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

ISSN 2349-8870
Volume: 11
Issue: 4
152-160
Year: 2024

FORMULATION AND EVALUATION OF DAPSONE-LOADED MICROSPHERE GEL FOR THE TREATMENT OF ACNE VULGARIS

Athira K.*, K. Vineetha, Krishnananda Kamath K. and A. R. Shabaraya

Department of Industrial Pharmacy, Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka, India-574143.

***Corresponding Author: Athira K.**
Department of Industrial Pharmacy, Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka, India-574143.

Article Received on 15/02/2024 **Article Revised on 06/03/2024** **Article Accepted on 26/03/2024**

ABSTRACT
Microspheres are solid spherical particles ranging in size from 1-1000µm consisting of proteins or synthetic polymers which are biodegradable in nature. The present study is to investigate the effectiveness of Dapsone microsphere gel for the treatment of acne vulgaris. Dapsone is a sulfone antibiotic with an anti-inflammatory effect, which is considered to be accountable for its effectiveness in the treatment of acne vulgaris. Six different(F1-F6) formulations of dapsone microspheres are prepared, F1-F3 with ethyl cellulose in different concentration and F4-F6 with Eudragit L 100 in different concentrations. The best formulation was chosen on the basis of their entrapment efficiency, which is then incorporated into the carbopol 934 gel. The prepared gel was then evaluated for its parameters like physical appearance, pH, drug content, spreadability, viscosity, *In vitro* diffusion studies and Kinetic studies. Results revealed that the entrapment efficiency of the F6 was found to be 80.14±0.36% which is higher than the other formulations which is in micro-size range (85.65 ± 6.57 µm) and showed cumulative drug release of 61.477%. SEM analysis illustrated spherical and uniform microparticles. The developed micro formulation showed improved stability and the obtained results corroborate the premise that dapsone loaded microsphere gel can be an effective strategy for acne management.

KEYWORDS: Dapsone, Microspheres, Solvent evaporation method, Carbopol gel.

INTRODUCTION
Microspheres can be referred as small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm).[1] Microspheres can also be called as microparticles. Microspheres had been explored significantly for their use in the subject of drug transport and various polymers had been utilized for the formulation of the microspheres, which in turn have been assessed for distinctive purposes. Eventually the whole dose and few adverse reactions can be decreased due to the fact that a steady plasma concentration is maintained.[2]

Dapsone microspheres are prepared by solvent evaporation method. Solvent evaporation is carried out in a manufacturing vehicle phase. The microcapsule coating is first dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. In a coating polymer solution, a core material to be microencapsulated is dissolved or dispersed. To obtain acceptable size microcapsule, agitation is performed so that the core material mixture is dispersed in the liquid manufacturing vehicle phase. If necessary, the mixture is heated to evaporate the solvent. If the core material is dissolved in the coating polymer, matrix-type microcapsules are formed. The core material may be either water soluble or water insoluble material. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.[3,4]

Topical route of drug delivery provides a high local concentration of the drug without affecting the general circulation. It is very convenient to use and ease of administration leads to higher patient compliance.[5] The potential of oral dapsone to treat acne vulgaris is well established, but the risks of serious side effects have made it an undesirable drug. It was hypothesized that a topical formulation of Dapsone may be appropriate for treating acne vulgaris while minimizing systemic exposure and hematologic risk.[6] Clinical studies indicate dapsone gel 5% is effective in treating mild to moderately severe acne. Because of unsuitability of dapsone for the oral route; topical route is another alternative.[7] Dapsone is marketed as gel, the bioavailabitlity of dapsone can be improved by incorporating drug in microsphere based gel.[8] Dapsone possesses potent anti-microbial activities and hence, it is

used to cure multiple inflamed acne lesions. The side effects like mild irritation and dryness are reported with normal topical gel. These problems, along with orange-brown discoloration on skin, restrict the topical application of this drug. The above-mentioned limitations like irritation, dryness, and skin discoloration of dapsone can be avoided by formulating microsphere based gel of this moiety.[9] The present study aims to prepare and evaluate the effectiveness of dapsone microspheres for topical application with an objective to control and prolong the release of the drug with improved skin penetration as a novel formulation for healing of mild to moderate acne vulgaris.[10]

MATERIALS AND METHODS

Pure drug dapsone, Ethyl cellulose, Eudragit L 100, Dichloromethane, Polyvinyl alcohol and Carbopol 934 was obtained from Yarrow chemicals, Mumbai. Dapsone microspheres are prepared by solvent evaporation method. Ethyl cellulose/ Eudragit L 100 was dissolved in dichloromethane. Dapsone was dissolved in the solution. The resulting solution was added to aqueous phase containing polyvinyl alcohol. The above mixture was agitated at 500 rpm. Then the drug and the polymer were transformed to fine droplets which solidified into rigid microsphere by solvent evaporation. Collected by filtration and washed and desiccated at room temperature.

Table No. 1: Formulation chart of microspheres.

Ingredients	F1	F2	F3	F4	F5	F6
Dapsone	100mg	100mg	100mg	100mg	100mg	100mg
Ethyl Cellulose	100mg	200mg	300mg	-	-	-
Eudragit L 100	-	-	-	100mg	200mg	300mg
Dichloromethane	qs	qs	qs	qs	qs	qs
Polyvinyl Alcohol(%w/v)	0.3	0.3	0.3	0.3	0.3	0.3
Purified Water	qs	qs	qs	qs	qs	qs

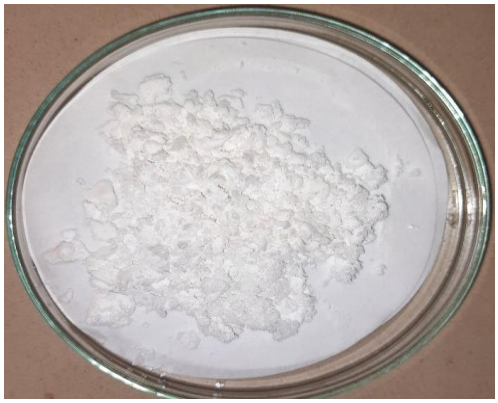

Fig. No. 1: Prepared microsphere formulation.

Evaluation of dapsone microspheres

1. Percentage yield

The dapsone microspheres obtained after drying was weighed.

$$\text{Percent Yield} = \frac{\text{Weight of microsphere}}{\text{Total expected weight of drug and polymer}} * 100$$

2. Particle size determination

The particle size of the microspheres was determined by using optical microscopy method. It was used to determine the shape and outer structure of the particles.

3. Entrapment efficiency

Microspheres equivalent to 10 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and were dissolved in Phosphate buffer (pH-7.4) repeatedly. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 291 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula.

$$\% \text{ Drug entrapment} = \frac{\text{Amount of drug actually present}}{\text{Total weight of microsphere}} \text{ X } 100$$

4. Scanning electron microscopy (SEM)

The morphological study was carried out by Scanning Electron Microscope (SEM). The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

5. *In vitro* drug release study

Dissolution profile of microspheres was studied using dissolution apparatus USP II with a modified basket consists of 5µm stainless steel mesh. The speed of the rotation was set at 150 rpm. The dissolution medium was phosphate buffer pH 7.4. Samples from the dissolution medium were analyzed by UV- Visible spectrophotometer (Jasco) at various intervals.

Formulation of gel containing microspheres

Based on the results of best entrapment efficiency of Microspheres, they are imcorporated into gel formulation using carbopol. Accurately weighed amount of Carbopol 934 (1% w/v) was taken and moistened in water. Prepared microsphere formulation was incorporated. Dispersed by constant stirring at 600rpm with the aid of magnetic stirrer to get smooth and uniform dispersion. The prepared dispersion was allowed to stand for 15 minutes to expel the entrapped air. 2% triethanolamine was added as pH neutralizer. Propylene glycol was added as permeability enhancer.

Fig. No. 2: Prepared microsphere gel formulation.

Characterization of microsphere gel

1. Measurement of pH of the microsphere gel

1g microsphere gel was mixed in 100 ml distilled water utilizing a homogenizer. Electrodes are then immersed in the developed gel solution and readings were documented using digital pH meter three times and mean value was estimated.

2. Viscosity determination

Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. 50 g of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated. The procedure was repeated three times and observations are recorded as mean.

3. Spreadability

The spreadability of the gel formulation was determined by taking two glass slides (14*5cm) of equal length. On one glass slide, 1gm gel was applied. To the other slide, weights (125g) are added and the time taken for the second glass slide to slip off from the first glass slide was determined. A shorter interval indicates better spreadability. Spreadability was calculated by using the formula,

S=M*L/T

Where, S = spreadability,
M = Weight kept on upper slide,
L = Length of glass slides,
T = Time taken to slip off the slides completely from each other.

Drug content

One gram of gel was dissolved in a 100 ml of phosphate buffer pH 7.4 stirred constantly for 10 minutes. From this 1 ml of solution was diluted to 100 ml of PB pH7.4. The resultant solution was filtered and was analysed by U.V spectrophotometer at 291 nm.

$$\text{Amount of drug} = \frac{\text{Concentration from the standard graph} \times DF}{1000}$$

Where DF = dilution factor

4. *In vitro* diffusion study

In vitro skin permeation studies were performed by using a franz diffusion cell with a receptor compartment capacity of 50ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated microsphere gel of 1gm was placed over the drug release membrane (In the donor compartment) and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor. And the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.5°C by surrounding water in jacket. The samples of 1ml were withdrawn at time interval of 1, 2, 3, 4, 6 and 8 hours and analyzed for drug content UV. Spectrophotometrically at 291nm against blank. The receptor phase was replaced with an equal volume of phosphate buffer at each time of sample withdrawal.

5. Kinetic studies

To analyze the mechanism of drug release from the topical gel, the release data were fitted to following equations.

a. Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that release the drug slowly, assuming that the area does not change and no equilibrium conditions and are represented by the equation

$Qt = Q_0 + K_0 t$

Where Qt is the amount of drug dissolved in time t,
Q_0 is the initial amount of drug in the solution
K_0 is the zero order release constant

b. First order model

The application of this model to the drug dissolution studies used to describe absorption and/or elimination of drugs. To study the first order rate kinetics the release rate data were fitted into the following equation.

$\text{Log } QT = \log Q_0 - K_1 t / 2.303$

Where Qt is the amount of drug released in time t,
Q_0 is the initial amount of drug in the solution,
K_1 is the first order rate constant

c. Higuchi model

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs

incorporated in semi-solid and/ or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the discussion media, the equation is

$Q = KH. t_{1/2}$

Where KH is the Higuchi dissolution constant,

Qt is the amount of drug released in time t

Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent.

d. Korsmeyer - Peppas model

This model is generally used to analyse the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomenon could be involved

$Mt / M = K.tn$

Where Mt / M is the fraction of drug released.

T is the release time

K is the release rate constant and 'n' is the release exponent for the drug release that is dependent on the shape of the matrix dosage form.

6. Stability studies

The success of an effective formulation can be evaluated only through stability studies. The prepared microsphere gel placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2 ^{0}C for a period of 3 months.

RESULTS AND DISCUSSION

1. Evaluation of microspheres loaded with dapsone

1. Percent yield

The percentage yield of the formulation F1, F2, F3, F4, F5 and F6 was found to be 57.01%, 52.02%, 54.06%, 63.83%, 68.91% and 77.85 % respectively. Dapsone microsphere using eudragit L100 in the ratio 1:3 gives the highest yield.

2. Entrapment efficiency(EE)

The EE of all the formulation F1, F2, F3, F4, F5 and F6 is in the range of 74.12±0.21 – 80.14±0.36 as shown in table No. 7 and figure No.10. The highest entrapment efficiency was found in the batch F6, consisted of Dapsone and Eudragit L-100 in the ratio of 1:3.

3. Particle Size and Size distribution

Average particle size of microspheres as determined by optical microscopy are shown in Table 11 and in Figure 8. The mean particle size for the formulation F1 to F3 containing Ethyl cellulose was found to be in range from 116.36±5.83µm to 142.68±9.36µm. For formulation F4 to F6 containing Eudragit L 100, the mean particle size was found to be in range from 85.65±6.57µm to 90.89±6.88µm. For formulation F4-F6, increase in polymers concentration in the microspheres the particle size of microspheres increases respectively. This is because the viscosity of the polymer solution increases with increasing polymer concentration, which in turn decreases the stirring efficiency.

4. SEM Analysis

The shape and surface morphology of the prepared microspheres were observed by scanning electron microscopy. SEM photogragh of F6 formulations revealed that microspheres were spherical in shape with smooth surface.

5. *In vitro* drug release from microspheres

Dissolution studies on all the six formulations of Dapsone microspheres were carried out using a USP dissolution apparatus Type II. Phosphate buffer pH 7.4 was used as the dissolution medium. The *in vitro* drug release data of different formulations are shown in Table. No.10 and Figure.No.16. The cumulative percent drug release after 8 hours was found to be 43.038%, 48.050%, 42.369% for the formulations F1, F2 and F3 respectively whereas cumulative percent drug release after 8 hours was 49.477%, 44.050% and 53.477% for formulations F4, F5 and F6 respectively. F6 shows the maximum drug release.

Evaluation of microsphere gel

1. Physical examination

The prepared microsphere gel formulations were colourless to white preparation with a smooth and homogeneous appearance and there was no phase separation in any of formulation. Physical examinations were shown in the table No 11.

2. Measurement of pH

pH of the formulations is shown in the table no.11. The pH values of the prepared formulation ranged from 7.4±0.05, which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

3. Viscosity study

Viscosities of the formulations were evaluated by using Brookfield viscometer at 27^{o}C using spindle no.64 at 6 rpm. The viscosity of the microsphere gel was found to be 1245 cps.

4. Spreadability

The values of spreadability were shown in table No. 11. The microsphere formulation showed 4.03±0.105g cm/ sec. The results indicate that the polymers used gave gels spread by small amount of shear and the spreadability of the microsomes decreases with the time.

5. *In vitro* diffusion study of microsphere gel

The *invitro* diffusion study of the drug is given fig no 18. The microsphere gel formulation with Dapsone and Eudragit L 100 in the ratio 1:3 polymer showed 57.54% within 8 hours.

6. Kinetic study

In order to study the exact mechanism of drug release from microsphere gel loaded with Dapsone, drug release data were fit into various mathematical models like zero

order , first order , higuchi matrix and peppas and were shown in fig no 20(a)-02(d).

Based on the highest regression values (r), the best fit model for the microsphere gel formulation is found to be zero order.

The data were processed for regression analysis using MS –EXCEL. The results of kinetics analysis of the *in vitro* drug release data for all formulation are given in table No12.

7. Stability studies

Stability study of the vesicles is the major determinant for the stability of the formulations. The study was carried to evaluate physical appearance, drug content and *in vitro* diffusion studies at accelerated condition [(25±2^{0}c at (60±5%RH].

The results of stability study of the formulation were depicted in table No 14.

- **Standard graph of dapsone**

Table No. 2: calibration data of Dapsone in phosphate buffer of pH 7.4.

Concentration(µg/ml)	Absorbance
0	0
2	0.2298
4	0.4587
6	0.6610
8	0.8799
10	0.9925

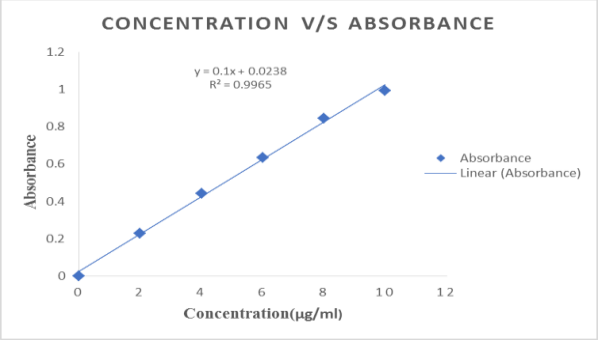

Fig. No. 3: Standard calibration curve of Dapsone in phosphate buffer pH 7.4.

The standard calibration curve of Dapsone was obtained in the range of 2-10µg/ml measured at the wavelength of 291nm

(a) Evaluation test for microsphere containing dapsone

1. Percentage yield:

Table No. 4: Percentage yield.

Formulation Code	Percent Yield (%)
F1	57.01
F2	52.02
F3	54.06
F4	63.83
F5	68.91
F6	77.85

2. Entrapment efficiency (EE)

Table No. 5: percentage entrapment efficiency (%EE) of microspheres F1 to F6.

Formulation	%EE*
F1	74.12±0.21
F2	77.56±0.43
F3	75.23±0.66
F4	77.55±0.54
F5	79.22±0.28
F6	80.14±0.36

*data expressed as mean

3. Average particle Size and Size distribution

Table No. 6: Average particle size of microspheres.

Formula	Particle size (µm)*
F1	116.36±5.83
F2	124.25±6.25
F3	142.68±9.36
F4	90.89±6.88
F5	87.95±8.37
F6	85.65±6.57

*Data expressed as mean±SD, n=3

4. Surface morphology

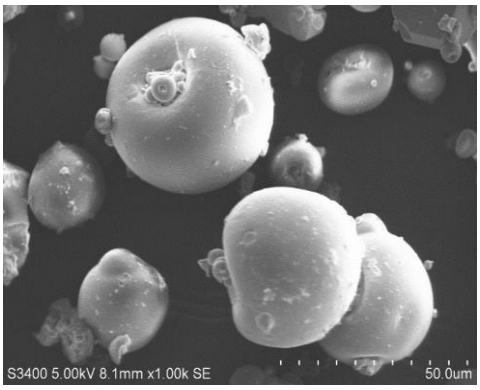

Fig. no. 4: SEM image of F5 formulation.

5. *In vitro* drug release from microspheres

Table No. 7: *In- vitro* drug release data of formulations (F1-F6).

Time (hrs)	Cumulative % Drug Release of Formulation*					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	14.21±0.2	14.55±0.5	15.47±0.1	16.95±0.4	16.21±0.2	17.95±0.1
2	28.41±0.3	28.75±0.2	25.43±0.4	32.55±0.2	32.76±0.2	35.5±0.3
4	32.71±0.2	34.40±0.4	31.73±0.1	39.14±0.3	39.40±0.3	42.14±0.4
6	38.37±0.4	40.38±0.2	37.07±0.5	46.81±0.1	46.38±0.4	48.81±0.1
8	43.03±0.2	48.05±0.4	46.36±0.3	52.47±0.4	53.05±0.2	61.47±0.1

*data expressed as mean±SD, n=3

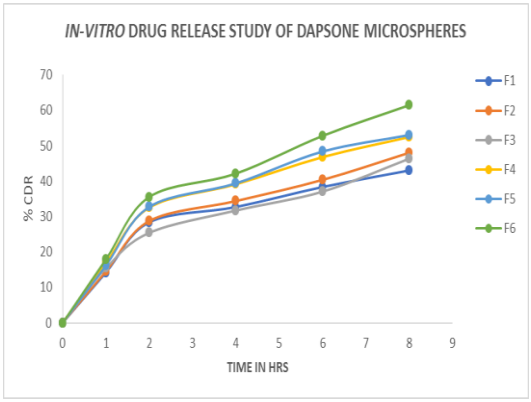

Figure No. 5: *In vitro* drug release profile of microspheres (F1-F6).

5.3. Evaluation test for formulated microsphere gel

1. Physical characteristics of dapsone microsphere gel

Table No. 8: Physical characteristics of Dapsone Microsphere gel*

Formulation code	Appearance	pH	Spreadability (gms.cm/sec)	Consistency	Drug content (%)	Viscosity (cps)
F6	Colourless-white	7.4±0.05	19.03±0.10	Good	84.98%	1245

*data expressed as mean±SD, n=3

2. *In vitro* diffusion study

Table No. 9: *In vitro* diffusion study of microsphere gel.

Time(hr)	% Cumulative drug release*
0	0±0.00
1	4.52±0.01
2	8.32±0.03
3	14.54±0.02
4	21.65±0.03
5	33.28±0.04
6	45.54±0.03
8	57.54±0.02

*data expressed as mean±SD, n=3

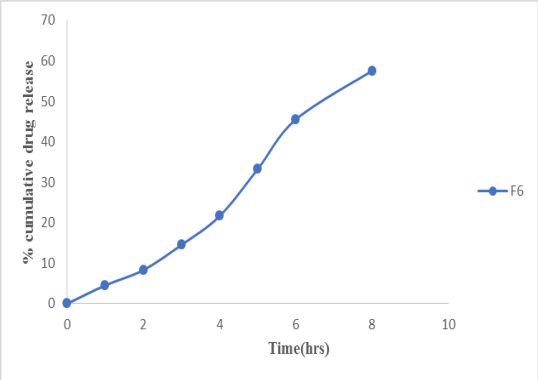

Fig. no. 6: Release profile of microsphere gel.

3. Kinetics of drug release of microsphere gel

Table No. 10: Kinetic release study of microsphere gel.

Time	Log time	Square root of time	% cumulative release of F6	Log % cumulative release of F6	% cumulative remaining	Log % cumulative remaining
0	0.000	0	0	0	100	2
1	0.000	1.000	4.52	0.65	95.48	1.97
2	0.301	1.414	8.32	0.92	91.68	1.96
3	0.477	1.732	14.54	1.16	85.46	1.93
4	0.602	2.000	21.65	1.33	78.35	1.89
5	0.698	2.236	33.28	1.52	66.72	1.82
6	0.778	2.449	45.54	1.65	54.46	1.73
8	0.903	2.82	57.54	1.75	42.46	1.62

Zero order kinetics release

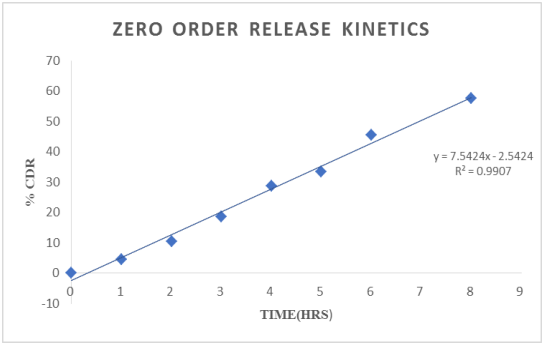

Fig. No. 7(a): Plot of Percentage CDR v/s Time (Zero order kinetics).

First order kinetics release

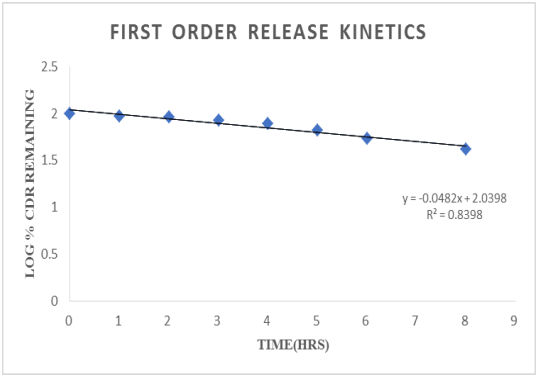

Fig. No. 7(b): Plot of Log Percentage CDR v/s Time (First order kinetics).

Higuchi model

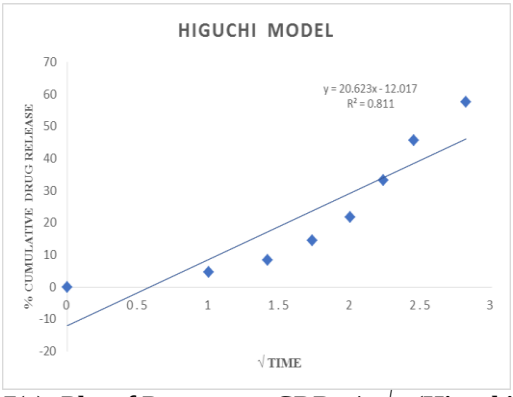

Fig. No. 7(c): Plot of Percentage CDR v/s √ t (Higuchi model).

Peppas Model.

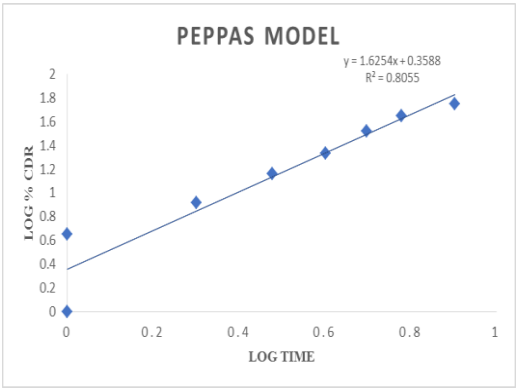

Fig. No. 7(d): Plot of Log Percentage CDRv/s Log Time (Peppas exponential equation).

4. Stability Studies

Table No 11: Evaluation of F6 for stability study.

Evaluation Parameters	Time (days) Accelerated condition 25±2°C at (60±5%RH)			
	0 day	30 days	45 days	90 days
Colour	Colourless	Colourless	Colourless	Colourless
Drug content(%)*	84.98±0.01%	84.32±0.03	83.95±0.02	84.71±0.01
In vitro diffusion in 8 hr	57.54±0.02	57.34±0.02	57.19±0.01	56.88±0.02

*data expressed as mean±SD, n=3

CONCLUSION

The Dapsone microspheres were prepared by solvent evaporation method using ethyl cellulose and Eudragit L 100 as polymers. Three formulations(F1-F3) were prepared using ethyl cellulose and the other three formulations(F4-F6) were prepared using Eudragit L 100. All the microsphere formulations were evaluated for percentage yield, micromeritic properties, SEM analysis, Entrapment efficiency and *in vitro* release studies. The entrapment efficiency of F6 formulation containing Dapsone and Eudragit L100 in 1:2 ratio was found to be 80.22±0.28% which is greater than other formulations. The F5 formulation was selected as best formulation and conveniently incorporated into carbopol 934 gel(1%). The gel formulation were evaluated for various parameters like appearance, viscosity, spreadability, drug content, pH, invitro diffusion studies, kinetic studies, stability studies and the results were found to be reproducible. *In vitro* release studies of microsphere gel showed that the drug release is maximum at 8 hr that is 57.54±0.02%. Stability studies as per the ICH guidelines of the formulation F6 were carried out for short duration of time (3 month). There was no significant changes found in the evaluation of microsphere gel which indicates that the formulation is fairly stable at storage conditions.

REFERENCES

1. Rajput S, Agrawal P, Pathak A, Shrivasatava N, Baghel SS, Baghel RS. A review on microspheres: methods of preparation and evaluation. World journal of pharmacy and pharmaceutical sciences, 2012; 1: 422-38.
2. Saini S, Kumar S, Choudhary M, Nitesh, Budhwar V. Microspheres as controlled drug delivery system: an updated review. International journal of pharmaceutical sciences and research, 2018; 9(5): 1760-8.
3. Patel KS, Patel MB. Preparation and evaluation of chitosan microspheres containing nicorandil. International journal of pharmaceutical investigation, 2014; 4(1): 32. doi: 10.4103/2230-973X.127738; PMID: 24678460
4. Dhadde Gurunath S, Mali Hanmant S, Raut Indrayani D, Nitalikar Manoj M, Bhutkar Mangesh M. A Review on Microsphere: Types, Method of Preparation, Characterization and Application. Asian Journal of Pharmacy and Technology, 2021; 11(2): 149-55. Doi: 10.52711/2231-5713.2021.00025
5. Kadam N. R. and Suvarna V, Microspheres: A Brief Review, Asian Journal of Biomedical and Pharmaceutical Sciences, 2015; 3(4): 13-15.
6. Pickert A, Raimer S. An evaluation of dapsone gel 5% in the treatment of acne vulgaris. Expert Opin Pharmacother, 2009; 10(9): 1515-21.
7. Deshkar SS, Bhalerao SG, Jadhav MS, Shirolkar SV. Formulation and optimization of topical solid lipid nanoparticles based gel of dapsone using design of experiment. Pharm. Nanotechnol, 2018; 6(4): 264-75.
8. Nithya R, Jerold P, Siram K. Cubosomes of dapsone enhanced permeation across the skin. J Drug Deliv Sci Technol, 2018; 48: 75-81.
9. El-Nabarawi MA, Shamma RN, Farouk F, Nasralla SM. Dapsone-loaded invasomes as a potential treatment of acne: preparation, characterization, and in vivo skin deposition assay. AAPS PharmSciTech, 2018; 19(5): 2174-84.
10. Dreno B. Topical antibacterial therapy for acne vulgaris. Drugs, 2004; 64(21): 2389-97.