Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 12(1):01-15

Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Preparation, Characterization, and *In Vitro* Evaluation of Parenteral Olanzapine Microspheres Loaded in Malaysian Mastic Polymer

Mohammed Gameel Al-Haddad[1*], Khaled Al-Tahami[2] and Gamil Qasem Othman[3]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, University of Science and Technology, Hodeida Branch, Yemen*

[2]*ALkhaled Pharma, Sana'a, Yemen*

[3]*Department of Pharmaceutical sciences, Faculty of Pharmacy, University of Science and Technology, Sana'a, Yemen*

ABSTRACT

The purpose of this study is to develop and evaluate olanzapine microspheres fabricated from biodegradable Malaysia mastic polymer to prolong release. Olanzapine microspheres have been prepared by oil/water solvent vaporization method with biocompatible Malaysia mastic polymer in different ratios with drug. The prepared microspheres were assessed for morphology, density, syringability, suspendability, percent yield, drug encapsulation efficiency and drug/polymer compatibility. In-vitro release, isothermal balance and accelerated studies were also studied. The percent yields and drug encapsulation efficiency of various formulations were within the acceptable range. IR spectra display no interplay between olanzapine and polymer used. Scanning electron microscopy of formulated microspheres showed spherical shape particles with small particles size. The release profile of olanzapine from the various microspheres was prolonged over 600 hours and the release obeyed Peppas diffusion for all formulations with different value that comply with Fickian law and showed acceptable stability and shelf life of selected formula. It was concluded that the formulated olanzapine microspheres by Malaysia mastic polymer was confirmed probable candidate for harmless and effective prolonged microspheres. It also concluded that the ratio of polymer/drug had main role in prolongation and the optimum formula was MF1:6 in which the drug amount was double the amount of polymer.

Keywords: Microspheres; Olanzapine; Malaysia mastic; Solvent vaporization method; *In-vitro* release

---

INTRODUCTION

Traditional therapeutic drug delivery system, although reasonably priced and easy to fabricate, show many therapeutic problems together with fluctuating plasma levels, inadequate amount, and terrible compliance *via* sufferers due to the repeated dosing instant release. Furthermore, conventional dosage systems are unpredictable and erratic; in many cases, an excessive quantity of drug is needed to reach the target location [1].
Tablet dosage form are used effectively in schizophrenia disease which is a chronic a mental illness over the debilitating life [2]. All over the world, schizophrenia affects more than 24 million human beings with the costs of remedy for as much as numerous billions yearly. Schizophrenia is a psychiatric disorder that often severely disabling that causes the patient to suffer hallucinations, delusions and cognitive deficits.

Non-adherence in schizophrenia also accounts for 40% of health spending for the disease. The rate of patients with schizophrenia who are partially or totally noncompliant has been estimated at 40-60% of all patients [3]. Finally, antipsychotic used to treat schizophrenia is an extremely resource-intensive disorder. The costs of relapses and rehospitalisation have significant effects on healthcare budgets [4].

The drug delivery systems offer numerous advantages as compared to conventional dosage forms, such as improved efficiency, reduced toxicity, improved patient compliance convenience and improves drugs' efficacy [5]. For patients with psychosis who will not or cannot take oral medication on a regular basis, a long-acting injectable antipsychotic may offer a solution [6]. Long-acting injectable antipsychotics may be used as an alternative to oral medication therapy in the case of patients with schizophrenia for whom adherence is a clinically significant problem [7]. Moreover, long-Acting Injectable antipsychotics (LAIS) should maximize pharmacokinetic coverage and minimize antipsychotic withdrawal symptoms resulting from partial compliance. Findings from many publications have frequently highlighted the importance of developing biodegradable microspheres as long-acting injectable dosage forms for atypical antipsychotic [8].

Microspheres are one of the multi particulate drug delivery systems that are employed for prolonged or controlled drug delivery [8]. Microspheres can be defined as solid approximately spherical particles ranging from 1 to 1000 μm containing dispersed drug [9]. They could be administered orally or injected into the body due to their small size. Gum mastic is a natural oleoresin exudate obtained from stem and broad-leaved variety of *Pistacia lentiscus* (Family Anacardiaceae) shown in Figure 1A. Microspheres have many advantages as facilitate accurate delivery of small quantities of potent drug and reduced concentration of drug at site other than the target organ or tissue. Several studies have shown that drug-loaded microspheres for parenteral administration offer specific advantages over the oral dosage forms in that they reduce dosing frequency and improve patient compliance, including assured bioavailability, elimination of uncertainty regarding compliance [10].

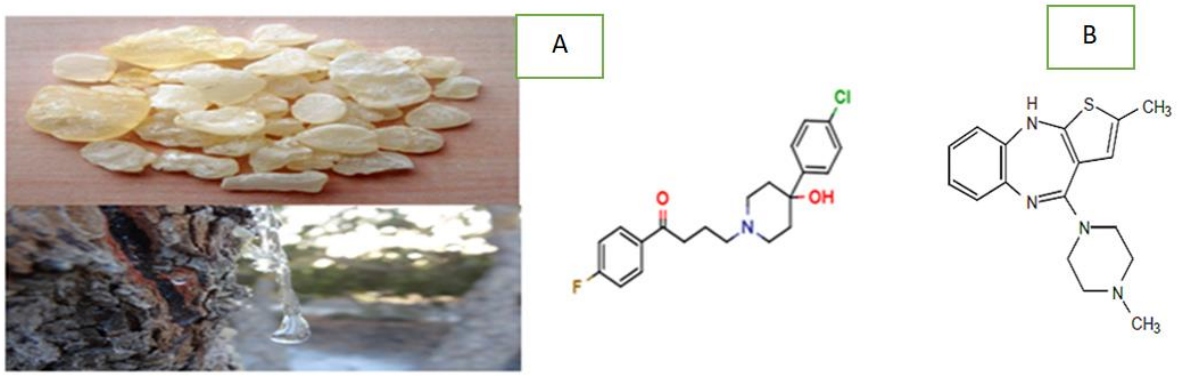

Figure 1. (A) Chemical structure of gum mastic resin, (B) Chemical Structure of olanzapine

Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H thieno [2,3][1,5]benzodiazepine) is a thienobenzodiazipine derivative (Figure 1B), which is an atypical antipsychotic effectively used in the treatment of schizophrenia and bipolar disorders [11,12]. Olanzapine has been shown to have affinity for dopamine, muscarinic and serotonin receptors [13]. Half-life of olanzapine is variable, which ranged from 21-54 hours. Olanzapine is highly metabolized with first pass effect and only 60% of the dose reaches the systemic circulation. Olanzapine is both excreted in urine and feces with 60% and 30% excretion, respectively [14]. Olanzapine drugs have a more potent dose with low strength and nature of the lipophilicity of candidate and encourages as parenteral dosage form.

The aim of this study was to develop and evaluate olanzapine microsphere delivery systems by single o/w solvent vaporization technique employing Malaysia mastic polymer as carrier to achieve sustained release.

MATERIALS AND METHODS

Materials

Olanzapine was provided as a gift from Rafa pharma, Yemen. Malaysia mastic was purchased from Bin Yasin stores, Yemen. Acetonitrile, Tween 80 and dichloromethane were purchased from HeMedia, India. Polyvinyl alcohol (PVA- Mw 140,000), methanol and sodium azide were purchased from Scharllau co, Spain. Disodium monohydrogen orthophosphate and mono potassium dihydrogen were purchased from Global Pharma, Yemen. All other chemicals were obtained commercially as analytical grade reagents from UST laboratory, Yemen.

Preparation of Olanzapine Loaded Microspheres

Treatment of mastic plant (Gum resin) polymer: 100 grams of mastic were weighed and immersed in 800 ml of aqueous solution and stirred for 4 hours, and then filtered. The formed precipitate was dissolved in 200 ml dichloromethane, then it was filtered again and the filtrate was collected with the solvent used and the undissolved part was discarded. The filtrate was spread on plates in the fume hood until dichloromethane was evaporated. Then the precipitate was collected and weighted. For the following six days of storage, the collected resin was weighed until the weight was constant to assure complete drying of the resin. The polymers obtained were stored for farther studies.

Preparation of olanzapine loaded microspheres: The polymeric microspheres of olanzapine were prepared by oil/water single emulsion solvent evaporation technique modified from the method showed by Tiwari et al. [15]. Certain amount of polymer was dissolved in organic solvent as Dichloromethane (DCM) to produce a polymeric microspheres solution. After complete dissolution of polymer in given solvent, the proper quantity of olanzapine

was added to the polymeric microspheres solution in different proportions as shown in Table 1. The high organic phase comprising olanzapine, polymer and DCM was gradually added drop wise into 100 ml of a water solvent containing concentration of the surfactant (polyvinyl alcohol) and by shaking (2000 rpm) at 25°C using a 3-blade homogenizer mixer, the emulsion was formed.

Table 1: Formulation of Malaysia mastic microspheres

Formulation Code	Drug: Polymer Ratio
M:F2:1	2:1
M:F1:1	1:1
M:F1:2	1:2
M:F1:4	1:4
M:F1:6	1:6

After the emulsion was formed, the shaking was continued for 3 hours in order to help the evaporation of the organic solvent (DCM) until microspheres were obtained. The formed microspheres were washed with deionized water for numerous periods using vacuum filtration system. The microspheres residue was then dried and stored in locked containers for further investigations [16]. The diagram representing the procedure carried out was shown in Figure 2.

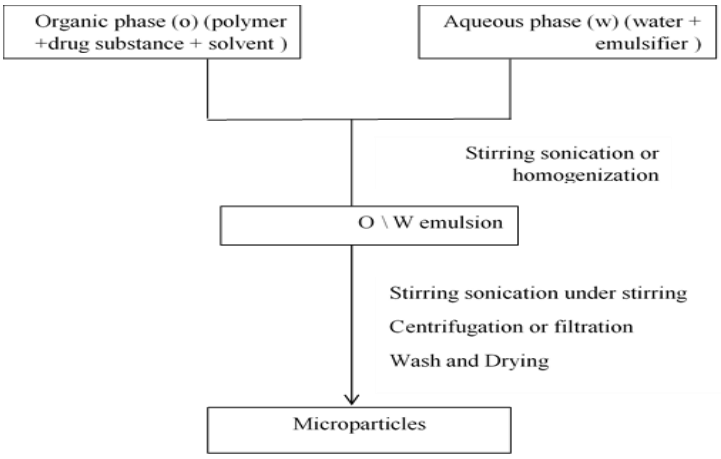

Figure 2. Experimental technique for production of olanzapine formulation

Characterization of Olanzapine Microspheres

Drug polymer compatibility study: The confirmation of olanzapine was analyzed using (FTIR PerkinElmer Spectrum-65, USA) by potassium bromide tablet method. Drug (2 mg) was mixed with KBr (40 mg) and prepared to a small clear tablet by implementation power in manual compares device. The IR spectrum was documented in the examination range of 4200-400 cm^{-1}. The IR spectrum of drug was compared to that present in literature and then the IR of mixture (drug and polymer) was compared to that of pure drug with noting any shifting or changes in the different peaks.

Microspheres morphology and size study: Certain amount (5 mg) of prepared microspheres were coated with palladium: gold in aluminum remains and was examined by Scanning Electron Microscopy (SEM) in appropriate magnification for superficial morphology [8].

Percentage yield measurement: The percentage yield of the prepared microspheres containing olanzapine was evaluated and determined by the following equation [17]:

%Yield (Recovery)={microspheres mass/mass of polymer + drug}*100

Drug entrapment efficiency and drug loading estimation: The olanzapine entrapment efficiency is defined as the proportion of the actually encapsulated quantity of olanzapine to that theoretically quantity used throughout the microsphere production. The capacity of the microsphere to load the olanzapine was estimated by accurately weighed amount of microsphere and then dissolved in the solvent [18,19]. Olanzapine was assessed by HPLC method in phosphate buffer solution (pH 6.6). The technique was validated for limit of quantitation, linearity and limit of detection. The method obeys standard calibration curve in the concentration range of 20 to 150 μg/ml [20]. The olanzapine encapsulation efficiency and drug loading was determined based to the next formulas:

Drug Encapsulation Efficiency%=Actual drug content/Theoretical weight of drug and polymer × 100

Loading%=Actual weight of drug/Weight of loaded microspheres (drug + polymer) × 100

Bulk density measurement: It is determined by density law from the weight and volume of microsphere formulations. Two grams of dry microspheres was examined for the bulk density test and the process was repeated three runs, then the average was calculated [21].

Suspendibility and syringability test: The suspendibility of microspheres in the vehicle is required for ease of administration and it was determined in an aqueous vehicle by accurately weighing microsphere that was suspended in 1.5 ml of vehicle. The syringability of the microspheres was being determined using 20 and 21 gauge needle [22].

***In vitro* release studies:** The release of olanzapine of the formulated microspheres was measured and calculated in buffered solution pH 7.4. The prepared microsphere (15 mg) was suspended in 15 ml screw-cap centrifuge tube containing in 10 mL of in buffered solution. Tween 80 (0.02%) and sodium azide (0.05%) was also added to above mixture. The samples were kept at 37 ± 0.7°C with continuous shaking (100 rpm), the samples were withdrawn from medium at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours in first day then after 1, 2, 3, 4, 7, 14, 21, 24, 28 days. The supernatant for each sample was collected by centrifuge at 2000 rpm, filtered through 0.45 μm filter paper and replaced with fresh buffer solution and analyzed by UV [23,24].

Furthermore, the amount of the olanzapine released (mg) at every time as follows:

Ar (mg)=Cr μg/ml × 10 ml/1000

where 10 was the volume of the drug release vehicle. The percent of olanzapine release (% Ar) at every time was determined from following equation:

%Ar=100 × Ar (mg)/15 (mg)

where 15 mg was the quantity of olanzapine present in microsphare was tested.

The cumulative olanzapine release% (Σ Ar% t) at every time was determined as follows:

(Σ Ar% t=Ar% t + Σ Ar% (0 to (t-672 hr)),

where Σ Ar% t, Ar% t, Σ Ar% (t2-t)) were the cumulative drug release at time (t), the percent of olanzapine release

at time and the cumulative of olanzapine release at previous times where 1 hr and also day was the time interval, respectively.

To determine olanzapine release kinetics, the cumulative release data were fitted to orders (mechanism) represent in, zero, first order and Higuchi equations. The mechanism of drug release was calculated by fitted data to Peppas equation [25]. In stability study, the shelf life (t90) was determined from Arrhenius modified equation [26].

Stability Study

Accelerated stability study: The ideal chosen formula (MF1:6) was investigated for stability tests. The stability tests were done by keeping the microspheres in a glass container at different temperature and humidity conditions as 25°C/60% RH, 30°C/65% RH and 40°C/75% RH for 3 months. The samples were collected at various periods (after 15, 45 and 90 days) of storage, tested to check of alterations in physical form of microspheres. Olanzapine amount was also evaluated and estimated by UV spectrophotometer at 256 nm [27].

Statistical analysis

All determinations and calculations were carried out by use of a Microsoft Excel 2010 program. Whenever necessary descriptive statistical limits including the average, standard deviation (SD), Range, 95% confidence limit were determined. With regard to calibration curve the validation of data depended on the linearity of the curve determined as regressions coefficient (R^2). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were also determined.

RESULTS AND DISCUSSION

Characterization of Olanzapine Microspheres

Drug polymer compatibility study: The FTIR spectrum was studied to examine olanzapine-polymer compatibility. The single spectra of the pure olanzapine, Malaysia mastic polymer and poly vinyl alcohol as individual and in mixture are shown in Figures 3 and 4. The IR spectrum of drug is shown in Figure 3A and was compared to that published in the literature Figure 3B. It was observed at 3219.2, 2932, 1585.5, 1558.6, 1412.3, 1224.1, 1179.1, 1143.1 and 745.7 centimeter^{-1}, indicating the existence of N-H stretching bond, aliphatic C-H stretching bound, C=N stretch, C=C stretching the thiophane and aromatic, C-H bending, C-H waging, C-C bond, C-N bond, C-S bond, respectively. The accuracy%, as shown in Table 2, of wave number between the two IR spectra was found to be in a range of 99.9-100.2% which confirmed the identity of the material including a research published by Pervaiz [23]. IR spectrum of Malaysia mastic polymer was shown in Figure 4B and it showed absorption bands at 3435, 2953 ,2870 ,1706 ,1456 and 1384 cm^{-1} because of stretch of aliphatic O-H bond, C-H aliphatic bond, C-H aliphatic, C=O vibrations bond, C-H bending and C-H bending, respectively. IR spectrum of PVA in Figure 4A that indicated the absorption bonds at 3436, 2924, 2853,1744,1464 cm^{-1} because of stretch the aliphatic O-H, C-H aliphatic bond, C-H aliphatic, C=O vibrations bond and C-H bending.

It was observed in Figure 4C no significant modifications on the site of the absorption spectrum of olanzapine and subsequently blends into PVA or Malaysia mastic polymer which showed no overlaps among olanzapine and mastic polymer and PVA that imply compatibility in all microsphere formulations.

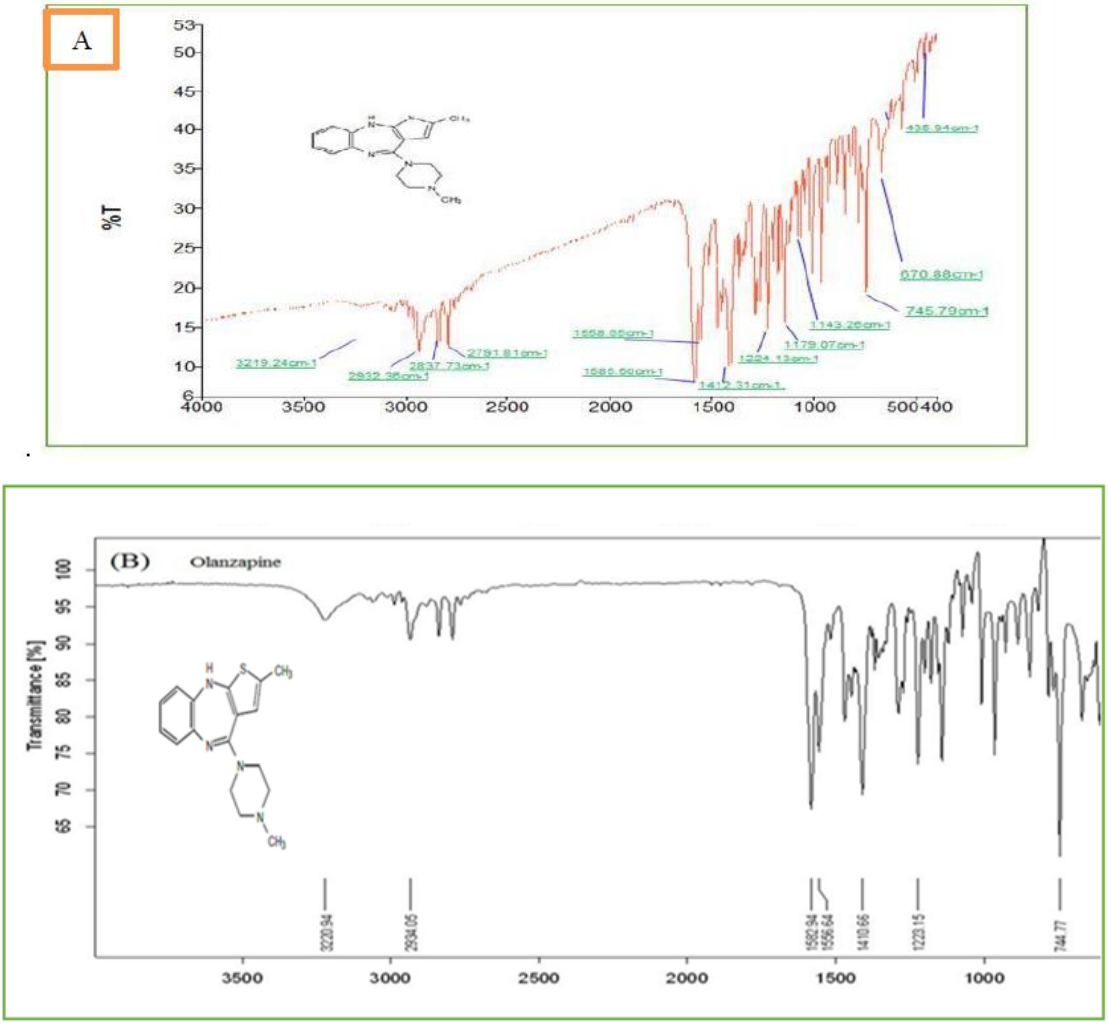

Figure 3. IR spectrum of (A) olanzapine used in this study (B) olanzapine published in the literature

Table 2. Comparison of wave numbers of olanzapine IR spectrum between the standard used in this study to that published by pervaiz [23]

Comparison Point	Standard used	Literature	(STD.W.N)/(Liter.W.N)x 100=%=A
N-H st	3219.2	3220.9	99.9
C-H alph:bond	2932.3	2934.1	99.9
C=N Bond	1585.5	1582.9	100.1
C=C Bond	1558.6	1556.6	100.1
C-H Bending	1412.3	1410.6	100.1
C-H wag	1224.1	1223.1	100.1
C-C bond	1179.1	1180.1	99.9
C-N bond	1143.1	1140.1	100.2
S-H bond	745.7	744.7	100.1
Mean		100.03	
SD		0.11	

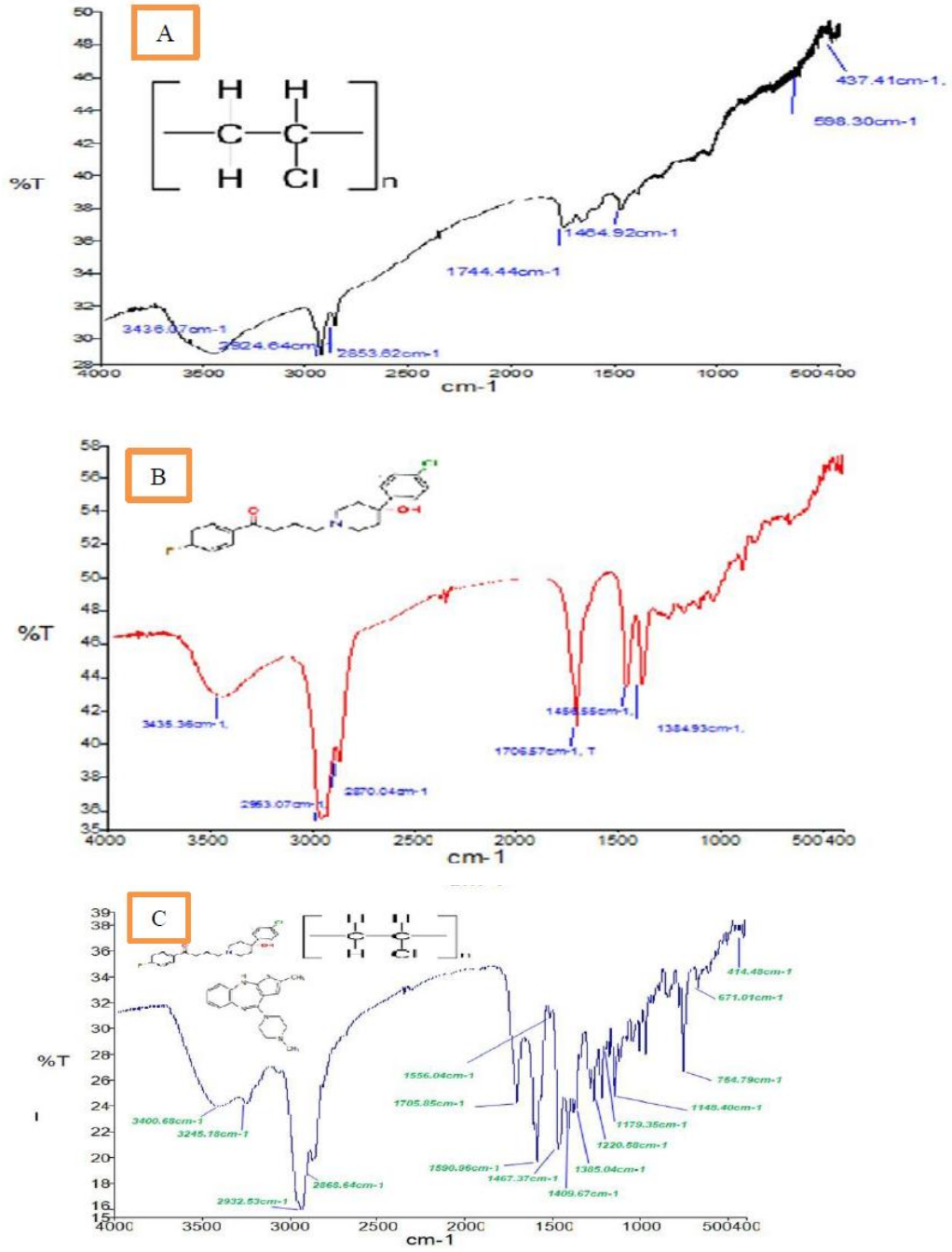

Figure 4. Spectrum of IR (A) polyvinyl alcohol (B) Malaysia mastic polymer (C) mixture of olanzapine, Malaysia polymer and polyvinyl alcohol

Microspheres Morphology and Size Study

- **Surface morphology of microspheres:** The superficial morphology of formulated microspheres was examined using Scanning Electronic Microscope (SEM). Scanning electron microphotographs of formulations are shown in Figure 5. All microscopical analyzes examined under the electronic microscope

emphasize and show all five microsphere formulas in a spherical shape with minimal porosity. It was found that all formulations loaded mastic polymer showed spherical morphology and aggregations with superior properties MF 1:2 formulations.

- **Microsphere particle size:** The size of the formula based on several elements inclusive of the kind and quantity of polymer in the organic solvent, mixer velocity, temperature through preparation moving frequency at the emulsification stage and quantity of emulsifying agent. The size range of olanzapine microsphere formulation was (7.75-18.93) µm. These are indicated in Table 3. It was noted that all five formulations were formed within the range of microsphere technique that was assumed of 1-100 µm range. In addition, the larger and smaller practical size of five formulations was within microsphere technique according to the shapes. The best formula was MF 1:2 and this result agrees with results obtained by published in the literature [28].

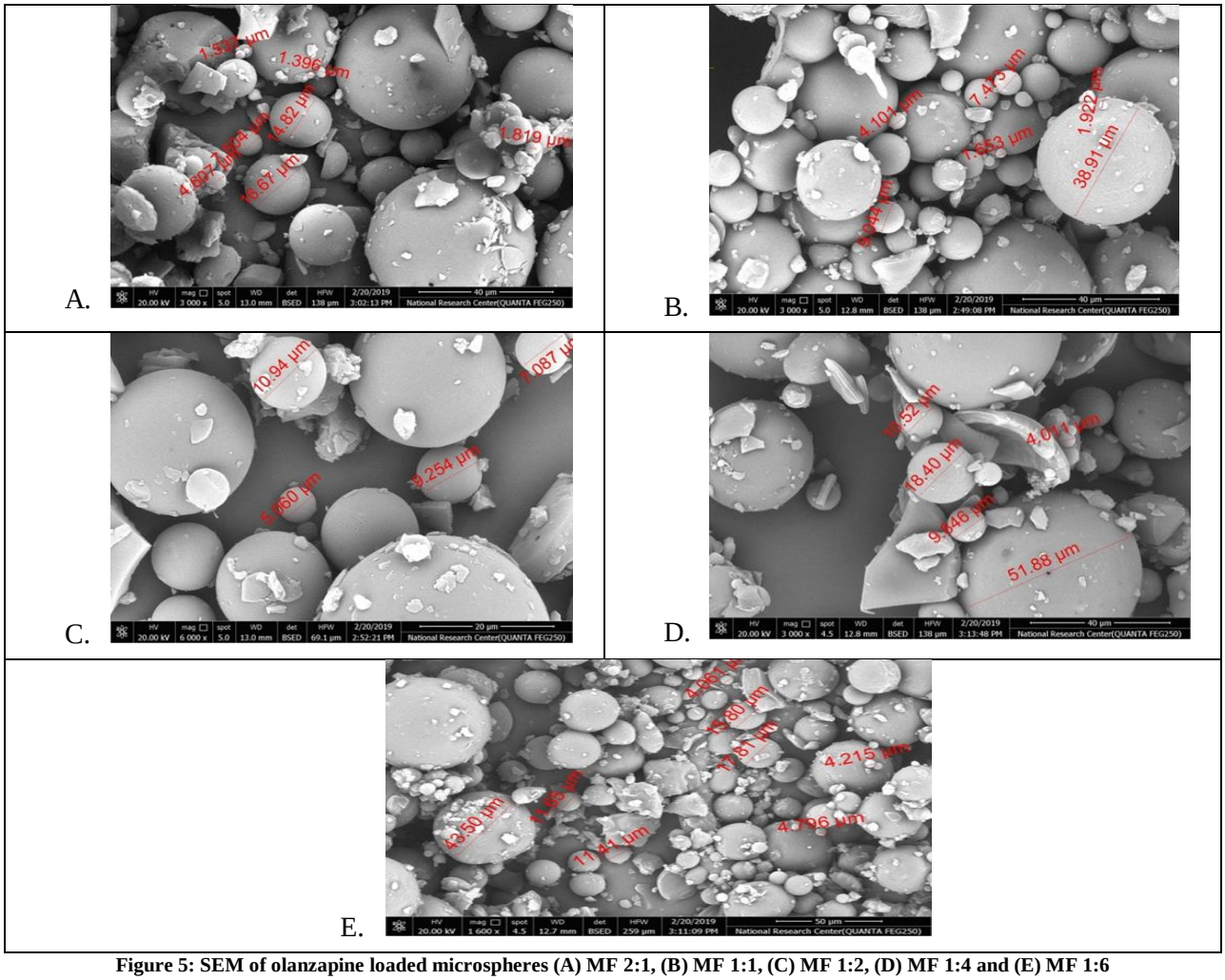

Figure 5: SEM of olanzapine loaded microspheres (A) MF 2:1, (B) MF 1:1, (C) MF 1:2, (D) MF 1:4 and (E) MF 1:6

Percentage yield: The percentage yield values of various preparations were variable and they were in the range of 94.1-98.3% as scheduled in Table 3. The highest was achieved with MF 2:1 (98.3%) in which the polymer ratio to olanzapine was 2:1 whereas the lowest value indicated with MF 1:6 (94.1%) with ratio of 1:6. [The data appeared in

all five formulations excess in% yield with decrease in polymer quantity in various microspheres that indicates a decrease in the formula lack throughout producer of the olanzapine found in formulation]. These outcomes resemble or comply with consequences found by using other research [29]. This decrease in% yield with excess ratio of olanzapine to mastic polymer might due to the lack of small microsphere throughout filtration and washing methods of formulation. In the latter, most of the results within the reasonable and acceptable range and the best formula was with MF 2:1.

Drug encapsulation efficiency and drug loading estimation: The actual olanzapine quantity in all formulas of microspheres was determined from the mean area under curve that was existed on the spectrum of HPLC Percentage of drug Encapsulation Efficiency (EE) for various microsphere preparations of olanzapine obtained to be various from 99.45% to 93.40% as reported in Table 3. Through this test it was confirmed that there is no overlap between the drug and mastic polymer and supports the compatibility test result obtained by IR which lead to the same path as indicated previously.

Table 3. Encapsulation efficiency (%), Drug loading (%), Yield (%), particles size of formulated microspheres

Formulation Code	Drug: polymer Ratio	Encapsulation efficiency (%)	Drug loading (%)	Mean Yield (%) ± SD	Mean particles size (µm) ± SD
MF 2:1	2:1	94.10 ± 0.23	66.41 ± 0.12	98.30 ± 0.21	7.75 ± 6.6
MF 1:1	1:1	95.12 ± 0.02	48.12 ± 0.34	97.60 ± 0.31	10.51 ± 14.22
MF 1:2	1:2	93.40 ± 0.34	30.23 ± 0.87	95.40 ± 0.43	8.08 ± 2.56
MF 1:4	1:4	99.45 ± 0.95	17.44 ± 0.98	97.20 ± 0.01	17.49 ± 13.50
MF 1:6	1:6	96.00 ± 0.56	13.56 ± 0.77	94.10 ± 0.51	18.93 ± 19.11

The maximum encapsulation efficiency (%) was observed in the microsphere preparations of MF 1:4 in which the olanzapine was found in ratio of 1:4 with polymer and was 99.45%. All the microspheres showed excellent% EE that can be due to the high water/oil partition coefficient of olanzapine. Additional factor affecting on encapsulation efficiency concerning the vehicle used in mastic polymers and olanzapine

Dichloromethane was employed as organic vehicle throughout produced olanzapine formulations and in this research and showed aqueous miscibility and needed little temperature to eliminate it by vaporization at 25°C [25]. The results showed that polymer quantity performs a main role in olanzapine EE%. While the ratio of olanzapine amount to polymer was reversely proportionally as shown in Table 3. The encapsulation efficiency decreased in MF 2:1 and also compared to the microsphere preparations which consist of excess ratio of polymer to olanzapine quantity as MF 1:6 observed.

The high encapsulation efficiency associated with increasing of polymer to olanzapine ratio may be due to the following reasons: by increasing the polymer concentration, we reduced the formation of microspheres particle of large size which may cause losing of olanzapine from the microsphere surface during the washing step. Fast precipitation of the polymer due to its high concentration which prevents diffusion of olanzapine across the phase boundary. In addition, as the polymer concentration increased, the viscosity of the solution increased and thus delayed the diffusion within the polymer droplets [30-32].

The drug loading percent of formulations was 66.41, 48.12, 30.23, 47.60, 17.44 and 13.56 for formulations MF 2:1 to MF 1:6, respectively as shown in Table 3. The best formulation near of true value encapsulation efficiency is MF 1:4 with 99.45% and loaded drug is MF 2:1 with 66.41%. These results acceptable and agree well with other results

published [27,33].

Bulk density measurement: The results of bulk density test were shown in Table 4. Bulk density for the formulations was calculated and the range of all formulations was 0.64 and 0.70 g/cc. This complies with Susan result [28].

Table 4. Bulk density of mastic polymer microspheres

Formula code	Bulk density	Syringeability 20-21	Suspendability
MF 2:1	0.67 ± 0.11	Yes	Yes
MF 1:1	0.66 ± 0.22	Yes	Yes
MF 1:2	0.64 ± 0.15	Yes	Yes
MF 1:4	0.69 ± 0.18	Yes	Yes
MF 1:6	0.70 ± 0.33	Yes	Yes

Suspendability and syringability test: This test was conducted for all mastic preparations and the results were observed in Table 4. The microspheres were easily and properly suspended into the aqueous vehicle. This showed that the administration of microspheres could easily be suspended into 1.5 ml of aqueous vehicle. Also, the suspension of microspheres was easily withdrawn from the vial into the syringe using 20 and 21 gauge needle that makes all formulations can be injected under the skin and comply with other drug depot as published (Figure 6) [22].

***In vitro*-release studies:** *In vitro*-release data of olanzapine microspheres was calculated from the percentage of the R% release of various formulations in buffer solution at pH 7.4 for different microspheres as indicated in Figure 7. Cumulative release of olanzapine through the various formulations was found extended olanzapine liberation and was summarized in Table 5.

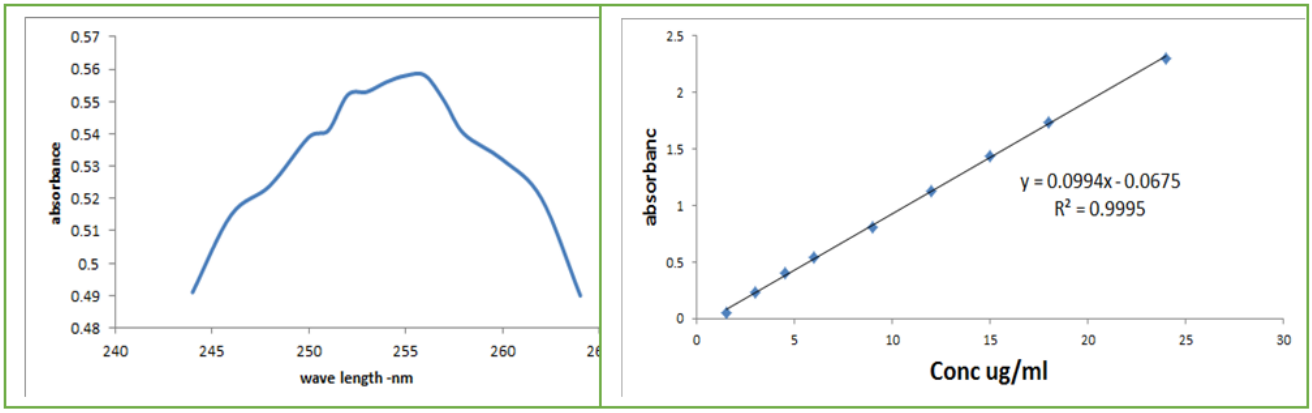

Figure 6. (A) UV Scan of λmax olanzapine (B) Standard UV calibration curve at 256 nm of olanzapine

It was investigated by UV and used regression equation derivative from calibration curve with standard concentration solution range (1.5-24) µg/ml with some validation parameters as LOC and LOQ as indicated 0.04 and 0.122 respectively.

Table 5. % Cumulative release of olanzapine Malaysia mastic microspheres

Time(hr)	%R ± SD MF 2:1	%R ± SD MF 1:1	%R ± SD MF 1:2	%R ± SD MF 1:4	%R ± SD MF 1:6
1	6.26 ± 0.29	5.92 ± 0.23	5.17 ± 0.19	5.18 ± 0.93	5.04 ± 0.13
2	6.94 ± 0.27	6.60 ± 0.28	6.34 ± 0.62	5.81 ± 0.36	5.84 ± 0.42
3	7.68 ± 0.64	7.28 ± 0.25	7.54 ± 0.78	6.60 ± 0.28	6.53 ± 0.88
4	8.36 ± 0.62	7.95 ± 0.23	8.38 ± 0.29	7.34 ± 0.65	7.34 ± 0.65
5	9.17 ± 0.39	8.63 ± 0.21	9.0 ± 0.26	8.02 ± 0.63	8.16 ± 0.43
6	9.92 ± 0.77	9.31 ± 0.19	9.70 ± 0.09	8.63 ± 0.21	8.90 ± 0.28
7	10.32 ± 0.32	9.99 ± 0.16	9.96 ± 0.66	9.24 ± 0.79	9.78 ± 0.97
8	10.60 ± 0.01	10.11 ± 0.01	10.11 ± 0.22	9.92 ± 0.77	10.36 ± 0.05
9	11.25 ± 0.67	11.33 ± 0.41	10.62 ± 0.21	10.52 ± 0.53	11.16 ± 0.19
10	12.03 ± 0.81	16.04 ± 0.82	12.72 ± 0.01	11.23 ± 0.93	11.81 ± 0.33
24	18.14 ± 0.39	21.55 ± 0.38	13.52 ± 0.41	14.04 ± 0.56	14.82 ± 0.14
48	35.06 ± 0.33	35.06 ± 0.33	13.83 ± 0.01	16.75 ± 0.59	17.83 ± 0.43
72	51.90 ± 0.27	55.37 ± 0.26	21.14 ± 0.58	22.86 ± 0.17	23.84 ± 0.27
96	65.53 ± 0.23	68.98 ± 0.22	55.05 ± 0.51	37.07 ± 0.53	34.67 ± 0.89
168	99.3 ± 0.18	92.69 ± 0.14	86.36 ± 0.53	42.59 ± 0.71	50.38 ± 0.52
336	99.3 ± 0.19	98.7 ± 0.72	90.14 ± 0.51	57.48 ± 0.46	60.35 ± 0.27
504	99.3 ± 0.14	98.7 ± 0.72	98.05 ± 0.32	58.75 ± 0.25	68.06 ± 0.26
624	99.3 ± 0.13	98.7 ± 0.72	100.0 ± 0.55	99.4 ± 0.12	100.2 ± 0.85
672	99.3 ± 0.12	98.7 ± 0.72	100.0 ± 0.55	99.4 ± 0.12	100.2 ± 0.35

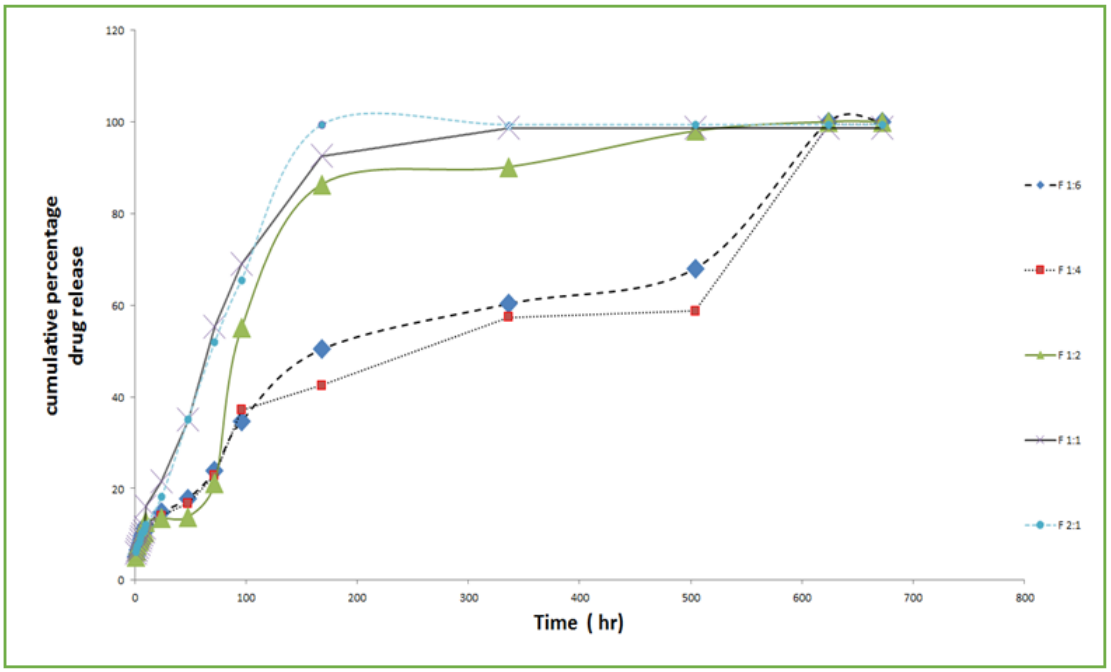

Figure 7.%Cumulative release of olanzapine of Malaysia mastic microspheres

In PBS of pH 7.4, the minimum and maximum percentage of cumulative liberation of olanzapine Malaysia mastic microsphere was indicated to be 98.7% for period 14 days and 100% for period 26 days individually, as shown in Table 5. This result complies with a previous published study [28]. The release studies showed that of olanzapine was released within 28 days. The liberation summary of the various microsphere preparations was distinguished by initial burst liberation of olanzapine between 5.04%-6.26% for five Malaysia mastic microsphere formulations as shown in Table 5. The formulation MF 1:6 showed the lowest burst release that 5.0% while the formulation MF 2:1 showed the highest burst release that 6.26% which may be decrease by excess the polymer concentrations as found in published studies [34,35]. In pH 7.4, all the produced Malaysia preparations dosage forms exhibit higher cumulative liberation of 100% indicated in MF 1:2 and MF 1:6 microspheres next transit of 26 days based on shown in the results values of the this research.

In-vitro release studies showed no relationship in the cumulative release of olanzapine with regard to excess of the polymer amount which lead to variation in the rate and quantity of olanzapine release between different formulations because of the modification of the ratio of olanzapine to polymer. These values observed means that the olanzapine liberation from polymeric formulation was sustained. Olanzapine release was found to be little amount released with time with regard to of Malaysia mastic according to preparation forms (MF 2:1-MF 1:1). The results of this released in first time high amount then decrease released gradually with time and carried out summarized all cumulative results. All formulations subjected to kinetic studies mathematically by kinetic order of drug release (Zero, First, Higuchi and Korsmeyer Peppas) and to done relation between the cumulative drug release% was determined for each formulation at intervals over time 0-672 hours. Results showed that formulation's drug had correlation coefficients for Pappas order greater than corresponding data others orders as shown Table 6. These findings indicated that the drug release obeyed Peppas equation for all formulations with different n value with respect to five formulations loaded with Malaysia mastic polymer comply with non-fickian law more than n=0.45 which mean not affected by factor of fickian diffusion law. This is the important different significant *in vitro*-release test between all microsphere formulations. General, all the preparations of olanzapine microspheres made of Malaysian mastic polymer with various ratios of olanzapine to polymer showed sustained olanzapine liberation in PBS at pH equal 7.4 for 28 days as maximum and this result consider preferred of research published [33,36]. The best formula was MF 1:6 with R^2 0.9809 with longest release then MF 1:4.

Table 6: Regression coefficient (R^2) values of various kinetic models of Malaysia mastic microspheres

Correlation coefficient (R^2)					
	Order		Dependence On particle size Higuchi	Mechanism Peppas	Peppas (n)
Code	Zero order	1st order			
MF 2:1	0.7435	0.6234	0.8947	0.9595	0.5176
MF 1:1	0.7452	0.6038	0.9007	0.9621	0.5225
MF 1:2	0.8356	0.7913	0.9253	0.9341	0.5009
MF 1:4	0.9526	0.7982	0.9536	0.9735	0.4613
MF 1:6	0.9607	0.8012	0.9743	0.9809	0.4709

Stability study: The ideal microsphere MF 1:6 was exposed for accelerated stability studies. Stability studies were investigated at 25°C/60% RH, 30°C/65% RH and 40°C/75% Relative Humidity (RH) for interval of three months (Table 7). It was concluded these condition didn't that don't modify physical properties injectability and Suspendability. It was shown that olanzapine was stable part and an excellent indicator of long shelf life according to results and this result comply with published study [27].

Table 7 shows the Stability studies of Malaysia mastic microsphere MF 1:6 for drug content, syingeability and suspedability and Physical appearance of microsphere. Values shown in the table mean percent of 3 batches (n=3), RH=Relative humidity.

Table 7. Data for construction of stability condition of MF 1:6 Malaysia mastic polymer

Stability condition	Sampling (days)	Drug content (mg) ± SD	Syringability and Suspendability	Physical appearance
25°C/60% RH	15	99.45 ± 0.41	Pass	No change
	45	99.33 ± 0.61	Pass	No change
	90	99.12 ± 0.71	Pass	No change
30°C/65% RH	15	99.43 ± 0.77	Pass	No change
	45	98.80 ± 0.69	Pass	No change
	90	97.91 ± 0.48	Pass	No change
40°C/75% RH	15	99.44 ± 0.22	Pass	No change
	45	98.60 ± 0.94	Pass	No change
	90	97.10 ± 0.43	Pass	No change

CONCLUSION

In the summary conclude in all the results in this research there was a better formula in each test carried out. Regarding the test of drug compatibility by IR ,the morphology and particle size test, the yield test, encapsulation efficiency, the load of drug test, In the HPLC chromatogram experiment and density test was The results were close to each other no variation significant. In respect to the *in vitro* release was the important pointed due to variation significant so the best formula based on of correlation coefficients R^2 was MF 1:6 then formula is MF 1:4.

The studies and results clearly indicate the utility of the tailored formulation approach to developing long-acting olanzapine injectable depot preparations. Thus, proper selection of polymer composition with suitable ratio will enable customizing drug release from Malaysia mastic polymer formulations. Additionally, this strategy depicts a reduction in the frequency of dosing that can prove to be of significant benefit in the development of novel therapy type drugs as we move from animal to human models. Preparation of injectable depot formulations of an atypical antipsychotic encapsulated within Malaysia mastic microspheres is an excellent delivery mechanism that offers the possibility of sustained drug release over a large duration of time.

ACKNOWLEDGMENTS

This research was performed with support of the University of Sciences and Technology, Yemen, Hodeida Branch.

REFERENCES

1. O Wichterle; D Lim. *Nature*. **1960**, 185: 117-118.
2. A Shrivastava; S Rao. *Indian J Psychiatry*. **1999,** 41: 329.
3. JM Olivares; B Pinal; C Cinos. *Neuropsychiatry*. **2011**, 1: 275.

4. T Dilla; J Möller; P O'Donohoe; M Álvarez; JA Sacristán; M Happich; A Tockhorn. *BMC psychiatry*. **2014**, 14: 298.
5. SP Vyas; RK Khar. *CBS publishers & distributors*. **2004**.
6. R Catalán; R Penadés. *Journal of central nervous system disease*. **2011**, 3: 95-105.
7. L Citrome. *Int J Clin Pract*. **2009**, 63: 140-150.
8. SD'Souza; S Murty; JA Faraj; PP DeLuca. *Advances in Pharmaceutics*. **2014**, 2014.
9. NSSD Vankayalu; M Madhuri; MD Dhanaraju. *Int J Pharm Life Sci*. **2014**, 4: 5.
10. S D'Souza; JA Faraj; S Giovagnoli; PP DeLuca. *Int J Biomater*. **2014**, 2014.
11. H Scherk; FG Pajonk; S Leucht. *Arch Gen Psychiatry*. **2007**, 64: 442-455.
12. N Moore. *Curr Opin Invest Drugs*. **1993**, 2: 281-293.
13. M van der Zwaal; MC Luijendijk; RA Adan; SE la Fleur. **2008**, *Eur J Pharm*. 585: 130-136.
14. JT Callaghan; RF Bergstrom; LR Ptak; CM Beasley. *Clinical Pharmacokinetics*. **1999**, 37: 177-193.
15. S Tiwari; P Verma. *Int J Pharm Life Sci*. 2: 2011.
16. J Naik; A Lokhande; S Mishra; R Kulkarni. *Int J Pharm Bio Sci*. **2012**, 3: 573-590.
17. A Neha; K Himanshi; P Deepa; A Nageen; G Tarun. *IOSR Journal of pharmacy*. **2012**, 2: 180-181.
18. K Vinod; A Sri; D Banji; S Anbazhagan; S Vasa; S Sandhya. *Der Pharma Chemica*. **2010**, 2: 500-506.
19. Y Wang; DJ Burgess. *Springer*. **2012**, 2012: 167-194.
20. C Singh; S Purohita; BL Pandeya; Priyankab. Int J Sci Innov Res Stud. **2014**, 1: 18.
21. B Ravivarapu; K Burton; PP DeLuca. *Eur J Pharm Biopharm*. **2000**, 50: 263-270.
22. A Saraf. *World J Pharm Pharm Sci*. **2014**, 3: 733-739.
23. F Pervaiz; M Ahmad; T Hussain; A Idrees; A Yaqoob; K Abbas. *Acta poloniae pharmaceutica*. **2016**, 73: 175-181.
24. F Pervaiz; M Ahmad; L Li; G Murtaza. *Current drug delivery*. **2019**, 16: 375-383.
25. SC Basak; KS Kumar; M Ramalingam. *Revista Brasileira de Ciências Farmacêuticas*. **2008**, 44: 477-483.
26. S Yoshioka; VJ Stella. *Springer Science & Business Media*. **2000**.
27. M Manjunath; A Aravindram; B Vishwanathan; FM Ahmed; DV Gowda; MS Khan. *Latin American J Pharm*. **2012**, 31.
28. S D'Souza; JA Faraj; S Giovagnoli; PP DeLuca. *Journal of pharmaceutics*. **2013**, 2013.
29. M Dixit; RN Charyulu; A Shetty; N Charyulu; M Rao; P Bengre; S Thomas. *J Appl Pharm Sci*. **2014**, 4: 81-86.
30. RC Dhakar; SD Maurya; S Aggarawal; G Kumar; VK Tilak. *Pharmacie Globale (IJCP)*. **2010**, 1: 1-5.
31. H Rafati; A Coombes; J Adler; J Holland; S Davis. *Journal of Controlled Release*. **1997**, 43: 89-102.
32. R Bodmeier; JW McGinity. *Pharm Res*. **1987**, 4: 465-471.
33. J Ruby; V Pandey. *J Appl Pharm Sci*. **2016**, 6: 34-40.
34. X Huang; CS Brazel. *J Control Release*. **2001**, 73: 121-136.
35. F Kang; J Singh. *Aaps Pharmscitech*. **2001**, 2: 86-92.
36. A Fini; C Cavallari; G Ceschel; AM Rabasco. **2010**, 99: 4251-4260.