CHAPTER- 6 SUMMARY AND CONCLUSION

Summary

Majority of new drug candidates have poor aqueous solubility. The poor water solubility and the oral delivery of such drugs is frequently associated with implications of low bioavailability, high intra- and inter subject variability, and lack of dose proportionality. Many advanced and effective approaches for drug delivering systems have emerged in recent years. Self Microenulsifying Drug Delivery System (SMEDDS) is one of the focused areas. SMEDDS typically comprises a mixture of surfactant, oil and drug (known as the concentrate) which when introduced into the body is rapidly dispersed to form droplets of approximately same size and peristaltic moment of GI tract provides necessary agitation to form in-vivo microemulsion. Due to small droplet size, drug entrapped in oil droplet easily diffuse through cell membrane of gastrointestinal tract. On dispersal in water, surfactants self-associate into a variety of equilibrium phases. Medium-chain glycerides induce structural and fluidity changes in the mucosal membrane thus resulting in significant permeability changes which improves bioavailability of drug.

Cardiovascular disorders are the world's most prevalent diseases. Various groups of antihypertensive agents are used today to treat high blood pressure either as single drug therapy or combination therapy. The angiotensin-receptor blockers (ARBs), originally indicated for hypertension, have shown themselves to have cardiovascular benefits. Most of the drugs are suffering with poor water solubility and less bioavailability problems. To enhance effectiveness of drug, a few issues should be carefully considered when designing drug delivery system. The formulation should be designed so as to provide rapid transport of drug across GI membrane. Microemulsions, by virtue of their lipophilic nature and having low globule size, are widely explored as a delivery system to enhance uptake across membrane. Presence of a medium chain and long chain triglycerides helps in diffusion of drug across the membrane. Evidences of self microemulsifying drug delivery systems formulated using triglyceride lipids and its benefits in enhancing drug bioavailability have been reported by many scientists.

The objectives of this investigation were to prepare and optimize self microemulsions drug delivery system (SMEDDS) of valsartan and olmesartan and evaluate their performance *in vitro* and *in vivo* in rabbits. Studies were focused on the preparation of SMEDDS, characterization, evaluation for bioavailability of developed formulations. It was hypothesized that SMEDDS of selected drugs will promote dissolution rate and bioavailability. Hence, this can help to maximize the therapeutic index of the drug, reduce side effects, reduce the dose and frequency of dosing and perhaps even the cost of the therapy.

6.1 Preparation and Evaluation of SMEDDS for Valsartan

Different analytical methods were developed to evaluate drug concentration in prepared formulation as well as in plasma matrix. Two photometric methods like UV spectroscopic and flurophtometric methods were developed for estimation of valsartan. Spectrophtometic methods were developed to estimate drug content in formulations. In UV spectroscopy was developed and absorbance maximum (λ max) was found at 248 nm. The method was linear for the concentration range of 7.5 µg/mL to 22.5 µg/mL. The minimum concentration that can be detected (LOD) by developed method was 0.042 µg/ml and minimum quantified concentration (LOQ) was 0.141. Developed method was validated for precision and accuracy. This developed method was applied for determination of concentration of valsartan in SMEDDS formulation as well as during in-vitro study.

Another spectroscopic method developed was based on fluorescence phenomenon called as spectrofluometric method. The emission was observed at 373 nm when excitation was given at 286 nm. The method was linear within the range of 0.75 µg/mL to 2.25 µg/mL. The method was validated for accuracy and precision. Market sample was analyzed by developed method.

Estimation of drug in plasma matrix requires very precise method and hence blood sample obtained during animal study were analysed by developed HPLC method. Method was linear within the range of 0.5 to 50 µg/mL. LOD and LOQ were found to be 0.125 µg/mL and 0.5 µg/mL respectivey. Method was validated for system suitability, accuracy, precision, robustness, ruggedness. All validation parameters meet

the acceptance criteria as per ICH guideline. Plasma samples were analyzed using validated HPLC method. All these developed methods were applied for estimation of drug in formulations or biological matrix.

In preparation of SMEDDS, selection of components is important aspect. Since oils, surfactants and cosurfactants are three constructing components of SMEDDS, the selection of components were based on solubility of drug in that mixture. Solubility of valsartan was determined in different natural and synthetic oils. Valsartan showed good solubility in Captex 200P, Capmul MCM and Capmul MCM C10 and hence they were selected as oil, Tween 80 and Cremophore EL were selected as surfactant and Plurole oleique and PEG 400 were used as cosurfactants. Three different systems VSMS 1, VSMS 2 and VSMS 3 were prepared using above components. From each system three formulations were prepared with surfactant: cosurfactant ratio of 1:1, 2:1 and 3:1. Each of formulation was evaluated to determine microemulsion region by pseudo-ternary phase diagram. VSMS 1 was prepared using Capmul MCM as oil, Tween 80 as a surfactant and PEG 400 as cosurfactant and it gave three formulation A, B and C with surfactant : cosurfactant ratio of 1:1, 2:1 and 3:1 respectively. Similarly VSMS 2 was prepared using Capmul MCM C10 as oil, Tween 80 as a surfactant and Plurole olieque as cosurfactant and it gave three formulation D, E and F with surfactant : cosurfactant ratio of 1:1, 2:1 and 3:1 respectively. Formulations G, H and I were prepared from VSMS 3 containing Captex 200 P as oil, Cremophore EL as a surfactant and PEG 400 as cosurfactant.

On comparing phase diagram for all formulations, formulation A showed maximum self-microemulsion region. On dilution with water SMEDDS of formulation A forms globule size less than 100 nm. After dilution the oil concentration was upto 10%, S/CoS concentration was reached to 50% and amount of water was 90%. In case of other formulations very less microemulsion region were observed. So it was decided that composition of formulation A was best suitable for formation of self-microemulsion. Further stability of this microemulsion was checked by determining different in vitro characteristics.

Valsartan SMEDDS were prepared using excipients mentioned above. In all

formulations oil concentration was kept constant to 10%. The concentration of valsartan was also fixed to 10 mg/0.2 mg of SMEDDS. Concentration of surfactant was increased as ratio of S/CoS was increased.

Each prepared formulation was checked for different characteristics like particle size, zeta potential, viscosity and refractive index, conductance, % transmission and assay content.

Particle size is main identifying tool to check the formation and stability of micoremulsion and it is reported below 100 nm. In our study, formulations A, B and C showed particle size less than 20 nm which clearly indicates the formation microemulsion. All other formulation showed particle size above 100 nm do not match with criteria of microemulsion.

Another characteristic which define stability of microemulsion was zeta potential. Zeta potential indicates repulsive forces between oil droplets which keep the system suspended. Ideal value of zeta potential is between -10 to -30 will impart maximum stability to microemulsion. Among all formulations, only formulation A showed value of -12.1 which meets with ideal value of zeta potential and provides good stability to microemulsion. The values for other formulations were near to zero which may give primary indication for instability of microemulsion.

Polydispersivity index (PDI) measures the uniformity of particle size in formulation. Higher the PDI, lower will be the uniformity of microemulsion and formulation will be unstable. Least PDI value of 0.196 of formulation A proves uniform droplet distribution and formation of stable microemulsion.

Microemulsion shows bluish transparent solution up on dilution. Clarity of solution can be measured by % Transmission (%T). If % T value is more than 98%, it can be considered as clear formulation. Formulations having less particle size showed more clear solution and their %T values were also observed more than 98%. Formulation A showed 98.6 %T which indicates clarity of microemulsion.

pH of all formulations were found same and that indicated the compatibility of drug with excipients. Further it also gives idea about stability of drug in formulation. As all SMEDDS form water continuous microemulsion viscosity of microemulsions, were

near to that of water. Variations in viscosities were due to presence of oil droplets in formulation. Similarly, conductivities of microemulsions were found high as all formulations contained water as external phase.

Based on results of characterization and phase diagrams mainly three formulations A, B and C were finalized and considered for stability study. Two types of studies were performed and that were physical and chemical stability study.

Physical stability decides stages of microemulsion during its storage. Stable formulation should have consistency in particle size after dilution and should not convert in emulsion during storage as well as phase separation should not occur. Measuring particle size gives exact idea about stability. Formulation A, B and C were centrifuged at high speed and particle size of solutions were measured collected from three different portions top, middle and bottom layer. Formulation A showed consistency in particle size in all three layers while formulation B and C showed continuous increase in drop size. This increase may be due to non uniformity of oil droplets. There may be presence of different particles which agglomerate and settled down on centrifugation which results in increased particles size in bottom layer. The results of physical stability indicate uniformity in formulation A.

Prepared formulations were observed for chemical reactions and thereby change in characteristics on storage by performing chemical stability study. Samples were kept at two conditions 30°C/65% RH and 40°C/75% and evaluated for different characteristics at specific time intervals. Formulation A was found stable at all intervals in both the conditions. After period of 6 months, particle size was below 20 nm and %T was more than 98% proves microemulsion with low size droplets and remains clear. The assay content was also found to 99% indicates no degradation of drug occurred at high temperature and humidity. Opposite to this continuous increase was observed in particles size for formulation B and C. Also decrease in drug content below 88% was indicative of drug degradation in the formulations.

Hence both stability studies showed formulation A has stable one compared to that of B and C. Therefore formulation A was further considered for *in vitro* diffusion study and *in vivo* study.

In vitro diffusion study gives release profile of drug form the prepared formulation. It was performed by two techniques i.e. dialysis bag method and intestinal permeability study. The release profile of valsartan was checked in three different medias of pH 1.2, pH 4.5, pH 6.8. Based on these results, pH 6.8 buffer was selected as diffusion media. Formulation A was compared with standard drug solution and suspension of marketed formulation. Formulation A showed maximum release to 90% while market formulation showed only 60% release. Another *in vitro* method adopted was intestinal permeability study. As in previous method, pH 6.8 media showed more release than pH 1.2 and pH 4.5, release of valsartan was studied for formulation A, market formulation and standard drug solution using intestine. In this study also formulation A showed maximum release up to 76.28% as compare to 52.86% for market formulation and 17.09% for standard drug solution. On comparing both the method, although release of valsartan was less in intestinal permeability method than that of dialysis bag method formulation A showed highest release.

Finally bioavailability of valsartan was checked in rabbit to evaluate efficiency of SMEDDS formulation. Valsartan was administered in rabbit with dose of 5.6 mg/kg body weight form SMEDDS and market formulation. The pharmacokinetic parameters were determined for valsartan found higher in case of SMEDDS formulation A. T_{max} was decreased and at the same time C_{max} was increased to 112 compared to 69 ng/mL for market formulation. Other parameters were also found increased. Relative bioavailability of SMEDDS formulation A was increased to 1.78 fold as compared to market formulation.

6.2 Preparation and Evaluation of SMEDDS for Olmesartan

Two spectroscopic methods were developed as part of analytical method development. UV spectroscopic method was developed and absorbance maximum (λ max) was found at 256 nm. Linearity was developed for the range of 6 µg/mL to 18 µg/mL. The minimum concentration that can be detected (LOD) by developed method was 0.053 µg/ml and minimum quantified concentration (LOQ) was 0.177. Developed method was validated for precision and accuracy. This developed method was applied for determination of concentration of olmesartan in SMEDDS formulation as well as

during *in vitro* study.

Spectrofluorometric method was also developed with excitation wavelength at 286 nm and emission wavelength at 360 nm. Linearity was between range of 0.75 to 2.5 µg/mL. The method was validated for accuracy and precision. Market sample was also analyzed by developed method.

HPLC method was developed and linearity was found in the concentration range of 0.25 µg/mL to 50 µg/mL. LOD and LOQ were found to be 0.0625 µg/mL and 0.25 µg/mL respectively. Method was validated for system suitability, accuracy, precision, robustness, ruggedness. All validation parameters meet the acceptance criteria as per ICH guideline. Plasma samples were analyzed using validated HPLC method. All these developed methods were applied for estimation of drug in formulations or biological matrix.

Solubility of olmesartan was determined in oils, surfactants and cosurfactants. Based on solubility Captex 200P, Labrafac PG and Labrafil M 2125 were selected as oils, Cremophore EL, Cremophore RH 40 and Tween 80 were selected as surfactant and Transcutol P was used as cosurfactant. Three different systems OSMS 1, OSMS 2 and OSMS 3 were prepared using above components. Fromulations A, B and C were prepared form OSMS 1 containing Labrafil M 2125, Cremophore RH 40 and Transcutol P with surfactant: cosurfactant ratio of 1:1, 2:1 and 3:1. Similarly OSMS 2 was prepared using Captex 200 P, Tween 80 and Transcutol P and it gave three formulation D, E and F with S/CoS ratio of 1:1, 2:1 and 3:1 respectively. Formulations G, H and I were prepared from VSMS 3 containing Labrafac PG, Cremophore EL and Transcutol P with S/CoS ratio of 1:1, 2:1 and 3:1 respectively.

Microemulsion regions were identified for all formulations using pseudo-ternary phase diagram. All formulations showed presence of microemulsion region except formulation D. Due to higher particle size formulation D formed emulsion instead of microemulsion. Ideal formulation was selected such that it covers maximum self-microemulsion region and could solubilize maximum amount of oil. It was clear form phas diagram study that formulation B covered maximum region and up to 30% of oil was could solubilized after dilution of SMEDDS. Moreover time required to form

microemulsion was less than 2 minutes. Hence formulation B with S/CoS ratio of 2:1 was considered as better formulation. Irrespective of oil concentration and S/CoS ratio, amount of olmesartan was kept constant to 13 mg/0.2 mg of SMEDDS.

Each prepared formulation was checked for different characteristics like particle size, zeta potential, viscosity and refractive index, conductance, % transmission and assay content.

As particle size is main character to decide formation of microemulsion, formulation B and C which showed least particle size of 23.5 and 15.3 respectively were considered as good formulations. But only that was not sufficient to decide stability of microemulsion and hence other characteristics were also determine which includes zeta potential, polydispersivity index % transmission, pH, viscosity and conductivity. Among results of all these formulation A, B and C were found better formulation and hence they were kept under stability studies.

Zeta potential values were observed almost same for all formulations which were near to zero. The use of non ionic surfactant and non ionic nature of drug may be responsible for this conclusion.

Low PDI vales were found for formulations A, B and C which proves uniformity of particle size in microemulsions.

Clarity of solution was checked by measuring % Transmission (%T). The formulations with % T value more than 98% were considered as clear solutions. Only formulations A, B and C showed %T values above 98%. %T values less than 98% for other formulations indicates formation of emulsion or phase inversion of formulation.

pH of all formulations were found to 6.8 same as that of diluting media. This represents that drug or any excipients do not contribute to ionic strength formulation.

As all SMEDDS forms water continuous microemulsion viscosity of microemulsions were near to that of water. Variations in viscosities were due to presence of oil droplets in formulation. Similarly conductivities of microemulsions were found high as all formulations contained water as external phase.

Based on results of characterization and phase diagrams mainly three formulations A, B and C were finalized and considered for stability study. Two types of studies were

performed and that were physical and chemical stability study.

To determine physical stability formulation A, B and C were centrifuged at high speed and collected from three different portions top, middle and bottom layer. Formulation B showed consistency in particle size in all three layers while formulation A and C showed continuous increase in droplet size. There may be presence of different size of particles which agglomerate and settled down on centrifugation which results in increased particles size in bottom layer. The results of physical stability indicate uniformity in formulation B.

Chemical reactions and thereby change in characteristics on storage were measured by performing chemical stability study. Samples were kept at two conditions 30°C/65% RH and 40°C/75% and evaluated for different characteristics at specific time intervals. Formulation B was found stable at all intervals in both the conditions. After period of 6 months, particle size was below 30 nm and %T was more than 98% proves microemulsion with low droplets size remains clear. The assay content was also found to 99% indicates no degradation of drug occurred at high temperature and humidity. Opposite to this significant changes were observed in all characteristics for formulation A and C. Also decrease in drug content below 95% was indicative of drug degradation in the formulations.

Hence both stability studies showed formulation B as stable one compared to that of A and C. Therefore formulation A was further considered for *in vitro* diffusion study and *in vivo* study.

Two techniques i.e. dialysis bag method and intestinal permeability study were performed for *in vitro* diffusion study to get release profile of olmesartan form the prepared formulation. The release profile of olmesartan was checked in three different medias of pH 1.2, pH 4.5, pH 6.8. Based on these results, pH 6.8 buffer was selected as diffusion media. Formulation B was compared with standard drug solution and suspension of marketed formulation. Formulation B showed maximum release to 91% while market formulation showed only 59% release. Another *in vitro* method adopted was intestinal permeability study. As in previous method, pH 6.8 media showed more release than pH 1.2 and pH 4.5, release for olmesartan was also studied for

formulation A, market formulation and standard drug solution using intestine. In intestinal permeability study SMEDDS formulation showed 73.50% release while market formulation showed only 44.3% release. In both studies formulation B showed maximum release as compare to market formulation and standard drug solution. On comparing both the method, although release of olmesartan was less in intestinal permeability method than that of dialysis bag method formulation B showed highest release.

Finally bioavailability of olmesartan was checked in rabbit to evaluate efficiency of SMEDDS formulation B. Olmesartan was administered in rabbit with dose of 2.8 mg/kg body weight form SMEDDS and market formulation. The pharmacokinetic parameters were determined for olmesartan found higher in case of SMEDDS formulation B. T_{max} was found equal and at the same time C_{max} was increased to 887 as compared to 328 ng/mL for market formulation. Other parameters were also found increased for SMEDDS formulation. Relative bioavailability of SMEDDS formulation B was increased to 2.16 fold as compared to market formulation.

Conclusion

In this study, SMEDDS of valsartan was successfully prepared and demonstrated in rabbits to deliver valsartan to the blood rapidly and more effectively following oral administration. Stability of formulation could increase as it was present in concentrate form. Increase in bioavailability of valsartan was due to promising role of medium chain triglycerides which alter permeability of cell membrane. The studies also demonstrated rapid and larger extent of drug from SMEDDS as compared to market formulation in rabbits. Larger extent of drug transport and wider distribution of the drug in the body is expected to maximize therapeutic index of the drug. SMEDDS of olmesartan was effectively prepared and demonstrated in rabbits to deliver olmesartan with enhanced rate and extent, quickly and effectively to the blood stream following oral administration. These studies aptly demonstrated the effectiveness of SMEDDS of valsartan and olmesartan. However, the ratio of benefits vs. the risks must be evaluated and clinical intricacies must be scientifically established for its efficacy in clinical practice, in the treatment of cardiovascular diseases.

Appendix-I Approval of Animal Ethics Committee for Animal Stu

Centre for Post-Graduate Studies &
Research in Pharmaceutical Sciences
The Maharaja Sayajirao University of Baroda
Shri G. H. Patel Pharmacy Building, Donors' Plaza, Nr. FDL, Fatehgunj, Vadodara-390002, India.
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(partment of Special Assistance, U.G.C; Q.I.P. Center, AICTE; Centre of Relevance & Excellence In New Drug Delivery Systems of TIFAC, DST, Government of India)

Date: 15/02/2008

The meeting of Institutional Animal Ethics Committee (IAEC) is being held for the approval of Research Project of Ph.D & M. Pharm students of pharmacy department M.S.University of Baroda on 15th February, 2008, 12 Noon, at G. H. Patel Pharmacy Building, Opp. M. S. University main building, Donor's Plaza, Fatehgunj, Vadodara. The following members are present for the approval of the enclosed projects.

SR NO	NAME	SIGNATURE
1	Mrs. Snehal Bhatt	
2	Dr. R. S. R. Murthy	
3	Dr. R Balaraman	
4	Dr. A. N. Misra	
5	Dr. (Mrs) R. Giridhar	
6	Dr. M.R Yadav	
7	Mr. Rajesh Bhavsar	
8	Mr. S. P. Rathod	

20	Mrs. Smita S. Pimple	Prof. R. S. R. Murthy	Parenteral colloidal delivery of combination anticancer drug	✓	
21	Mr. Hamsaraj Karanth	Prof. R. S. R. Murthy	Formulation, characterization and evaluation of pH sensitive, serum stable, long circulating liposomes containing anticancer drugs	✓	
22	Mr. Suresh Kamath B.	Prof. R. S. R. Murthy	Formulation, characterization and evaluation of Gold nanoparticles containing anticancer drugs for tumor targeting	✓	
23	Mr. Ronak Patel	Prof. R. S. R. Murthy	Formulation development and evaluation of oral extended release formulation for highly water soluble drugs (Venlafaxine HCl and Tramadol HCl)	✓	
24	Mr. Yogesh Raichandani	Prof. A. N. Misra	Delivery of chemotherapeutic agents for effective treatment of brain tumor	✓	
25	Mr. Kamal kumar Upadhyay	Prof. A. N. Misra	Cancer nanotechnology: Ligand mediated nano-construct for the enhancement of antitumor activity of anticancer drugs in breast cancer	✓	
26	Mr. Sachin P. Naik	Prof. A. N. Misra	Nanopaticulate drug delivery for cancer therapy	✓	
27	Mr. Nirav I. Parmar	Prof. A. N. Misra	Liposomal dry powder inhaler	✓	
28	Mr. Yogesh K. Wagh	Prof. A. N. Misra	Trans nasal microemulsion in emergency contraception	✓	
29	Mr. Bhavik Shah	Prof. A. N. Misra	Vaginal spray of Clotrimazole	✓	
30	Mr. Adhvait Dixit	Prof. S. J. Rajput	Study on self-microemulsifying systems	✓	
31	Mr. Nitin Dobaria	Dr. R. C. Mashru	Development and evaluation of bioadhesive vaginal formulations of some drugs	✓	
32	Mr. Atul C. Badhan	Dr. R. C. Mashru	Design-development and evaluation of gastroretentive drug delivery system	✓	

33	Mr. Vishal Parmar	Prof. K. K. Sawant	Oral delivery of low molecular weight heparin for colon specific delivery (Enoxaparin HCl)	✓	
34	Mr. Darshit Desai	Prof. K. K. Sawant	Novel buccal adhesive system of Prochlorperazine Maleate	✓	
35	Mr. Krishnakumar Patel	Prof. K. K. Sawant	Development of cefdinir nanosuspension for enhancement of oral bioavailability	✓	
36	Mr. Ripal Shah	Prof. K. K. Sawant	Development of microemulsion of Buspirone HCl for nose to brain delivery	✓	
37	Miss Nisha B. Prajapati	Prof. K. K. Sawant	Development of microemulsion containing Famotidine for improvement of oral bioavailability	✓	
38	Mr. Vijay Patel	Prof. K. K. Sawant	Development of multiple emulsion for enhancement of oral bioavailability of Lisinopril Dihydrate	✓	
39	Mr. Nihar Ranjan Kar	Prof. R. Giridhar	Estimation of curcumin and some of its derivatives in biological fluid	✓	
40	Ms. K. Kiruba Florence	Prof. A. N. Misra	Novel intranasal approaches for brain targeting for the treatment of CNS disorders (stroke & epilepsy)	✓	

Mrs. Snehal Bhatt
(CPCSEA Government Nominee)

Dr. R. S. R. Murthy
Prof of Pharmaceutics

Dr. R Balaraman
Prof. of Pharmacology

Dr. M.R Yadav
Prof.of Pharm.Chemistry

Dr. (Mrs) R. Giridhar
Co-ordinater

Dr. A. N. Misra
Head of Pharmacy Dept.

Mr. Rajesh Bhavsar
Sociologist

Mr. S. P. Rathod
Reader in Pharmacology

Appendix II: Abbreviations Used

LC - Liquid Crystal

ME – Microemulsion

SMEDDS – Sel-Microemulsifying Drug Dilivery System

SEDDS – Self-Emulsifying Drug Dilivery System

SNEDDS – Self- Nano Emulsifying Drug Dilivery System

ACE – Angiotensine Converting Enzyme

AUC – Area Under Curve

C_{max} – Peak Plasma Concentration

t_{max} – Time Required For Peak Plasma Concentration

PO – Per Oral

GIT – Gastro Intestinal Tract

MCT – Medium Chain Tryglycerides

HLB – Hydrophillic Lipophillic Balance

PEG – Polyethylene Glycol

HCTZ – Hydrochlorothiazide

HPLC – High Performance Liquid Chromatography

LCMS – Liquid Chromatography Tendum Mass Spectroscopy

ARAII – Angiotensine II Receptor Antagonist

λ_{max} – Absorbance Maximum

GC – Gas Chromatography

UPLC - Ultra Performance Liquid Chromatography

UV – Ultra-Violate

GLC – Gas Liquid Chromatography

HPTLC – High Performance Thing Layer Chromatography

LQC – Low Quantifying Concentration

MQC – Middle Quantifying Concentration

HQC – High Quantifying Concentration

RSD – Relative Standard Deviation

LOD – Limit of Detection

LOQ – Limit of Quantification

SEM – Standard Error of Mean

λ_{ex} – Excitation Maxima

λ_{em} - Emission Maxima

WRS – Working Reference Standard

S/N – Signal to Noise Ratio

ACN – Acetonitrile

R_t – Retention Time

SD – Standard Deviation

VSMS – Valsartan Self Microemulsifying System

S/CoS – Surfactant/Cosurfactant

S_{mix} – mixture of surfactant and cosurfactant

%T – % Transmission

PDI – Polydispersivity Index

R.H. – Relative Humidity

AUMC – Area Under Moment Curve

MRT – Mean Residance Time

Fig. – Figure

TEA – Triethyle Amine

OSMS – Olmesartan Self-Microemulsifying Sysem

S_{mix} – Mixture of Surfactant and Cosurfactant

CMC – Critical Micelles Concentration

PIT – Phase Inversion Temperature

VMD – Volume Mean Diameter

Appendix III: Profile of the Excipients Used

CAPMUL MCM

Synonyms:

Medium chain mono- & diglycerides

Glyceryl mono- & dicaprylo / caprate

Glycerol monocaprylocaprate

Glycerides C8-10 mono-, di-, tri-

Description:

Capmul MCM is a mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). It is an excellent solvent for many organic compounds, including steroids. It is also a useful emulsifier for water-oil (w/o) systems. to 21 CFR § 184.1505 mono and diglycerides prepared from edible fats and oils or fat-forming acids are affirmed GRAS.

Physical and Chemical Properties:

Appearance / Form at 25 °C: Liquid / Semi-solid Visual

Odor: Mild, fatty or grease smell

Color: Off-white

Solubility in Water: Partially Soluble

Residue on Ignition: 0.5%

Heavy Metals, as lead: 10 ppm max

Specific Gravity: 0.97 - 1.02

Toxicology Information:

Acute Oral Toxicity (Oral LD50): > 5 gm / kg in rats

Primary Dermal Irritation (rabbit): Non-irritant

Acute Eye Irritation (rabbit): Non-irritant

Acute Inhalation Toxicity (LC50): > 20 mg / L in rats

Comedogenicity (rabbit): Slight to moderate comedogenic

Dermal Sensitization (guinea pig): Non-sensitizing

Pharmaceutical Applications:

- Carrier (vehicle)
- Solubilizer

• Emulsifier / Co-emulsifier
• Bioavailability enhancer
• Penetration enhancer (dermatological applications)

Storage and Handling:

• Store in a dry location at 68-77 °F
• Retest and re-qualify 20 months from the date of manufacture
• Contents of package must be heated slightly with agitation to ensure uniformity prior to use

CAPMUL MCM C 10

Synonyms:

Glyceryl monocaprate
Medium chain mono- & diglycerides
Glyceryl mono- & dicaprate

Product Description:

Capmul MCM C10 is a mono-diglyceride of medium chain fatty acids (mainly capric). It is a useful emulsifier for water-oil systems. It has an excellent skin feel and has been utilized in lip products to reduce sweating at elevated temperatures and improve gloss. to 21 CFR § 184.1505 mono and diglycerides prepared from edible fats and oils or fat-forming acids are affirmed GRAS.

Physical and Chemical Properties

Appearance / Form at 25 °C: White Solid
Acid Value (mg KOH/g): 2.5 max.
Moisture, Karl Fischer: 1.0% max.

Toxicology Information:

Acute Oral Toxicity (Oral LD50): > 5 gm / kg in rats
Primary Dermal Irritation (rabbit): Non-irritant
Acute Eye Irritation (rabbit): Non-irritant
Acute Inhalation Toxicity (LC50): > 20 mg / L in rats
Comedogenicity (rabbit): Slight to moderate comedogenic
Dermal Sensitization (guinea pig): Non-sensitizing

Pharmaceutical and Applications:

- Carrier (vehicle)
- Solubilizer
- Emulsifier / Co-emulsifier
- Bioavailability enhancer
- Penetration enhancer (dermatological applications)

Storage and Handling:

- Store in a dry location at ambient temperature
- Retest and re-qualify 12 months from the date of manufacture
- Contents of package must be heated slightly with agitation to ensure uniformity prior to use(maximum of 3 heat cycles)

CREMOPHORE RH 40

Chemical nature

The Cremophor® grades are nonionic solubilizers and emulsifying agents obtained by reacting hydrogenated castor oil with ethylene oxide.

Composition

The main constituents of both Cremophor® grades are glyceryl poly ethylene glycol oxystearate, which, together with fatty acid glyceryl poly glyceryl esters, forms the hydrophobic part of the product. The hydrophilic part consists of polyethylene glycols and glyceryl ethoxylate.

State Viscous liquid or soft paste

Specifi cation Limits

Sulphated ash:0.25 g/100 g

Iodine value:1.0/100 g

Saponification value: 50-60 mg KOH/g

Hydroxyl value: 60-75 mg KOH/g

1,4-Dioxane: 10 mg/kg

Acid value:1.0 mg KOH/g

pH value: 6-7

Water content, K. Fischer:2.0 g/100 g

Heavy Metals:10.0 ppm

Solubility

Cremophor® RH 40 form nearly clear solution in water, ethanol, isopropanol, with essential oils and fragrance oils and other hydrophobic compounds, e. g. vitamins and alpha-bisabolol.

Stability

Cremophor® RH 40 are chemically very stable. Prolonged exposure to heat can cause physical separation into a liquid and a solid phase on cooling but the product can be restored to its original form by homogenization.

Applications

The Cremophor® grades are used to solubilize ethereal oils, perfume compositions, vitamins and hydrophobic active substances in aqueous-alcoholic and purely aqueous solutions. The fi nished preparations are particularly stable.

Toxicity

An investigation of the raw material gave no indication of harmful effects to health if the substance is used for the stated applications and con centrations. Due to the large variety of applications and possible combinations with other products, users are responsible for their own safety assessment of their products.

Storage

The Cremophor® grades are stable for at least 2 years if stored in the original sealed containers in a dry place at room temperature.

CREMOPHORE EL

Common names

Polyoxyethylenglyceroltriricinoleat 35 (DAC), Polyoxyl 35 Castor Oil.

Nature

Cremophor EL is a non-ionic solubilizer and emulsifier obtained by causing ethylene oxide to react with castor oil of German Pharmacopoeia (DAB 8) quality in a molar ratio of 35 moles to 1 mole.

Composition

The main component of Cremophor EL is glycerol-polyethylene glycol ricinoleate, which, together with fatty acid esters of polyethyleneglycol, represents the

hydrophobic part of the product. The smaller, hydrophilic part consists of polyethylene glycols and ethoxylated glycerol.

Properties

Cremophor EL is a pale yellow, oily liquid that is clear at temperatures above 26 °C. It has a slight but characteristic odour and can be completely liquefied by heating to 26 °C. The hydrophilic-lipophilic balance (HLB) lies between 12 and 14.

Viscosity (Höppler) at 25 °C: 700 – 850 mPa· s

Mass density at 25 °C: 1.05 –1.06 g/ml

Refractive index at 25 °C: 1.465 –1.475

Saponification value: 63 – 72

Hydroxyl value: 65 – 78

Iodine value: 28 – 32

Acid value ≤ 2

Water content (K. Fischer) ≤ 3%

pH value of 10 % aqueous solution: 6 – 8

Sulfated ash ≤ 0.2%

Heavy metals (USP XX method) ≤ 10 ppm

Solubility

Cremophor EL forms clear solutions in water. It is also soluble in ethyl alcohol, n-propyl alcohol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene and xylene. In contrast to that of anionic emulsifying agents, the solubility in water decreases with rising temperature. Thus, aqueous solutions become turbid at a certain temperature. Cremophor EL is miscible with all other Cremophor grades and, on heating, with fatty acids, fatty alcohols and certain animal and vegetable oils. It is thus miscible with oleic and stearic acids, dodecyl and octadecyl alcohols, castor oil, and a number of lipid-soluble substances.

Stability

Cremophor EL in aqueous solutions is stable towards electrolytes, e. g. acids and salts, provided that their concentration is not too high. Mercury (II) chloride is an exception and forms a precipitate with the product. Some organic substances may cause precipitation at certain concentrations, especially compounds containing phenolic

hydroxyl groups, e. g. phenol, resorcinol and tannin. Cremophor EL can be sterilized by heating in an autoclave for 30 minutes at 120 °C. It may thus acquire a deeper shade. During sterilization, Cremophor EL should not be heated together with substances that are strongly acidic or alkaline and would thus saponify it.

Application

In aqueous solution, Cremophor EL emulsifies or solubilizes the fat-soluble vitamins A, D, E and K. In aqueous-alcoholic solutions, it very readily solubilizes essential oils. Other hydrophobic drugs can also be converted into aqueous solutions with Cremophor EL (e. g. Miconazole, Hexedetine, Clotrimazole, Benzocaine).

Acute toxicity

LD 50 (7 days follow-up period):

Rat oral > 6.4 ml/kg

Rabbit oral > 10.0 ml/kg

Cat oral > 10.0 ml/kg

Mouse i. v. 2.5 – 4 ml/kg

Rat percutaneous > 4.0 ml/kg (maximum applicable dose)

No characteristic toxic symptoms were observed after oral doses or application to the skin, and no pathological changes of the inner organs were discernible with the naked eye during autopsy.

Acute inhalation toxicity

Cremophor EL is pratically non-volatile. In tests, rats have inhaled air saturated at 20 °C with the volatile components of the product for over eight hours without suffering any irritation of respiratory tract or any injury by absorption.

Irritation of skin and mucous membranes

Contact for more than 20 hours between the undiluted product and the highly sensitive skin on the backs and ears of white rabbits caused only slight or insignificant inflammation that disappeared rapidly. This instillation of 0.05 ml of Cremophor EL in the rabbit's conjunctival sac only caused slight reddening of the conjunctiva that disappeared within a few hours. The application of a 50 % aqueous solution of the product caused slight irritation and lachrymation, both of which disappeared rapidly; 30 % aqueous solutions had no irritant effect.

Storage

Cremophor EL should be stored in tightly closed containers and protected from light. Prolonged storage is not advisable unless the containers are completely full.

POLYOXYETHYLENE GLYCOL 400 (The Merck Index, 11th Edition)

Synonym: PEG-8; PEG 400; Poly(oxy-1,2-ethanediyl).alpha.-hydro-.omega.-hydroxy-

Description:

PEG 400 (Polyethylene Glycol 400) is a low molecular weight grade of Polyethylene glycol. It is a clear, colorless, viscous liquid. Due in part to its low toxicity, PEG 400 is widely used in a variety of pharmaceutical formulations.

Physical and Chemical Properties:

IUPAC name: Polyethylene glycol

Molecular formula: $C_{2n}H_{4n+2}O_{n+1}$, n = 8.2 to 9.1

Molar mass: 380-420 g/mol

Density: 1.128 g/cm^3

Melting point: 4-8 °C

Viscosity: 90.0 cSt at 25 °C, 7.3 cSt at 99 °C

Appearance: Liquid. (viscous)

Odor: Odorless.

Color: Clear

Melting Point: 4°C (39.2°F) - 6 C.

Specific Gravity: 1.1254 (Water = 1)

Solubility: Soluble in cold water, hot water. Readily soluble in aromatic hydrocarbons. Slightly soluble in aliphatic hydrocarbons.

Toxicological Information

Acute oral toxicity (LD50): 26800 mg/kg [Rabbit].

Acute dermal toxicity (LD50): >20000 mg/kg [Rabbit].

Acute toxicity of the vapor (LC50): >13 8 hours [Rat].

Handling and Storage

Precautions:

Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as oxidizing agents, acids, alkalis.

Storage: Keep container tightly closed. Keep container in a cool, well-ventilated area.

TWEEN 80 (*Merck Index*, 13th Edition, 7664.)

Tween 80 (Polysorbate 80) is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid, and is often used in foods. Polysorbate 80 is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups which are polymers of ethylene oxide. In the nomenclature of polysorbates, the numeric designation following polysorbate refers to the lipophilic group, in this case the oleic acid.

IUPAC Name: Polyoxyethylene (20) sorbitan monooleate

Molecular formula: $C_{64}H_{124}O_{26}$

Molar mass: 1310 g/mol

Appearance: Amber colored viscous liquid

Density: 1.06-1.09 g/mL, oily liquid

Boiling point: > 100°C

Solubility: In water, very soluble, in other solvents soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene

Viscosity: 300-500 centistokes (@25°C)

Appendix IV: Some of the Commercial Formulations available based on SMEDDS

Name of Molecule/Trade Name	Company	Type of formulation	Lipid excipients used
Cyclosporin A/ Neoral®	Novartis, U.K.	Soft Gelatin Capsule, Oral Solution	dl-tocopherol, corn oil-mono-diglyceride, polyoxyl 40, hydrogenated castor oil (Cremophore RH 40)
Cyclosporin A/ Sandimmunine®	Novartis	Soft Gelatin Capsule, Oral Solution	dl-tocopherol, corn oil-mono-diglyceride, polyoxyl 40, hydrogenated castor oil
Cyclosporin A/ Panimun Bioral™	Panacea Biotech	Soft Gelatin Capsule, Oral Solution	NA
Sequnavir/ Fortovase®	Roche	Soft Gelatin Capsule	NA
Danozol/ Danozol	Barr Laboratories	Soft Gelatin Capsule	soybean oil, maisine 35-I, cremophor El and ethanol
Ritonavir®/Norvir	Abott Laboratories	Soft Gelatin Capsule, Oral Solution	NA
Tipanavir/Aptivus®	Boehringer Ingelheim	Soft Gelatin Capsule	Polyoxyl 35 castor oil (Cremophare EL), medium chain mono-and diglycerides

Appendix V: List of Publications

1. **Paper Published**

   A. Based on Thesis

   1. Spectrofluorimetric Estimation of Valsartan in Marketed Formulation. *Indian Drugs*. 8 (7): 34.

   B. Other Publications

   1. Application of HPLC Techniques as stability indicating method for determination of Gatifloxacin sesquihydrate in pharmaceutical preparation and bioanalysis in human plasma. *Research J Pharmacy and Technology*. (In Press).

2. **Paper Presented**

   1. Development of Self-Microemulsifying Drug Delivery System of Valsartan. AAPS 2007, CA, USA.

ERRATA

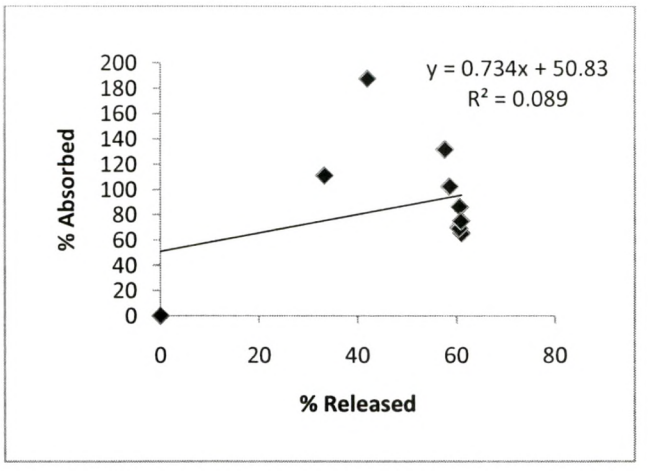
Sr No.	Page No/ Line No:	Uncorrected	Corrected
1	2/32	those of lyotropic liquid crystals depend concentration.	lyotropic liquid crystals depends on concentration.
2	3/3	formation of microemulsion is depends on properties	formation of microemulsion depends on properties
3	5/29	Supporting to this, several in vitro….	Supporting to this, several *in vitro*….
4	10/12	capacity is desired inn	capacity is desired in
5	11/27	evaluation of of the resulting	evaluation of the resulting
6	17/23	Cmax	C_{max}
7	23/30	prolongation if its normally	prolongation of its normally
8	26/9	microemulsions may some time suffers	microemulsions may some time suffer
9	30/9	The in vitro	The *in vitro*
10	32/28	all formulation suffers	all formulations suffer
11	46/13	water soluble hypertensive drugs	water soluble antihypertensive drugs
12	46/20	dissolution rate of these drugs are	dissolution rates of these drugs are
13	51/10	Primary stock solutions are	Primary stock solutions were
14	51/18	Primary stock solutions are	Primary stock solutions were
15	56/11	The similar results are	The similar results were
16	58/12	1.75, 2.0, 2.25 were	1.75, 2.0, 2.25mL were
17	59/18	RSD	% RSD
18	84/7	Valsartan (mg) 10	Valsartan 5
19	84/15	Valsartan (mg) 10	Valsartan 5
20	84/23	Valsartan (mg) 10	Valsartan 5
21	145/21	Olmesartan (mg) 13	Olmesartan 6.5
22	146/2	Olmesartan (mg) 13	Olmesartan 6.5
23	146/10	Olmesartan (mg) 13	Olmesartan 6.5
24	95/7	82.2 form original value	82.2 from original value
25	100/11	The data of stability studies is	The data of stability studies are
26	101/3	viscosity and conductivity confirms	viscosity and conductivity confirm
27	109/20	The study suggest	The study suggests
28	119/29	volume were	volumes were
29	129/2	combinations of Mobile phase	combinations of mobile phases
30	137/13	formulation forms each system	formulation from each system
31	144/7	Therefore to prepare SMEDDS form	Therefore to prepare SMEDDS from

ADDENDA

- Page: 75/Line:28, The phase diagrams were constructed using Chemix software.
- *IN VITRO -IN VIVO* CORRELATION (IVIVC) FOR VALSARTAN

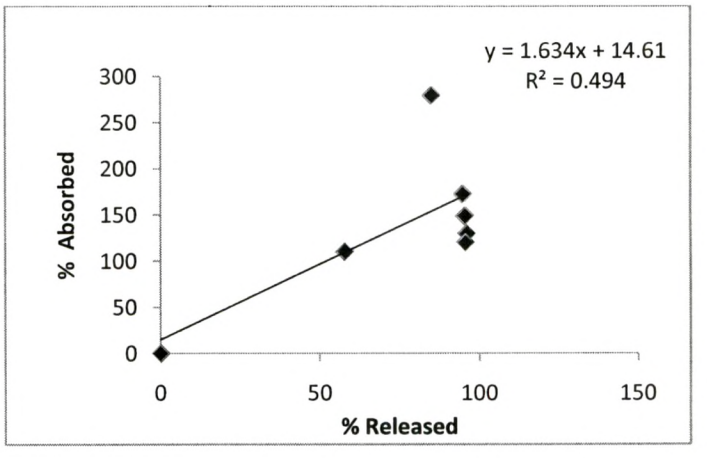
Reference

Time(hr)	% Released	% Absorbed
0	0	0
0.5	33	111
1	42	187
2	58	132
3	59	103
4	61	86
5	60	70
6	61	69
8	61	65
12	61	69
24	61	75



Test

time(hr)	% Released	% Absorbed
0	0	0
0.5	58	110
1	85	279
2	95	172
3	95	149
4	96	129
5	96	120
6	95	111
8	95	87
12	96	78
24	96	91

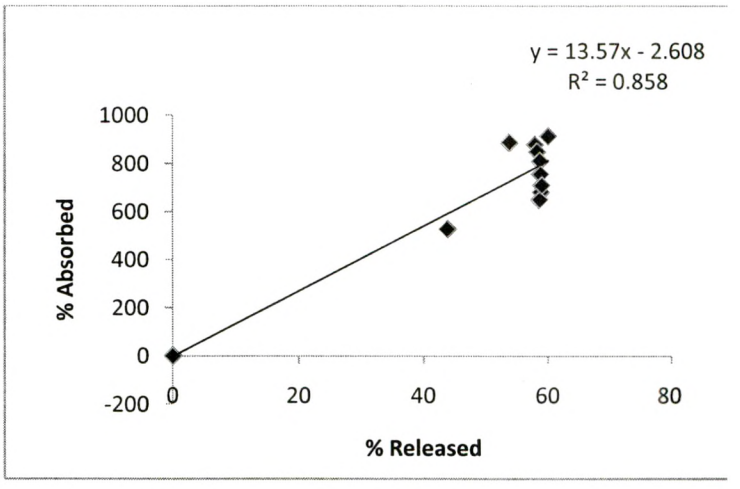


IVIVC found was not significant.

- *IN VITRO -IN VIVO* (IVIVC)CORRELATION FOR OLMESARTAN

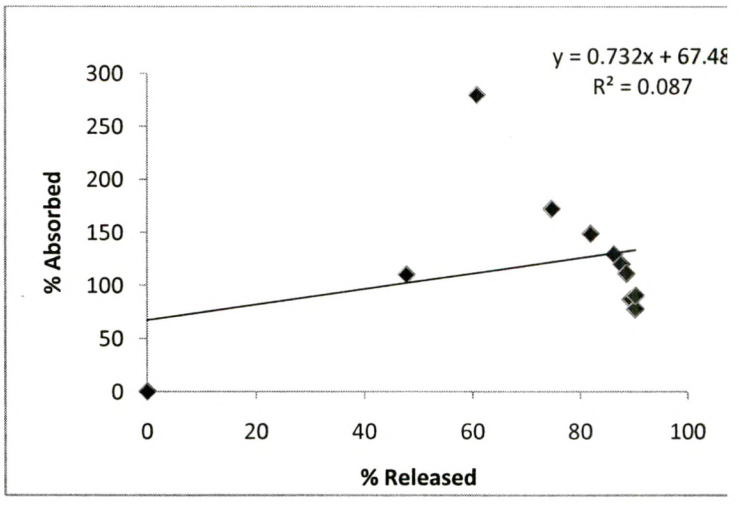
Reference

Time(hr)	% Released	% Absorbed
0	0	0
0.5	44	528
1	54	888
2	58	879
3	58	850
4	59	811
5	59	756
6	59	683
8	59	651
12	59	710
24	60	914



Test

Time(hr)	% Released	% Absorbed
0	0	0
0.5	48	110
1	61	279
2	75	172
3	82	149
4	86	129
5	88	120
6	89	111
8	89	87
12	90	78
24	90	91



IVIVC found was not significant.