FORMULATION AND EVALUATION OF SUSTAINED RELEASE MICROSPHERES OF VENLAFAXINE HYDROCHLORIDE

Dissertation Submitted to

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In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by

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Accredited By "NAAC" with a CGPA of 2.74 on a Four point Scale at B Grade

MELMARUVATHUR - 603 319

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CERTIFICATE

This is to certify that the research work entitled **"FORMULATION AND EVALUATION OF SUSTAINED RELEASE MICROSPHERES OF VENLAFAXINE HYDROCHLORIDE"** Submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the award of the Degree of the Master of Pharmacy (Pharmaceutics) was carried out by **SABARINATHAN.R** (**Register No. 26106006)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2011-2012.

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This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF SUSTAINED RELEASE MICROSPHERES OF VENLAFAXINE HYDROCHLORIDE"** the bonafide research work carried out by **SABARINATHAN.R** (**Register No. 26106006)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai under the guidance of **Prof. K. SUNDARAMOORTHY, B.Sc., M.Pharm., Department of Pharmaceutics, Adhiparasakthi College of Pharmacy,** during the academic year 2011-2012.

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SABARINATHAN R.

My Heartful Dedication To

My Beloved Parents and

My beloved ones...✍

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LIST OF ABBREVIATIONS USED

%	-	Percentage
&	-	And
°C	-	Degree Celsius
µg	-	Microgram
µm	-	Micrometer
λ_{max}	-	Absorption Maximum
i.e	-	That Is
AUC	-	Area Under Curve
BP	-	British Pharmacopoeia
CDDS	-	Controlled Drug Delivery System
CM	-	Centi meter
CNS	-	Central Nervous System
CR	-	Controlled Release
CRDDS	-	Controlled Release Drug Delivery System
SRDDS	-	Sustained Release Drug Delivery System
DE	-	Dissolution Efficiency

DR	-	Drug Release
DSC	-	Differential Scanning Calorimetry
Eg	-	Example
ER	-	Extended Release
F	-	Formulation
FDA	-	Food and Drug Administration
FTIR	-	Fourier Transform Infra Red
GIT	-	Gastrointestinal tract
gm	-	Gram
h	-	Hour
HBS	-	Hydro Dynamically Balanced System
HCL	-	Hydrochloride
ICH	-	International Conference of Harmonization
IP	-	Indian Pharmacopoeia
IR	-	Infra Red
Kv	-	Kilo volt
LOD	-	Loss on Drying
MAOIs	-	Mono Amino Oxidase Inhibitors

M	-	Molarity
m	-	Slope, Units Of Response
MDT	-	Mean Dissolution Time
Mg	-	Milligram
Min	-	Minute
ml	-	Milliliter
Mp	-	Melting point
MRT	-	Mean Residence Time
N	-	Normality
NDDS	-	Novel Drug Delivery System
Nm	-	Nanometer
PBS	-	Phosphate Buffer Saline
pH	-	Negative Logarithm of Hydrogen ion
ppm	-	Parts Per Million
RH	-	Relative Humidity
rpm	-	Revolutions Per Minute
S.D.	-	Standard Deviation
SEM	-	Scanning Electron Microscopy

SR	-	Sustained Release
t	-	Time
$t_{1/2}$	-	Biological half-life
UK	-	United Kingdom
USP	-	United State Pharmacopoeia
UV-VIS	-	Ultraviolet-Visible spectrophotometer
Vs	-	Versus
V/V	-	Volume By Volume
W/W	-	Weight By Weight
5-HT	-	5-Hydroxy Triptamine

INTRODUCTION

1. INTRODUCTION

1.1. NOVEL DRUG DELIVERY SYSTEM *(Bankar G.S and Rhodes C.T., 2009; Brahmankar D.M and Jaiswal S.B., 2005; Chein Y.W., 2002)*

The goal of a sustained release dosage form is to maintain therapeutic blood or tissue levels of the drug for an extended and specified period of time. This is generally accomplished by attempting to obtain "zero-order" release from the dosage form. Zero-order release constitutes drug release from the dosage form which is independent of the amount of drug in the delivery system (i.e. a constant release rate). Sustained-release systems generally do not attain this type of release and usually try to mimic zero-order release by providing drug in a slow first-order fashion (i.e., concentration release dependent). Systems that are designated as prolonged release can also be considered as attempts at achieving sustained-release delivery.

The term “Controlled- release drug product” has been used to describe various types of oral extended release rate dosage forms, including sustained release (SR), sustained action, prolonged action, long action and retarded release. These terms for extended release dosage forms were introduced by drug companies to reflect a special design for producing an extended release (ER) dosage form or used as a marketing term.

In the last two-three decades interest in sustained release drug delivery systems is remarkably increasing. This has been due to various factors viz.

- Developing new drug entities.
- Expiration of international patents
- Discovery of new polymeric materials suitable for prolonging the drug release.

- Need of therapeutic efficacy and safety achieved by sustained release drug delivery.

The subject of sustain release has been reviewed by various authors. Several books have been published on it. These reviews and books provide not only the mechanisms and technology of production of dosage forms but also the information on clinical evidence and performance.

There are many definitions of sustained release but the simplest definition is "Any drug or dosage form or medication that prolongs the therapeutic activity of drug''. The overall objective is that, once the drug-carrier material has been injected or otherwise implanted or taken orally into the body, the drug is released at a predetermined rate for some desired period of time. Controlled release technology is relatively new field and as a consequence, research in this field has been extremely fertile and has produced many discoveries.

Non-immediate release delivery systems may be divided conveniently into 4 categories,

A. Delayed release

B. Sustained release

a) Controlled release

b) Prolonged release

C. Site- specific release

D. Receptor release

Delayed – release systems are those that use repetitive, intermittence dosing of a drug from one or more immediate release units incorporated into a single dosage forms to make delayed action. Example: Repeat- action tablets and capsules, enteric coated tablets where timed release achieved by a barrier coating.

Sustained- release systems includes any drug delivery system that achieves slow release of drug over an extended period of time.

Controlled release systems are those systems which are successful maintaining constant drug levels in blood or target release (i.e.) release rate of drug occurs in controlled manner.

Prolonged released systems only extends the duration of action and drug release that achieved by conventional drug delivery.

Site specific and receptor release refers to targeting of drug directly to a certain biological location. In the case of site- specific release, the target is a certain organ or tissue, for receptor release, the target is the particular receptor for a drug within an organ or tissue.

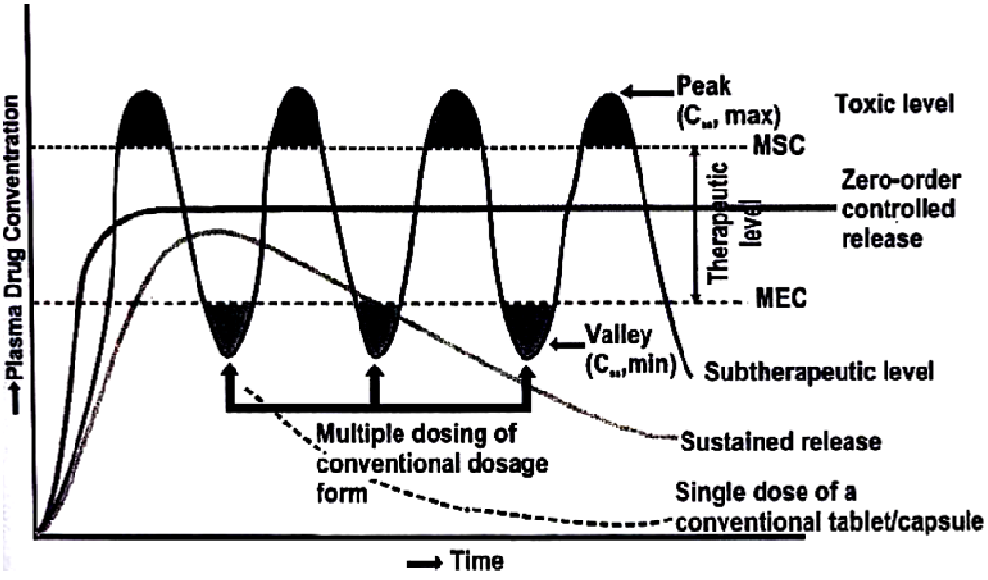


Figure 1.1: A hypothetical plasma concentration vs time profile from conventional multiple and single doses of sustained release drug delivery formulations.

Control release system differs from Sustain release system which simply prolongs the drug release and hence plasma drug levels for an extended period of time (i.e. not necessarily at a predetermined rate). Thus the chief objective of most products should be controlled delivery to reduce dosing frequency to an extent that once daily dose is sufficient for therapeutic management through a uniform plasma concentration at steady state.

Advantages of sustained release drug delivery system *(Bankar G.S and Rhodes C.T., 2009; Chein Y.W., 2002)*

Some advantages are as follows

1. Reduction in dosing frequency.
2. Reduced fluctuation in circulating drug levels.
3. Increased patient convenience and compliance.
4. Avoidance of night time dosing.
5. More uniform effect.
6. Maximum utilization of drug.
7. Reduction in GI irritation and other side effects.
8. Reduction in health care cost through improved therapy.
9. Improve bioavailability of some drugs.

Disadvantages of sustained drug delivery system

1. Decreased systemic availability in comparison to immediate release conventional dosage form. This may be due to
    - Incomplete release,
    - Increased first-pass metabolism, Increased instability,
    - Insufficient residence time for complete release,
    - Site specific absorption, pH dependant solubility, etc.

2. Poor *in vitro-in vivo* correlation.
3. Possibility of dose dumping.
4. Retrieval of drug is difficult in case of toxicity, poisoning, or hypersensitivity reactions.
5. Higher cost of formulation.

PREREQUISITES FOR A DRUG TO BE SUITABLE FOR DESIGN OF ORAL SUSTAINED RELEASE DOSAGE FORM *(Bandyopadhyay A .K., 2008)*

Some characteristics make a drug more suitable for extended release dosing, such as

1. Elimination half-life between 2 to 8 hours.
2. Broader therapeutic index.
3. Moderate unit dose.
4. Significant extent of absorption in GIT.
5. Optimum solubility characteristics.
6. Minimal first-pass clearance.

FACTORS INFLUENCING THE DESIGN AND PERFORMANCE OF ORAL SUSTAINED RELEASE PRODUCTS

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products in different dosage forms. Irrespective of their mode of delivery (immediate, sustained or controlled release) and the design of dosage forms (either solid or liquid) they must be developed within the intrinsic characteristics of GIT physiology. Therefore a fundamental understanding of pharmacokinetics, pharmacodynamics and formulation design is essential to achieve a systematic approach to the successful development of an oral pharmaceutical dosage form. A number of variables such as drug properties, route of delivery, target sites, duration of

therapy, the disease state and patient variables must be considered. The formulation and performance of sustained release products are greatly influenced by the physicochemical and biological properties of drug.

1.2. DRUG PROPERTIES RELEVANT TO SUSTAINED RELEASE FORMULATIONS

(Bankar G.S and Rhodes C.T., 2009; Robinson J.R and Lee V.H.L., 2005)

During design of sustained release delivery systems, variables such as the route of drug delivery, the type of delivery system, the disease being treated, the patient, the length of therapy and the properties of the drug, are considered of particular interest to the scientist designing the system are the constraints imposed by the properties of the drug. These properties are classified as,

A) Physicochemical properties

B) Biological properties

These properties have the greatest effect on the behavior of the drug in the delivery system and in the body. There is no clear-cut distinction between these two categories since the biological properties of a drug are a function of its physicochemical properties. By definition, physicochemical properties are those that can be determined from *in vitro* experiments and biological properties will be those that result from typical pharmacokinetic studies of the absorption, distribution, metabolism, and excretion (ADME) characteristics of a drug and those resulting from pharmacological studies.

A) Physicochemical Properties

a) Dose Size

b) Aqueous Solubility and pKa

c) Partition Coefficient

d) Drug Stability

e) Molecular Size and Diffusivity

f) Drug Protein Binding

B) Biological Properties

a) Absorption

b) Distribution

c) Metabolism

d) Elimination and Biological Half-Life

e) Margin of Safety (Toxicity).

A. Physiochemical properties

a) Dose size: For orally administered drugs, there is an upper limit to the bulk size of the dose to be administered. In general, a single dose of 0.5 – 11.0 gm for conventional dosage form is considered maximal.

b) Aqueous Solubility: Extremes in aqueous solubility are under desirable in the preparation of a SR product. For drug with low water solubility, it will be difficult to incorporate into a SR formulation. The lower limit of solubility for such product has been reported to be 0.1 mg/ml.

c) Partition Co-efficient: Drug that are very lipid soluble or water soluble i.e. extremes in partition co-efficient, will demonstrate either low flux in to the tissues or rapid flux followed by accumulation in the tissues. Both extremes are

undesirable for a SR system Eg: Phenothiazines class of compounds is highly lipid soluble.

d) Drug Stability : since most oral SR systems, by necessity are designed to release their contents over much of the length of the GIT, drugs which are unstable in the environment of the intestine might be difficult to formulate into prolonged release systems.

Eg: Propanthdine and Probanthine.

B. Biological properties

a) Absorption: Drugs that are slowly absorbed or absorbed with variable absorption rate are poor candidates for SR systems. For oral dosage forms the lower limit on the absorption rate constant in the range of 0.17 to 0.23 hr^{-1}(assuming GI transit time of 8-12 hr^{-1}).

b) Metabolism: Drugs that are significantly metabolized, especially in region of small intestine, can show decreased bioavailability from SR dosage forms, because less total drug is presented to enzymatic process during a specific period. This allows more complete conversion of drug to its metabolite.

c) Therapeutic Index: Drugs with a narrow therapeutic range which require precise control over the blood levels of the drug are unsuitable for SR dosage forms.

d) Half Life: The biological half life and duration of action of drug obviously plays a major role in considering a drug for SR systems. Drugs with a very short half life (>2 hours) require large amounts of drug to maintain sustained effects and drugs with longer life (<8hours) because their effects are already sustained.

1.3. PRINCIPLE BEHIND SR/CR DRUG RELEASE *(Bankar G.S and Rhodes C.T., 2009; Brahmankar D.M and Jaiswal S.B., 2009; Robinson J.R and Lee V.H.L., 2005)*

Dissolution and diffusion controlled systems have classically been of primary importance in oral delivery of medication because of their relative ease of production and cost compared with other methods of sustained or controlled delivery. Most of these systems are solids, although a few liquids and suspension have been recently introduced.

The classification of such systems is as follows:

1. Diffusion controlled systems.
2. Dissolution controlled systems.
3. Dissolution and Diffusion controlled systems.
4. Osmotically controlled systems.
5. Ion exchange systems.

1. Diffusion Controlled Systems

Diffusion systems are characterized by the release rate being dependent on its diffusion through an inert membrane barrier. Usually this barrier is an insoluble polymer. In general two types of sub classes of diffusion systems are recognized they are

a. Reservoir devices.
b. Matrix devices.

a. Reservoir devices

Reservoir devices are characterized by a core drug reservoir surrounded by a polymeric membrane.

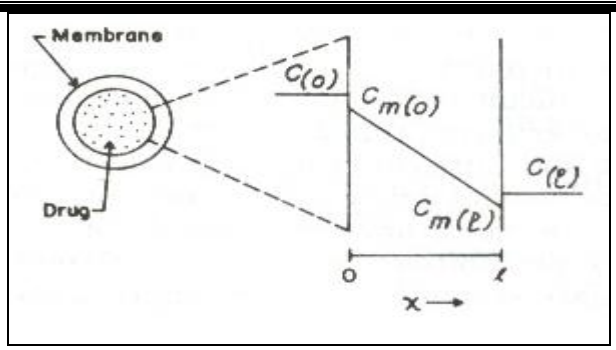


Figure 1.2: Systematic representation of diffusion controlled drug release matrix system.

Figure 1.2 Schematic representation of reservoir diffusion device Cm (o), and Cm (d) represent concentration of drug inside surfaces of membrane and C (o) & C (d) represents concentration in adjacent regions.

If it is assumed that the drug on the both side of membrane is in equilibrium with its respective membrane surface which in equilibrium between the membrane surfaces and their bathing solutions as shown in Figure 1.2

Therefore the concentration just inside the membrane surface can be related to the concentration in the adjacent region by following expression.

$K = Cm(o) / C(d)$ at $X = o$ (2)

$K = Cm(d) / C(d)$ at $X = d$ (3)

Where K = partition coefficient. If we consider K & D are constants then equation (1) becomes,

$$J = D K \Delta C/d \quad (4)$$

Where Δc is the concentration difference across the membrane and d is path length of diffusion. The simplest system to consider is that of slab, where drug release is from only one surface as shown Figure 1.2 in this case equation (4) becomes

$$dMt/dt = ADK \Delta C/d \quad (5)$$

The process of diffusion is generally described by Ficks equation,

$$J = -D\, dc/dx$$

Where, J -- Flux (amount/ area –time)

D -- Diffusion co-efficient of drug in the membrane (area / time)

Dc/dx -- rate of exchange in concentration C, with respect to a distance X in the membrane.

Advantages

1. Zero order delivery is possible.
2. Release rate variable with polymer type.

Disadvantages

1. Potential toxicity if system fails.
2. System must be physically removed from implant sites.
3. Difficult to deliver high molecular weight compounds.
4. Generally increased cost per dosage units.

b. Matrix Devices

It contains of drug dispersed homogeneously throughout a polymer matrix. In this model, drug in the outside layer exposed to bath solution is dissolved first and then diffuses out of the matrix. The following equation describe the rate of release of drug dispersed in an inert matrix system have been derived by Higuchi.

$$Dm/d_h = Cod_h – Cs/2.$$

Where, dm = Change in the amount of drug released per unit area.

d_h = Change in the thickness of the zone of matrix that have been depleted of drug.

Co = Total amount of drug in unit volume of matrix.

Cs = Saturated concentration of drug within the matrix.

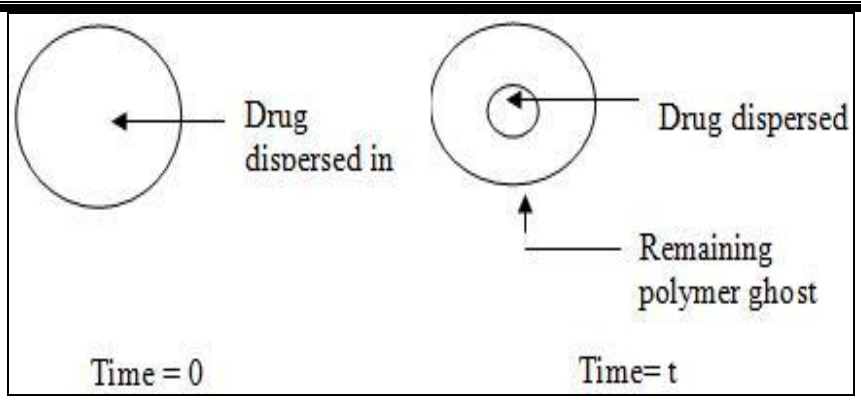


Figure 1.3: Release of drug dispersed in an inert matrix system.

Advantages

1. Can deliver high molecular weight compounds.
2. Easier to produce than reservoir devices.

Disadvantages

1. Removal of remaining matrix is necessary for implanted systems.
2. Cannot obtain zero order release.

Diffusion Rate Modifications

Modification of the following will change the rate of diffusion

(a) Thickness of the separating layer

(b) Porosity

(c) Partition coefficient

(d) Modification of the diffusion co-efficient.

(e) Modification of efficient molecular size.

(f) Modification of viscosity.

(g) Modification of concentration.

2. Dissolution-controlled Systems

Drug with a slow dissolution rate will demonstrate sustaining properties, since the release of the drug will be limited by rate of dissolution. This being the case, SR preparations of drugs could be made by decreasing their dissolution rate. This includes preparing appropriate salts or derivatives, coating the drug with a slowly dissolving material, or incorporating it into a tablet with a slowly dissolving carrier.

The dissolution process at steady state, is described by Noyes-Whitney equation,

$dc/dt = K_D A(Cs-C) = D/h\ A(Cs-C)$

Where,

dc/dt = Dissolution rate.

K_D = Diffusion co-efficient

A = surface area of the dissolving solid

Cs = Saturation solubility of the solid.

C = Concentration of solute in bulk solution.

H = Thickness of diffusion layer.

Principles of dissolution rate modification

The following are may affect dissolution rate modification of

(a) Solubility,

(b) Specific area,

(c) Particle shape and surface structure,

(d) Dissolution conditions (contact of solid particles with the Solvent) and

(e) Crystallographic modification.

3. Dissolution and Diffusion - Controlled release system

Normally, therapeutic systems will never be dependent on dissolution only or diffusion only. In practice, the dominant mechanism for release will over shadow other processes enough to allow classification as either dissolution rate limited or diffusion controlled.

The mechanism of release from simple erodible slabs, cylinders and spheres has been described by Hopenberg are described as

$$Mt/M = 1 - (1- K_o t/C_o a)^n$$

Where, n = 2 for cylinder and

n = 1 for a slab.

a = Radius of sphere or cylinder or half height of a slab.

M_t = Mass of drug release at time t

M = Mass released infinite time.

Advantages

1. Easier to produce than reservoir devices.
2. Can deliver high molecular weight compounds.
3. Removal from implant sites is not necessary.

Disadvantages

1. Difficult to control kinetics owning to multiple process of release.
2. Potential toxicity of degraded polymer must be considered.

4. Osmotically controlled systems

This device is fabricated as tablet that contains water soluble osmotically active drug, of that was blended with osmotically active diluents by coating the tablet with a cellulose triacetate barrier which functions as a semi permeable membrane. A laser is used to form a precision orifice in the barrier, through which the drug is released due

to development of osmotic pressure difference across the membrane, when this was kept in water.

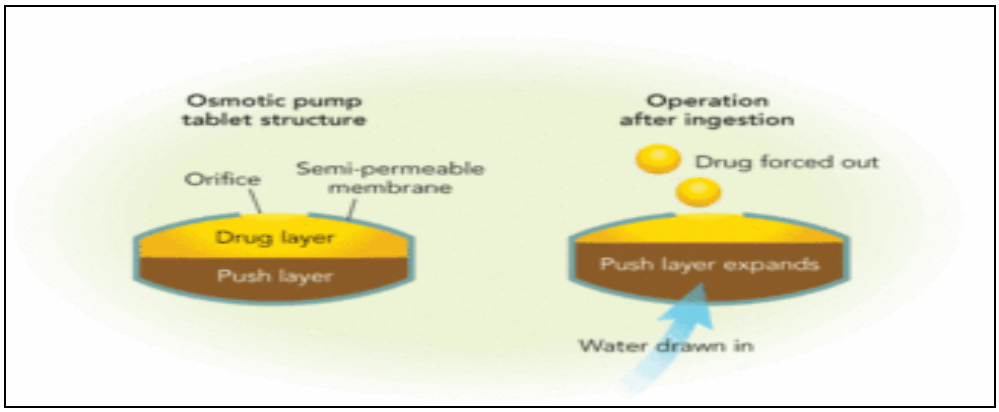


Figure 1.4: Osmotically controlled release systems.

5. Ion Exchange Systems

These are salts of cationic or anionic exchange resins or insoluble complexes in which drug release results from exchange of bound drug ions that are normally present in GI fluids.

1.4. MICROSPHERES

(shobharani., 2008; Kedar Prasad Meena and Danji J.S., et al., 2011)

Microspheres are solid, approximately spherical particles ranging 1-1000µm in size. They are made up of polymeric substances, in which the drug is dispersed throughout the microsphere matrix. The substances used in the formulation are biodegradable synthetic polymers and natural products. The natural polymers of choice are albumin and gelatin, the synthetic ones being polylactic acid and polyglycolic acid. The polymers used to manufacture microspheres are chosen according to their solubility, stability profile, process, safety and economic suitability.

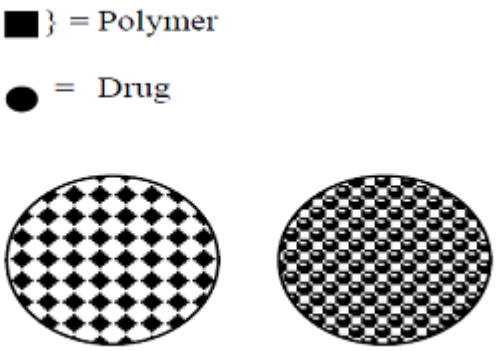


Figure 1.5: Microsphere

Advantages of microsphere delivery system

- Protection of unstable, sensitive materials from their environments prior to use.
- Better processability (improving solubility, dispersibility, flowability)
- Self-life enhancement by preventing degradative reactions.
- Safe and convenient handling of toxic materials.
- Masking of odor or taste.
- Enzyme and microorganism immobilization.
- Controlled and targeted drug delivery.
- Handling liquids as solids.
- To improve bioavailability
- To improve the stability
- Limiting fluctuation within therapeutic range

Applications of microspheres

Some of the applications of microspheres can be described in detail as given below

1. Prolonged release dosage forms. The microsphere drug can be administered, as microsphere is perhaps most useful for the preparation of tablets, capsules or parenteral dosage forms.

2. Microsphere can be used to prepare enteric coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.

3. It can be used to mask the taste of bitter drugs.

4. From the mechanical point of view, microsphere has been used to aid in the addition of oily medicines to tablet dosage forms. This has been used to overcome problems inherent in producing tablets from otherwise tacky granulations. This was accomplished through improved flow properties. For example, the non-flowable multicomponent solid mixture of niacin, riboflavin, and thiamine hydrochloride and iron phosphate may be encapsulated and made directly into tablets.

5. It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat. Microsphere does not yet provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however a great degree of protection against these elements can be provided. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microsphere**.**

6. Microsphere can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.

7. Microsphere has also been used to decrease potential danger of handling of toxic or noxious substances. The toxicity occurred due to handling of fumigants, herbicides, insecticides and pesticides have been advantageously decreased after microencapsulation.

8. The hygroscopic properties of many core materials may be reduced by microsphere.

9. Many drugs have been microsphere to reduce gastric irritation.

10. Microsphere method has also been proposed to prepare intrauterine contraceptive device.

1.5. Classification of polymers used for preparation of microspheres

(Vyas S.P., 2002)

A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of microspheres. These materials include the polymers of natural and synthetic origin and also modified natural substances. Some of the polymers used in the preparation of the microspheres are classified and listed in Table 1.

Table 1.1: Classification of polymers

Nature of polymer	Classification	Examples
A. Synthetic polymer	Non biodegradable	Acrolin Glycidal methacrylate Epoxy polymers
	Biodegradable	Lactides and glycosides and other copolymers Polyalkyl cyano acrylates Polyanhydride
B. Natural polymer	Protein	Albumin Gelatin Collagen
	Polysaccharides	Chitosan Agarose Carragenen Starch Sodium alginate
	Chemically modiffied carbohydrates	Poly(acryl)dextran Poly(acryl)starch

Prerequisites for ideal micosphere carriers

The polymer utilized for the preparation of microspheres should ideally fulfill the following prerequisites:

- ✓ Longer duration of action
- ✓ Control of content release
- ✓ Increase of therapeutic efficacy
- ✓ Protection of drug
- ✓ Reduction of toxicity
- ✓ Biocompatibility
- ✓ Sterilizability
- ✓ Relative stability
- ✓ Water solubility or dispersability
- ✓ Bioresorbability
- ✓ Targetability
- ✓ Polyvalent

1.6. PREPARATION METHOD OF MICROSPHERE

(Kedar Prasad Meena and Danji J.S., et al., 2011)

Preparation of microspheres should satisfy certain criteria

- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and dispersability in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale.
- Biocompatibility with a controllable biodegradability.

Preparation of microspheres by any one of the suitable methods like:

1. Protein gelation technique

2. Single Emulsion polymerization technique

3. Double Emulsion polymerization technique

4. Multiple emulsion polymerization technique

5. Solvent evaporation technique

6. Sonication technique

7. Spray and freeze drying technique

8. Emulsification-heat stabilization technique

9. Quasi-emulsion solvent diffusion method of the spherical crystallization technique

10. Spray congealing

11. Phase separation coaservation method

12. Polymerisation technique

13. Solvent extraction method

1. Protein gelation technique

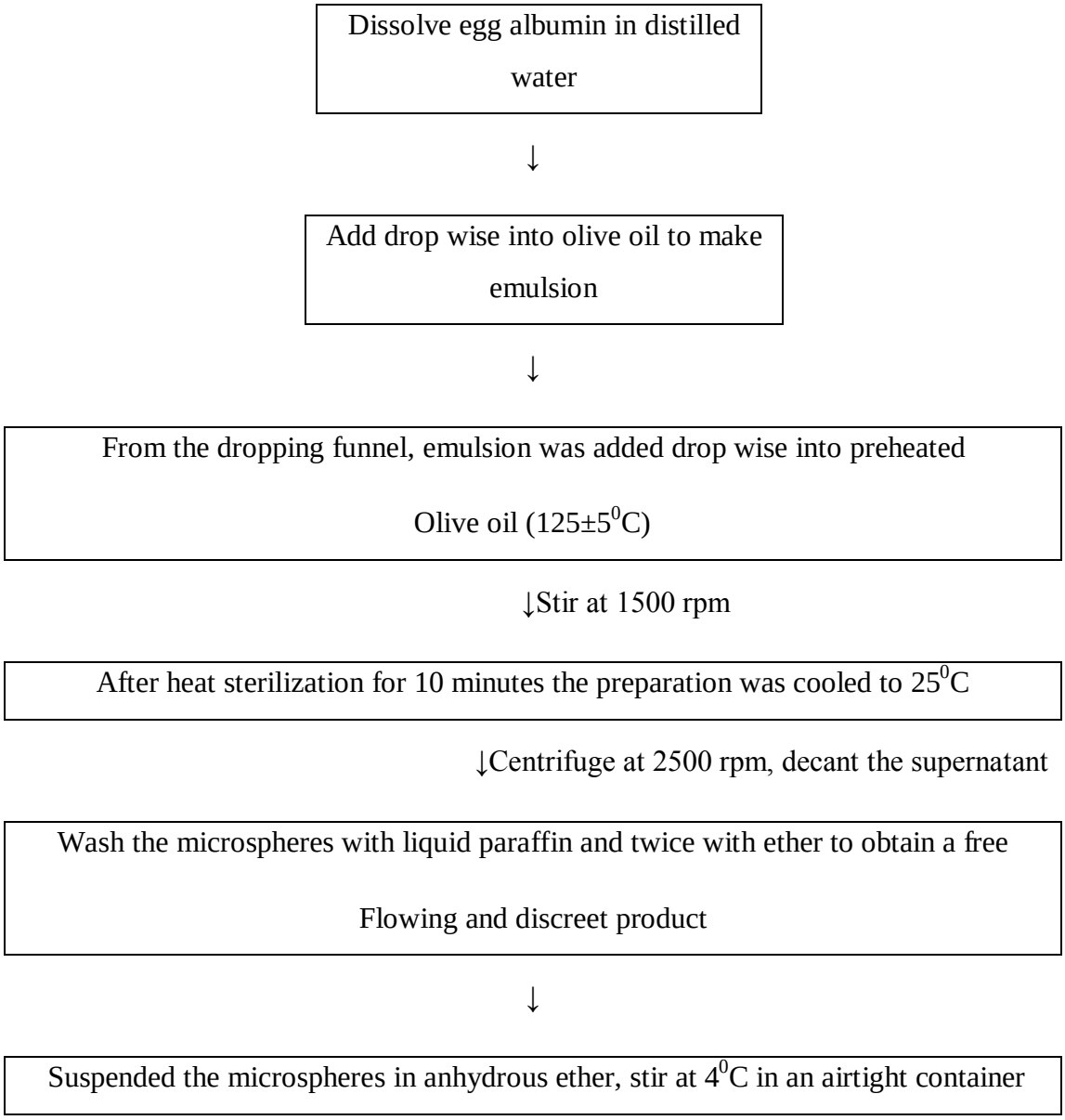


Figure 1.6: Preparation of microspheres by Protein gelation technique.

2. Single Emulsion polymerization technique

Take 100ml of light paraffin oil in glass beaker , mix with 0.4% w/v span 60

↓Stir and heat at 70^0C until complete solubilisation

Cool the mixture at room temperature

↓

Drop wise add 10ml of egg albumin aqueous solution of different drug to polymer ratio (1:1, 1:5, 1:2) using a 22-gauge hypodermic syringe into external phase

↓ Stir light paraffin at 600 rpm for 10 minutes

A w/o emulsion was formed

↓Raise the temperature of oil bath to 95^0C, stir until microspheres completely dehydrate

Microspheres thus obtained were decanted, washed 6 times with 20ml petroleum ether for 2 minutes at 700 rpm

↓ finally wash 3 times with 60 oz of distilled water for 2 minutes at 700 rpm, dry at room temp for 24 hour

After drying, a fine yellow free flowing powders of microsphere were obtained that was stored in desiccators at room temperature.

Figure 1.7: Preparation of microspheres by Single emulsion polymerization technique.

3. Double Emulsion polymerization technique

A double emulsion is usually prepared in two main modes

Mode 1: One-step emulsification

In one step emulsification mode a strong mechanical agitation is used for the water phase containing a hydrophilic surfactant and an oil phase containing large amounts of hydrophobic surfactant. Due to this a W/O emulsion is formed which quickly inverts to form a W/O/W double emulsion.

Mode 2: Two-step emulsification

A two-step procedure is reported where the primary emulsion can be formed as a simple W/O emulsion which emulsion can be formed as a simple W/O emulsion which is prepared using water and oil solution with a low HLB (hydrophilic-lipophilic balance) surfactant. In the second step, the primary emulsion (W/O) is re-emulsified by aqueous solution with a high HLB surfactant to produce a W/O/W double emulsion.

4. Multiple emulsion polymerization technique

Multiple emulsion method involves formation of (o/w) Primary emulsion (non aqueous drug solution in polymer solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of cross linking agent (glutaraldehyde) and evaporation of organic solvent. This method of preparation is ideal for incorporating poorly aqueous soluble drug, thus enhancing its bioavailability.

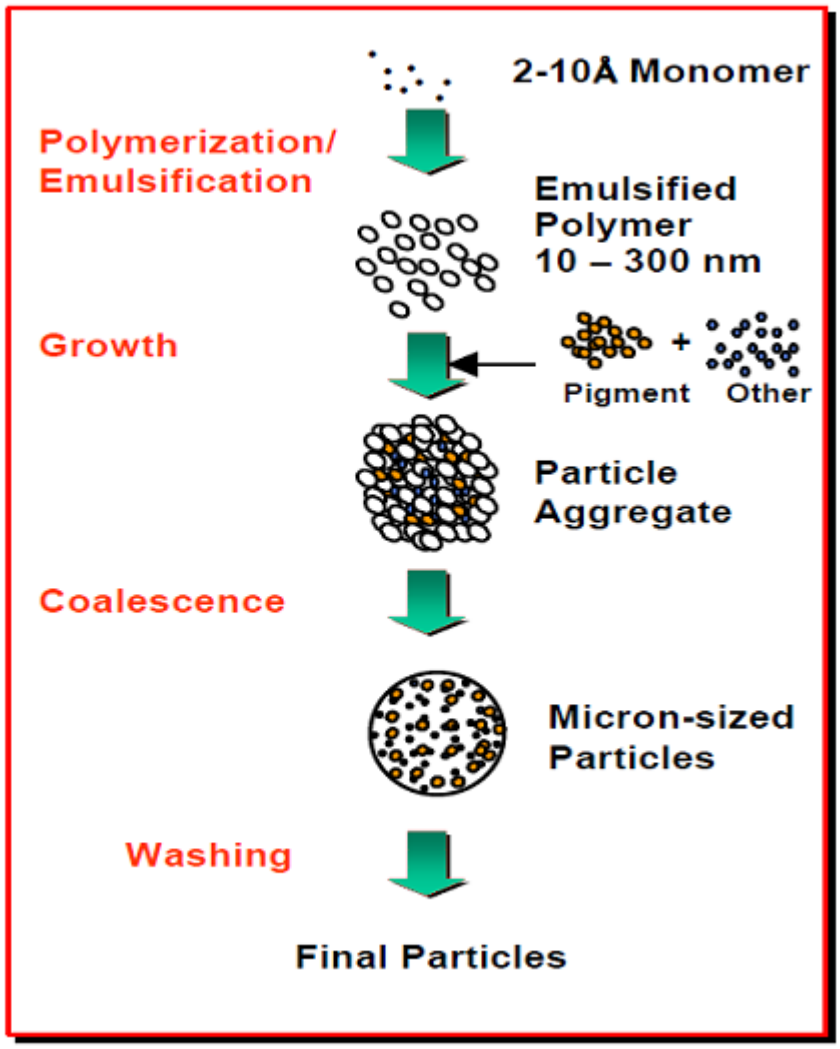


Figure 1.8: Microsphere preparation by multiple emulsion method

5. Solvent evaporation technique

A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microsphere. The mixture is then heated if necessary to evaporate the solvent. The solvent Evaporation technique to produce microspheres is applicable to wide variety of core materials. The core materials may be either water soluble or water insoluble materials. Solvent

evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

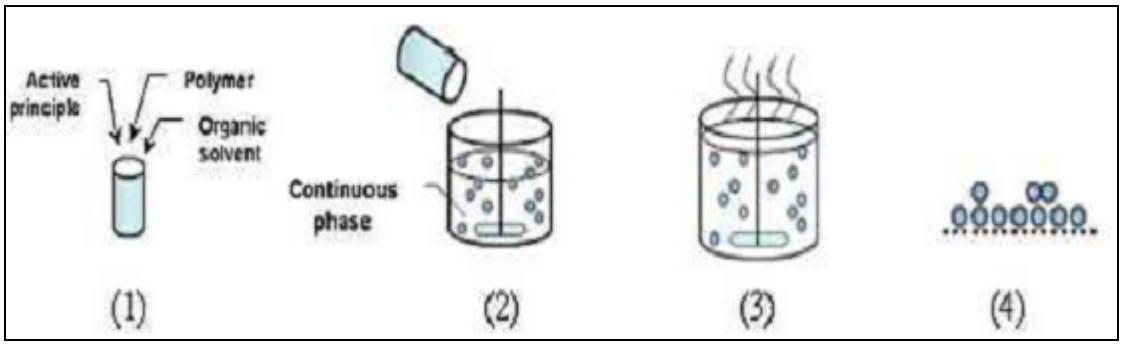


Figure 1.9: Basic steps of microsphere by solvent evaporation method

6. Sonication technique

As the technique name itself is self explanatory, it just involves a simple sonication for certain period of time till a desired size of microspheres are obtained. The polymer solution of desired concentration is taken which is sonicated. To this add the drug which will then form intrachain cross-link with cysteine residues of polymer chains. Prepared a stable preparation of air filled human polymer microspheres (Albunex) by sonication technique. The microspheres ranged in size from 1-10m with 99% of particles smaller than 10 m. The mean size was 5 m, which is small enough to pass freely through the pulmonary capillary circulation.

7. Spray drying technique

In Spray Drying the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, Acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the

small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100m.

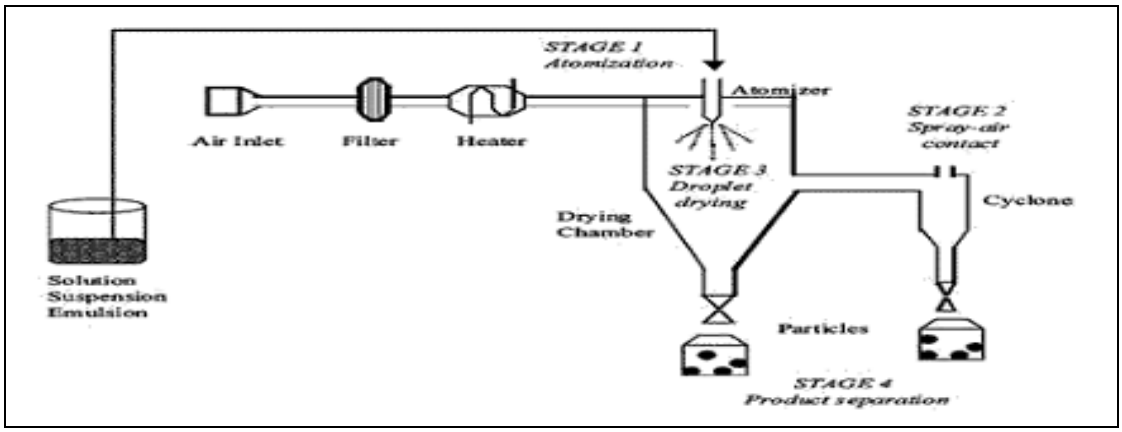


Figure 1.10: Main process stages involved in spray drying process.

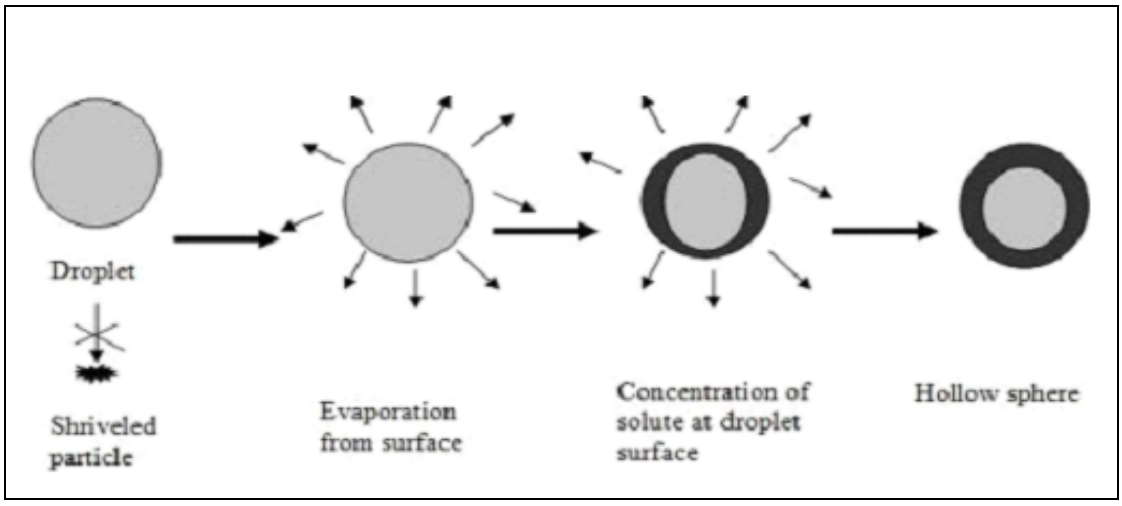


Figure 1.11: Formation of product in spray drying.

Principle

There are three fundamental steps involved in spray drying

1) Atomization of a liquid feed into fine droplets.

2) Mixing of these spray droplets with a heated gas stream, allowing the liquid to evaporate and leave dried solids.

3) Dried powder is separated from the gas stream and collected. Spray drying involves the atomization of a liquid feedstock into a spray of droplets and contacting the droplets with hot air in a drying chamber.

The sprays are produces by either rotary (wheel) or nozzle atomizers. Evaporation of moisture from the droplets and formation of dry particles proceed under controlled temperature and airflow conditions. Powder is discharged continuously from the drying chamber. Operating conditions and dryer design are selected according to the drying characteristics of the product and powder specification.

8. Emulsification-heat stabilization technique

5% solution of BSA containing 0.1% Tween80 was made, to which 4% drug was added and used as the aqueous phase. The oil phase composed of 30 ml maize oil and 10 ml petroleum ether with 1% Span 80 as emulsifier were mixed together and allowed to stir for 10 min at 1000 rpm. The aqueous phase was added drop wise to the oil phase and stirred on a magnet stirrer at 1000 rpm for 30 min to form the initial emulsion. This emulsion was then added to 40 ml of maize oil preheated to 120° C and stirred at 1000 rpm for 15 min to allow the formation and solidification of microspheres. The microsphere suspension was centrifuged at 3500 rpm for 30 min and the settled microspheres were washed three times with ether to remove traces of oil on microsphere surfaces. The microspheres were vacuum dried in desiccators overnight and stored were vacuum dried in a desiccators overnight and stored at 4°C in dark. The microspheres had mean diameters between 1-25 m of which more than 50 percent were below 5 m.

9. Quasi-emulsion solvent diffusion method of the spherical crystallization technique.

Development and characterization of sustained release microspheres by quasi emulsion solvent diffusion method. The microspheres were prepared using the quasiemulsion solvent diffusion method of the spherical crystallization technique. Drug and polymer were dissolved completely in the acetone–dichloromethane mixture. Then Aerosil was suspended uniformly in the drug– polymer solution under vigorous agitation. The resultant drug–polymer–Aerosil suspension was poured into the distilled water (150 ml) containing 0.08% of SDS (i.e. poor solvent) under a moderate agitation (450–750rpm) and thermally controlled at 0–38°C. The suspension was finely dispersed into quasi-emulsion droplets immediately under agitation, and the drug and polymers co precipitated in the emulsion droplets. After agitating the system for 20 min, 150 ml of poor solvent was added slowly to promote the diffusion of the good solvent from emulsion droplets into poor solvent resulting in enhancement of the solidification of quasiemulsion droplets.

10. Spray congealing

The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of cold air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 m.

11. Phase separation coacervation technique

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment

12. Polymerization techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

I. Normal polymerization

II. Interfacial polymerization. Both are carried out in liquid phase.

- **Normal polymerization**

It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers.

- **Interfacial polymerization**

It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase

13. Solvent extraction

The contaminants are separated from the solvent either by changing the pressure and temperature, by using a second solvent to pull the first solvent out of the solvent/contaminant mixture, or by other physical separation processes. At the completion of this step, concentrated contaminants result. Concentrated contaminants are removed during the separation process, and the solvent is sent to a holding tank for reuse. The contaminants are then analyzed to determine their suitability for recycle/reuse, or need for further treatment before disposal.

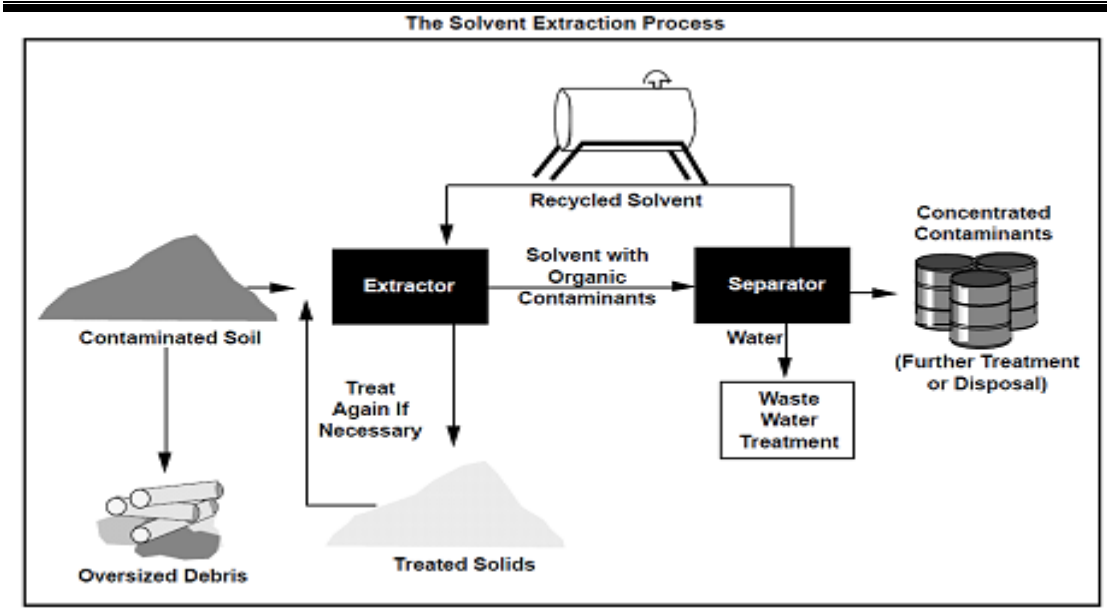


Figure 1.12: Microsphere preparation by solvent extraction.

Loading of drug *(vyas s.p., 2002)*

The active components are loaded over the microspheres principally using two methods. I.e. during the preparation of the microsphere or after the formulation of the microsphere by incubating them with the drug/protein. The active component can be loaded by means of the physical entrapment, chemical linkage and surface absorption. The entrapment largely depends on the method of preparation and nature of the drug or polymer.

Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives, heat of polymerization, agitation intensity, etc.

The loading is carried out in pre-formed microspheres by incubating them with high concentration of the drug in a suitable solvent. The drug in these microspheres loaded via penetration or diffusion of the drug through the pores in the microspheres as well as adsorption on the surface. The drugs and protein can also be

incorporated by physical or chemical linkage. The absorption of the drug/proteins depends on the nature of the polymers.

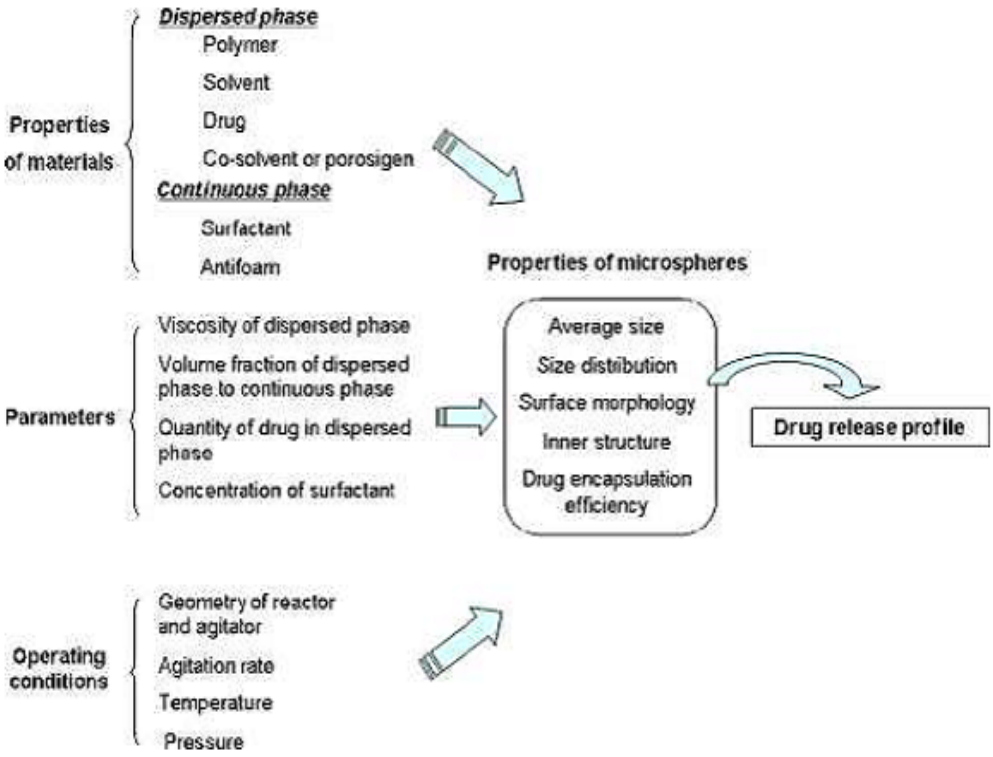
1.7. DRUG RELEASE KINETICS

Release of the active constituent is an important consideration in case of microspheres. Many theoretically possible mechanisms may be considered for the release of drug from the microspheres.

1. Liberation due to polymer erosion or degradation,
2. Self diffusion through the pore,
3. Release from the surface of the polymer,
4. Pulsed delivery initiated by the application of an oscillating or sonic field.

FACTORS INFLUENCING PROPERTIES OF MICROSPHERES.

(Kedar Prasad Meena and Danji J.S.,et al., 2011)



(A) Dispersed phase

1. Polymers commonly used to form microspheres

2. Choice of solvent

(1) Should be able to dissolve the chosen polymer;

(2) Poorly soluble in the continuous phase;

(3) High volatility and a low boiling point;

(4) Low toxicity.

(5) Alternative components (dispersed phase)

(a) Co-solvent:- organic solvents miscible with water such as methanol and ethanol.

(b) Porosity generator:- increases the degradation rate of polymer and improves drug release rate. E.g. Incorporating sephadex (cross-linked dextran gel) into insulin–pla microspheres significantly increases microsphere porosity.

(B) Continuous phase

(a) Surfactant:-

- It reduces the surface tension of continuous phase.
- Avoids the coalescence and agglomeration of drops.
- Stabilizes the emulsion.
- Widely used stabilizers include:

i. Non-ionic: partially hydrolyzed poly vinyl alcohol, methylcellulose, tween, span

ii. anionic: sodium dodecyl sulphate (sds), sodium lauryl sulphate.

iii. cationic: cetyltrimethyl ammonium bromide (ctab).

(b) Alternative component:-

- Antifoaming agent - foaming problem will disturb the formation of microspheres.
- Anti-foams of silicon and non-silicon constituents are used.

(C) Impact of parameters and operating conditions on the properties of microspheres.

Technology limitations in preparing microspheres

- Residual solvents
- Stability
- Non availability of degradable, synthetic polymers
- Encapsulation efficiency
- Limitation of manufacturing process
- Sterilization

1.8. DEPRESSION *(http://www.about.com)*

Depression makes a person feel sad, hopeless, worthless, pessimistic and guilty. Often the sufferer has difficulty concentrating and making decisions, has a loss of appetite and weight or a weight gain, has difficulty sleeping, has a lack of energy and sometimes physical symptoms such as slow movement and speech. Depression must be taken seriously because of the high rate of suicide associated with it.

Most common types of depression

The following are descriptions of the most common types of depression.

1. Major Depressive Disorder

When people use the terms depression or clinical depression, they are generally referring to major depressive disorder. Major depressive disorder is a mood disorder characterized by a depressed mood, a lack of interest in activities normally enjoyed, changes in weight and sleep, fatigue, feelings of worthlessness and guilt, difficulty concentrating and thoughts of death and suicide. If a person experiences the

majority of these symptoms for longer than a two-week period, they may be diagnosed with major depressive disorder.

2. Dysthymic Disorder

Dysthymia (pronounced Dis-THIGH-me-uh) comes from the Greek roots dys, meaning "ill" or "bad," and thymia, meaning "mind" or "emotions." The terms dysthymia and dysthymic disorder refer to a mild to moderate, chronic state of depression.

3. Bipolar Disorder

Bipolar disorder is an illness that consists of alternating periods of elevated moods, called manic episodes, and depression. Mood swing run on a spectrum from mild mania (called hypomania) to more severe, debilitating highs. Periods of mania can last for hours, days, weeks or even months before depression returns.

4. Postpartum Depression

Pregnancy brings about many hormonal shifts. These dramatic shifts can sometimes affect mood. This is commonly known as the "baby blues." Postpartum depression can be more than just a case of the blues, however. It can range from mild symptoms that go away without treatment all the way up to postpartum psychosis, which left untreated, may be responsible for tragic murders of children.

5. Seasonal Affective Disorder

If you experience depression, sleepiness, weight gain and carbohydrate cravings during the winter months, but feel great as soon as spring returns, you may have a condition called Seasonal Affective Disorder (SAD).

6. Premenstrual Dysphoric Disorder

The most frequently reported symptoms of premenstrual syndrome (PMS) include irritability, fatigue, anxiety, nervous tension, mood swings, depression, feeling overwhelmed or out of control, physical symptoms of swelling or bloating of the abdomen or extremities, appetite changes and food cravings, aches, and breast tenderness. These symptoms may occur for several days to 2 weeks before menses but subside with the onset of the menstrual period. When these symptoms, especially those of mood, are severe, a diagnosis of premenstrual dysphoric disorder (PMDD) may be made.

7. Atypical Depression

Do you experience symptoms such as improved mood when good things happen, overeating, sleeping too much or sensitivity to rejection? These are symptoms characteristic of atypical depression, which is a type of depression which does not follow the "typical" set of depression symptoms, such as a lack of appetite and insomnia. It is actually more common than the name might imply.

Etiology of depressions

Though there are several theories about what cause classical depressions, 5-HT plays an important role in the genesis of depressive psychosis as evidenced by increasing in 5-HT (or) its precursors in the brain in patients of depressive psychosis. Many drugs (antidepressants) based on 5-HT metabolization and activations are now available. They prevent the uptake of 5-HT from serotonergic nerve endings, thereby increasing endogeneous 5-HT levels.

Similarly, noradrenaline (NAD) also plays an important role in the genesis of depression as its absence in various areas of the CNS leads to the development of depressions. Antidepressant like MAO and SNRI act by inhibiting the metabolism of NAD (or) preventing its reuptake into the adrenergic nerve ending.

Symptoms and Signs of Depression

Depression is not something you feel for a day or two before feeling better. In true depressive illnesses, the symptoms last weeks, months, or sometimes years if you don't seek treatment. If you are depressed, you are often unable to perform daily activities. You may not care enough to get out of bed or get dressed, much less work, do errands, or socialize.

Adults: You may be said to be suffering from a major depressive episode if you have a depressed mood for at least two weeks and have at least five of the following clinical symptoms:

- Feeling sad or blue
- Crying spells
- Loss of interest or pleasure in usual activities
- Significant increase or decrease in appetite
- Significant weight loss or weight gain
- Change in sleep pattern: inability to sleep or excessive sleeping
- Agitation or irritability
- Fatigue or loss of energy
- A tendency to isolate from friends and family
- Trouble concentrating
- Feelings of worthlessness or excessive guilt

- Thoughts of death or suicide

Facts/Statistics of Depression

Depression affects about 19 million Americans annually. It is estimated to contribute to half of all suicides. About 5%-10% of women and 2%-5% of men will experience at least one major depressive episode during their adult life. Depression affects people of genders, as well as all races, incomes, ages, and ethnic and religious backgrounds. However, it is twice as common in women compared to men and three to five times more common in the elderly than in young people

1.9. DIAGNOSIS OF DEPRESSION

Many providers of health care may help diagnose clinical depression: licensed mental-health therapists, family physicians, or other primary-care providers, specialists whom you see for a medical condition, emergency physicians, psychiatrists, psychologists, psychiatric nurses, and social workers.

If one of these professionals suspects that you have depression, you will undergo an extensive medical interview and physical examination. As part of this examination, you may be asked a series of questions from a standardized questionnaire or self-test to help assess your risk of depression and suicide.

Depression may be associated with a number of other medical conditions or can be a side effect of various medications. For this reason, routine laboratory tests are often performed during the initial evaluation to rule out other causes of your symptoms. Occasionally, an X-ray, scan, or other imaging study may be needed

Treatment of Depression

If your symptoms indicate that you have clinical depression, your health-care provider will strongly recommend treatment. Treatment may include addressing any medical conditions that cause or worsen depression. For example, an individual who is found to have low levels of thyroid hormone might receive thyroid hormone replacement with levothyroxine (Synthroid, Levoxyl). Other components of treatment may be supportive therapy, such as changes in lifestyle and behavior, psychotherapy, complementary therapies, and may often include medication. If your symptoms of depression are severe enough to warrant treatment with medication, you are most likely to feel better faster and for longer when medication treatment is combined with psychotherapy.

Most practitioners will continue treatment of major depression for six months to a year. Treatment for teens with depression can have a significantly positive effect on the adolescent's functioning with peers, family, and at school. Without treatment, your symptoms will last much longer and may never get better. In fact, they may get worse. With treatment, your chances of recovery are quite good.

Prevention of Depression

People who have risk factors for depression should be "screened" regularly by their health-care provider. This means that when they see their health-care provider, questions should be asked that might indicate depression.

If identified early, those who are at risk for depression are more likely to benefit from treatment.

NEED
AND
OBJECTIVE

2. NEED AND OBJECTIVE

There are various approaches in delivering a therapeutic substance to the target site in a sustained or controlled release fashion. One such approach is using microspheres as carrier for drugs. Microspheres of biodegradable and non-biodegradable polymers have been investigated for sustained release depending on the final application. In the case of non-biodegradable drug carriers, when administered parenterally, the carrier remaining in the body after the drug is completely released possess the possibility of carrier toxicity over a long period of time. Biodegradable carriers which degrade in the body to non-toxic degradation products do not possess the problem of carrier toxicity and are more suited for parenteral application. Thus, emphasis in this review is on the approach of using biodegradable microspheres for achieving sustained release.

Microsphere formulation offers a number of advantages in therapeutics where the sustained release of drugs as well as predictable and reproducible drug release kinetics is important factors among them. Venlafaxine Hydrochloride is the most widely used anti- depressant agent in the treatment of major depression disease has a low bioavailability, because of its poor absorption. It undergoes hepatic metabolism and its mean elimination half life 5 hours. The aim of the present work was to formulate by using natural gum like chitosan, sodium alginate and xanthan gum microsphere containing Venlafaxine Hydrochloride in order to provide a prolonged effect and relatively constant effective level of these drugs in the treatment of major depression disease.

Objectives

- To reduced dosing frequency and improved patient compliance.
- To optimize the concentration of drug-polymer ratio for suitable formulation.
- To characterize the microsphere for its physiochemical properties.
- To evaluate the microsphere for surface characterization with DSC for drug-polymer compatibility study and FTIR for identification of drug.
- To determine the effect of enteric coated microspheres in protecting drug release in acidic media (pH1.2) using 0.1 HCL.
- To determine the *invitro* release efficiency of microspheres for releasing at buffer 7.4 pH determine the percentage of drug release.
- In- vitro drug release kinetic study for release mechanism by the best fit model.
- To perform stability studies as per ICH guidelines.

PLAN OF WORK

3. PLAN OF WORK

- **LITERATURE REVIEW**
- **SELECTION OF DRUG AND EXCIPIENTS**
- **EXPERIMENTAL WORK**

A) PREFORMULATION STUDY

- **Identification of drug**
  - By FTIR spectroscopy
  - By melting point
- **Physicochemical parameters**
  - Organoleptic properties
  - Solubility profile
  - Loss on drying
- **Analytical methods**
  - Determination of λ_{max}
  - Development of standard curve of venlafaxine Hydrochloride
  - Determination of percentage purity of drug
- **Determination of compatibility for drug with polymer**
  - By DSC thermal analysis

B) PREPARATION OF SUSTAINED RELEASE MICROSPHERES

C) EVALUATION OF SUSTAINED RELEASE MICROSPHERES

- **Percentage yield**
- **Drug content**
- **Entrapment efficiency**
- **Particle size analysis**
- **SEM**
- ***In vitro* dissolution studies**
- **Kinetics of *In vitro* drug release**

D) STABILITY STUDY

- **RESULTS AND DISCUSSION**
- **CONCLUSION**
- **FUTURE PROSPECTS**
- **BIBLIOGRAPHY**

REVIEW OF LITERATURE

4. REVIEW OF LITERATURE

Adimoolam Senthil *et al.*, (2010) reported that was to formulate and evaluate the mucoadhesive Venlafaxine HCl microspheres using Hydroxy propyl methyl cellulose K4M as polymer. Venlafaxine HCl is a new generation serotonin reuptake inhibitor drug showing effective antidepressant properties, having a short bioavailability of 12.6% and biological half-life of 5 hours. Venlafaxine HCl microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a crosslinking agent. From those fifteen preliminary trial batches, the optimized formulation was selected based on the percentage of mucoadhesion and sphericity of microspheres. On the basis of the preliminary trials, 32 full factorial design was employed to study the effect of independent variable X1 (polymer-to- drug ratio 1:1, 3:1 and 6:1) and the stirring speed X2 (500, 1000 and 1500rpm) on the dependent variables like percentage mucoadhesion, drug entrapment efficiency, particle size and t80. The drug polymer compatibility studies were carried out using FTIR. As the concentration of glutaraldehyde increased, the mucoadhesiveness decreases and there was no significant effect in time. Stirring speed has negative effect on t80.

Anita Verma., *et al.*, (2010) have studied preparation of methacrylate for delivery of ketorolac tromethamine via the oral route. Drugs were encapsulated within polymethacrylate copolymer Eudragit RS100 and Eudragit RL100, by solvent evaporation method. Magnesium sterate was used as droplet stabilizer in concentration of 0.3(v/v). Selected formulations were characterized for their

entrapment efficiency, particle size, surface morphology and release behaviour. *Invitro* dissolution tests were performed by using dissolution media with two different pH. All the selected formulations exhibited a prolonged release for almost 24 hour. This study indicated that Eudragit microspheres containing ketorolac tromethamine could be prepared successfully by using an emulsion solvent evaporation technique, which would not only sustain the release of drug but also minimize the side effects of this drug.

Barish.*, et al.*, (2010) have developed oral sustained release microspheres of Cefazolin sodium to attain prolonged action, which can reduce its side effects and provide more safety and efficacy. The microspheres were formulated by solvent evaporation method using the polymer Guar gum. Five formulations were prepared with 1:1, 1:2, 1:3, 1:4, 1:5 drugs: polymer ratio. They were comparatively evaluated for their better release profile.

Bindhu madhavi.*, et al.*, (2009) have reported a new class of antidepressants. Its higher solubility in water results in burst effect with sudden peak levels of drug in blood. The half lives of venlafaxine and its active metabolite O-desmethyl venlafaxine are 5 hour and 11 hour respectively. The micro beads were prepared by the ionotropic gelation of sodium alginate in calcium chloride solution. The prepared micro beads were evaluated mainly for the sustain release of the drug apart from the other tests like, % drug encapsulation, particle size and drug polymer compatibility by the FTIR studies.

Bindhu Madhavi B., *et al.*, (2011) reported that venalafaxine hydrochloride is the free soluble with antidepressant activity. Being highly soluble drug there is

possibility of burst effect which causes sudden peak level of drug in blood. To reduce the adverse effects due to burst effect and to have the sustain action of the drug we have prepared the ethyl cellulose microsphere of the drug. The drug release was found to be sustained for 16 hours and was found to follow the peppas kinetics.

Bipul N., *et al.,*(2009) have developed microencapsulated controlled release preparations of a highly water/soluble drug, salbutamol sulphate by (water in oil) in oil emulsion technique using ethyl cellulose as the retardant material. Various processing and formulation parameters such as drug/polymer ratio, stirring speed, volume of processing medium were optimized to maximize the entrapment. The prepared microspheres were characterized for their micromeritic properties and drug loading, as well as compatibility by infrared spectroscopy, differential scanning calorimetry (DSC), X-ray powder diffractometry and scanning electron microscopy (SEM).

Dhanraju M.D., *et al.*, (2010) concluded that the microspheres were evaluated based on average particle size by optical microscopy, surface morphology by scanning electron microscopy (SEM) and *in vitro* drug release. The drug - polymer interactions and the effect on drug crystalline due to the incorporation of Indomethacin in polymer matrix have been evaluated by differential scanning calorimetry (DSC) and thin layer chromatography studies. The *in vitro* release studies were performed in phosphate buffer medium pH 7.2. The microspheres fabricated by emulsion solvent evaporation method had average particle size of about 35µ and observed to have a spherical shape with smooth surface.

Kannan., *et al.*, (2009) have developed sustained release microcapsules of Acetazolamide, a short half life carbonic anhydrase inhibitor, was developed to reduce the frequency of drug administration, ease of dose adjustment and improve patient compliance. The result showed that the encapsulation efficiencies were desired for all the formulations of microcapsules developed. Particle sizes of the microcapsules were influenced by the concentration of Eudragit and stirring speed.

Kedar Prasad Meena., *et al.*, (2011) concluded that Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. There are various approaches in delivering a therapeutic substance to the target site ina sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. It is there liable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor. In future by combining various other strategies, microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective *in vivo* delivery and supplements as miniature versions of diseased organ and tissues in the body.

Kishore Kumar Reddy k., *et al.*, (2011) have studied the microspheres have been utilized to obtain prolonged and uniform release in the stomach for development of once daily a formulation. The major advantage of the preparation technique

includes, Short processing time, the lack of exposure of the ingredients to high temperature, high encapsulation efficiencies. The formulated metformin loaded microspheres by solvent evaporation method by using aqueous medium as processing and tried to evaluate them for various in vitro characterization parameters.

Kumar Darapu B.N., *et al.*, (2011) prepared floating microspheres of Ranitidine Hydrochloride with HPMC K 100, Xanthan gum and Eudragit S100 and in various ratios of 1: 1, 1: 2, and 1: 3. The formulations were evaluated for FTIR, drug loading, % entrapment, particle size, SEM, buoyancy, dissolution study and the drug release kinetics. The enhanced floatability of the formulation and its retention in GIT may attribute for the increased bioavailability and decrease in frequency of administration. Comparison of three polymers revealed HPMC to be a suitable candidate for sustained release.

Lakshmana Prabu., *et al.*, (2009) have developed Aceclofenac was micro encapsulated using rosin o/w emulsion solvent evaporation technique. The effect of three formulation variables including the drug: polymer ratio, emulsifier (polyvinyl alcohol) concentration and organic solvent (dichloromethane) volume were examined. The prepared batches were characterized for microspheres particle size distribution, encapsulation efficiency and *invitro* release behavior. The study reveals that drug: polymer ratio had considerable effect on the entrapment efficiency, however particle size distribution of microspheres rather than on the drug: polymer ratio.

Mohanraj Palanisamy., *et al.*, (2009) have reported metoprolol tartrate formulated as biodegradable microspheres using chitosan by the phase separation emulsification technique. Microspheres of 1:0.5 to 1:2 drugs to carrier ratios were

prepared and thermally cross linked. Drug to carrier ratio 1:1 showed maximum percentage yield and highest drug entrapment. The size range of the microspheres varies from 3.5 to 31.5µm. UVand DSC studies were carried out to confirm the presence and stability of the drug in the microsphere short time stability studies were carried out different temperatures. *Invitro* release studies were carried out different pH for period of 10 hour and compare with pure drug. The release of metoprolol tartrate from the chitosan microsphere was found to be sustained.

Nair Sagjisha ., ***et al.,*** **(2010)** have developed formulation and evaluation of microspheres with repaglinide as model drug for prolongation of drug release time. An attempt was made to prepare microspheres of repaglinide by quasi emulsion solvent diffusion technique with a view to deliver the drug at sustained or controlled manner in gastrointestinal tract and consequence in systemic circulation. The microspheres were formulated by using various concentrations of HPMC, Ethyl cellulose and Eudragit RSPO as a retarding agent to control the release rate. *Invitro* release studies indicated that, as the concentration of retarding agent increase the formulation become more sustained.

Nappinai M., ***et al.,*** **(2011)** have developed using combination of polyethylene glycol 6000 and Eudragit RS 100 and Eudragit RS 100 alone by solvent evaporation method and non-solvent addition method with an aim to prolong its release. The microspheres were spherical, discrete and compact and size distribution was between 4 to 24µm. *Invitro* studies are carried out at different pH for a period of 12 hours and compared with marketed formulation. The analysis of regression values of Higuchi plot and Korsemeyer-peppas plot and n values of Korsemeyer-peppas

model suggested a combination of diffusion and dissolutional mechanism indicating the drug release from the formulations was controlled by more than one processes.

Nikil Karani A., *et al.*, (2009) have reported new, simple and cost effective uv-spectrophotometric method was developed for the estimation of venlafaxine hydrochloride in bulk and pharmaceutical formulation venlafaxine hydrochloride was estimated at 225.27 nm in distilled water. Linearity range was found to be $3.4x10^4$ mol^{-1} cm in distilled water. These methods were tested and validated for various parameters according to ICH guidelines and USP. The results demonstrated that the procedure is accurate, precise and reproducible while being simple, cheap and less time consuming and can be suitably applied for the estimation of venlafaxine hydrochloride in different dosage form and dissolution studies.

Phalguna Y., *et al.*, (2010) have developed in the present study was made to formulate zidovudine as microparticulate drug delivery system in order to localize drug at the absorption site, enhance its bioavailability, reduce dose, thereby improving patient compliance. Microparticulate system of zidovudine was formulated using HPMC as carrier by emulsification heat stabilizing method. Preformulatuion studies were carried out in order to establish compatability between drug and polymer by IR spectroscopy. These formulation were subjected to various evaluation parameters like % practical yield, drug entrapment efficiency, particle size distribution, in-*vitro* release studies and stability studies.

Prasant K., *et al.*, (2009) have reported the microspheres were found to be discrete, spherical with free flowing properties. The morphology (Scanning Electron Microscopy), particle size distribution, entrapment efficiency and their

release profiles were investigated. The percentage yield was found to be maximum in case of solvent evaporation method. The mean geometric particle size of microspheres prepared by solvent evaporation method was found in the ranges of 40-50µm and the microspheres prepared by W/O emulsion solvent evaporation method was found in a ranges of 126-150 µm, respectively.

Rajamanikam Daveswaran., *et al.*, (2010) have reported sustained release microspheres of Aceclofenac using egg albumin as release retarding agent. The results of FTIR spectral and DSC studies showed that there was no significant interaction between the drug and polymer. The maximum yield of the microspheres was found to be 96.99% and the encapsulation efficiency was found to be 65.2%. The prepared albumin microspheres released the drug completely within 10 hours at lower drug to polymer ratio. The prepared microspheres showed minor change in drug content proving good stability of the product conducted both in accelerated and long term stability studies.

Senthil A., *et al.*, (2011) have reported mefenamic acid is a potent non-steroidal anti-inflammatory drug, has low oral bioavailability due to poor aqueous solubility and insufficient dissolution, so it is used in high dose which leads to adverse drug reactions (ADRS) associated with NSAIDS such as gastrointestinal (GI) effects and renal effects of the agents. The formulations were characterized for their entrapment efficiency, particle size, surface morphology and release behaviour. The mean particle size of microspheres ranged from 75 to 225 µm and encapsulation efficiency ranged from 72.72 to 95.88% (w/w).

Shivakumar H.N., *et al.,* (2011) investigated the microcapsule size was found to increase with increased polymer loads and decrease with decreased polymer loads and size decrease with increase span-80 concentrations. The microencapsulation efficiency was found to depend on the initial polymer loads and the concentration of span-80 employed during emulsification. The increase in the drug encapsulation with increase in the span-80 level could also ascribed to the emulsifying effect of the surfactant which helped the drug to be finely dispersed and embedded in the polymer matrix prior encapsulation.

Sindhuri P., *et al.,* (2011) have reported Norfloxacin a fluroquinoline derivative used as antibiotic requires multiple administration of drug, leading to fluctuation in plasma concentration. Norfloxacin microsphere by using various polymers like carbopol 934, sodium carboxy methyl cellulose (scmc) using different drug: polymer ratios. The prepared Norfloxacin microspheres showed good flow properties showed sustained release of the drug from the formulations for a period of 12 hours.

Sudhamani., *et al.,* (2010) have developed as microspheres by using ethyl cellulose as carrier. These ethyl cellulose microspheres were prepared by the solvent evaporation method. The prepared microspheres were subjected to various evaluation and *Invitro* release studies. Highest percentage of loading was obtained by increasing the amount to polymer. The particle sizes of the prepared microspheres were determined by optical microscopy and SEM analysis. The *Invitro* release studies showed that ibuprofen microspheres of 1:2 ratios showed better sustained effect over a period of 8 hours.

Sunitha S., *et al.*, (2010) have been reported sustained release microspheres of tramadol hydrochloride were formulated using a enteric polymers like cellulose acetate phthalate (CAP),cellulose acetate (CA) and ethyl cellulose (EC) by emulsion solvent evaporation technique. The microspheres were characterized for particle size, flow properties, percentage yield, scanning electrom microspcopy (SEM) and drug entrapment. The results obtained were found in desired range where the drug entrapment efficiency of microspheres was found to be ranging from 61.54% to 86.95%, size of microspheres found in the range 341µm to 608µm. The drug release was extended up to 12 hours with CAP and EC and maximum release retardation was found in the formulation with EC(formulation F3) FTIR and XRD results showed that tramadol is compatable with excipients.

Tamizharasi S., *et al.*, (2011) designed drug therapy is to achieve a desire concentration of the drug in blood or tissue which is therapeutically effective and nontoxic for extended period of time, and this goal can be achieved by proper design of sustain release dosage regimen of the various biodegradable polymers used for the development of sustained release formulations, poly caprolactone has been reported to be advantageous since they are biocompatible and suitable for controlled drug delivery due to high permeability to many drugs and at the same time being free from toxicity.

Thomas L., *et al.*, (2009) have reported the antibiotic drug (erythromycin) to provide sustained release delivery system. Coarse containing Erythromycin as a model drug was prepared by emulsion solvent evaporation technique using different ratios of drug and polymer (ethyl cellulose). The prepared microspheres were subjected to drug loading, *In-vitro* drug release as well as scanning electron

microscopy. The drug loaded microspheres shows 70-85% drug entrapment. *In-vitro* drug release studies were carried out up to 12-18hours in phosphate buffer (pH-7.4) at 480nm.

Venkatesan P., *et al.*, (2011) concluded that formulate and evaluate the loxoprofen loaded Sustained release microspheres by emulsion solvent evaporation technique. Ethylcellulose, a biocompatible polymer is used as the retardant material. The prepared microspheres were characterized for their particle size and drug loading and drug release. The *in-vitro* release studies were carried out in phosphate buffer at pH 7.4. The prepared microspheres were white, free flowing and spherical in shape. The drug-loaded microspheres showed 71.2% of entrapment and the *in-vitro* release studies showed that Loxoprofen microspheres of 1:3 ratios showed better sustained effect over a period of 8 hours.

Vikram Deshmukh., *et al.*, (2011) have reported the present investigation is aimed to prepare the sustained release beads of carbamazepine using different proportions of talc and sodium alginate for sustained delivery of carbamazepine. The proposed beads were formulated using the iontropic gelation method. The drug to talc to polymer ratio in optimized batch S2 was kept at 1: 0.5: 0.3. The obtained beads were characterized for its particle size distribution, percent drug content, mean diameter and crushing strength, thermal analysis (DSC), crystallinity, surface morphology (SEM), and in vitro drug release. Further investigations are required to reduce the amount of polymer in microspheres that can provide maximum drug loading and acceptable dosage form.

Vimala Shirvi D., *et al.*, (2010) have developed for the estimation of venlafaxine hydrochloride in raw material and pharmaceutical dosage form. In this method venlafaxine hydrochloride showed zero crossing at 274 nm, with a sharp peak at 285 nm. Beers law was obeyed in the concentration range of 40-120 µg/ml. The limit of detection and limit of quantification were found to be 1.82µg/ml and 5.49µg/ml respectively. The method was successfully applied to the determined of venlafaxine hydrochloride in tablet.

Yadav N., *et al.*, (2011) have reported the micro particles can be prepared by using any one of the several techniques but choice of the technique mainly depends on the nature of the polymer used, the drug and the duration of the therapy. Atenolol microspheres synthesized by solvent evaporation method in which the different concentration ranges of Atenolol and ethyl cellulose polymer were taken. The reaction mechanism to form sustained release Atenolol microspheres were determined by optical microscopic method and evaluated by using FT-IR spectroscopy. The particle size of Atenolol ranged from 80 to 186 µm. The size of particle was observed to increase with increasing concentration.

DRUG
AND
EXCIPIENTS
PROFILE

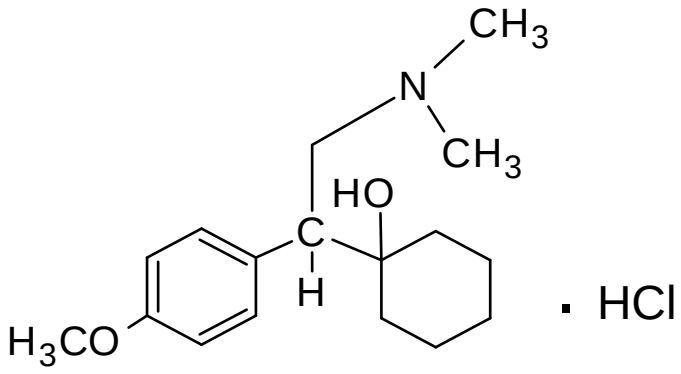
5. DRUG AND EXCIPIENTS PROFILE

5.1. DRUG PROFILE

VENLAFAXINE HYDROCHLORIDE *(The Merck Index)*

A. IUPAC-Name **:** Dimethyl {5-[2-(1-methylamino-2nitrovinylamino) ethylthiomethyl] furfuryl}-amine hydrochloride.

B. Structural formula **:**



C. Molecular formula **:** $C_{13}H_{27}NO_2$, HCl

D. Molecular weight **:** 313.86

E. Physicochemical properties *(www.Chemicalbook.com)*

Description **:** White to off white crystalline solid.

Solubility **:** Freely soluble in water and methanol, insoluble (or) slightly soluble in acetone.

Category **:** serotonin-norepinephrine reuptake inhibitor(SNRI) class

Dose **:** 75-225 mg/day (divided in 2-4 doses)

F. Pharmacology *(www.About CNSforum.com/venlafaxine)*

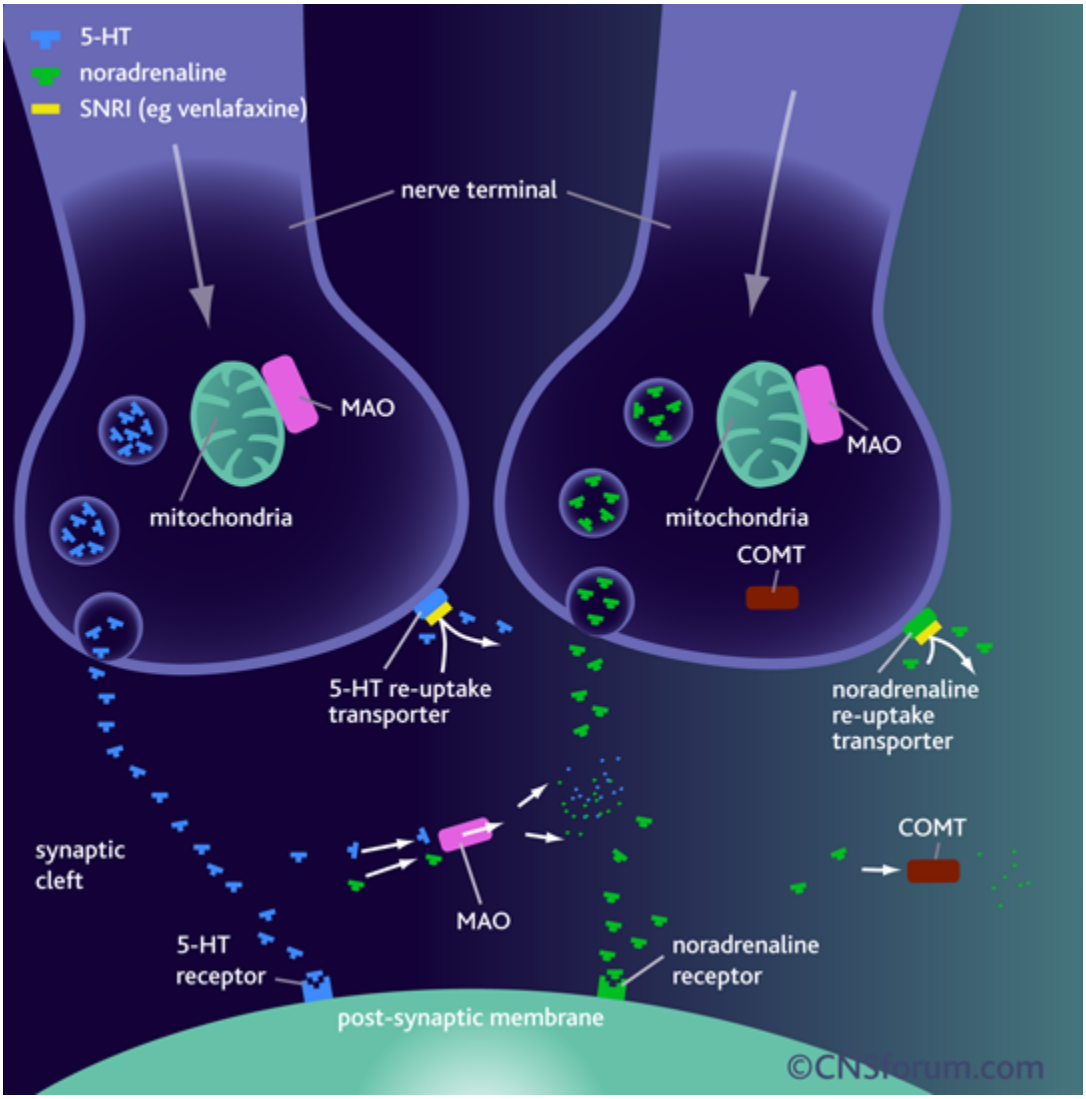


Figure 5.1: The mechanism of action of specific 5-HT and noradrenaline re-uptake inhibitors

Venlafaxine is a bicyclic antidepressant, and is usually categorized as a serotonin-norepinephrine reuptake inhibitor (SNRI), but it has been referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI). Depression is associated with reduced levels of the monoamines in the brain, such as 5-HT. The selective 5-HT and noradrenaline re-uptake inhibitors (SNRIs) are thought to restore

the levels of 5-HT and noradrenaline in the synaptic cleft by binding at their re-uptake transporters preventing the re-uptake and subsequent degradation of 5-HT and noradrenaline. This re-uptake blockade leads to the accumulation of monoamines in the synaptic cleft and the concentration returns to within the normal range. This action of SNRIs is though to contribute to the alleviation of the symptoms of depression. In the presence of the SNRIs, small amounts of 5-HT and noradrenaline continue to be degraded in the synaptic cleft.

G. Pharmacokinetics (*goodman and gilman, 2006)*

Absorption **:** Venlafaxine is well absorbed and extensively metabolized in the liver. O-desmethylvenlafaxine (ODV) is the only major active metabolite.

Volume of distribution **:** 7.5±3.7 L/kg

Partition coefficient **:** octanol/water- 0.43

Oral bioavailability **:** 10-45%

Plasma half life **:** 5hrs.

Plasma protein binding **:** 27%

H. Indications *(Tripathy K.D., 2008)*

- In endogeneous (major) depression.
- Obsessive-compulsive and phobic states
- Anxiety disorder
- Neuropathic pain
- Attention deficit-hyperactive disorder in children

- Enuresis
- Migrain
- Pruritus.

I. **Adverse Effect** *(www.drugbank.ca)*

Venlafaxine hydrochloride is generally well tolerated, with a low incidence of adverse effects. The most common side effects of venlafaxine are nausea, somnolence, headache, dry mouth, sweating, hypotension, nervousness and abnormal ejaculation, dizziness, insomnia, sedation and constipation. At high doses, there may be an increase in blood pressure.

J. Drug Interaction

Venlafaxine should not be used with MAOIs and at least 14 days should elapse between stopping an MAOI and starting treatment with venlafaxine. Although the synergistic effects may not be as bad as with other antidepressants, it is still not recommended to take venlafaxine with alcohol. Venlafaxine may lower the seizure threshold, and co administration with other drugs that lower the seizure threshold such as bupropion and tramadol should be done with caution and at low doses. Although cimetidine inhibits the hepatic metabolism of venlafaxine, it has no effect on the active metabolite of venlafaxine, o-desmethylvenlafaxine

K. Dosage Forms *(http://en.wikipedia.org/wiki/Venlafaxine)*

- Tablets : 25 mg, 37.5 mg, 50 mg, 75 mg, 100 mg and 225mg.
- Tablet : ER- 37.5 mg, 75 mg, and 150 mg and 225mg.
- Capsules ER : 7.5 mg (gray/peach), 75 mg (peach), and 150 mg (brownish red).

L. Contraindications

Venlafaxine is not recommended in patient with hypersensitive, Glaucoma, Pregnant women, Heart disease and hypertension. It should never be used with a monoamine oxidase inhibitor (MAOI), as it can cause potentially fatal serotonin syndrome. Caution should also be used in those with a seizure disorder.

5.2. EXCIPIENTS PROFILE

5.2.1. Chitosan100cps *(Raymond C Rowe., et al., 2003)*

Chitosan is one of naturally available product and is a very versatile compound. Although studies on chitin and chitosan were initiated in the early nineteenth century, most of the reports available today on its medical and pharmaceutical applications have been obtained only during the last couple of decades. Despite the considerable research carried out on chitosan over recent decades, new registered products have failed to reach the market

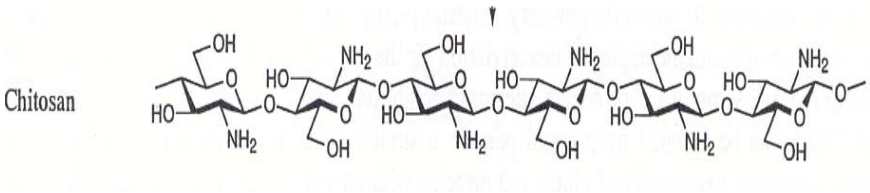
- **Sources and synthesis of chitosan**

It is derived from a material called chitin, which is an amino polysaccharide, extracted from the powdered shells of crustaceans like shrimps and crabs. Chitosan is prepared by partial deacetylation of chitin. To prepare chitin, crab and shrimp shells

are demineralised in dilute, HCl deproteinated in dilute NaoH, and then decolourised in potassium permanganate. The chitin is then deacetylated to become chitosan by boiling it in a concentrated NaoH solution. Biochemical grade/purified chitosan is prepared by repeating the deacetylation process. Pharmaceutical grade chitosan is deacetylated between 90 and 95% and food grade between 75 and 80%.

❖ **Structural nature**

- **Chemical Formula** $(C_6H_{11}O_4N)_n$.
- **Structural Formula**



❖ **Bio physicochemical Properties Of Chitosan**

- **Colour:** Yellowish white in colour.
- **Physical Appearance:** Occurs as flakes.
- **Viscosity:** 100cps
- **Degree Of Deacetylation:** > 85%
- **Molecular Weight:** <10,000.
- **Heavy Metals:** Maximum 40 ppm.
- **Loss On Drying:** Maximum 10 percent, determined on 1 gram by drying in an oven at 100-105°C.

- **Storage:** A t a temperature of 2-8°C, protected from moisture and light.

- **Solubility:** Sparingly soluble in water and practically insoluble in ethanol. Freely Soluble in dilute acids. pH: 4.0 – 6.0. pKa: 6.2-7.0. Chitosan salts are soluble in water; the solubility depends on the degree of deacetylation and the pH of the solution.

- **Pharmaceutical Requirements of chitosan**

particle size <30 μm, density between 1.35 and 1.40 g/cm3, pH 6.5-7.5, in soluble in water, and partially soluble in acids. The chemical and biological properties of chitosan are summarized in below Chemical and biological properties of chitosan.

Chemical and biological properties of chitosan

Chemical Properties of Chitosan

- Cationic polyamine
- High charge density at pH <6.5
- Forms gels with polyanions
- Viscosity, high to low
- Chelates certain transitional metals
- Amiable to chemical modification
- Reactive amino/hydroxyl groups
- High molecular weight, linear Polyelectrolyte
- Adheres to negatively charged surfaces

Biological Properties of Chitosan

- Biocompatible
- Natural polymer
- Biodegradable to normal body
- Constituents
- Safe and non-toxic
- Used as Haemostatic, bacteriostatic and fungi static
- Spermicidal
- Anti-cancerogen
- Anti-cholesteremic

❖ **Safety**

Presently used is approved as an food additives in most of the countries is being considered and approved as safe polymer in humans. The oral toxicity of chitosan has been reported to 1g/kg weight.

❖ **Pharmaceutical applications**

- **Colon targeted drug delivery**

Chitosan is a promising polymer for colon drug delivery since it can be biodegraded by the colonic bacterial flora and it has mucoadhesive character. In vitro release study revealed no release of the drug in gastric pH for 3 hrs and after the lag-time, a continuous release for 8– 12 h was observed in the basic pH.

- **Mucosal delivery**

Nowadays, mucosal surfaces such as nasal, per oral and pulmonary are receiving a great deal of attention as alternative routes of systemic administration. Chitosan has mucoadhesive properties and therefore, it seems particularly useful to formulate the bioadhesive Dosage forms for mucosal administration (ocular, nasal, buccal, gastro-enteric and vaginal-uterine therapy). Nasal mucosa has high permeability and easy access of drug to the absorption site.

- **Cancer therapy**

Gadopentetic acid-loaded CS nanoparticles have been prepared for gadolinium neutron-capture therapy. Their releasing properties and ability for long term retention of gadopentetic acid in the tumor indicated that these nanoparticles are useful as intratumoral inject able devices for gadolinium neutron capture therapy.

Biopharmaceutical applications

- **Oral drug delivery**

The bioavailability of drugs has been improved by the use of mucoadhesive dosage forms. This is because by prolonging the residence time of drug carriers at the absorption site, improved absorption of drugs can be achieved. It is acknowledged that higher molecular weight chitosan possesses better mucoadhesion as compared to lower molecular weight chitosan. Insulin loaded liposome's coated with chitosan were developed to improve the enteric absorption of insulin. The results of the above-mentioned study indicated that as chitosan concentration increased in the coating, the mucoadhesion improved, resulting in an increased absorption of insulin. This muco-adhesive property makes chitosan an ideal candidate for buccal delivery of drugs.

- **Ocular drug delivery**

The poor bioavailability of topically applied ophthalmic drugs implies a necessity for frequent instillation to achieve therapeutic effect (Paul and Sharma, 2000:9). This inconvenience may be overcome by a prolonged release of the drug in the corneal area. The use of chitosan based colloidal suspensions during *in vivo* studies were investigated and showed a significant increase in ocular drug bioavailability.

- **Nasal drug delivery**

Effective nasal drug delivery depends on increased absorption of the drug through the nasal mucosa without undesired sideeffects. However, a nasal formulation with improved absorption of macromolecules is still a challenge because of the short retention time in the nasal cavity due to the efficient physiological clearance mechanism. Chitosan may be a good option in nasal drug delivery as it binds to the nasal mucosal membrane with an increased retention time and is therefore potentially a good absorption enhancer for nasal drug delivery.

- **Parenteral drug delivery**

In controlled release technology, biodegradable polymeric carriers offer potential advantages for the prolonged release of low molecular weight compounds. The susceptibility of chitosan to liposome makes it biodegradable and an ideal drug carrier. Molecules such as bovine serum albumin, diphtheria toxoid and bisphosphonates have been successfully incorporated into chitosan microspheres.

- **Gene delivery**

The development of new carrier systems for gene delivery represents an enabling technology for treating many genetic disorders. Since, a critical barrier to successful gene therapy remains in the formulation of an efficient and safe delivery vehicle, non-viral delivery systems have been increasingly proposed as alternatives to viral vectors owing to their safety, stability and ability to be produced in large quantities. Chitosan not only increases transformation efficiency but also through the addition of appropriate ligands 32 to the DNA-chitosan complex, facilitates more efficient gene delivery via receptor-mediated endocytosis without cytotoxic effects

5.2.2. SODIUM ALGINATE (*Raymond C Rowe., et al., 2003)*

- **Nonproprietary Names:**

  BP: Sodium alginate, PhEur: Natrii alginas, USPNF: Sodium alginate

- **Synonyms:**

  Algin; alginic acid, sodium salt; E401; Kelcosol; Keltone; Protanal; sodium polymannuronate.

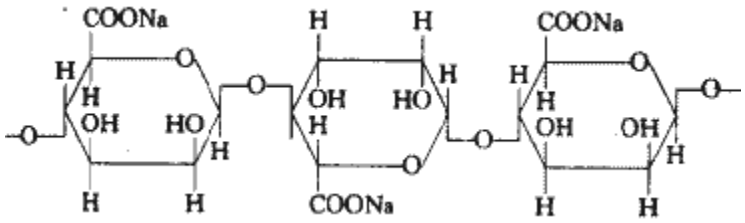
- **Chemical Name and CAS Registry Number:**

  Sodium alginate [9005-38-3]

- **Empirical Formula :**$(C_6H_7O_6Na)_n$

- **Molecular Weight:** The block structure and molecular weight of sodium alginate samples has been investigated.

- **Structural Formula:**



- **Functional Category:**

Stabilizing agent; suspending agent; tablet and capsule disintegrant; tablet binder; viscosity-increasing agent.

Grades:

Various grades of sodium alginate are available yielding aqueous solutions of varying viscosities within a range of 20 to 400 centipoises in 1% solution at 20 °C.

- **Applications in Pharmaceutical Formulation or Technology:**

o Sodium alginate for oral and topical pharmaceutical formulations.

o In tablet formulations, sodium alginate may be used as both a binder and disintegrant, diluents in capsule formulations. Sustained release oral formulations are prepared by using, since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions. In topical formulations, sodium alginate is mainly used as a thickening and suspending agent in product such as variety of pastes, creams, and gels, and as a stabilizing agent for oil-in-water emulsions

o Recently, sodium alginate has been used mostly for microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organic solvent systems. It has also been used in the formulation of nanoparticles. Other NDDS containing sodium alginate include ophthalmic solutions that form a gel in situ when administered to the eye.

- **Description:**

  Sodium alginate occurs naturally as an odorless and tasteless, white to pale yellowish-brown colored powder.

- **Typical Properties:**

  Acidity/alkalinity: pH 7.2 for a 1% w/v aqueous solution

- **Solubility**:

  Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, the pH is less than 3. Slowly soluble in water, forming a viscous colloidal solution.

- **Stability and Storage Conditions:**

  Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humanities and a cool temperature.

5.2.3. Xanthan Gum (*Raymond C Rowe., et al., 2003)*

Nonproprietary Names

BP **:** Xanthan gum

PhEur **:** Xanthani gummi

USPNF **:** Xanthan gum

Synonyms

Corn sugar gum; E415; Keltrol; polysaccharide B-1459; Rhodigel; Vanzan NF; Xantural

Chemical name and CAS registry number

Xanthan gum [11138-66-2]

Molecular weight

Approximately 2000000.

Functional category

Stabilizing agent; suspending agent and viscosity-increasing agent.

Description

Xanthan gum occurs as a cream- or white-colored, odorless, free-flowing, fine powder.

Solubility

It is practically insoluble in ethanol and ether, soluble in cold or warm water.

Viscosity (dynamic)

1200-1600 m Pas (1200–1600 cP) for a 1% w/v aqueous solution at 25°C.

Applications in pharmaceutical formulation or technology

Xanthan gum is widely used in oral and topical pharmaceutical formulations, cosmetics and foods as a suspending and stabilizing agent. It is also used as a thickening and emulsifying agent. It is nontoxic, compatible with most other pharmaceutical ingredients, and has good stability and viscosity properties over a wide pH and temperature range. When xanthan gum is mixed with certain inorganic suspending agents, such as magnesium aluminum silicate, or organic gums, synergistic rheological effects occur. It is used as for preparation of sustained release matrix tablets and also used as thickening agent in shampoo.

Stability and storage conditions

Bulk material should be stored in a well closed container in a cool, dry place.

MATERIALS AND EQUIPMENTS

6. MATERIALS AND EQUIPMENTS

6.1. RAW MATERIALS

Table 6.1: List of raw materials with name of the supplier

S. No.	Name of raw Material	Name of the Supplier
1	Venlafaxine Hydrochloride	Orchid Pharmaceticals., Chennai
2	Chitosan 100cps	Micro laboratories Pvt. Ltd., Hosur
3	Sodium alginate	Merk Chemicals, Chennai
4	Xanthan gum	Merk Chemicals,Chennai
5	Dichloromethane	Qualigens fine chemicals, Mumbai
6	Ethanol	Qualigens fine chemicals, Mumbai
7	Span 80	Loba Chemical Pvt. Ltd. Mumbai,
8	n-Hexane	Loba Chemical Pvt. Ltd. Mumbai,
9	Liquid paraffin	Loba Chemical Pvt. Ltd. Mumbai,
10	Petroleum ether	S d fine-chem limited, Mumbai.
11	Sodium hydroxide	Qualigens fine chemicals, Mumbai
12	Ethanol (95%)	S d fine-chem limited, Mumbai.
13	Potassium dihydrogen orthophosphate	Fischer scientific chemicals, Mumbai.
14	Acetone	Fischer scientific chemicals, Mumbai.
15	Hydrochloric acid	S d fine-chem limited, Mumbai.
16	Dialysis bag	Himedia labaratories ,Mumbai.

6.2. EQUIPMENTS

Table 6.2: List of equipments with company name

S. No.	Name of the Equipments	Company
1.	Over head Propeller Agitator	REMI
2.	Electronic Balance	Shimadzu BL-220H
3.	Rotary shaker	RS-12R, remi equipments limited, vasai
4.	pHmeter	LI120 pHmeter, ELICO LTD
5.	UV Spectrophotometer	Shimadzu-1700 Pharmaspec UV-Visible spectrophotometer
6.	Differential scanning calorimeter	Shimadzu DSC 60, Japan
7.	FTIR Spectrophotometer	Shimadzu S4008
8.	Scanning Electronic Microscopy	JEOL, JSM-6360, Japan
9.	USP Tablet Dissolution Apparatus Type II	USP XXIV (Electro Lab, Mumbai).

EXPERIMENTAL WORK

7. EXPERIMENTAL WORK

7.1. PREFORMULATION STUDY

Preformulation testing was an investigation of physical and chemical properties of a drug substance alone. It is the first step in rational development of dosage form.

7.1.1. Identification of drug

7.1.1.1. Identification by FTIR spectroscopy *(Skoog D.A.,et al.,1996;)*

Venlafaxine Hydrochloride discs were prepared by pressing the Venlafaxine Hydrochloride with potassium bromide and the spectra between 4000^{-1} to 500^{-1} cm was obtained under the operational conditions. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented in Table 8.1 and Figure 8.1 respectively.

7.1.1.2. Identification by melting point *(www.Chemicalbook.com/venlafaxine)*

Melting point of the drug was determined by capillary tube method.

7.1.2. Physicochemical parameters

7.1.2.1. Organoleptic properties

(Lachman L., et al., 1991; Bankar G.S. and Rhodes C.T., 1996)

The color, odor and taste of the drug were recorded using descriptive terminology.

7.1.2.2. Solubility study

It was important to know about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic

response. The solubility of drug was recorded by using various descriptive terminology specified in drugs.com.

7.1.2.3. Loss on drying

Loss on drying was the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified condition. The accurately weighed 1gm of sample was transferred in glass-stoppered, shallow weighing bottle and accurately weighed the bottle. The bottle was transferred in oven and substance was dried at 105°C for 3 hours. The bottle was removed from oven and reweighed; loss on drying was calculated by following equation,

$$\text{LOD} = \frac{\text{Initial weight of substance} - \text{Final weight of substance}}{\text{Initial weight of substance}} \times 100$$

7.1.3. Analytical methods

7.1.3.1. Determination of λ max *(Sundraganapahty., et al., 2011)*

The absorption maximum of the standard solution was scanned between 200-400 nm regions on Shimadzu-1700 Pharmaspec UV-Visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum represented in Figure 8.2 and 8.4.

7.1.3.2. Development of standard curve of Venlafaxine Hydrochloride in 0.1N HCl: *(Nikhil Karani., et al., 2009)*

Preparation of 0.1N HCl: 0.1N HCl was prepared according to I.P. 1996. 8.5 ml of HCl was diluted with fresh distilled water to produced 1000 ml.

Preparation of stock solution of Venlafaxine Hydrochloride in 0.1 N HCl

Accurately weighed 100 mg of Venlafaxine Hydrochloride was dissolved in little quantity of 0.1 N HCl and volume was adjusted to 100 ml with the same to prepared

standard solution having concentration of 1000 µg/ ml then further diluted to get the concentration of 100 µg/ml.

Procedure: From the stock solution, aliquots of 1, 2, 3, 4 and 5 ml were transferred to 25 ml volumetric flasks and final volume was made to 25 ml with 0.1N HCl. Absorbance values of these solutions were measured against blank (0.1N HCl) at 225 nm using UV-Visible spectrophotometer. The data was given in Table 8.4 and 8.5; also shown in Figure 8.3 respectively.

7.1.3.3. Development of standard curve of Venlafaxine Hydrochloride in pH 7.4 phosphate buffer

Preparation of phosphate buffer pH 7.4: pH 7.4 phosphate buffer was prepared by according to I.P 1996. Placed a 50 ml of 0.2M potassium di hydrogen phosphate in a 200ml volumetric flask. Added the specified volume of 0.2M sodium hydroxide and required volume was made up with distilled water.

Preparation of stock solution: Accurately weighed 100 mg of Venlafaxine Hydrochloride was dissolved in little quantity of pH 7.4 phosphate buffer and volume was adjusted to 100 ml with the same to prepared standard solution having concentration of 1000 µg/ml, then further diluted to get the concentration of 100 µg/ml.

Procedure: From the stock solution, aliquots of 1, 2, 3, 4 and 5 ml were transferred to 25 ml volumetric flasks and final volume was made up to 25 ml with pH 7.4 phosphate buffer solution. Absorbance values of these solutions were measured against blank (pH 7.4 phosphate buffer solution) at 225.5 nm using UV-VISIBLE spectrophotometer. The data was represented in Table 8.6 and 8.7; also shown in Figure 8.5 respectively.

7.1.3.4. Determination of Percentage purity of Drug

(Bindhu Madhavi., et al., 2011)

Accurately weighed 100 mg of Venlafaxine Hydrochloride was dissolved in little quantity of pH 7.4 Phosphate buffer and volume was adjusted to 100 ml with the same to prepared standard solution having concentration of 1000 µg/ml. From the above solution, aliquots of 3 ml were transferred to 25 ml volumetric flasks and final volume was made up to 25 ml with pH 7.4. Absorbance values of these solutions were measured against blank (pH 7.4) at 225.5 nm using UV-VISIBLE spectrophotometer. The percentage purity of drug was calculated by using calibration graph method (least square method).

7.1.4. Determination of drug-polymer compatibility ***(****Aulton M.E., 2007)*

The proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients to be used in the fabricating the product. Each polymer used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form. Each polymer was thoroughly blended with drug to increase drug-polymer molecular contacts to accelerate the reactions if possible.

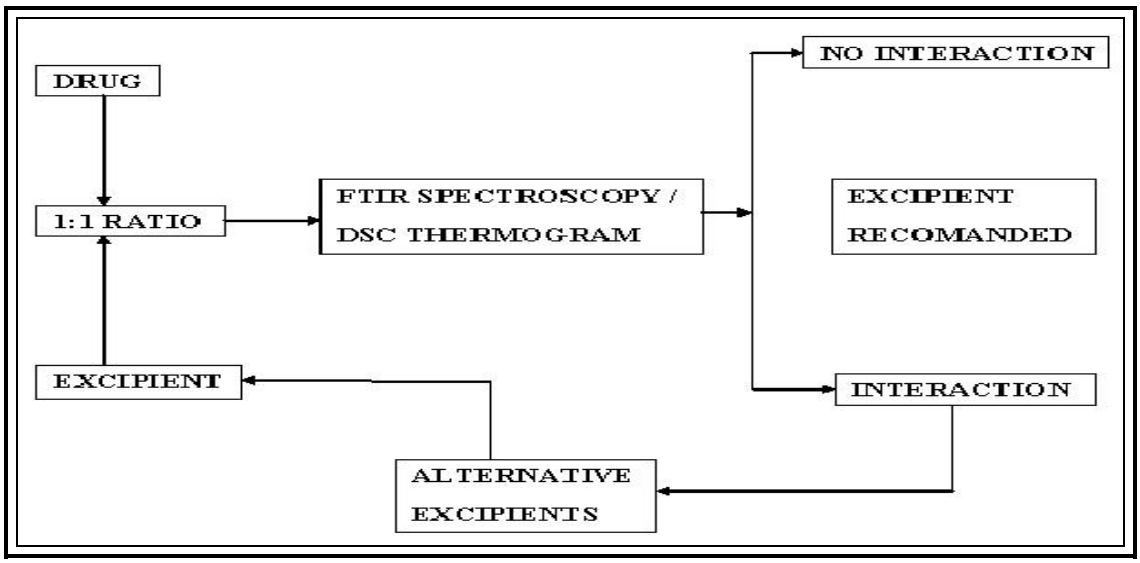


Figure 7.1: Schematic representation of compatibility studies

7.1.4.1. Fourier transform Infra-Red (FTIR) spectroscopy *(Sunitha., et al., 2010)*

FTIR study was carried out to check compatibility of drug with polymers. Infrared spectrum of Venlafaxine Hydrochloride was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug with various polymers by FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented in Table 8.7 and was shown in Figure 8.6 to 8.9.

7.1.4.2. Differential scanning calorimetry (DSC) *(Bindhu Madhavi., et al., 2011)*

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure drug, drug+ Chitosan, drug+Sodium alginate and drug+ Xanthan gum. The study was carried out using a Shimadzu DSC 60, (Japan). The 2 mg of sample were heated in a hermetically sealed aluminum pans in the

temperature range of 25-300ºc at heating rate of 10ºc /min under nitrogen flow of 30ml/min.

7.2. PREPARATION OF MICROSPHERES *(Kumar Darapu B N.,et al., 2011)*

Nine formulations were prepared by using drug and three different polymers in the ratios of 1:1, 1:2 and 1:3 respectively shown in Table 7.1. And represented in Figure 7.2. The powder blend (drug with polymer) was dissolved at room temperature in ethanol and dichloromethane (1:1% v/v) with vigorous agitation to form uniform drug-polymer dispersion. This was slowly poured into the dispersion medium consisting of heavy liquid paraffin (50ml) containing 0.5% span 80. The system was stirred by using over head propeller agitator at speed of 750 rpm for 5 hours, to ensured complete evaporation of the solvent. The liquid paraffin was decanted and the microspheres were separated by filtration through a whatmann filter paper. The microspheres were separated and washed thrice with 180ml of n-Hexane and air dried for 24 hours.

Table.7.1: Formulation table for sustained release microspheres of Venlafaxine Hydrochloride

S. No	INGREDIENS	FORMULATIONS								
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1	Venlafaxine Hydrochloride*	1	1	1	1	1	1	1	1	1
2	Chitosan*	1	2	3	-	-	-	-	-	-
3	Sodium Alginate*	-	-	-	1	2	3	-	-	-
4	Xanthan gum*	`	-	`	-	-	-	1	2	3
5	Heavy Liquid Paraffin#	50	50	50	50	50	50	50	50	50
6	Dichloromethane #	5	5	5	5	5	5	5	5	5
7	Ethanol#	5	5	5	5	5	5	5	5	5
8	Span 80	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %
9	n-Hexane#	180	180	180	180	180	180	180	180	180

* All values are in grams. # All values are in ml

(Formulations F1, F2, and F3 – Venlafaxine Hydrochloride + Chitosan

Formulations F4, F5, and F6 – Venlafaxine Hydrochloride + Sodiumalginate

Formulations F7, F8, and F9 – Venlafaxine Hydrochloride +Xanthan gum)

Figure 7.2: Sustained release Microspheres

7.3. EVALUATION OF MICROSPHERES

7.3.1. Percentage yield *(Venkatesan P et al., 2011; Bindhu Madhavi., et al., 2011)*

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula.

Percentage yield = (Weight of Microspheres/Weight of Polymer + drug) X 100

7.3.2. Drug content estimation *(Prasant K., et al., 2009)*

Drug loaded microspheres (100mg) were powdered and suspended in 100ml methanolic : water (1:99 v/v) solvent. The resultant dispersion was kept for 20min for complete mixing with continuous agitation and filtered through a 0.45µm membrane filter. The drug content was determined spectrophotometrically (UV-1700, Shimadzu Japan) at 225.5nm using a regression equation derived from the standard graph (r^2=0.9954).

7.3.3. Entrapment efficiency of the drug ***(****Bindhu Madhavi B ., et al.,2011)*

The micro beads equivalent to 10mg of Venlafaxine Hydrochloride were weighed and dispersed in PBS of pH 7.4. The resulting mixture was agitated on mechanical shaker for 24 hours. The solution was then filtered and drug content was estimated by UVspectrophotometry.

$$\text{Encapsulation Efficiency} = (W_1/W_2) \text{ X } 100$$

W_1 = Actual Weight of Drug in Sample

W_2 = Microspheres Sample Weight

7.3.4. Size distribution of microspheres *(Kishore Kumar Reddy k., et al.,2011)*

Microspheres were separated into different size fractions by sieving for 10 minutes using a Mechanical shaker (geologists Syndicate pvt Ltd, India) containing standard sieves having Apertures of 710, 500, 355, 250, 125, 80, 60, 45, 30 and 20mm (Indian Pharmacopoeia 1996). The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula.

$$\text{Mean Particle Size} = \sum (W_1 \text{ X } W_2) / (W_2)$$

W_1 = Mean Particle Size of the Fraction

W_2 = Weight Fraction

7.3.5. Scanning electron microscopy (SEM) *(Kishore Kumar Reddy k., et al.,2011)*

The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of a double adhesive stub. The stub was then coated with gold (Fine coat, Ion sputter, JFC-1100). The microspheres were then observed with the scanning electron microscope (JEOL, JSM-6360, scanning electron microscope, Japan) at 15kv. The samples include blank microspheres, drug loaded microspheres.

7.3.6. In Vitro Release Study ***(****Bindhu Madhavi B ., et al.,2011)*

In vitro drug release from microspheres was performed using the rotating basket method as specified in USP XXIV (Electro Lab, Mumbai). Microspheres equivalent to 100 mg of venlafaxine hydrochloride was tied in a Cellophane membrane and put in basket, immersed in 900 ml of 0.1 N HCl (pH 1.2) dissolution medium and allowed to rotated at 100 rpm. Operating temperature was maintained at $37^{o} \pm 1^{o}C$. Sample (5ml) was withdrawn at predetermined time hour intervals up to 2hr. same volume was replaced to maintain sink condition. After 2hr, dissolution medium was changed by 900ml of fresh phosphate buffer of pH 7.4 and study was continued upto 16^{th} hour. The samples were analyzed by UV-spectrophotometer at 225.5 nm.

7.3.7. Kinetic of *In vitro* drug release ***(****Mohanraj Palanisamy., et al.,2009)*

Five kinetic models including the zero order (Equation 1), first order (Equation 2), Higuchi matrix (Equation 3) and Peppas-Korsmeyer (Equation 4) release equations were applied to process the in vitro released data to find the equation with the best fit using Microsoft Office Excel 2007.

$$R = k_1 t \quad \text{----------} > \quad \text{Equation 1}$$

$$\log UR = \frac{k_2 t}{2.303} \quad \text{----------} > \quad \text{Equation 2}$$

$$R = k_3 t^{0.5} \quad \text{----------} > \quad \text{Equation 3}$$

$$R = k_4 t^n \text{ or } \log R = \log k_4 + n \log t \quad \text{----------} > \quad \text{Equation 4}$$

Where R and UR are the released and unreleased percentages, respectively, at time (t); k1, k2, k3 and k4 are the rate constants of zero-order, first-order, Higuchi matrix, Peppas-Korsmeyer respectively.

Table 7.2: Diffusion exponent and solute release mechanism

Diffusion exponent (n)	**Overall solute diffusion mechanism**
< 0.5	Quasi-Fickian diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Anomalous (non-Fickian) diffusion
1.0	Case-II transport
> 1.0	Super case-II transport

7.4. STABILITY STUDY *(Manavalan R. and Ramasamy S., 2004)*

In any rational drug design or evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection. Stability of a drug can be defined as the time from the date of

manufacture and the packaging of the formulation, until its chemical or biological activity was not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

- **Objective of the study**

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. The International Conference on Harmonization (ICH) Guidelines titled Stability testing of New Drug Substances and Products describes the stability test requirements for drug registration application in the European Union, Japan and the States of America.

ICH specifies the length of study and storage conditions

- **Long-Term Testing:** 25^{0} C $\pm 2^{0}$ C at 60% RH ±5% for 12 Months
- **Accelerated Testing:** 40^{0} C $\pm 2^{0}$ C at 75% RH ±5% for 6 Months

The selected formulation F3 was exposured up to 3 months of stability studies at accelerated condition (40^{0} C $\pm 2^{0}$ C at 75% RH ± 5%RH) to find out the effect of percentage yield, drug content, entrapment efficiency and *in vitro* drug release.

- **Procedure**

Stability studies were carried out accelerated for the selected formulation F3. The sustained release microspheres were stored at for accelerated temperature in closed in a Aluminium foil for 3 months. The samples were withdrawn after periods of 1 month, 2 month and 3 month. The samples were analyzed for its percentage yield, drug content, entrapment efficiency and *in vitro* drug release.

RESULTS AND DISCUSSION

8. RESULTS AND DISCUSSION

8.1. preformulation parameters

8.1.1. Identification of drug

8.1.1.1. Identification by FTIR spectroscopy

The FTIR spectrum of Venlafaxine Hydrochloride was showed in Figure 8.1 and the interpretations of IR frequencies were represented in Table 8.1.

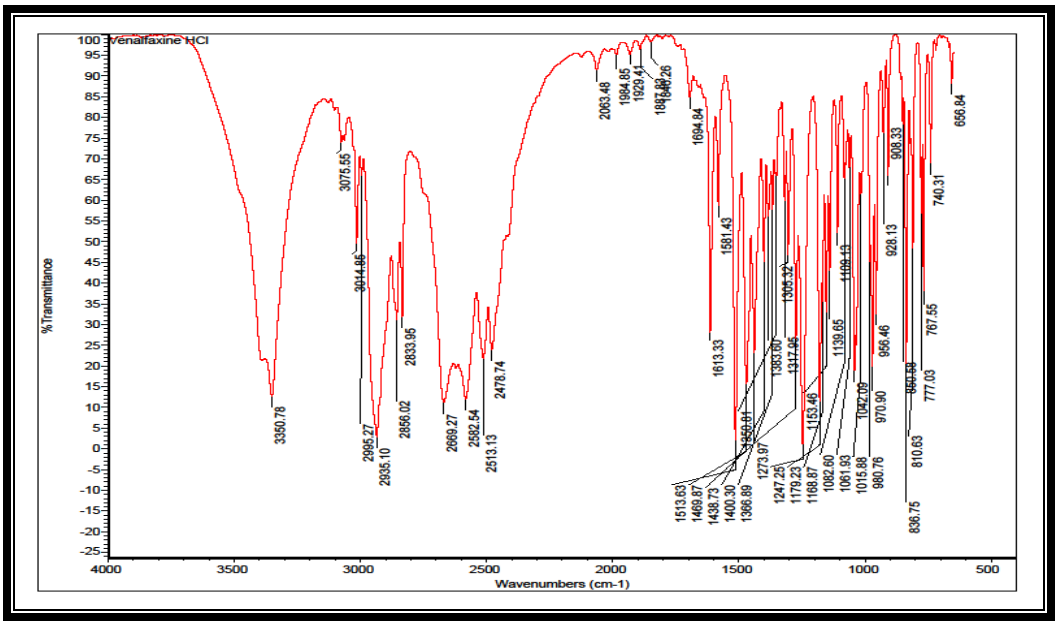


Figure 8.1: FTIR spectrum of Venlafaxine Hydrochloride

- **Interpretation of FTIR Spectrum**

Major functional groups methoxy, amine and alcohol groups Present in Venlafaxine Hydrochloride were showed at 2935 cm^{-1}, 1317.95 cm^{-1} and 1153.46 cm^{-1} respectively. These peaks were identical to functional group of Venlafaxine Hydrochloride. Hence, the sample was confirmed as Venlafaxine Hydrochloride.

Table 8.1: Characteristic frequencies in FTIR spectrum of Venlafaxine Hydrochloride

Wave No.(cm^{-1})	**Inference**
3075.55	C-H stretching
2935.13	R-O-CH_3 stretching
1317.95	NH_2 stretching
1153.46	OH stretching
1042.09	CH_2 stretching
836.5	C-H bending
740.31	OH bending

8.1.1.2. Melting point

Melting point values of Venlafaxine Hydrochloride sample was found to be in range of 215°C to 216°C. The reported melting point for Venlafaxine Hydrochloride was 215.3°C. Hence, experimental values were in good agreement with official values.

8.1.2. Physicochemical parameters of drug

8.1.2.1. Organoleptic properties

Colour: White to pale off white crystalline powder

Odour: Odourless

Nature: Crystalline powder

8.1.2.2. Solubility study

Solubility of Venlafaxine Hydrochloride was shown in Table 8.2.

Table 8.2: Solubility of Venlafaxine Hydrochloride in various solvents

Name of solvent	Solubility
Distilled water	Freely Soluble
Methanol	Freely Soluble
Acetone	Sparingly Soluble

8.1.2.3. Loss on drying

The percentage loss on drying after 3 hours was represented in Table 8.3.

Table 8.3: Percentage loss on drying for Venlafaxine Hydrochloride

S. No.	Percentage LOD	Average percentage LOD
1	0.3	0.533
2	0.6	
3	0.7	

The sample passes test for loss on drying as per the limit specified (N.M.T.1%).

8.1.3. Analytical methods

8.1.3.1. Determination of λ max in 0.1N HCl:

The absorption maximum for Venlafaxine Hydrochloride in 0.1N HCL was found to be 225 nm and absorption maximum was shown in Figure 8.2.

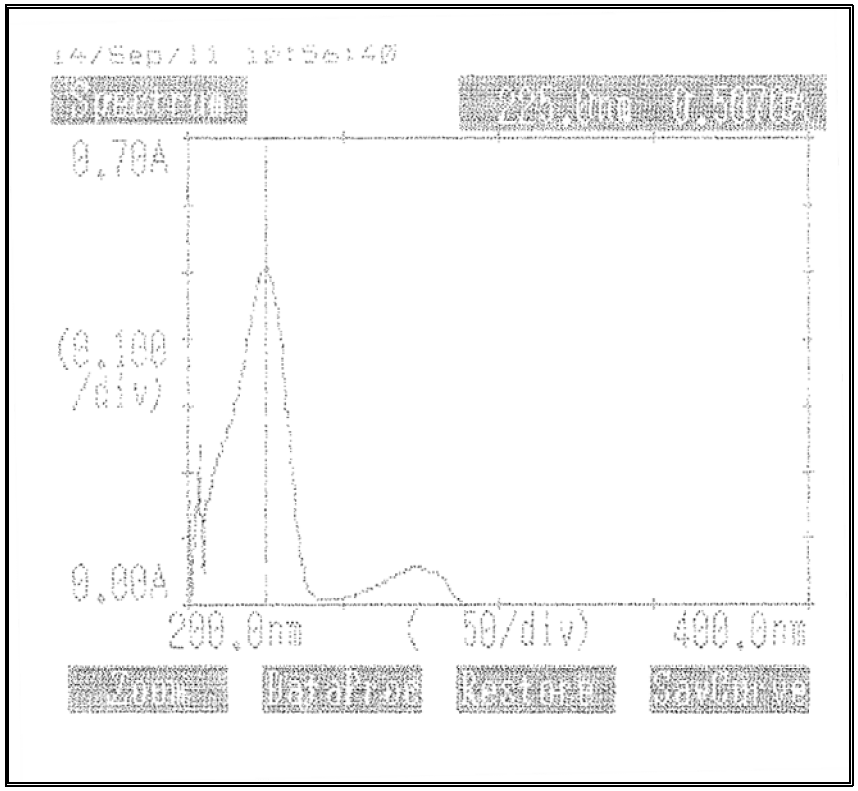


Figure 8.2: λ max observed for Venlafaxine Hydrochloride in 0.1N HCl

8.1.3.2. Preparation of standard graph of Venlafaxine Hydrochloride in 0.1N HCl:

Absorbance was obtained in various concentrations of Venlafaxine Hydrochloride in 0.1N HCl were given in Table 8.4 and shown in Figure 8.3. The graph of absorbance vs. concentration for Venlafaxine Hydrochloride was found to be linear in the concentration range of 4-20 µg /ml. The calibration curve parameters shown in Table 8.5. So the drug obeys Beer- Lambert's law in the range of 4-20 µg/ml.

Table 8.4: Data of concentration and absorbance for Venlafaxine Hydrochloride in 0.1N HCl

S. No	Concentration (µg/ml)	Absorbance
1	0	0.000
2	4	0.114
3	8	0.221
4	12	0.329
5	16	0.437
6	20	0.543

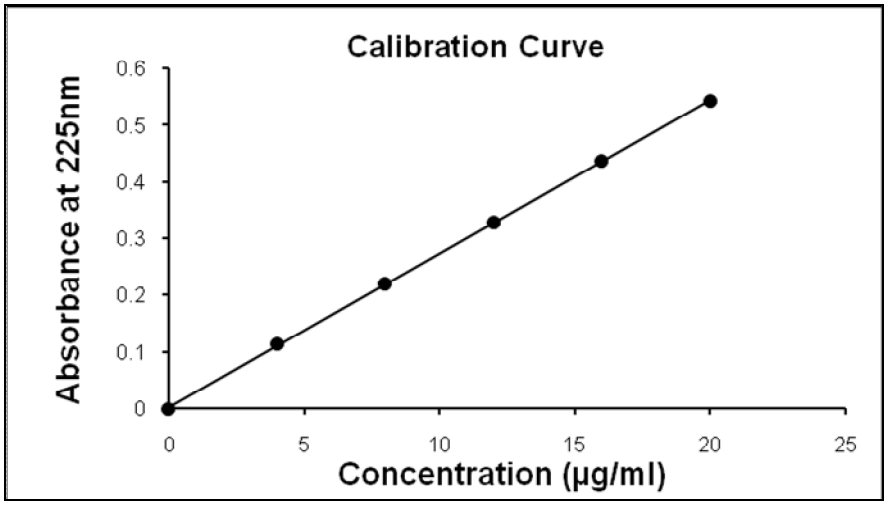


Figure 8.3: Standard graph of Venlafaxine Hydrochloride in 0.1N HCl

Table 8.5: Data for calibration curve Parameters for 0.1 N HCl

S. No	Parameters	Values
1	Correlation coefficient (r)	0.9999
2	Slope (m)	0.02708
3	Intercept (c)	0.003143

8.1.3.3. Determination of λ max in pH 7.4 phosphate buffer saline:

The absorption maximum for Venlafaxine Hydrochloride in pH 7.4 phosphate buffer saline was found to be 225 .5nm and absorption maximum was shown in Figure 8.4.

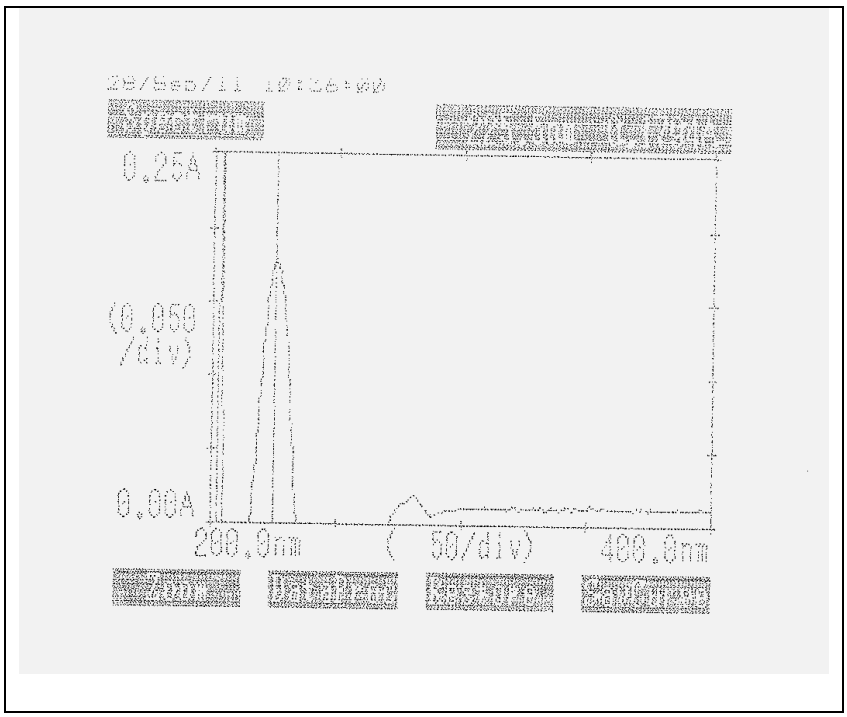


Figure 8.4: λ max observed for Venlafaxine Hydrochloride in PBS

8.1.3.4. Preparation of standard graph of Venlafaxine Hydrochloride in PH 7.4 phosphate buffer saline:

Absorbance obtained for various concentrations of Venlafaxine Hydrochloride in PH 7.4 phosphate buffer saline were given in Table 8.6 and shown in Figure 8.5. The graph of absorbance vs concentration for Venlafaxine Hydrochloride was found to be linear in the concentration range of 4–20 µg /ml. The calibration curve parameters shown in Table 8.7.So the drug obeys Beer- Lambert's law in the range of 4–20 µg /ml.

Table 8.6: Data of concentration and absorbance for Venlafaxine Hydrochloride in phosphate buffer saline

S. No	Concentration (µg/ml)	Absorbance
1	0	0.0000
2	4	0.113
3	8	0.229
4	12	0.340
5	16	0.461
6	20	0.571

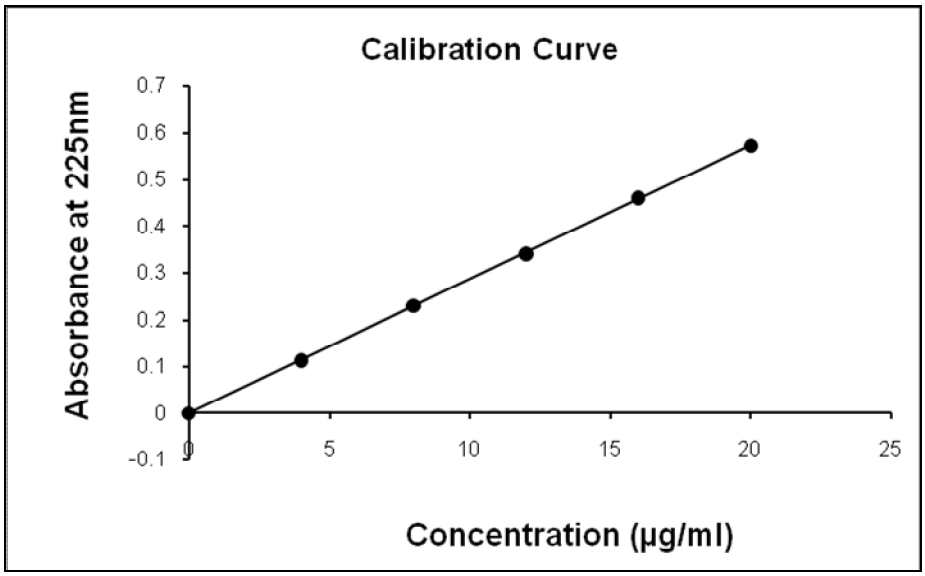


Figure 8.5: Standard graph of Venlafaxine Hydrochloride in PBS

Table 8.7: Data for calibration curve Parameters for Venlafaxine Hydrochloride in phosphate buffer saline

S. No	Parameters	Values
1	Correlation coefficient (r)	0.9999
2	Slope (m)	0.0287
3	Intercept (c)	-0.0016

8.1.4.3. Percentage purity of drug

The percentage purity of drug was calculated by using calibration graph method and represented in Table 8.8.

Table 8.8: Percentage purity of drug

S. No.	Percentage purity (%)	Avg. percentage purity (%)
1	99.75	101.14
2	101.49	
3	102.19	

The reported percentage purity for Venlafaxine Hydrochloride was 98 to 102%.

8.1.5. Determination of compatibility for drug with polymer

8.1.5.1. By FTIR spectroscopy

The FTIR spectrums of Venlafaxine Hydrochloride with different polymers were used in formulation was showed in Figures 8.6 to 8.9. And represented in Table 8.9.

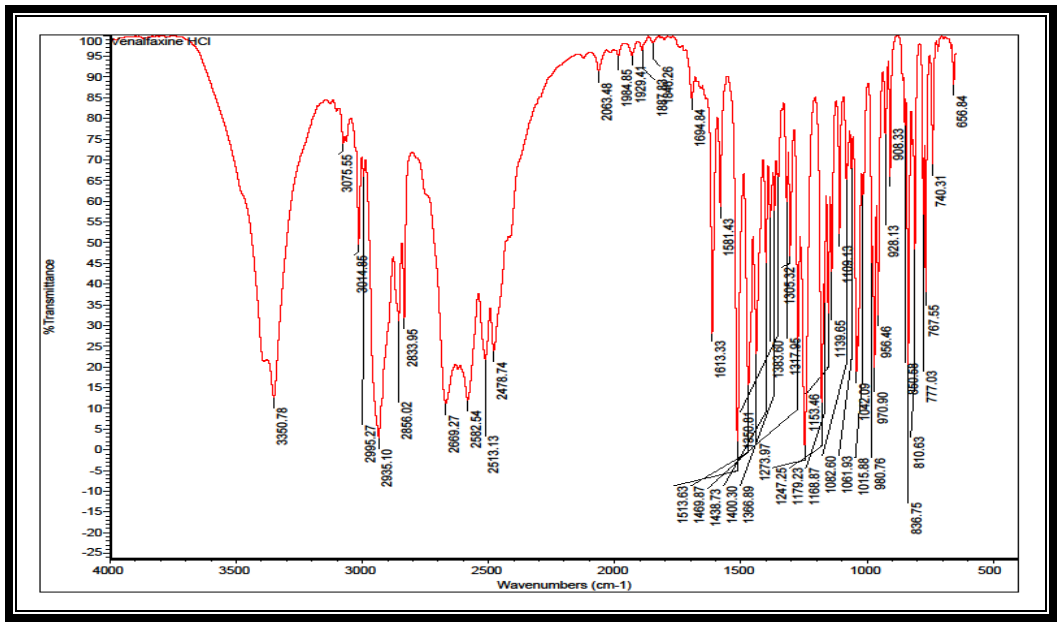


Figure 8.6: FTIR spectrum of Venlafaxine Hydrochloride

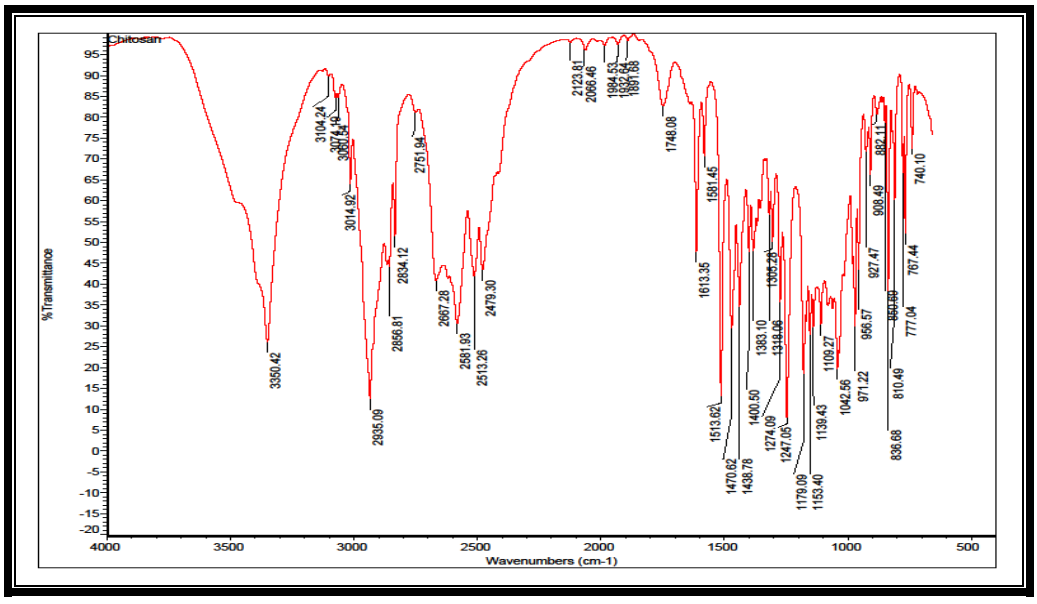


Figure 8.7: FTIR spectrum of Venlafaxine Hydrochloride with Chitosan

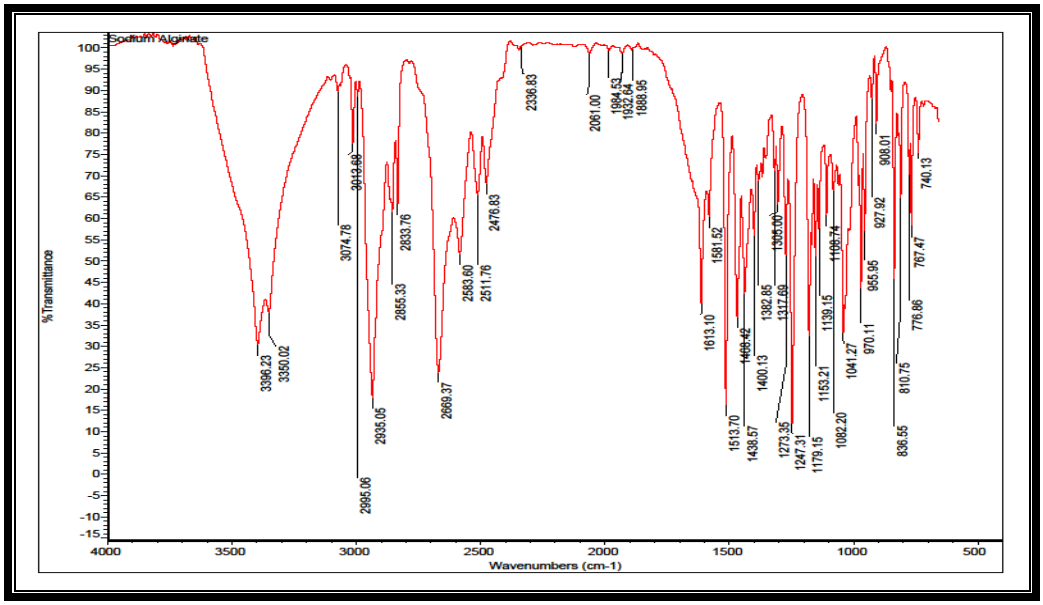


Figure 8.8: FTIR spectrum of Venlafaxine Hydrochloride with Sodium alginate

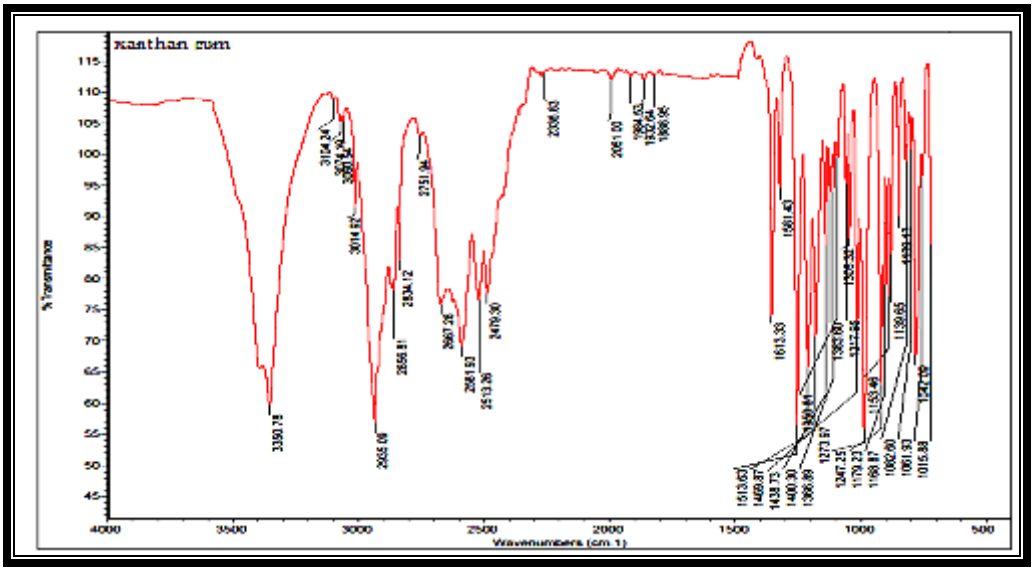


Figure 8.9: FTIR spectrum of Venlafaxine Hydrochloride with Xanthan gum

Table 8.9: Interpretation of FTIR spectrum

Functional groups	Peaks observed (wave no. (cm^{-1}))			
	Venlafaxine hydrochloride	Venlafaxine hydrochloride + chitosan	Venlafaxine hydrochloride + Sodium alginate	Venlafaxine hydrochloride + Xanthan gum
C-H stretching	3075.55	3074.24	3074.78	3074.55
R-O-CH_3 stretching	2935.13	2935.09	2935.05	2935.03
NH_2 stretching	1317.95	1318.06	1317.69	1317.75
OH stretching	1153.46	1153.40	1153.21	1153.04
CH_2 stretching	1042.09	1042.56	1041.27	1042.05
C-H bending	836.5	836.68	838.55	836.35
OH bending	740.31	740.10	740.13	740.19

FTIR spectrums were compared, it could indicate that there was no incompatibility between drug and polymer.

8.1.5.2. By DSC thermal analysis

The compatibility and interactions between drug and polymer were observed using DSC studies; results obtained were showed in Figure 8.10 to 8.13.

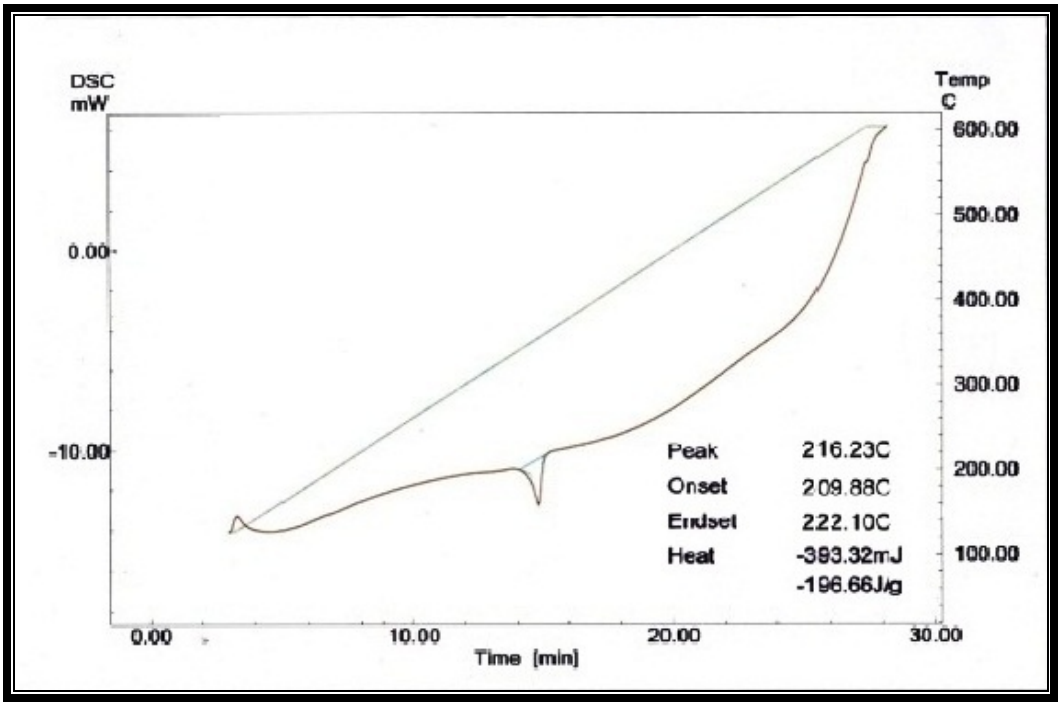


Figure 8.10: DSC thermogram of Venlafaxine Hydrochloride

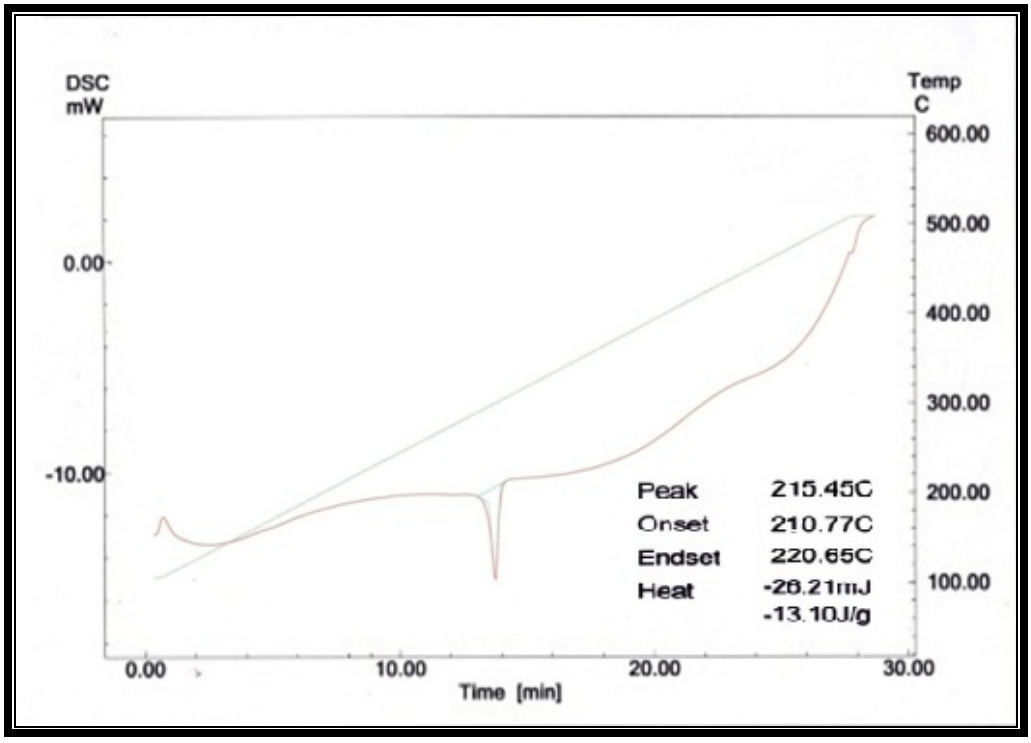


Figure 8.11: DSC thermogram of Venlafaxine Hydrochloride with Chitosan

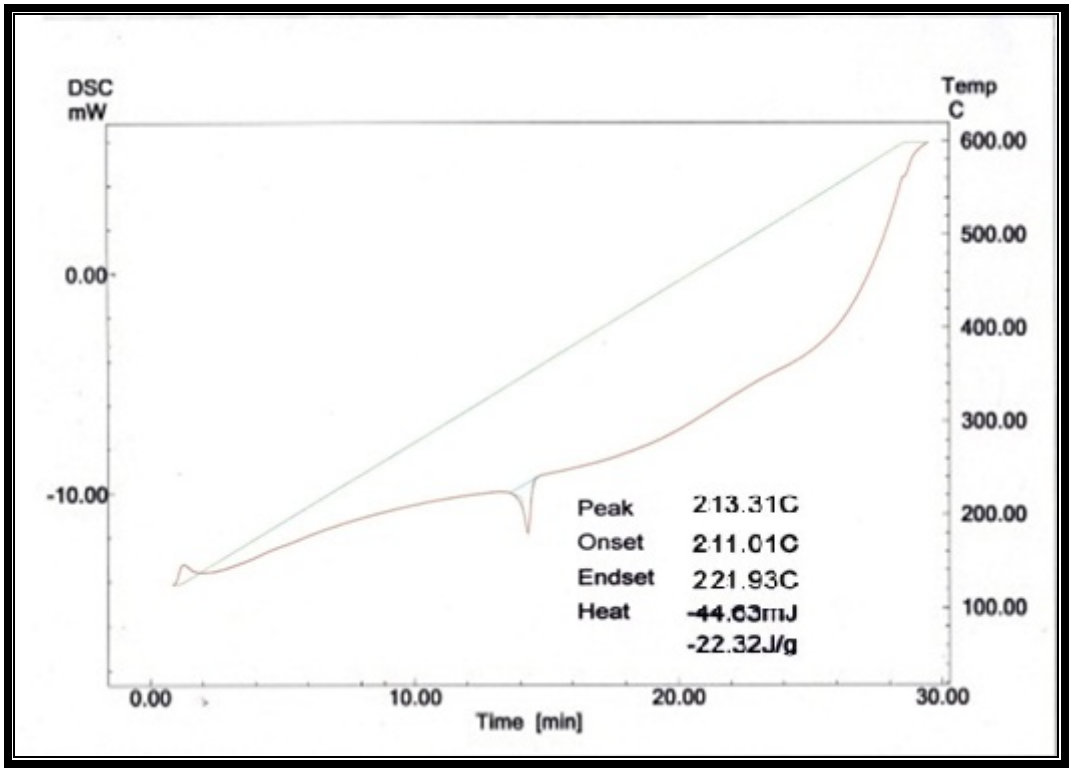


Figure 8.12: DSC thermogram of Venlafaxine Hydrochloride with Sodium alginate

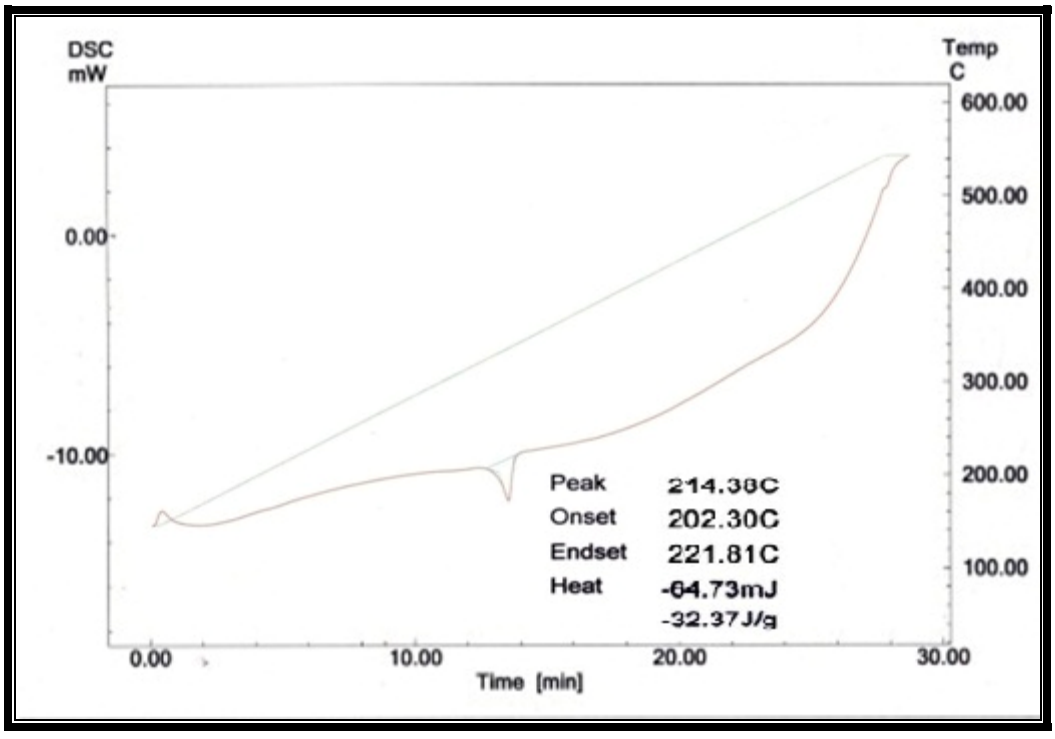


Figure 8.13: DSC thermogram of Venlafaxine Hydrochloride with Xanthan gum

Table 8.10: Various DSC thermogram parameters

S. No.	DSC thermogram	Onset temperature (°C)	Peak temperature (°C)	Endset temperature (°C)
1	Venlafaxine Hydrochloride	209.88	216.23	222.10
2	Venlafaxine Hydrochloride + Chitosan	210.77	215.45	220.65
3	Venlafaxine Hydrochloride + Sodium alginate	211.01	213.31	221.93
4	Venlafaxine Hydrochloride + Xanthan gum	202.81	214.36	221.81

According to Figures 8.10 to 8.13 and Table 8.10, DSC thermogram showed that there was no major difference in onset temperature, peak temperature and end set temperature when compared with pure drug's thermogram. No interaction was found between drug and polymers.

8.2. PREPARATION OF MICROSPHERES

Nine formulations of sustained release microspheres of Venlafaxine Hydrochloride were prepared by solvent evaporation method. Formulations F1, F2 and F3 were formulated and prepared Venlafaxine HCL with Chitosan polymer in the ratio of 1: 1, 1: 2 and 1: 3. Similarly, formulations F4, F5 and F6 were formulated and prepared Venlafaxine HCL with Sodium alginate polymer in the ratio of 1:1, 1:2 and 1:3; and formulations F7, F8 and F9 were formulated and prepared Venlafaxine HCL with Xanthan gum polymer in the ratio of 1: 1, 1: 2 and 1: 3.All the formulations (F1 to F9) of prepared microspheres were taken for further evaluation studies.

8.3. EVALUATION OF MICROSPHERES

8.3.1. Percentage Yield, Drug Content and Entrapment Efficiency

The percentage yield, Drug Content and Entrapment Efficiency of Sustained release microspheres were found to increased as the polymer ratio was increased. The maximum yield of microspheres was 87.16% in Chitosan polymer, 80.48% in Sodium alginate polymer and 83.48% in Xanthan gum. Better yield of microspheres was obtained from Chitosan. Drug content and Entrapment efficiency was high in Chitosan containing formulations when compared to Sodium alginate and Xanthan gum formulations. All the formulations were Percentage Yield, Drug content and Entrapment efficiency data was showed in Table 8.11 and showed in Figure 8.14.

Table 8.11: Percentage yield, Drug content and Percentage Entrapment efficiency

S. No.	FORMULATIONS	% YIELD	% DRUG CONTENT	% ENTRAPMENT
1	F1	81.01	43.83	76.41
2	F2	84.60	57.49	84.77
3	F3	87.16	69.26	92.09
4	F4	73.14	43.31	67.35
5	F5	78.20	49.40	78.50
6	F6	80.48	53.29	81.11
7	F7	75.26	39.82	72.75
8	F8	78.35	52.36	74.8
9	F9	83.48	62.64	82.33

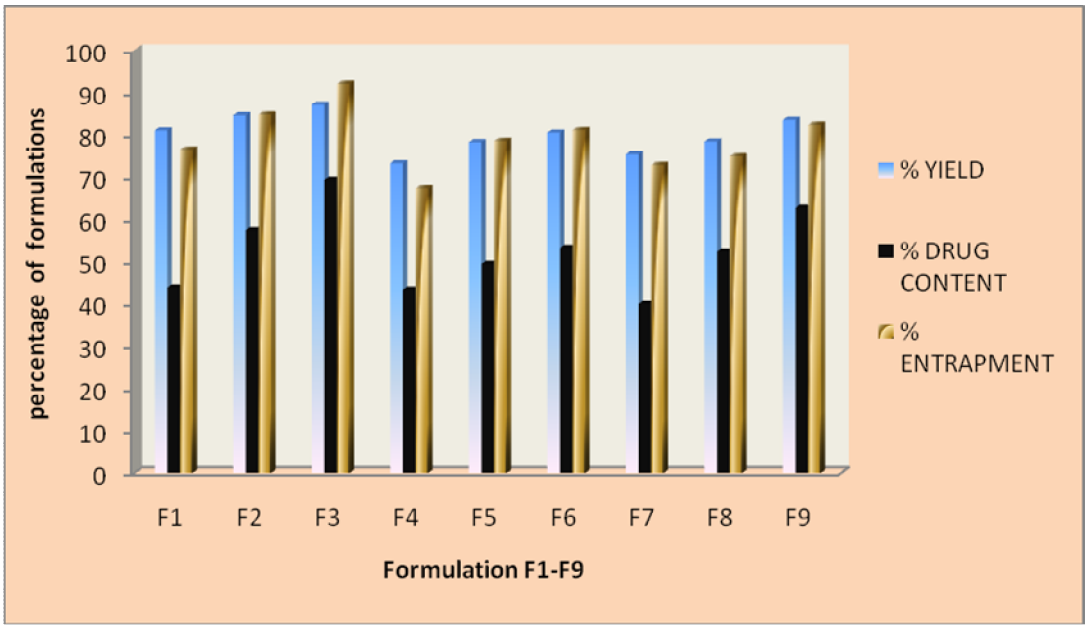


Figure 8.14: Percentage Yield, Drug Content and Entrapment Efficiency

8.3.2. Particle size analysis

Particle size of prepared microspheres was determined by optical microscopy method and the average particle sizes of all batches of microspheres were represented in Table. 8.12.

All the batches of microspheres were prepared by keeping the drug amount and solvent volume constant. The particle sizes of the sustained release microspheres were found to be in the range of 32.72 µm – 62.57 µm. The results were represented graphically in Figure 8.15.

Table 8.12: Percentage Mean Particles size

S. No.	FORMULATIONS	MEAN PARTICLE SIZE (µm)
1	F1	43.10
2	F2	49.25
3	F3	62.57
4	F4	35.19
5	F5	37.05
6	F6	44.91
7	F7	32.72
8	F8	39.78
9	F9	41.16

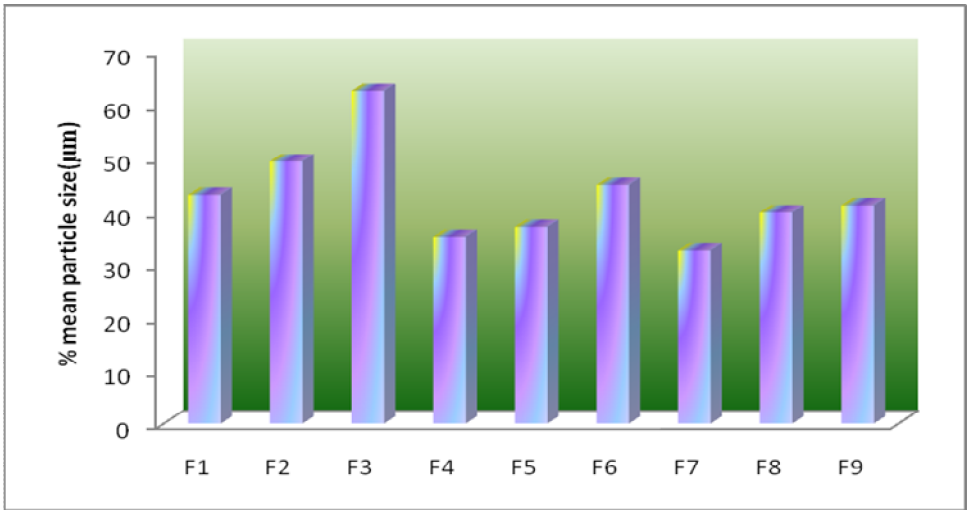


Figure 8.15: Percentage Mean Particles size

8.3.3. Scanning Electron Microscopy Analysis (Surface morphology)

Surface morphology and shape characteristics of microspheres were evaluated by means of scanning electron microscopy. The SEM photographs of the

microspheres revealed that the microspheres were spherical with rough, hollow surface and slightly aggregated were showed in Figure 8.16 and 8.17.

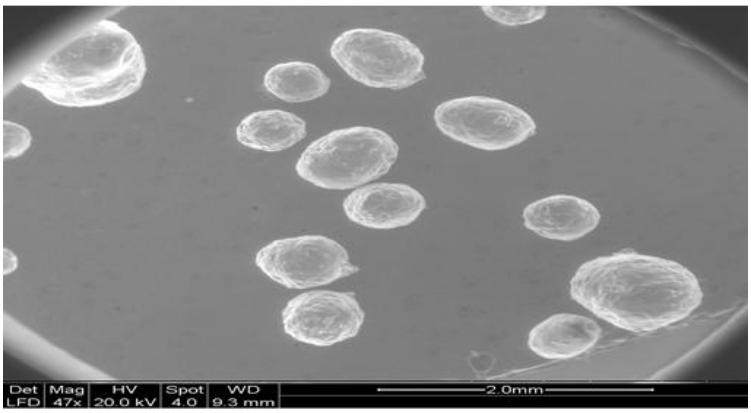


Figure 8.16: Scanning electron microphotograph of formulation F3 at lower magnification

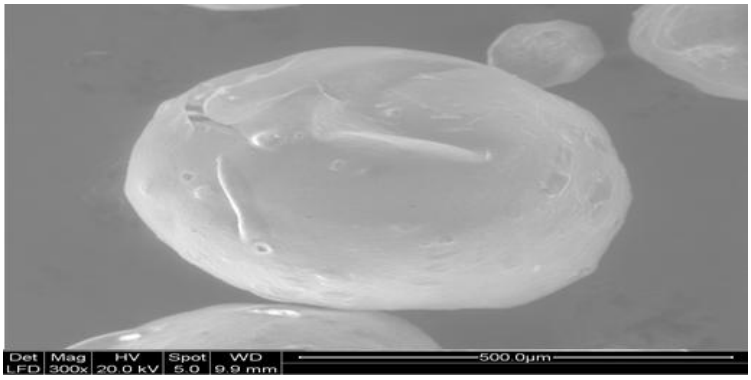


Figure 8.17: Scanning electron microphotograph of formulation F3 at higher Magnification

8.3.4. *In Vitro* Dissolution studies

In-vitro drug released profiles of Venlafaxine Hydrochloride microspheres were performed in each formulation up to 2 hours in 0.1N HCl followed by phosphate buffer (pH 7.4) up to 16 hours. It was represented in Table 8.13 and showed in Figure 8.18 to 8.21.

Table 8.13: Cumulative percentage *in-vitro* drug released from the formulations F1-F9

Time in hours	pH Medium	Formulations								
		F1 %	F2 %	F3 %	F4 %	F5 %	F6 %	F7 %	F8 %	F9 %
1	pH 1.2 (simulated gastric fluid)	9.63±1.33	9.07±1.54	8.99±0.56	12.48±1.04	10.98±0.64	9.16±1.01	12.38±0.83	10.98±0.80	9.87±1.43
2		18.75±0.38	16.2±1.39	14.92±0.56	20.9±1.20	19.63±0.84	17.28±0.73	21.63±1.22	19.81±1.18	18.36±1.43
3	pH 7.4 (simulated intestinal fluid)	26.79±0.65	25.77±1.23	22.19±0.79	28.48±0.65	27.81±0.78	25.93±0.95	29.38±2.05	27.14±1.84	24.1±0.95
4		34.98±1.04	29.61±0.87	28.37±1.35	38.49±0.96	35.61±1.13	31.28±0.95	37.85±1.48	37.28±2.30	33.93±1.48
5		41.28±1.06	38.05±0.72	31.27±0.72	48.36±0.79	45.25±0.52	37.47±1.28	48.29±0.56	46.41±0.61	42.18±0.78
6		49.48±1.21	46.57±1.39	37.85±1.18	54.83±1.76	50.94±0.69	49.29±1.23	54.38±1.12	51.16±0.70	48.32±0.78
7		55.82±0.96	51.33±0.69	42.18±1.05	57.74±0.46	54.81±2.07	53.74±0.56	58.29±1.84	57.32±0.87	55.29±0.96
8		59.43±0.46	56.04±0.62	47.33±0.96	61.29±0.78	59.17±1.41	56.49±0.56	62.16±1.31	60.27±1.15	59.69±0.95
10		68.72±0.87	65.21±0.96	55.83±0.95	71.82±1.65	66.38±0.62	65.38±1.05	70.29±0.87	69.38±1.08	67.12±1.05
12		76.84±1.23	70.41±0.78	64.72±1.13	79.09±0.62	75.48±0.53	74.09±1.13	78.27±1.46	75.93±0.78	73.26±1.31
16		91.74±0.89	86.84±0.79	83.14±1.04	96.86±0.70	93.28±1.12	89.03±0.69	97.14±0.95	95.39±0.79	91.97±2.21

* All the values expressed as mean ± S.D., n=6

Table 8.14: Cumulative percentage *in-vitro* drug released from formulation F1

Time (hours)	**pH Medium**	**DR* (%)**	**Amount (mg)**	**% DE**	**MDT**
1	pH 1.2 (simulated gastric fluid)	9.63±1.33	9.63	4.79	0.50
2		18.75±0.38	18.75	9.49	0.99
3	pH 7.4 (simulated intestinal fluid)	26.79±0.65	26.79	13.88	1.43
4		34.98±1.04	34.98	18.02	1.90
5		41.28±1.06	41.28	21.86	2.28
6		49.48±1.21	49.48	25.72	2.91
7		55.82±0.96	55.82	29.60	3.30
8		59.43±0.46	59.43	33.14	3.57
10		68.72±0.87	68.72	39.39	4.28
12		76.84±1.23	76.84	44.93	4.95
16		91.74±0.89	91.74	54.70	6.44

* All the values expressed as mean ± S.D., n=6

Table 8.15: Cumulative percentage *in-vitro* drug released from formulation F2

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
1	pH 1.2 (simulated gastric fluid)	9.07±1.54	9.07	4.86	0.50
2		16.2±1.39	16.2	11.82	1.15
3	pH 7.4 (simulated intestinal fluid)	25.77±1.23	25.77	16.75	1.02
4		29.61±0.87	29.61	19.43	1.37
5		38.05±0.72	38.05	22.26	2.04
6		46.05±1.39	46.05	25.52	2.68
7		51.33±0.69	51.33	28.84	3.09
8		56.04±0.62	56.04	31.97	3.44
10		65.21±0.96	65.21	37.60	4.14
12		70.41±0.78	70.41	42.66	4.87
16		86.84±0.79	86.84	51.80	6.43

* All the values expressed as mean ± S.D., n=6

Table 8.16: Cumulative percentage *in-vitro* drug released from formulation F3

Time (hours)	**pH Medium**	**DR* (%)**	**Amount (mg)**	**% DE**	**MDT**
1	pH 1.2 (simulated gastric fluid)	8.99±0.56	8.99	4.44	0.50
2		14.92±0.56	14.92	7.93	0.86
3	pH 7.4 (simulated intestinal fluid)	22.19±0.79	22.19	11.24	1.45
4		28.37±1.35	28.37	14.66	1.91
5		31.27±0.72	31.27	17.58	2.11
6		37.85±1.18	37.85	20.18	2.64
7		42.18±1.05	42.18	22.77	3.07
8		47.33±0.96	47.33	25.06	3.19
10		55.83±0.95	55.83	29.95	4.78
12		64.72±1.13	64.72	35.01	5.35
16		83.14±1.04	83.14	44.71	7.54

* All the values expressed as mean ± S.D., n=6

Table 8.17: Cumulative percentage *in-vitro* drug released from formulation F4

Time (hours)	**pH Medium**	**DR* (%)**	**Amount (mg)**	**% DE**	**MDT**
1	pH 1.2 (simulated gastric fluid)	12.48±1.04	12.48	6.84	0.50
2		20.9±1.20	20.9	12.38	0.88
3	pH 7.4 (simulated intestinal fluid)	28.48±0.65	28.48	16.78	1.26
4		38.49±0.96	38.49	21.18	1.87
5		48.36±0.79	48.36	26.24	2.53
6		54.83±1.76	54.83	30.98	2.69
7		57.74±0.46	57.74	34.77	2.86
8		61.29±0.78	61.29	37.92	3.04
10		71.82±1.65	71.82	43.48	3.81
12		79.09±0.62	79.09	48.72	4.65
16		96.86±0.70	96.86	58.61	6.33

* All the values expressed as mean ± S.D., n=6

Table 8.18: Cumulative percentage *in-vitro* drug released from formulation F5

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
1	pH 1.2 (simulated gastric fluid)	10.98±0.64	10.98	4.79	0.50
2		19.63±0.84	19.63	9.98	1.04
3	pH 7.4 (simulated intestinal fluid)	27.81±0.78	27.81	14.40	1.32
4		35.61±1.13	35.61	18.50	1.94
5		45.25±0.52	45.25	22.92	2.47
6		50.94±0.69	50.94	27.34	2.94
7		54.81±1.76	54.81	31.29	3.12
8		57.74±0.46	57.74	34.47	3.17
10		61.29±0.78	61.29	39.47	3.62
12		71.82±1.65	71.82	43.94	4.54
16		93.28±1.12	93.28	53.17	6.65

* All the values expressed as mean ± S.D., n=6

Table 8.19: Cumulative percentage *in-vitro* drug released from formulation F6

Time (hours)	**pH Medium**	**DR* (%)**	**Amount (mg)**	**% DE**	**MDT**
1	pH 1.2 (simulated gastric fluid)	9.16±1.01	9.16	4.86	0.50
2		17.28±0.73	17.28	9.17	0.94
3	pH 7.4 (simulated intestinal fluid)	25.93±0.95	25.93	13.08	1.40
4		31.28±0.95	31.28	16.60	1.77
5		37.47±1.28	37.47	20.28	2.47
6		49.29±1.23	49.29	24.69	3.23
7		53.74±1.12	53.74	28.95	3.35
8		56.49±0.56	56.49	32.42	3.52
10		65.38±1.05	56.49	38.20	4.10
12		74.09±1.13	74.09	43.19	4.75
16		89.03±0.69	89.03	52.16	6.37

* All the values expressed as mean ± S.D., n=6

Table 8.20: Cumulative percentage *in-vitro* drug released from formulation F7

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
1	pH 1.2 (simulated gastric fluid)	12.38±0.83	12.38	6.98	0.50
2		21.63±1.22	21.63	12.91	0.91
3	pH 7.4 (simulated intestinal fluid)	29.38±2.05	29.38	17.48	1.22
4		37.85±1.48	37.85	22.05	1.90
5		48.29±0.56	48.29	27.13	2.44
6		54.38±1.12	54.38	31.83	2.69
7		58.29±1.84	58.29	35.66	2.81
8		62.16±1.31	62.16	38.90	3.10
10		70.29±0.87	70.29	44.16	3.41
12		78.27±1.46	78.27	49.00	4.60
16		97.14±0.95	97.14	58.98	6.41

* All the values expressed as mean ± S.D., n=6

Table 8.21: Cumulative percentage *in-vitro* drug released from formulation F8

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
1	pH 1.2 (simulated gastric fluid)	10.98±0.80	10.98	5.92	0.50
2		19.81±1.18	19.81	11.01	0.92
3	pH 7.4 (simulated intestinal fluid)	27.14±1.84	27.14	15.29	1.33
4		37.28±2.30	37.28	19.64	1.93
5		46.41±0.61	46.41	24.28	2.45
6		51.16±0.70	51.16	28.66	2.79
7		57.32±0.87	57.32	32.50	3.04
8		60.27±1.15	60.27	35.92	3.38
10		69.38±1.08	69.38	41.88	3.95
12		75.93±0.78	75.93	46.97	4.55
16		95.39±0.79	95.39	56.59	6.50

* All the values expressed as mean ± S.D., n=6

Table 8.22: Cumulative percentage *in-vitro* drug released from formulation F9

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
1	pH 1.2 (simulated gastric fluid)	9.8±71.43	9.8	5.29	0.50
2		18.3±61.42	18.3	9.84	0.92
3	pH 7.4 (simulated intestinal fluid)	24.1±0.95	24.1	13.22	1.18
4		33.93±1.48	33.93	16.52	1.87
5		42.18±0.78	42.18	20.42	2.51
6		48.32±0.72	48.32	24.04	2.67
7		55.29±0.96	55.29	27.69	3.53
8		59.69±0.95	59.69	31.72	4.04
10		67.12±1.05	67.12	38.56	4.32
12		73.26±1.31	73.26	43.84	4.76
16		91.97±2.21	91.97	53.32	6.61

* All the values expressed as mean ± S.D., n=6

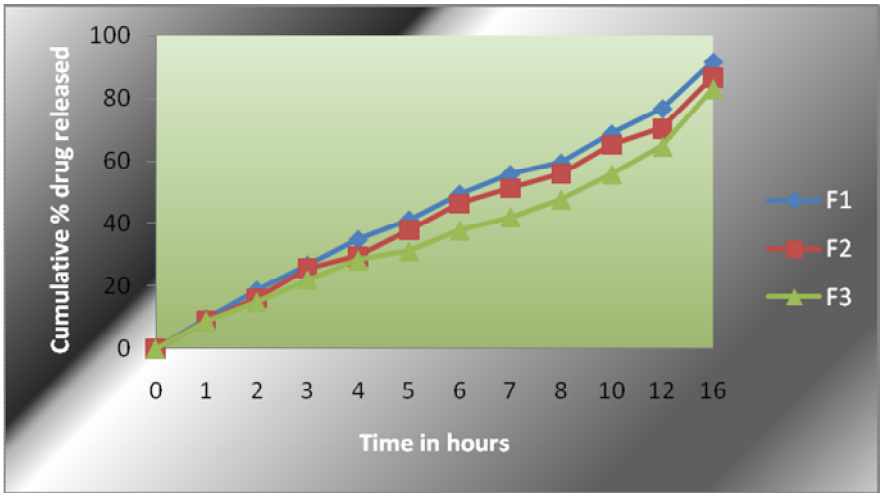


Figure 8.18: Graphical representation of Cumulative % in-*vitro* drug released in formulations F1-F3

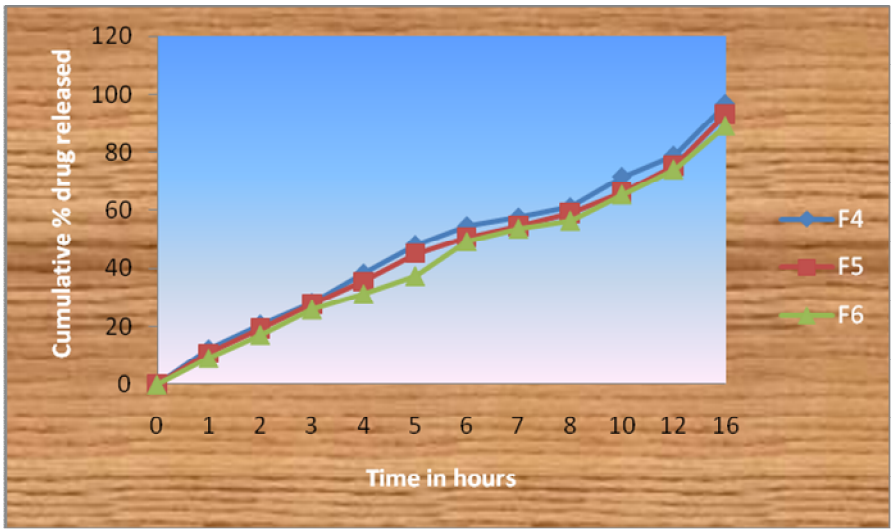


Figure 8.19: Graphical representation of Cumulative % in-*vitro* drug released in formulations F4-F6

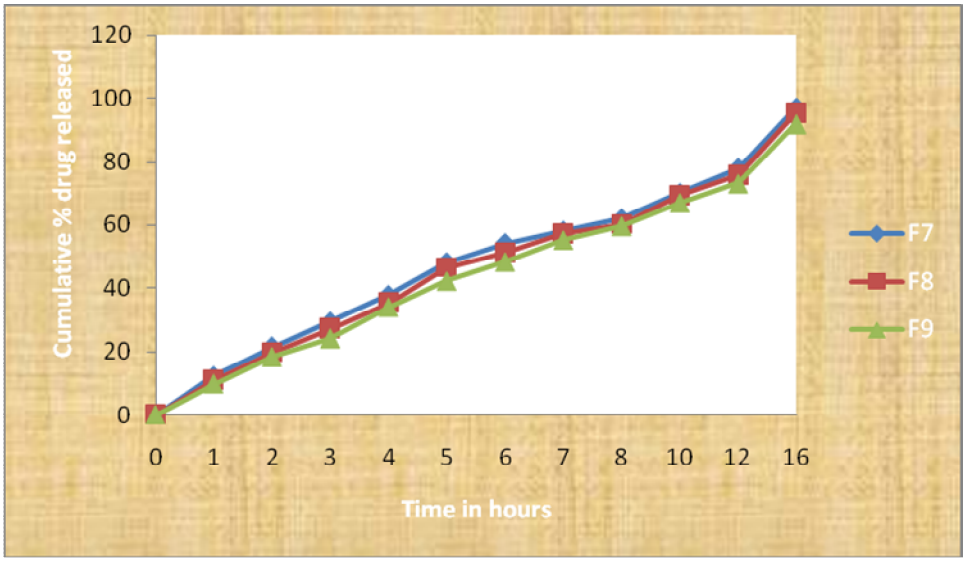


Figure 8.20: Graphical representation of Cumulative % in-*vitro* drug released in formulations F7-F9

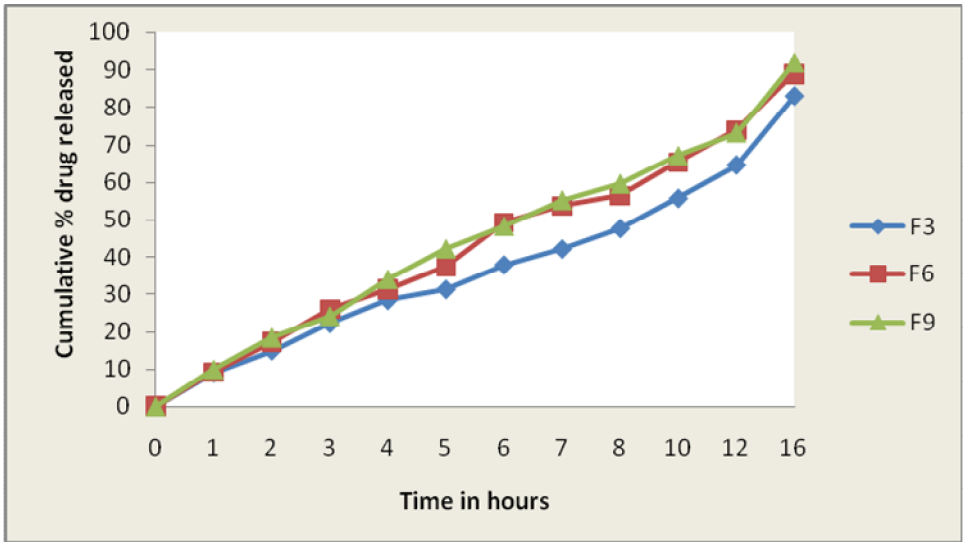


Figure 8.21: Graphical representation of Cumulative % in-*vitro* drug released from formulations F3, F6 and F9

According to *in-vitro* drug released profiles of the Venlafaxine microspheres formulation F1showed that 91.74±0.89% in 16hrs, formulation F2 showed that 86.84±0.79% in 16hrs, formulation F3 showed that 83.14±1.04% in 16hrs, formulation F4 showed that 96.86±0.70% in 16hrs, formulation F5 showed that

93.28±1.12% in 16hrs and formulation F6 showed that 89.03±0.69%, formulation F7 showed that 97.14±0.95% in 16hrs, formulation F8 showed that 95.39±0.79% in 16hrs and formulation F9 showed that 91.97±2.21%. Solvent evaporation method formulation F3 showed better sustained release (83.14%) at the end of the 16th hour as compared to other formulations (F1, F2, F4, F5, F6, F7, F8 and F9).

8.5. Release Kinetics of *In-vitro* Drug Release

The kinetics of *in-vitro* drug released was determined by applying the drug release data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas. The results obtained were represented in Table 8.23 and shown in Figures 8.22 to 8.30.

Table 8.23: Release kinetics of *in-vitro* drug release

Formulation code	Zero order	First order	Higugi	Peppas		Best model
	R^2	R^2	R^2	R^2	n	
F1	0.9621	0.9501	0.9723	0.9908	0.432	Peppas
F2	0.9650	0.9705	0.9663	0.9909	0.456	Peppas
F3	0.9900	0.9471	0.9530	0.9985	0.402	Peppas
F4	0.9503	0.8439	0.9782	0.9908	0.485	Peppas
F5	0.9592	0.8811	0.9729	0.9903	0.365	Peppas
F6	0.9622	0.9307	0.9684	0.9903	0.462	Peppas
F7	0.9540	0.8611	0.9770	0.9901	0.398	Peppas
F8	0.9597	0.9162	0.9756	0.9907	0.441	Peppas
F9	0.9647	0.9609	0.9663	0.9907	0.356	Peppas

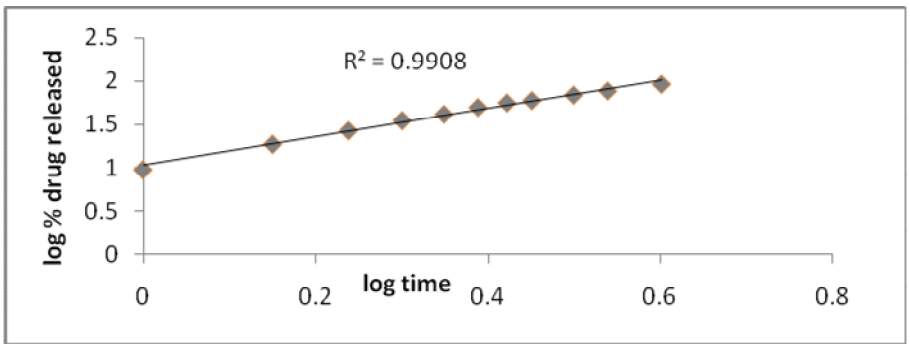


Figure 8.22: Best fit model (korsemeyer peppas) of formulation F1

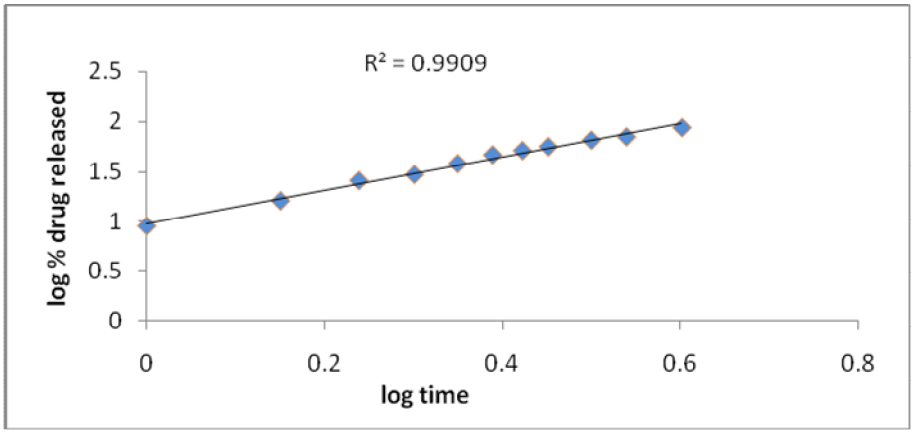


Figure 8.23: Best fit model (korsemeyer peppas) of formulation F2

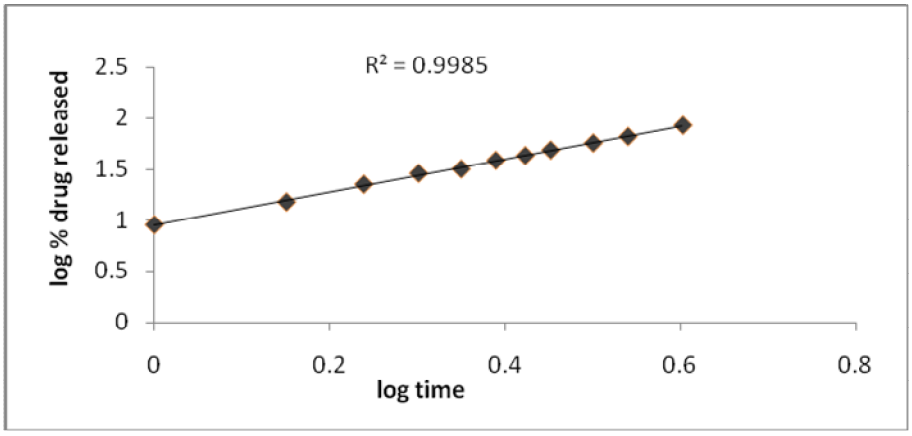


Figure 8.24: Best fit model (korsemeyer peppas) of formulation F3

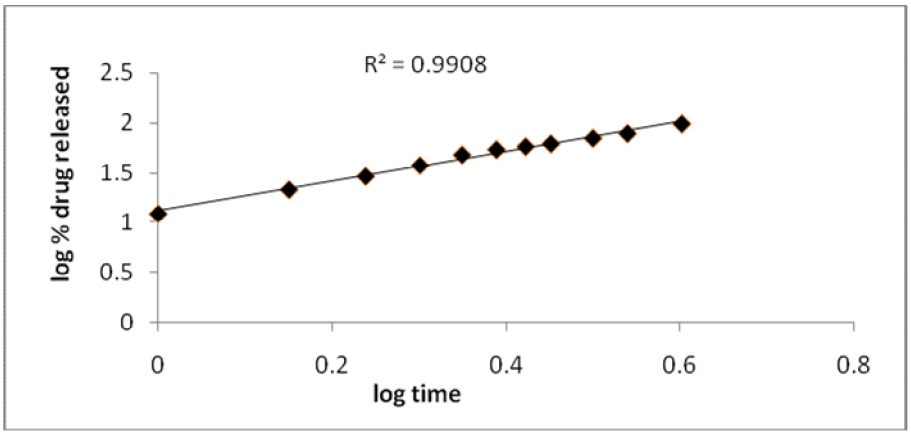


Figure 8.25: Best fit model (korsemeyer peppas) of formulation F4

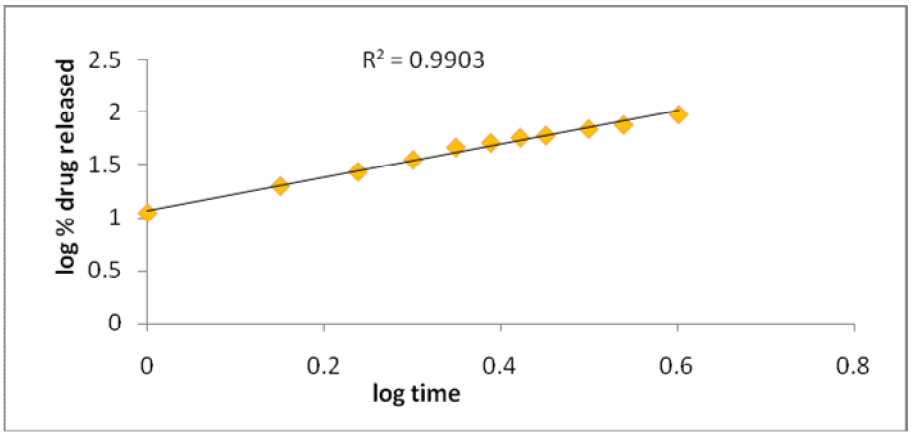


Figure 8.26: Best fit model (korsemeyer peppas) of formulation F5

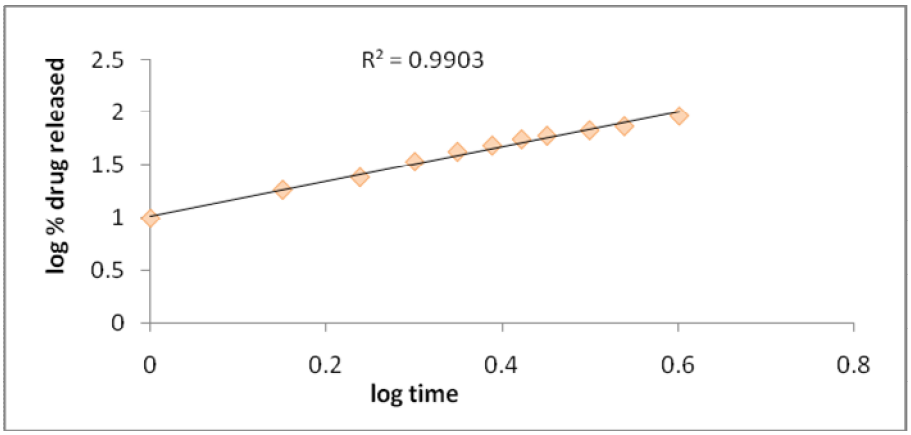


Figure 8.27: Best fit model (korsemeyer peppas) of formulation F6

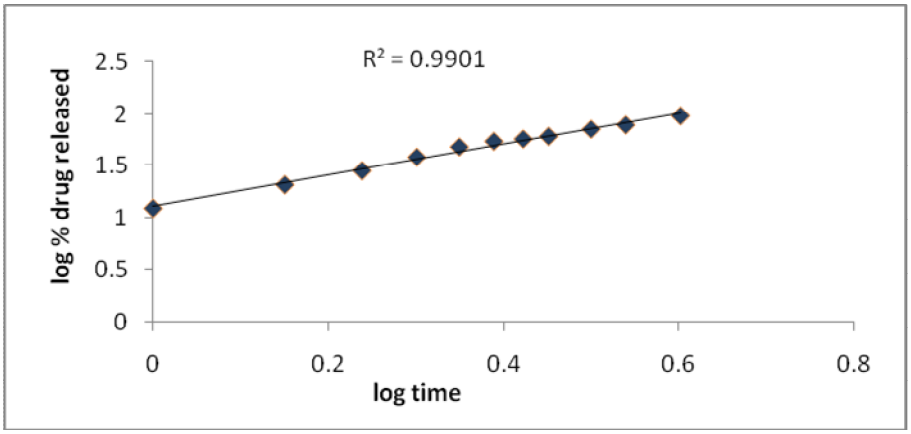


Figure 8.28: Best fit model (korsemeyer peppas) of formulation F7

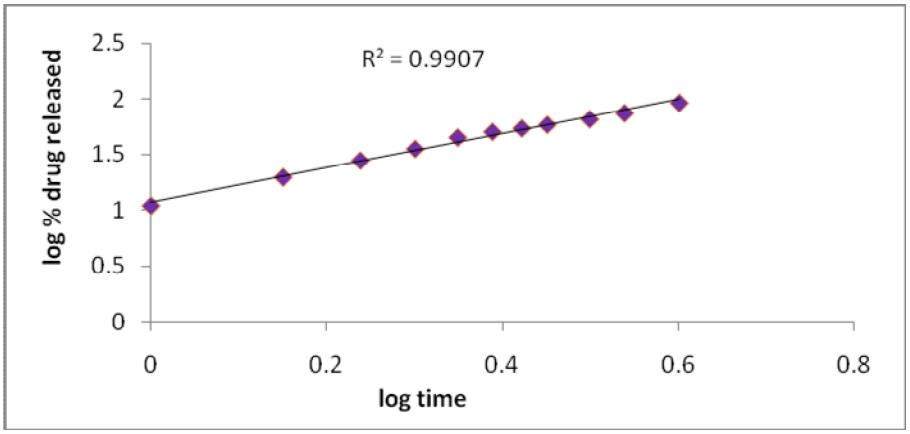


Figure 8.29: Best fit model (korsemeyer peppas) of formulation F8

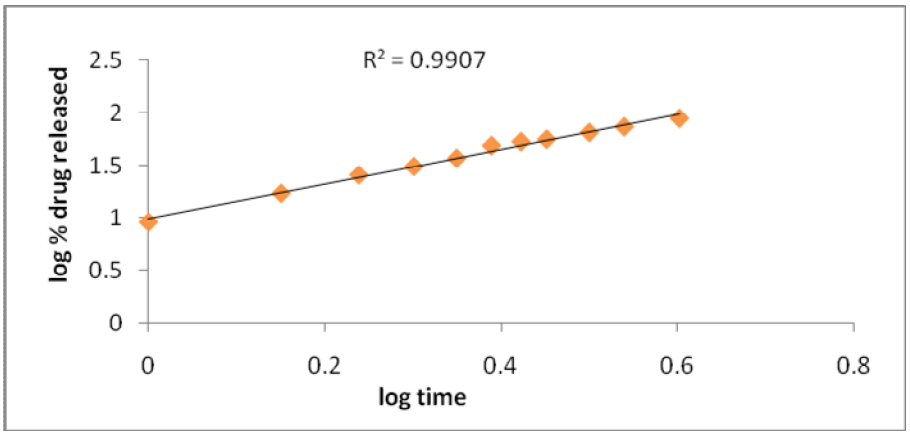


Figure 8.30: Best fit model (korsemeyer peppas) of formulation F9

From the data and graphical representations, the Venlafaxine Hydrochloride microspheres formulations were showed well fitted korsemeyer peppas kinetics and formulation F3 was showed best among the formulations were prepared based on Percentage Yield, Drug content, Percentage Entrapment and *in-vitro* drug released profiles and also well fitted the korsemeyer peppas kinetics

Table 8.24: Time of drug released values of t_{25}, t_{50} and t_{90} for Venlafaxine Hydrochloride in sustained release microspheres for formulations F1 to F9

Formulations	Time of percentage drug released (hours)		
	25%(t_{25})	**50%(t_{50})**	**90%(t_{90})**
F1	2.8	6.0	15.8
F2	3.0	7.0	>16
F3	3.5	9.0	>16
F4	2.5	5.5	14.0
F5	2.8	6.0	15.0
F6	3.0	6.1	>16
F7	2.5	5.3	14.0
F8	2.8	5.8	15.0
F9	3.2	6.5	15.8

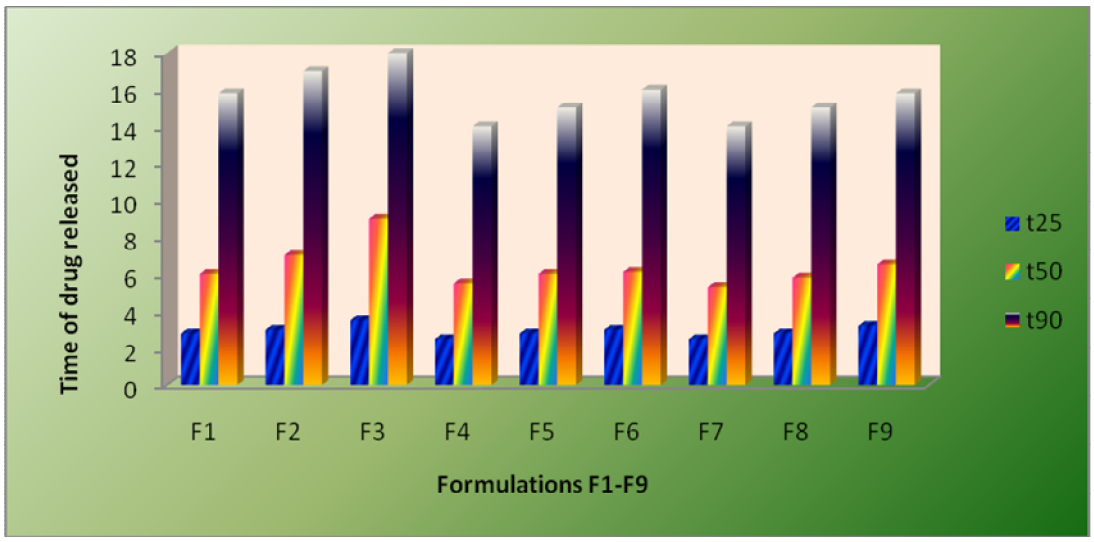


Figure 8.31: Histogram for time of drug released values of t_{25}, t_{50} and t_{90} of Sustained release microspheres of Venlafaxine hydrochloride formulations F1 to F9

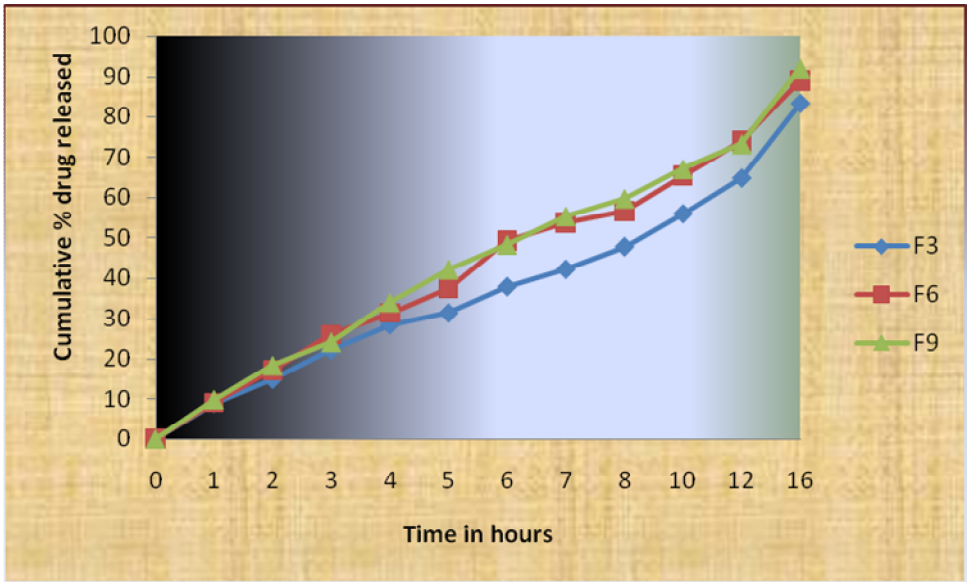


Figure 8.32: *In vitro* drug released profile of selected formulations (F3, F6 and F9)

The time required to released 25% of drug (t_{25}), time required to released 50% of drug (t_{50}) and time required to release 90% of drug (t_{90}). The results of t_{25}, t_{50} and t_{90} are showed in Table 8.24 and Figure 8.31. According to the time of drug released values of t_{25}, t_{50} and t_{90} the formulations F3, F6 and F9 were selected as the best formulation of Chitosan, Sodium alginate and Xanthan gum polymers respectively. The drug released profile of selected formulations was showed in Figure 8.32, it was observed that formulation F3 was the sustained drug released profile up to 16 hours as compared to formulations F6 and F9. Hence the formulation F3 was selected for further stability study.

8.4. STABILITY STUDIES

From the results it was found that formulation F3 was the best formulation amongst the 9 formulations. Thus formulation F3 was selected for stability studies. Formulation F3 was analyzed for Percentage yield, Drug content, Entrapment

Efficiency and *in vitro* drug released at the end of each month up to three months, results are shown in Table 8.25 and Figure 8.33 to 8.36.

Table 8.25: Stability studies of selected formulation F3

EVALUATION PARAMETERS	Initial	1st Month	2nd Month	3rd Month
Percentage yield %	87.16	87.10	87.04	86.98
Drug content %	69.86	69.84	69.81	69.77
% Entrapment efficiency	92.09	92.05	92.01	91.96
% drug release	83.14	83.15	83.15	83.19

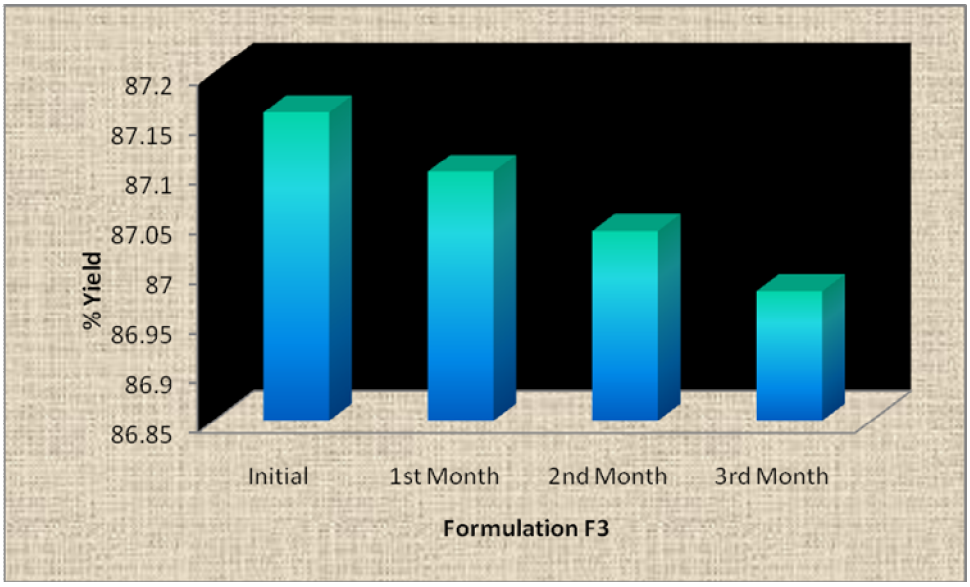


Figure 8.33: comparisons of percentage release profile of before and after stability studies for optimized formulation F3.

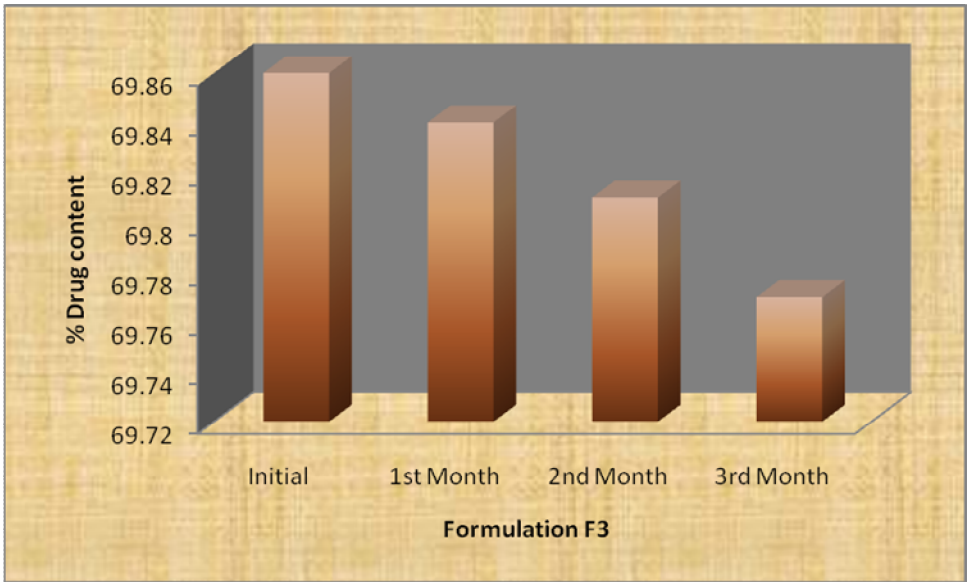


Figure 8.34: comparisons of drug content profile of before and after stability studies for optimized formulation F3.

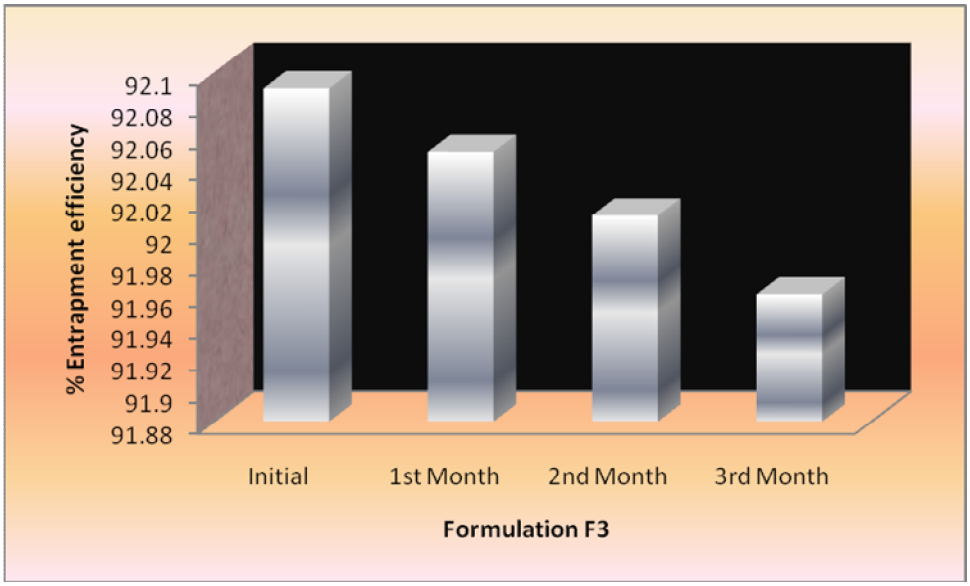


Figure 8.35: comparisons of entrapment efficiency profile of before and after stability studies for optimized formulation F3.

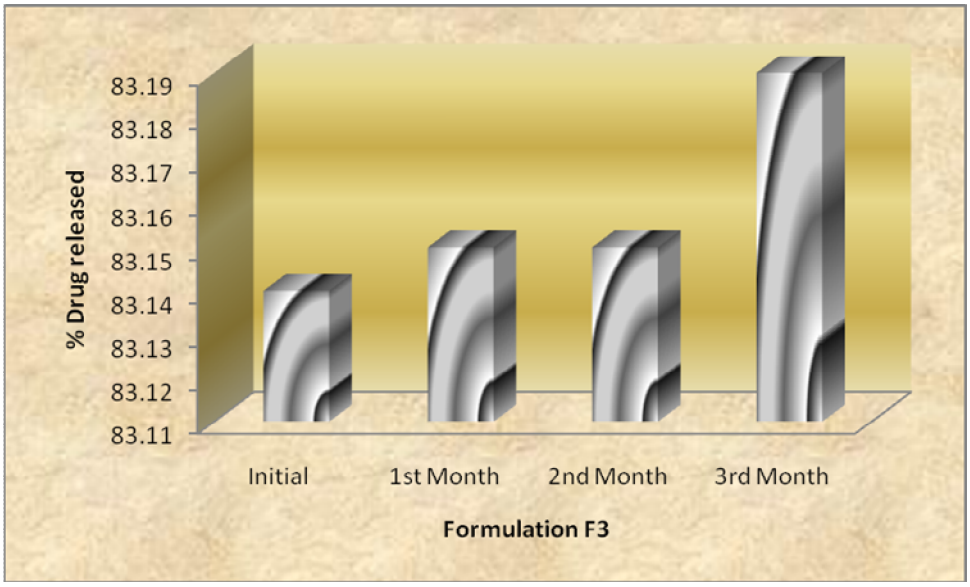


Figure 8.36: Comparison of drug released profile of before and after stability studies for formulation F3

No major variation was found between evaluated parameters before and after stability studies. The sustained release microspheres were showed satisfactory physical stability at 40^{0} C ± 2^{0} C at 75 % ± 5% RH.

SUMMARY
AND
CONCLUSION

9. SUMMARY AND CONCLUSION

The goal of any drug delivery system was to provide the therapeutic amount of drug to the proper site in the body also to achieve and maintain the desired drug concentration in blood. Improving the therapeutic efficacy of existing drugs has been tried by different technologies. One of the effective technologies exiting in recent years of pharmacy is Microspheres.

Sustained release drug delivery system was developed in pharmacy field and drug retention for a prolonged time has been achieved. Hence, it was made an effective attempt to formulate the Sustained release by used Venlafaxine hydrochloride as the model drug.

Venlafaxine hydrochloride was the serotonin-norepinephrine reuptake inhibitors used for the treatment of depression and anxiety disorders. Venlafaxine hydrochloride possess the mean half life of five hours and bioavailability was found to be only 27% and high water solubility Hence, it was chosen as the good candidate for the sustained release microspheres in order to improve the bioavailability and prolong period of drug released.

Sustained microspheres of Venlafaxine Hydrochloride were successfully prepared by solvent evaporation technique and confirmed that it was a best method for preparing microspheres from its higher percentage yield.

The identification of drug was carried out by FTIR spectroscopy and melting point. The physicochemical parameters such as appearance, solubility study and loss on drying were performed by suitable methods. The analytical profile of drug was

evaluated for determination of absorption maximum, development of standard curve and percentage purity of drug.

Compatibility of drug and polymer mixture was done by performing DSC study. It was concluded that there was no interaction between the drug and polymer. Sustained release microspheres were obtained by solvent evaporation method for all the formulations from F1 to F9. Formulations F1, F2 and F3 were prepared Venlafaxine HCL with Chitosan polymer in the ratio of 1: 1, 1: 2 and 1: 3. Similarly, formulations F4, F5 and F6 were prepared Venlafaxine HCL with Sodium alginate polymer in the ratio of 1:1, 1:2 and 1:3; and formulations F7, F8 and F9 were prepared Venlafaxine HCL with Xanthan gum polymer in the ratio of 1: 1, 1: 2 and 1: 3. All formulations were evaluated for the Percentage yield, Entrapment efficiency, Particle size, Scanning electron microscopy, and *in vitro* drug released profile.

On comparing the major criteria in evaluation such as percentage yield, drug content, entrapment efficiency and *In-Vitro* drug released profile, the **formulation F3** was selected as the best formulation, as it showed the percentage yield as 87.16%, drug content as 69.26% and Entrapment efficiency as 92.09% and showed a good sustained release nature in the *In-Vitro* drug released was nearly 83.14% upto 16 hrs. Based on all the above evaluation parameters it was concluded that the formulation F3 was found to be best formulation among the formulations from F1 to F9. The *in-vitro* drug released data was applied to various kinetic models such as zero order kinetics, Higuchi plot, first order kinetics and Peppas plot by predict the drug release kinetics mechanism. The formulation F3 was best fitted with Peppas kinetics and it undergoes fickian diffusion mechanism ($n \geq 0.5$).

Based on the *in vitro* drug released characteristics, entrapment efficiency and t_{25}, t_{50} and t_{90} values, the **formulation F3** was found to be best formulation. By increasing the concentration of polymer, decreased the rate of drug released.

According to stability study it was found that there was no variation in Percentage yield, Entrapment efficiency, and *in vitro* drug released profile of selected formulation F3 at specified period.

The **formulation F3** was concluded best formulation among the formulations were prepared.

FUTURE PROSPECTS

10. FUTURE PROSPECTS

- Once the technology is fully accepted, these systems will probably increase with new pipeline drugs that need enhancement to their bioavailability.
- The sustained release can also be formulated for advanced drug delivery other than oral administration.
- In the present work the sustained microspheres were formulated using natural polymers such as Chitosan, Sodium alginate and Xanthan gum by solvent evaporation method. In this work only physiochemical property, formulation and *in vitro* evaluation of sustained release microspheres of Venlafaxine hydrochloride was done. The study requires attention of researcher to develop sustained drug delivery systems using other synthetic polymers. Furthermore, the study can be extended to evaluate *in-vivo* performance and also *In-vitro-In-vivo* correlation of the microspheres.

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ANNEXURE

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