Formulation and Evaluation of Deflazacort Loaded Solid Lipid Microparticles

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Abstract **- Micro particulates are the colloidal system ranges from 1- 1000 micrometer they can be erodible, non-erodible or ion-exchange resins. These systems were developed to conquer solubility problems of poorly soluble drugs as well as for long-acting injectable depot formulations and specific drug targeting options. The drug is released from the particles through diffusion, chemical reaction, polymer degradation, or ion exchange mechanism. The main objective for these systems was to improve the bioavailability and enhance the sustainability of drug within the body. The in vitro and in vivo studies have shown that this dosage form holds great promise for sustained drug release. The thesis summarizes the present manufacturing methods and the materials used for these delivery systems. The purpose of the present study was to develop solid lipid microparticles (SLMs) of Deflazacort by investigating the relationship between drug/lipid ratios. SLMs were successfully prepared by Solvent emulsification-based method followed by ultrasonication using. Morphology, particle size, entrapment efficiency, drug content, and drug release behavior were investigated, respectively. As a result, the microparticle designed showed nearly spherical particles. The EE (%) and DC (%) could reach up to 89.5% and 93.3%, respectively. In vitro release studies show as lipid concentration increases prolonged release of drug from SLM up to 24 hours was observed. The distribution and penetration pathways of the particulate delivery systems are also described. The applications of drug-loaded particles are presented with focus on enhancing the bioavailability. Productive goals of innovative difficulties will bring about a predominant measurements structure for desired site of action like topical and intraocular ophthalmic application.**

Index Terms **- Colloidal drug carrier system, SLM's, Deflazacort, entrapment efficiency, drug content, drug delivery.**

INTRODUCTION

In today's world there is increasing need of developing suitable drug carrier systems in order to control, localize and improve drug delivery. Many different drug carriers can be used depending on the administration route, the chosen drug properties and the intended drug release profile. The carriers that have been the most often studied in the controlled release of the incorporated substances are:
The two basic deliveries are
1. Lipid based drug delivery
2. Polymeric drug delivery

Drug delivery based on lipid
The lipid-based drug delivery system is the method in which pharmaceutical dosage are designed in such a way that their therapeutic efficacy will be enhanced. In this method the lipid acts as a component of different oily liquid and dispersion that are projected to enhance drug solubility and bioavailability. The most common role of lipid-based formulation has conventionally been to improve the solubility of sparingly water-soluble drug.[1]

Advantage of lipid drug delivery system
a) Controlled and targeted release of drug.
b) pharmaceutical stability.
c) Better and higher drug content as compared to other carriers.
d) Biodegradable and Biocompatible action.
e) Capable to transport hydrophilic and lipophilic drugs.
f) Versatility of the Excipients.

Different kinds of lipid-based drug delivery system: -
Emulsion- An emulsion (oil/water or water/oil) is a dispersion of two immiscible phases in which an emulsifier (surfactant) is added to the external phase to stabilize the dispersed droplets. This system is thermodynamically unstable as the immiscible phases have a tendency to separate with time.

Microemulsion

These are optically isotropic system of water and oil and are stabilized by an interfacial film of co-surfactant and surfactant in which the droplet size is <100nm which have no coalescence tendency, these are less viscous systems and are thermodynamically stable, formed by simple mixing of the constituents and require low shear rate. Microemulsions aids to enhance the bioavailability, solubility and permeation of drugs.

Nano-emulsion

These are biphasic systems (oil and water) that are stabilized by alcohol and surfactants and are metastable preparation which are diluted using water with no variation in droplet, and size of droplet ranges from 200-600nm.

Self-emulsifying drug delivery systems (SEDDS)

These systems have the ability to emulsify due to existence of one or more surfactants in addition to oily phase. The lipophilic drug gets dissolved in the oily phase and the oily phase gets dispersed in the gastrointestinal fluid with the help of surfactants which gives micro-emulsion. These systems are categorized as self-nano emulsifying drug delivery and self-micro emulsifying drug delivery system, which depends on the size of the emulsion particles. SMEDDS is anisotropic mixture of surfactant, co-surfactant, lipids and drug.

Microparticle

Microparticulate drug delivery is one of the procedures to give the sustained and controlled conveyance of drug to significant stretches of time. They are small particles of solids or liquid encompassed by natural or synthetic polymeric films and there diameter ranges from 1 micrometer to 1000micrometer.9 At first use of albumin microspheres in drug delivery system was recommended by Kramer in 1974. In 1997, Java Krishna and Catha proposed the use of microspheres as sustained release vehicles. There additionally reports about using hemoglobin as natural biodegradable carriers for microparticulate drug delivery 10. Microparticles have been end up being a perfect method of getting sustained and controlled release dosage form. They are likewise a gainful method of delivering APIs which are pharmacologically active but are tough to deliver because of low water solubility. In such drugs the accomplishment of high Cmax, Tmax, and Area under the curve is problematic.[3] Microsphere-based formulations can be figured to give a steady drug concentration in the blood or to target site [4-5]

MORPHOLOGY OF MICROPARITCLE

Two general morphologies of microparticles are their - microcapsules and microspheres. Microcapsule (Fig.1.1) is a system in which drug containing core is completely surrounded by a polymer shell. The core can be solid, liquid or gas; the shell is a continuous, porous or non-porous polymeric layer.[6-7]

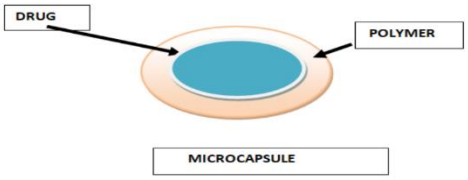


Fig. 1.1 microcapsule

However, the morphology of the internal structure of a micro particle depends on the shell materials and the micro encapsulation methods that are employed. Microsphere (Fig.2) is a system in which the drug substance is either homogenously dissolved or dispersed in a polymeric matrix. Microspheres show different release properties compared to true microcapsules.

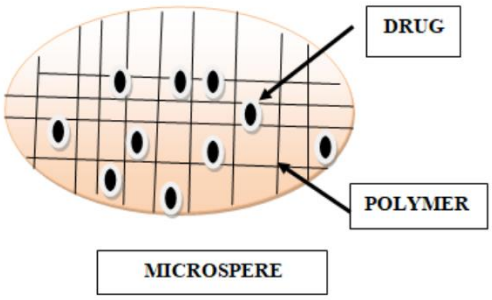


Fig.2: Microsphere

Advantages of microparticles in drug delivery:[8-9]

- Microparticles can be effectively managed and enter inside the film.
- Microparticles can control the arrival of the medication during the transportation and at the site of confinement.
- Controlled discharge and molecule corruption qualities can be promptly tweaked by the decision of framework constituents. Medication stacking is generally high and medication can be

consolidated into the frameworks with no synthetic response; this is a significant factor for saving the medication action.

- Site-explicit focusing on can be accomplished by appending focusing on legends to surface of particles or utilization of attractive direction.
- The system can be used for various routes of administration such as oral, nasal,
- parenteral, intra-ocular and topical etc.[10-11]

Types of microparticles:

- Microparticles
  - Polymeric microparticles
  - Solid lipid microparticles
- Microcrystals
  - Microsuspensions
  - Microemulsion [12]

MATERIALS AND METHODS

Powder sample of *Deflazacort* was received as a gift sample from IPCA LABORATORIES, Dehradun.

Table-1: List of chemicals used and its manufacturers:

INGREDIENTS	PURPOSE	SOURCE
Deflazacort	Drug (API)	IPCA Laboratories
Glyceryl mono stearate	Lipid	Yarrowpharma
Tween 80	Emulsifier	Finar limited
Ethanol	Solvent	Finar limited
Potassium Dihydrogen phosphate	Buffer	Finar limited
Disodium Hydrogen phosphate	Buffer	Finar limited
Carbopol 940	Gelling agent	Otto chemika
Sodium alginate	Gelling agent	SD fine-chem limited
Poloxamer-188	Gelling agent	Yarrowpharma
Dialysis membrane-70	Drug release	HiMedia, Mumbai

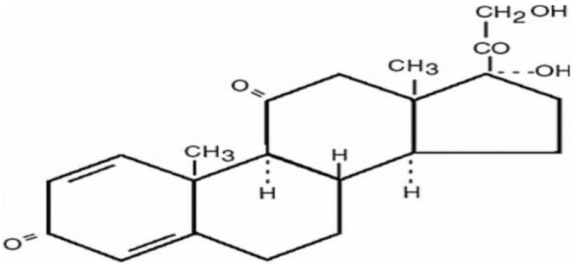
Table -2: List of instruments & equipment's used and its manufacturer:

INSTRUMENTS	MODEL	MANUFACTURER
Digital balance	CX 220	Citizen scale, Delhi
Melting point apparatus	-	Scientech
Magnetic stirrer	-	REMI
Mechanical stirrer	-	REMI
Ultrasonic probe sonicator	DP 120	PCI Analytes
Cooling centrifuge	412 LAG	REMI instruments division
pH meter	-	HANNA Instrument
UV spectrophotometer	UV-1800	Shimadzu, Delhi
Brookfield viscometer	LVDVE	Brookfield engineering lab.
Microscope	-	Teknik
Humidity chamber	-	Scientech
Bath sonicator	-	Scientech

DRUG PROFILE- DEFLAZACORT

Chemical name: 11β,16β)-21-(acetyloxy)-11-hydroxy-2'-methyl-5'H-pregna-1,4-dieno[17,16 d]oxazole-3,20-dione3.

Structure



Molecular formula: C25H31O6

Molecular Weight: 441.57 gm/mol

Melting Point: 252.0 to 258.0℃40 40

Description: White to off-white crystalline solid powder.

Solubility: It is soluble in dichloromethane, poorly soluble in water and in methanol.

Dose : 6mg-30 mg

Storage Conditions: Store at room temperature between 20-250C.

Pharmacology: Deflazacort significantly inhibits proliferation of human peripheral blood mononuclear cells and also inhibits release of certain cytokines in these cells. Deflazacort administration has shown depletion of CD4+ lymphocytes along with increase in the CD8+ subset. Deflazacort also has potent immunosuppressive action. Half life : 1.5 to 2 hrs

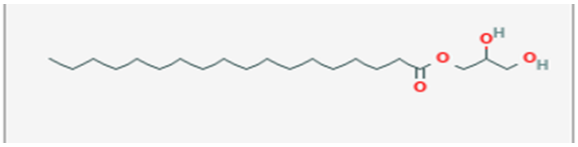
Bioavailability : 70%

Protein binding : 40%

Mechanism of Action: It acts by preventing the release of certain chemicals producing immune and allergic responses, resulting in inflammation. It also decreases the numbers of white blood cells circulating in the blood. This, along with the decrease in inflammatory chemicals, can prevent the rejection of organ transplants, as it prevents the body from attacking foreign tissue,

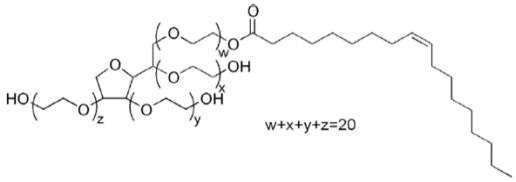
Use: Deflazacort is an oxazoline derivative of prednisolone with anti-inflammatory and immunosuppressive activity.
Deflazacort is used in rheumatoid arthritis, nephritic syndrome, organ transplantation rejection and juvenile chronic arthritis, among other diseases.

EXCIPIENT PROFILE:
Synonyms: Glycerylmonostearate, Glycerin monostearate, Monostearin. Glycerol monostearate, commonly known as GMS.
Chemical structure:



Molecular formula: C21H42O4
IUPAC Name: 2,3-Dihydroxypropyl octadecanoate
Description: White to cream-colored, wax-like solid in the form of flakes.
Molar mass: 358.56 g/ mol
Melting point: 58 to 59 °C
Boiling point: 238 to 239 °C
Density: 0.97 g/cm3
Specific gravity: 0.92
Solubility: Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, and fixed oils, practically insoluble in water, but it may be dispersed in water with the help of a small amount of soap or surfactant.
HLB value: 3.8
Stability and storage: it should be stored in a light resistant, tightly closed container in a cool and dry place.

POLYSORBATE 80:
SYNONYM: Tween 80
Structure:



Chemical name: Polyoxyethylene (20) sorbitanmonooleate
Molecular formula: C64H124O26
Molecular mass: 1310 g/mol
HLB value: 15
Solubility: soluble in ethanol and water, insoluble in mineral oil and vegetable oil.

METHOD AND MATERIAL

METHODS
PREFORMULATION STUDIES:
Pre-formulation studies are the initial phase in rational improvement of dosage form of drug substances. Before the development of the dosage form. It can be characterized as determination of physicochemical properties of a drug substance alone and with different Excipients the principle of Pre-formulation studies is to produce data which was useful to the formulator for the development of the stable and bio available dosage forms. The objectives of the program are as follows:

- To establish the necessary physicochemical characteristics of new drug substances.
- To determine its kinetics release rate profile.
- To establish its compatibility with different Excipients.

Solubility studies:
Solubility study was done by taking water, ethanol, methanol, phosphate buffer (7.8, 7.4) as a solvent and drug was added in the solvent. Absorbance was taken by using UV- visible spectroscopy.[13]

Fig. 3 Solubility study

Determination of absorbance maxima of drug in Methanol:
A reference drug solution of *Deflazacort* was prepared by dissolving 10mg of *Deflazacort* power in 10ml volumetric flask. It was dissolved and diluted to volume with methanol to give stock solution containing 1mg/ml and further dilutions were made of 10microgram/ml (until the absorbance comes below 1). UV- Spectrophotometer scan was taken in the range of wavelength 200-400nm, it give a peak at 244nm.
Partition coefficient:
Partition coefficient provides a means of characterizing lipophilic/hydrophilic nature of the

drug which affect the rate and extent of drug absorption. Partition coefficient (oil phase/aqueous phase) is measure of drug's lipophilicity and its ability to cross cell membrane.[14]

- Drug having value of log P >1 are classified as lipophilic.
- Log P<1 are indicated of hydrophilic drug.

Po/w = (Coil /Caq) Equilibrium

The partition coefficient study was performed using n-octanol as oil phase and water as aqueous phase. *Deflazacort* power (100mg) was added to 50ml each of oil phase and aqueous phase in separating funnel. The mixture was shaken in alpha direction motion continuously until equilibrium was reached and stands for overnight. The two phases were separated within themselves. Both phases were analyzed for respective drug content by measuring the absorbance using a UV Spectrophotometer.

Fig. 4 Partition coefficient

FORMULATION OF *DEFLAZACORT* LOADED SLMS:

Table- 3 Composition of SLMs of *Deflazacort:*

S. No.	Formulation code	Lipids (GMS)	Poloxamer	Solvent(ml) Ethanol	Distilled water (ml)
1	F1	200	200	10	200
2	F2	400	200	10	200
3	F3	600	200	10	200
4	F4	800	200	10	200
5	F5	1000	200	10	200
6	F6	1200	200	10	200
7	F7	1400	200	10	200
8	F8	1600	200	10	200
9	F9	1800	200	10	200
10	F10	2000	200	10	200

PREPARATION OF SOLID LIPID MICROPARTICLES OF DEFLAZACORT:

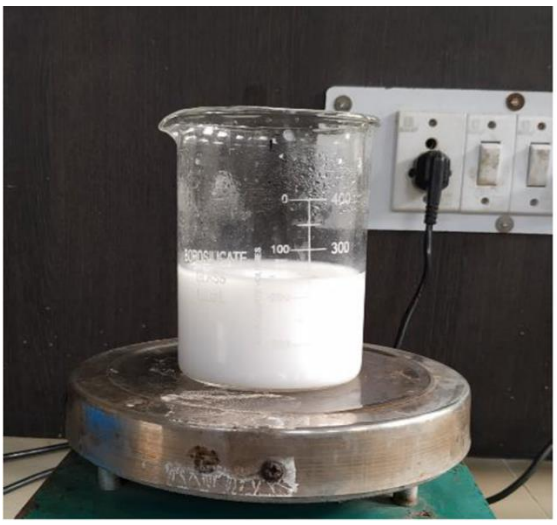


Fig. 5 formulation of SLM

In-Vitro Drug Release Study of SLMs Dispersion:

For in-vitro release study, the batches of *Deflazacort* loaded SLM dispersion (5ml) was added to a dialysis bag (12,000 Da molecular weight, Sigma) using dialysis system comprising of HI Media Dialysis membrane- 70 mounted over jacketed Dialysis tube, which was previously soaked in medium overnight. The dialysis bag was clipped and exposed into500ml conical flask containing 250ml of a phosphate buffer solution (pH 7.4) as a medium. The flask was kept in magnetic stirrer; the solution in receptor side was maintained at 370C ± 0.50C and stirred at 100 rpm with Teflon-coated magnetic stirring bars. The receptor compartment was covered to prevent the evaporation of dissolution medium. At predetermined time intervals (0.5, 1, 2, 3, for 24 hrs.), 5ml aliquots was taken and replaced with the same amount of fresh medium. Sink condition was maintained throughout the experiment by used phosphate buffer (pH 7.4) in release media. The amount of *Deflazacort* released from the SLM was measured by UV spectrophotometer at 244nm.

Fig. 6 in-vitro drug release study

Drug Release Kinetics:
In order to investigate the mechanism of release, the release data were analyzed with the following mathematical model: Zero-order kinetic, First order kinetics, and Higuchi kinetics.

Zero-order model:
In the drug dissolution study, the release of drug is slow; it can represent by the equation:
Qt = Q0 + K0 t
Qt – amount of drug dissolved in time t
Q0 – initial amount of drug in the solution
K0 – zero order release constant.
The graph was plotted as cumulative amount of drug release versus time.

First order model:
This model has shows absorption and elimination of some drugs. The release of the drug which followed first order kinetics can be expressed by the equation:
Log Qt = Log Q_0 – K_1t / 2.303
Q_0 – Initial concentration of drug
K_1 – first order rate constant
t – Time
The plot was developed by log cumulative percentage drug remaining vs. time.

Higuchi model:
It is a hypothetical model based on:
I. Primary concentration of drug in the matrix is more than drug solubility.
II. Diffusion of drug takes place in the one direction.
III. Drug particles are very small than system thickness.
IV. Dissolution rate and swelling properties are negligible
V. Diffusion coefficient is constant.
VI. Skin conditions are maintained. The model expression is given by the equation:
Q = KH t1/2
Where, KH is the Higuchi dissolution constant, t1/2 is square root of time.
The plot was developed by cumulative percentage drug release vs. square root of time.

Preparation of calibration curve of Deflazacort in methanol:
Calibration curve of Deflazacort was prepared in methanol at λmax 244 nm. Deflazacort was found to obey Beer-Lambert's law in the concentration range of 0-20µg/ml with regression coefficient (R2) values 0.998. The absorbance values with their standard deviations at different concentrations in the range of 0-20µg/ml are tabulated in (table 4) and represented in (figure 7).
Table 4. Calibration Curve of Deflazacort in methanol n-3 (Mean±S.D.):

S. No.	Concentration (µg/ml)	Absorbance
1	0	0 ± 0
2	5	0.03 ± 0.0054
3	10	0.059 ± 0.004
4	15	0.086 ± 0.0054
5	20	0.12 ± 0.005

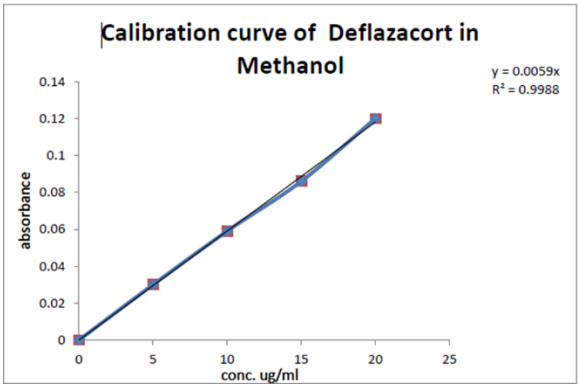


Fig-7 Calibration Curve of Deflazacort in methanol:
Preparation of calibration curve of Deflazacort in phosphate buffer (pH 7.8):
The UV absorption data at 244 nm and concentration estimates of pure Deflazacort showed linearity (r2 = 0.999) over the concentration range of 0-20µg/ml passing through origin and it follows the Beer-Lambert law. The absorbance shown by standard solution is given in (table no. 5) and the standard curve is shown in (figure no. 8).

Table 5 Calibration Curve of Deflazacort in phosphate buffer (pH 7.8) (Mean±S.D.):

S. No.	Concentration (µg/ml)	Absorbance
1	0	0 ± 0

2	5	0.021 ± 0.002
3	10	0.043 ± 0.0023
4	15	0.066 ± 0.0030
5	20	0.088 ± 0.0023

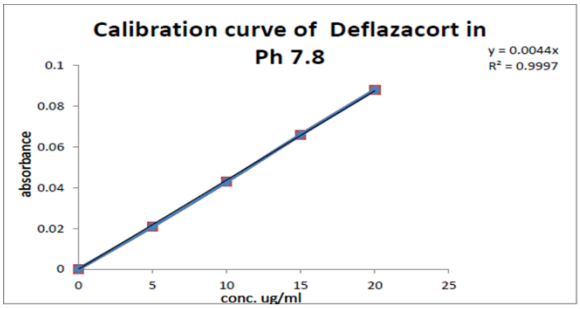


Fig- 8 : Calibration Curve of Deflazacort in phosphate buffer (pH 7.8):

In-vitro release studies

In-vitro drug releases from the solid lipid microparticles (SLMs) formulation were studied by the dialysis membrane. The diffusion medium was 250 ml of phosphate buffer pH (5.5) stirred at 50 rpm at 37ºC±0.5ºC.*In-vitro* drug release of Deflazacort and all the formulations were shown in table. It was concluded that the *in-vitro* drug release of Deflazacort were higher in comparison to the solid lipid microparticles formulations.

Table-6 *In-vitro* drug release profile of Pure Drug:

Time (Hrs)	Absorbance	Concentration	Amount (1ml)	Amount (5ml)	Amount (250ml)	CDR	Percentage CDR
0	0	0	0	0	0	0	0
0.5	0.314	11.61654	0.011616541	0.058083	2.904135	2.904135	81.11
1	0.456	16.95489	0.016954887	0.084774	4.238722	4.296805	90.22
2	0.589	21.95489	0.021954887	0.109774	5.488722	5.573496	97.54
3	0.654	24.3985	0.024398496	0.121992	6.099624	6.209398	99.32
4	0.745	27.81955	0.027819549	0.139098	6.954887	7.07688	100
5	0.745	27.81955	0.027819549	0.139098	6.954887	7.07688	100

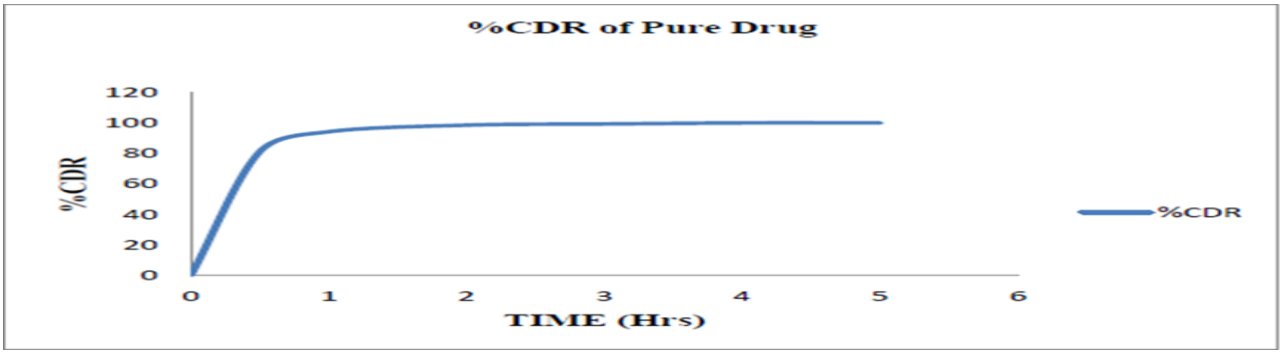


Fig-9 *In Vitro* Drug Release Profile of Pure Drug

Table-7 *In-vitro* drug release profile for F1:

Time(Hrs)	Absorbance	Concentration	Amount (1ml)	Amount (5ml)	Amount (250ml)	CDR	Percentage CDR
0.5	0.0221	0.20301	0.00020301	0.00102	0.05075	0.05075	7.38346
1	0.282	0.43233	0.00043233	0.00216	0.10808	0.1091	12.27318
2	0.0296	0.48496	0.00048496	0.00242	0.12124	0.1234	18.3402
3	0.0389	0.83459	0.00083459	0.00417	0.20865	0.21107	25.1071
4	0.0436	1.01128	0.00101128	0.00506	0.25282	0.25699	32.6992
5	0.0496	1.23684	0.00123684	0.00618	0.30921	0.31427	43.4267
6	0.0569	1.51128	0.00151128	0.00756	0.37782	0.384	54.4004
7	0.0600	1.62782	0.00162782	0.00814	0.40695	0.41451	65.4511
8	0.0667	1.8797	0.0018797	0.0094	0.46992	0.47806	76.8064
9	0.0727	2.10526	0.02105263	0.10526	1.05263	1.06203	85.1015
10	0.0786	2.32707	0.02327068	0.11635	1.16353	1.2688	89.4398
11	0.0812	2.42481	0.02424812	0.12124	1.21241	1.32876	95.438
12	0.0873	2.65414	0.02654135	0.13271	1.32707	1.44831	98.4154

CONCLUSION

In the present study an attempt was made to enhance the solubility and sustainability of *Deflazacort.* The SLM dispersion was prepared by solvent emulsification followed by ultrasonication technique by using glycerol mono stearate (GMS) as lipid matrix. The prepared SLM dispersion were characterized for various parameters such as particle size, percentage entrapment efficiency, percentage drug loading, assessed for physical properties, in-vitro drug release study. SLM also have the potential to localize the drug at the site and could be useful for site-specific delivery of drugs. Initially the project goal was to make a microparticle loaded deport system and target to the back of the eye, but due to COVID-19 the final portion of study could not be completed.

REFERENCE

[1] Adeyeye M.C. and Adebayo A., 2017. Hard shell capsules in clinical trials. In *Pharmaceutical Dosage Forms*, pp. 31-74.

[2] Attia Shafie, M.A. and Fayek H.H.M., 2013. Formulation and evaluation of betamethasone sodium phosphate loaded nanoparticles for ophthalmic delivery. *Journal of Clinical & Experimental Ophthalmology*, *4*(2), pp.1-11.

[3] Ekambaram P., Sathali A.A.H. and Priyanka K., 2012. Solid lipid nanoparticles: a review. *Scientific Review Chemical Communication*, *2*(1), pp.80-102.

[4] Gaddam N., and Aukunuru J., 2010. systemic delivery of diclofenac sodium after topical application of gels incorporated with drug-loaded solid lipid nanoparticles (SLNs),

[5] *Joanne Penner Ricardo Hoar Christian Jacob Department of Computer Science*, Vol. 2, (2), pp.177-181.

[6] Gardouh A. R., Gad S., Ghonaim H. M. and Ghorab M. M., 2013. Design and Characterization of Glyceryl Monostearate Solid Lipid Nanoparticles Prepared by High Shear Homogenization, *British Journal of Pharmaceutical Research (BJPR),* 3(3), pp.326-346.

[7] Government of India Ministry of Health and Social Welfare, Indian Pharmacopoeia. Delhi: 2007. The Controller of Publication, pp.2226-7.

[8] Gupta A. K., Madan S., Majumdar D. K., and Maitra A., 2009. Ketorolac entrapped in polymeric micelles, Preparation, characterization and ocular anti-inflammatory studies. *International Journal of Pharmaceutics*, pp. 1-14.

[9] Harde H., Das M., Jain S., 2011.Solid lipid nanoparticles. An oral bioavailability enhancer vehicle. Expert Opinion Drug Delivery;8:1407-24

[10] Jain S., Mistry M.A. and Swarnakar N.K., 2011. Enhanced dermal delivery of acyclovir using solid lipid nanoparticles. *Drug Delivery and Translational Research*, *1*(5), pp.395.

[11] Joshi M. and Patravale V., 2006. Formulation and evaluation of nanostructured lipid carrier (NLC)–based gel of Valdecoxib. *Drug Development and Industrial Pharmacy*, *32*(8), pp.911-918.

[12] Kaur L.P., 2013. Microparticle: a recent approach for novel drug delivery. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *3*(17), pp.1.

[13] Kumar D.P., Dinda S., Chakraborty S., 2012.Formulation and Evaluation of Solid Lipid Nanoparticles of a Poorly Water-Soluble Model Drug Ibuprofen, *International Research Journal of Pharmacy (IRJP)*, pp. 3(12).

[14] Palei N. N, Das M. K., 2013. preparation and characterization of Lornoxicam loaded solid lipid nanoparticles made from different lipids, *International Journal of Pharmaceutical Science,* 5(4), pp.438-442.

[15] Verma S., Kumar A., Malik V. K. and Kumar V., 2013. Compritrol ATO 888 based solid lipid nanoparticles of cefixime: Formulation and evaluation, *Der Pharmacia Sinica,* 4(3), pp.8-13.