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Preparation, Evaluation & Optimization of Nanoparticles Composed of Pyridostigmine

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Abstract: ***The purpose of this study was to prepare Pyridostigmine nanoparticles for control release of Pyridostigmine to improve the oral bioavailability, enhance the solubility and dissolution rate by decreasing particle size of drug. Infrared spectroscopic studies confirmed that there was no interaction between drug and polymers. The controlled release Pyridostigmine nanoparticles were prepared by Solvent evaporation by using Ethyl cellulose, Chitosan & HPMC K100 at different ratios. The production yield of the formulated controlled release nanoparticles (F1 to F16) in the range of 76.11 % to 83.58 %. The drug content of the formulated controlled release nanoparticles (F1 to F16) in the range of 82.56 %to 98.20%. The Theoretical loading of the formulated controlled release nanoparticles (F1- F16) in the range of 24.43 % to 64.24%. The entrapment efficiency increased with increasing the concentration of polymers and the formulations containing chitosan nanoparticles F6 (1:2) showed better entrapment (90.94%) among all formulation. The solubility of selected formulation (F6) in 0.2 M Phosphate buffer pH 6.8 increased when compared to pure drug. Particle size distribution was determined by Malvern zeta size, the size range for produced nanoparticles in the range of 200 nm to 400 nm. The Polydispersity index of selected nanoparticle formulation (F6) was indicated a narrow range and a homogeneous size distribution of particles. The in vitro dissolution study was carried out in 0. 2N PBS for 2 hours and phosphate buffer pH 6.8 for 10 hours. The formulations shows controlled release of drug up to 12 hrs and all formulations showed more than 75% of drug release. The release kinetics showed that the formulations were complies with Zero order kinetics followed by diffusion controlled mechanism. The best formulation F6 was evaluated by infrared spectroscopy, particle size, Polydispersity index & zeta potential and Scanning Electron microscopy. Best formulation of nanoparticles shown the extent of drug release was found to be F6 (96.93%) in 12 hrs. SEM studies confirmed the morphology of the nanoparticle formulation.***

Keywords: Polydispersity index, Zeta potential, Scanning Electron microscopy, Pyridostigmine

I. INTRODUCTION

Due to swift industrialization and urbanization, our environment is undergoing huge smash up and a large amount of perilous and superfluous chemical, gases or substances are released, and so now it is our need to learn about the secrets that are present in the Nature and its products which leads to the growth of advancements in the synthesis processes of nanoparticles. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which was found to be reliable and eco-friendly. The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies. The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticle show completely new or improved properties, such as size, distribution and morphology of the particles etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly on various fields [1]**.**
Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-eco-friendly by-products. The need Rfor environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approach [2]**.** Nanotechnology refers to an emerging field of science that includes synthesis and development of various nano-materials. Nanoparticles can be defined as objects ranging in size from 1- 100 nm that due to their size may differ from the bulk material. Presently, different metallic nano-materials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver. Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes [3-5]**.**

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In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally, the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for [6].

II. MATERIALS AND METHODS

A. *Preformulation Study*

The basic purpose of the preformulation activity are to provide a rational basis for the formulation approaches, to maximize the chances of success in formulating an acceptable product and to ultimately provide a basis for optimizing drug product quality and performance. Preformulation is defined as an investigation of physical & chemical properties of sustained release matrix tablet substance alone and when combined with excipient. A step in time saves nine, so the Preformulation studies of the new product can away the disaster that is disasters are prevented in advance. [7]

B. *Organoleptic Parameter*

It is the initial evaluation during preformulation studies which assess the colour, odor and taste of the substance. The appearance was checked visually for color, homogeneity and transparency. The appearance was checked visually for color, homogeneity and transparency [8].

C. *Solubility*

Aqueous solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of drug was determined in water and methanol, ethanol, chloroform and ethyl acetate [9].

D. *Melting Point Determination*

Melting point of drug was determined by Open capillary method. The melting point of a drug is one of the first and more reliable physical properties measured; it can be advantageously used as a guide in early drug discovery and development [10]

E. *Determination of Partition Coefficient*

50 mg of drug was taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phases was calculated by using formula: K_{PC} = Concentration of Drug in Oil Phase/ Concentration of Drug in Water Phase

F. *Determination of λ max*

The solution was scanned in the range of 200 – 400 nm UV spectrum using Systronic double beam spectrophotometer. Prepared diluted solution (in water) to determine the λ max for the detection of Nanoparticle using pH 7.4 Phosphate buffer as blank. Five different concentrations (240, 260, 280, 300, 320 µg/ml) were prepared after withdrawing five different aliquots from the stock solution and diluted up to 10ml with same diluents. Prepared diluted solution is used to determine the λ max for the detection of nanoparticle using pH 7.4 Phosphate buffer as blank [11].

G. *Standard Calibration Curve of Pyridostigmine*

1) *Preparation of Dissolution Medium:* pH 7.4 phosphate buffer is used as a dissolution medium.
2) *Determination of Absorption Maximum (λ max) :* 100 mg of Pyridostigmine was accurately weighted into 100 ml volumetric flask, dissolved in water and volume was made up with water. Pipette 1ml of this solution into another 10 ml volumetric flask and the volume was made with water and marked as Stock. The resultant solution is scanned in the range of (200-400nm) by UV Spectrophotometer (Systronic (2202) to get absorption maximum (λ max).
3) *Preparation of Standard Calibration Curve:* From this Pyridostigmine standard stock solution (1000µg/ml), 1ml solution was diluted to 10 ml using water solution to get concentrations of 100 µg/ml. from this solution, aliquots of, 20 ml, 40 ml, 60 ml,80 ml,100 ml, from standard drug solution were diluted to 10 ml with water. The absorbance of these solutions was measured at 270 nm water as a blank. A standard curve is plotted using concentration on X-axis and the absorbance obtained on Y-axis. And also a standard curve is prepared similarly using water.

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H. Formulation of Pyridostigmine Nanoparticles Solvent Evaporation Method (Ansari et al., 2011)

Nanoparticles prepared by polymers like chitosan, ethyl cellulose, hydroxyl propyl methylcellulose; and polyvinyl alcohol by solvent evaporation method. Disperse phase consisting of Pyridostigmine (100mg) and requisite quantity of polymers dissolved in 20 ml solvent (dichloromethane) was slowly added to a definite amount of PVA in 100 ml of aqueous continuous phase. There action mixture was stirred at 1000 rpm for two-three hours on a magnetic stirrer. The nanoparticles formed were collected by filtration through whatman filter paper and dried in oven at 50^0C for 2 hours. The dried nanoparticles were stored in vacuum desiccater to ensure the removal of residual solvent [12].

I. Characterization of Nanoparticles

All the formulations are evaluated for its production yield, particle size, polydispersity index, zeta potential, drug content, entrapment efficiency, solubilisation efficiency and *in-vitro* drug release studies and Kinetics of drug release studies [13].

J. Production Yield

The production yield of the Nanoparticles is calculated for each batch by dividing the total weight of product (M) by the total expected weight of drug and polymer.

K. Theoretical Drug Loading

Theoretical drug loading in nanoparticles is estimated by using the following formula,

$$\text{Drug Loading Content (\%)} = \frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles}} \times 100$$

L. Determination of Drug Content

Sample containing 100 mg equivalent Pyridostigmine nanoparticles are dissolved and the volume is made up to 100 ml with pH 6.8 phosphate buffer. From the above solution 10 ml is pipette out and made upto100ml with phosphate buffer. The absorbance of resulting solution is determined at λ max (270nm) using UV Spectrophotometer (Systronic 2022) and the drug content is estimated.

M. Entrapment Efficiency

For the drug entrapment efficiency tests, the nano particles of F1-F16 were performed. Before starting the chemical (spectrophotometric) analyses for the drug entrapment efficiency, the repeatability of measurements between different batches was ensured by repeated analyses. The 10 mg of the nanoparticles was analyzed by dissolving ample in 10 ml of distilled water. After the drug was dissolved, 10 ml of clear layer of dissolved drug is taken [14].

Their after, the amount of drug in the water phase was detected by a UV-spectrophotometric method at 270 nm (U.V Spectrophotometer, Systronics 2022). The test was repeated with another sample. The amount of the drug in the suspension was analyzed by centrifugation at 500 rpm for 5 minutes and by measuring the concentration of the drug in the clears up supernatant layer by the UV-spectrophotometric method. The test was again repeated with another sample. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticles suspension. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\%\ \textbf{Entrapment Efficiency} = \frac{\textbf{Total amount of drug} - \textbf{Concentration of drug} \times 100}{\textbf{Total amount of drug}}$$

N. Determination of Particle size and Zeta Potential

The mean particle size (z-average), poly dispersity index (PI) and zeta potential of Pyridostigmine nano particles formulations are determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS90, Malvern Instruments Ltd., UK). The freeze dried powders are redispersed with water to obtain a proper scattering intensity before measurement [15].

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O. Morphological studies of nanoparticles by using Scanning Electron Microscopy (SEM)

Morphological evaluation of the selected Nanoparticles formulation is carried out in scanning electron microscope (SEM) (Hitachi X650, Tokyo, Japan). All samples are examined on a brass stub using carbon double-sided tape. Powder samples are glued and mounted on metal sample plates. The samples are gold coated (thickness ≈ 15–20 nm) with a sputter coater (Fison Instruments, UK) using an electrical potential of 2.0 k Vat 25 mA for 10 min. An excitation voltage of 20 kV was used in the experiments [16].

P. In vitro Dissolution Studies

In-vitro release studies were carried out by using dissolution apparatus (II type). The dissolution medium will be water. The rpm of dissolution apparatus will set 50 rpm. A sample of 5 ml was withdrawn from the dissolution setup at regular intervals for 24 hours and an equal volume of water was replaced to maintain a sink condition. Samples were analyzed by using UV spectrophotometer at 270 nm and the amount of drug release was calculated.

Absorbance values of sample solutions are measured at λ max (270 nm) in UV Spectrophotometer (Systronic 2022). The cumulative percentage drug release is calculated.

Q. In vitro Kinetic Analysis

In order to investigate the drug release mechanism from Controlled release nanoparticles formulations, the percentage cumulative drug release data is analyzed with following mathematical model [17].

The zero order rate Equation describes the systems where the drug release rate is independent of its concentration.

$$Qt=Q0+K0t$$

Where, Qt is the amount of drug dissolved intimet, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero order release constant expressed in units of concentration/ time. To study the release kinetics, data obtained from *in-vitro*drug release studies are plotted as cumulative amount of drug released versus time.

The first order Equation describes the release from a system where the release rate is concentration dependent.

$$Log\ C = log\ C0 – kt/2.303$$

Where C is the concentration of the drug at time (t), C0 is the initial concentration of the drug and k is the first- order release rate constant. The data obtained are plotted as log cumulative percentage of drug remaining vs time.

Higuchi described the release of drugs from porous, insoluble matrix as a square root of time dependent process based on Fickian diffusion as shown in following Equation.

$$F\ t=Q=KH\times t1/2$$

Where Q is the amount of drug released in time t. This model is based on the hypotheses that initial drug concentration in the matrix is much higher than drug solubility drug diffusion takes place only in one dimension (edge effect must be negligible) drug particles are much smaller than system thickness matrix swelling and dissolution are negligible drug diffusivity is constant and perfect sink conditions are always attained in the release environment.

The data obtained are plotted as cumulative percentage drug release\versus square root of time.

Hixson and Crowell (1931) recognized that the Particles' regular are a isproportional to the cube root of its volume. They derived the equation

$$W01/3-Wt1/3=\kappa t$$

Where W0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the remaining amount of drug in the pharmaceutical dosage format time t and κ (kappa) is a constant incorporating the surface volume relation. The equation describes the release from systems where there is a change in surface are and diameter of particles. To study the release kinetics, data obtained from in vitro drug release studies are plotted as cube root of drug % remaining in matrix vs. time.

Korsmeyer–Peppas model describes the fraction of drug release relates exponentially with respect to time [18-19].

$$Mt / M\infty = K\ tn$$

Where Mt/M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. In this model, the value of n characterizes the release mechanism of drug. To study the release kinetics, data obtained from In vitro drug release studies are plotted as log cumulative percentage drug release versus log time [19].

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Table 1: Kinetic Standards For Different Models

Release exponent (n)	Drug transport	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45< n-0.89	Non-fickian transport	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super case II	t^{n-1}

III. RESULTS AND DISCUSSION

A. *Preformulation Studies*

1) Description: The colour, odour, nature and taste of the API were evaluated. It was found to be as per the monograph.

Table 2: Description of Pyridostigmine

S. No.	Drug	Tests	Results
1	Pyridostigmine	Color	White
2	Pyridostigmine	Odor	Agreeable odor
3	Pyridostigmine	Appearance	Crystalline powder

2) Solubility Study: Solubility study of Pyridostigmine is reported in table

Table 3: Solubility Study of Pyridostigmine

S. No.	Solvent	Solubility
1.	Water	Freely soluble
2.	Alcohol	In soluble
3.	Ether	In soluble
4.	Chloroform	Slightly soluble
5.	Acetone	In soluble
6.	Benzene	In soluble
7.	Hexane	Slightly soluble

3) Melting Point Determination: The melting point of Pyridostigmine was found to be 153 ^{O}C.

4) Determination of λ max: Solution was scanned under UV-Vis Spectrophotometer and λ max was determined. It was found to be as per the monograph.

Table 4: Partition coefficient of Pyridostigmine in water

S. No.	Solvent	Partition coefficient
1	n-octanol/water	1.574 Octanol/water partition coefficient

Table 5: Standard calibration Curve data of Pyridostigmine in water

S. No	Concentration (µg/ml)	Absorbance at λ max 270 nm
1	00	00
2	20	0.214
3	40	0.422
4	60	0.642
5	80	0.884
6	100	1.01

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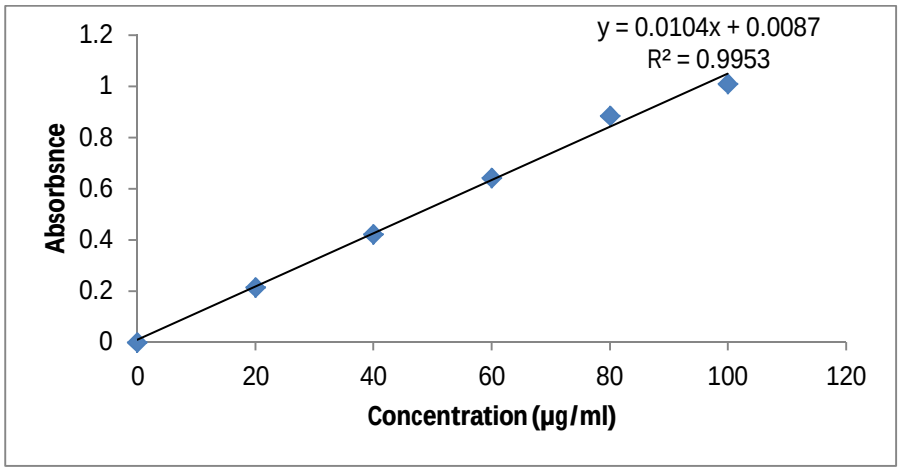

Fig. 1: Standard calibration curve of Pyridostigmine in water

5) *FTIR Study:* From the spectra of Pyridostigmine physical mixture of drug and selected ingredients it was observed that all characteristic peaks of Pyridostigmine were present in the combination spectrum, thus indicating compatibility between drug and selected ingredients.

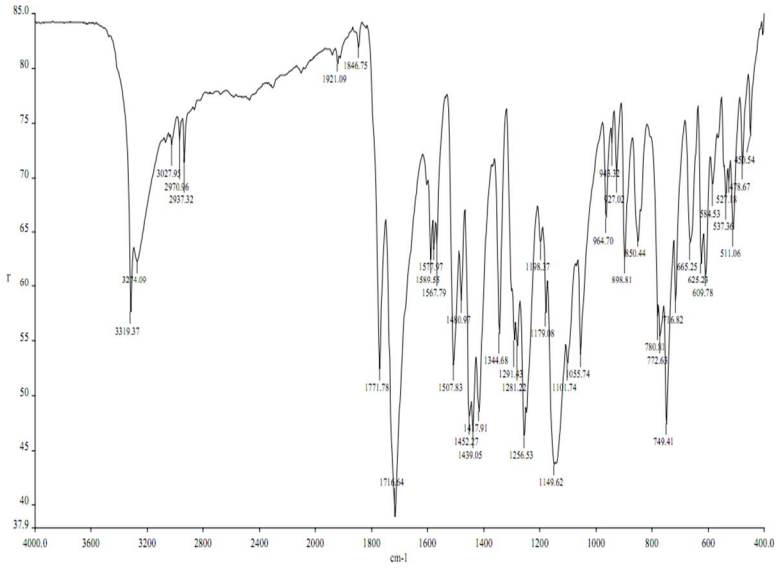

Fig. 2: FTIR of Pyridostigmine

Table 6: FTIR Spectral Data of Pyridostigmine

S. No	Wave Number (cm-1)	Functional Group
1.	3630.8	–CO- stretching of ester
2.	3317.4	–CO- stretching of acid
3.	1770.2, 1716.9	N-H stretching vibrations and O-H stretching vibrations
4.	1580.6, 1505.4, and 1445	C=C ring stretching
5.	1252 and 1145.9	C-N stretching and O-C=C stretching

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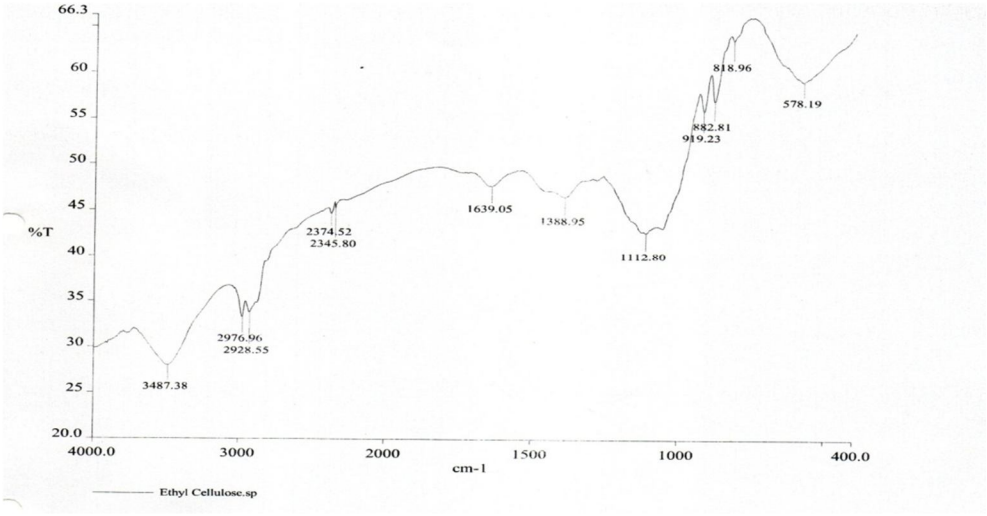

Fig. 3: FTIR of Ethyl cellulose

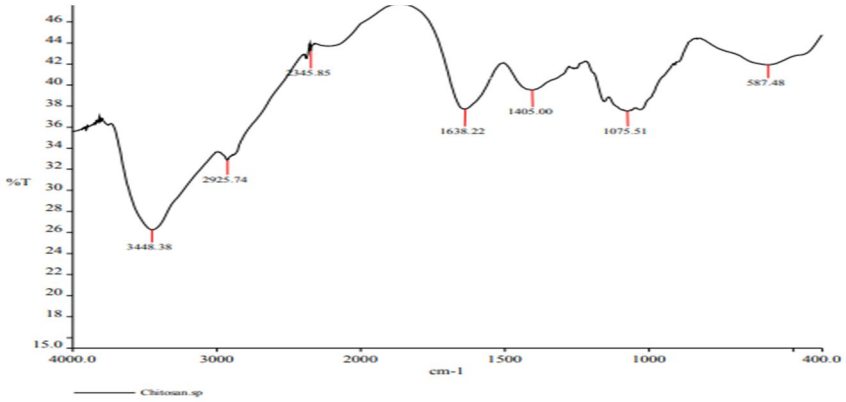

Fig. 4: FTIR OF Chitosan

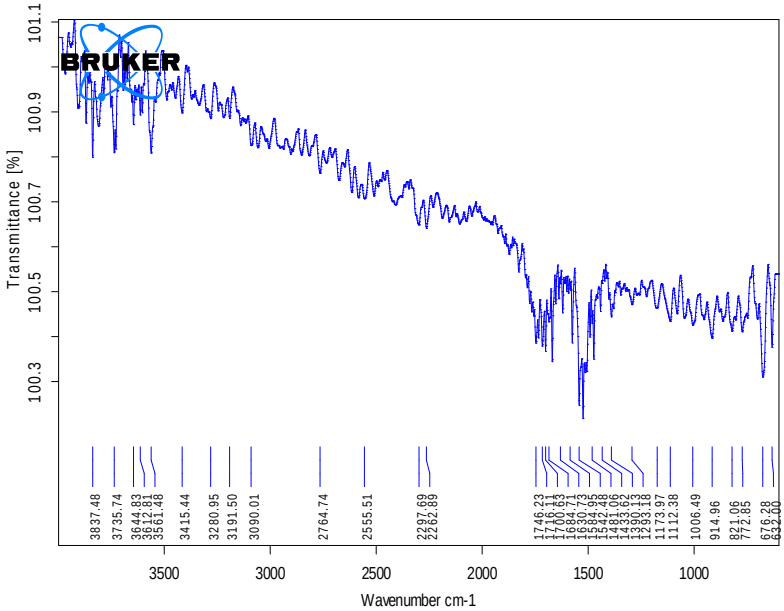

Fig. 5: FTIR of HPMC K100

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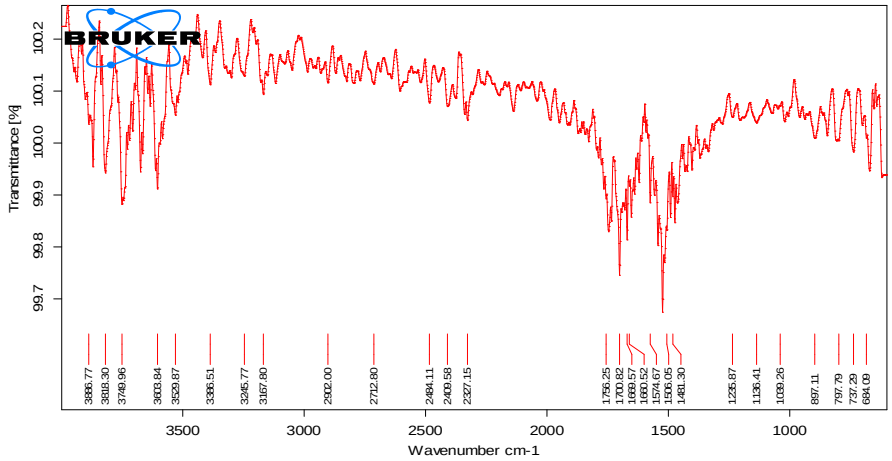

Fig. 6: FTIR OF Polyvinyl alcohol

B. Preparation of Pyridostigmine Nanoparticles

1) Solvent Evaporation Method: Controlled release nanoparticles of Pyridostigmine were prepared by using Solvent evaporation method using different ratio of polymers Table7.7 shows the composition of various prepared controlled release nanoparticles. The Drug (Pyridostigmine) and polymer (Ethyl cellulose) was in the ratio of 1:1,1:2,1:3 and 1:4 for F1, F2, F3 and F4 respectively, Pyridostigmine and polymer (Chitosan) in the ratio of 1:1,1:2,1:3 and 1:4 for F5, F6, F7 and F8 respectively, Pyridostigmine and polymer (HPMCK100M) in the ratio of 1:1, 1:2, 1:3 and 1:4 for F9, F10, F11, and F12 respectively. Disperse phase consisting of Pyridostigmine (100mg) dissolved in methanol and requisite quantity of polymers dissolved in 20 ml solvent (dichloromethane) was slowly added to a definite amount of PVA in100ml of aqueous continuous phase. There action mixture was stirred at 1000 rpm for two to three hours on a magnetic stirrer. The nanoparticles formed were collected by filtration through whatman filter paper and dried in oven at 50^0C for 2 hours. The dried nanoparticles were stored in vacuum desiccators to ensure the removal of residual solvent **(Umar *et al.*, 2015).**

Table 7: Formulation Designing of Pyridostigmine Nanoparticles

Formulation Code	INGREDIENTS						
	Pyridostigmine (mg)	Ethyl cellulose (mg)	Chitosan (mg)	HPMC K100 (mg)	Polyvinyl alcohol (%w/v)	Dichlro Methane (ml)	Distilled water (ml)
F1	200	200			0.2	20	100
F2	200	400			0.2	20	100
F3	200	600			0.2	20	100
F4	200	800			0.2	20	100
F5	200		200		0.2	20	100
F6	200		400		0.2	20	100
F7	200		600		0.2	20	100
F8	200		800		0.2	20	100
F9	200			200	0.2	20	100
F10	200			400	0.2	20	100
F11	200			600	0.2	20	100
F12	200			800	0.2	20	100

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C. Characterization of Nanoparticles

1) *Production Yield:* The Production yield of prepared controlled release nanoparticlesF-1 to F-12 was shown in Table: 6.8Increasing polymer ratio in the formulation led to increase the product yield). The low percent yield in some formulations may also due to nanoparticles lost during successive decantation during drying process. The percentage yield of produced nanoparticles F1 to F12 is shown
2) *Determination of Drug Content:* Sample containing 100 mg equivalent Pyridostigmine Nanoparticles are dissolved and the volume is made up to 100ml with water. From the above solution 10 ml is pipette out and made up to 100 ml with phosphate buffer. The Absorbance of resulting solution is determining at λ max 270 using UV spectrophotometer (Systronic 2022) and the drug content is estimated shown in
3) *Entrapment Efficiency:* Drug entrapment efficiency for prepared controlled release nanoparticles was observed in the range 59.15% to 91.88%.. Among the different drug polymer ratios investigated Formulation F6, showed the maximum capacity for drug entrapment efficiency as shown (Wang *et al*., 2015). Drug entrapment efficiency was increased with increasing polymer concentration in Nanoparticle. Production yield (%) and Theoretical Loading (%) were also shown.

Table 8: Characterization of Pyridostigmine Nanoparticles Formulations

S. No.	Formulation Code	Production yield (%)	Theoretical Loading (%)	Drug Content (%)	Entrapment Efficiency (%)
1.	F1	79.28±1.29	60.09±0.93	89.22±1.37	66.56±1.03
2.	F2	74.22±2.34	41.61±1.65	92.01±1.41	73.96±1.14
3.	F3	82.11±1.26	27.71±0.45	86.57±1.33	62.53±0.97
4.	F4	80.56±1.24	22.43±1.37	83.88±2.29	65.84±1.02
5.	F5	76.55±1.18	62.51±0.97	82.56±1.27	66.66±2.03
6.	F6	79.12±2.22	39.51±2.62	95.20±1.46	89.88±1.38
7.	F7	79.22±1.22	30.42±0.49	89.17±0.37	72.93±1.12
8.	F8	77.38±1.19	23.06±1.38	89.34±1.37	53.36±0.83
9.	F9	78.55±2.21	60.11±0.93	89.03±1.37	66.85±3.03
10.	F10	74.18±1.14	42.79±1.67	93.03±2.43	73.57±1.13
11.	F11	81.76±1.26	29.07±0.47	89.46±1.37	59.33±1.92
12.	F12	78.14±2.32	23.87±2.39	85.33±1.31	57.15±0.89

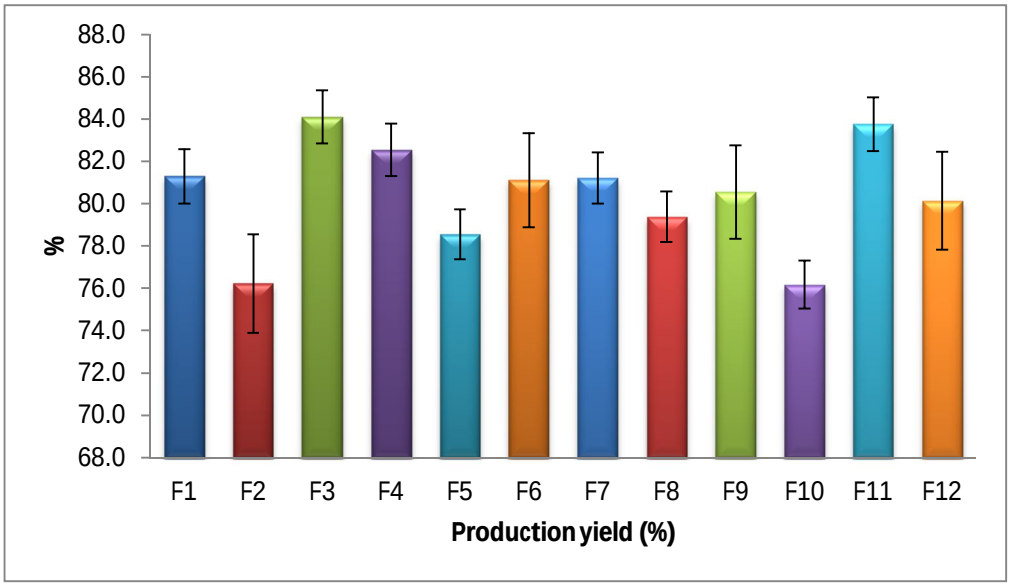

Fig. 7:% Practical Yield of Pyridostigmine Nanoparticles Formulations

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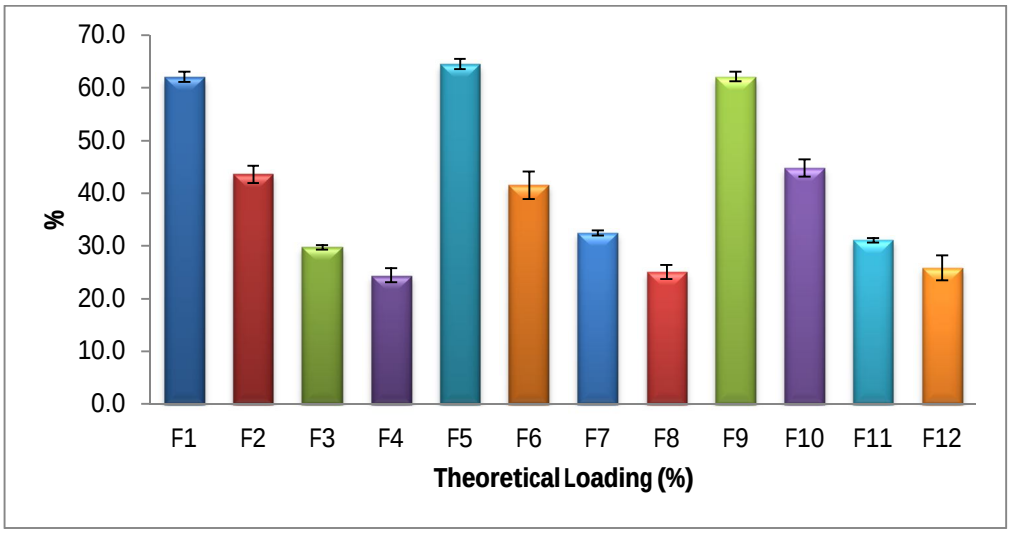

Fig. 8: Theoretical Loading of Pyridostigmine Nanoparticles Formulations

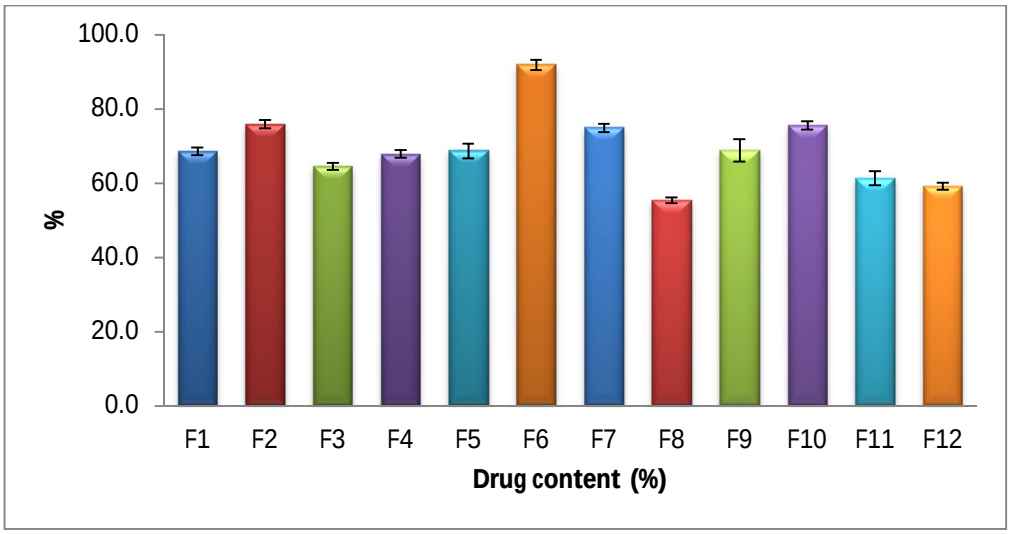

Fig. 9: Drug content of Pyridostigmine Nanoparticles Formulations

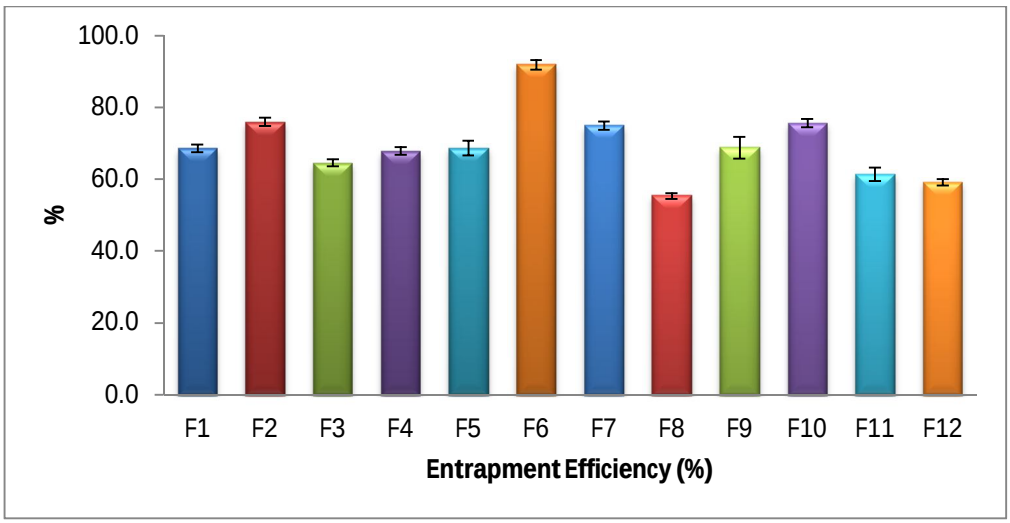

Fig. 10: Entrapment Study of Pyridostigmine Nanoparticles Formulations

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4) *In vitro Release Studies:* The dissolution study was carried out in 0.1N Hydrochloric acid for 2 hours and phosphate buffer of pH 6.8 for the 10 hours. The results of *in-vitro* drug release studies from the controlled release Nanoparticles of Pyridostigmine are shown. The cumulative percentage drug release after 12 hours was found to be 2.84±0.35, 12.32±2.05, 21.81±0.23, 26.95±0.26, 32.96±1.48, 37.39±1.55, 44.87±0.65, 52.41±0.93, 58.28±0.68, 64.38±1.14, 80.49±1.84 and 87.07±1.07for the formulations of F1 to F12. It was found that the drug release was prolonged up to 12 hrs. Pyridostigmine loaded chitosan nanoparticles F6 formulation exhibited good controlled release characteristics. The release rate was related to drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. The initial burst effect of some formulations may be due to presence of drug particle on the surface of the nanoparticles, this initial drug release may also attribute as a desired effect to ensure Minimum effective concentration of drug to produce pharmacological action (Umar *et al.*, 2015).

Table 9: Cumulative % Drug Release of Pyridostigmine Nanoparticles Formulations

S. No.	Time in hours	Cumulative % Release		
		F2	F6	F10
1.	1	2.84±0.35	2.77±0.25	2.08±0.06
2.	2	12.32±2.05	17.05±0.71	10.65±0.15
3.	3	21.81±0.23	20.91±0.68	22.47±2.02
4.	4	26.95±0.26	29.57±0.33	32.03±0.46
5.	5	32.96±1.48	35.95±0.91	37.09±0.92
6.	6	37.39±1.55	42.96±1.14	43.55±0.94
7.	7	44.87±0.65	49.73±0.81	50.22±0.85
8.	8	52.41±0.93	57.59±1.16	58.46±1.11
9.	9	58.28±0.68	67.51±1.03	65.99±0.85
10.	10	64.38±1.14	77.28±0.47	72.55±1.21
11.	11	80.49±1.84	88.37±1.81	80.48±1.01
12.	12	87.07±1.07	98.07±0.73	90.02±0.77

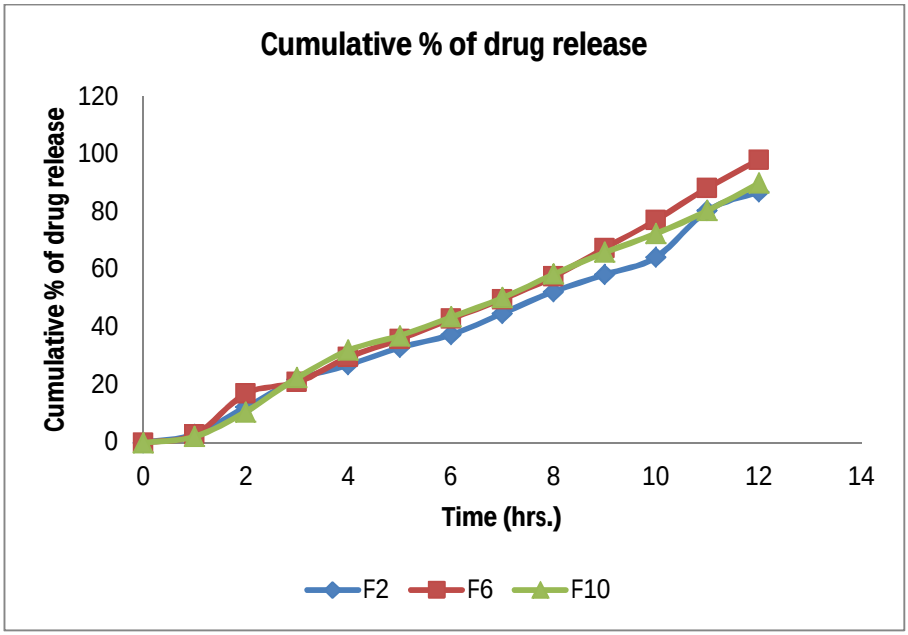

Fig. 11: Cumulative % Drug Release Study of Pyridostigmine Nanoparticles Formulations

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5) *Kinetic Analysis:* The release data was modeled for Zero order, First order, Higuchi model, Hixson- Crowell model, Korsmeyer-Peppas model.

Table 10: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulations

S. No.	Model	F2	F6	F10
1.	Zero order Kinetics	0.9918	0.9924	0.9921
2.	First order Kinetics	0.9843	0.9371	0.98
3.	Higuchi Model	0.9577	0.9413	0.9488
4.	Korsemeyer- peppas model	0.9876	0.971	0.991

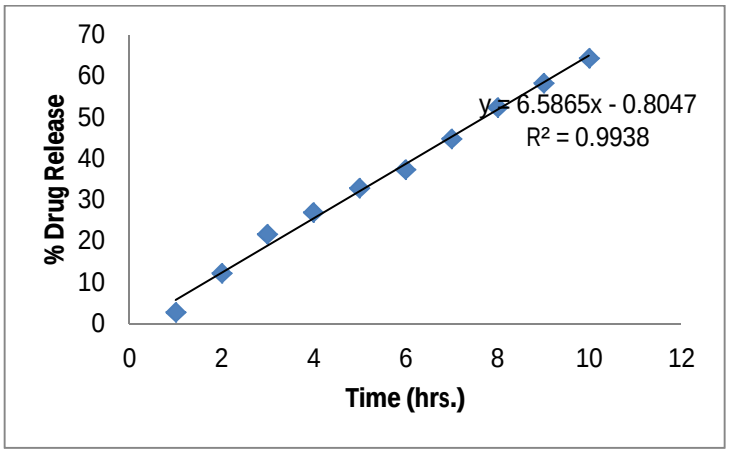

Fig. 12: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F2- Zero Order)

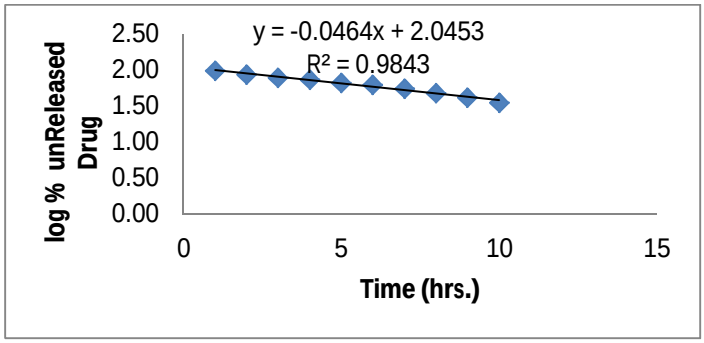

Fig. 13: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F2- First Order)

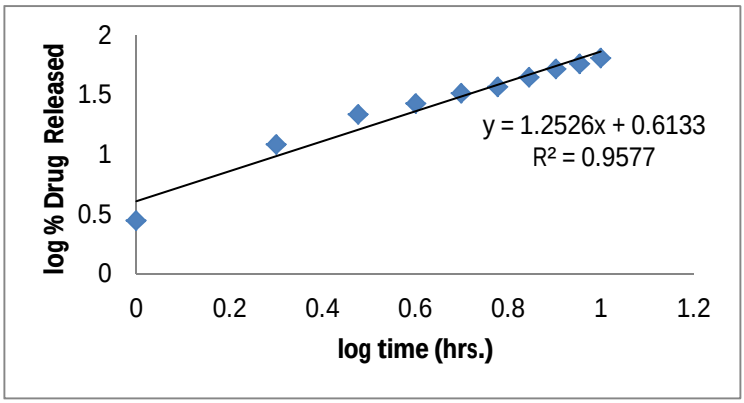

Fig. 14: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F2- Korsemeyer- peppas Model)

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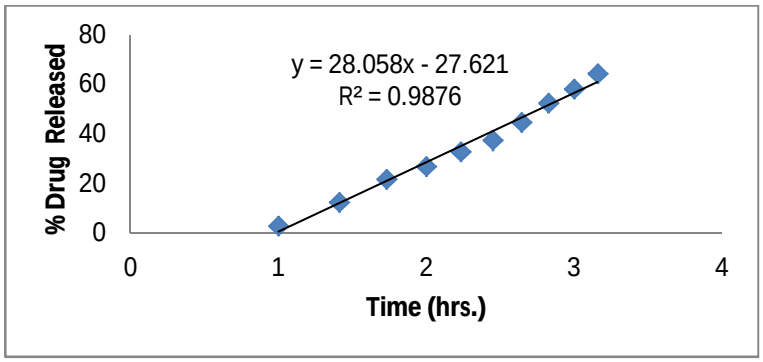

Fig. 15: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F2- Higuchi Model)

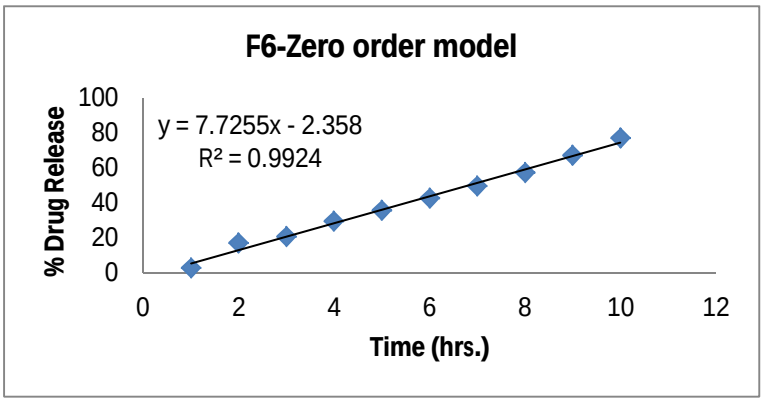

Fig. 16: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F6- Zero Order)

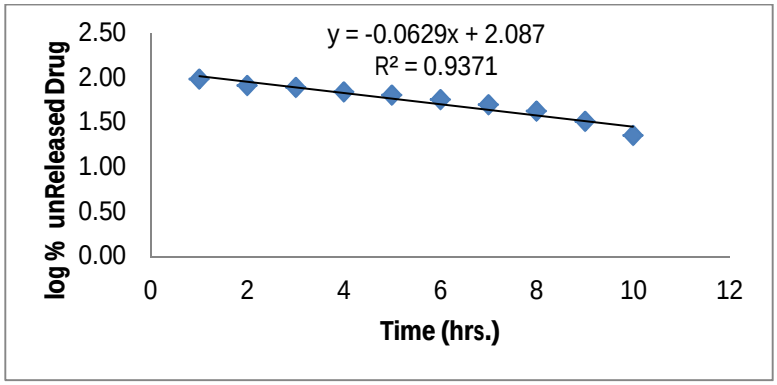

Fig. 17: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F6- First Order)

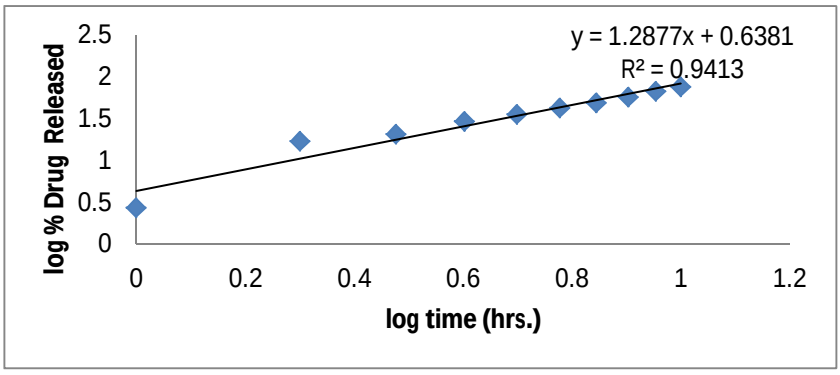

Fig. 18: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F6- Korsemeyer- peppas Model)

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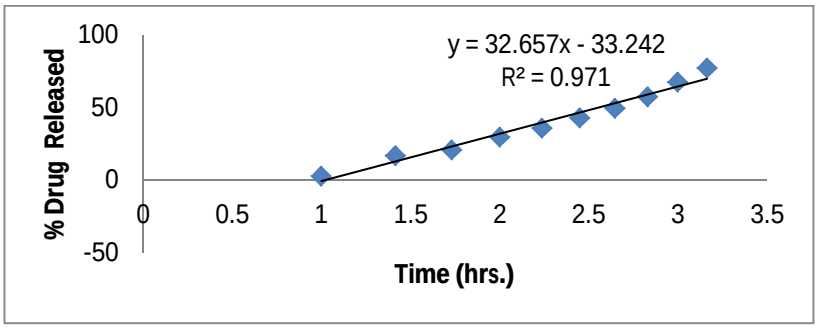

Fig. 19: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F6- Higuchi Model)

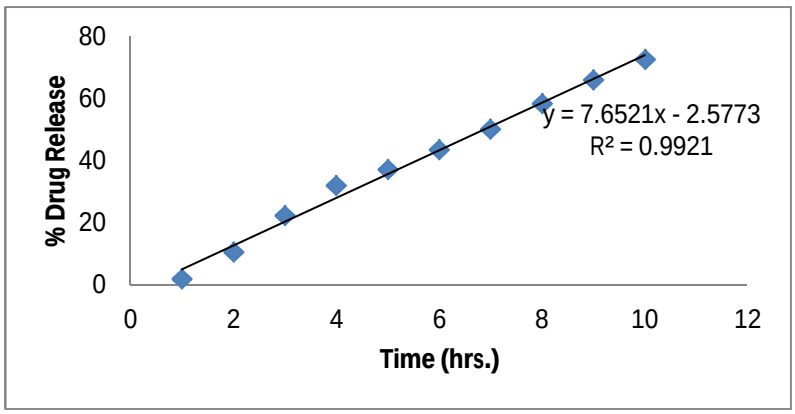

Fig. 20: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F10- Zero Order)

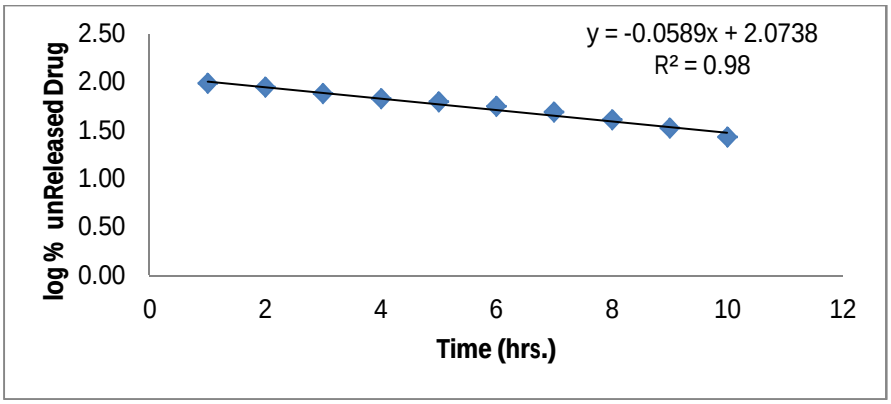

Fig. 21: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F10- First Order)

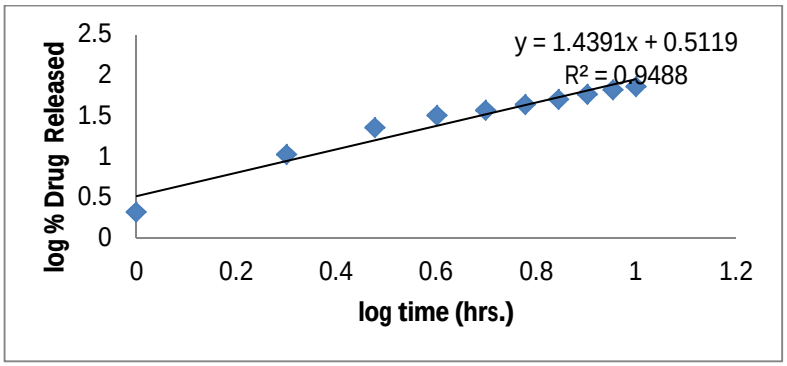

Fig. 22: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F10- Korsemeyer- peppas Model)

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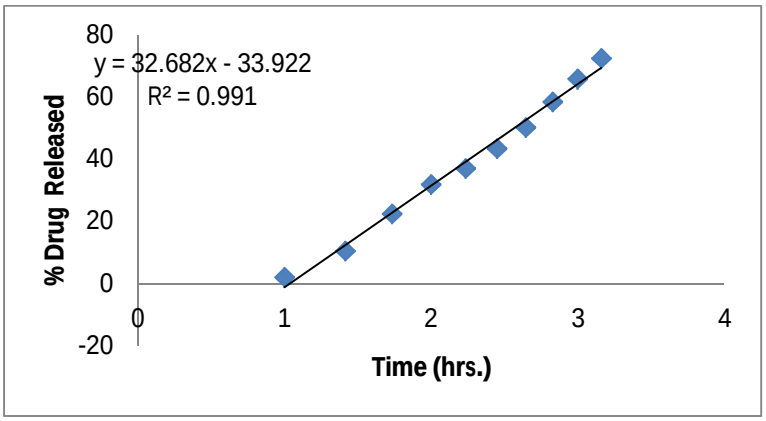

Fig. 23: Kinetic Drug Release Study of Pyridostigmine Nanoparticles Formulation (F10- Higuchi Model)

6) *FTIR Spectroscopic Study:* The results obtained from FTIR study of best formulation was shown in Figure 25.The peaks obtained in the pure Pyridostigmine were also found in final formulation, which indicates that there is no interaction between the drug and excipients.

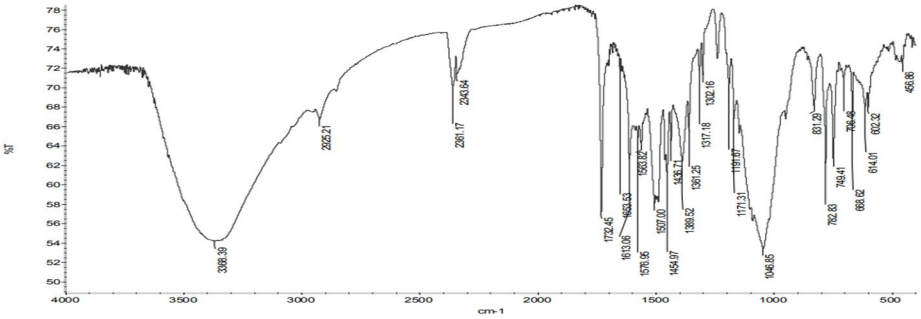

Fig. 24: FTIR Spectra of Pyridostigmine containing Formulation

7) *Particle Size & Zeta Potential Study:* The particle size, polydispersity index and zeta potential for nanoparticle formulation were studied. The particle size analysis revealed that the particle size measured by laser light scattering method is around 200-400 nm with low poly dispersity index values as known, the polydispersity index is a parameter used to define the particle size distribution of nanoparticles. It is a dimension less number and it values ranges from 0.5-0.7 for mono dispersed particles, values greater than 0.7 are characteristic of samples with a broad size distribution. Therefore, it can be stated that the particle size distribution is unimodel, having a narrow range and a homogenous size distribution. Particle size and zeta potential of nanoparticles showed in figure 7.23 and 7.24 respectively. When the polymer to drug ratio was increased, the proportion of larger particles was high, because the viscosity of the polymer and drug dispersion was increased with increase of polymer to drug ratio.

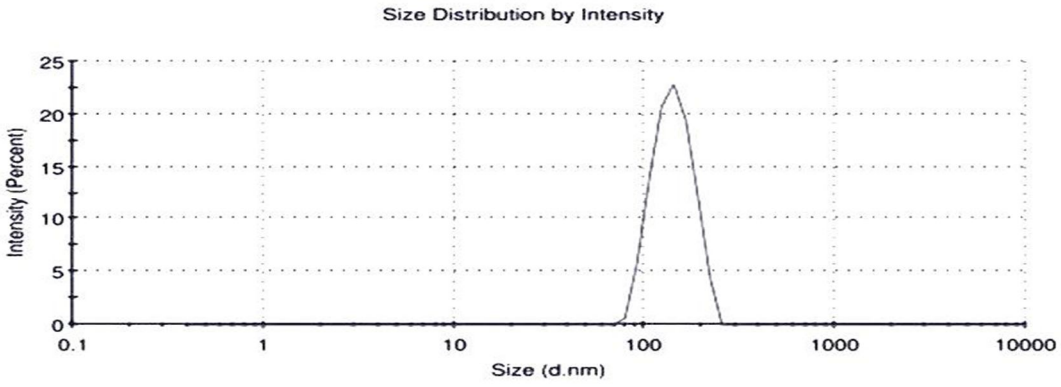

Fig. 25: Particle Size Analysis of Pyridostigmine containing Optimized Formulation

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8) Zeta Potential

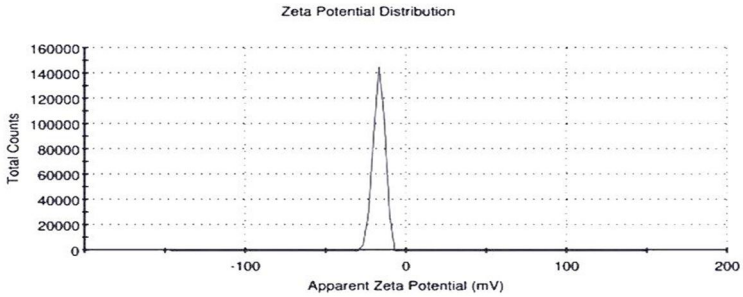

Fig. 26: Zeta Potential Study of Pyridostigmine containing Optimized Formulation

9) Scanning Electron Microscopy (Ansari KA et al., 2011): The morphology of the nanoparticles by solvent evaporation method was investigated by Scanning electron microscopy (SEM). The particle and porous nature of the nanoparticles with rough surface and presence of holes /hollow cavity due to the collapse of the wall of the nanoparticles during in situ drying process. Thus the rate of solvent removal from the embryonic nanoparticles exerts an influence on the morphology of the end product. Porous structure was observed on the surface due to the rapid diffusion of the solvent, there is a possibility of rupture of the capsule wall.

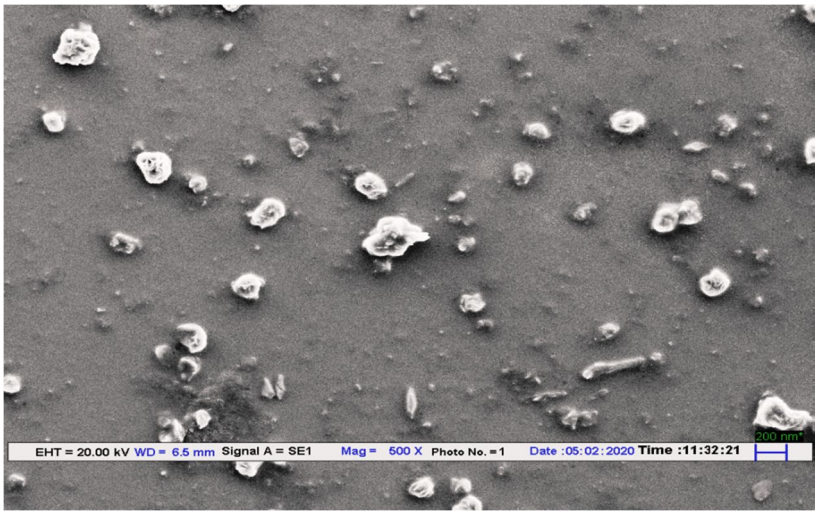

Fig. 27: Scanning Electron Microscopy of Pyridostigmine containing Optimized Formulation

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also to achieve and maintain the desired drug concentration. The λ max of Pyridostigmine as found to be 270 nm in pH 7.4 in PBS 10µg/ml solution. Pyridostigmine is a highly potent SSRI was used as a model drug to develop a controlled release formulation. Pyridostigmine designed to prolong the release increase the drug bioavailability, diminish the side effects of irritating drugs and also reducing the frequency of administration, thereby improving the patient compliance and therapeutic efficacy. The Pyridostigmine obeys the Beers law within the concentration of 5 to 35µg/ml. Pyridostigmine is having low solubility the drug displays extensive first pass metabolism. The purpose of this study was to prepare Pyridostigmine nanoparticles for control release of Pyridostigmine to improve the oral bioavailability, enhance the solubility and dissolution rate by decreasing particle size of drug. Infrared spectroscopic studies confirmed that there was no interaction between drug and polymers. The controlled release Pyridostigmine nanoparticles were prepared by Solvent evaporation by using Ethyl cellulose, Chitosan & HPMC K100 at different ratios. The production yield of the formulated controlled release nanoparticles (F1toF16) in the range of 76.11% to 83.58%. The drug content of the formulated controlled release nanoparticles (F1toF16) in the range of 82.56 %to 98.20%. The Theoretical loading of the formulated controlled release nanoparticles (F1- F16) in the range of 24.43 % to 64.24%. The entrapment efficiency increased with increasing the concentration of polymers and the formulations containing chitosan nanoparticles F6 (1:2) showed better entrapment (90.94%) among all formulation.

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The solubility of selected formulation (F6) in 0.2 M Phosphate buffer pH 6.8increased when compared to pure drug. Particle size distribution was determined by Malvern zeta size, the size range for produced nanoparticles in the range of 200 nm to 400 nm. The Polydispersity index of selected nanoparticle formulation (F6) was indicated a narrow range and a homogeneous size distribution of particles. Zeta potential value of Pyridostigmine nanoparticles showed a positive surface charge this is because of more anion on the surface of the particles higher the charge higher is the stability of the nanoparticle. The *in vitro* dissolution study was carried out in 0. 2N PBS for 2 hours and phosphate buffer pH 6.8 for 10 hours. The formulations shows controlled release of drug upto1 2 hrs and all formulations showed more than 75% of drug release. The release kinetics showed that the formulations were complies with Zero order kinetics followed by diffusion controlled mechanism and Korsemeyar-peppas n values were more than 0.4 indicating Non fickian diffusion. The best formulation F6 was selected based on production yield, entrapment efficiency, solubilization efficiency, particle size, Polydispersity index & zeta potential and *in-vitro* drug release and release kinetics. The best formulation F6 was evaluated by infrared spectroscopy, particle size, Polydispersity index & zeta potential and Scanning Electron microscopy. Best formulation of nanoparticles shown the extent of drug release was found to be F6 (96.93%) in 12hrs. SEM studies confirmed the morphology of the nanoparticle formulation.

IV. CONCLUSION

Hence, it was concluded that nanoparticle a good approach to release the drug in a controlled manner to the targeted site and enhance the solubility and dissolution property of Pyridostigmine by solvent evaporation method for the successful incorporation of Pyridostigmine with high entrapment efficiency. The solubility studies suggested that the nanoparticle formulations enhanced the bioavailability of Pyridostigmine by improving its solubility and dissolution rate when compared to pure drug. Furthermore, it could be presumed that if then a no meter range of particles were obtained, the bioavailability might be increased. Thus the nanoparticles as controlled release formulations can be useful for delivery of short elimination half life, low bioavailability through orally. Thus nanoparticle drug delivery system provides site specific drug delivery and prolongs dosage interval and thus improving patient compliance.

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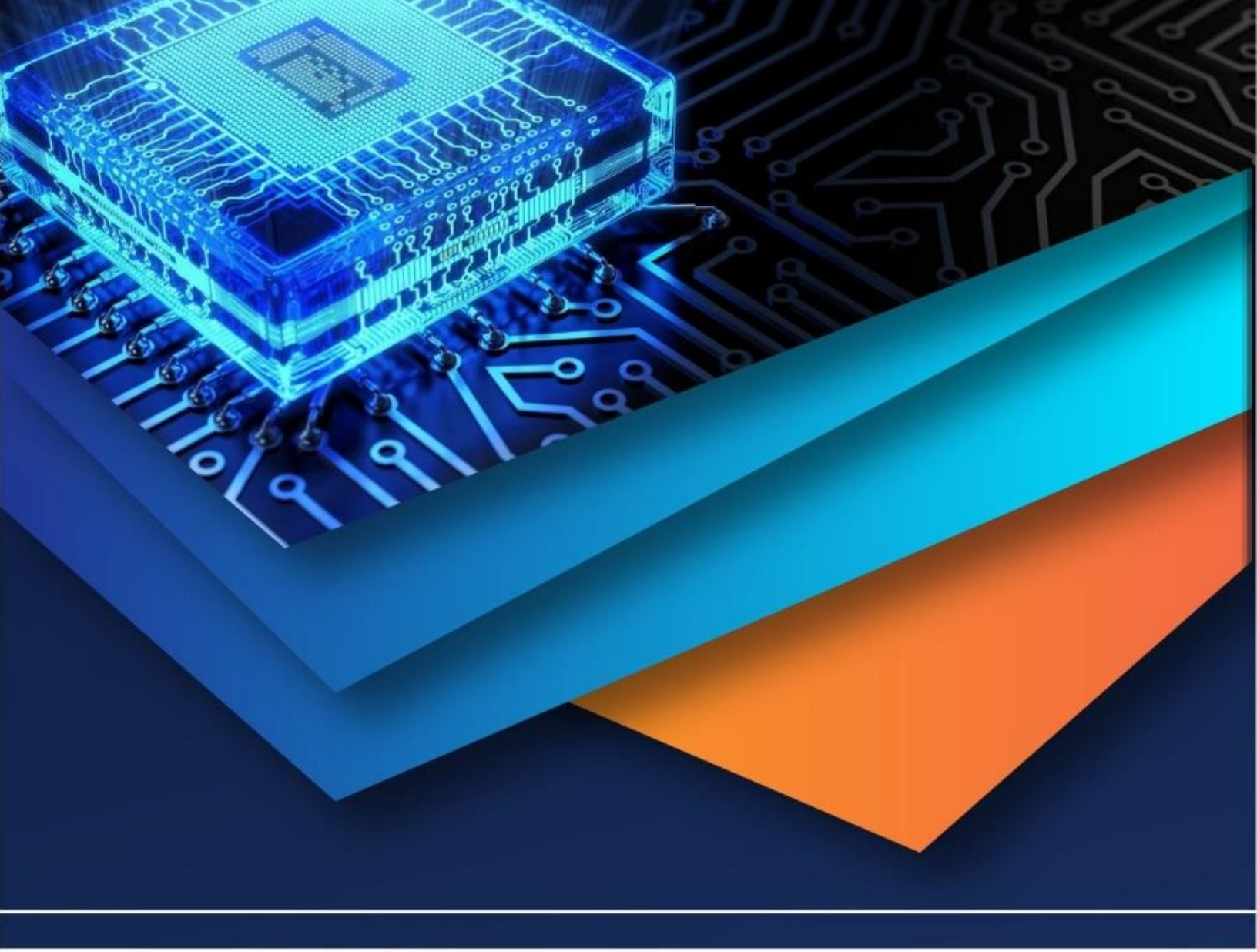

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