IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2016 Vol.:6, Issue:2

Formulation and Evaluation of W/O/W Multiple Emulsions with Diclofenac Sodium

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Sagar Savale[1*], Uday Mali[2], Patil Prafulla[2]

*[1]*Department of Pharmaceutics, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, 425405, Dist. Dhule, Maharashtra State, India.*

[2]*Department of Quality Assurance, R. C. Patel Institute of Pharmaceutical Education and*

Research, Shirpur, 425405, Dist. Dhule, Maharashtra State, India.

Submission: 2 May 2016
Accepted: 7 May 2016
Published: 25 May 2016

www.ijppr.humanjournals.com

Keywords: Multiple emulsions, Non-ionic surfactants, Diclofenac sodium, Bioavailability, Stability, W/O/W multiple emulsions

ABSTRACT

Multiple emulsion is novel approach of drug delivery system for enhancement of bioavailability and pharmacological activity. It is important to prevent the problem of oral drug delivery system and they are stabilized by using of combination of hydrophilic and lipophilic surfactant. The specific ratio of surfactant concentration is responsible for maintaining the stability of multiple emulsions, the importance of this study was to prepare multiple emulsion of Diclofenac sodium by using two step emulsification process, by using the non-ionic surfactant units. In multiple emulsion, the stability of multiple emulsion was evaluated, percent entrapment efficiency as well as *in vitro* studies are conducted. The process of primary and secondary emulsification was optimized to get the stable multiple emulsion with the high entrapment efficiency. Multiple emulsion to improve bioavailability with the hypothesis that improvement of drug release profile will reflect the enhancement of bioavailability of the drug.

INTRODUCTION

Multiple emulsion is novel approach on drug delivery system in which the emulsion system can exist both types of emulsion (water in oil (O/W) and oil in water (W/O)) exist simultaneously. The combination of both properties of emulsion are known as Multiple Emulsion. Multiple emulsion is mainly divided into two types, first is W/O/W (water in oil emulsion dispersed in water) and another is O/W/O (oil in water emulsion dispersed in oil). In W/O/W multiple emulsion is polydispersible vesicular system in which the dispersed oil drops contain even smaller dispersed droplets, which generally consist of liquid identical to continuous phase. The presence of reservoir phases inside the droplet system can be used for prolonged release of active ingredients, Multiple emulsion is having the many applications such as used in pharmaceutical industries and cosmetic industries. The most common method for the preparation of multiple emulsions is the two step emulsification method by using mechanical stirrer and High pressure homogenizer (HPH). In case of Diclofenac sodium multiple emulsion is applicable for various applications such as it acts as Anti-inflammatory action to prevent the inflammation. It is applicable to give antipyretic as well as analgesic activity, it is used for treatment for musculoskeletal complaints, especially arthritis, rheumatoid arthritis, polymyositis, dermatomyositis, osteoarthritis, dental pain, TMJ pain, spondylarthritis, ankylosing spondylitis, gout attack sand pain management in cases of kidney stones and gallstones. It is also applicable to treat mild to moderate postoperative or posttraumatic pain, in particular when inflammation and is effective against menstrual pain and endometriosis. It can give local as well as systemic activity for prevention of infection as well as toxicity and enhanced bioavailability. [1, 2, 3, 4, and 5]

MATERIALS AND METHODS

Materials

Diclofenac sodium and all formulation excipients (Paraffin oil, Span 80, Tween 20, Water) were obtained from Pharmaceutics Laboratory of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur 425405, Maharashtra State, one of the NBA and NAAC accredited and AICTE Approved institutes in India.

Methods

The parameters of authentication and preformulation is carried out by pure drug Diclofenac sodium for maintaining their quality, purity and standard.

Authentication Parameters[5, 6, 7]

Melting Point Method

Melting point determination is one of the pre-formulation property in which the temperature at which it changes state from solid to liquid at atmospheric pressure. At the melting process, the solid and liquid can exist equilibrium. The melting point of Diclofenac sodium pure drug is determined by using two types of methods. One is Conventional method and another is Digital method.

Log P Value

Log p value is determined by using Partition Coefficient phenomenon. In which 1gm of drug is added in separating funnel containing equal portion of 25ml of Octanol and 25ml of Water. The separating funnel is shake 20 – 25 min. and stabilized the mixture. After stabilizing the mixture to remove water phase from separating funnel and filter it. Take Absorbance of Filtrate and calculate the log p value.

Solubility Studies

The term solubility is defined as maximum amount of solute that can be dissolved in a given amount of solvent to form a homogenous system at specified temperature and specific pressure to form saturated solution.

Procedure

- To prepare a different solutions water, pH 1.2 acidic buffer, pH 6.8 phosphate buffer, pH 7.4 phosphate buffer.
- The drug material is added into above solutions till supersaturated solution forms.
- The mixture is placed in Orbital Shaker for 24 hrs. After 24 hrs. filter the mixture. Take filtrate and give absorbance.

- To detect the concentration of drug in different solutions.

Calibration Curve of Diclofenac sodium in water

Calibration curve is determined by using UV Spectrophotometric methods. In which 10mg drug is added in 100ml of water (100µg/ml solution). To prepared different dilutions (0, 2, 4, 6, 8, 10, 12) of above solution (100µg/ml solution). Take absorbance in respective λ_{max} 275nm.

PREFORMULATION STUDIES[8]

Drug-Excipient Compatibility Studies

Drug is an active part of dosage forms and it is mainly responsible for the therapeutic value and excipient substances which are included along with drugs being formulated in a dosage form so as to impart specific qualities to them. It is important for the determination of stability of the dosage. It's also used for development of new drug delivery system as well as investigation of new drug product.

Procedure

The equal portion of Drug and Excipient (1:1 ratio) is added in ampules and the ampules are placed in stability chamber for one week, After one week the drug excipient compatibility study is determined by using TLC (Thin Layer Chromatography) (Methanol: Water: Glacial acetic acid (7:2.5:0.5)), IR (Infrared Spectroscopy).

Methods of Formulation

Multiple emulsion is prepared by two step emulsification process 1. Preparation of Primary emulsification 2. Secondary emulsification.

1. Primary emulsification: 20ml of distilled water containing 28mg of drug was gradually added to 40ml of oil phase (Paraffin oil) containing primary emulsifier (Span 80 (con. 10%) and 56mg of drug with continuous stirring at 5000r/min for 15min. (Using Mechanical Stirrer and High Pressure Homogenizer (HPH) having continuous stirring). (Total Quantity of Primary emulsion is 60ml).

2. Secondary emulsification: 60ml previously prepared viscous primary emulsion was emulsified further with an external aqueous phase (water (40ml)) containing secondary emulsifier (Tween 20 (con. 10%)) and 140mg drug with continuous stirring at 5000r/min for 30min. (Using Mechanical Stirrer and High Pressure Homogenizer (HPH) having continuous stirring). After continuous stirring to form homogenous or uniform W/O/W type of Multiple Emulsion (Total quantity of Multiple Emulsion is 100ml).

Evaluation of Multiple Emulsion[9, 10, 11, 12]

Entrapment Efficiency

The % entrapment efficiency is important for determination % content of active ingredient. The percentage entrapment efficiency (% ee) was determined by taking freshly prepared W/O/W Multiple Emulsions and immediately centrifuged at 4000rpm for 10min. Then 1ml of the aqueous phase (the lower layer) was precisely withdrawn through 2ml hypodermic syringe and diluted properly with phosphate buffer 6.8. The solution was filtered through a millipore filter (0.22mm in pore size) and drug content was analysed on UV spectrophotometer at 275nm. The Encapsulation Efficiency was determined by following equation:

% EE = (Total drug incorporated – Free Drug) / Total drug * 100

Globule Size or Particle Size

In this study, globule sizes of the multiple emulsions were determined by using Zeta Analyser apparatus, Zeta Analyser is an apparatus mainly used for determination of particle size, Zeta potential, molecular weight are mainly based on Light diffraction and Scattering Phenomenon.

***In Vitro* Release Studies**

The *in vitro* drug release study was carried out on a simple dissolution cell using cellophane membrane (thickness-200mm, breaking strength-2.7kgf/cm). Prior to release studies, the cellophane membrane was soaked in distilled water for 6 hours, washed frequently 4 times by changing distilled water, then immersed in 5% v/v glycerol solution for at least 60min and washed finally with 5 portions of distilled water. 15ml freshly prepared multiple emulsion was added to donor chamber, made up of a hollow glass tube (2.5cm in diameter and 10cm in length)

and membrane was tied on bottom end of the tube with a nylon string. This tube was dipped into 250ml vessel containing 100ml of PBS pH 6.8 and was stirred at 100 rpm on a magnetic stirrer and maintained at 37°C which acted as receiving chamber. Aliquots of 1ml were collected from receiving chamber at predetermined time intervals and the drug content was determined on UV spectrophotometer at 275nm after suitable dilution.

RESULTS AND DISCUSSION

Authentication Parameters

Melting Point Method

The melting point of Diclofenac sodium is determined by Conventional and Digital Method and melting point of Diclofenac sodium is reported in Table 1.

Log P Value

Log P Value is determined by Partition Coefficient Phenomenon and Log P Value of Diclofenac sodium is reported in Table 1.

Solubility Studies

The solubility of Diclofenac sodium in given solution. (Water, pH 1.2 acidic buffer, pH 6.8 phosphate buffer, pH 7.4 phosphate buffer) is reported in Table 2 and concentration of drug soluble in different solution is shown in Figure 1.

Calibration Curve of Diclofenac in Water

The calibration curve of Diclofenac sodium is determined by using U.V. Spectroscopic method. In which the absorbance of Diclofenac sodium in different concentration (0, 2, 4, 6, 8, 10, and 12) is reported in Table 3. And the calibration curve is shown in Figure 2.

Preformulation Studies

The drug and excipient compatibility studies determined by TLC (Thin Layer Chromatography) and IR (Infrared Spectroscopy) method. In which TLC of drug, drug and excipient before stability chamber and after stability chamber is reported in Table and shown in Figure 3. The IR

of drug, drug and excipient spectra is taken. The IR spectra of pure drug Diclofenac sodium is shown in Figure 4.

Evaluation of Multiple Emulsion

Entrapment Efficiency

The Entrapment Efficiency of Multiple Emulsion is reported in Table 7.

Globule size or Particle Size

The particle size globules of Multiple Emulsion is reported in Table 7, the peak of size distribution report is shown in Figure 5. (By using Zeta Analyser Apparatus)

***In Vitro* Release Studies**

The *in vitro* release of multiple emulsion, in which the standard calibration curve of Diclofenac in phosphate pH 6.8 is reported in Table 5 and shown in Figure 6. The *in vitro* release of Diclofenac sodium Multiple emulsion is reported in Table 6 and shown in Figure 7. The *in vitro* release study is reported in Table 7.

Table 1: Melting point and Log P Value

Sr.No.	Parameters	Result	Std.
1	Melting Point (˚C)	283 – 285˚C	284 -285˚C
2	Log p Value	4.50	4.51

Table 2: Solubility Studies of Pure Drug in Different Solvent

Sr. No.	Medium	Concentration of drug Soluble (mg /ml)
1	Water	1.36
2	pH 1.2 Acidic Buffer	2.96
3	pH 6.8 Phosphate Buffer	3.69
4	pH 7.4 Phosphate Buffer	5.20
Result	**Class of drug**	**BCS Class II**

Table 3: Calibration Curve of Diclofenac sodium in Water

Concentration	Absorbance
0	0
2	0.087
4	0.161
6	0.238
8	0.298
10	0.388
12	0.481

Table 4: Standard Calibration Curve of Diclofenac in Phosphate pH 6.8

Concentration	Absorbance
0	0
2	0.223
4	0.375
6	0.579
8	0.763
10	0.935

Table 5: The TLC of Drug, Drug and Excipient before Stability Chamber and After Stability Chamber

Sr. No.	Samples (Pure From of Drug material) (Drug + Excipient Mixture)	Retention factor of drug Before the Stability Chamber	Retention factor of drug After the Stability Chamber
1	Pure Drug Diclofenac sodium	0.77	0.81
2	Diclofenac + Paraffin oil	0.80	0.84
3	Diclofenac + Span 80	0.74	0.78
4	Diclofenac + Tween 20	0.72	0.75

Table 6: *In vitro* Drug Release of Multiple Emulsion

Time (min.)	Abs	Conc µg/ml	DF	Conc µg/ml	Conc mg/ml	Conc mg/5ml	Conc mg/900ml	CDR	%CDR
0	0	0	0	0	0	0	0	0	0
15	0.082	0.72	10	7.21	0.0072	0.036	6.49	6.49	32.48
30	0.084	0.74	10	7.43	0.0074	0.037	6.69	6.72	33.63
45	0.086	0.76	10	7.65	0.0076	0.038	6.88	6.92	34.61
60	0.088	0.78	10	7.86	0.0078	0.039	7.079	7.11	35.58
75	0.121	1.14	10	11.42	0.011	0.057	10.28	10.31	51.59
90	0.145	1.40	10	14.00	0.014	0.070	12.60	12.66	63.32
105	0.161	1.57	10	15.73	0.015	0.078	14.15	14.22	71.14
120	0.189	1.87	10	18.75	0.018	0.093	16.87	16.95	84.93

Table 7: Evaluation of Multiple Emulsion

Sr. No.	Parameters	Result
1	Entrapment Efficiency (%)	95.85
2	Globule size or Particle Size (d. nm), [PDI: 1.000, Intercept: 1.02]	1047
3	*In vitro* studies (%)	84.93

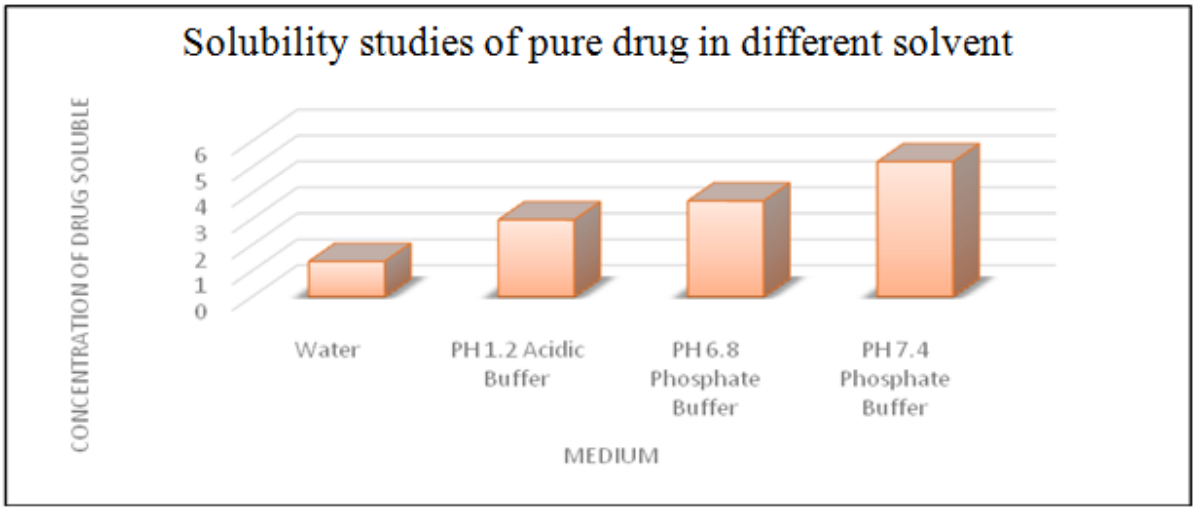

Figure 1: Solubility Studies of Pure Drug in Different Solvent

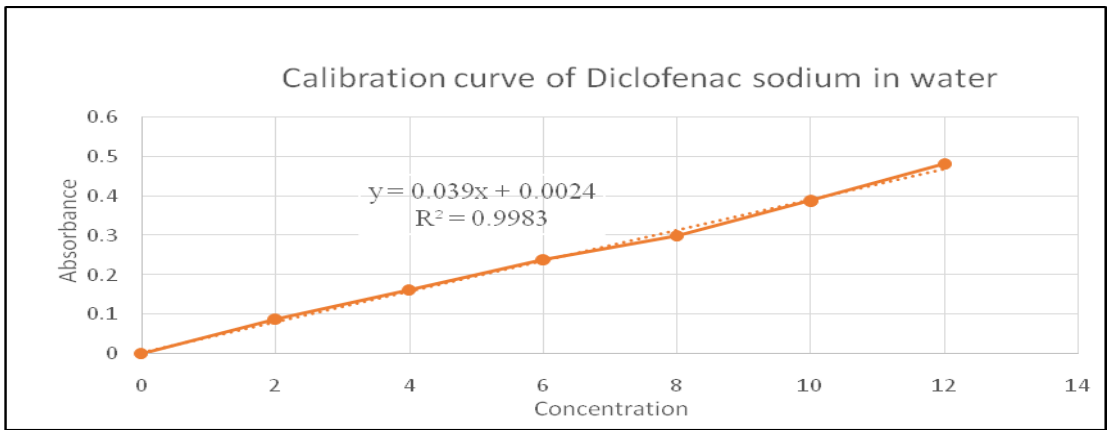

Figure 2: Calibration Curve of Diclofenac Sodium in Water

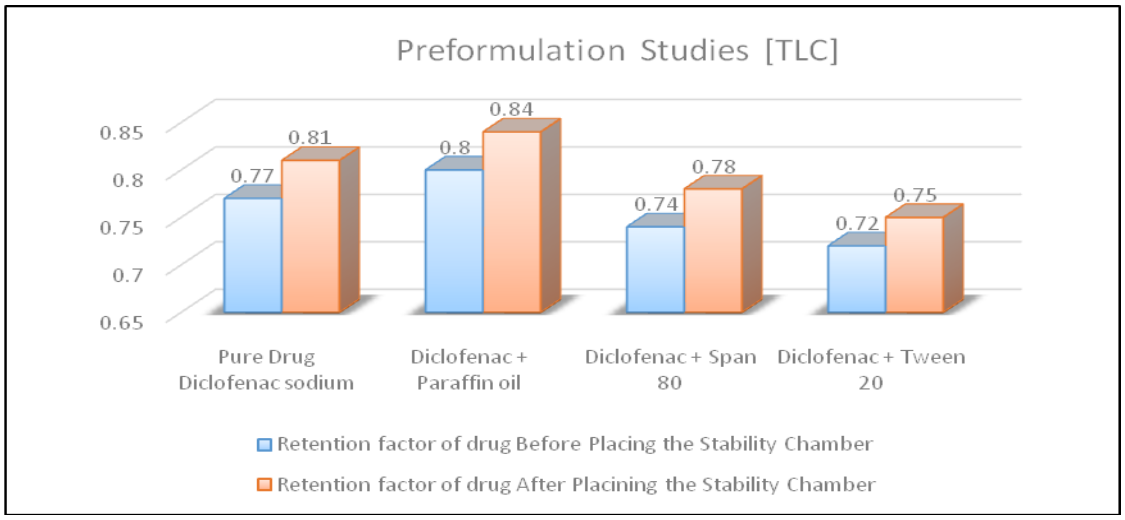

Figure 3: TLC Studies (Drug: Excipient Compatibility)

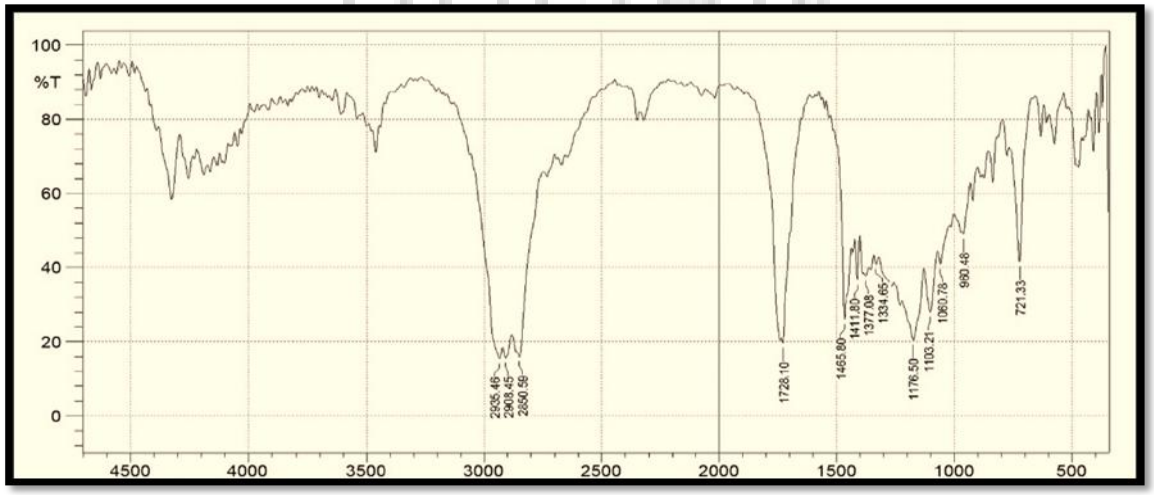

Figure 4: The IR Spectra of Pure Drug Diclofenac sodium

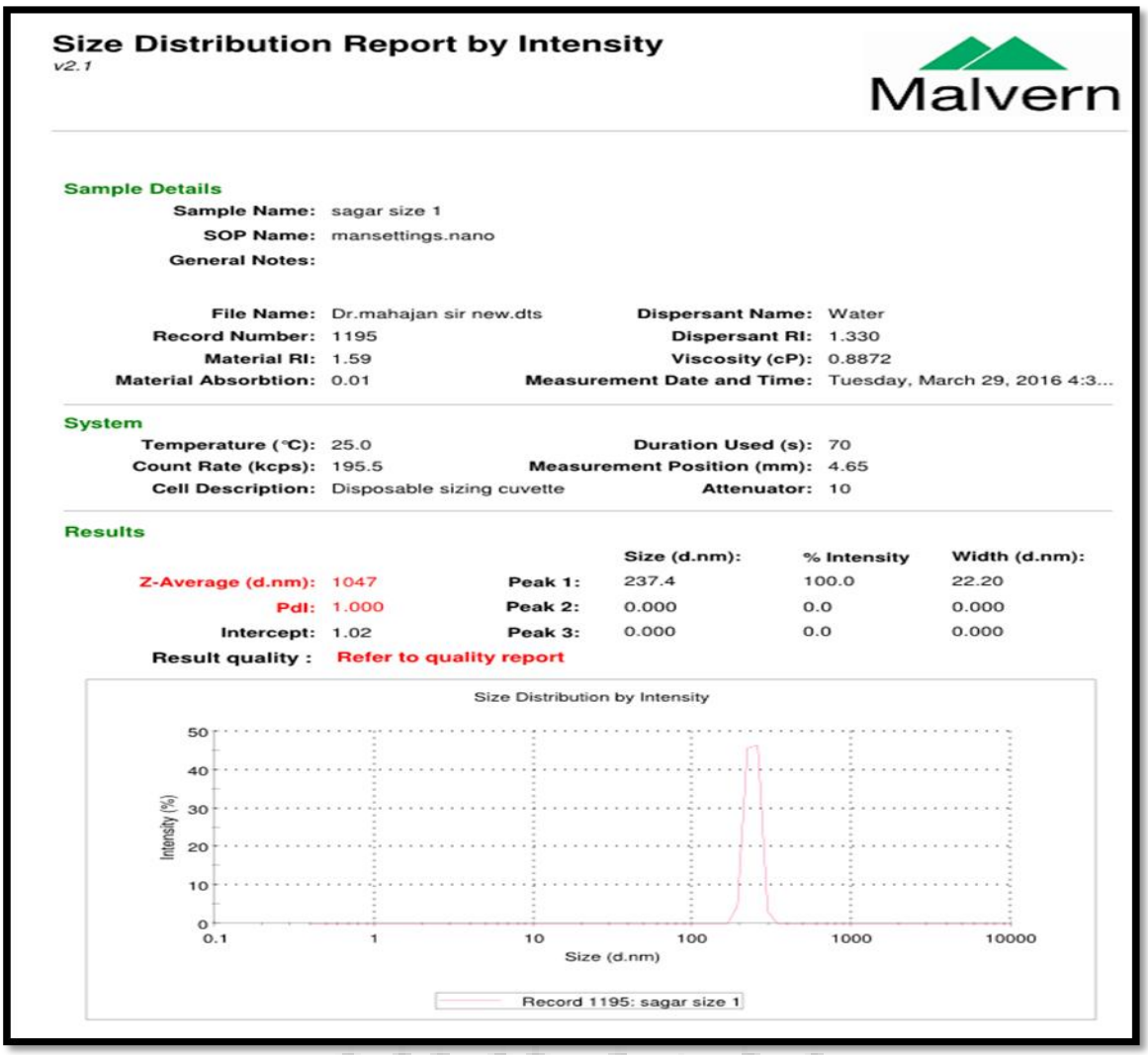

Figure 5: Peak Size Distribution Report (Zeta Analyser)

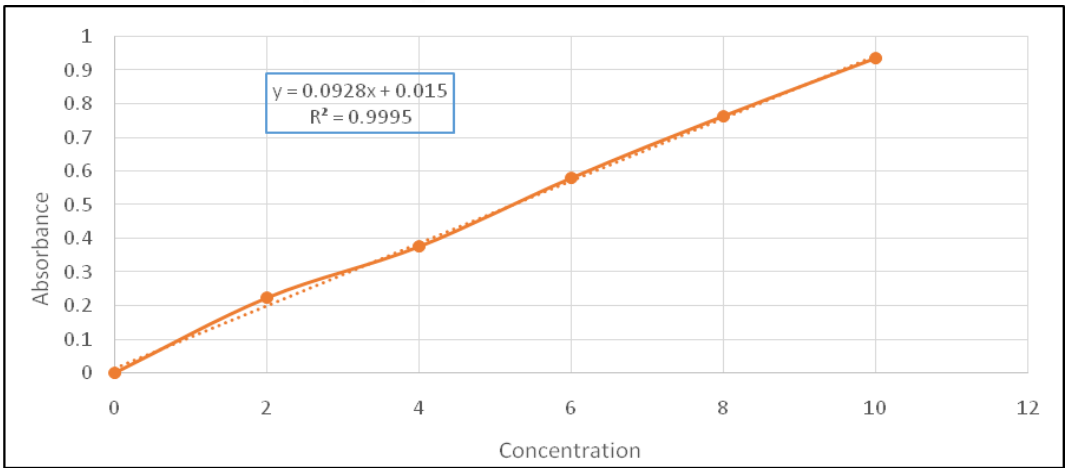

Figure 6: Std. Calibration Curve of Diclofenac Sodium in pH 6.8 Phosphate Buffer

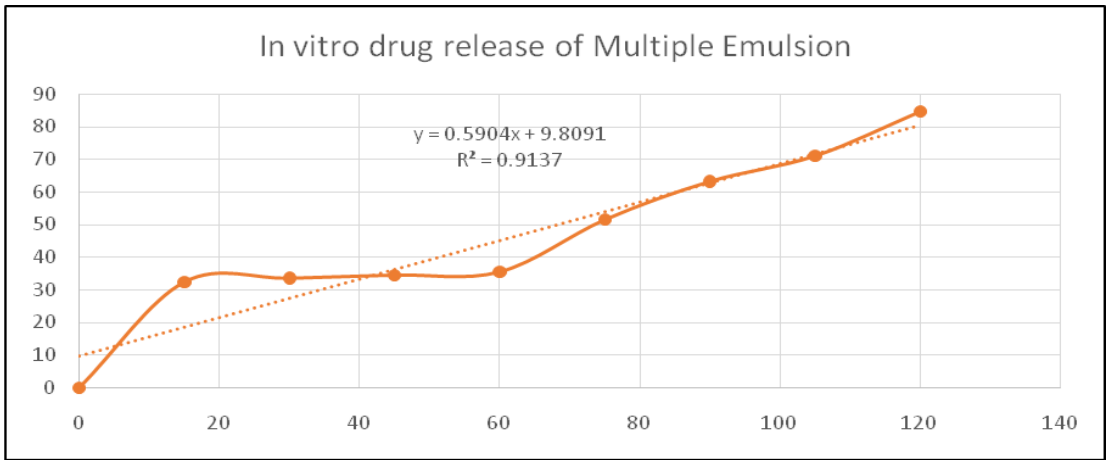

Figure 7: *In Vitro* release of Multiple Emulsion

CONCLUSION

Multiple Emulsion is prepared by two step emulsification method in which first step under formation of pre-emulsion (W/O) and second step under treatment of aqueous water phase emulsified with previously prepared W/O emulsion to form W/O/W type of multiple emulsion. Using different W/O emulsions for the second emulsification step the finest W/O emulsion led to W/O/W emulsions with the highest encapsulation rate. To prevent a production-induced reduction of encapsulation rate the inner water droplets have to be much smaller than the oil droplets in the Multiple Emulsion. Diclofenac sodium is in the inner water phase of a multiple Emulsion. Due to the same reason Diclofenac sodium is unsuitable as marker substance, if the inner W/O emulsion. The main purpose was to develop stable multiple emulsion with higher Entrapment Efficiency. The study revealed that Multiple Emulsion can be optimized for good stability and higher entrapment efficiency by optimizing different formulations variables like type & proportion of primary & secondary emulsifier.

ACKNOWLEDGEMENT

The authors are grateful to Hon. Principal, SES's, R. C. Patel Institute of Pharmaceutical Education and Research, Dr. S. J. Surana sir. A special gratitude to Dr. H.S. Mahajan sir Head, (HOD) Dept. of Pharmaceutics and Quality assurance. Without whom and their constant caring and loving support, we would be unable to achieve this advancement and precious stage of our life.

REFERENCES

1. Vyas S. P. And Khar R. K., Targeted and Controlled Drug Delivery System, 1st Edition, 2002, CBS Publication; 303-329.
2. Omotosho JA, Florence AT, and Whateley T L. (1990). Absorption and lymphatic uptake of 5-flourouracil in the rat following oral administration of w/o/w multiple emulsions. Int J Pharm: Vol. 61, 51-56.
3. Florence AT., Whitehill D. (1982). The formulation and stability of multiple emulsions. Int J Pharm: Vol. 11, 277 - 308.
4. Jain N. K., Controlled and Novel Drug Delivery, 1st Edition 2001, CBS Publication; 381-399.
5. Matsumoto S, Kita Y, Yonezawa D. (1976). An attempt of preparing water-in-oil-in-water multiple phase emulsions. J Colloid Interface Sci: Vol. 57, 353-361.
6. Cunha AS, Cheron M, Grossiord JL, Puisieux F and Seiller M. (1998). W/O/W multiple emulsions of insulin containing a protease inhibitor and an absorption enhancer: biological activity after oral administration to normal and diabetic rats. Int J Pharm: Vol. 169, 33-44.
7. Yazan Y and Puisieux F. (1993). Multiple emulsions. Boll Chim Fharmaceutico: Vol. 132, 187–196.
8. De Luca M, Rocha-Filho P, Grossiord JL, Rabaron A, Vaution C, Seiller M. (1991). Les emulsions multiples, Int J Cosmet Sci: Vo.\l. 13, 1-21.
9. Raynal S, Grossiord JL, Seiller M, Clausse DA. Topical W/O/W multiple emulsion containing several active substances: formulation, characterization and study of release. J Controlled Release: Vol. 26, 129-140.
10. Jong-wook Ha And Seung-man Yang., Breakup Of A Multiple Emulsion Drop In A Uniform Electric Field., Journal Of Colloid And Interface Science 213, 92–100 (1999).
11. Jim Jiao and Diane J. Burgess., Rheology and Stability of Water-in-Oil-in-Water Multiple Emulsions Containing Span 80 and Tween 20., AAPS Pharm Sci 2003; 5 (1) 252-259
12. Abraham Aserin., Multiple Emulsions Technology And Applications, Wiley-interscience, A John Wiley & Sons, 2007, Inc., Publication; 111-324