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# Design and Evaluation of Entecavir Sustained Release Microspheres

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**A B S T R A C T**

The present research work is aimed to design oral twice a daily sustained release microspheres of Entecavir, used for the treating or preventing Hepatitis B which can release the drug for 10 to 12 hours. The microspheres were prepared by the Quasi emulsion solvent diffusion method using varying concentrations of sustained release polymers Eudragit S 100, Eudragit RS 100 and Eudragit RLPO. The compatibility of the polymers was ruled out by FT-IR studies and found to be compatible. Total nine formulations were prepared. The Entecavir were evaluated for their micromeritic properties and found to have good flow property. The prepared microspheres were evaluated for particle size analysis, determination of drug entrapments efficiency, percentage yield and in vitro drug release. The dissolution medium used was pH 7.4 phosphate buffer. All formulations showed acceptable pharmaco-technical properties and complied with in-house specifications for tested parameters. The results of dissolution studies indicated all formulations released up to 12hours and F7 was the most successful formulation with 99.43 % drug release at the end of 12 hours.

**Keywords:** Quasi emulsion solvent diffusion method, Entecavir, Sustained release, Microspheres.

**A R T I C L E I N F O**

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## 1. Introduction

Controlled release dosage form is a dosage form that releases one or more drugs continuously in predetermined pattern for a fixed period of time, either systemically or locally to specified target organ. Controlled release dosage forms provide better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects [1].

There are various approaches in delivering a therapeutic substance to the target site in a controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm.

The range of techniques for the preparation of microspheres offers a variety of opportunities to control aspects of drug administration. This approach facilitates the accurate delivery of small quantity of the potent drugs, reduced drug concentration at the site other than the target site and the protection of the labile compound before and after the administration and prior to appearance at the site of action. The behavior of the drugs in vivo can be manipulated by coupling the drug to a carrier particle. The clearance kinetics, tissue distribution, metabolism and cellular interaction of the drug are strongly influenced by the behavior of the carrier. The exploitation of these changes in pharmacodynamics behavior may lead to enhanced therapeutic effect [2,3].

Microspheres are one of the multiparticulate delivery system and are prepared to obtain controlled drug release from the dosage form to improve bioavailability reduce the adverse action and prolong the action of drug, reduce absorption difference in patients, reduce the dosing frequency and adverse effects during prolong treatment. It is needed to formulate in long acting dosage form, reaching to effective biological site rapidly. This type of drug delivery systems mainly provides the encapsulated material to reach the area of action without getting adversely affected by the environment through which it passes. Microparticulate drug delivery offers several applications for drugs having poor bioavailability. A number of pharmaceutical encapsulated products are currently in the market, such as aspirin, theophylline and its derivatives, vitamins, antihypertensive, potassium chloride, progesterone and contraceptive hormone combinations [4].

Hepatitis B virus causes inflammation to the liver. This virus may leads to cirrhosis and hepatocellular carcinoma. It is mainly present in liver, blood and certain body fluids. Anti-viral drugs such as lamivudine, telbivudine, Entecavir, adefovir and tenofavir are used for the treatment. Vaccines are used to enhance the immunity against the virus. Interferons are used in long term therapy for chronic hepatitis B virus. Oral solutions and tablets are available for the treatment of hepatitis B virus.

Though the above drugs are used to cure the disease they have side effects like swelling of face, lips and throat and severe side effects like liver damage, nausea, stomach pain and loss of appetite. There is a chance of taking heavy dosing which may lead to toxicity. In order to overcome the above problems the micro encapsulation technique is done by formulating microspheres of anti-viral drugs by using different polymers such as cellulose derivatives, Eudragit, poly lactones, poly anhydrides and poly ortho esters and natural polymers like albumin, starch and amylose etc [5-7].

The main objective of the present study outlines a systematic approach to design and evaluate Entacavir sustained release microspheres. Using different polymers like Eudragit S100, Edragit RLPO, Eudragit RS100, in different ratios to enhance the gastric retention time and oral bioavailability of the drug.

## 2. Materials and Methods

The pure drug entacavir was obtained as a gift sample from Hetero Pharma Hyd limited. Eudragit S100, Eudragit RS100 and Eudragit RLPO were obtained from Evonik Industries. Aerosil, Acetone and Di Chloromethane were purchased from SD Fine Chem Pvt, Mumbai.

**FTIR Studies:**

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of Entacavir, polymer and physical mixtures of samples were weighed and mixed properly with Potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a thin semi transparent pellet by applying pressure. The IR spectrum of the pellet from 450-4000$cm^1$ was recorded taking air as the reference and compared to study any interference.

**Preparation of the Entecavir microspheres: [8,9]**

The microspheres have been prepared by using different polymers in the ratios as given in the table 1. The drug–polymer Aerosil suspension was poured into poor solvent with stirring, the finely dispersed gel like emulsion droplets were formed immediately. As the polymer have good affinity to the organic solvent, good solvent (acetone) and bridging liquid (dichloromethane) in the droplets could not be diffused into poor solvent at once. But as stirring going on good solvent, which is discretionarily miscible with poor solvent, was diffused out from the quasi-emulsion droplets under the agitation, drug and polymer in the droplets were supersaturated, precipitated, and deposited on the Aerosil gradually. As a result, the droplets were consolidated into microspheres by the linkage action of bridging liquid. The diffusion of good solvent into poor solvent can make a part of the bridging liquid in droplets diffuse into poor solvent to

achieve diphase equilibrium between the droplets and poor solvent. After the process for 20 min, 150 ml of poor solvent was added into the system to promote the diffusion speed of good solvent and part of bridging liquid. Further solidification of the droplets led to production of the microspheres. The solidified microspheres was filtrated, washed, and dried to eliminate the residual organic solvent. The Aerosil was introduced in this microspheres formulation as an inert solid dispersing carrier to improve the dissolution rate of Entacavir. Due to its large surface area, high porosity, and unique adsorption properties, Aerosil has been successfully used as a dispersing agent to increase the dissolution rate of sparingly soluble drugs. At the same time, Aerosil was an effective anti adhesion agent, and it could accelerate the solidification of droplets and be packed in the microspheres well. These suggested that the higher recovery of microspheres could be obtained comparing with other conventional methods of microspheres. In this formulation, Eudragit RS was used as a bonding and retarding agent in order to bind the Aerosil into microspheres and control the release rate.

**Table 1: Formulation development**

| Drug/Excipients (Mg) | Formulation Code | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
| Entacavir | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Eudragit S100 | 25 | 50 | -- | -- | -- | -- | 25 | 25 | -- |
| Eudragit RS100 | -- | -- | 25 | 50 | -- | -- | -- | 25 | 25 |
| Eudragit RLPO | -- | -- | -- | -- | 25 | 50 | 25 | -- | 25 |
| Aerosil | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Acetone | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Dichloromethane | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Sodium Dodecyl Sulphate | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Drug:Polymer | 1:0.5 | 1:1 | 1:0.5 | 1:1 | 1:0.5 | 1:1 | 1:1 | 1:1 | 1:1 |

**Characterization of microspheres:[10-12]**

**Micromeritic properties:**

**Angle of repose:**

Angle of repose of different formulations was measured according to fixed funnel standing method ( $n = 3$).

$$\theta = \tan^{-1} h / r$$

Where θ is the angle of repose, $r$ is the radius, and $h$ is the height.

**Bulk density & Tapped density:**

Bulk and tapped densities were measured by using 10 ml of graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times, then tapped volume was noted down and bulk density and tapped density were calculated. Each experiment for micromeritic properties was performed in triplicate manner and reported.

**Carr's index:**

Compressibility index (Ci) or Carr's index value of micro particles was computed according to the following equation:

$$\text{Carrs index (\%)} = \frac{\text{Tapped density} - \text{Bulk Density}}{\text{Tapped density}} \times 100$$

**Hausner's ratio:**

Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation:

$$\text{Hausner}' \text{ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

**Particle size analysis:** The particle size of microspheres was determined using optical microscopy method approximately 100 microspheres were counted for particle size using a calibrated optical microscope (Magnus MLX-DX).

**Determination of drug entrapments efficiency and yield:**

Microspheres (25 mg) were suspended in 25 ml of methanol. After 24 hrs, the solution was filtered and the filtrate was analyzed for drug content; this filtrate was diluted up to appropriate dilution; and for the determination of drug entrapment efficiency, the following formulas were used:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

$$\text{Yield(\%)} = \frac{\text{Weight of microparticle}}{\text{Total expected weight of drug and polymer}} \times 100$$

**Scanning electron microscopy analysis (SEM)**

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan) using gold sputter technique. The particles were Vacuum dried, coated to 200A$^{o}$ thicknesses with gold palladium prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.

***In vitro* dissolution analysis**

In vitro drug release from Entacavir microspheres was performed using USP Apparatus 1 in 900 ml of 0.05 M potassium phosphate buffer pH 7.2 stirred at 37 °C and 50 rpm maintaining sink conditions. The accurately weighed Entacavir microspheres were enclosed in a sieve, placed in the basket, and processed for dissolution testing. All the Entecavir microspheres stayed in the basket during 24-h dissolution testing (i.e., no particles diffused out of the sieve). Dissolution samples (5 ml) were withdrawn at regular intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16,

18, 20, 22 and 24h) using an auto sampler with replacement of equal volumes of fresh medium. The samples were filtered through a 0.45-µm filter and analyzed spectrophotometrically at 254 nm in triplicate. Drug concentration was calculated using a calibration curve.

**Kinetic-models:**

In order to describe the drug release kinetics from individual tablet formulations, the corresponding dissolution data were fitted in various kinetic dissolution models:

Zero order, first order, and Higuchi respectively.

$Q_t = Q_0 + K_0 t$…….. (3)

Where, Qt is the amount of drug released at time t; Q0 the amount of drug in the solution at t = 0, (usually, Q0 = 0) and K0 the zero order release constant.

$\log Q_t = \log Q\alpha + (K1 /2.303) t$…….. (4)

Qα being the total amount of drug in the matrix and K1 the first order kinetic constant.

$Q_t = KH. t ½$……. (5)

where, KH is the Higuchi rate constant.

Further, to better characterise the mechanism of drug release from matrices, dissolution data were analyzed using the equation proposed by Korsmeyer and Peppas.

$Q (t-l) /Q\alpha = KK (t-l)n$……. (6)

where, $Q_t$ corresponds to the amount of drug released in time t, *l* is the lag time (*l* = 2 hours), Qα is the total amount of drug that must be released at infinite time, KK a constant comprising the structural and geometric characteristics of the tablet, and n is the release exponent indicating the type of drug release mechanism. To the determination of the exponent n, the points in the release curves where Q (t-*l*)/Qα>0.6, were only used. If n approaches to 0.5, the release mechanism can be Fickian. If n approaches to 1, the release mechanism can be zero order and on the other hand if 0.5<n<1, non-Fickian (anomalous) transport could be obtained. Anomalous (non-Fickian) transport generally refers to the drug release by the summation of both diffusion and erosion of the polymeric matrix. The criteria employed to select the ‘‘best model’’ was the one with the highest coefficient of determination.

**Stability studies:**

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. $25^0$C/60% RH analyzed every month for period of three months.
2. $30^0$C/75% RH analyzed every month for period of three months.
3. $40^0$C/75% RH analyzed every month for period of three months.

## 3. Results and Discussion

**FT-IR studiess:** Pure Entacavir showed peaks at 3099.18 cm-1 (O-H stretching), 1622.30cm-1 (O-H deformation), 3358.54 cm-1 (N-H stretching) , 2963.81 cm-1 (aliphatic C-H stretching anti symmetric), 1452.96 cm-1 (aliphatic C-H deformation), 1715.93 cm-1 ( C=O stretching) and 1056.35 cm-1 (C-O stretching). Infrared absorption spectrum of formulation F7 shows peaks at 3003.29cm-1(O-H stretching),1622.27cm-1 (O-H deformation),3426.22 cm-1 (N-H stretching) , 2970.34 cm-1 (aliphatic C-H stretching anti symmetric), 1456.37 cm-1 (aliphatic C-H deformation), 1715.85 cm-1 ( C=O stretching) and 1064.19 cm-1 (C-O stretching). As the sharp characteristic peaks of Entacavir did not change in physical mixture with polymer and different excipients, indicating no possible interaction.

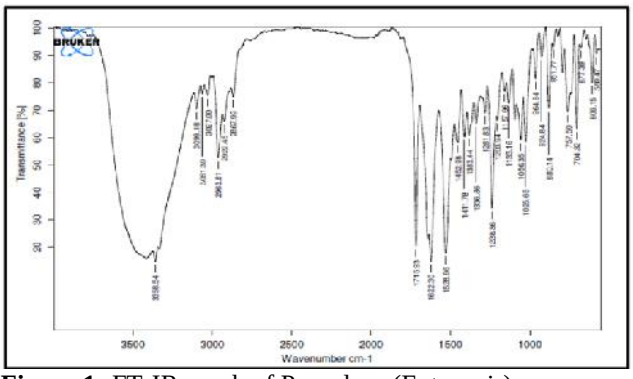


**Figure 1:** FT-IR graph of Pure drug (Entacavir)

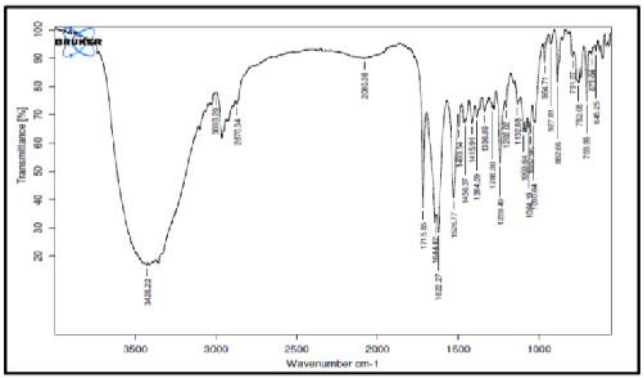


**Figure 2:** FT-IR graph of Optimized formula

**Table 2:** Results of Micromeritic properties of Microspheres

| Parameter | Angle of repose (Θ) | Bulk density (gm/cc) | Tapped density (gm/cc) | Carrs index (%) | Hausner's ratio | Drug content (%) |
|---|---|---|---|---|---|---|
| F1 | 25.43±0.1 | 1.041±0.3 | 1.16±0.1 | 11.4 | 1.114 | 98.56 |
| F2 | 26.46±0.2 | 1.02±0.4 | 1.12±0.2 | 9 | 1.09 | 98.48 |
| F3 | 23.31±0.1 | 1.01±0.2 | 1.11±0.1 | 9 | 1.09 | 97.59 |
| F4 | 26.89±0.7 | 1.02±0.28 | 1.11±0.21 | 8 | 1.08 | 98.64 |
| F5 | 29.14±0.1 | 0.96±0.24 | 1.03±0.27 | 7 | 1.07 | 98.46 |

| | | | | | | |
|---|---|---|---|---|---|---|
| F6 | 28.14±0.2 | 0.95±0.24 | 1.03±0.27 | 9.5 | 1.095 | 98.78 |
| F7 | **29.1±0.1** | **0.94±0.2** | **1.03±0.2** | 9 | **1.095** | **99.89** |
| F8 | 28.2±0.1 | 0.96±0.2 | 1.04±0.2 | 8 | 1.08 | 97.46 |
| F9 | 27.1±0.4 | 1.04±0.3 | 1.16±0.1 | 10.35 | 1.12 | 99.74 |

**Table 3:** Determination of drug entrapments efficiency, and yield

| Formulation Code | Particle Size (µm) | % Yield | Entrapment Efficiency (%) |
|---|---|---|---|
| F1 | 106.5±2.3 | 93.70±1.28 | 87.04±1.92 |
| F2 | 110.2±2.21 | 87.82±2.01 | 78.68±2.1 |
| F3 | 103.4±1.42 | 92.70±1.19 | 85.04±1.87 |
| F4 | 102.5±1.3 | 85.95±1.98 | 76.87±1.91 |
| F5 | 103.2±0.9 | 94.82±2.16 | 88.35±2.67 |
| F6 | 103±2.8 | 86.90±3.05 | 75.69±1.91 |
| F7 | **108.6±1.7** | **98.25±1.37** | **89.98±2.08** |
| F8 | 106.8±2.35 | 85.82±2.01 | 76.68±2.1 |
| F9 | 103.8±1.8 | 93.70±1.28 | 87.04±1.92 |

Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity; this influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. If there was increase in the amount of polymer concentration, there was increase in relative viscosity so as a result increases in mean particle size. And 7th formulation shows good micromeritic properties when compared to other formulations.

**Scanning electron microscopy analysis (SEM)**

The shape and surface characteristics were determined by scanning electron microscopy using gold sputter technique and the photomicrographs are given.

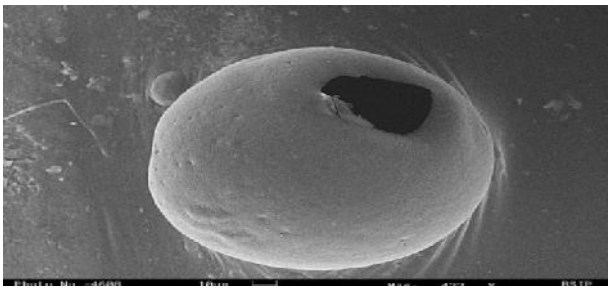

**Figure 3:** Scanning electron photomicrographs of Entacavir based microsphere

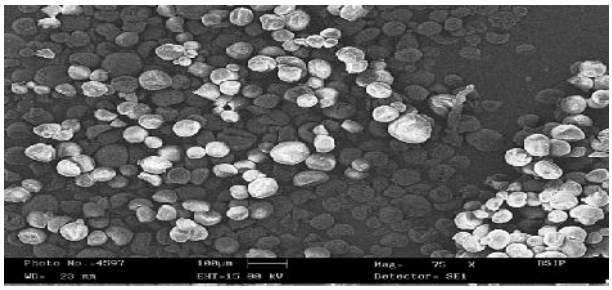

**Figure 4:** Scanning electron photomicrographs of population of microspheres

**In-vitro dissolution studies:**

Formulations F1, F2, F3, F4, F5 and F6 drug release not achieved to expected time release i.e released in 12 hrs. Formulation F8 and F9 showed a unsatisfactory release than F7, but the optimum level of sustained release effect will be observed in the batch F7 containing combination of Eudragit polymers.

**Table 4:** Dissolution profile of prepared formulations

| TIME (hrs) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 |
| 1 | 30.6 | 10.94 | 19.7 | 29.5 | 29.5 | 10.8 | **18.96** | 10.8 | 20.2 |
| 2 | 38.9 | 21.6 | 31.5 | 38.59 | 38.59 | 15.3 | **22.25** | 15.3 | 35.1 |
| 3 | 43.8 | 30.1 | 42.6 | 42.53 | 42.53 | 21.6 | **27.85** | 21.6 | 41.7 |
| 4 | 50.6 | 42.6 | 53.6 | 54.55 | 54.55 | 31.45 | **32.58** | 28.45 | 58.48 |
| 5 | 64.9 | 49.8 | 61.8 | 62.23 | 62.23 | 34.8 | **39.89** | 34.81 | 64.73 |
| 6 | 68.6 | 59.3 | 80.7 | 69.45 | 69.45 | 51.3 | **46.53** | 42.34 | 72.32 |
| 7 | 70.3 | 68.7 | 91.7 | 72.55 | 72.55 | 58.9 | **50.23** | 49.82 | 89.88 |
| 8 | 99.8 | 87.6 | 99.5 | 79.88 | 79.88 | 67.4 | **52.25** | 55.97 | 92.92 |
| 10 | - | 93.45 | - | 85.23 | 85.23 | 73.25 | **57.25** | 62.81 | 94.81 |
| 12 | - | 98.99 | - | 89.99 | 89.99 | 82.45 | **64.55** | 69.18 | 95.28 |
| 14 | - | - | - | 94.88 | 94.88 | 90.99 | **72.89** | 77.29 | 97.56 |
| 16 | - | - | - | 99.87 | 99.81 | 97.92 | **80.12** | 82.42 | 98.37 |
| 18 | - | - | - | - | - | 99.79 | **84.89** | 86.44 | 98.53 |
| 20 | - | - | - | - | - | - | **88.93** | 89.51 | 98.87 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 22 | - | - | - | - | - | - | **94.35** | 91.48 | 99.43 |
| 24 | - | - | - | - | - | - | **99.43** | 93.71 | 99.76 |

On the performance with respect to drug release characteristics, the formulation F7 was selected as the best formulation after optimization. This formulation showed that showed 99.43% release at 24$^{th}$ hour. The study has revealed that by increasing the polymer concentration, drug released rate of drug was improved and results confirmed that the drug released rate from microspheres depends on type and concentration of polymer used.

**Kinetic Models:**

**Table 5:** kinetic release data for formulations $F_1$ to $F_7$

| Formulation Code | Zero order | First order | Higuchi model | Koresmayer peppas model | | Best fit model |
|---|---|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ | Slope (n) | |
| F1 | 0.924 | 0.054 | 0.93 | 0.569 | 1.706 | Zero order & Higuchi |
| F2 | 0.97 | 0.01 | 0.934 | 0.299 | 1.187 | Zero order & Higuchi |
| F3 | 0.992 | 0.069 | 0.939 | 0.509 | 1.617 | Zero order & Higuchi |
| F4 | 0.866 | 0.011 | 0.988 | 0.238 | 1.001 | Zero order & Higuchi |
| F5 | 0.866 | 0.009 | 0.987 | 0.238 | 1.001 | Zero order & Higuchi |
| F6 | 0.953 | 0.103 | 0.955 | 0.034 | 0.339 | Zero order & Higuchi |
| **F7** | **0.93** | **0.995** | **0.978** | **0.982** | **0.73** | **First order & Peppa's** |
| F8 | 0.958 | 0.803 | 0.992 | 0.987 | 0.563 | Zero order & Higuchi |
| F9 | 0.666 | 0.973 | 0.86 | 0.88 | 0.481 | First order & Peppa's |

Dissolution data of above two methods was fitted in Zero order, First order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation. From the above kinetic data we conclude that the drug release follows zero order and mechanism of drug release is diffusion controlled because it shows higher regression value in Higuchi model. And that koresmayer peppas model shows that the drug release indicates analomous super case-II transport.

**Stability Study**

There was no significant change in physical and chemical properties of the tablets of formulation F-7 after 3 Months.

## 4. Conclusion

Entacavir microspheres were prepared successfully by using the Quasi-emulsion solvent diffusion method. Polymer-drug ratio influences the particle size as well as drug release pattern of microsphere. The yield was high and encapsulation efficiency was good for all the preparation, and was highest for F7 formulation. As the polymer concentration increases, the particle size increases. Initially at gastric medium (pH 1.2), very less release of drug (Entacavir) from microspheres was found, but pH 7.4 all formulations (percentage drug entrapment efficiency-89.98±2.08, percentage yield-98.25±1.37,particle size-108.6±1.7,and in-vitro drug release optimized value-99.43) showed burst release initially and then tend to release at constant rate. As per our aim, formulation does not show release in gastric medium for desired period of time and releases the drug at pH 7.4, which is the pH of colon and their allied areas; the prepared microspheres proved to be good candidate for site-specific drug delivery.

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