DEVELOPMENT OF METHOTREXATE LOADED PRONIOSOMAL FORMULATION FOR THE TREATMENT OF CANCER

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ABSTRACT

BACKGROUND:

Methotrexate is an antineoplastic antimetabolite with immunosuppressant properties. It is used in the treatment of certain cancers like breast, skin, and lung. It also used to treat severe psoriasis or rheumatoid arthritis. Major problem associated with methotrexate is its adverse effects such as Immunosuppression, Bone marrow suppression, Diarrhea, Pneumonitis, Hepatic fibrosis, Pruritic rash. So, in order to avoid these adverse effects of methotrexate there is a need to develop novel drug delivery system for this drug.

METHODS:

Methotrexate loaded proniosomes were prepared by slurry method. Total twelve formulations (P1-P12) were prepared, in which first three formulations were prepared by altering carrier concentration, four formulations were prepared by varying drug to surfactant ratio and five formulations were prepared by altering lipid concentration. All the formulations are evaluated for drug content, entrapment efficiency and drug release studies.

RESULTS:

Among all the prepared proniosomal formulations the P3 formulation having 500mg as a carrier was considered to be the best formulation because of its highest drug content of 60.9%, highest entrapment efficiency of 87.6%, mean particle diameter of 706.9nm, zeta potential value of -32.9mV and Invitro drug release data showed 68% of drug release sustained upto 12hrs and followed zero order kinetics with non fickian diffusion mechanism.

CONCLUSIONS:

In this study methotrexate loaded proniosomes were prepared by slurry method. The best formulation was exhibiting good entrapment efficiency with sustain release property.

KEYWORDS: Methotrexate, Proniosomes, Span 60, cholesterol, Slurry method.

BACKGROUND:

The aim of this study was to develop and characterize methotrexate loaded proniosomes by slurry method by using dextrose as carrier, cholesterol as lipid, span 60 as surfactant (1,2).

The Controlled drug delivery systems are often formulated to permit the establishment and maintainence of drug concentrations at target site for longer interval of time in order to impove therapeutic efficacy and to reduce the side effects by preventing undesired drug localization in healthy tissue site and decreasing rapid degradation or elimination of drugs. The Nanotechnology is an advancing technology which helps to prepare new formulations covering various routes of administration to achieve their controlled or target drug delivery (3,4). In this, the preparation of proniosomes derived niosomes which are superior to conventional niosomes provide convenience in dosing, transport and storage. The aim of novel drug delivery system is maintainence of constant and effective drug level in the body by using drug carriers such as polymeric micelles, niosomes, liposomes, proniosomes, nanoparticles, microspheres. Proniosomal systems are microscopic lamellar structures which consist of non- ionic surfactant of alkyl or dialkyl polylgycerol ether class of cholesterol followed by hydration in aqueous media. The surfactant molecule here consists of hydrophilic end of non ionic surfactant which orient outwards, while the hydrophobic end is in opposite direction to form bilayer. In proniosomes, this bilayer is made up of non ionic surface active agent which may be unilamellar or multilamellar based on method of preparation of proniosomeS(5,6). The Proniosomes are dry powder formulation which provides optimal flexibility, unit dosing and makes further processing and packaging possible. This Proniosomal system serves as a rate limiting barrier for absorption of drugs. These systems also overcome the permeation barrier of the skin and act as a penetration enhancers for the drugs because of the ampiphillic nature of vesicles; they are more stable and compatible with the skin. Provesicular system can be simply converted into vesicular system,

which presents a useful vesicular delivery concept with potential to deliver drugs via transdermal drug delivery(7,8).

DRUG: METHOTREXATE

Methotrexate is an antineoplastic antimetabolite antifolate drug with immunosuppressant properties. It is used in the treatment of certain cancers like breast, skin, and lung cancer. It also used to treat severe psoriasis or rheumatoid arthritis that has not responded to other treatments. Methotrexate is also an inhibitor of tetrahydrofolate dehydrogenase and also prevents the formation of tetrahydrofolate, necessary for the synthesis of thymidylate, an essential component of DNA[14,15]. It is a BCS class IV drug with low solubility, low permeability. As it is a low soluble drug we can improve the solubility and enhance the permeability by preparing the proniosomal formulation. Methotrexate is available as niosomal formulation in the form of tablets, solutions. As methotrexate loaded niosomal formulation are suffering with stability issues, these proniosomes are considered to be a novel carrier to improve the stability of niosomal formulation. Major problem associated with methotrexate is its adverse effects such as Immunosuppression, Bone marrow suppression, Diarrhea, Pneumonitis, Hepatic fibrosis, Pruritic rash. So, in order to avoid these adverse effects of methotrexate there is a need to develop novel drug delivery system for this drug. The proniosomes because of its vesicular structure are capable of penetrating more into the systemic circulation. So, in the present study attempts have been made to prepare and evaluate methotrexate loaded proniosomes(9,10).

METHODS:

The aim of this study was to develop and characterize methotrexate loaded proniosomes by slurry method. All the formulations are evaluated for drug content, entrapment efficiency and drug release studies (11,12).

- **Materials:**
  Drug: Methotrexate
  Surfactant: Span 60
  Lipid: Cholesterol
  Carrier: Dextrose
  Solvent: Chloroform and Methonol

SLURRY METHOD:

Proniosomes can be prepared by using a carrier and surfactant solution in a round bottomed flask which is fitted to rotary flash evaporator and vacuum was applied to form a dry and free flowing powder. Finally, the formulation should be stored in tightly closed container under refrigeration in light. The time required for proniosomes production is independent of the ratio of surfactant solution to carrier material and appears to be stable. The proniosomal powder formed is collected and sealed in containers and stored at 4°C. The required volume of surfactant and cholesterol stock solution per gram of carrier and drug should be dissolved in the solvent in 100 ml round bottom flask containing the carrier. Additional chloroform can be added to form slurry in case of lower surfactant loading. The flask has to be attached to a rotary flash evaporator at a temperature of 64˚C and a reduced pressure of 600mm Hg. Then above mixture was dried for overnight. Proniosomes formed were observed under projection microscope (13,14).

OPTIMIZATION PARAMETERS:

Slurry method were optimized for stirring speed and stirring time. Total six formulations were prepared in which three formulations were prepared by varying stirring speed and other three formulations were prepared by varying stirring time (15).

Optimization of stirring speed:

Three formulations were prepared by varying stirring speed at 400 rpm, 500 rpm, 600 rpm. It was found that stable proniosomes were formed at a speed of 600 rpm in slurry method (16).

Optimization of stirring time:

Three formulations were prepared by varying stirring time at 30min, 45min and 1hr. It was found that stable proniosomes were formed at a time of 1hr in slurry method as shown in table 1.

Table 1: Parameters optimized for the preparation of Methotrexate loaded proniosomes by slurry method.

Optimized parameters	Formulation variables
Stirring speed	400 rpm 500 rpm 600 rpm
Stirring time	30 min 45 min 1 hr

Experimental Methodology:

Preparation of methotrexate loaded proniosomes by slurry method:

Weighed quantity of drug was dissolved in methanol, then cholesterol and span60 were dissolved in chloroform separately and then both were mixed. Carrier dextrose was taken in rota flask and slowly above mixture was added to form slurry, further it was kept on rota for solvent evaporation at 64˚c and a pressure of 600mm of Hg. Then above mixture was dried for overnight. Next day proniosomal powder is removed and dried in oven. Proniosomes formed were observed under projection microscope (17). The formulations were shown in table 2 table 3 and table 4

Table 2: Formulations by changing amount of carrier

Formulation	Drug	Surfactant	Drug: surfactant ratio	Lipid	Carrier	Solvent used (methanol: chloroform)
P1	40mg	80mg	1:2	100mg	300mg	2:1
P2	40mg	80mg	1:2	100mg	400mg	2:1
P3	40mg	80mg	1:2	100mg	500mg	2:1

Table 3: Formulations by changing drug to surfactant ratio

Formulation	Drug	Surfactant	Drug: Surfactant ratio	Lipid	Carrier	Solvent used (methanol: chloroform)
P4	40mg	40mg	1:1	100mg	500mg	2:1
P5	40mg	60mg	1:1.5	100mg	500mg	2:1
P6	40mg	80mg	1:2	100mg	500mg	2:1
P7	40mg	100mg	1: 2.5	100mg	500mg	2:1

Table 4: Formulations by changing Lipid ratio

Formulation	Drug	Surfactant	Drug: surfactant	Lipid	Carrier	Solvent used (methanol: chloroform)
P8	40mg	80mg	1:2	50mg	500mg	2:1
P9	40mg	80mg	1:2	100mg	500mg	2:1
P10	40mg	80mg	1:2	150mg	500mg	2:1
P11	40mg	80mg	1:2	200mg	500mg	2:1
P12	40mg	80mg	1:2	250mg	500mg	2:1

Evaluation and Characterization of Methotrexate Loaded Proniosomes:

Optical microscopy:

Morphology was determined for all formulations using optical microscopy. The photo microscopic pictures of the preparation was obtained from the microscope by using a digital SLR camera (18).

Drug Content:

Proniosomal suspension equivalent to 10mg was separately taken in 10ml volumetric flask and the volume was made with methanol and sonicated for 10 min to disrupt the vesicles. From this 1 ml of solution was taken and suitable dilutions was made and the concentration of drug was analysed using UV-Spectrophotometer at 303nm (19).

Entrapment Efficiency:

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation at 17000 rpm for 40 min was determined (w) by UV-Spectrophotometry. Amount of the drug in supernatant was subtracted from the total amount of drug added during the preparation (W) effectively, (W-w) will give the amount of drug entrapped in the pellet. The entrapment efficiency is given by the following formula(20)

$$\text{Entrapment efficiency (\%)} = \frac{W-w}{W} \times 100$$

Vesicle size Distribution

The prepared formulations were characterized for vesicle size using zetasizer (Malvern Instruments Ltd). The analysis was performed at a temperature of 25˚C with double distilled water as dispersion medium.

Zeta Potential

The prepared formulations were characterized for zeta potential value in order to know the stability of the formulations. The analysis was performed at a temperature of 25˚C with double distilled water as dispersion medium(21).

Drug Diffusion Studies:

Drug diffusion studies was performed by using the Franz diffusion cell. A known amount of proniosomal suspension was separately pippeted out and transferred to the donor compartment and 50 ml of the pH 7.4 phosphate buffer was taken in the receptor medium. The temperature and stirring speed was adjusted to 37°C and 100 rpm, respectively. At predetermined time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours aliquots of 1ml of samples were withdrawn and same volume was replaced with the fresh medium to maintain the sink conditions. The samples were further analysed using UV Spectrophotometer at 303nm(22).

RESULTS:

Methotrexate loaded proniosomes were prepared by using the slurry method. By altering carrier concentration, by varying drug to surfactant ratio and by altering lipid concentration, total twelve formulations P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, P11, P12 were prepared. They were evaluated and characterized in order to determine the best formulation for the preparation of Methotrexate loaded proniosomes by slurry method.

EVALUATION PARAMETERS:

Optical Microscopy: The prepared proniosomes were evaluated for lamellar structures

Fig. 1 Photomicrographic images of P3 formulation of Methotrexate loaded proniosomes prepared by slurry method (magnification 10x).

They were observed as multi lamellar vesicles as shown in figure 1

DETERMINATION OF EFFECT OF CARRIER CONCENTRATION UPON FORMULATION OF PRONIOSOMES:

Three formulations were prepared by changing carrier concentration from 300mg to 500mg and keeping all other parameters constant. All the obtained formulations has been evaluated for drug content, entrapment efficiency, invitro diffusion studies, particle size and zeta potential.

Results and Discussion of Proniosomal Formulation Prepared by Changing Carrier Concentration:

Vesicle size Distribution

The prepared formulations were characterized for vesicle size using zetasizer (Malvern Instruments Ltd). The analysis was performed at a temperature of 25˚C with double distilled water as dispersion medium.

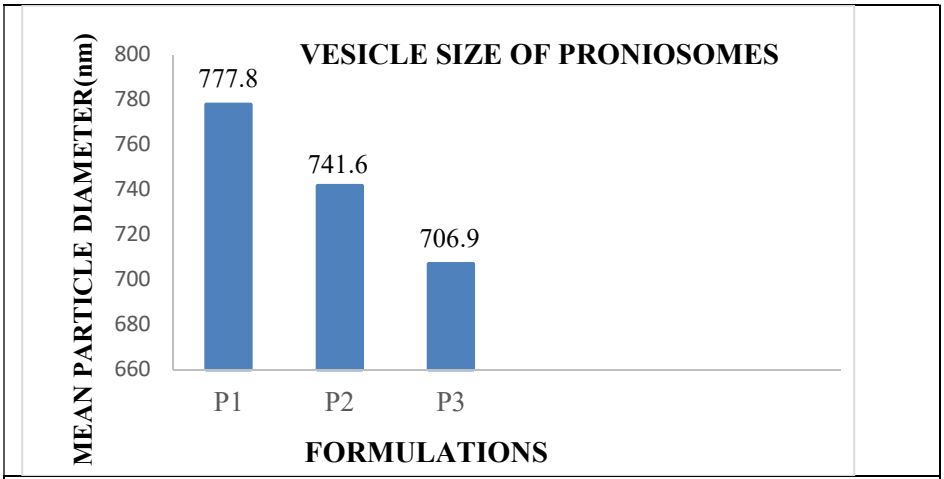


Fig. 2 Comparision of mean vesicle diameter of three formulations of methotrexate loaded proniosomes by slurry method.

All three formulations were in Nano size range as shown in figure 2. The mean vesicle diameter of P1, P2 and P3 formulations was found to be between 706.9nm to 777.8nm respectively. Out of the three formulations the P3 formulation (5:1 carrier to lipid ratio) was found to be best formulation

Zeta Potential

The prepared three formulations were characterized for zeta potential value in order to know the stability of the formulations. The analysis was performed at a temperature of 25˚C with double distilled water as dispersion medium.

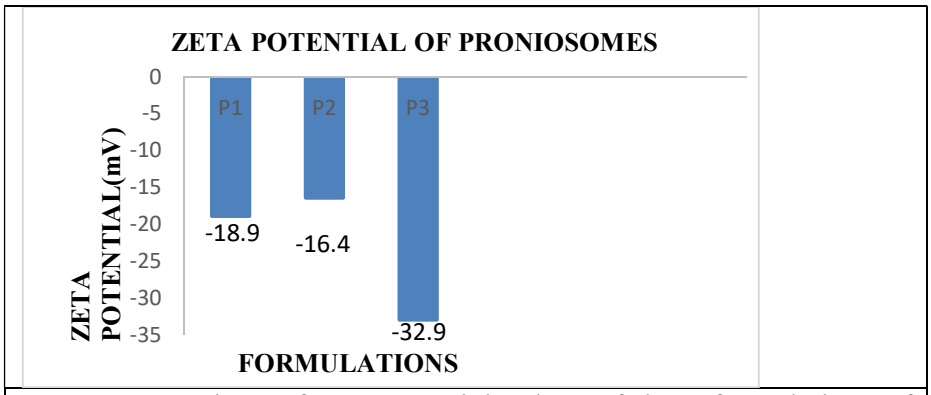


Fig. 3 Comparison of zeta potential values of three formulations of Methotrexate Loaded Proniosomes by Slurry method.

The zeta potential values of P1, P2, P3 formulations was found to be between -18.9 to -32.9mV respectively. Out of the three formulations the P3 formulation (5:1 of carrier to lipid ratio) was found to be stable formulation with highest zeta potential value of -32.9mV as shown in figure 3.

Drug Content:

The prepared three formulations were evaluated for drug content.

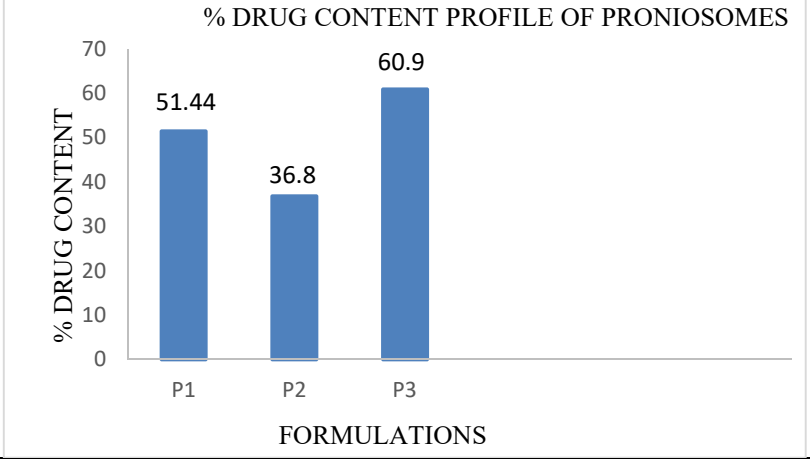


Fig. 4 Comparison of drug content among the three formulations of methotrexate loaded proniosomes by slurry method.

Drug content of P1, P2 and P3 was found to be 51.44%, 36.8% and 60.9% respectively. Out of the three formulations the P3 formulation containing 5:1 ratio of carrier to lipid was considered the best formulation because of its highest drug content of 60.9% as shown in figure 4.

Entrapment Efficiency:

The prepared three formulations were evaluated for entrapment efficiency.

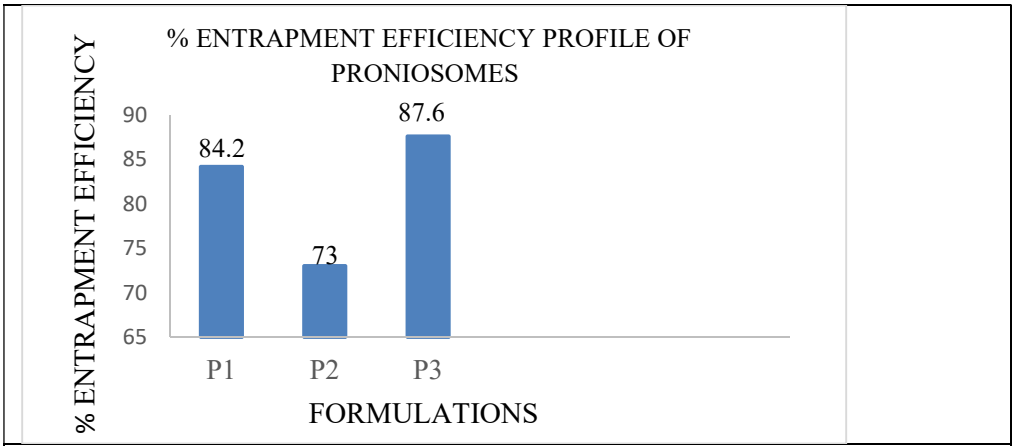


Fig. 5 Comparison of entrapment efficiency among the three formulations of methotrexate loaded proniosomes by slurry method.

Entrapment efficiency of P1, P2 and P3 was found to be 84.2%, 73% and 87.6% respectively. Out of three formulations the P3 formulation containing 5:1 ratio of carrier to lipid was considered the best formulation because of its highest entrapment efficiency of 87.6% as shown in figure 5

In Vitro Diffusion Studies:

The prepared three formulations were evaluated for invitro drug diffusion studies.

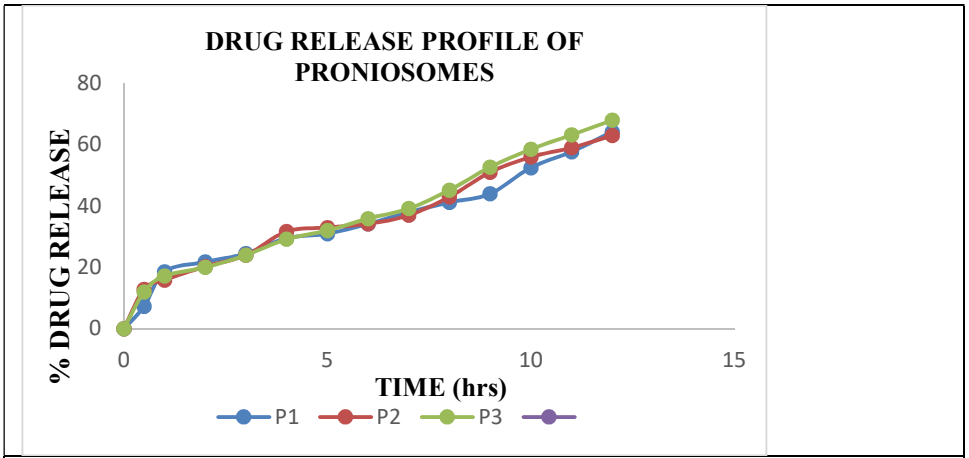


Fig. 6 Comparison of invitro drug diffusion studies among the three

formulations of methotrexate loaded proniosomes by slurry method.

The prepared three formulations were evaluated for invitro drug diffusion studies using Franz diffusion cell. In vitro drug diffusion studies were conducted for a time period of 12 hrs. The percentage of drug release of P1, P2 and P3 formulations was found to be 64.2%, 63% and 68% respectively. P3 formulation containing 5:1 ratio of carrier to lipid was considered the better formulation because of its highest drug release of 68% as shown in figure 6.

DETERMINATION OF EFFECT OF SURFACTANT CONCENTRATION UPON FORMULATION OF PRONIOSOMES:

Four formulations were prepared by altering drug to surfactant ratio i.e., by increasing surfactant concentration from 40mg to 100mg and keeping all other parameters.

Results and Discussion of Proniosomal Formulation Prepared by Changing Drug to Surfactant Ratio:

Vesicle Size Distribution:

The prepared four formulations were characterized for vesicle size using Zetasizer (Malvern Instruments Ltd.). The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

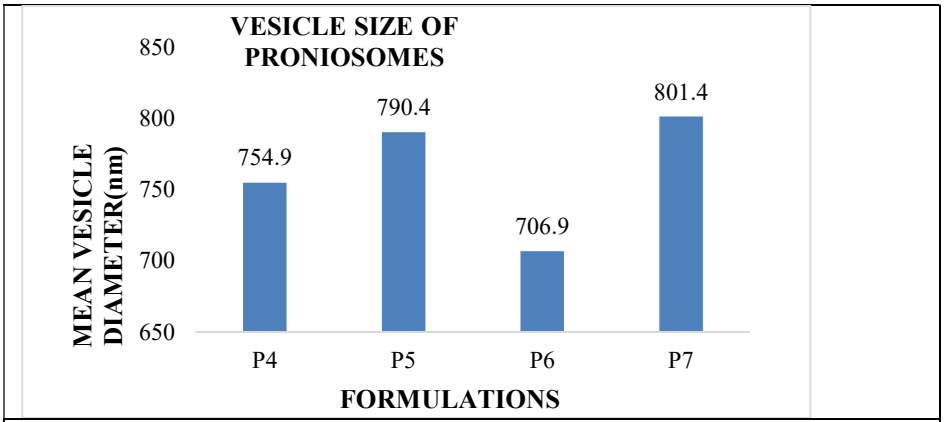


Fig. 7 Comparison of mean vesicle diameter of four formulations of Methotrexate loaded proniosomes by slurry method.

All four formulations were in Nano size range. The mean vesicle diameter of P4, P5, P6 and P7 formulations was found to be between 706.9nm to 801.4nm respectively. Out of the four formulations the P6 formulation (1:2 of drug to surfactant ratio) was found to be the best

formulation with its small mean vesicle diameter of 706.9nm as shown in figure 7.

Zeta Potential:

The prepared four formulations were characterized for zeta potential value in order to know the stability of the formulations. The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

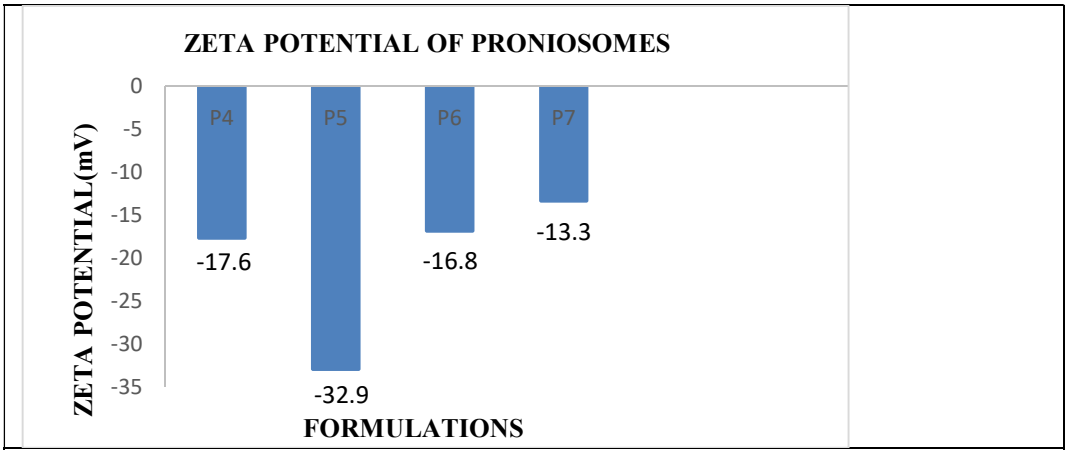


Fig. 8 Comparison of zeta potential values of four formulations of Methotrexate Loaded Proniosomes by Slurry Method.

The zeta potential values of P4 to P7 formulations was found to be between -13.3mV to -32.9mV. Out of the four formulations the P6 formulation (1:2 drug to surfactant ratio) was found to be stable formulation with highest zeta potential value of -32.9mVas shown in figure 8.

Drug Content:

The prepared four formulations were evaluated for drug content.

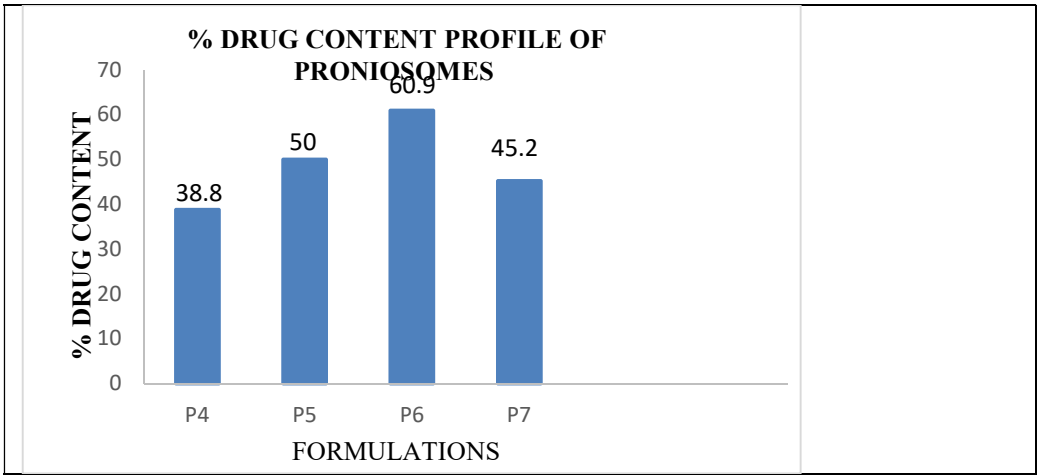


Fig. 9 Comparison of drug content among the four formulations of methotrexate loaded proniosomes by slurry method.

Drug content of P4, P5, P6 and P7 was found to be 38.8%, 50%, 60.9% and 45.2% respectively. Out of the three formulations the P6 formulation containing 1:2 ratio of drug to surfactant was considered the best formulation because of its highest drug content of 60.9%.

Entrapment Efficiency:

The prepared four formulations were evaluated for drug entrapment efficiency using ultracentrifuge.

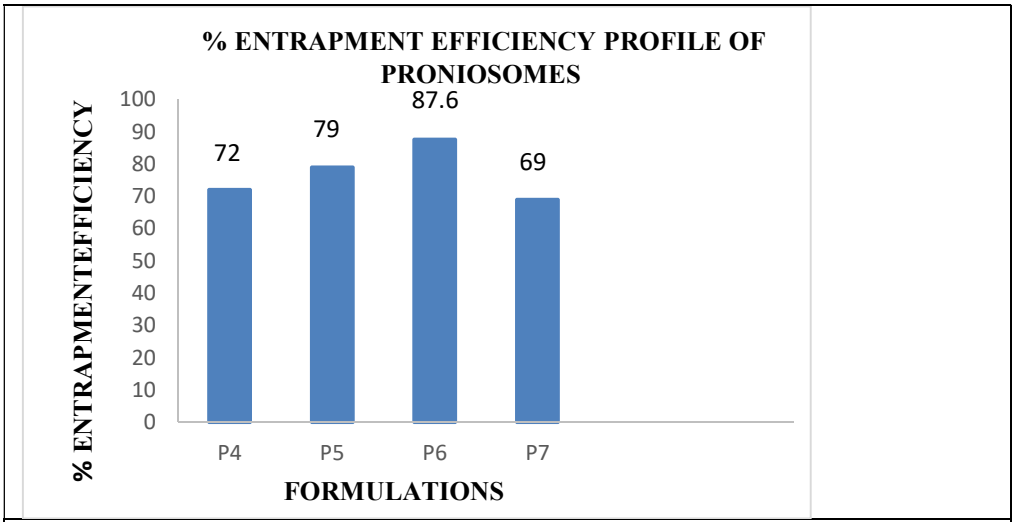


Fig. 10 Comparison of entrapment efficiency among the four formulations of methotrexate loaded proniosomes by slurry method.

Entrapment efficiency of P4, P5, P6 and P7 was found to be 72%, 79%, 87.6% and 69% respectively. Out of the four formulations the P6 formulation containing 1:2 ratio of drug to surfactant was considered the best formulation because of its highest entrapment efficiency of

87.6% as shown in figure 10.

In Vitro Diffusion Studies:

The prepared formulations were evaluated for invitro drug diffusion studies.

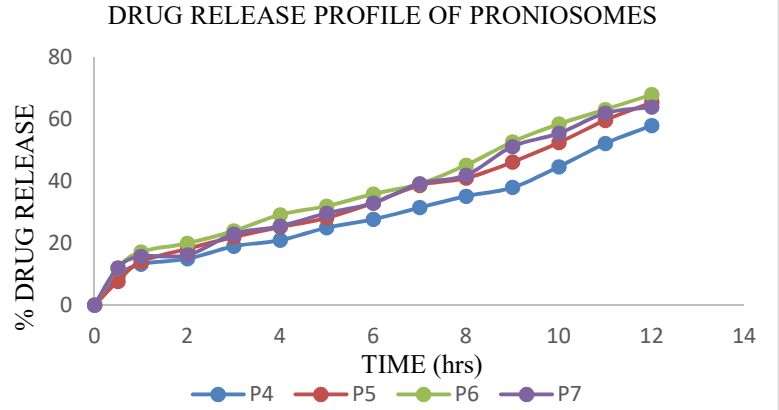


Fig. 11 Comparison of invitro drug diffusion studies among the four formulations of Methotrexate Loaded Proniosomes by Slurry Method.

The prepared four formulations were evaluated for invitro drug diffusion studies using Franz diffusion cell. In vitro drug diffusion studies were conducted for a time period of 12hrs. The percentage of drug release of P4, P5, P6 and P7 formulations was found to be 58%, 65.5%, 68% and 64% respectively. P6 formulation containing 1:2 ratio of drug to surfactant was considered the better formulation because of its highest drug release of 68% as shown in figure 11.

DETERMINATION OF EFFECT OF LIPID CONCENTRATION UPON FORMULATION OF PRONIOSOMES:

Five formulations were prepared by changing lipid concentration from 50mg to 250mg and keeping all other parameters constant.

Results and Discussion of Proniosomal Formulation Prepared by Changing Lipid concentration:

Vesicle Size Distribution:

The prepared five formulations were characterized for the vesicle size using the Zetasizer (Malvern Instruments Ltd.). The analysis was performed at a temperature of 25°C with double

distilled water as dispersion medium.

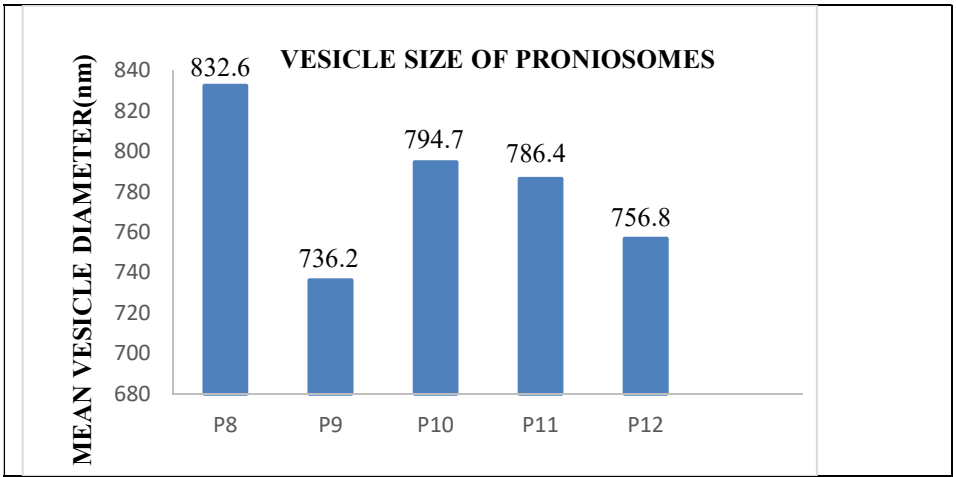


Fig. 12 Comparison of mean vesicle diameter of five formulations of Methotrexate Loaded Proniosomes by Slurry Method.

All five formulations were in nano size range. The mean vesicle diameter of P8, P9, P10, P11 and P12 formulations was found to be between 736.2nm to 832.6 nm respectively. Out of the five formulations the P9 formulation (100mg of lipid concentration) was found to be the best formulation with its small mean vesicle diameter of 736.2nm as shown in figure12.

Zeta Potential:

The prepared five formulations were characterized for zeta potential in order to know the stability of the formulations. The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

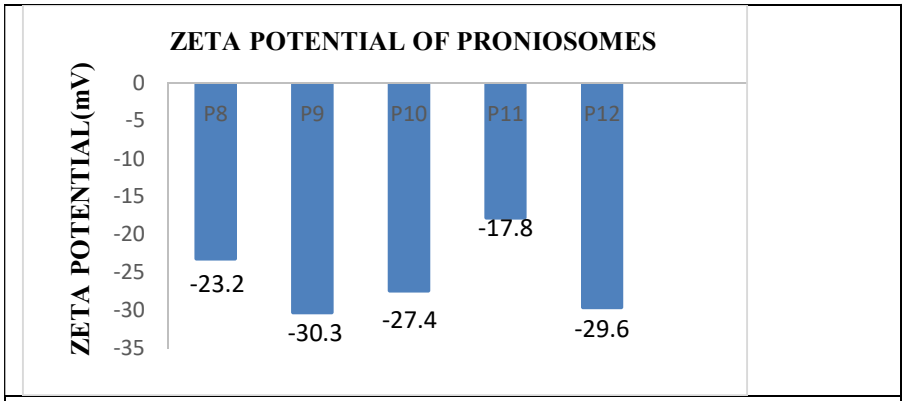


Fig. 13 Comparison of zeta potential of five formulations of Methotrexate Loaded Proniosomes by Slurry Method.

The zeta potential values of P8 to P12 formulations was found to be between -13.3mV to -32.9mV. Out of the five formulations the P9 formulation (100mg of lipid conc.) was found to be stable formulation with highest zeta potential value of -32.9mV as shown in figure 13.

Drug Content:

The prepared five formulations were evaluated for the drug content.

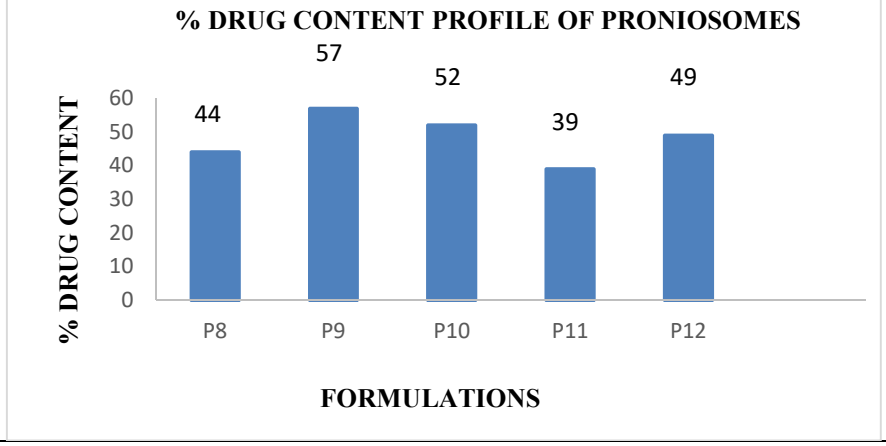


Fig. 14 Comparison of drug content among the five formulations of methotrexate loaded proniosomes by slurry method.

Drug content of P8, P9, P10, P11 and P12 was found to be 44%, 57%, 52%, 39% and 49% respectively. Out of the five formulations the P9 formulation containing 100mg of lipid concentration was considered the best formulation because of its highest drug content of 57% as depicted in figure 14.

Entrapment Efficiency:

The prepared five formulations were evaluated for the drug entrapment efficiency using ultracentrifuge.

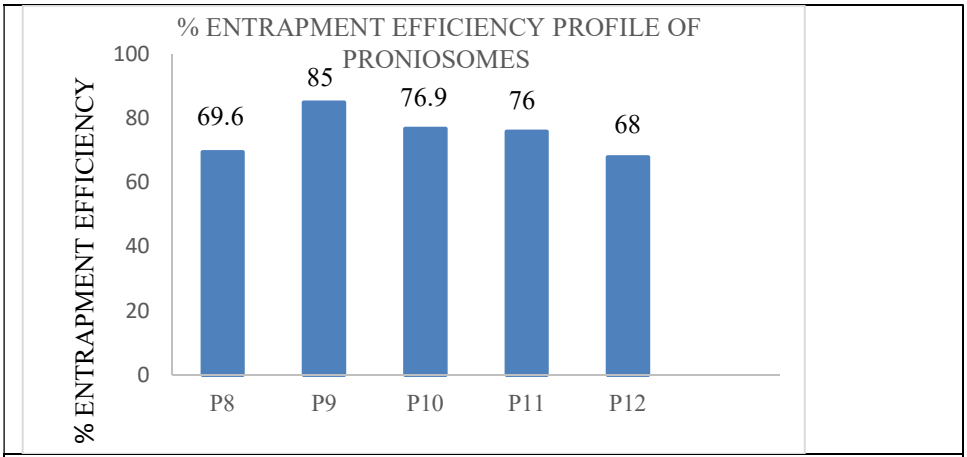


Fig. 15 Comparison of entrapment efficiency among the five formulations of Methotrexate Loaded Proniosomes by Slurry Method.

Entrapment efficiency of P8, P9, P10, P11 and P12 was found to be 69.6%, 85%, 76.9%, 76% and 68% respectively. Out of the five formulations the P9 formulation containing 100mg of lipid concentration was considered the best formulation because of its highest entrapment efficiency of 85% as shown in figure 15.

In Vitro Diffusion Studies:

The prepared five formulations were evaluated for the invitro drug diffusion studies.

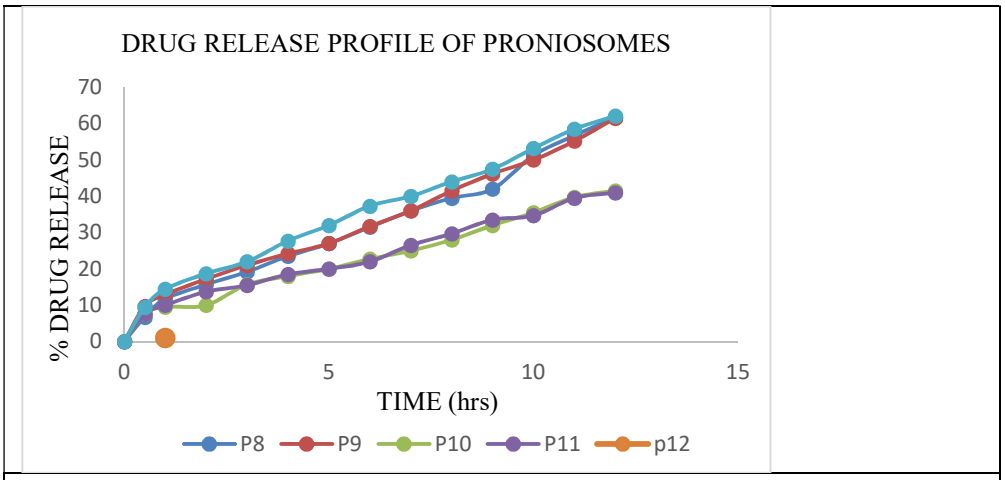


Fig. 16 Comparison of invitro drug diffusion studies among the five formulations of methotrexate loaded proniosomes by slurry method.

The prepared five formulations were evaluated for in-vitro drug diffusion studies using Franz diffusion cell. In vitro drug diffusion studies were conducted for a time period of 12hrs. The percentage of drug release of P8, P9, P10, P11 and P12 formulations was found to be 61.7%,

62%, 61.5%, 41.5% and 41% respectively. P9 formulation containing 100mg of lipid concentration was considered the better formulation because of its highest drug release of 62%.

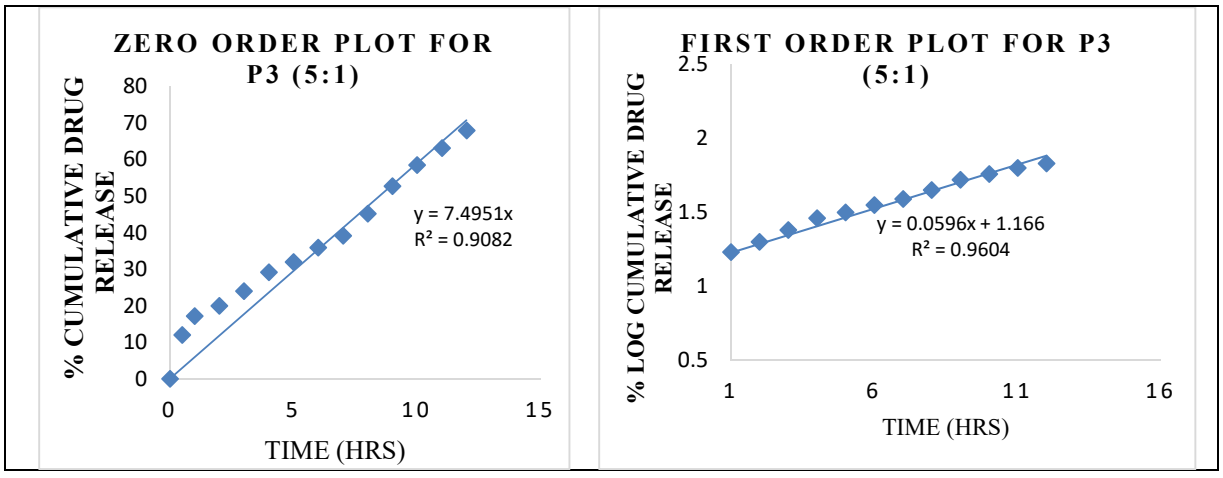
Table 5: In-vitro drug release data of P3 best formulation of Methotrexate Loaded Proniosomes by Slurry method.

Time (hr)	Cumulative drug release(%) n=3	Drug remaining (%)	Log drug remaining (%)	$\sqrt{T}$	Log T	Log cumulative drug release (%)
0.5	12	88	1.94	0.70	-0.30	1.07
1	17.2	82.8	1.91	1	0	1.23
2	20	80	1.90	1.41	0.30	1.30
3	24	76	1.88	1.73	0.47	1.38
4	29.2	70.8	1.85	2	0.60	1.46
5	32	68	1.83	2.23	0.69	1.50
6	35.9	64.1	1.80	2.44	0.77	1.55
7	39.2	60.8	1.78	2.64	0.84	1.59

8	45.2	54.8	1.73	2.82	0.90	1.65
9	52.7	47.3	1.67	3	0.95	1.72
10	58.5	41.5	1.61	3.16	1	1.76
11	63.2	36.8	1.56	3.31	1.04	1.80
12	68	32	1.83	3.46	1.07	1.83

Drug Release Kinetic Plots for P3 Proniosomal Formulation:

Several plots (zero order plot, first order plot, higuchi plot and peppas plot) were drawn for the optimized proniosomal formulation in order to know the release kinetics and drug release mechanism.



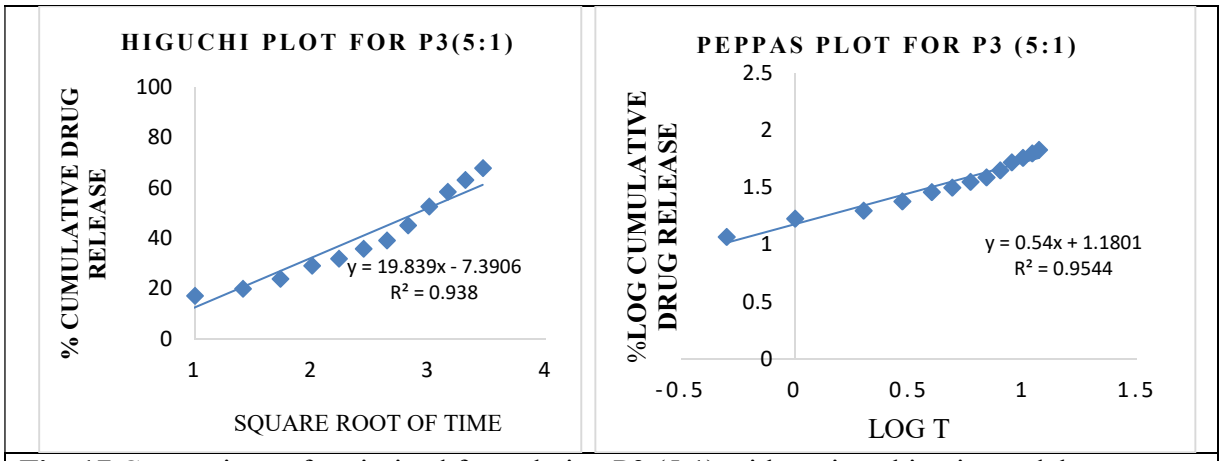


Fig. 17 Comparison of optimized formulation P3 (5:1) with various kinetic models.

Table 6: Kinetic data of P3 formulation

Formulation	The Zero order plot (R^2)	The First order plot (R^2)	The Higuchi plot (R^2)	The Peppas plot (n)
P3	0.908	0.960	0.938	0.54

From the result it was concluded that the drug release was following zero order kinetics and fitted into korsmeyer equation revealing non fickian diffusion mechanism.

DISCUSSION

Methotrexate known as amethopterin, is an antineoplastic antimetabolite antifolate drug with immunosuppressant properties(23,24). It is used in the treatment of certain cancers like breast, skin, and lung cancer. It also used to treat certain types of cancer or to control severe psoriasis or rheumatoid arthritis that has not responded to other treatments. Methotrexate is also an inhibitor of tetrahydrofolate dehydrogenase and also prevents the formation of tetrahydrofolate, necessary for synthesis of thymidylate, an essential component of DNA. Major problem with this methotrexate is its side effects. So, in order to avoid adverse effects of methotrexate there is a need to adopt novel drug delivery approaches in the design of dosage form. In the present study vesicular drug delivery system is designed for methotrexate. Attempts have been made to prepare and characterize methotrexate loaded proniosomes (25, 26).

Methotrexate loaded proniosomes were prepared by slurry method. Total twelve formulations (P1-P12) were prepared. The first three formulations (P1, P2 and P3) were prepared by varying carrier concentration (300mg, 400mg, 500mg) by keeping all the other parameters constant. Among the three formulations the P3 formulation having 500mg as carrier is giving the better result with good drug content, entrapment efficiency, zeta potential, mean particle diameter and invitro drug diffusion.

After optimizing the carrier concentration four formulations were prepared by varying drug to surfactant concentration. Keeping the concentration of carrier and lipid constant, the surfactant concentration was varied from 40mg to 100mg in the P4-P7 formulations. On comparison the P6 formulation containing 1:2 ratio having 80mg of surfactant was showing good drug content and highest entrapment efficiency and better in-vitro drug diffusion.

After optimizing the surfactant concentration five formulations were prepared by altering lipid concentration. Keeping the concentration of drug and surfactant constant, the lipid concentration was varied from 50mg to 250mg in the P8-P12 formulations. On comparison the P9 formulation having 100mg cholesterol was showing good drug content and highest entrapment efficiency and better in-vitro drug diffusion.

From this work the concentration of carrier, surfactant and lipid required for the preparation of proniosomal formulation of Methotrexate were optimized. From the study 500mg carrier, 80mg of surfactant and 100mg lipid were considered to be the optimum concentrations for the preparation of proniosomes. By increasing the surfactant concentration, the drug content and entrapment efficiency was reduced, it may be because of drug-surfactant complex formation. By increasing the lipid concentration from 50mg to 250mg, initially increase lipid concentration is increasing the entrapment efficiency. Once reaching the particular point further increase of lipid concentration is decreasing the entrapment efficiency because of higher concentration of lipid is showing the opposing effect, but initially up to 100mg concentration it is showing positive effect.

Successful proniosomal formulations were prepared for Methotrexate. On comparison of all the prepared proniosomal formulations (P1- P12) the P3 formulation having 500mg of carrier is showing good result having drug content of 60.9%, entrapment efficiency of 87.6%, mean particle diameter of 706.9 nm, zeta potential of -32.9 mV and in-vitro drug release of 68% in time period of 12 hrs.

CONCLUSIONS

Methotrexate known as amethopterin, is an antineoplastic antimetabolite antifolate drug with immunosuppressant properties. Methotrexate is an inhibitor of tetrahydrofolate dehydrogenase and also prevents the formation of tetrahydrofolate, necessary for synthesis of thymidylate, an essential component of DNA. Major problem with this methotrexate is its side effects. So, in order to avoid adverse effects of methotrexate there is a need to adopt novel drug delivery approaches in the design of dosage form. In the present study vesicular drug delivery system is designed for methotrexate. Attempts have been made to prepare and characterize methotrexate loaded proniosomes.

For the preparation of methotrexate loaded proniosomes by slurry method, the process parameters such as optimization of stirring speed and stirring time were optimized. Formulations were evaluated for product yield, drug content, entrapment efficiency, and loading capacity. Mean particle diameter and zeta potential values were also compared.

Total twelve formulations (P1-P12) of proniosomes were prepared, out of this twelve formulations P3 formulation having 500mg as carrier was found to be the best formulation and based on the results it was concluded that slurry method was considered as the desirable and ideal technique for the preparation of methotrexate loaded proniosomes.

LIST OF ABBREVIATIONS

PRN	Proniosomes
TDDS	Transdermal Drug Delivery Systems
PS	Particle Size
EE	Entrapment Efficiency
ZP	Zeta Potential
MTX	Methotrexate
FTIR	Fourier Transform Infrared Spectroscopy
RPM	Rotation Per minute

nm	Nanometer
g/mol	Gram per mole
mV	Milli volts
μm	Micrometer
mg	Microgram
Log	Logarithm
Hrs	Hours
min	Minutes
sec	Seconds
%	Percentage

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIAL
The data supporting this findings of the study are available within the article.

COMPETING INTERESTS
The authors declares no competing interest, financial or otherwise.

FUNDING
None.

AUTHORS CONTRIBUTIONS
All authors contributed to the study conception and design.

ACKNOWLEDGEMENTS
Declared none.

REFERENCES

1. Alsarra, A.A. Bosela, S.M. Ahmed, and G.M. Mahrous, 2004."A Proniosome as a drug carrier for transdermal delivery of ketorolac". Eur. J.Pharm. and Biopharm. **2**(1): 1-6.
2. Biju SS, Misra PR, Khar RK Talegoankar S.2006. "Vesicular systems : An overview". Ind. J. Pharm. Sci, **68**: 141-153.
3. Baillie AJ, Florence AT, Hume LR, Muirhead GT and Rogerson A.1985."Preparation and properties of the niosomes non ionic surfactant vesicles". Journal of pharmaceutics and pharmacology,**37**, 863-868.
4. Brahmankar, D.M., and Jaiswal, S. B.1995. "In; The Biopharmaceutics And Pharmacokinetics A Treatise", 1st Edn., Vallabh Prakashan Publications, Delhi, 50-57.
5. Blazek- Welsh AI, Rhodes DG. 2001."Scanning Electron Microscopy Imaging predicts quality of niosomes from maltodextrin based proniosomes".Pharm Res. **18**:656-661.
6. Chain YW.1992. "Oral Drug Delivery And Delivery System", In novel drug delivery system, 2nd edition (James Swarbrick, ed.), Marcel Dekker, New York, 139-196.
7. Fulzele SV, Satturwar PM, Nande VS and Khandare JN.2002. "Formulation And Evaluation Of Ketoconazole Niosomes",Indian J. Pharm, **64**(2): 155-158.
8. Hu C and Rhodes D.1999. "Proniosomes : the novel drug carrier preparation". International Journal of Pharmaceutics, **185**, 23-35.
9. Malhotra M. and Jain N.K. 1994."Niosomes as drug carriers". Indian Drugs, **31**(3), 81-86.
10. Nagasamy Venkatesh D, Swetha Priyanka, Tulasi, K. Kalyani, Sheik Abid Ali, Harikrishna Jilakara.2014. "Proniosomes: A superior drug delivery system". Int. J. Pharm. Sci. Drug Res, **6** (3), 178-182.
11. Rhodes DG , Almira I, Blazek- Welsh.2001. "Maltodextrin based proniosomes". AAPS PharmsciTech, **3**(1) ,article 1
12. Radha GV, Rani TS, Sravani B.2013. "A review on the proniosomal drug delivery system for targeted drug action". J Basic Clin Pharm, **4**, 42-8.
13. Santosh Kumar, Rishikesh Gupta, S.K. Prajapati, Nikhil Gupta, Snigdha Patnaik and Ashu Agarwal.2014. "The Proniosomes formulation and evaluation by slurry method as an emerging provesicular drug carrier in NDDS". A World Journal of Pharmacy and Pharmaceutical Science, **3**(9), 1486-1501.

14. Suresh P. Vyas., Ijeoma F.,1998. Uchegbu.“Non- ionic surfactant based vesicles (Niosomes) in drug delivery”. International Journal Of Pharmaceutics, **172**, 33-70.
15. T. Sudhamani, V.Ganesan, N. Priyadarsini, M. Radhakrishnan, 2010,“Formulation of evaluation of Ibuprofen Loaded Maltodextrin Based Proniosome”. International Journal of Biopharmaceutics, **1**(2), 75-81.
16. Berenguer D, Alcover MM, Sessa M, Halbaut L, Guillen C, Boix-Montanes A, Fisa R, Calpena Campmany AC, Riera C, Sosa L.2020.Topical amphotericin B semisolid dosage form for cutaneous leishmaniasis: physicochemical characterization, ex vivo skin permeation and biological activity. Pharmaceutics,12(2),1-16. doi: 10.3390/pharmaceutics12020149, PMID 32059430
17. Cohen BE.2016. The role of signaling via aqueous pore formation in resistance responses to amphotericin b. Antimicrob Agents Chemother. 60(9),5122-9.
18. Venkatesh Gavini, M Srinivasa Murthy, K Srinivas Rao, P Kiran Kumar.2014.“Formulation and the Invitro Evaluation of the Buoyant Drug Delivery System of Glipizide using Acrylic Polymer”, Journal of Drug Delivery and Therapeutics, **4**(6), 107-113.
19. Lu C.T., Zhao Y.Z., Wong H.L., Cai J., Peng L., Tian X.Q. “Current approaches to enhance CNS delivery of drugs across the brain barriers”. Int. J. Nanomed.2014;9:2241–225 .
20. Okafor N.I., Nkanga C.I., Walker R.B., Noundou X.S., Krause R.W.M.2020. “Encapsulation and physicochemical evaluation of efavirenz in liposomes”. J. Pharm. Investig, 50,201–208.
21. Sargazi S., Hosseinikhah M., Zargari F., Pal N., Chauhana S., Hassanisaadi M., Amani S. 2021.“pH-responsive cisplatin-loaded niosomes: Synthesis, characterization, cytotoxicity study and interaction analyses by simulation methodology” . Nanofabrication 6,1–15.
22. Marzoli F., Marianecci C., Rinaldi F., Passeri D., Rossi M., Minosi P., Carafa M., Pieretti S. 2019.Long-lasting, antinociceptive effects of pH-sensitive niosomes loaded with ibuprofen in acute and chronic models of pain. Pharmaceutics, 11,62.
23. Barani M., Hajinezhad M.R., Sargazi S., Rahdar A., Shahraki S., Lohrasbi-Nejad A., Baino F. 2021.“In vitro and in vivo anticancer effect of pH-responsive paclitaxel-loaded niosomes”. J. Mater. Sci. Mater. Med, 32,147.

24. Rinaldi F., Forte J., Pontecorvi G., Hanieh P.N., Carè A., Bellenghi M., Tirelli V., Ammendolia M.G., Mattia G., Marianecci C., et al.2022. “pH-responsive oleic acid based nanocarriers: Melanoma treatment strategies”. Int. J. Pharm. 613, 121391.
25. Jamshidifar E., Eshrati Yeganeh F., Shayan M., Tavakkoli Yaraki M., Bourbour M., Moammeri A., Akbarzadeh I., Noorbazargan H., Hossein-Khannazer N.2021. “Super magnetic niosomal nanocarrier as a new approach for treatment of breast cancer: A case study on sk-br-3 and mda-mb-231 cell lines”. Int. J. Mol. Sci, 22,7948.
26. Maurer V., Altin S., Seleci D.A., Zarinwall A., Temel B., Vogt P.M., Strauß S., Stahl F., Scheper T., Bucan V., et al.2021. In-vitro application of magnetic hybrid niosomes: Targeted sirna-delivery for enhanced breast cancer therapy.Pharmaceutics,13,94.