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PRE-FORMULATION, CHARACTERIZATION STUDIES OF ACTIVE PHARMACEUTICAL INGREDIENT (API) AND POLYMER.

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INTRODUCTION

It has been established that a medication's physicochemical characteristics, main burst, and excipients all have a direct impact on how quickly the drug leaves the microsphere[3].Injectable microsphere systems were developed in late 70's. Poly (lactide) and/or poly (lactide-co-glycolide) was used to encapsulate an ester of norethisteron calledNET via oil-in-water emulsion/solvent evaporation process. These microcrystals ofNET were encapsulated into microspheres of PLA and PLGA. Since then PLGA has been the first choice of polymer for preparation of microspheres to encapsulateAPIs using w/o or o/w emulsion/solvent evaporation technique. Poly (ε-caprolactone) is the secondly most accepted polymer for preparation of microspheres to encapsulate APIs. Moreover PLGA, PCL and PLA, diblock copolymers of lactide, ethylene glycol, L-lactide and ε-caprolactone, triblock co polymers of caprolactons, lactides and glycolides, have also been used to encapsulate APIs via o/w emulsion / solvent evaporation. [(3)]The composition of the microspheres and the rate at which they degrade have an impact on the release profiles of the API. For instance, the hydrolytic breakdown rate of PGA, PLA, and PCL is in the following order: PGA > PLA >> PCL. In contrast to PLA and PGA, which had inferior permeability in the case of steroidal pharmaceuticals but showed uniform biodegradation, PCL has great permeability in the case of steroidal medications but exhibits sluggish biodegradation. With high patient compliance, microspheres are now regarded as a dependable and efficient release mechanism that lowers doses, frequency of dosing, and the risk of dosage dumping (2). For example, polyesters employed in microspheres have the advantage of employing water as a vehicle to generate suspensions which allows consistent release of desired medicine. Microspheres prepared by natural or synthetic polymers provides sustained release.

Key Words-: Microspheres, Active PharmaceuticalIngredient (API) And Polymer

Materials and Methods

Methods
Preformulation

Following Preformulation parameters were performed on Active PharmaceuticalIngredient (API) and polymer.

Characterization of API

Characterization of API Olanzapine was performed on following parameters:

FTIR Analysis

FTIR of Olanzapine was performed on Perkin Elmer FTIR spectrometer shown inFigure no. 5.1

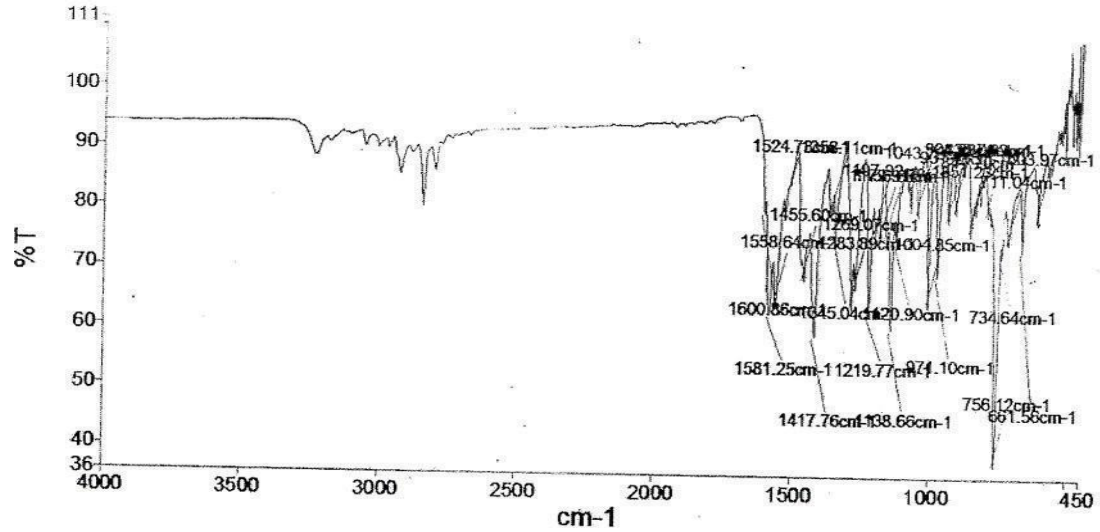


Figure 5.1: FTIR spectra of Olanzapine

DSC Analysis

Olanzapine had a DSC study, which is displayed below, on a Mettler Toledo. A slandered metal sample holder was loaded with 2.0 mg of olanzapine, which was weighed beforehand. A 50 mL per minute air flow was used to raise the sample holder's temperature from 50°C to 250 Cat at a rate of 10°C per minute.

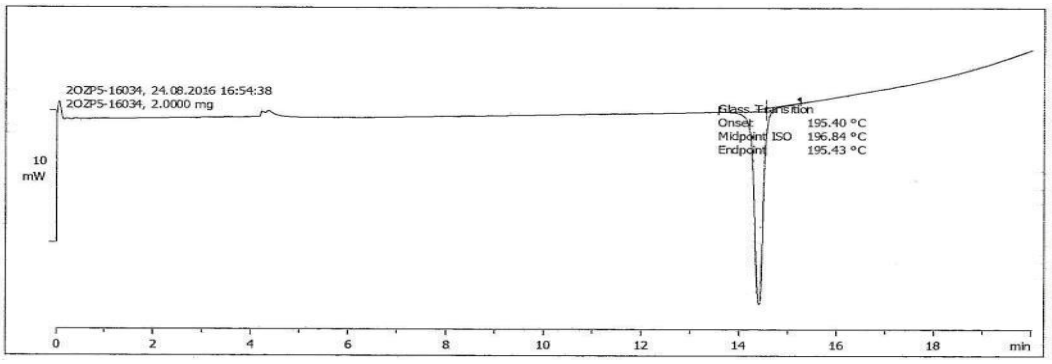


Figure 5.2: DSC thermogram of Olanzapine

Solubility analysis

Solubility was checked using different solvents like n-propanol, acetonitrilemethanol, absolute alcohol and water. Results of the study are given in Table 5.2

Table 5.2: Solubility Analysis of Olanzapine

Sr.No.	Solvent	Quantity of Olanzapine (In mg)	Amount of Solvent (in mL)	Observation
1	n-propanol	100	1	Soluble
2	Benzyl Alcohol	100	1	Soluble
3	Acetonitrile	100	1	Sparingly Soluble
4	Methanol	100	1	Slightly Soluble
5	Absolute alcohol	100	1	Slightly Soluble
6	Ethyl acetate	100	1	Slightly Soluble
7	Water	100	1	Practically insoluble

Characterization of Polymer

Characterization of polymer PLGA was performed on following parameters.

Molecular weight analysis

Molecular weight of polymer was determined with the help of HPLC using GPCsoftware. Following reagents and instrument accessories were used.

Table 5.3: Chromatographic conditions for molecular weight determination

Column:	Styragel HR-4 THF (300 mm x 4.6 mm) + Styragel HR-4 THF (300mmx4.6mm) + Styragel 30mm 4.6mm Guard (Part no. WAT045895)
Flow rate:	0.4mL/min
Injection volume:	50 µL
Column Temperature:	35 ˚C
Sensitivity:	32
Sampling (Hz):	10
Response(s):	1.5
Polarity:	(+)Positive
Cell Temperature:	35 ˚C
Mobile phase:	THF (Tetrahydrofuran)
Diluent:	THF (Tetrahydrofuran)
Run Time:	40 minutes
Rinsing Solution:	Isopropyl alcohol

Standard stock for mixture

Stock-1: About 40mg of 5.6K polymer standard was weighed and dissolved in 10mL volumetric flask, 5mL THF was added , dissolved and volume was made upto the mark with THF and mixed well.

Stock-2: 40mg of 33k polymer standard was weighed and dissolved in 10mL volumetric flask, 5mL THF was added , dissolved and volume was made up to the mark with THF and mixed well.

Stock-3: 40mg of 100k polymer standard was weighed and dissolved in 10mL volumetric flask, 5mL THF was added, dissolved and volume was made to the mark with THF and mixed well.

Stock-4: 40mg of 320k polymer standard was weighed and dissolved in individual10mL volumetric flask, 5mL THF was added , dissolved and volume was made up to the mark with THF and mixed well.

Standard mixture

1.0mL stock-1, 1.0mL stock-2, 1.0mL stock-3 and 1.0mL, stock-4 was pipette in 5mL volumetric flask and mixed well. Volume was not made up in this case.

Sample preparation

Standard mixture was mixed well to form an uniform solution, 74.64mg accurately weighed sample (equivalent to 60 mg PLGA) was taken in 10mL volumetric flask. 8mL THF was added and sonicated for 5 minutes. It was vortexed to dissolve the contents of solution and volume was made up to the mark with THF and wasmixed well. Solution was centrifuged for 5 minutes at 4500 rpm. Supernatant was collected and transferred to HPLC vial and injected.

Table 5.4: Order of injections for molecular weight determination

Sr. No.	Solution	Function	No of Injections
1	Blank	Inject control	1
2	Standard mixture	Inject narrow standard	1
3	Standard preparation	Inject board sample	6
4	Sample preparation	Inject board sample	1
5	Standard preparation (BKT)	Inject board sample	1

System suitability

Chromatographic system was equilibrated until reproducible baseline was achieved. Results were interpreted and molecular weight was calculated by using GPC software.

Table 5.5: Percent relative deviation of molecular weight

Sr.	Parameter	Acceptance Criteria
1	% relative standard deviation of molecular weight (Mw) of six replicates of standard preparation	Not more than 5.0

Table 5.6 : GPC result for system suitability

Sr. No.	Sample name	Mn	Mw	Polydispersity
1	System suitability-1	75502	124570	1.650
2	System suitability-2	77777	124699	1.603
3	System suitability-3	77764	124632	1.603
4	System suitability-4	77558	124917	1.611
5	System suitability-5	78327	126569	1.616
6	System suitability-6	76899	124920	1.624
7	System suitability-bkt-1	80185	127011	1.584
Mean		77716	125331	1.613
% RSD		1.8	0.8	1.3

FTIR Analysis

FTIR of PLGA was performed on Perkin Elmer FTIR spectrometer and is shownin figure 5.3

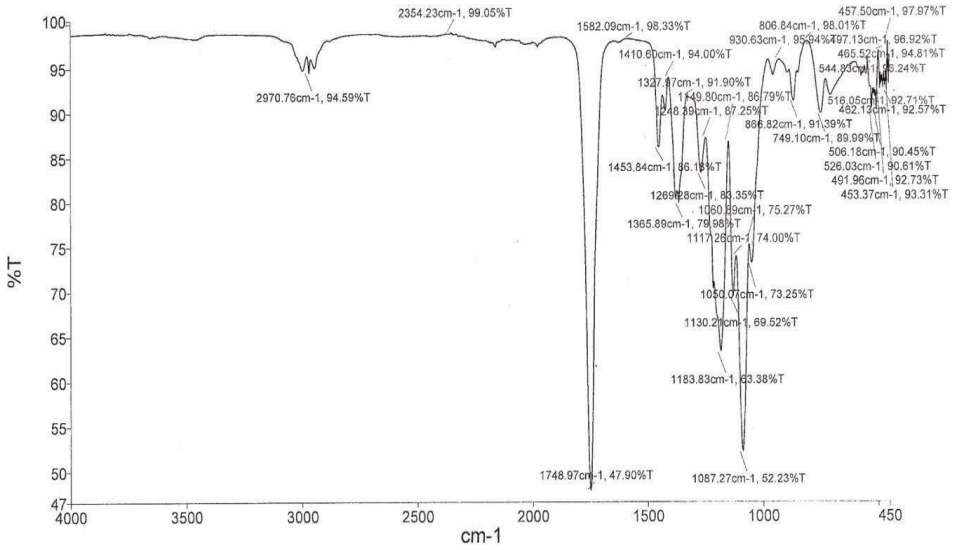
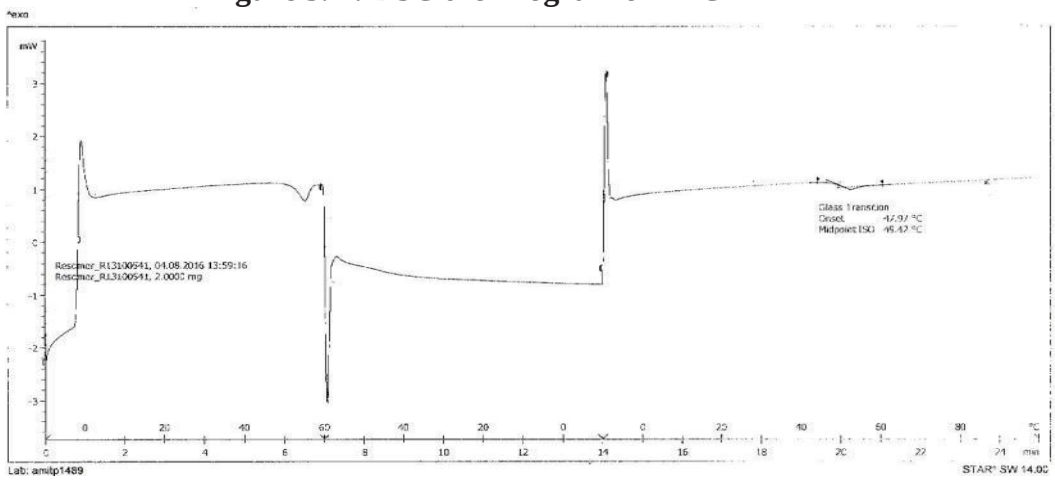


Figure 5.3 : FTIR spectra of PLGA

DSC Analysis

DSC analysis of PLGA was performed on Mettler Toredo. Approximately, 2 mg ofpolymer was weighed in standard aluminum sample holder. In first cycle thetemperature sample holder was brought to 10 ˚C. Then the temperature of sample holder was raised at the rate of 10 ˚C per minute up to 60˚C. In the second cycle, the temperature sample holder was decreased to -10 ˚C at rate of 10 ˚C per minute. In third cycle temperature of sample holder again raised from -10 ˚C up to 100˚C atrate of 10˚C per minute. Air flow of 50mL per minute was maintained throughout the experiment. Figure 5.4

Figure 5.4 : DSC thermogram of PLGA



RESULT & DISCUSSION

Pre-formulation Studies
Characterization of API

Olanzapine API was characterized and their analytical results are shown below.

FTIR Analysis of olanzapine

The FTIR spectra of olanzapine showed 30 peaks, which are recorded in table 6.1. Based on the wave number and peak transition percentage, an inference was made and recorded in table number 6.1. Peaks were seen on the same wave number in both the literature-reported and experimental study-recorded FTIR spectra of olanzapine, which helped to confirm the drug's identity.

Table 6.1: FTIR Interpretation for olanzapine

Sr. No.	Wave number cm^{-1}	Interpretation
1	1600.86	C-N stretching
2	1581.025	C-N stretching
3	1558.64	C-N stretching
4	1524.73	N-H deformation
5	1455.6	CH_2 deformation
6	1417.76	CH_3 deformation
7	1358.11	CH_2 wagging
8	1345.04	CH_2 wagging
9	1283.89	CH_2 twisting
10	1269.07	CH_2 twisting
11	1219.77	C-N stretching, C-S stretching
12	1197.92	CH_2 twisting
13	1179.92	CH deformation
14	1156.66	Aromatic ring stretching
15	1138.66	Aromatic ring stretching CH deformation
16	1120.9	C-N stretching CH_2 twisting
17	1043.78	Aromatic ring stretching CH deformation
18	1004.85	Aromatic ring deformation

19	971.1	C-S stretching
20	931.49	Aromatic ring deformation
21	904.99	Aromatic ring deformation
22	851.23	C-H out of plane banding
23	844.7	C-H out of plane banding
24	833	C-N stretching
25	787.93	Aromatic ring deformation
26	756.12	C-H out of plane deformation
27	734.64	C-H out of plane banding
28	711.04	Aromatic ring deformation
29	661.56	Aromatic ring stretching
30	603.97	Aromatic ring deformation

DSC Analysis of olanzapine

Olanzapine glass transmission temperature and DSC analysis were in agreement. The average glass transition temperature for three observations was 195.89 C.

Table 6.2: DSC thermogram results for olanzapine

Sr. No.	Glass Transition Temperature	Mean	Reported melting point of olanzapine
1	195.4	195.89	195.0
2	196.84		
3	195.43		

Solubility analysis

API solubility analysis N-propanol, benzyl alcohol, acetonitrile, methanol, absolute alcohol, ethyl acetate, and water were used to perform the olanzapine. The experimental solubility data matched the information found in the literature. Olanzapine was found to be almost insoluble in water, barely soluble in acetonitrile, methanol, pure alcohol, and ethyl acetate. Olanzapine's lipophilic character was demonstrated by its solubility in benzyl alcohol

and n-propanol. The olanzapine solubility profiles were used to choose the solvent for the drug's dissolution during the manufacture of microspheres as well as the quenching media for the microspheres.

Characterization of PLGA 750S Polymer

Molecular weight Analysis

The chosen polymer PGLA's molecular weight was determined using the HPLC technique. The criteria for chromatography are shown in table number -. To create standard solutions, polymers with molecular weights of 5.6K, 33K, and 100K were chosen. The table number gives the injection order for the standard solution and sample solution. The appropriateness of the system was further examined and is shown in table no. Table 6.3 provides the results of the GPC software's calculation of the molecular weight.

The polymer sample was determined to have a molecular weight of 1.23031 and a molecular ID of 78575. During formulation and development, the molecular weight of the polymer was found to be adequate because a higher molecular weight could result in a more consistent release of the API. Yet, throughout the polymer degradation process, an ideal molecular weight between 90,000 and 100,000 was obtained in the final formulation, which was determined to be suitable to accomplish the necessary rate of API release.

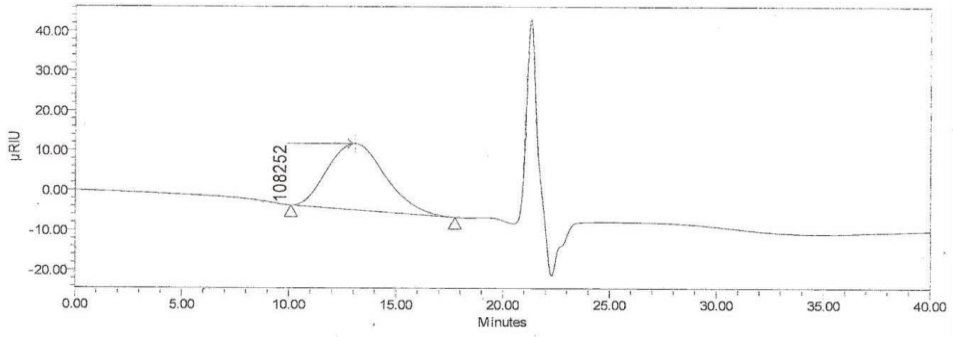


Figure 6.1 : Chromatogram of polymer PLGA for molecular weight determination

Table 6.3: GPC result for molecular Weight determination of polymerPLGA 750S

Sr. No.	Sample name	MP (Daltons)	Mn	Mw	Polydispersity	Retention time (Min)
1	R13000541_polymer	108252	78575	123031	1.566	13.085

Mean	123031	1.566	

FTIR Analysis of PLGA 750S Polymer

The PLGA 750S polymer's FTIR spectra revealed 21 peaks, which are listed in Table 6.4. Table 6.4 records inference based on peak transition percentage and wave number. It was determined that the FTIR spectra of the PLGA 750S polymer provided in literature and those obtained during experimentation were consistent and displayed peaks at the same wave number, so confirming the identity of the PLGA 750S polymer.

Table 6.4 : FTIR Interpretation for PLGA 750S Polymer

Sr.	Wave Number (cm^{-1})	Interpretation
1	2970.76	CH_2, CH_3 Asymmetric stretching
2	2354.23	O-H stretching
3	1748.97	C=O stretching
4	1582.09	C-H stretching
5	1453.84	CH_2 deformation
6	1410.6	OH bending
7	1365.89	CH2 wagging
8	1327.97	C-O stretching
9	1269.28	CH_2 twisting
10	1248.39	CH_2-C-O-CH_2 stretching
11	1183.83	CH deformation
12	1149.8	C-O Stretching
13	1130.21	C=O stretching
14	1117.26	CH_2 Twisting
15	1087.27	C-O Stretching

16	1060.89	C=O stretching
17	1050.07	CH deformation, C-O Stretching, straight chine anhydrides C-(C=O)-O-(C=O)-C
18	930.63	C-H out of plane bending
19	866.82	C-H out of plane bending
20	806.84	C-H out plane bending
21	749.1	C-H out of plane deformation

DSC analysis of PLGA 750S polymer

DSC of PLGA 750S was performed using Mettler Toledo. The glass transition wasinitiated at a temperature 47.97 C and midpoint was recorded at 49.42C which is inconfirmation of reported value for PLGA 750S polymer. The thermogram is shownin figure 6.2

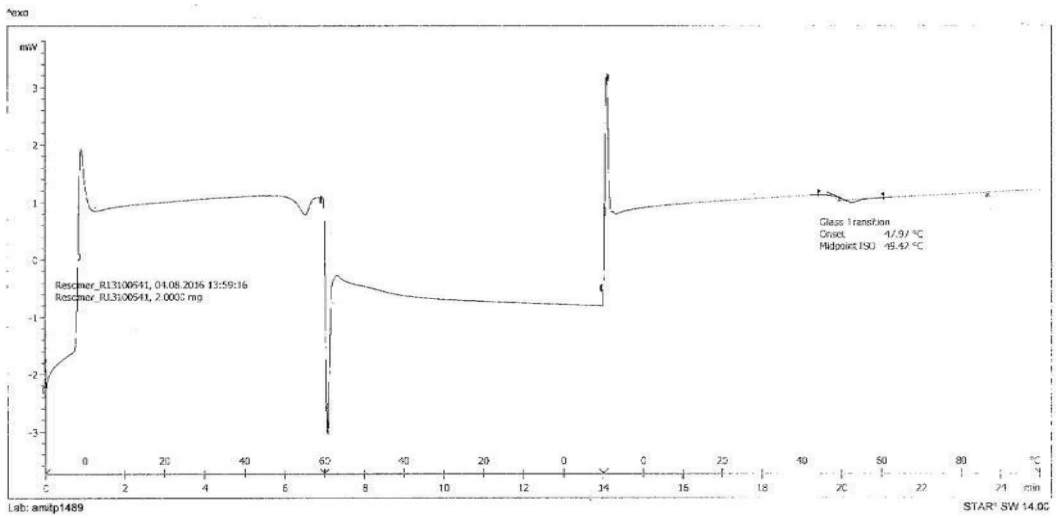


Figure 6.2 : DSC thermogram of PLGA 750S

CONCLUSIONS

The principal oral dose, which can be given as a single therapeutic agent or as part of a combination therapy, is 10 mg

per day. Degradation of PLGA depends on percentage of glycolic monomer in polymer. Higher the quality of glycolic acid lower is the time for degradation. Hence PLGA with 75:25 lactic acid : Glycolic acid ratio will take higher time for degradation in comparison to 50:50 lactic acid to glycolic acid ratio. Keeping in mind required properties of polymer PLGA 755 (lactic acid: Glycolic 75:25) was selected in present study to prepare microsphere of olanzapine. Olanzapine is a second-generation analytical antipsychotic medication. It is used to treat mild to severe mania associated with bipolar illnesses as well as schizophrenia.

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