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DEVELOPMENT AND EVALUATION OF MUCOADHESIVE NANOPARTICLES A NOVEL DRUG DELIVERY FOR THE MANAGEMENT OF PARKINSONISM DISEASES

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ABSTRACT

Anti Parkinson's drugs are used for the management of a neurodegenerative disorder characterized by a multifactorial nature because of reduction in dopamine level in the human brain called Parkinsonism. Normally a combination of two drugs namely Levodopa and Carbidopa is used in a ratio of 4:1 for the management of parkinsonism. Subsequently, strict adherence to the dosing frequency is strongly emphasised to attain therapeutic effects of the drug, in this research the efforts have been taken to develop a mucoadhesive drug delivery systems using nanoparticle technology. Nanoparticles were prepared by spray drying techniques. The drugs [Levodopa and Carbidopa (4:1)] was fused into the nanoparticles, the percentage yield, loading efficiency, surface characteristic studies, particle size, swelling index, drug content, ex vivo, drug release, drug distribution were carried out. Results: The yield percentage and drug loading were determined as 98.5 ± 0.5% and 84.06 ± 0.5%, respectively. The microphotographs revealed that the nanoparticles possess smooth surface and were spherical in shape, with particle size about 460 nm with 390 - 540 range of scattering and swelling Index was found to be 185.6±12%. The mucoadhesive strength was revealed to excellent (9.08± 05 hr) during ex vivo studies. The release of drug was prolonged to about 12 hr from the developed formulation. The prolonged retention in GIT (~12 h) also proves the benefits associated with oral delivery of nanoparticles with higher efficiency. Conclusion: The developed formulation of mucoadhesive nanoparticles may possibly help as the perfect technology for the effective

and safe administration of anti- parkinsonism drugs for the management of Parkinsonism disease.

KEYWORDS: Parkinsonism, Levodopa, Carbidopa, Nanoparticle, Mucoadhesive, Spray drying, Burst release.

INTRODUCTION

The Parkinson's Disease Foundation has reported that, seven to ten million people are affected by Parkinson's disease worldwide, and this number will increase considerably in near future.[1] Parkinson's is a devastating neurodegenerative disorder occurs due to reduction in dopamine level in the brain. The dopaminergic neurons in the substantia nigra are progressively destroyed leading to a continuous decline in the amount of dopamine in the basal ganglia, still the exact pathological reasons of Parkinson's Disease are unclear. The drugs generally treat symptoms associated with Parkinsonism disease.

The oral route of administration is the most convenient, easy and preferred routes for drug administration. Even though, the orally administered drugs are exposed to hepatic first-pass metabolism or metabolism in GI tract or both. The delivery through nasal, rectal, vaginal, ocular and oral mucosa may be the potential alternative solution for delivery of such classes of drugs. The mucoadhesive nanoparticle drug delivery systems enhance the bioavailability of the drugs by avoiding the first pass effects and escaping the pre systemic elimination of the drug within the GI tract.[2]

In order to reduce drug related side effects and to enhance therapeutic effects of a drug mucoadhesive nanoparticle drug delivery systems was developed.[3,4] A mixture of the mucoadhesive polymer to form mucoadhesive devices which adhere to mucous intestinal lining and releasing fused drug gradually was used. The advantages of mucoadhesive drug delivery systems over the other existing conventional drug delivery systems is that they not only target and localise dosage but also result in higher drug flux at the absorption site in the Gastrointestinal tract (GIT) specifically, they further reduce the drug associated side effects by stabilising the drug fluctuations in the plasma as well.[5] Thus, this research work was designed to evaluate the properties of drug combination [Levodopa – Carbidopa (4:1)] release in GIT using a rat model. Also, a mucoadhesive formulation of drug combination [Levodopa–Carbidopa (4:1)] nanoparticle drug delivery system was optimized and established. On the basis of the published literature and reports albumin was used as bioadhesive which has

controlled release properties and also significantly increases the delivery of active constituents to various sites.

MATERIALS AND METHODS

Materials: Levodopa and Carbidopa was procured as gift sample (Divi's Laboratories, Hyderabad, India.), bovine serum Albumin was obtained as gift sample from Modern laboratories, Indore, M.P. India. Other chemicals and reagents used were of analytical grade.

Drug and excipient compatibility

The IR spectroscopy was performed and the spectrum was recorded in the wavelength region of 4000m – 400cm-1 for Levodopa and Carbidopa alone and along with the excipients used in the present research in order to detect any type interaction between the drug and the excipients.

Preparation of Nanoparticles: Albumin fused drug [Levodopa – Carbidopa (4:1)] nanoparticles was prepared by nano-spray drying technique. Concisely, a spray nozzle of 5µm was used at 100% relative fixed spray rate with a 125 L/min flow rate. The temperature of inlet and outlet was set at 100°C, Feed flow was set between 20% respectively. Nano-spray drying technique for preparing nanoparticles is widely used in pharmaceutical and food science.[6,7]

A solution of 1000mg of polymer was suspended in 25 ml of acetone, a combination of drug equivalent to 500mg was dissolved in 10 ml of dimethyl sulfoxide both the solution was then filtered to prevent nozzle blockage and spray dried. Successively, the chamber was scraped and dried particles collected by using a powder scraper and the product was collected and stored at 25°C in a vacuum desiccator for further studies.[8]

Characterization of Formulated Nanoparticles

Percentage Yield: it is the ratio of actual theoretical yield and the experimental yield obtained. Normally the percentage yield is always less due to incomplete reaction or the loss of sample during recovery. The percentage yield was calculated using equation.

$$\text{Percentage Yield} = \frac{\text{Amount of nanoparticles collected}}{\text{Total amount of the polymer and drug}} X100$$

Drug Loading Efficiency: 50 mg of formulated nanoparticles were dissolved in 50 ml of 0.1 M Hydrochloric acid: it was filtered through a Millipore filter and the drug contents were determined at 280 nm, using UV spectrophotometer, and the loading efficiency was calculated using equation.

$$\text{Percentage drug Loading} = \frac{\text{weight of nanoparticles}}{\text{Amount of drug present in the nanoparticles}} X100$$

Drug content: A weighed quantity of formulated nanoparticles were triturated into fine powder using mortar and pestle and 100 ml of Phosphate buffered saline (PBS) pH 7.4 was added and mixed properly and kept aside for 24 hrs. After 24 hr the solution obtained was agitated for 15 min and filtered using whattman filter paper and was analysed using by UV-spectrophotometer at a wavelength of 280 nm. The % drug content was calculated using equation.

$$\%\ \text{drug content} = \frac{\text{Estimated Drug content}}{\text{Total drug amount taken}} X100$$

Surface Characterization: The formulated nanoparticles batches were evaluated using scanning electron microscopy (SEM) at 20 kV viewed at 1,000x to 95,000x range of magnifications followed by platinum sputtering for surface characteristic studies.[9,10] Further the laser light diffraction and ultrasonic techniques were applied to investigate the distribution of the nanoparticles.[11]

Particle size measurement: The formulated nanoparticles were observed using Malvern Zetasizer for particle size determination. The glass cuvette were used for the purpose, the 3/4th cuvette was with organic solvent and the sample and ultra- sonicated, further the obtained suspension, were suspended and used for the measurement of particle size.[12]

Swelling Index: A 1:1 solution of ethanol: methanol was prepared freshly and used to measure the degree of swelling of the nanoparticles, Baseline measurements and after incubation with ethanol/methanol solution for 0.25, 0.50, 1.0, 2.0, 4.0, 0.6, 8.0, 10.0 and 12.0 hrs. The swelling index was calculated using microscopy techniques. The rate of nanoparticle swelling at different time interval was determined by difference in particle diameter at time given time and the initial time using equation:

$$\text{Percentage Swelling Index} = \frac{\text{Diameter of nanoparticles at a specific time}}{\text{Initial diameter of the nanoparticles}} X100$$

Mucoadhesive Strength: An in vitro wash-off method was employed to evaluate the mucoadhesion characteristics of formulated nanoparticles.[13,14] the study involves stimulation of an artificial biological flow to wash test sample fixed to a mucous membrane. During experiment a 10 cm^2 freshly cut rat stomach mucosa was cleaned in isotonic saline solution and tied onto a glass slide with a thread. A weighed quantity of formulated nanoparticles was spread over the mucous membrane; the relative humidity was maintained at 85% for 30 min. using desiccator. Phosphate buffer solution (PBS) at pH 7.4 was poured over the mucosa accurately at a flow rate of 10 ml/min using a peristaltic pump. Drug content was then measured using spectrophotometer at 280 nm at different time intervals and the amount of nanoparticles conforming to the differences in drug content levels was observed. An assessment of adhered nanoparticles and the flowed nanoparticle amounts was calculated from the difference between the applied nanoparticles and flowed nanoparticles. The mucoadhesive strength was then estimated from the comparison of actually applied nanoparticles and adhered nanoparticles.

Drug release studies: The *in vitro* release study was carried out in USP XXIV six station dissolution apparatus type 1 with 900 ml pH 7.5 phosphate buffer solution (dissolution medium) was used. The nanoparticle was weighed equivalent to 100mg of drug [Levodopa – Carbidopa (4:1)]. During the release study the temperature and rotation speed of the apparatus was maintained at 37±0.5° and 50 rpm, respectively. The in vitro release study was carried out for 12 h. First sample was taken at 30 min. after that every hour, samples were withdrawn from each station, filtered, diluted suitably and then analysed spectrophotometrically at 280 nm.

RESULTS AND DISCUSSION

The IR spectrum of the drugs and excipients was taken individually and in combination. There was no interaction found between the drug and excipients (Figure 1, 2, 3 & 4).

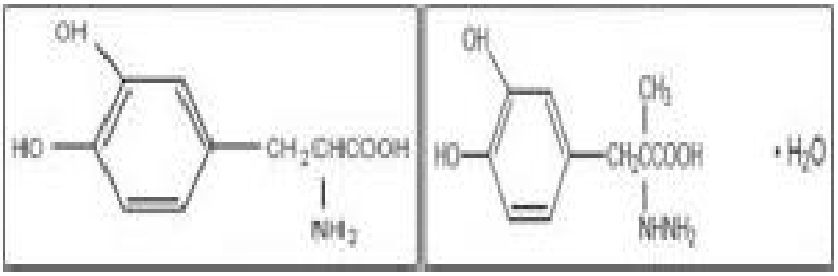

Figure 1: Molecular Structure of Levodopa and Carbidopa.

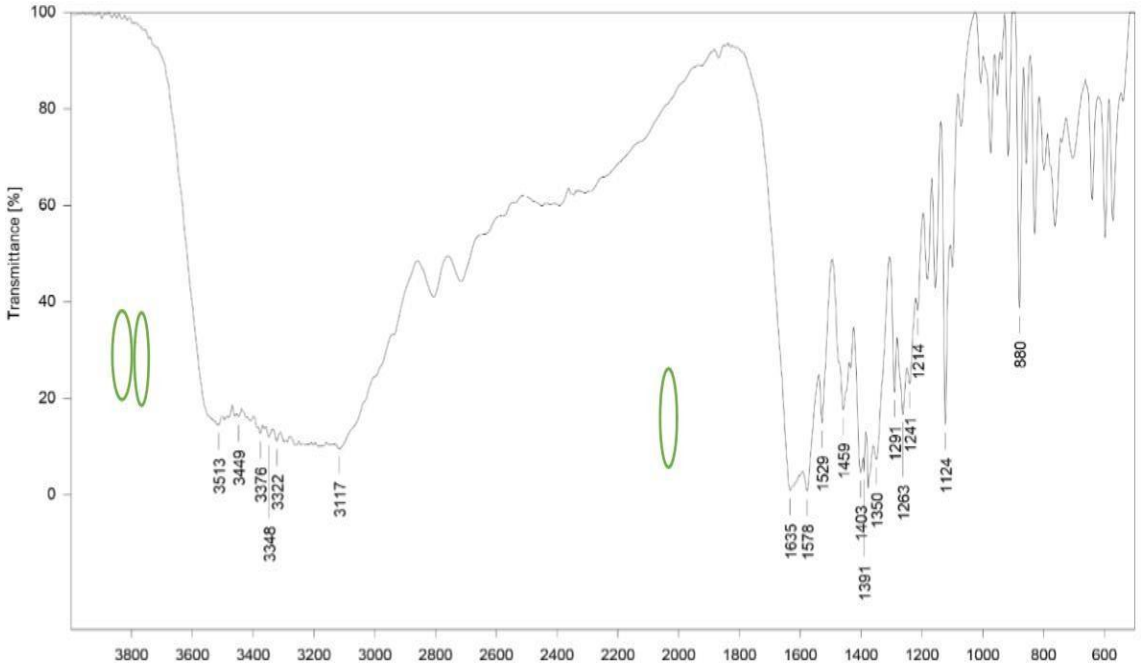

Figure 2: IR Spectra of Carbidopa.

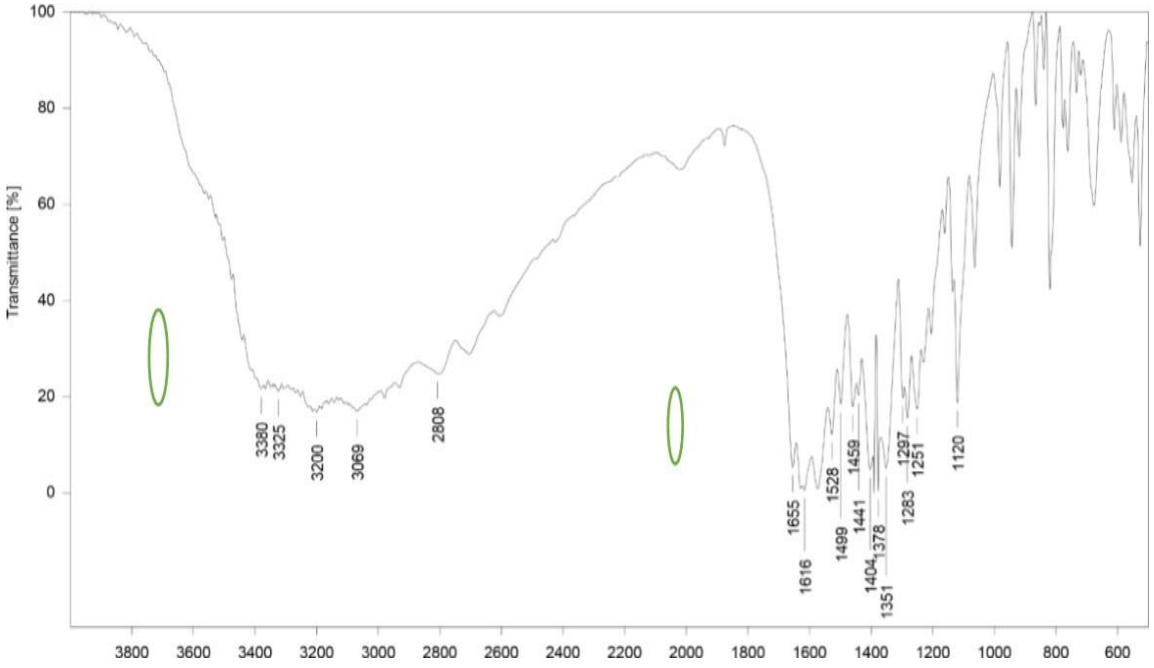

Figure 3: IR Spectra of Levodopa.

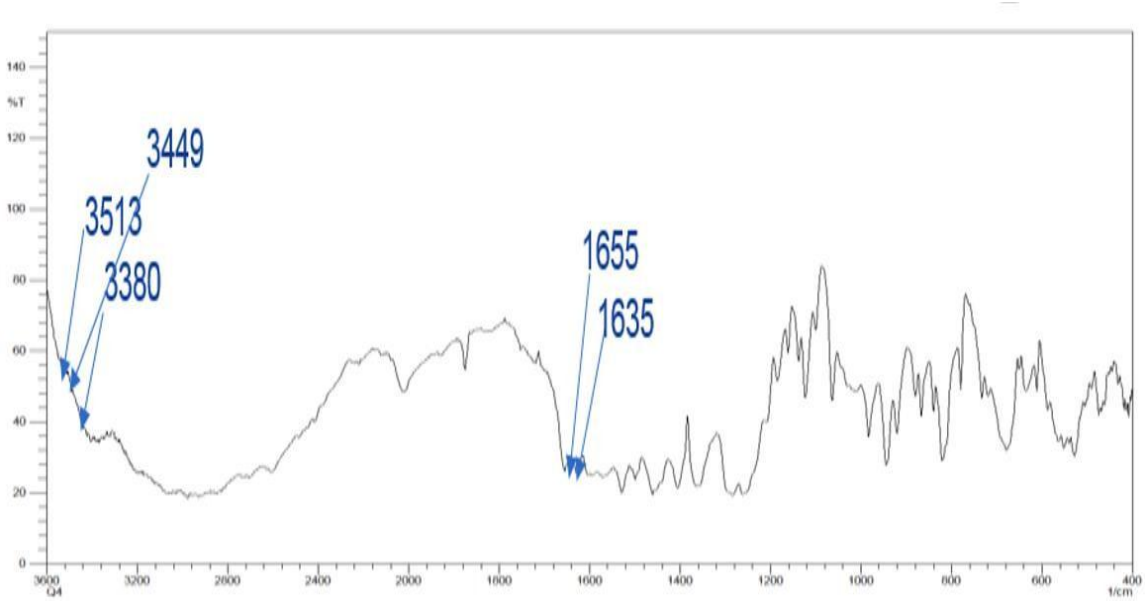

Figure 4: IR Spectra of Levodopa, Carbidopa and Excipients.

Spray-drying is commonly used technique for nanoparticle formulation. The percentage yield was found to be 98.5 ± 0.5% and the drug loading efficiency of nanoparticles was estimated as 84.06 ± 0.3%. The results indicates that this technique can be an important in pharmaceutical application.[15] the technique has excellent yield and drug loading efficiency hence attracted much attention for mass production. The nanoparticles prepared by spray drying technique revealed smooth to minor wrinkled surfaces (Figure 5) maybe due to contractions throughout the drying process in the drying compartment.[16]

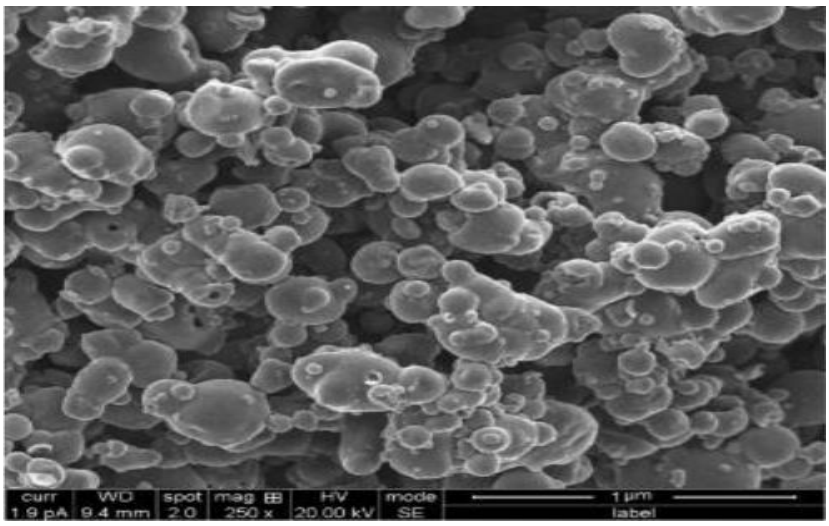

Figure 5: Scanning Electron Microscopy of spray-dried nanoparticles.

The formulated nanoparticles were about 460 nm with 390-540 range of scattering (Figure 6). Therefore advocates that the obtained particle size may allow sustain release of the drug from

the nanoparticles at the site of absorption.[17]

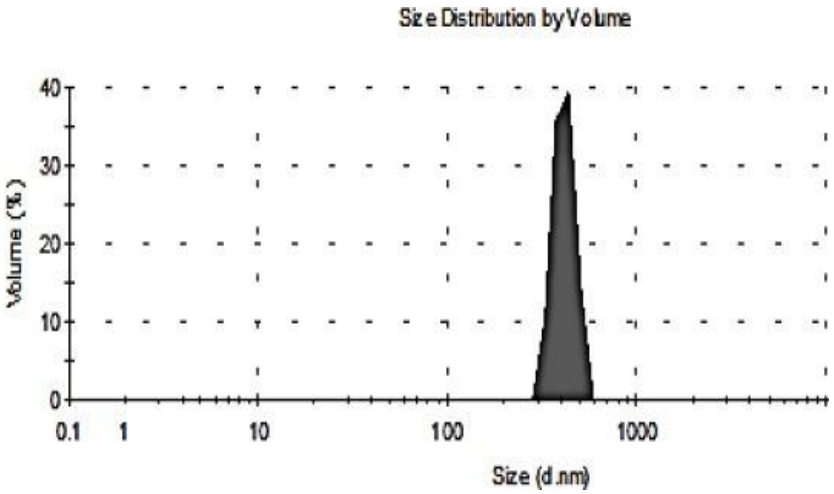

Figure 6: Particle size and distribution of spray-dried nanoparticles.

The swelling index of formulated nanoparticles at different time intervals is shown in Figure 7. The results show that the soaking of the nanoparticles in phosphate buffer solution at pH 7.4 was taken place rapidly. Earlier, it was shown that the swelling effect the adhesion and cohesion activities of mucoadhesive agents.[18] As expected, the capillary action was used to withdraw water from the underlying mucosa which is a tendency of formulated mucoadhesive nanoparticles this helps in rapid swelling ensuing stronger adhesion.[19] The swelling index of nanoparticles was found as 185.6±12%.

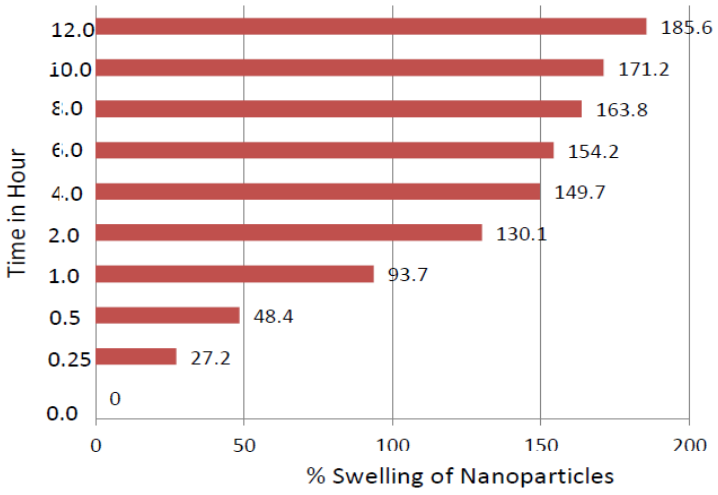

Figure 7: Swelling index of nanoparticles.

The wash-off technique for mucoadhesion shows greater mucoadhesive strength of nanoparticles (9.02 ± 05 hr) during ex vivo studies. The electrostatic forces and hydrogen

bonding between mucus membrane and molecules of nanoparticles was responsible for the formation of strong adhesive bonding. In turn, drug release into the GIT was made possible due to mucoadhesive strength of the nanoparticles.

During in vitro release studies the formulated nanoparticle exhibits initial rapid release (Figure 8) in first one hour of study, almost 30.63 ± 5% of the total drug was released. It indicates that the adsorption of weakly fused drug on to the surface of nanoparticles. This can be illuminated as rapid initial burst release following extend release of drug up to 12 hrs (98.9 ± 5.0. The drug release pattern describes that the nanoparticles initially swells together with the surface-adsorbed drug which lead to a 'burst' release. Afterward, owing to diffusion activity, the drug release displays a better control release of drug over longer period of time.

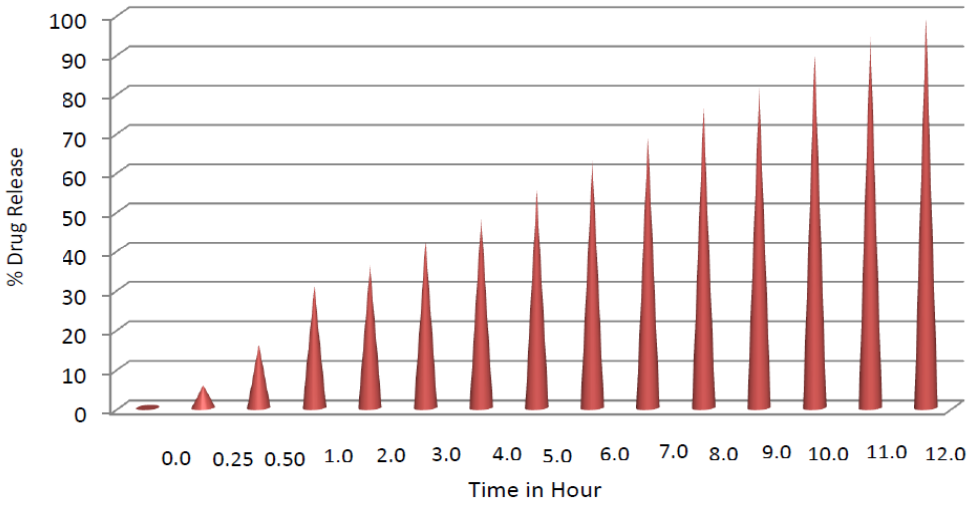

Figure 8: In-vitro Drug release.

The finding of the formulated nanoparticles shows that the presence of Albumin is responsible for the results due to inherent chemical nature. Also the albumin is free from immunogenicity and possesses a half-life of 19 days with much stronger adhesive property under acidic environmental conditions.

CONCLUSION

During the current research work the mucoadhesive nanoparticles of anti-parkinsonism drug was developed and characterised. The formulation was evaluated and it was found that the preparation can be promising for effective and safe management of Parkinsonism disease. In addition, the nanoparticles exhibit significantly stronger and greater mucoadhesive bonds lasting approximately 12 hrs. Prolonged retention in GIT (~12 h) also shows the benefit,

associated with oral administration of nanoparticles with constant drug release. It is therefore concluded that the mucoadhesive nanoparticles formulated in present study may potentially serve as the ideal technology for the effective management of patients suffering from Parkinsonism.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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