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Formulation and Characterization of Repaglinide Chitosan Nanoparticles for the Treatment of Diabetes Mellitus Type II

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ABSTRACT

The aim of this study was to prepare and characterize the nanoparticle formulation of Repaglinide to facilitate the development of a novel drug delivery system with improved efficacy and bioavailability. These particles were prepared by Ionotropic gelation method. Prepared formulations were evaluated for Zeta potential, Particle size Polydispersibility index, entrapment efficiency, production yield and *in vitro* drug release. The formulation batch F4 was found to be best formulation among all the prepared formulations because it showed least particle size, better poly dispersibility index, entrapment efficiency and *in vitro* drug release.

Key words: Repaglinide, polymer, nanoparticles, ionotropic gelation, *in vitro* drug release.

1. INTRODUCTION

Nanotechnology has shown tremendous potency of extensive research in many fields due to the peculiar properties of nanoparticles and nano drug delivery system. Nanoparticles have applicability in many applications in the field of medicine, which including diagnostics, vaccination, gene delivery, Drug delivery, Targeting of Drug and many more to reveal.[1] Research on nanoparticles is currently an area of intense scientific interest due to its wide range of applications.[2] Nanoparticles have gained much interest as a drug delivery system of ant diabetic drugs due to many advantages of nano sized formulations .Nanoparticles reduce the side effects of administration of conventional dosage form especially for the treatment of diabetes mellitus. [3]

Diabetes mellitus is a metabolic disorder which characterized by insufficient secretion or action of Endogenous insulin resulting in the elevation of Blood glucose and numerous other complications.[4] As per latest report of International Diabetes Federation, the number of people with diabetes is set to rise beyond 592 million in the next 25 years. It is also a fact that 47 % of the population goes undiagnosed and progresses toward diabetic implication unaware. [5]

To treat this type of diabetes, different classes of Oral antidiabetic drugs have been used routinely in the market. Repaglinide is an antidiabetic agent which is approved by FDA as an oral hypoglycemic drug in 1998. It works by reducing the fasting glucose concentration by stimulating insulin release from the pancreatic beta cells. It has peculiar specialty than other antidiabetic drug in term of structure, duration of action, binding profile, mode of excretion, practically insoluble in water but capable of being absorbed from the gastrointestinal tract after administering oral route. It also undergoes extensive first pass metabolism. It has 56% oral bioavailability. It causes hypoglycemia following oral administration although the extent of this feature is less than sulphonylureas. These properties make this drug as a good candidate for the selection. [5-7]

2. MATERIALS AND METHODS

2.1 Materials

Drug Repaglinide was obtained as a gift sample. Polymers Chitosan, Sodium Alginate, calcium chloride were purchased from Central drug house Delhi, rest of the chemicals were of analytical grade.

2.2 Preparation of Nanoparticles

Nanoparticle were prepared by Ionotropic gelation method. Nanoparticles of Repaglinide were prepared by Ionotropic gelation method by using chitosan Sodium Alginate and calcium chloride, nanoparticles can be obtained easily by inducing gelation with calcium ion followed by the addition of an aqueous polycationic solution to make a polyelectrolyte complex coating. Inotropic gelation is based on the ability of polyelectrolyte to cross link in the presence of the counter ion to form hydrogel.

Both the sodium alginate and calcium chloride solution were prepared by dissolving the chemicals in distilled water, the pH of the sodium alginate solution was adjusted to 5.1 using hydrochloric acid. After that a known amount of Chitosan was dissolved in 1% Acetic acid solution and pH was modified to 5.4 using sodium hydroxide solution.

Aqueous calcium chloride solution was added drop wise to 10 ml of sodium alginate with continuous stirring for 30 min and then chitosan solution was added to the resultant calcium alginate pre gel and stirred for an additional 1 hour, the resultant suspension was then sonicated for 30 min with probe sonicator.

2.3 Characterization of Nanoparticle

2.3.1 Zeta Potential

Zeta potential is used as a surrogate for surface charge and is often measured by observing the oscillation in signal that result from light scattered by particles located in an electric field. Though there are other approaches, it is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particles and the medium in which it is dispersed. The Zeta potential of the formulation was determined by Malvern Zeta Sizer.
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2.3.2 Particle size

The particle size of the various formulations were determined with the help of Malvern Zeta Sizer.

2.3.3 Polydispersity Index

The Polydispersity index together with the Z average diameter is calculated from the cumulants analysis as defined in ISO13321 and is a dimensionless estimate of the width of the distribution scale from 0 to 1. A PDI value of 1 indicates that the sample has a very broad size distribution and may contain large particles or aggregates that could be slowly sedimenting. The polydispersity Index is dimensionless and scaled such that value smaller than 0.05 are rarely seen other than with highly monodisperse standards.Value greater than 0.7 indicate that the sample has very broad size distribution and is probably not suitable for the dynamic light scattering technique.

The size distribution uniformity of nanoparticles was measured by polydispersity Index .it is a measure of Molecular mass in a given polymer sample. The width of size distribution was indicated by the polydispersity index.

2.3.4 Differential Scanning calorimetry (DSC)

Thermal Analysis comprises a group of techniques in which a physical sample property is measure as a function of temperature while the sample is subjected to a predefined heating or cooling programme. In differential thermal analysis the temperature difference between a sample and an inert reference material is measure when both are subjected to identical heat treatments .the related techniques of differential scanning calorimetry relies on difference in enerty required to maintain the sample and reference at an identical temperature.

Differential scanning calorimetry monitors heat effects associated with phase transition and chemical reaction as a function of temperature In a DSC the difference is hear flow to the sample and a reference at the same temperature is recorded as a function of temperature .The reference is an inter material such Alumina or just an empty aluminum Pan .The temperature of both the sample and reference are increased at a constant rate.

The DSC thermograms of pure Repaglinide, Chotsan, Sodium Alginate and the blend mixture of all i.e. Repaglinide, chitosan and sodium alginate.

2.3.5 Entrapment Efficiency

Entrapment efficiency was determined by Gravimetry method. 2ml of nanosuspension was taken and centrifuges at 14000g for 45 min, the supernant was collected and analyzed spectrophotometrically at 247nm for the determination of drug content which was unentrapped. From this value, the entrapped

value of drug was determined and percentage entrapment efficiency was calculated by the following formula:

Entrapment Efficiency = total amount of drug- Free amount of drug X 100 / total amount of drug.

2.3.6 Production yield

Production yield was determined by gravimetry method. 2 ml of suspension was taken and centrifuges at 14000g for 45 min, the supernant was collected and the sediment obtained was dried. The amount of dried sediment was weighed accurately and production yield was calculated by using following formula

Production yield = total weight obtained X100 / Total solid weight taken

2.3.7 In vitro Drug release study

The *in vitro* release of Repaglinide loaded chitosan nanoparticles was performed by using diffusion cell by using Phosphate buffer 7.4 at 37 $\pm$ 0.5 at 100rpm, the Himedia membrane (100 Kilo Dalton) utilized for the release study was soaked overnight in the dissolution medium. Nanoparticles equivalent to 18mg of drug were placed in the dialysis bag prepared by tying the membrane on both sides, the dialysis bag was hang in the dissolution media. Aliquotes were collected periodically and replace with the equal volume of fresh dissolution on each withdrawal. After filtration through whatsman filter paper.

3. RESULT AND DISCUSSION

Repaglinide chitosan nanoparticles were prepared by ionotropic gelation method after selection and optimization of various process variables.

3.1 Effect of polymer concentration

The polymer concentration was varied from 0.1 to 0.2 % (w/v) keeping other process variable constant. As the concentration of chitosan increases it result in gradual increase in nanoparticle diameter. It was observed that increase in chitosan concentration leads to an increase in the viscous forces resisting droplet break down by stirring and leads to increase in particle size. The increase in polymer viscosity increases the diffusional resistance to drug molecule thereby entrapping more drug.

3.2 Effect of cross linking Agent concentration

Sodium alginate concentration was varied from 0.5 -1%(w/v) while keeping other processing parameters at standard conditions, as the sodium alginate concentration was increased. The particle size also increased due to increased viscosity of the aqueous phase. The increase in viscosity reduces the net shear stress available for droplet break down by stirrer.

3.3 Particle size and size distribution

Smaller particle have higher surface area/volume ratio, which makes it easier from the encapsulated drug to be released from it. It was also reported in literature that smaller nanoparticles will have greater case of entry and durability in the gastric mucosa, The particle size of F1 and F2 was found in nm but larger value of particle size was obtained. The Z-average value for F3 was found to be 560.44 nm. In case of F4 the z-average was found to be 421.95nm F5 formulation showed particles in micro meter range.

But in case of F6 aggregates of microparticles were formed as the concentration of acetic acid was increased. As the acetic acid concentration increases the chitosan solution was not able to pass through the syringe needle, because of increase in the viscosity, no particle were obtained in 2% âcetic acid concentration because of the precipitation of polymer. Thus F4 was selected as optimized formulation on the basis of the particle size.

3.4 Poly dispersibility index

It should be as less and it is considered as less than one is better. In all the nanoparticle formulation all the prepared batch showed less than 1 value of PDI. In prepared formulation batches F1 and F2 showed its less value but since small size is the best criteria for better formulation Formulation F5 showed higher PDI value but it was lesser than formulation code F3.

Entrapment Efficiency of Batch F4 was found to be good due to optimum concentration of crosslinking agent and Chitosan.

Nanoparticles having zeta potential value more than +25mv or less than -25mv typically have high degree of stability. Though, in prepared formulation all the formulation revealed variation in the result here formulation F1 showed zeta potential -9.4mv.

3.5 Entrapment Efficiency

The optimized formulation F4 was taken to determine the entrapment efficiency. The percent entrapment efficiency was determined by using formula.

3.6 Production Yield

The production yield of the formulation F4 was calculated and it was found to be 78.6%.

3.7 *In vitro* release

The in vitro release study of Formulation F4 batch was done and it was observed that the cumulative percent release after 11 hour was found to be 68.8%

Kinetic of Drug release was studied for the optimized formulation that is formulation code F4.

4. CONCLUSION

Chitosan is an dacetylated derivative of chitin which is used in therapeutic used in owing to its biodegrability and biocompatibility. Chitosan is cationic poyamine adhere to negatively charged surface and chelates metal ions. It is a linear polyelectrolyte with reactive hydroxyl and amino groups. The properties of chitosan relates to its polyelectrolyte and polymeric carbohydrate character. Due to the above chitosan has been selected for present research work for development of biodegradable polymeric nanoparticles of Repaglinide ,Preformulation studies of drug was performed in terms of identification by physical appearance ,melting point ,solubility and absorption maxima determination along with the preparation of Calibration curve in 0.1NHCL and PBS 7.4.

Present work summaries the nanoparticles technology and describes the systemic study of formulation, optimization and characterization of Repaglinide chitosan nanopaprticles with emphasis mainly on particle size. The observation from present work shows that the ionotropic gelation cab be used for the preparation of nanoparticles.

Different chitosan concentration, sodium alginate concentration and acetic acid concentration were used to prepare the various formulation and their effect on particle size was observed, on the basis of particle size F4 was selected as the optimized formulation with the average particles size of 421.9 nm. Also 1% acetic acid was suitable concentration for the preparation of nanoparticles because of ease of solubility of chitosan in acidic solution.

It was concluded that the optimized formulation F4 showed the particle size of 421.9 nm, PDI 0.678, Zeta potential 3.09 mV, Entrapment efficiency 74.03 % and production yield was 78.61%.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 1. Different formulation batches of Nanoparticles prepared

S. No	Formulation code	Chitosan (% w/w)	Acetic Acid (% v/v)	Sodium Alginate (% w/w)	Drug (mg)	Calcium Chloride (% w/w)
1	F1	0.1	1	0.5	18	0.1
2	F2	0.2	1	0.5	18	0.1
3	F3	0.1	1	1	18	0.1
4	F4	0.2	1	1	18	0.1
5	F5	0.1	1	1	18	0.1
6	F6	0.2	1	1	18	0.1

Table 2. Average size of different batches of Nanoparticles prepared

S. No	Formulation Code	Z average value	Inference
1	F1	735.45	Larger particles
2	F2	654.89	Larger particles
3	F3	560.44	Smaller particles
4	F4	421.95	Smaller particles
5	F5	Particle in micrometer size	Microparticles obtained
6	F6	Aggregates of microparticles were obtained	Microparticles aggregates were obtained

Table 3. The PDI value of various formulation were obtained from Zeta Analyzer

Formulation Code	PDI
F1	0.348
F2	0.545
F3	0.721
F4	0.678
F5	-
F6	-

Table-4 Percent Entrapment Efficiency and Production yield of Optimized batch

S. No	Formulation Code	Percent Entrapment Efficiency	Production Yield %
1	F4	74.03	78.6

Table 5. Zeta Potential of Different Prepared batch

S. No	Formulation Code	Zeta Potential
1	F1	-9.4mv
2	F2	18.5mv
3	F3	11.0mv
4	F4	3.09mv

Table 6. Kinetic treatment of Release of Best batch F4

S No	Regression Value	Kinetic model
1	0.954	Zero Order
2	0.900	First order
3	0.913	Higuchis
4	0.967	Peppas

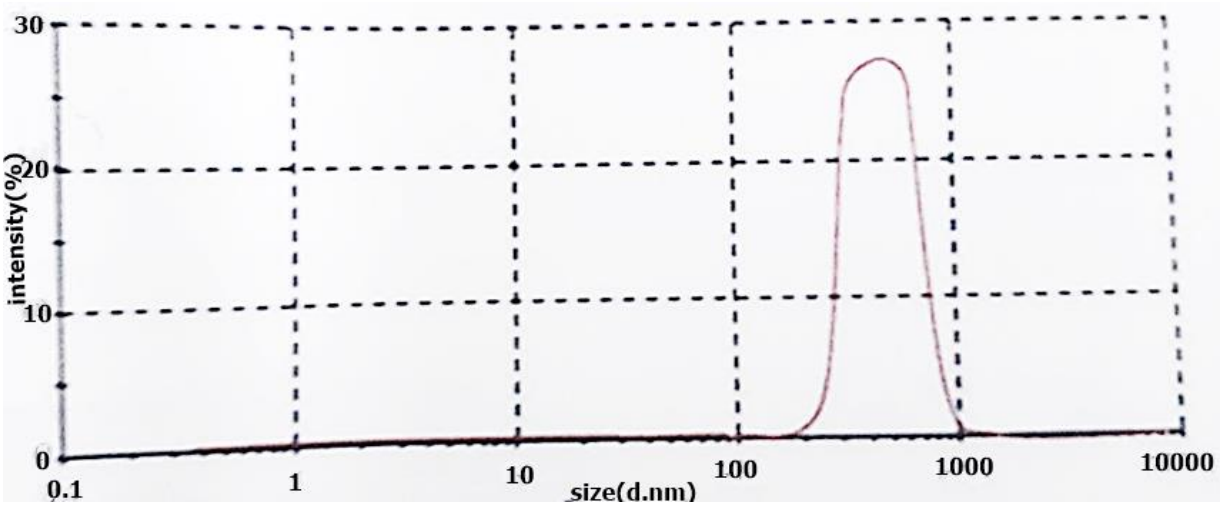

Fig.1: Formulation F1 Size distribution by intensity using Malvern at 25°C

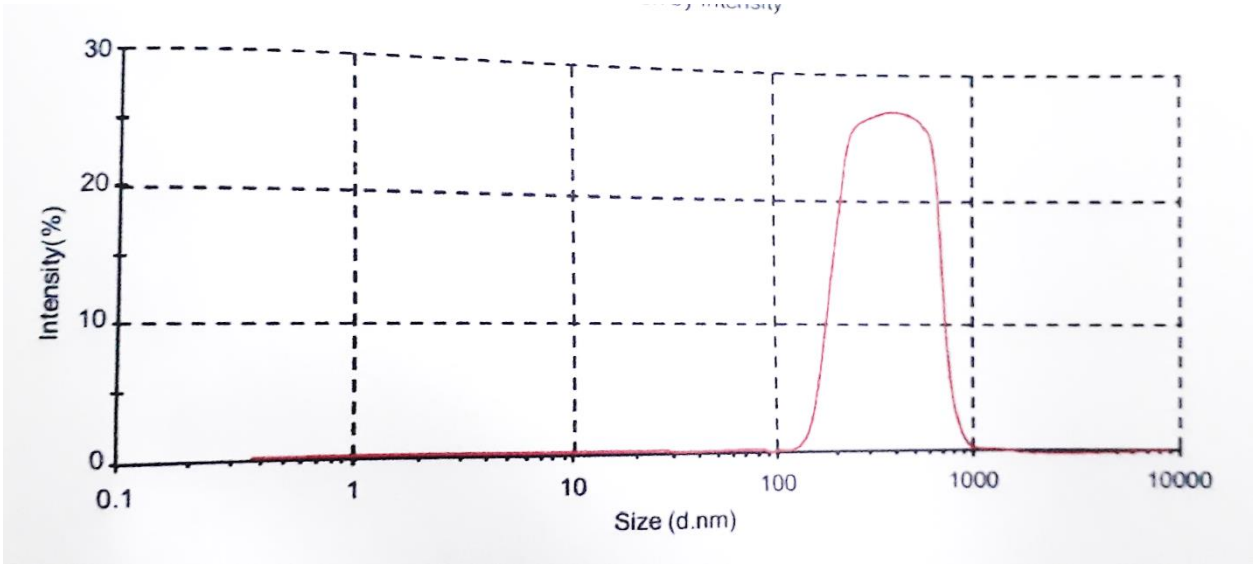

Fig.2: Formulation F2 Size distribution by intensity using Malvern at 25°C

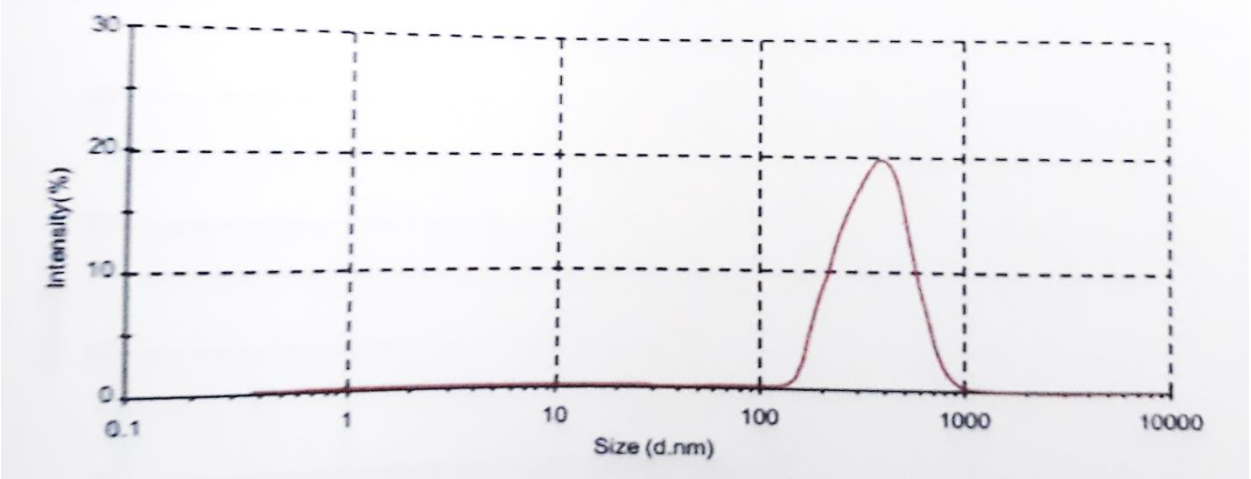

Fig.3: Formulation F3 Size distribution by intensity using Malvern at 25°C

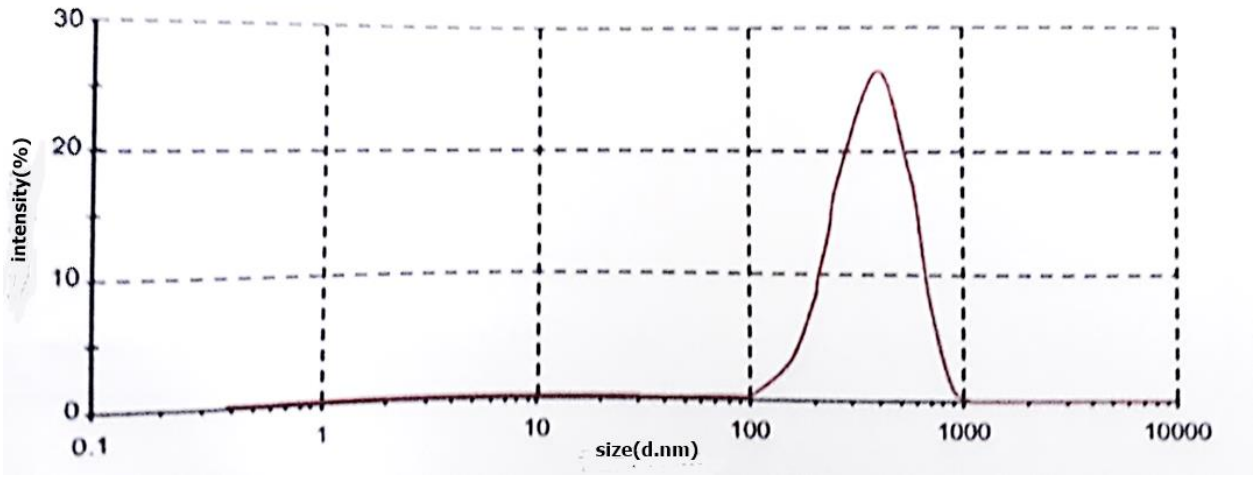

Fig. 4: Formulation F4 Size distribution by intensity using Malvern at 25°C

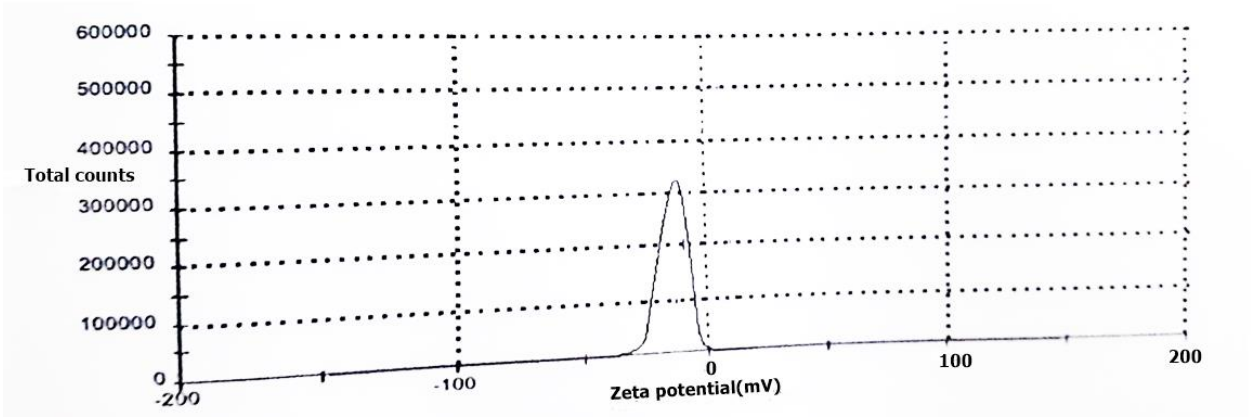

Fig.5: Formulation F1 Zeta potential

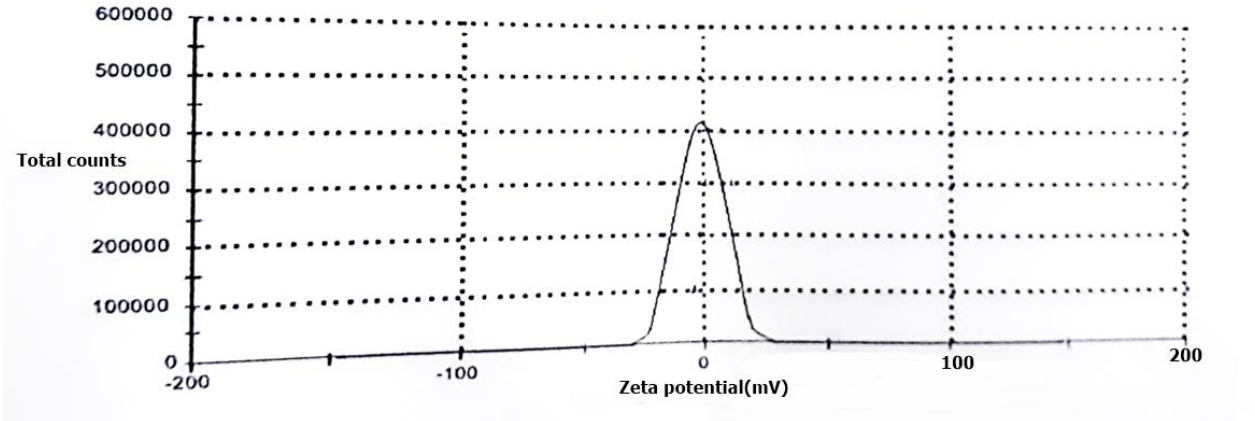

Fig. 6: Formulation F2 Zeta potential

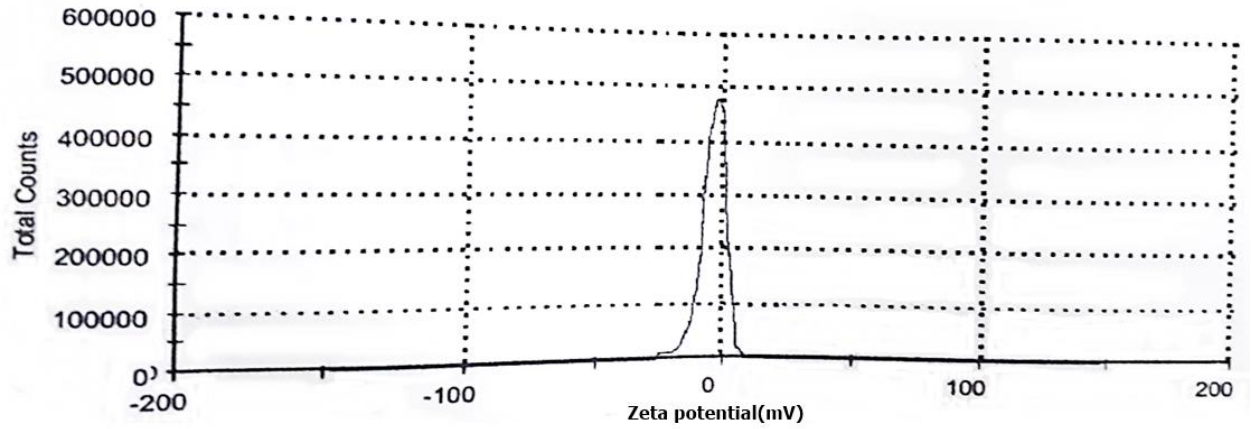

Fig.7: Formulation F3Zeta potential

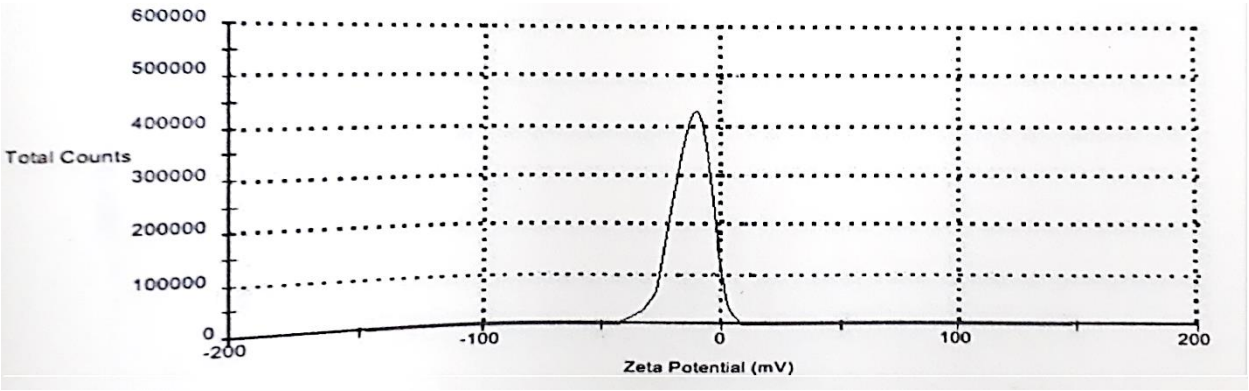

Fig. 8: Formulation F4 Zeta potential

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