 145-148°C. Methoxsalen was soluble in methanol and ethanol, sparingly soluble in chloroform and insoluble in water. Identification of methoxsalen was done by FTIR spectroscopy with respect to marker compound. It was identified from the result of IR spectrum as per specification fig. 1. The calibration curve of methoxsalen was found to be linear in the concentration range of 2-12 µg/ml at 249 nm fig. 2. Partition coefficient and moisture content of methoxsalen was found to be 1.98K and 0.082 respectively. Nanogel formulations were white viscous creamy preparation with a smooth homogeneous texture and glossy appearance. Results have been discussed in table 2. The results of washability, extrudability and spreadability of all formulation were given in table 3. From the result it was found that formulation F1-F4 has good washability ability, formulation F3, F4 has good extrudability and spreadability of all formulation was found to in range of 11.37±1.20 to 23.84±1.25. It was concluded that all the developed formulation showed acceptable spreadability. The viscosity of the nanogel was obtained by using Brookfield digital viscometer. The viscosity of the formulations increases as concentration of polymer increases and pH of prepared nanogel were measured by using pH meter (Orion Research, Inc., USA). The pH of the nanogel formulation was in the range of 4.9-6.9 which considered acceptable to avoid the risk of skin irritation upon application to skin. The drug content of methoxsalen from its various nanogel formulations are represented in the table 4. F3 and F4 showed better drug content as compared to other formulations. The percent drug content of these formulations was 94% and 91% respectively. The *in-vitro* release profiles of various nanogel formulations are represented in table 5 & fig. 3. The better release of the drug from all nanogel formulation can be observed and nanogel formulation can be ranked in the following descending order F3> F4> F2> F5 >F1 >F6. Where the amounts of the drug released after 24 hours were 98.13, 94.15, 92.03, 90.26, 88.26 and 86.26% respectively. The higher drug release was observed with the formulation F3. Various kinetic models were used to describe the release kinetics of prepared nanogel. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of F3 formulation showed the constant drug release from the nanogel, the results of the zero order model was found to be $y = 0.048x + 0.440$, $R^2 = 0.902$. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be $y = -0.071x + 1.971$, $R^2 = 0.995$. It can be observed in the figure that graphical representation of cumulative % of drug release against time represents that drug release of methoxsalen from the nanogel is perfectly following first order drug release model as the drug release profile is very closest to regression line and the highest value of coefficient of correlation values (R^2) was in the range table 6 & fig. 4-6.

Table 2: Physical parameter of formulation batches.

Formulation	Colour	Homogeneity	Consistency	Phase separation
F1	White	Excellent	Excellent	None
F2	White	Excellent	Excellent	None
F3	White	Excellent	Excellent	None
F4	White	Excellent	Excellent	None
F5	White	Excellent	Excellent	None
F6	White	Excellent	Excellent	None

Table 3: Result of washability, extrudability and spreadability study.

Formulation	Washability	Extrudability	Spreadability (gcm/sec)
F1	+++	0.2 cm	20.26±1.25
F2	+++	0.5 cm	15.76±1.25
F3	+++	0.6 cm	12.74±1.05
F4	+++	0.4 cm	11.37±1.20
F5	++	0.5 cm	23.84±1.25
F6	++	0.8 cm	17.47±1.20

Excellent: +++, Good: ++, Average: +, Poor: -

Table 4: Viscosity, pH and % drug content.

Formulation	Viscosity (cps)	pH	Drug content (%)
F1	23,486	6.2	90
F2	43,486	6.3	88
F3	35,476	4.9	94
F4	27,587	5.5	91
F5	51,486	6.9	89
F6	48,396	5.9	86

Table 5: Cumulative drug release of all the formulations.

Time (hours)	Formulation code					
	F1	F2	F3	F4	F5	F6
2	28.23±0.97	31.24±0.54	35.17±0.44	24.13±0.48	27.03±0.50	20.25±0.45
4	39.22±0.53	44.19±0.54	48.22±0.52	42.12±0.50	43.26±0.56	40.43±0.33
6	54.07±0.48	57.08±0.46	61.10±0.42	58.12±0.48	55.23±0.42	52.23±0.48
8	69.15±0.30	73.09±0.33	77.14±0.37	74.02±0.32	60.01±0.33	57.22±0.28
12	80.02±0.28	84.02±0.27	88.07±0.26	85.12±0.23	81.05±0.21	84.08±0.23
24	88.26±0.15	92.03±0.12	98.13±0.10	94.15±0.15	90.26±0.18	86.26±0.17

Table 6: Release kinetics study of F3 formulation.

Formulation	Model	Kinetic parameter values
F3	Zero Order	y =0.048x + 0.440, R^2 = 0.902
	First Order	y =-0.071x + 1.971, R^2 = 0.995
	Higuchi	Y=18.69x - 14.83, R^2 = 0.902

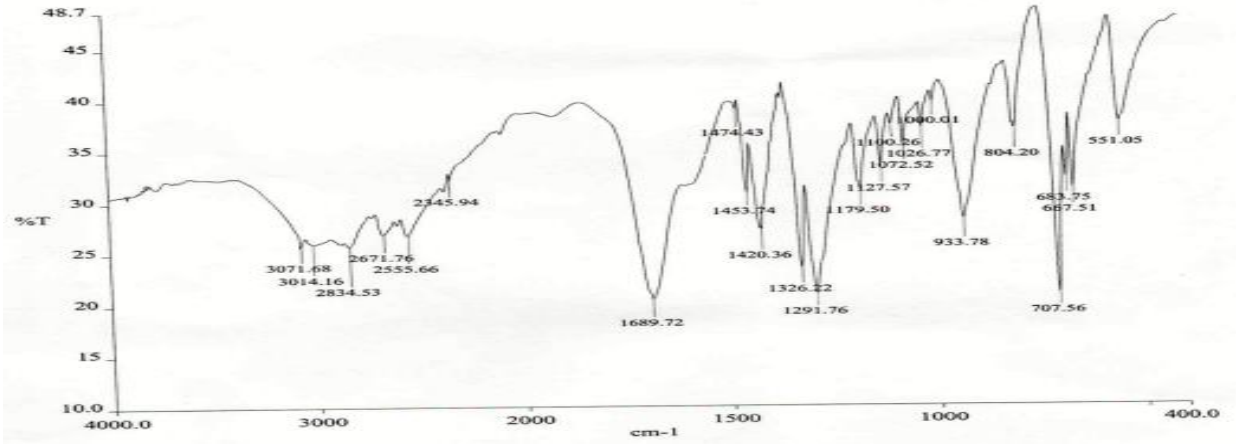

Fig. 1 FT-IR spectrum of pure drug (Methoxsalen).

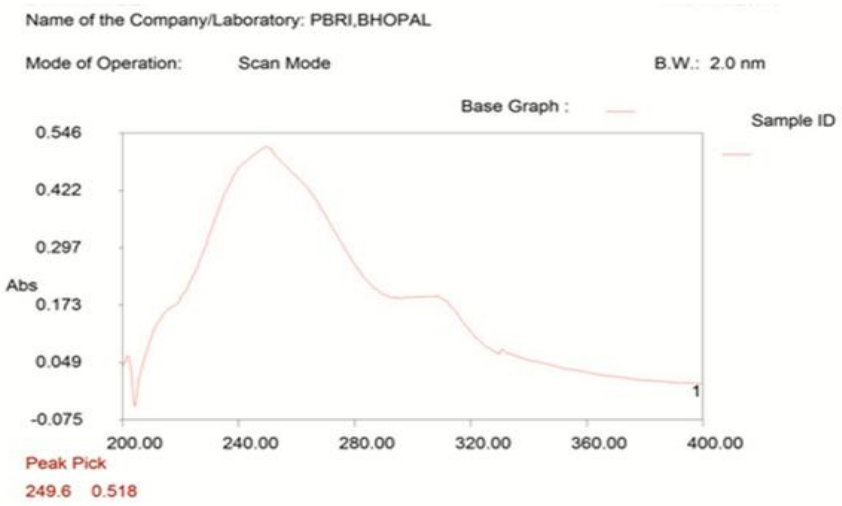

Fig. 2 Wavelength maxima of esomeprazole in methanol.

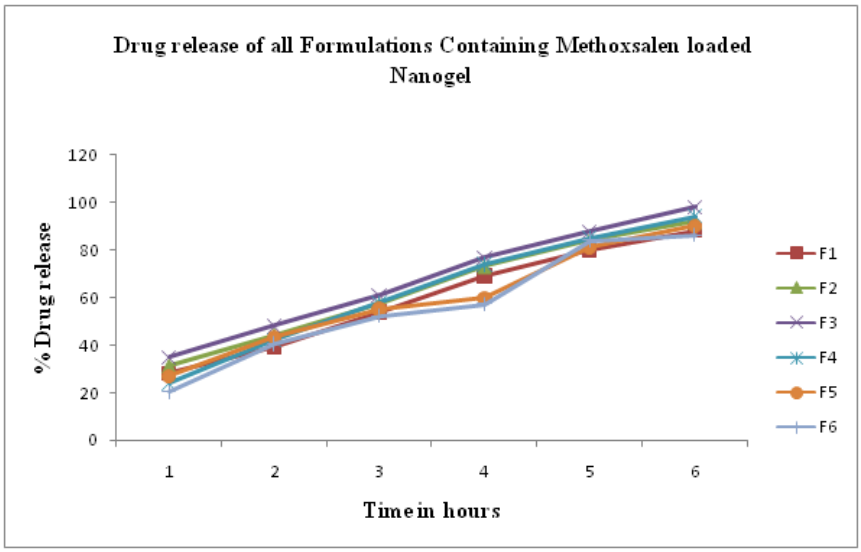

Fig. 3 Cumulative % drug release of all the formulations.

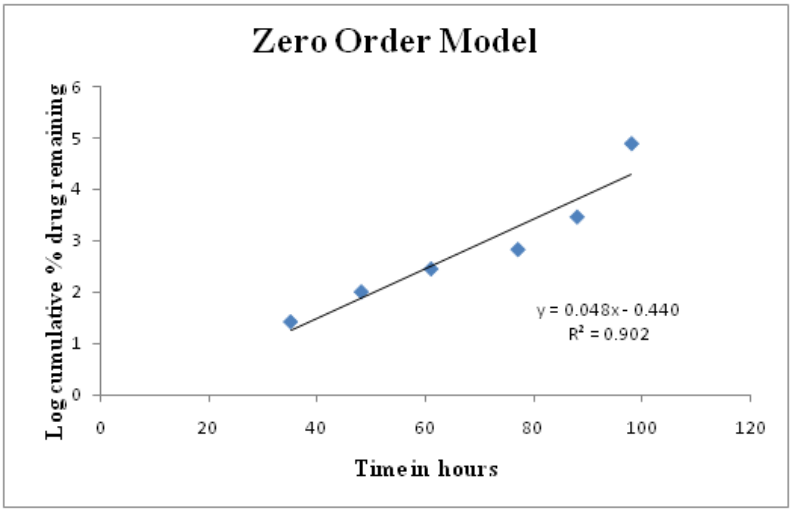

Fig. 4 Zero order kinetic model.

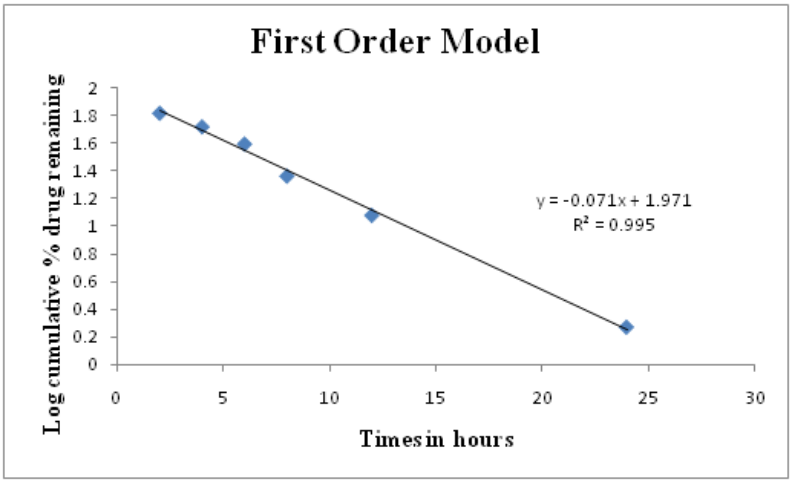

Fig. 5 First Order kinetic model.

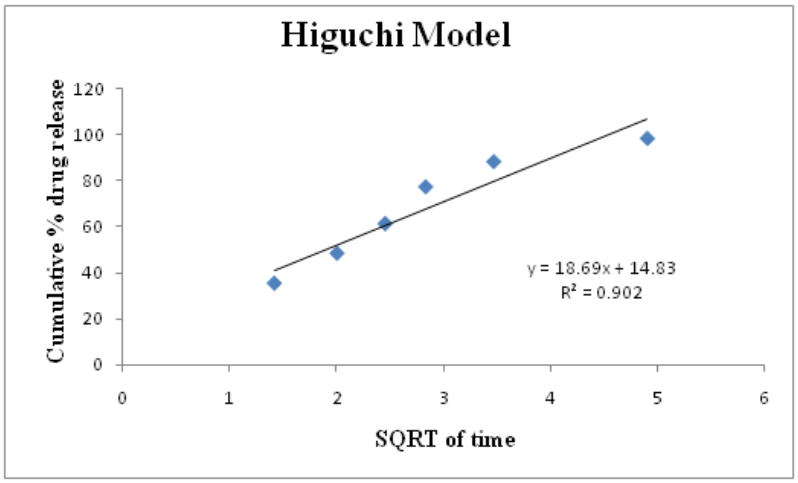

Fig. 6 Higuchi model.

CONCLUSION

In the coming years, topical drug delivery will be used extensively to impart better patient compliance. Since nanogel is helpful in enhancing spreadability, adhesion, viscosity and extrusion, this novel drug delivery become popular. Moreover, they will become a solution for loading hydrophobic drugs in water soluble gel bases for the long term stability. In present investigation topical methoxsalen nanogel was prepared by using carbopol 940 showed acceptable physical properties, pH, drug content, viscosity. In vitro releases of nanogel were also performed to determine drug release from nanogel and duration of drug release. From the in vitro studies, formulation F3 showed maximum release of 98.13±0.10% in 24hrs. So methoxsalen nanogel can be used as an psoriasis for topical drug delivery.

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