"OPTIMIZATION OF RESIDUAL SOLVENTS OF PEPTIDE CONTAINING MICROSPHERES"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfilment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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Under the guidance of

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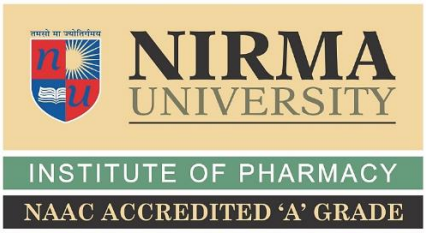

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May 2019

CERTIFICATE

*This is to certify that the dissertation work entitled "**Optimization of Residual Solvents of Peptide Containing Microspheres**" submitted by Ms. Preksha Vinchhi with Regn. No. (17MPH110) in partial fulfilment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my/our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.*

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We wish her better achievements in her future endeavors

Thanking You.

Yours faithfully,

For, Cadila Healthcare Ltd.

Nikul Bhatt
Deputy General Manager
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CERTIFICATE OF ORIGINALITY OF WORK

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I hereby declare that the dissertation entitled "Optimization of Residual Solvents of Peptide Containing Microspheres" is based on the original work carried out by me under the guidance of Dr. Mayur Patel, Assistant Professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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Lastly would like to share an amazing and inspirational quote by Paul J. Meyer-

"Productivity is never an accident.
It is always the result of a commitment
towards excellence, intelligent planning
and focused efforts!"

Regards
Preksha Vinchhi

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

PLGA	Poly (lactic-co-glycolic acid)
PVA	Polyvinyl alcohol
PLA	Polylactic acid
PLLA	Poly-L-lactic acid
GC	Gas chromatography
HPLC	High performance liquid chromatography
ICH	International conference on harmonization
ppm	Parts per million
MDC	Methylene dichloride
BCS	Biopharmaceutical classification system
RLD	Reference listed drug
Tg	Glass transition temperature
$CHCl_3$	Chloroform
HFIP	Hexafluoroisoproanol
EtOAc	Ethyl acetate
GRAS	Generally regarded as safe
FDA	Food and drug administration
CAS	Chemical abstracts service
cSt	Centistokes
SPG	Shirasu porous glass
API	Active pharmaceutical ingredient
ANFD	Agitator nutch filter dryer
WFI	Water for injection
µl	Microlitre
gb	Gram batch
mbar	Milibar
IVR	Invitro release

ABSTRACT

In recent times, proteins are proved to be an imperative class of therapeutically active agents. Owing to limitations in oral route including enzymatic degradation, poor bioavailability, short half lives, and multiple injections are to be given to provide efficacy. Long acting parenteral formulation is beneficial to surmount the limitations of traditional therapy. Polymeric microspheres have received extensive interest in delivery of protein and peptide molecules in recent years. Biodegradable microspheres dwell in a significant position due to various aspects like prevents protein degradation, sustained and desired release profile, improves patient compliance etc. Among various synthetic polymers, poly lactic co glycolic acid (PLGA) loaded microspheres is having a successful market because PLGA is approved by USFDA as biocompatible and biodegradable polymer. Amongst various formulation techniques for microspheres, organic solvents are an innate part of the process involved. Organic solvents when present in unacceptable limit in finished product are highly toxic. Since some of the organic solvents are carcinogenic, teratogenic as well as neurotoxic, residual solvent contect is a safety concern among various regulatory bodies. According to ICH Q3C guideline they are classified in four categories based on their toxicity. The residual solvent content of finished product should comply with the limits according to ICH guideline. In this study the effect of various formulation components, washing and drying techniques on residual solvents is investigated. When microspheres were formulated by multiple emulsion technique high impurity, high residual methylene dichloride and variability in particle size was observed. Phase separation coacervation technique was employed which resulted in a reduced amount of impurity, uniform particle size distribution and reduced residual solvent. Various strategies were applied to reduce the residual solvent. Large amount of washing solvent, increase in washing time, addition rate of coacervationg agent, increase in temperature, changing polymer solvent: coacervating agent ratio were the strategies applied. There was significant impact of polymer solvent: coacervating agent ratio on residual hardening solvent and residual polymer solvent. Various changes in formulation conditions alter the residual solvent and can be beneficial in complying the limits according to guideline.

1.

INTRODUCTION

1.1 INTRODUCTION TO PROTEIN AND PEPTIDE DRUG DELIVERY

Proteins and peptides are progressively more acknowledged as impending leads for development of new therapeutics for many human diseases. [1] Higher target specificity and pharmacological potency are therapeutically beneficial properties of proteins when compared to traditional small molecule drugs. Hence, in recent years they become a very significant category of therapeutic agents.[2] Main drawback of protein drugs is that they are of high molecular weight, prone to degradation in Gastrointestinal tract, low bioavailabilty,enzymatic degradation, poor gastrointestinal absorption.[3] They are sensitive to environmental conditions like pH, temperature, solutes, salt, surfactants as well. Lack of desired pharmacokinetic properties are limiting factor for its clinical use. [4] They are generally administered via injections. Multiple injections are administered due to short in vivo life which creates poor patient compliance. [5]

Sustained-release system provides the potential for minimizing dosing frequency, maximizing the efficacy–dose relationship, eliminating adverse effects, prevent degradation of protein and improves stability. Many controlled release formulations are developed like micelles, liposomes, niosomes, microspheres etc. Among them polymeric microspheres and microcapsules have exhibited noteworthy benefits. [2, 5]

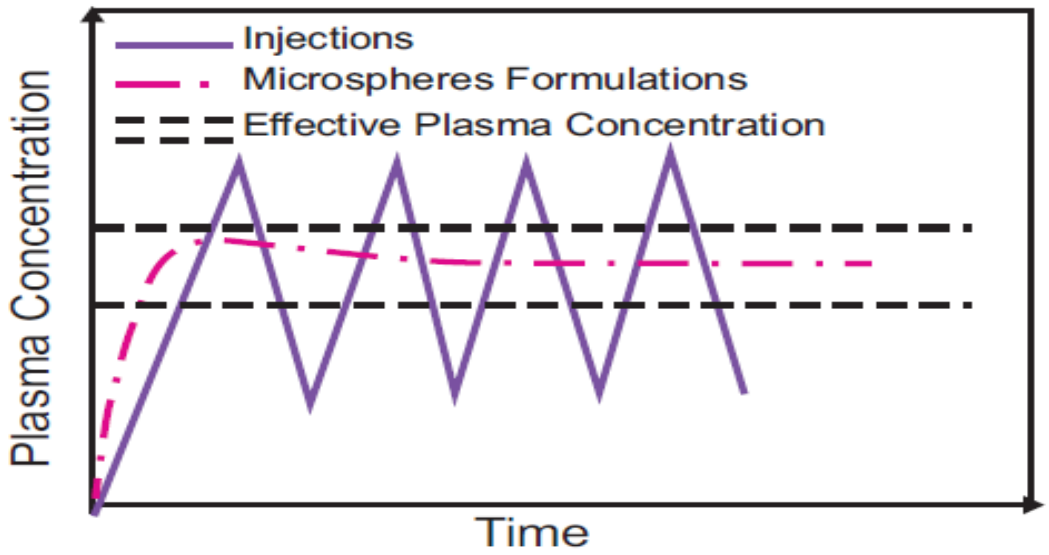


Figure 1.1 : *Comparision of plasma concentration vs. time profile of multiple injections and microspheres/ microcapsules formulation* [2]

1.2 INTRODUCTION TO MICROPARTICLES

Principally, a particle having a diameter of 1-1000 µm are termed as "microparticle". Microcapsules and microspheres are two types of microparticles. Microcapsules are reservoir type and microspheres are matrix type of system. [6] Microcapsules are microparticles having a core enclosed by a material that is markedly different from the core. Core might be solid liquid as well as gas. They are typically free-flowing powders comprising of spherical particles used for different drug delivery.

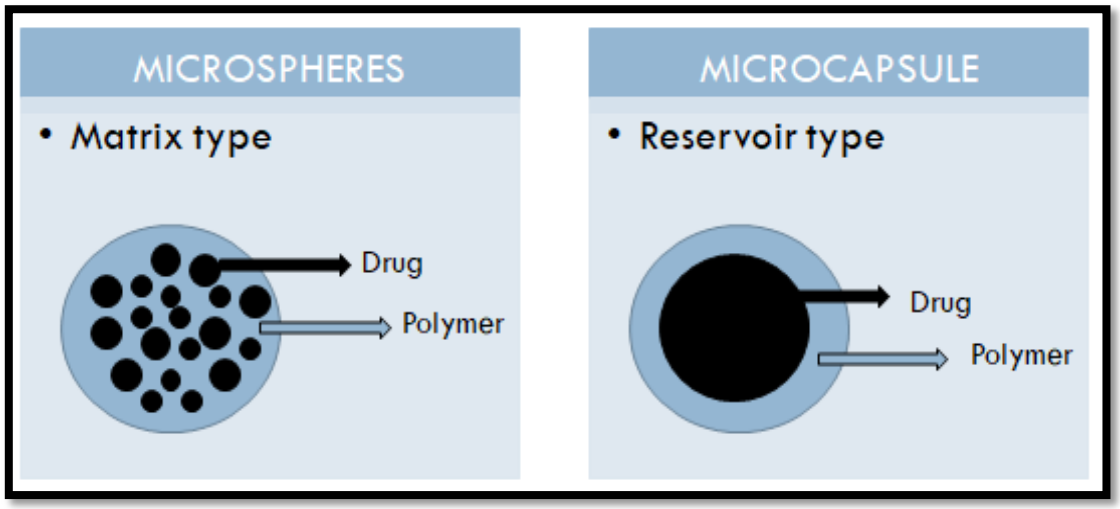


***Figure 1.2** : Difference between microspheres and microcapsules*

There are numerous advantages of using microparticles for delivery of protein and peptides.

(1) Protection of proteins against rapid degradation and clearance.

(2) Multiple injections can be avoided as they provide desired controlled release.

(3) Desired release profile can be achieved by changing particle size and formulation Components.

(4) Improved patient compliance.

(5) Easy administration.

(6) Pre-desired drug release profile. [2]

Some of the limitations are as follows:

(1) Difficulty in scale up.

(2) Variability in size and size distribution.

(3) Poor Invitro-in vivo correlation (IVIVC).

(4) Slow market introduction.

(5) Possibility of protein denaturation because of factors like mechanical stress during manufacturing, contact of protein at oil/water interface causes coagulation.

(6) Retrieval of drug is difficult in case of toxicity or hypersensitivity reaction.

1.2.1 POLYMERIC MICROSPHERES

- Polymeric microspheres can be employed to deliver therapeutic agents in targeted and rate controlled manner. Mechanism of release of active pharmaceutical ingredient is either leaching out of drug from polymer matrix or degradation of polymer. [7]
- Due to disadvantages of non biodegradable polymers like polyetherurethane, polypropylene, polysiloxanes etc, natural and synthetic biodegradable polymers came into picture. Selection of appropriate biodegradable polymer is an important factor in designing controlled release injectable formulation. As they are temporarily going to remain in body they should be safe, biocompatible, nontoxic and should not be carcinogenic, teratogenic, cytotoxic or mutagenic. Along with biocompatibility the properties of polymers like good tensile strength, thermoplasticity, regulated degradation rates promotes their use. Polymers can be categorized as natural and synthetic polymers. [6]

  (1) Natural polymers:

  They have been preferred because of its abundance in nature and biocompatibility.

  They are derived from plants, animals, microbial or marine sources. Majority of them are proteins or polysaccharides. Dextran, alginic acid, chitin, chitosan and starch are widely used in preparation of microspheres.

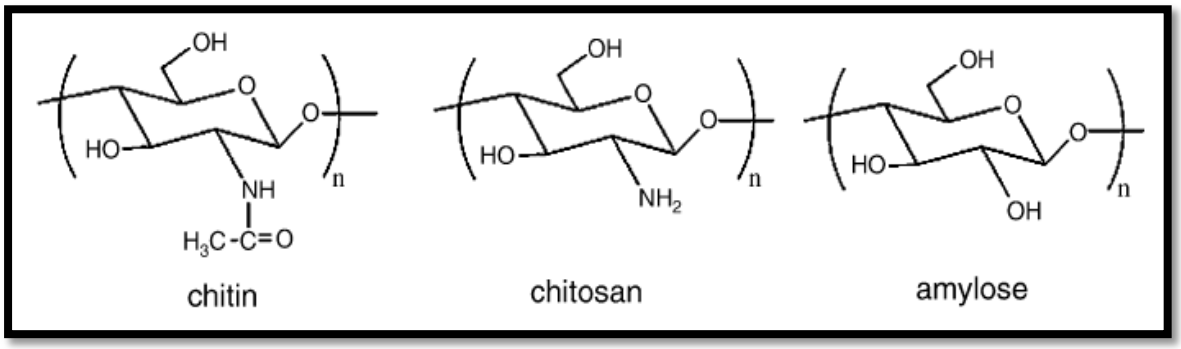


***Figure 1.3** : Structure of natural polymers* [7]

(2) Synthetic polymers:

They are easily reproduced and modified to achieve desired physico-chemical and mechanical properties. There molecular weights can be adjusted as high or low by appropriate reaction conditions. Biodegradability of polymers depends on factors like its structure, molecular weight, form etc. Among various synthetic polymers PLGA has great potential as drug delivery carrier. It is approved by FDA as safe and biocompatible. [6]

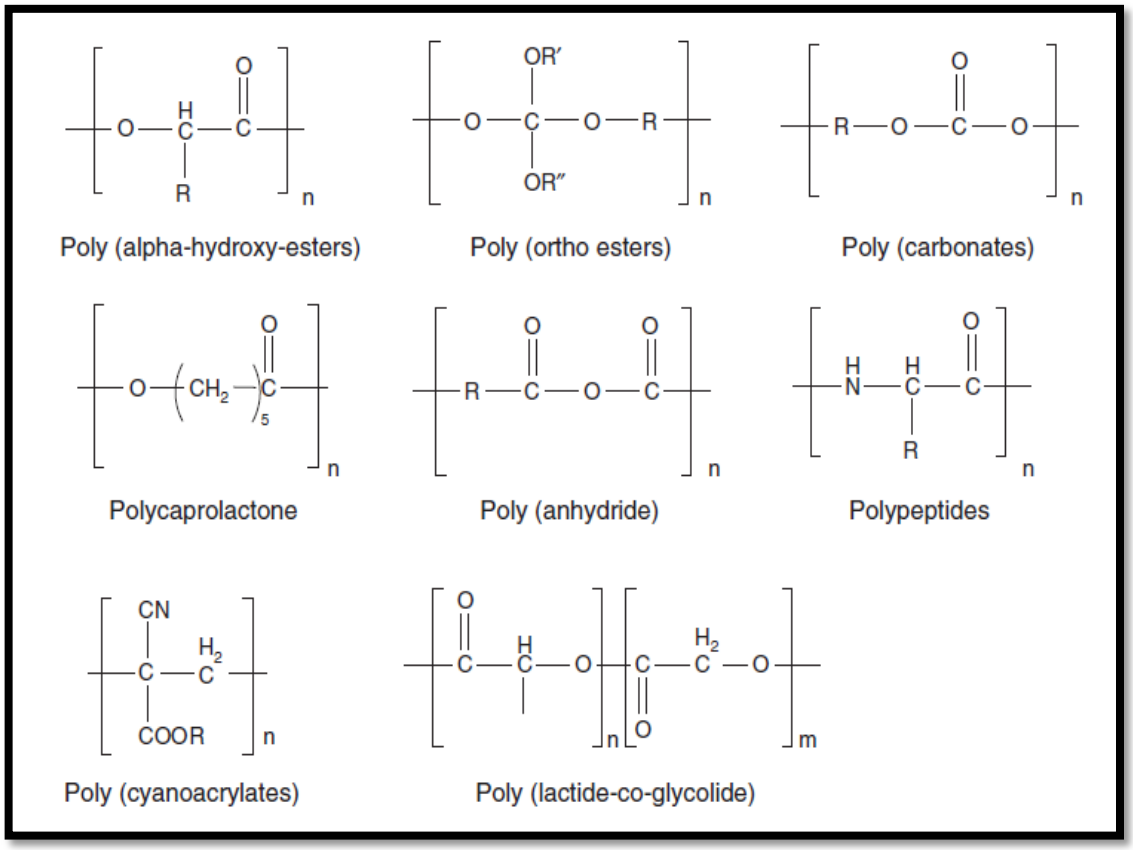


Figure 1.4 *: Structure of synthetic polymers*

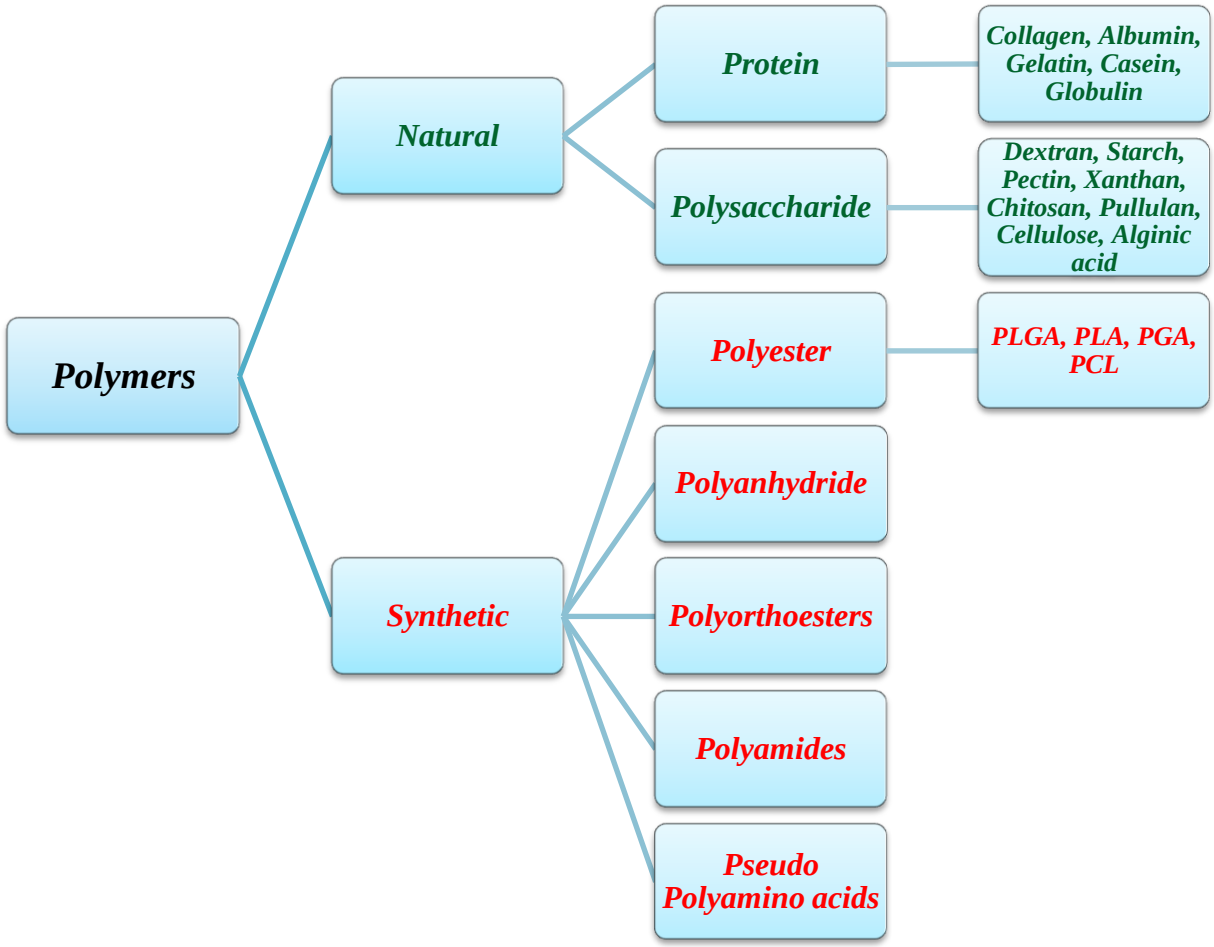


Figure 1.5 *:Classification of polymers*

The degradation mechanism is different for various biodegradable polymers. Following table represents mechanism of degradation due to which controlled delivery is achieved.

Table 1.1: Degradation mechanism of various polymers

Material	Degradation mechanism
Alginate	Alginase, pH, enzymes
Starch	Amylase
Proteins	Enzymes, proteases
Collagen	Collagenase
Polyesters	Ester hydrolysis
Polyanhydrides	Hydrolysis
Polyiminocarbonates	Hydrolysis
Polyamino acids	Enzymes, proteases
Polyphosphazenes	Hydrolysis, dissolution
Polycaprolactones	Hydrolysis

1.2.2 FORMULATION TECHNIQUES

During selection of method of preparation desired properties of the product must be kept in consideration because the method of preparation has great impact on product. The method which provides biological and chemical stability of the encapsulated therapeutic agent, high yield and high encapsulation efficiency, uniform and free flowing microspheres, and reproducible release profile of therapeutic agent should be selected. The method should be reproducible and easily scalable. For parenteral formulation product should be sterile so terminal sterilization or aseptic condition has to be maintained. The toxic residual solvent should be removed in the final step. Following are the widely used method of preparation for polymeric microspheres. [8, 9]

1) Single emulsion method

The Oil in water (o/w) method is used for water in soluble drugs while water in oil (w/o) method is used for peptide like water soluble agents. In the first step drug solution is added to polymer solution. In the second step evaporation or extraction of organic solvent is done. In extraction emulsion is transferred in huge quantity of quenching medium while in evaporation method emulsion is added in water or co solvent under high temperature and reduced pressure. [10] This method is widely used for hydrophobic drugs because addition of emulsion to aqueous phase in second step leads to leaching of drug. O/o emulsion method is used for hydrophobic drugs now a days. There are many disadvantages of this method like low drug loading, low encapsulation efficiency, time consuming process etc. [8]

2) Double emulsion method

Primary emulsion is prepared by adding aqueous phase of water soluble drug in organic phase of polymer. Generally using stirrer or homogenizer. This primary emulsion is added to excess amount of water containing stabilizer (example PVA) to form w/o/w emulsion. Solvent removal is done by evaporation or extraction. Advantages include high encapsulation efficiency, high yield. This process is widely used to develop protein drug delivery system. Characteristics of product depend on stirring speed during emulsification, emulsifier used, polymer: drug ratio, type of polymer used. [8, 9]

3) Phase separation coacervation

This method includes addition of organic non solvent (example silicone oil, light liquid paraffin, vegetable oil etc) which in turn results in phase separation of polymer solution. Therapeutic agent is dissolved in polymer solution and then non solvent is added under continuous stirring. This results in formation of soft coacervates which are hardened by adding it into excess amount of non solvent like heptane, hexane and diethyl ether. Parameters like addition rate of organic non solvent, stirring rate of drug and polymer dispersion, affects the formulation characteristics like size and encapsulation efficiency. [8, 10]

4) Spray drying

Primarily the polymer is dissolved in volatile organic solvents like dichloromethane or acetone and drug is dispersed in it under homogenizer. This mixture is atomized in stream of hot air which results in formation of microspheres. By subjecting these microspheres to vacuum drying residual solvents can be removed. Parameters like inlet temperature, outlet temperature, aspiration rate, flow rate of pump affects the characteristics of microspheres. Advantages like uniform particle size, good reproducibility, ease of scalability, control of release profile of drug are the positive aspects of this method. While there are several disadvantages as well like denaturation of proteins at high temperature, loss of product because of adhesion in inner wall of drier, aggregates due to incomplete removal of solvents. [8, 9, 10]

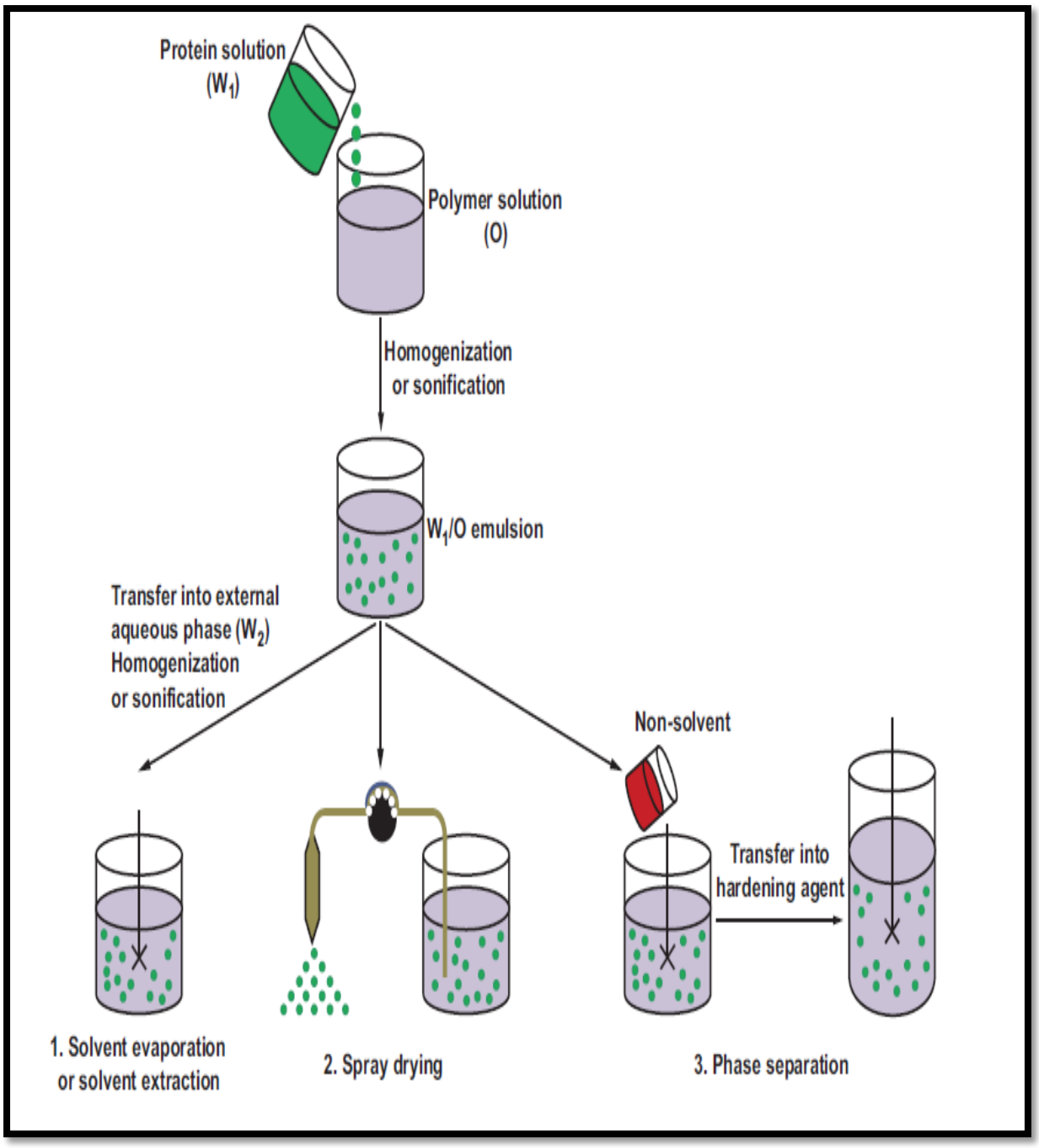


Figure 1.6 *:Various formulation techniques*

Factors influencing protein stability [2]

Above mentioned preparation methods are successful and advantageous for proteins in improving bioavailability but may also lead to disadvantages like protein denaturation, deamidation and aggregation. Factors during manufacturing process which leads to instability are shear forces, heat, water/oil interface etc. Protein might adsorb, unfold, and aggregate at interface during formation of emulsion. Homogenization speed or ultrasound, sonication involves cavitation stress or heat production which leads to loss of protein activity.

In spray drying technique heat produced of physical stress also leads to protein instability.

1.2.3 GENERAL RELEASE MECHANISM FROM BIODEGRADABLE MICROSPHERES [11, 12, 13]

The main mechanism of drug release from biodegradable microspheres is diffusion, dissolution and erosion. Basically degradation is of 2 types' surface degradation and bulk degradation. The mechanism can be understood by flick's diffusion equation, vergnaud and hopfenberg represents erosion controlled system. The degradation is dependent on scission, erosion and enzymatic activity.

(1) Diffusion:

Diffusion controlled system includes penetration of fluid in polymeric spheres, where it creates channels or pores and dissolution of drug occurs. The rate at which fluid penetrates microspheres, drug gets dissolved in dissolution fluid and rate at which it leaks out outlays the drug release rate. It obeys Higuchi's equation.

$$Q = [D/J \ (2A - \varepsilon \ C_S) \ C_S \ t]^{1/2}$$

Where,

Q- Amount of drug diffused per unit area in time t;

D- Diffusion coefficient of solute;

A- Amount of API per unit volume;

Cs- solubility of drug in dissolution fluid;

ε- Wall porosity;

J- Tortuosity of wall capillary system.

(2) Dissolution:

Here the polymer is soluble in dissolution fluid thus release rate depends on rate of polymer dissolution. Polymer dissolution involves solvent diffusion and chain entanglement and in turn leads to loss of bulk material. Thickness of coat influences rate of release.

(3) Erosion:

Erosion of coat in presence of enzymes or particular pH causes drug release. Erosion occurs as surface (heterogeneous) or bulk (homogenous) erosion. Hydrolytic or enzymatic degradation defines that bulk erosion or surface erosion will occur. Surface erosion occurs in case of enzymatic degradation while in case of hydrolytic degradation bulk erosion occurs.

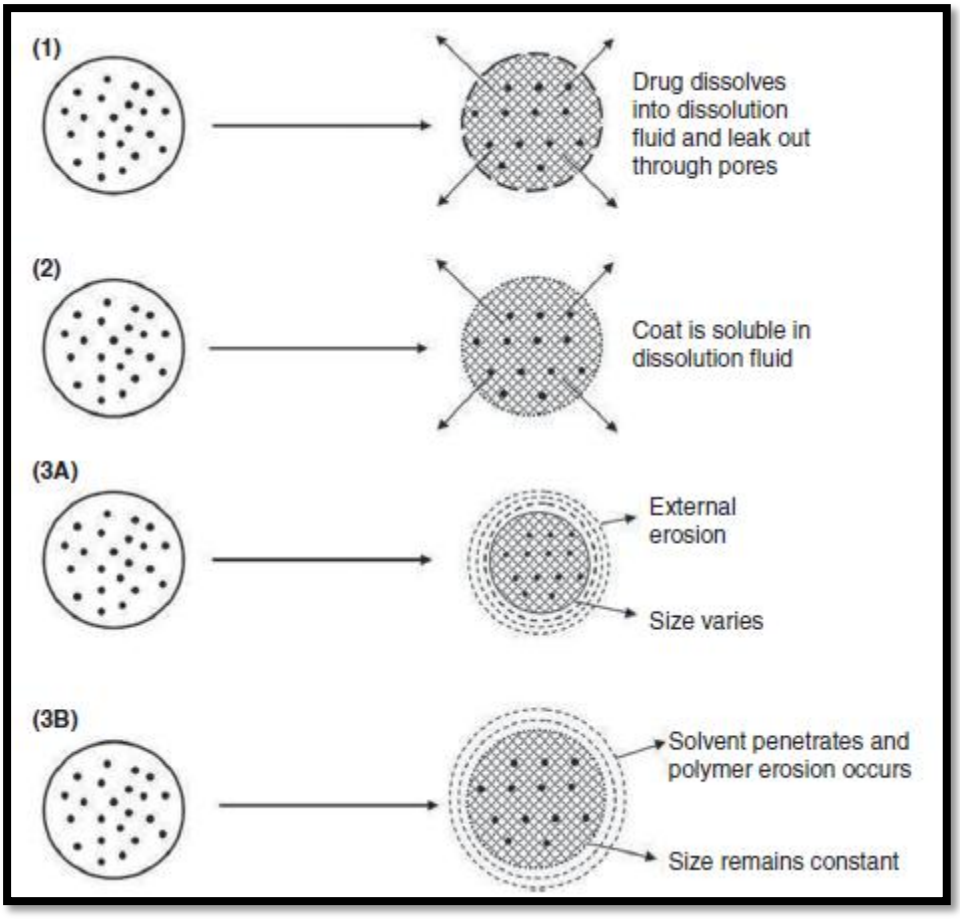


***Figure 1.7** : Mechanism of drug release from biodegradable microspheres*

1.2.4 CHARACTERIZATION TECHNIQUES [14]

1) Particle size and morphologic characterization:

There are numerous methods to characterize size and shape of microspheres.

1) Scanning electron microscopy (SEM)

2) Light microscopy

3) Coulter counter

4) Laser light scattering

5) Fluorescence microscopy

6) Invitro release

1) Scanning electron microscopy (SEM):

SEM is widely used to evaluate the surface morphology, cross section to determine internal structure. Compared to light microscopy SEM gives detailed three dimensional structure and higher resolution. Light scattering principle is used to determine particle size. To achieve accurate results, combination of various methods is used because of some of the limitations by using single method. Many of the factors are dependent on size of microspheres foe example its entrapment efficiency, syringebility, targeting, release rate of drug etc. Any of the change in size leads to change in penetration of water, diffusion of drug and release of the drug from matrix. Smaller the particle size faster is the penetration of diffusion fluid and faster the polymer erosion leading to comparative faster release than larger sized particles. If the microspheres are stored below Tg of polymer than aggregation does not occur.

2) Entrapment efficiency:

Amount of drug entrapped in the microspheres refers to entrapment efficiency. First of all to remove free drug known quantity of microspheres is added to solvent in which drug gets dissolved. Then by lysis of microspheres in appropriate solvent in which polymeric matrix and drug gets dissolved. Drug content is analysed by high performance liquid chromatography or uv spectrophotometric analysis. Entrapment efficiency can be calculated by following formula:

$$\% \text{ Entrapment} = (\text{actual content} / \text{theoretical content}) \times 100$$

3) Residual solvent analysis:

Majority of preparation method involves use of toxic organic solvents. It is necessary to assure that the residual solvent content is within the acceptable quantity. Widely used method to determine amount of residual solvent is by gas chromatography. Various sample introduction techniques are used like static head space injection, direct injection, solid phase micro extraction. For complex samples generally head space injection method is used. Flame inonization detector is also used to determine residual solvent. But it is usually observed that gas chromatography gives appropriate results of sample preparation, detects at lowest limits, specific and accurate results.

4) Flow properties:

To check uniformity and type of flow of the formulation flow properties are to be checked. Tapped density, bulk density, hausners ratio, compressibility index etc are the common methods to evaluate the flow of microspheres. Generally flow properties depend on size and shape, moisture content, chemical composition, temperature and humidity of the formulation.

5) Stability testing:

Stability study is done at different temperature and humidity conditions. It helps in defining the storage condition as well as shelf life of product. Accelerated stability testing is done at extreme conditions for understanding effect in a short term. Intermediate testing is done at 6 months while long term at 12 months. In microspheres physicochemical properties like particle size, drug loading, Tg, molecular weight of polymer etc changes due to high temperature and humidity conditions.

6) Invitro drug release:

USP type 2 and type 4 i.e. rotating paddle and flow through cell are used for release study of microspheres. Various factors like sink conditions, media, drug and polymer properties, etc affects the release profile. The most reliable method currently used for microspheres is USP type 4 apparatus. Some non official methods like dialysis bag method, reverse dialysis bag method are also used but justification is required by regulatory bodies for not using type 4 apparatus.

Characterization of microspheres includes following parameters:

Table 1.2: Characterization techniques of microspheres

Sr.No	Evaluation parameters	Method of determination
A. Physical Characterization		
1.	Particle size and size distribution	Transmission electron microscopy, optical microscopy, laser light scattering, sieve analysis, free-flow electrophoresis.
2.	Surface morphology	Scanning electron microscopy, transmission electron microscopy, freeze fracture, electron microscopy.
3.	Surface charge	Free flow electrophoresis.
4.	Density determination	Multivolume pycnometer or hydrometer.
5.	Isoelectric point	Microelectrophoresis.
B. Chemical characterization		
1.	Drug concentration	Different for different drugs.
2.	Surface degradation	Electron spectroscopy for chemical analysis attenuated total reflectance, Fourier transform infrared spectroscopy.
3.	Surface carboxylic acid and amino acid residue.	Liquid scintillation counter.
4.	pH	PH meter.
5.	Osmolarity	Osmometer.
6.	Residual solvent	Gas chromatography.
7.	Invitro drug release	HPLC analysis.
8.	Entrapment efficiency	Drug found in microsphere/drug loaded*100
C. Biological characterization		
1.	Sterility	Aerobic or anaerobic cultures
2.	Pyrogenicity	Limulus amebocyte lysate test
3.	Animal toxicity	Monitoring survival test, histology and pathology.

1.2.5 MARKETED PRODUCTS

Table1.3: List of marketed products (Polymeric microspheres)

Sr.No.	Drug	Polymer Used	Trade Name	Company	Indication
1.	Leuprolide acetate	PLGA	Luprondepot ®	Takeda - Abott	Prostate cancer
2.	Triptorelin Pamoate	PLGA	Trelstar LA	Watson Pharma	Palliative treatment of advanced prostate cancer
3.	Risperidone	PLGA	Risperdol Consta®	Janssen	Antipsychotic
4.	Buserelin acetate	PLGA	Profact®	Sanofi Aventis	Prostate cancer
5.	Naltrexone	PLGA	Vitrol®	Alkermes	Alcohol dependence opoid dependence
6.	Exenatide	PLGA	Bydureon	Alkermes	Type 2 diabetes
7.	Lanreotide	PLGA	Somatuline	Ipsen Beafour	Acromegaly
8.	Goserelin acetate	PLA	Zoladex®	Astrozeneca	Prostate cancer
9.	Vitamin B12	PLGA	Smart shot B12®	Stock guard	Cobalt deficiency in lambs and calves
10.	Octreotide acetate	PLGA	Sandostatin LAR depot	Novartis	Acromegaly

1.3 INTRODUCTION TO DEPOT FORMULATIONS [15, 16]

Depot formulations are long acting injectable formulations which include formulations like microspheres, liposomes, micelles, dendrimers, nanoparticles etc. Improved patient compliance, prolonged release, controlled release have led to successful market of depot formulations. With the advent of biodegradable and biocompatible polymers depot formulations are widely used. The fact that they are not to be removed from body, they get degraded in readily excreted compounds in body. Additionally encapsulation protects drug from degradation and in turn improves its bioavailability. PLGA and PLA microspheres are effective and important non oral controlled release formulations in market. From 1 week to month release of large molecular weight or small molecules release is achieved by PLA and PLGA. Depot formulations exhibits certain advantages over conventional oral formulations like lesser side effects, avoid multiple injections, improved patient compliance, reduced dose etc. Basically prefilled syringe are available in which the diluent is filled. At the time of administration this diluent is added in microspheres to prepare a suspension and then injected intramuscularly or by subcutaneous route. Generally the kit contains vial containing microspheres, prefilled syringe containing diluent, vial adapter, safety injection needle.

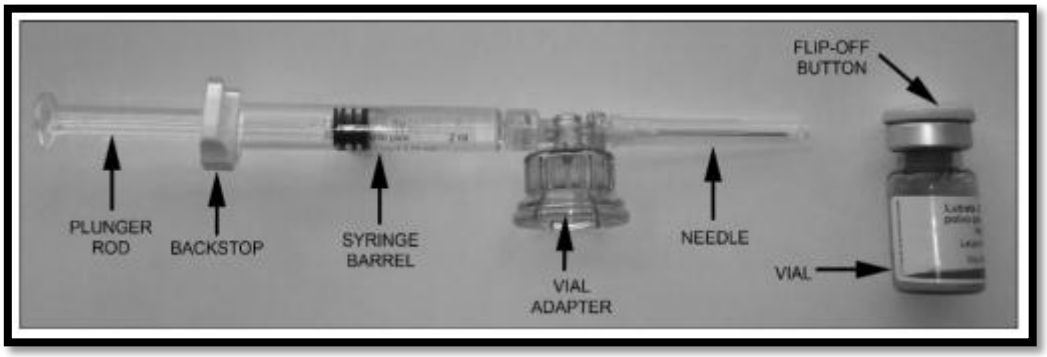


***Figure 1.8** : Schemetic representation of depot formulation kit*

Procedure for administration:

Step 1: Fix vial adapter on vial.

Step 2: Remove prefilled syringe cap and add the diluent in vial.

Step 3: Turn vial upside down and transfer content back in syringe.

Step 4: Immediately administer the suspension. Make sure that sedimentation is not there.

1.4 INTRODUCTION TO RESIDUAL SOLVENTS

Organic volatile impurities are generally regarded as residual solvents. Solvents are generally used in many manufacturing processses. Organic solvents are important in pharmaceutical formulations but their toxicity is a major concern. Various new formulation techniques like emulsion solvent evaporation, phase separation coacervation, supercritical fluid technology, etc newer possibilities of preparing desirable microspheres have developed but these methods still have drawback of high residual solvents on final product which is a major concern for formulation developers. Process parameters includes many solvents like polymer solvent, non solvent which are complicated to remove from final product. Since residual solvent are hazardous they should be within safe limits in final formulation. United States pharmacopoeia (USP, 1990: 22 edition) was first to add residual solvent testing. Then other pharmacopoeia like British, Chinese, and European also included it. International harmonisation was done by ICH in July 1997 Q3C guideline was established on basis of safety and toxicity data of various organic solvents and limits were established. [17] It involves acceptable quantity of residual solvents for safety purpose. To attain good manufacturing practices, quality requirements residual solvents should be removed to maximum possible extent. The solvents which are highly prone to cause intolerable toxicities should be avoided in production until it have strong risk benefit evaluation. Residual solvent are mainly analyzed by gas chromatography technique. Non specific method like loss on drying can be used for safer limits solvents. According to ICH Q3C guideline solvents are divided into 4 categories: [18]

Class 1: Solvents to Be Avoided

These solvents should be avoided because they are proved to have intolerable toxicity and have detrimental environmental effect. If the use is mandatory then its level is restricted between 2-8 ppm.

Class 2: Solvents to Be Limited

They are neurotoxic or teratogenic and carcinogenic so its concentration is limited between 50-3880 ppm.

Class 3: Solvents with Low Toxic Potential

They are less toxic has no major harmful effects. In short term studies no genotoxic effect was determined. Long term carcinogenicity and toxicity studies are not done for much class 3 solvents. Limits of up to 5000ppm are established.

Class 4: Solvents for which No Adequate Toxicological Data was found

1.4: Limits of Residual Solvents

Solvent	Concentration Limit (ppm)
Class 1 : Solvents that should be avoided	
Benzene	2
Carbon tetrachloride	4
1,2-Dichloroethane	5
1,1-Dichloroethene	8
1,1,1-Trichloroethane	1500
Class 2 : Solvents to be limited	
Acetonitrile	410
Chlorobenzene	360
Chloroform	60
Cumene	70
Cyclohexane	3880
1,2-Dichloroethene	1870
Dichloromethane	600
1,2-Dimethoxyethane	100
N,N-Dimethylacetamide	1090
N,N-Dimethylformamide	880
1,4-Dioxane	380

2-Ethoxyethanol	160
Ethyleneglycol	310
Formamide	220
Hexane	290
Methanol	3000
2-Methoxyethanol	50
Methylbutyl ketone	50
Methylcyclohexane	1180
Methylisobutylketone	4500
N-Methylpyrrolidone	530
Nitromethane	50
Pyridine	200
Sulfolane	160
Tetrahydrofuran	720
Tetralin	100
Toluene	890
1,1,2-Trichloroethene	80
Xylene	2170

Class 3: Solvents with low toxic potential (corresponding to 5000 ppm)	
Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone

tert-Butylmethyl ether	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Triethylamine
Class 4: Solvents for which No Adequate toxicological data was found	
1,1-Diethoxypropane	Methylisopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

1.5 INTRODUCION TO DRUG PROFILE

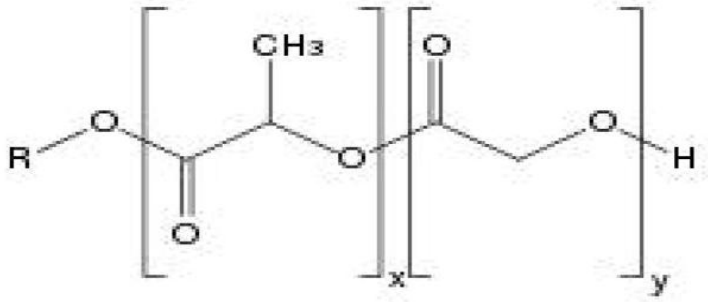
Table 1.5: Drug Profile

1.	**Physiological properties**	Appearance	:	White powder
		Solubility	:	Soluble in water, acetone, methanol, ethyl acetate
		Log P	:	1
		BCS class	:	Class III (high solubility, low permeability)
		Storage condition	:	-20ºC
		Melting point	:	153-156 ºC
2.	**Indication**	• To treat acromegaly and reduce side effects of chemotherapy in cancer patients. • In treatment of patients symptoms with metastatic carcinoid tumors like diarrhoea and flushing, watery diarrhoea.		
3.	**Mechanism of action**	• Inhibits growth hormone such as insulin and glucagon, suppresses leutenizing hormone, and inhibits serotonin, vasoactive intestinal peptide, gastrin, motilin release. • It is a somatostatin receptor agonist.		
4.	**Adverse effect**	Gall stones, nausea, flatulence, vomiting, fatigue, dizziness, and headache.		
5.	**Pharmacokinetics**	Absorption	:	Absorbs rapidly after injection
		Distribution	:	Binds to plasma albumin and lipoprotein
		Metabolism	:	Half-life : 1.7-1.9 hours
		Excretion	:	35% unchanged in urine

1.6 INTRODUCTION TO EXCIPIENTS

PLGA [19, 20]

Table1.6: Excipient profile - PLGA

1.	**Structure**	
2.	**Description**	• Polyester PLGA is co-polymer of: 1) Polylactic acid (PLA) 2) Polyglycolic acid (PGA) • Ratio of PLA: PGA and molecular weight controls the release rate. • PLA is low hydrophilic compared to PGA. • It is biocompatible and biodegradable

3. Chemical Names and CAS registry no. of PLGA

Generic name	Lac-tide %	Glyco-lide %	Syn	Trade name	Manufacturer	CAS No.
Poly(L-lactide-co-glycolide	85	15	-	Resomer LG 855 S,857 S.	Boehringer ingelheim	30846-39-0
Poly(L-lactide-co-glycolide	82	18	-	Resomer LG 824 S	Boehringer ingelheim	30846-39-0
Poly(L-lactide-co-glycolide	10	90	-	Resomer GL 903	Boehringer ingelheim	30846-39-0
Poly(DL-lactide-co-glycolide	85	15	PLGA (85:15)	Lactel 85:15 DL-PLG	Durect	30846-39-0 26780-50-7
				8515 DLG 7E	lakeshore	
				Resomer RG 858 S	BI	
Poly(DL-lactide-co-glycolide	75	25	PLGA (75:25)	Lactel 75:25 DL-PLG	Durect	26780-50-7
				Purasorb PDLG 7502A,7502,7507	Purac	
				Resomer RG 752 H,752 S, 753 S, 755 S, 756 S.	BI	
				7525 DLG 7 E	lakeshore	
Poly(DL-lactide-co-glycolide	65	35	PLGA (65:35)	Lactel 65:35 DL-PLG	Durect	26780-50-7
				Resomer RG 653 H	BI	
				6535 DLG 7E	lakeshore	

	Poly(DL-lactide-co-glycolide	50	50	PLGA (50:50)	Lactel 50:50 DL PLG	Durect	26780-50-7
					5050 DLG 7E,5E,1A,2A,3A,4A,4.5A	lakeshore	
					Purasorb PDLG 5002A,5002,5004A,5004,5010	Purac	

4. Physical and mechanical properties

Properties	50/50 DL-PLG	65/35 DL-PLG	75/25 DL-PLG	85/15 DL-PLG
Molecular weight	40,000- 1,00,000	40,000- 1,00,000	40,000- 1,00,000	40,000- 1,00,000
Inherent viscosity [dl/g]	0.5-0.8[(b)]	0.5-0.8[(b)]	0.5-0.8[(b)]	0.5-0.8[(b)]
Melting point [ºC]	45-50	45-50	50-55	50-55
Glass transition temperature[ºC]	Amorphous	Amorphous	Amorphous	Amorphous
Color	White to light gold	White to light gold	White to light gold	White to light gold
Solubility [at 5%w/w][(d)]	$MeCl_2$, THF, EtOAc, C_3H_6O, $CHCl_3$, HFIP	$MeCl_2$, THF, EtOAc, C_3H_6O, $CHCl_3$, HFIP	$MeCl_2$, THF, EtOAc, C_3H_6O, $CHCl_3$, HFIP	$MeCl_2$, THF, EtOAc, C_3H_6O, $CHCl_3$, HFIP
Approx. Resorption [months]	1-2	3-4	4-5	5-6
Specific gravity	1.34	1.30	1.30	1.27
Tensile strength [psi]	6000-8000	6000-8000	6000-8000	6000-8000
Elongation [%]	3-10	3-10	3-10	3-10
Modulus [psi]	$2\text{-}4 \times 10^5$	$2\text{-}4 \times 10^5$	$2\text{-}4 \times 10^5$	$2\text{-}4 \times 10^5$

Note: **DL-PLG : DL- poly(lactide-co-glycolide)**

(a) Specifications obtained from Durect.
(b) [HFIP] hexafluoroisopropanol.
(c) [$CHCl_3$] chloroform
(d) Partial listing only: [$MeCl_2$] methylene chloride; [THF] tetrahydrofuran; [EtOAc] ethyl acetate; [HFIP] hexafluoroisopropano; [C_3H_6O] acetone.

5. Glass transition temperature and melting point of selected biodegradable polymers

Polymer	Composition	Glass transition temperature [ºC]	Melting point [ºC]
9010 G/L	Poly(L-lactide-co-glycolide) (10:90)	35-45	180-200
8515 DL/G	Poly(DL-lactide-co-glycolide) (85:15)	50-55	Amorphous
7515 DL/G	Poly(DL-lactide-co-glycolide) (75:25)	48-35	Amorphous
6535 DL/G	Poly(DL-lactide-co-glycolide) (65:35)	45-50	Amorphous
5050 DL/G	Poly(DL-lactide-co-glycolide) (50:50)	43-48	Amorphous

6. Solubility of various grades of PLGA

Polymer	Solvent						
	Ethyl acetate	Methylene chloride	Chloroform	Acetone	Dimethyl formamaide	Tetrahydro furan	Hexafluoro-isopropanol
PLGA 85:15	S	S	S	S	S	S	S
PLGA 75:25	S	S	S	S	S	S	S
PLGA 65:35	S	S	S	S	S	S	S
PLGA 50:50	SS	S	S	SS	S	SS	S

	Note: S= Soluble; SS= Slightly Soluble	
7.	**Storage condition**	It is easily susceptible to hydrolysis so it should be protected from moisture. Hence stored in airtight container and below 0ºC temperature.
8.	**Regulatory status**	GRAS listed. Approved by FDA to be used in medical products and medical devices.
9.	**Application**	Used in • Injectable delivery system like microspheres, microcapsules, nanoparticles; • Implantable delivery system like cylinders, rods, films, pellets, beads, etc.

Silicone oil 1000 CST [anti-solvent] [20]

Table1.7: Excipient profile - Silicone oil

1.	**CAS No.**	63148-62-9
2.	**Molecular formula**	$C_6H_{18}OSi_2$
3.	**Molecular weight**	162.378
4.	**Composition**	Polydimethylsiloxane polymers
5.	**Appearance**	Colourless to slightly yellowish liquid
6.	**Boiling point**	> 65 ºC
7.	**Viscosity**	1000cst at 25 ºC
8.	**Refractive index**	1.4013
9.	**Specific gravity**	0.973
10.	**Storage condition**	Store at cool, dry, dark location in sealed container at or below 25 ºC temperature
11.	**Application**	Used as cosmetic ingredient, lubricant, defamers, topical formulation, etc.

2.

AIM AND OBJECTIVE

AIM AND OBJECTIVE

- **To optimize residual solvent content within ICH limits.**
- **To evaluate the impact of different manufacturing process parameters on residual solvent levels in the peptide containing microspheres.**

Polymeric microspheres are widely used for delivering various therapeutically important molecules due to controlled release, biocompatibility, less dosing frequency, high bioavailability and improved patient compliance. There are various manufacturing techniques for the preparation of microspheres. Organic solvents are the inherent part in the formulation techniques of microspheres. They are exceedingly toxic if present in intolerable limit in the finished product. While some of the organic solvents are neurotoxic, carcinogenic and teratogenic their concentration needs to be optimized in final pharmaceutical product. According to ICH Q3C guideline 69 organic solvents are classified in four categories on the basis of their toxicity. The amount of residual solvent of the final product should comply the limits specified in the guideline. The formulation components, process parameters, washing solvents and drying conditions influences on the residual solvent content in microspheres. The work represents various aspects influencing residual solvent content, parameters for lowering the residual solvent content which can be beneficial to fulfil the regulatory limits and manufacturing a therapeutically safe and effective product.

3.

LITERATURE REVIEW

3.1 LITERATURE REVIEW ON PLGA MICROSPHERES

Dunne et al investigated that for release of drug polymer degradation plays a critical role. To interpret the mechanism governing release in-vitro degradation behavior is to be analyzed. Effect of particle characterization, processing conditions and release media temperature on degradation of PLGA were studied. Three size range <50, <20 and <1 μm diameter particles were prepared. The larger particles degraded faster. The degradation path of large particle is larger due to which auto catalytic degradation might be occurred. With increase in temperature rate of polymer degradation was found to be increasing. (21)

Duncan et al demonstrated that protein encapsulation is known to cause insoluble aggregates in PLGA microspheres by emulsion technique. For α helical protein partial structure loss was observed while in β sandwich protein tremendous loss occurred. Addition of sucrose was not beneficial to prevent loss. Sucrose is added as lyoprotectant and it does not show any effects which concludes that changes seen are from emulsification. It was concluded that emulsion denatured insoluble protein represents the loss. (22)

Keels et al studied the effect of various processing and sterilization parameters which influences the physicochemical properties of PLGA and indirectly affect the release rate of API due to hydrolysis of PLGA. Effect of comonomer ratio, gamma radiation, supercritical carbon dioxide and temperature are investigated. SEM, gel chromatography, ATR-FTIR, DSC are used for analysis. The results described that on increase of lactide due to increase in hydrobhobicity which leads to decrease in hydrolysis rate. Due to gamma radiation molecular weight of polymer decreases by chain scission mechanism and thus increase in hydrolysis rate is observed. Supercritical carbon dioxide increases the pores of microspheres thus increase in hydrolysis rate occurs. Thus influence of many factors can change the desired release of API. (23)

Gasmi et al studied the effect of swelling on release rate from PLGA microspheres. Ketoprofen loaded microspheres were fabricated using O/W emulsion solvent evaporation method. Drug loading ranged from 0.6 – 45.2%. Size of microsphere was kept constant. Lower dose of ketoprofen showed three phases of release including initial burst release, second constant release and third phase rapid release. For high drug loading biphasic or monophasic release was observed. At lower drug loading swelling was seen in third phase of release. As soon as molecular weight of PLGA reached 20kDa swelling started. The reason of rapid release in third phase might be penetration of more amount of water in particles. In second phase the chain entanglement might be high which leads to slow drug release. [24]

3.2 LITERATURE REVIEW ON PROTEIN AND PEPTIDE MICROSPHERES

Chen et al developed a microsphere formulation of octreotide acetate which is incorporated in PLGA using double emulsification method. Brust release was decreased and loading capacity increased. Wagner nelson method was applied to co relate invitro and in vivo release profile. Result revealed close release profiles. Accelerated release method was developed by considering fact that pH effects degradation rate of most of biodegradable polymers. By optimization of variables a rapid release method was developed. [25]

Qi et al formulated exenatide loaded PLGA microspheres can be beneficial to overcome the limitations of exenitide like short half life. The conventional method of preparation has limitations like broad site range, poor drug loading, etc. Here shirasu porous glass (SPG) technique was used to develop uniform sized particles. Uniform microspheres of 20 μm size were obtained. Experimental trials showed that using ultrasonication to prepare primary emulsion, high entrapment efficiency was obtained but poor invitro release was there. By homogenization with optimized speed and time proper entrapment efficiency as well as invitro release was observed. It was proved that stability of exenatide was not hindered. [26]

Su et al described preparation and evaluation of resperidone loaded PLGA microspheres of lower molecular weight. It was compared to marketed formulation Resperidal Consta. Oil in water emulsion solvent evaporation method was used for preparation. It was observed that encapsulation efficiency was highly dependent on molecular weight of polymer, intrinsic viscosity, terminal group etc. Using 5050 4A PLGA the release order followed zero order kinetics for 14 days and microspheres were smooth and spherical in shape with high entrapment efficiency. This approach can be a great potential compared to marketed formulation.

3.3 LITERATURE REVIEW ON RESIDUAL SOLVENTS IN POLYMERIC MICROSPHERES

Herberger et al investigated a procedure for minimizing residual solvents in poly (lactide-co-glycolide) (PLGA)–darbepoetin alfa microspheres prepared by spray drying was done by using carbon dioxide as extraction solvent. Two phases of carbon dioxide i.e. liquid and gas were selected for reduction in residual solvent. Unfavourable effects on protein integrity and morphology of microspheres was observed using liquid carbon dioxide. Various pressure conditions were applied and checked for prevention of agglomeration of microspheres. At 100 psig minimum agglomeration and low residual solvent was obtained. With higher carbon dioxide pressures agglomeration of particles increased. The pressure below which agglomeration didn't occur was checked and extraction cycles were developed. These particles were evaluated and residual solvent reduced up to 200 ppm with no major change in morphology and integrity of protein. [28]

Jang et al studied isopropyl based plga microspheres advantageous then methylene chloride because of its nonhalogenated and safe properties. Along with numerous drawbacks linked with solvent evaporation process, the incorporation of halogenated organic solvent has issues regarding safety and environmental hazard. Methylene chloride is widely used solvent but has carcinogenic properties and is environmental hazard. There is a demand of developing microspheres using nonhalogenated organic solvents. This article is focused on employing isopropyl formate to develop plga based microspheres. It was reacted with ammonia to provide water soluble product. This study for the first time proposed isopropyl formate as dispersed solvent. Astonishingly, isopropyl formate had comparable evaporating tendency to

methylene chloride. Optimization of this solvent evaporation technique was followed by encapsulation of progesterone into microspheres. Encapsulation efficiency was 75 to 95 %. Progesterone amount had an influence on morphology of microspheres. Gas chromatography analysis described 1.8 to 4 % residual isopropyl formate. It can be concluded that isopropyl formate has an edge over halogenated organic solvents. (29)

Zielhuis et al studied that PLLA microspheres preparation includes use of organic solvents like chloroform. For preparation of Ho-PLLA-MS also chloroform is widely used. Conversely, they are difficult to remove and according to ICH limit of chloroform is 60 ppm. At present methods employed to minimize residual solvent includes extraction with supercritical carbon dioxide, drying at increased temperature and reduced pressure. It is recognized that chloroform is prone to decompose with high energy radiation. This concept was utilized to reduce residual chloroform. But it was to be studied as well that radiolysis didn't lead to any harmful component in microspheres which are hazardous to patient. It is reported that UV and gamma radiation leads to end product chloride which is safe but phosgene which is toxic. In this article it is studied that removal of residual chloroform with neutron or gamma irradiation occurs or not. In neutron irradiation rough surface was observed while gamma radiation didn't result in surface changes. Phosgene was not detected which shows safety for patient. The level of chloroform significantly reduced. (30)

3.4 PATENTS

Patent No.: US 8,187.672 B2

Title of patent: RESIDUAL SOLVENT EXTRACTION METHOD AND MICROPARTICLES PRODUCED THEREBY

Summary: this patent describes preparation methods having less residual solvent levels. To lessen the amount of residual solvent non washing aqueous system are used. Ethanol or mixture of heptane and ethanol are used. Within the hardening solvent also washing solvent can be added to avoid post hardening washing step. It also includes newer improved methods for microsphere formation. In further aspect formulation useful for therapy or diagnosis can be prepared. The method involves dissolution of peptide in aqueous phase and dissolution of polymer in organic halogenated solvent. To this blend coacervating agent is added. The extraction washing solvent of polymer solvent should be solvent for halogenated solvent but

non solvent for polymer. Then further washing includes 100% ethanol or heptane and ethanol mixture.

Patent No.: US 8,617,613 B2

Title of patent: POLYMER-BASED SUSTAINED RELEASE DEVICE

Summary: this patent includes composition of long acing peptide formulations and their method of preparation for sustained release. The sustained release is achieved by biodegradable polymer in which peptide and sugar is dispersed. Optimization of silicone oil to polymer ratio can be helpful to achieve low pore volume. The embedded peptide is an antidiabetic agent. Mannitol/sucrose is the preferred sugar. The peptide is 3%w/w and sucrose is 2%w/w in concentration. The preferred polymer is PLGA. Method includes dissolution of peptide and sugar in water and polymer in organic phase forming w/o emulsion. Hardening of embryonic microspheres is done by addition into a quenching solvent thereby washing and drying. Silicone oil: polymer solvent ratio was 1.5:1. Polymer concentration is 10%w/v or less. Route of administration can be intramuscular, intracranial, intradermal, intraperitoneal, intranasal, intrapulmonary, and intranasal. The formulation improves bioavailability of peptide and minimizes the stability concerns and chemical interactions of peptide.

Patent No.: US 8,728,528 B2

Title of patent: PROCESS FOR PREPARING MCROPARTICLES HAVING ALOW RESIDUAL SOLVENT VOLUME

Summary: The patent discloses various emulsion techniques to form microspheres utilizing reduced volume of water. The disclosed process results in microspheres having low residual solvent volume. The particles have residual solvent less than 3% by weight. Extraction ratios are predetermined to produce low residual solvent microspheres. Use of copolymers, homopolymers is used that results in lower residual solvent. Extraction solvent value calculated is helpful to provide minimal amount of water required for extracting solvent amount.

Patent No. : US 9, 943, 483 B2

Title of patent: PREPARATION OF PEPTIDE LOADED PLGA MICROSPHERES WITH CONTROLLED RELEASE CHARACTERISTICS

Summary: The patent involves a novel method for preparation of peptide containing long acting injectable microspheres. It describes single or double emulsion technique for preparation. The polymer use preferably is PLGA. The peptide drug particularly is octreotide. Other drugs are goserelin, exenatide, liraglutide and leuporelin. The process is done at low temperature in which rise in temperature is during evaporation which solidifies microspheres. Collection is by seiving, washing and drying under vacuum. Peptide is dissolved in aqueous phase /organic solvent which are miscible in water. Polymer is added in organic phase. Addition of water and oil phase is done. Evaporation of any one phase is done at controlled temperature to form microspheres. Temperature range is 15-20°C, preferably 20°C. Rate of temperature increase during drying is 0.1 °C/min or 1°C/min.

Patent no: US 2016/0120935 A1

Title of patent: SUSTANNED RELEASE FORMULATION COMPRISING OCTREOTIDE AND TWO OR MORE POLYACTDE-CO-GLYCOLIDE POLYMERS

Summary: the patent relates to formulation of sustained release injectable by two or more PLGAs in which the active moiety is octreotide or its salt. The formulation is for treatment of acromegaly, diarrhoea associated with cancer, VIP tumors. This invention overcomes the multiple injections required for treatment. Particle size influences the release profile. Drug is crystalline or amorphous form. Blends of PLGA are used in this formulation. The formulation provides sustained release over period of 3 months to 6 months. By using 2 grades of plga plasma level fluctuation can be reduced. The drug content is 15-20%.

4.

EXPERIMENTAL WORK

4.1 MATERIAL

Table4.1: List of materials

Sr. No.	Materials used	Company Name
1	API	Bachem
2	PLGA55:45 GLU	RESOMER® Select
3	Dichloromethane	Finar India
4	Methanol	Finar India
5	Heptanes	Finar India
6	Span 80	SEPPIC Germany
7	Potassium phosphate	Finar India
8	Ethanol	Merck India
9	Silicone oil 1000 CS	Dowcorning India

4.2 EQUIPMENTS

Table 4.2: List of equipments

Sr. No.	Equipments used	Company Name
1	Magnetic Stirrers	IKAC MagHS7
2	Overhead Stirrer	IKA Euro star
3	Peristaltic Pump	Electro lab
		Master flex Easy Load
4	Pocket Filter	Rosenmund
5	Rotating Evaporator	Heidolph
6	Microscope	Nikon Eclipse
7	ANFD (Agitator nutch filter dryer)	Rosenmund

4.3 EXPERIMENTAL TRIALS

To begin with, it was decided to employ the following manufacturing method for the preparation of peptide microspheres

1) Preference 1: Multiple emulsification method
2) Preference 2: Phase separation coacervation
3) Not preferred: Spray drying (Because of known thermal stability concerns/ issues/ facts.)

❖ Batch 1: Preparation method- Double emulsification method:

Formula:

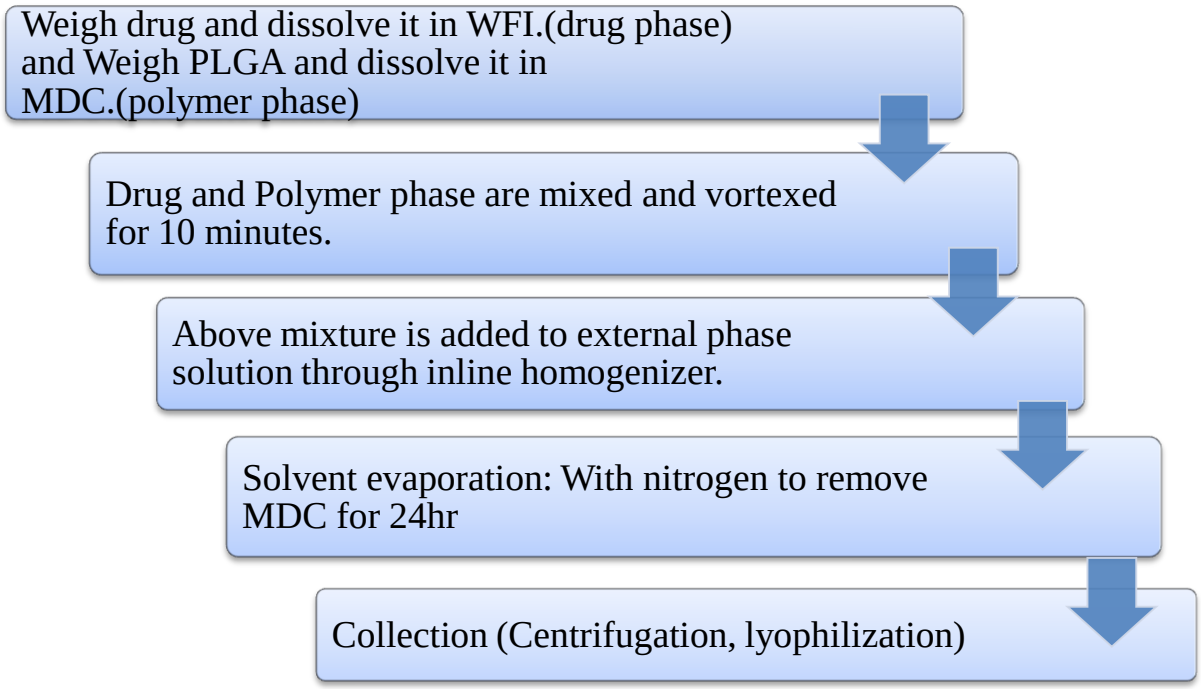
Batch size: 3g

Table 4.3: Formula for Double emulsification method

Content	Standard quantity	Actual quantity
1. Drug Phase		
Drug	5.6%	0.168 g
WFI	125 µl/gb	375µl/gb
2. Polymer phase		
PLGA	94.4%	2.832 g
Methylene Chloride	3g/gb	9 g
3. External phase		
Polyvinyl alcohol	0.05%	0.015 g
Nacl	2%	0.06 g
KH_2PO_4	8.16 g/ gb	24.48 g
WFI	300ml / gb	900 ml

* **gb: gram batch**

Method:



❖ Batch 2: Preparation method- Phase separation coacervation:

Formula:

Batch size: 3.5g

Table 4.4: Formula for Phase separation coacervation method

Content	Standard Quantity	Actual Quantity
Phase 1: Drug		
Drug	5.6%	0.243 g
Methanol	500 µl/gb	1.384 g
Phase 2: Polymer Phase		
PLGA 55:45	94.4%	0.243g
Methylene chloride	15ml/gb	69.56g
Phase 3: Antisolvent		
Silicon oil(1000 cs)	15 ml/gb	51.08g
Phase 4: Hardening Phase		
N-Heptane	400ml/gb	950.6g

Span 80	6ml/gb	19.74g
Phosphate buffer PH 4	100ml/gb	350ml
Silicone oil (350cs)	40ml/gb	135.94g
Phase 5: Washing solution		
Ethanol	50ml/gb	175ml
Span 80	0.07ml/gb	0.23g

Method:

- Step 1: Hardening phase is prepared and stirred under overhead stirrer at 450rpm at 14°C.
- Step 2: Meanwhile weigh drug and add methanol by manual vortexing and kept on stirrer for 30 minutes at 14°C.
- Step 3: Weigh PLGA and add methylene chloride by manual vortexing and kept on stirrer for 30 minutes at 14°C.
- Step 4: Add polymer phase to drug phase by manual vortexing and kept on stirrer for 30 minutes at 14°C.
- Step 5: Add antisolvent with flowrate of 8ml/min and stir for 40 minutes.
- Step 6: Add the above solution to the hardening phase and stir for 90 minutes.
- Step 7: Microspheres are filtered and added to washing solution ethanol+ span 80 and stirred for 90 minutes and then filtered and added to N heptane and stirred for 90 minutes.
- Step 8: Drying is done in rotating evaporator at 39°C temp 60 rpm and 5-10 mbar pressure for 24 hours.

➢ Phase separation coacervation method was selected over double emulsification method. Various strategies were applied to reduce the residual solvent level of employed methanol, ethanol, methylene chloride and N heptane.

4.4 STRATEGIES APPLIED TO OPTIMIZE RESIDUAL SOLVENT

Table 2: Strategies applied to reduce residual solvents

Batch No.	Process parameter
Strategy 1 : Different ratio of washing solvents	
3	Ethanol:Heptane (1:1)
4	Ethanol:Heptane (3:1)
Strategy 2 : Change in manufacturing steps	
5	Hardening and ethanol wash in single step
6	Hardening and ethanol washing as single step without buffer
Strategy 3 : Large volume of washing solvent	
7	75ml/gb washing solvent
8	100ml/gb washing solvent
Strategy 4: High temperature during manufacturing	
9	Washing at 25°C
10	Washing at 30°C
11	Hardening and washing at 25 °C
12	Phase separation, hardening and washing at 25°C
Strategy 5: Effect of addition rate of coacervation agent	
13	Direct addition
14	16ml/min
15	8ml/min
Strategy 6: Stirring time after addition of silicone oil	
16	2-5 min stirring
17	40 min stirring
Strategy 7: MDC:Silicone oil ratio impact (high MDC:low silicone oil)	
18	1.5:1 (15ml:10ml)
19	1.2:1 (17ml:14ml)
20	1.1:1 (16.5ml:15ml)
Strategy 8: MDC:Silicone oil ratio impact (low MDC: high silicone oil)	
21	1:1.33(15ml:20ml)
22	1:1.45 (11ml:16ml)

23	1:1.82 (11ml:20ml)
Strategy 9: Washing time impact on MDC:Silicone oil (1:1.82)	
24	1.5 hours washing
25	3 hours washing
Strategy 10: Effect of high vacuum on MDC:Silicone oil (1:1.82)	
26	70mTorr, 24 hours at 39°C
Scale up batch (batch size 120g)	
27	1:2.1 (MDC:Silicone oil)

5.

RESULT AND DISCUSSION

Batch 1:

Table 5.1: Result of evaluation parameters (Batch 1)

Evaluation parameter	Result			
Entrapment efficiency	98.15%			
Residual MDC	30,259 ppm			
Particle size	D10: 9.6µm	D50: 37.1µm	D90: 82µm	Span value: 1.95
IVR real time 1 day	16.8%			
Impurity	Single max-0.33%		Total – 3.4%	

Result and discussion:

- The residual MDC with this method was high; it was decided to evaluate the second preferred method.
- Other limitations of this method were:
    - As the method is aqueous in nature, there is chance of peptide hydrolysis & manufacturing need to be done at lower temperature.
    - Non-uniform particles were also observed (in optical microscope); Broader particle size distribution; high impurity was also obtained.

Batch 2:

Table 5.2: Result of evaluation parameters (Batch 2)

Evaluation parameter	Result			
Entrapment efficiency	102.1%			
Particle size	D10: 36µm	D50: 51µm	D90: 73µm	Span value:0.72
IVR real time 1 day	19.55%			
Impurity	Single max-0.14%		Total – 1.5%	
Residual methanol	32 ppm			
Residual ethanol	2103 ppm			
Residual MDC	7880 ppm			
Residual heptane	65528			

Result and discussion:

- Uniform particle size distribution, less impurity, higher entrapment efficiency compared to double emulsification method.
- This method was selected considering above mentioned parameters.

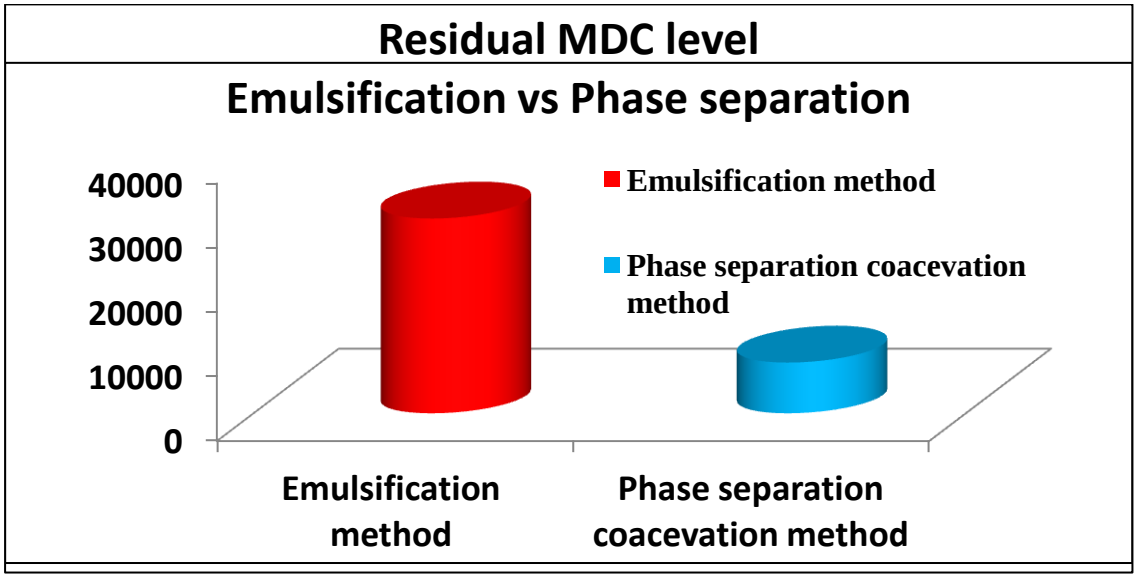


Figure 5.1: Graphical representation of Residual MDC (Batch 1 vs Batch 2)

- Residual solvent of RLD sample is mentioned below, reduction of residual solvent is to be done same as RLD sample.

Table 5.3: Residual solvent of RLD samples

Residual Solvent	ppm
Methanol	24
Ethanol	0
MDC	676
Heptane	18,942

Strategy 1: Different ratio of washing solvents

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
3	Ethanol:Heptane (1:1)	0	1395	5909	62508
4	Ethanol:Heptane (3:1)	0	1631	5603	57630

- According to known literature washing steps play an important role to lessen residual solvent.
- Changing ratio of washing solvents was done to check the impact on residual solvent but there was no impact observed.

Strategy 2: Change in manufacturing steps:

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
5	Hardening and ethanol wash in single step	0	2439	3558	63882
6	Hardening and ethanol washing as single step without buffer	0	3901	2626	53256

- Addition of washing solvent in hardening phase was done and batch without phosphate buffer was formulated but it did not give any impact on residual solvents.

Strategy 3: Large volume of washing solvent

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
7	75ml/gb washing solvent	17	2280	7171	57944
8	100ml/gb washing solvent	31	1576	7201	58137

- Washing solvents helps to remove the residual solvents so large volume of washing solvent was subjected but desired results were not obtained.

Strategy 4: High temperature during manufacturing

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
9	Washing at 25°C	31	6462	320	56338
10	Washing at 30°C	Polymer agglomeration			
11	Hardening and washing at 25 °C	31	5772	1515	55652
12	Phase separation, hardening and washing at 25°C	0	4991	1992	47638

- High temperature during manufacturing lead to decrease in MDC but impurity increased . At 30°C agglomeration also occured.
- So high temperature cannot be given to reduce residual solvent level.

Strategy 5: Effect of addition rate of coacervation agent

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
13	Direct addition	Non spherical particles			
14	16ml/min	17	8814	5821	68628
15	8ml/min	32	2103	7880	65528

- If direct addition of coacervating agent was done then non spherical particles were formed. 8ml/min flow rate of addition of silicone oil gave uniform spherical particles.
- Addition rate of coacervating agent had no significant effect on reduction of residual solvent.

Strategy 6: Stirring time after addition of silicone oil

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
16	2-5 min stirring	56	1010	283	65424
17	40 min stirring	32	2103	7880	65528

Strategy 7: MDC: Silicone oil ratio impact (high MDC: low silicone oil)

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
18	1.5:1 (15ml:10ml)	56	1010	283	65424
19	1.2:1 (17ml:14ml)	32	2103	7880	65528
20	1.1:1 (16.5ml:15ml)	17	3597	3560	59849

- High MDC: low silicone oil results in reduced MDC but high heptane, methanol and ethanol. It might be a possibility that low amount of silicone oil allowed close contact of microspheres with hardening solvent. And thus results in lower polymer residual solvent.

Strategy 8: MDC: Silicone oil ratio impact (low MDC: high silicone oil)

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
21	1:1.33(15ml:20ml)	31	2991	8928	39260
22	1:1.45 (11ml:16ml)	23	2182	9978	34459
23	1:1.82 (11ml:20ml)	30	2202	10847	22640

- Low MDC: high silicone oil results in reduced N heptane but in turn increase in residual MDC. As the silicone oil increases close contact between silicone oil and polymer solvent occurs. So in this case hardening solvent does not bind very closely so low residual heptane is obtained.

Strategy 9: Washing time impact on MDC: Silicone oil (1:1.82)

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
24	1.5 hours washing	0	1689	9357	23884
25	3 hours washing	24	1200	8590	21500

- Impact of washing time on the previous batch was checked but there was no significant reduction in residual solvent.

Strategy 10: Effect of high vacuum on MSC: Silicone oil (1:1.82)

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
26	70mTorr, 24 hours at 39°C	0	431	2451	23888

- By providing high vacuum for 24 hrs at 39°C all solvents gets reduced significantly in selected batch .

Scale up batch (batch size 120g)

Batch No.	Process parameter	Results			
		Methanol	Ethanol	MDC	N-Heptane
27	1:2.1 (MDC:Silicone oil)	0	1397	1258	19501

- Scale up batch was formulated in agitator nutch filter dryer in which hardening, washing steps are performed. Scale up batch selected was having MDC: silicone oil ratio (1:2.1) and there was significant decrease in residual solvent.

6.

CONCLUSION

CONCLUSION

Polymeric microspheres in which peptide drug was encapsulated successfully. Various methods of preparation were utilized to optimize the microspheres. Double emulsification solvent evaporation method was employed which lead to high impurity and wide range of particle size distribution. So phase separation coacervation method was employed which lead to decrease in impurity and uniform particles with narrow particle size distribution. Further the residual solvent was to be optimized. The present study shows that various processing parameters have an impact on residual solvent in microspheres. According to ICH guidelines for safety of patients the amount of harmful organic solvents in the final product should be in specified limits according to its toxicity. Formulation parameters like washing and drying conditions are known to reduce residual solvent. Various strategies were applied in which high temperature, rate of addition of coacervating agent, tome of washing, ratio of washing solvent ethanol:heptane, polymer solvent:coacervationg agent ratio were checked. In this method the ratio of polymer solvent to the antisolvent has a significant impact on residual solvent. MDC: silicone oil (1:1.82) gave desired limits of residual solvent. Drying under high vacuum is also beneficial to reduce residual solvent. Hence, the amount of residual solvent was optimized same as that of RLD product.

7.

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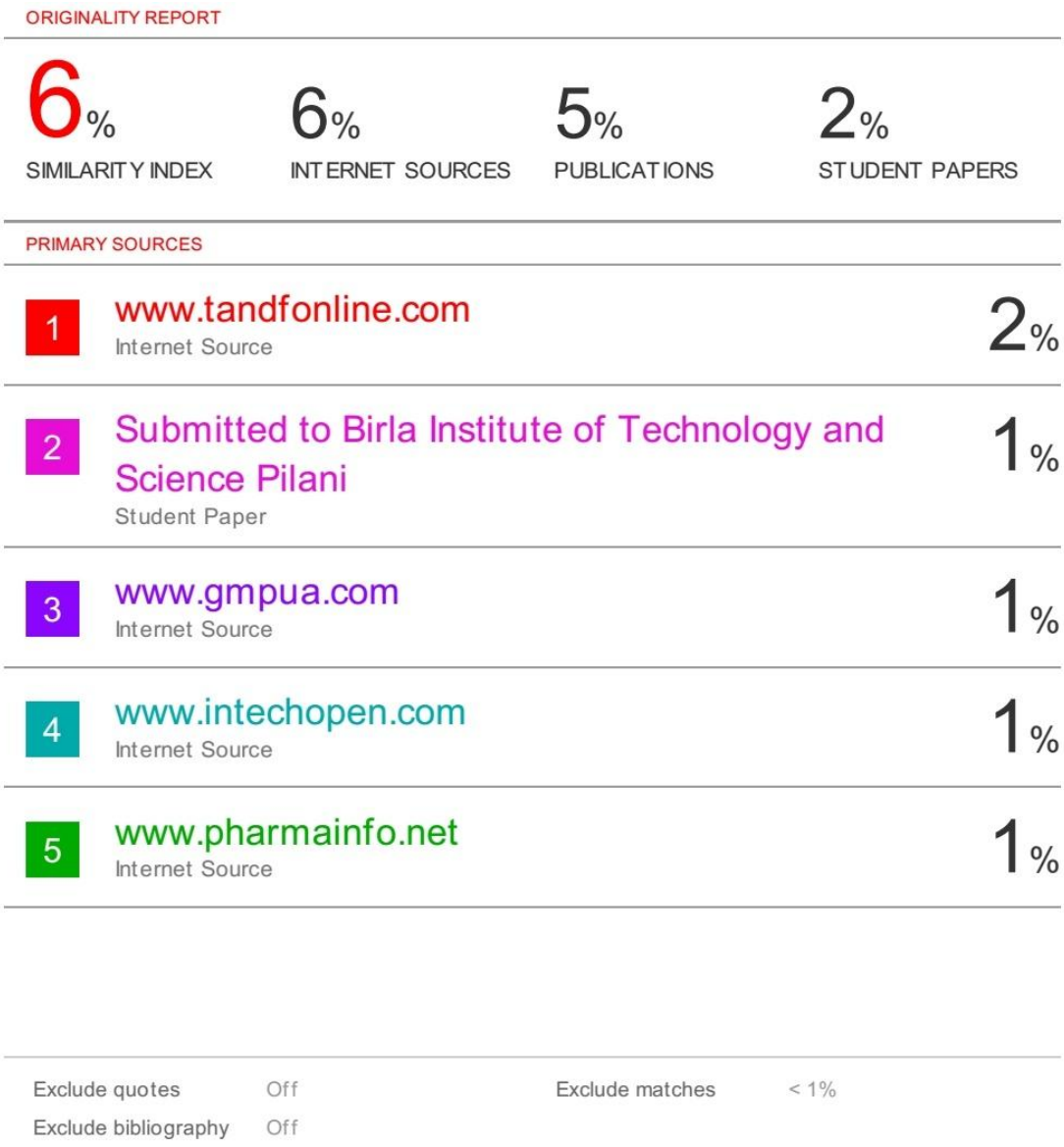

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