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# Formulation development and characterization of selegiline loaded nanoparticles For the effective management of parkinson's disease

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## ABSTRACT

Controlled drug release system is one of the most favorable techniques of novel drug delivery system owing to its reproducibility and ease of formulation. Nanotechnology is very useful for controlling the drug release and thus improving the pharmacokinetic and pharmacodynamic properties of the drug. The technique improves patient compliance by reducing both dose and the frequency of administration and thus minimizing the local as well as systemic toxic effects. The aim of the present research work was to formulate and evaluate nanoparticles of Selegiline, an Anti-Parkinson Drug by using the Emulsion solvent evaporation method. Selegiline has a very short half-life of 1.5-3.5 hour with bioavailability oral 10%. Sustained release nanoparticles of Selegiline were prepared to increase the drug residence time in gastrointestinal tract and thus improving the bioavailability of drug. The nanoparticles were prepared by using Chitosan and Carbopol 940 as polymers. Different formulations were prepared with varying concentrations of Chitosan and Carbopol 940 in order to achieve the optimum particle size and maximum encapsulation efficiency. The particle size of nanoparticles was found to be in the range of 0.181±0.051 nm to 0.390±0.101 nm. Drug encapsulation efficiency ranged between 58.1±0.651 percent to 82.9±1.216% with controlled drug release up to 99.29% in phosphate buffer pH 6.8, 12 hrs. FT-IR studies showed that the drug and polymers were compatible. The results of Nanoparticles indicated that optimized formulation exhibited excellent properties.

**Keywords:** Nanoparticles, Chitosan, Carbopol 940, Selegiline and Emulsion solvent evaporation method.

## INTRODUCTION

The development of dosage form is often necessary to achieve the desired release pattern and effective therapeutic response to improve patient compliance. During the last two decades, advanced drug delivery research and development activity has surged because of the afore mentioned medical and economic driving forces.

Nanotechnology is emerging as a promising tool for the twenty first century. Pharmaceutical nanotechnology is the application of nanoscience to pharmacy. A concept of novel drug delivery approach using design of nanomedicine has been now well established in current pharmaceutical scenario. Pharmaceutical nanotechnology has provided fine- tuned diagnosis and treatment of disease at molecular level using various nanomedicine like liposomes, dendrimer, polymeric micelles, nanospheres, niosomes; and more recently, carbon nanotubes and quantum dots etc.

Nanoparticles based drug delivery systems have created great impact on practically every branch of medicine including cardiology, ophthalmology, endocrinology, oncology, pulmonology, immunology and also on highly specialized areas like gene delivery, targeting to brain, tumor targeting, oral vaccine formulations and other areas. The pharmaceutical nanotechnology market especially in diagnostic and carrier for drug has been rapidly growing over last two decades. Some pharmaceutical nanotechnology based products liposome, nanoparticles, polymer micelles, dendrimer, monoclonal antibody, modified nanosystems have been approved by US-FDA and have entered the market. However, some unknown health risk, unpredictable and undefined safety issues and some clinical as well as regulatory issues still pose formidable challenges.

The emergence of nanotechnology and microtechnology and the growing capabilities of proteomics, genomics, and combinatorial chemistry have provided scientists and engineers with new techniques for creating novel advanced drug delivery technologies. Commonly accepted criteria of advanced drug delivery systems include maximal drug bioavailability, tissue targeting, controlled release kinetics, minimal immune response, ease of administration for patient compliance, and the ability to deliver traditionally difficult drugs such as lipophilic, amphiphiles and biomolecules. Modern drug delivery requirements are the targeted and controlled delivery of drugs, proteins, and genes into cells with reduced side effects and easier administration.

Therapeutic efficacy of the drug depends on four fundamental pathways of drug transport and modification within the body: absorption into the plasma from the administration site; distribution between the plasma and tissues; metabolism within the tissues and elimination from the body.

Absorption rate depends on many factors such as hydrophobicity, chemical environment, particle size, crystallinity, blood flow, absorptive surface area, and residence time at an absorptive surface. Drug distribution largely depends on blood flow, capillary permeability such as in the blood-brain barrier, ligand binding, and hydrophobicity. Drug metabolism and elimination primarily depend on the afore mentioned properties.[1]

The drug delivery system can greatly impact each pathway and therefore, the delivery system is a critical design component in pharmaceutical sciences. Despite the intense research efforts spanning several decades, targeted and controlled delivery of poorly water soluble drugs remains one of the more elusive objectives in the pharmaceutical sciences.

### *Controlled Drug Delivery*

A typical controlled release system is designed to provide constant or nearly constant drug levels in plasma with reduced fluctuations via slow release of drug over an extended period of time. Over the last two decades, controlled technology has received increasing attention from the pharmaceutical industry and academia. As new technologies emerge, they not only open up a wide range of new therapeutic opportunities, but also offer the benefits of product differentiation, market expansion, and patent extension. An ideal drug delivery system should deliver the drug at required by the needs of the body over the specified period of treatment. In many cases, it would be desired to deliver drugs at a specific site inside the body to a particular diseased tissue or organ. This kind of regional therapy mechanism would reduce systemic toxicity and achieve a peak drug level directly at the target site. The basic rationale of controlled drug delivery concept is to change the pharmacokinetic and pharmacodynamics of bioactive either by modifying the molecular structure or physiological parameters by an alternative selected route of administration or by using novel drug delivery.

The primary goal of controlled drug delivery is to ensure safety and enhancement of drug with better modification and control of plasma drug levels and reduction in dosing frequency. Controlled release therapeutic system may be either passive programmed or active programmed or active self-programmed. Most of the rate-controlled delivery system belongs to passive pre-programmed in which the release rate is predetermined and is not influenced or determined by the external biological environment. Many times, sustained release and controlled release terms are interchangeable. However, a sustained release system delivers the active ingredients at a slower rate than a conventional formulation but the release is substantially affected by external environment. At the other extreme controlled release systems provide a release profile predominantly controlled by the design of the system.

### *Advantages of Controlled drug delivery system:*

- Controlled delivery agent at programmed design to deliver at constant rate.
- Maintenance of optimum and effectual drug level for prolonged action.
- Reduction of unwanted side effects.
- Increase in patient fulfillment.
- Reduction in regularity of dosing.
- Release of drug in the vicinity of site of action.
- More efficient consumption of active agent.

### *Nanoparticles*

Colloidal particles range in size between 10 and 1000 nm are identified as nanoparticles. Although mainstream nanotechnology explores particles between 1 to 100 nm in diameter, the size of the individual particles tested for drug delivery of Introduction….. 5 therapeutic and imaging agents may range from 2 to 1000 nm.4 They are classified into two groups:

Nanosphere defined as solid core spherical particulates, which are nanometric in size. They contain drug embedded within the matrix or adsorbed on to surface.

Nanocapsules are vesicular system in which drug is essentially encapsulated within the central volume surrounded by an embryonic continuous polymeric sheath. They are manufactured from synthetic or natural polymers and ideally suited to optimize drug delivery and reduce toxicity. Nanoparticles have been successfully used for systemic, oral, pulmonary and transdermal routes. The successful operation of nanoparticles for drug release depends on their ability to penetrate through several anatomical barrier, sustained release of drug and stability in the nanometric size. However, the lack of safe polymers with rigid approval and their high cost have limited the extensive application of nanoparticles in clinical medicine.5 Biodegradable polymers from natural or synthetic origin are degraded either enzymatically or non-enzymatically or both to produce biocompatible, toxicologically safe by-products which are further eliminated by the normal metabolic pathways.

## MATERIALS

Selegiline Provided by Sura Labs, Dilsukhnagar, Hyderabad, Chitosan Chemical Drug House, New Delhi , Carbopol 940 Chemical Drug House, New Delhi, Tween 80 Merck, Span
60 Merck, Distilled water ,Rankem, Dichloromethane, Rankem, Methanol, Merck

## METHODOLOGY

### *Analytical Method Development Determination of absorption maxima*

Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of methanol (1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate buffer (5.5pH). From this stock solution pipette out 1ml and dilute to 10 ml with phosphate buffer and subject for UV scanning in the range of 200-400 nm using double beam UV spectrophotometer. The absorption maxima were obtained at 252 nm with a characteristic peak.

### *Preparation of calibration curve*

It is soluble in Methanol; hence Methanol was used for solubilizing the drug. Stock solution (1 mg/mL) of Selegiline was prepared in Dichloromethane and subsequent working standards (5,10,15,20 and 25 µg/mL) were prepared by dilution with phosphate buffer of pH-5.5. These solutions were used for the estimation Selegiline by UV method. The whole procedure was repeated three times and average peak area was calculated. Calibration plot was drawn between concentrations and peak area. Calibration equation and $R^2$ value are reported.

### *Preparation of nanoparticles*

### *Preparation of Selegiline loaded nanoparticles*

Polymeric Nanoparticles were prepared by Emulsion solvent evaporation method. Required quantity of polymer and drug were weighed & dissolved in 12ml of dichloromethane. Quantity of Tween 80 and Span 60 was mixed with of water & this solution was kept in another beaker. Both the phases were kept for sonication for 15 min. until it become clear. Solution containing drug and polymer were added drop wise to aqueous phase under continues stirring. The formed nanoparticles suspension were homogenized at 18000 rpm for 15min then followed by magnetic stirring for 3hr. The suspension was centrifuged at 9,000 rpm for 45 min. The samples were added to glass vials & freeze-dried with mannitol 2% (w/v) as cryprotectant in a lyophilizer.

**Table 1: Composition of nanoparticles formulations (F1 to F8)**

| Excipients | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| Selegiline | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Chitosan % | 1 | 2 | 3 | 4 | - | - | - | - |
| Carbopol 940 % | - | - | - | - | 1 | 2 | 3 | 4 |
| Tween 80 (mL) | 0.5 | 1 | 1.5 | 2 | - | - | - | - |
| Span 60 (mL) | - | - | - | - | 0.5 | 1 | 1.5 | 2 |
| Distilled water (ml) | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| Dichloromethane (ml) | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Methanol | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

## RESULTS AND DISCUSSION

### *Preparation of Standard Graph Determination of absorption maxima*

The standard curve is based on the spectrophotometry. The maximum absorption was observed at 252nm.

### *Calibration curve*

Graphs of Selegiline was taken in 6.8 Phosphate buffer

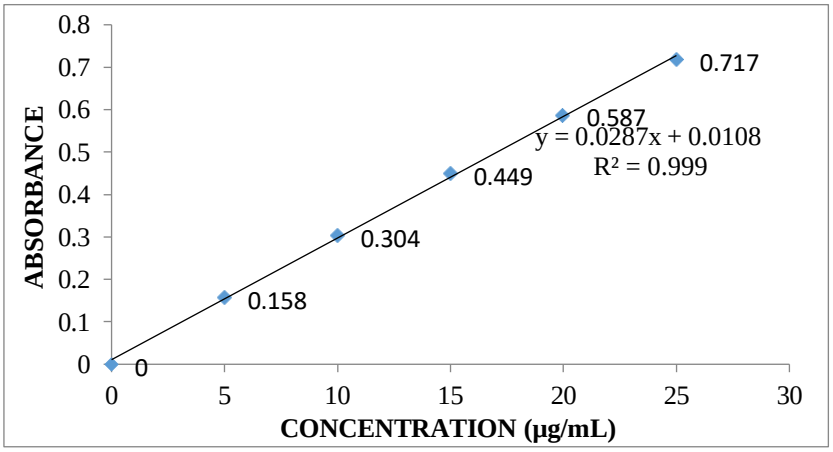


**Fig 1: Standard graph of Selegiline in pH 6.8 Phosphate buffer**

Standard graph of Selegiline was plotted as per the procedure in experimental method and its linearity is shown in Table 8.1 and Fig 1. The standard graph of Selegiline showed good linearity with $R^2$ of 0.999, which indicates that it obeys "Beer- Lamberts" law.

## *Evaluation Ofselegiline Loaded Nanoparticles*

**Table 2: Evaluation of Nanoparticles**

| Batch No | Mean Particle size (nm) | %Yield | Drug Content | Drug encapsulation efficiency | PDI | Zeta Potential (mV) |
|---|---|---|---|---|---|---|
| **F1** | 0.164±0.09 | 62.14 | 93.69 | 58.1±0.651 | 0.280±0.036 | -25.1±0.301 |
| **F2** | 0.171±0.025 | 78.92 | 95.33 | 61.6±0.215 | 0.293±0.028 | -23.5±0.810 |
| **F3** | 0.290±0.061 | 81.64 | 97.62 | 63.1±5.621 | 0.241±0.040 | -23.0±8.641 |
| **F4** | 0.181±0.051 | 90.94 | 98.99 | 82.9±1.216 | 0.421±0.015 | -35.9±1.824 |
| **F5** | 0.230±0.089 | 64.82 | 90.44 | 81.1±2.356 | 0.432±0.021 | -32.7±0.581 |
| **F6** | 0.376±0.101 | 72.61 | 92.65 | 84.9±2.306 | 0.380±0.035 | -30.6±0.301 |
| **F7** | 0.223±0.081 | 81.94 | 93.55 | 73.9±3.219 | 0.590±0.031 | -42.4±0.302 |
| **F8** | 0.390±0.101 | 86.39 | 94.05 | 75.6±2.603 | 0.643±0.025 | -36.2±0.392 |

Percentage yield of formulations F1 to F8 by varying drug was determined and is presented in Table 2. Highest drug content, Highest Entrapment efficiency observed for F4 formulation.

PDI observed in the F4 formulation i.e., 0.403 respectively. The Zeta potential range from -23.5±0.810 mV to -42.4±0.302 mV to all the formulations.

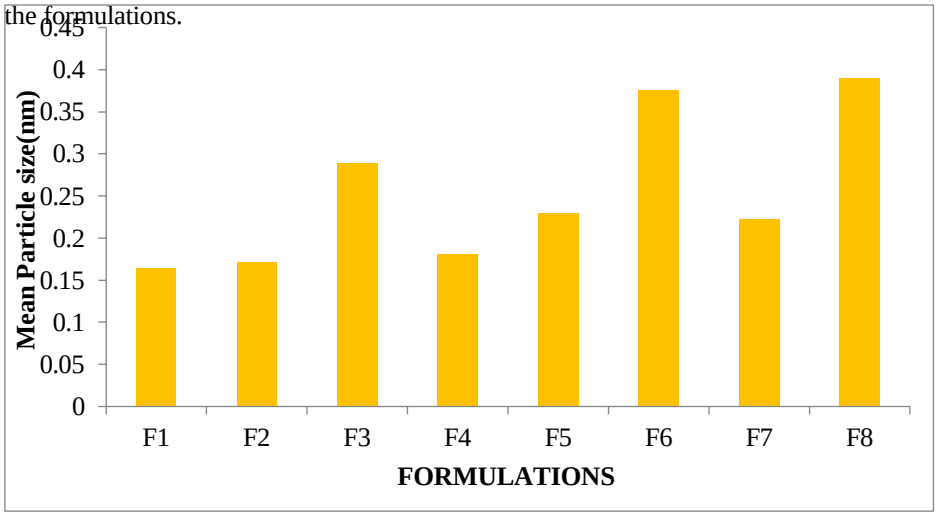


**Fig2: Mean Particle size (nm)**

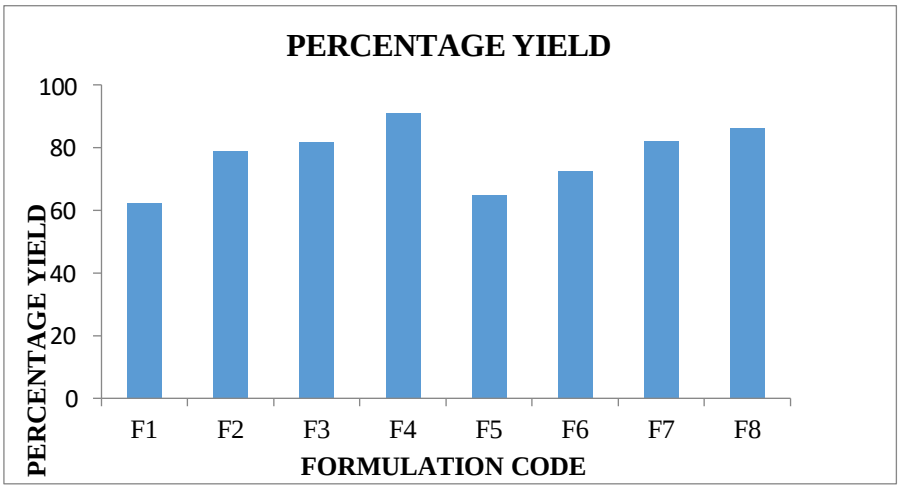


**Fig3:%Yield**

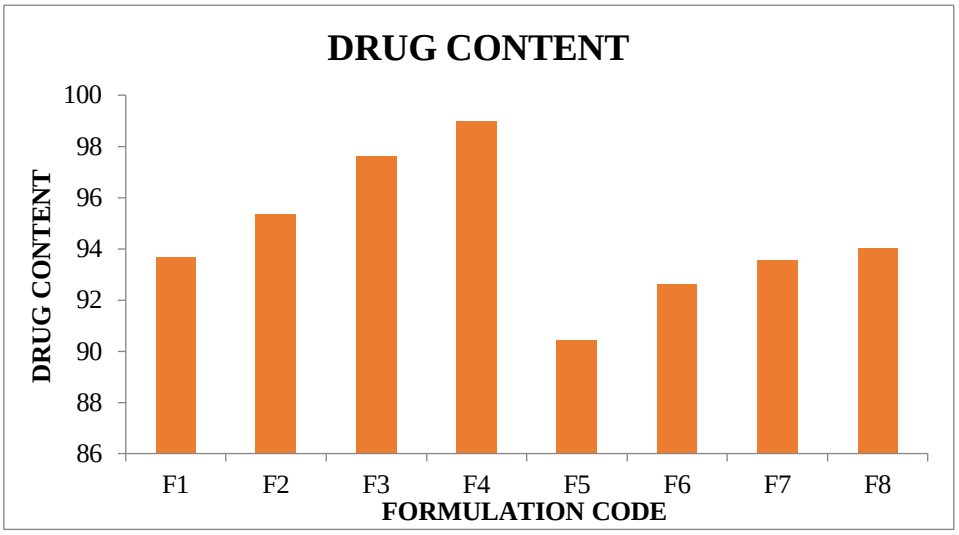


**Fig4:Drug content**

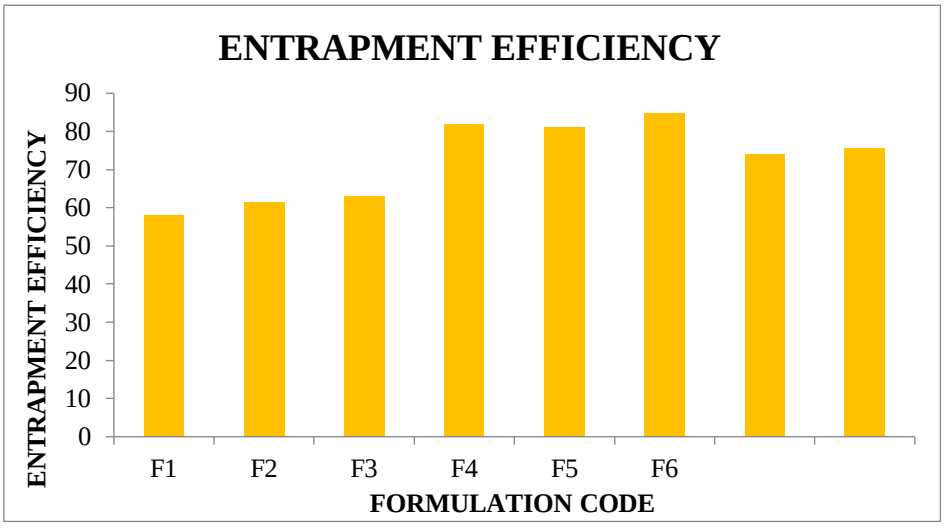


**Fig 5:Drug encapsulation efficiency**

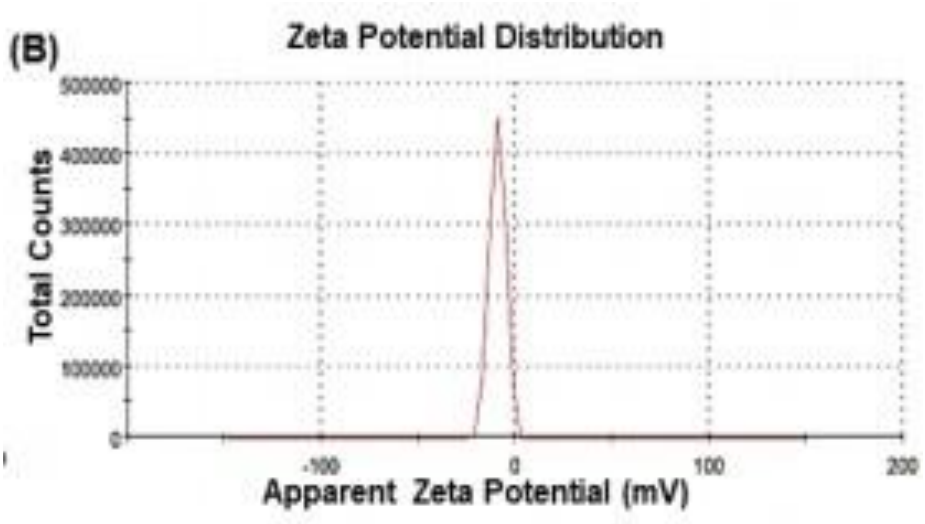


**Fig 6: Zeta Potential of F4 Formulation**

## *In vitro Drug release studies*

**Table 3: *In vitro* Drug release studies of Selegiline**

| TIME (hr) | CUMULATIVE PERCENT OF DRUG RELEASED | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 36.15 | 29.10 | 24.98 | 21.57 | 45.63 | 35.96 | 27.56 | 23.61 |
| 2 | 49.62 | 34.53 | 28.60 | 27.49 | 50.75 | 45.48 | 35.43 | 30.26 |
| 3 | 56.98 | 48.86 | 33.54 | 32.26 | 67.14 | 56.41 | 42.29 | 37.14 |
| 4 | 60.83 | 57.54 | 49.72 | 40.52 | 73.60 | 63.24 | 52.59 | 43.11 |
| 5 | 76.47 | 65.99 | 55.34 | 56.14 | 77.59 | 68.67 | 65.63 | 50.14 |
| 6 | 89.68 | 77.42 | 60.75 | 65.38 | 80.37 | 73.68 | 70.15 | 54.33 |
| 7 | 97.89 | 84.27 | 76.18 | 78.89 | 95.10 | 86.11 | 85.66 | 68.91 |
| 8 | | 91.38 | 82.26 | 91.14 | | 96.93 | 90.34 | 79.14 |
| 10 | | 95.18 | 95.74 | 95.72 | | | 95.24 | 88.63 |
| 12 | | | 97.51 | 99.29 | | | 97.14 | 93.25 |

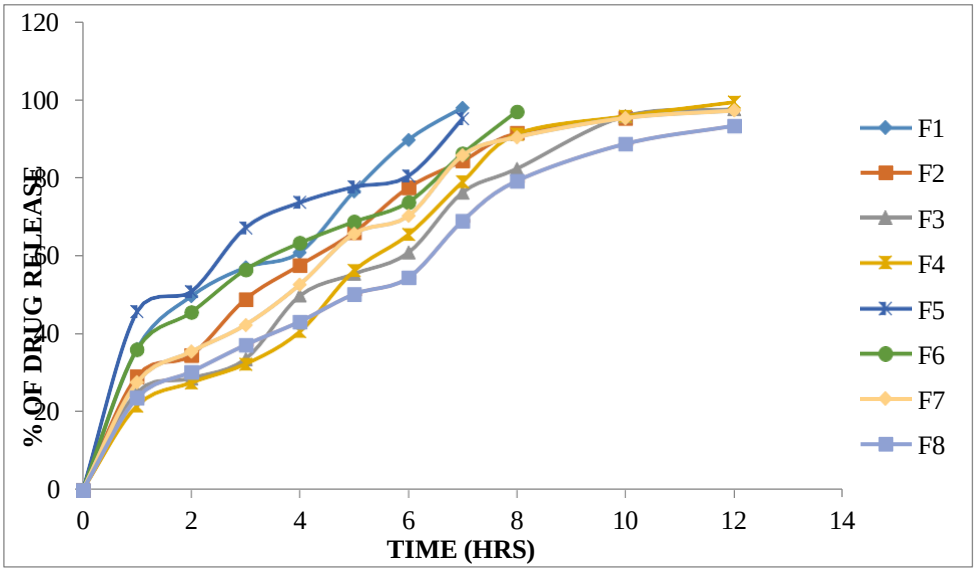


**Fig 7: Dissolution study of Selegiline Nanoparticles**

Hence based on dissolution data of 9 formulations, F4Chitosan (1:4 (4%)) formulation showed better release (99.29%) up to 12 hours. So F4 formulation is optimised formulation.

## *Application of Release Rate Kinetics to Dissolution Data*

Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of drug release from Nanoparticles. The data was fitted into various kinetic models such as zero, first order kinetics; higuchi and korsmeyerpeppas mechanisms and the results were shown in below table it follows the zero order kinetics.

**Table 4: Release kinetics data for optimized formulation (F4)**

| CUMULATIVE (%) RELEASE Q | TIME ( T ) | ROOT (T) | LOG( %) RELEASE | LOG ( T ) | LOG (%) REMAIN | RELEASE RATE (CUMULATIVE % 1/CUM% RELEASE | PEPPAS log Q/100 | % Drug Remaining | Q01/3 | Qt1/3 | Q01/3-Qt1/3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | | | 2.000 | | | 100 | 4.642 | 4.642 | 0.000 |
| 21.57 | 1 | 1.000 | 1.334 | 0.000 | 1.894 | 21.570 | 0.0464 | -0.666 | 78.43 | 4.642 | 4.280 | 0.361 |
| 27.49 | 2 | 1.414 | 1.439 | 0.301 | 1.860 | 13.745 | 0.0364 | -0.561 | 72.51 | 4.642 | 4.170 | 0.472 |
| 32.26 | 3 | 1.732 | 1.509 | 0.477 | 1.831 | 10.753 | 0.0310 | -0.491 | 67.74 | 4.642 | 4.076 | 0.565 |
| 40.52 | 4 | 2.000 | 1.608 | 0.602 | 1.774 | 10.130 | 0.0247 | -0.392 | 59.48 | 4.642 | 3.904 | 0.738 |
| 56.14 | 5 | 2.236 | 1.749 | 0.699 | 1.642 | 11.228 | 0.0178 | -0.251 | 43.86 | 4.642 | 3.527 | 1.115 |
| 65.38 | 6 | 2.449 | 1.815 | 0.778 | 1.539 | 10.897 | 0.0153 | -0.185 | 34.62 | 4.642 | 3.259 | 1.382 |
| 78.89 | 7 | 2.646 | 1.897 | 0.845 | 1.324 | 11.270 | 0.0127 | -0.103 | 21.11 | 4.642 | 2.764 | 1.878 |
| 91.14 | 8 | 2.828 | 1.960 | 0.903 | 0.947 | 11.393 | 0.0110 | -0.040 | 8.86 | 4.642 | 2.069 | 2.572 |
| 95.72 | 10 | 3.162 | 1.981 | 1.000 | 0.631 | 9.572 | 0.0104 | -0.019 | 4.28 | 4.642 | 1.624 | 3.018 |
| 99.29 | 12 | 3.464 | 1.997 | 1.079 | -0.149 | 8.274 | 0.0101 | -0.003 | 0.71 | 4.642 | 0.892 | 3.749 |

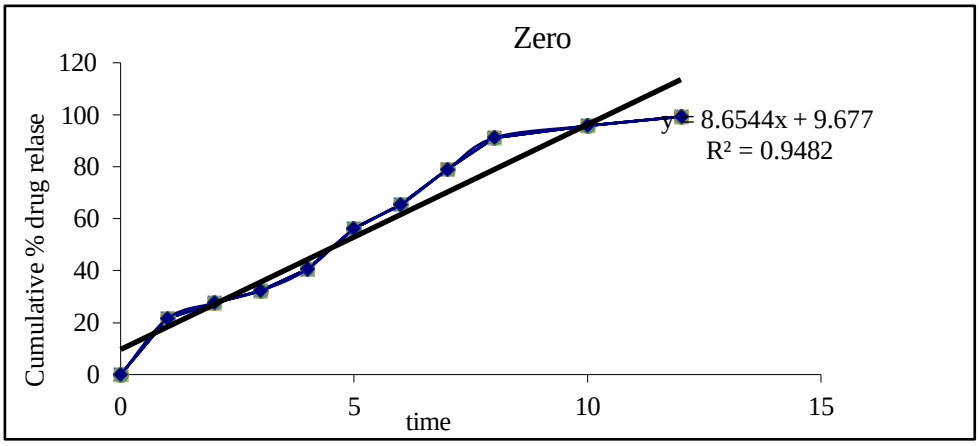


**Fig 8: Graph of zero order kinetics**

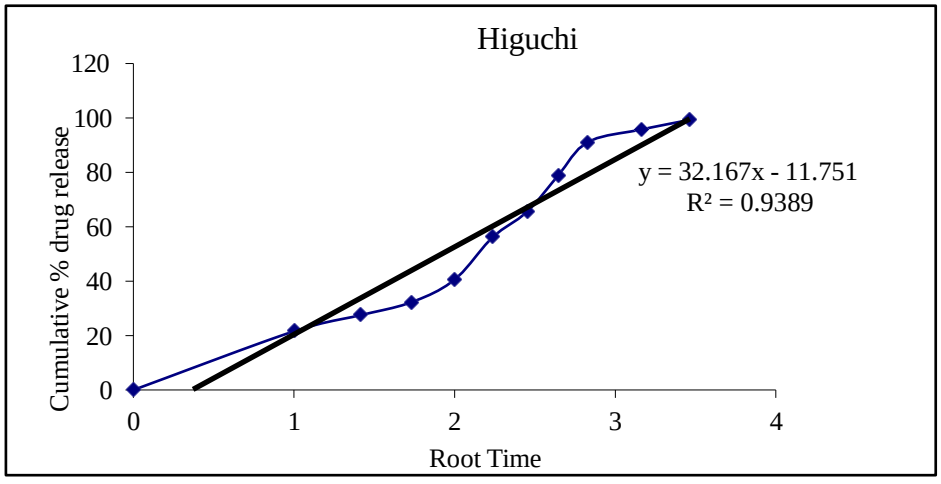


**Fig 9: Graph of higuchi release kinetics**

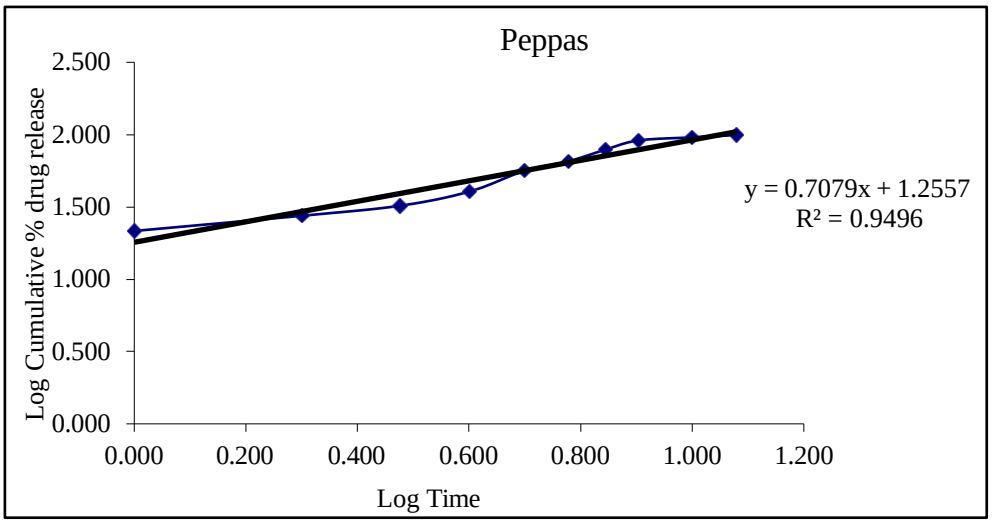


**Fig 10: Graph of peppas release kinetics**

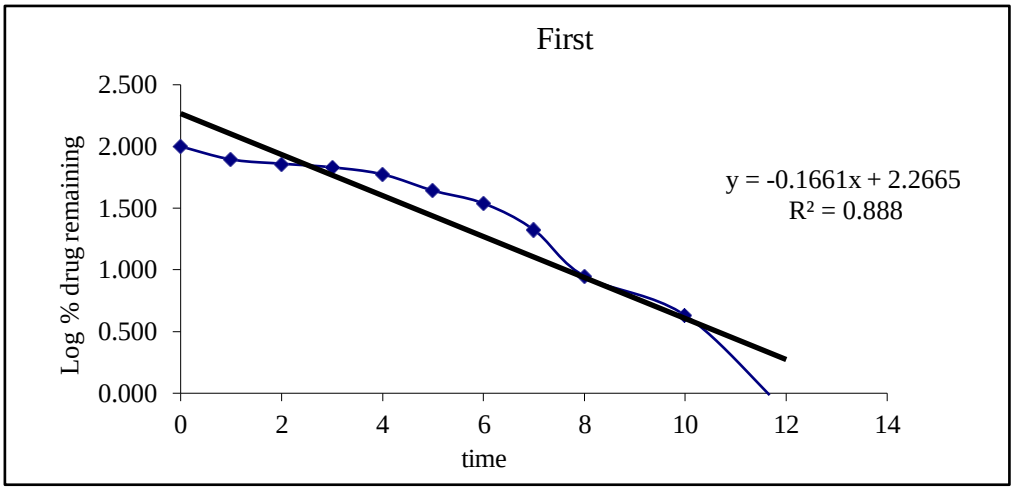


**Fig 11: Graph of first order release kinetics**

Based on the data above results the optimized formulation followed **Peppas release** kinetics.

## *Drug – Excipient compatibility studies*
## *Fourier Transform-Infrared Spectroscopy*

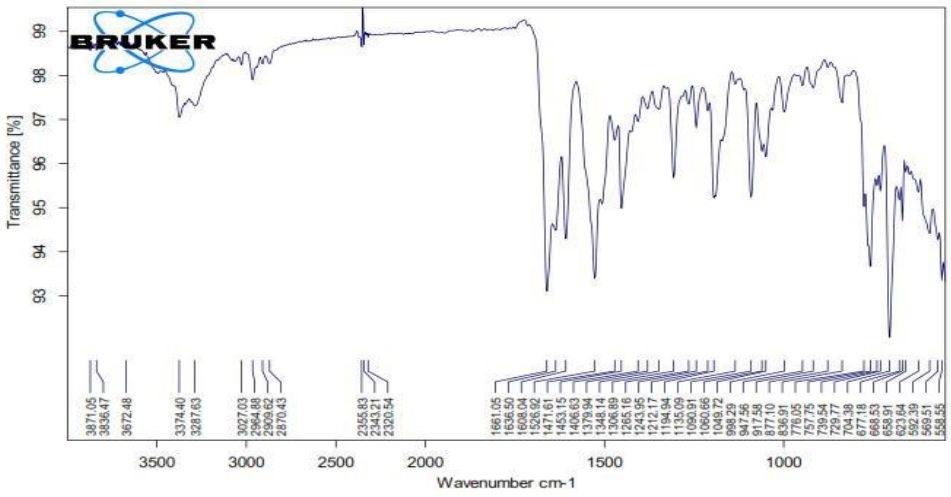


**Fig 12: FT-TR Spectrum of Selegiline pure drug.**

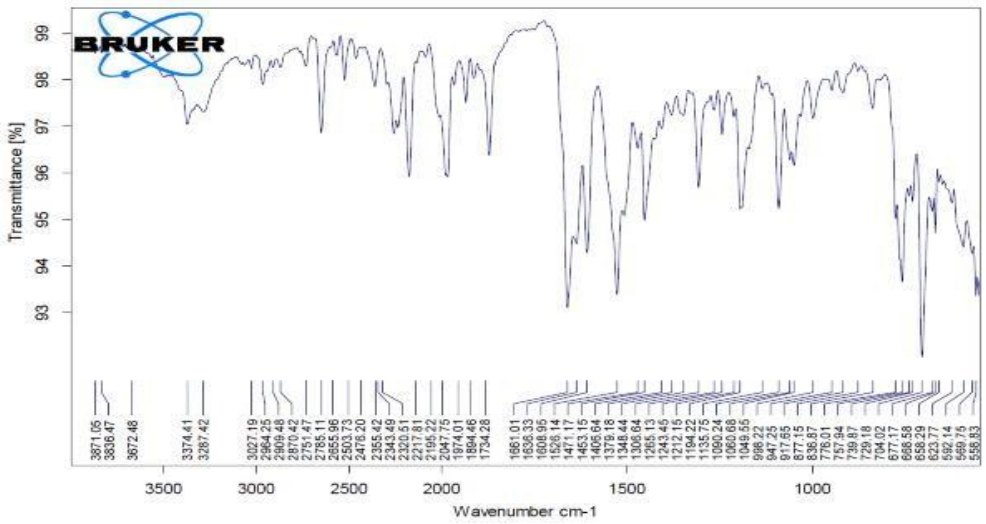


**Fig 13: FT-IR Spectrum of Optimised Formulation**

There is no incompatibility of pure drug and excipients. There is no disappearance of peaks of pure drug and in optimised formulation.

***SEM***

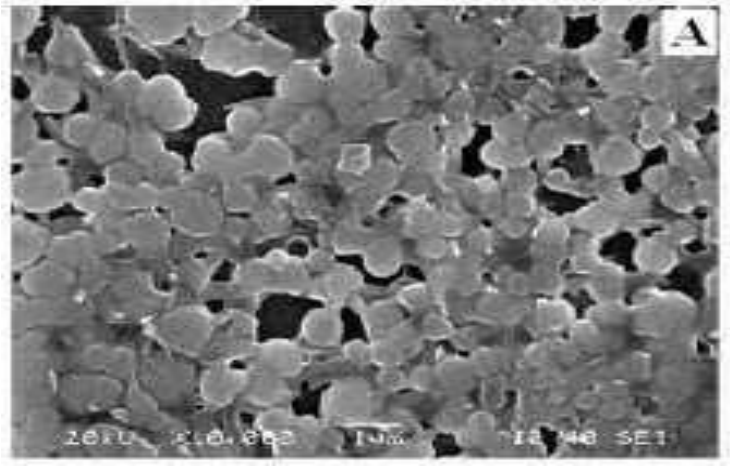


**Fig 14: SEM graph of optimized formulation**

SEM studies showed that the Selegiline - loaded nanoparticles had a spherical shape with a smooth surface as shown in Figure.

***XRD***

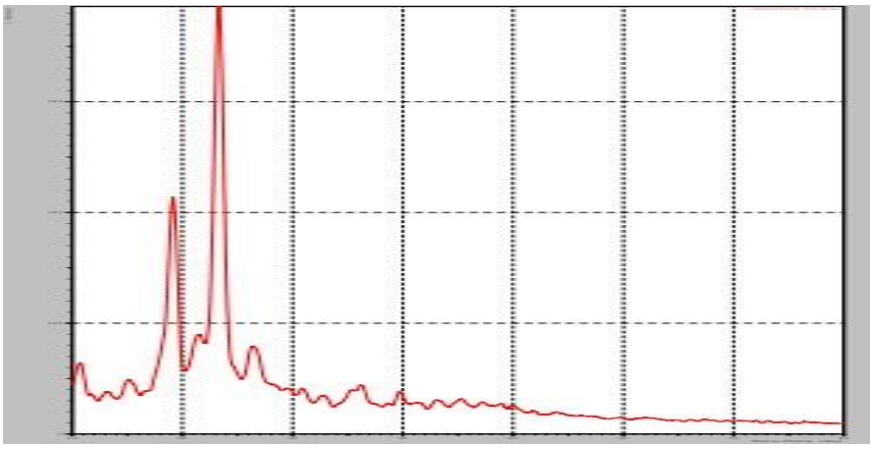

**Fig 15: Selegiline F4 optimised formulation**

## CONCLUSION

The method used for preparation of nanoparticles of Selegiline was found to be simple and reproducible. The slow and constant release of Selegiline from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. The developed formulation overcome and alleviates the drawbacks and limitations of Selegiline sustained release formulations. The development

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of effective nano delivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators. On the basis of different parameters i.e. physicochemical and *in-vitro* release study, nanoparticles of batch F4 are concluded as optimum formulations. Further, it can be concluded that the nanoparticulate formulation can be an innovative and promising approach for the delivery of Selegiline.


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