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Development and characterisation of methylphenidate hydrochloride loaded nanoparticles

Anju Singh
Department of Biotechnology, Faculty of Engineering and Technology, Rama University, G.T. Road, Kanpur - 209217, India

Ajay Kumar
Department of Biotechnology, Faculty of Engineering and Technology, Rama University, G.T. Road, Kanpur - 209217, India
Corresponding Author Email: ajaymtech@gmail.com

***Abstract*---**Preparation of Methylphenidate Hydrochloride nanoparticles was the goal of this study, which aimed to minimise the dosage frequency. Polymeric nanoparticles are gaining in popularity due to their inertness, solubility in non-toxic solvents, and ability to be used to create sustained-release dosage forms, among other characteristics. Melphenidate hydrochloride, a diuretic, is used to treat congestive heart failure, edoema, and renal failure among other conditions. Polymeric nanoparticles are one of the most effective platforms for long-term release because of their high stability. To put it another way, because its half-life (2.4 hours for children and 2.1 for adults) is short, methylphenidate hydrochloride is only effective for an extremely short period of time. This study's major focus is on nanoparticles. Eudragit RL 100 can be used as a release retardant. Formulation of Methylphenidate Hydrochloride and Eudragit RL 100 nanoparticles in order to provide long-term activity and hence boost bioavailability. The steady release of Methylphenidate Hydrochloride from nanoparticles increases therapeutic efficacy by maintaining a constant drug plasma concentration. The new formulation of methylphenidate hydrochloride sustained release proved successful in alleviating the problems associated with the old one.

***Keywords*---**Synthesis, Nanoparticles, Eudragit RL 100, Methylphenidate Hydrochloride.

Introduction

Throughout the last 50 years, nanotechnology has become an integral part of our daily life, from electrical devices to medications, cosmetics, and the food and beverage industries. Traditional medications are supplied excessively and inefficiently because they cannot reach certain cell compartments or are not formulated for the context in which they will be employed [1]. Polymeric nanoparticles were first studied for their potential to increase the bioavailability, manage the release of drugs from a single dosage, and preserve drugs until they reach their intended destination [2]. Creating polymeric nanoparticles may be done in several ways. Synthesis methods are critical to determining important features like particle diameter and polydispersity in the application and how the medication is incorporated into nanotransporters [3, 4].

In addition to the synthesis technique, polymer, whether synthetic or natural, should be taken into account. The biocompatibility, biodegradability, and surface modification capabilities of natural polymers make them a standout in the field [5]. Collagen, albumin, and gelatin have all been extensively studied because of their high application potential as biopolymers [6]. Specific properties of synthetic polymers can be modified to satisfy specific demands, such as optimising a compound's specificity or increasing its bioavailability or decreasing its toxicology [7]. New and improved technologies are being spurred on by interactions between these polymers and biological systems have different compositions and surface characteristics. Active vectoring is possible because to polymers known as smart, which respond to physiological events including pH and temperature changes and/or external stimuli [8]. Vinyl esters, for example, are hydrophilic polymers that breakdown in acidic conditions, double esters and hydrazones, are commonly used to respond to inflamed or cancerous tissues or lysosomal conditions in order to release the active principle [9].

The pharmaceutical sciences have benefited greatly from the development of nanoparticles [10]. Biological pathways can be better understood thanks to improved delivery systems, diagnostic and treatment techniques, and the development of new diagnostic and therapeutic methods [11]. As a result, the focus of this review was on polymeric nanoparticle production methods and mechanisms of controlled release for biomedical use [12].

Materials and Methods

Methylphenidate Hydrochloride was supplied Sun Pharma, Mumbai., Polymer Eudragit RL 100 were received from yarrow chem pvt ltd. All reagents were used as received.

Construction of standard curve for methylphenidate hydrochloride

A. By UV spectroscopy Method

Methylphenidate hydrochloride has a spectrophotometric absorption maximum of 210 nm, and its concentration ranges from 1-10 ng/ml according to Beer-Law. Lambert's

Determination of absorbance maximum (λ_{max}):
Methylphenidate hydrochloride solution of pH 7.4 in phosphate buffer saline was made by appropriate diluting to a concentration of 20 g/ml. Phosphate buffer saline pH 7.4 was used as a blank to scan the solution in a UV spectrophotometer between 200 and 400 nm. The 210 nm value was found to be the highest possible value for absorbance. The drug's absorbance at 210 nm in phosphate buffer saline pH 7.4 was used to determine its exact concentration.

Preparation of pH 7.4 phosphate buffer saline
Distilled water is used to make up the volume of a 1000-milliliter volumetric flask containing 2.38 grammes of disodium hydrogen phosphate, 0.19 grammes of potassium phosphate, and 8.3 grammes of sodium hydroxide. If necessary, the pH was tinkered with.

Preparation of stock solution
For the principal stock solution, 100mg of Methylphenidate hydrochloride medicines were dissolved in 100-ml of solvent medium to produce the 1000 mg/ml solution. 1ml was collected from a standard flask and diluted to 100 ml with solvent medium PBS 7.4 (secondary stock solution) to get a concentration of 1-10mcg/ml.

Preparation of standard solution
It is possible to achieve concentrations between one and ten micrograms per millilitre (mg/ml) from the secondary stock solution. Measurement of UV spctrophotometric absorption of the solution was performed at 210 nm against a blank of drug-free PBS pH 7.4 medium.

Table 1
Calibration curve of methylphenidate hydrochloride

S. no.	Concentration (µg/ml)	Absorbance at 210 nm
1	10	0.115
2	20	0.208
3	40	0.459
4	60	0.587
5	80	0.753
6	100	0.898

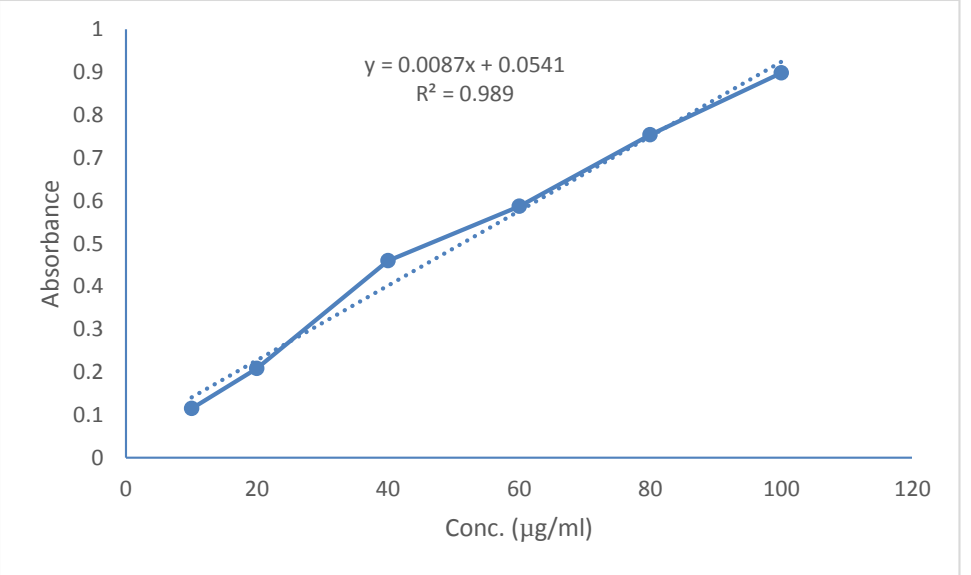

Fig. 1: Standard curve for methylphenidate hydrochloride

Drug and polymer compatibility study by FTIR

Choosing an excipient (or carrier) for pharmaceutical formulations that is compatible with the rest of the ingredients is an important consideration. The FT-IR spectrophotometer (perkin elmer) was used in the current investigation to confirm any probable chemical interaction between Methylphenidate hydrochloride and Eudragit RL 100 [13].

The pure drug (Methylphenidate hydrochloride, Eudragit RL 100) and their physical combination were studied using the potassium pellet technique of infrared spectroscopy. A hydraulic press at a pressure of 15 tonnes compresses them into a clear pellet. A spectrophotometer scanned the particle from 4000 to 400 cm^{-1}.

It was determined whether or not there were any molecular interactions between the medication and polymer by comparing the mixture's spectrum to the original spectra. The vibration modes of certain chemical bonds in a sample are measured using FTIR spectroscopy, a Fourier transform technique. The vibration spectrum of an encapsulated medicine may be used to determine the kind of interaction between the drug and polymer.

To acquire FTIR spectra, researchers used Methylphenidate hydrochloride and Eudragit RL 100 pure drugs. A hydraulic press compresses them to a translucent pellet under 15 tonnes of pressure. From 4000 to 400 cm^{-1}, a spectrophotometer measured the pellet's light absorption.

Method of preparation of methylphenidate hydrochloride nanoparticles
Solvent Evaporation Method

Solvent evaporation was used to make all of the nanoparticle batches. I portion: 5 ml ethanol, 50 mg sodium dodecyl sulphate diluted in 5 ml water, and the needed amount of medicine and polymer dissolved in this combination (II portion). Finally, the drug and polymer combination was combined with sodium dodecyl sulphate solution via injection. After being homogenised for one minute using a vortex mixture, the mixture was sonicated for size reduction at a power output of 90W. The flash evaporator was used to collect the dried nanoparticles following solvent drying [14].

Table 2
Methylphenidate hydrochloride nanoparticle production method

S. no.	Formulation code	Drug (Methylphenidate hydrochloride) in mg	Polymer Eudragit RL 100
1	F1	20	10
2	F2	20	20
3	F3	20	30
4	F4	20	40
5	F5	20	50
6	F6	20	60
7	F7	20	70
8	F8	20	80
9	F9	20	90
10	F10	20	100

Evaluation of nanoparticles
Drug entrapment study

Following centrifugation at 15,000 rpm for 20 minutes at 0°C using an ultra centrifuge, the free drug concentration in the supernatant was assessed by UV spectrophotometrically measuring the absorbance of the 210 nm absorbance peak [15].

Invitro drug release studies
By UV spectrophotometric method

The diffusion membrane method was used to conduct the in vitro drug release investigation. Diffusion medium (PBS 7.4) was kept at 37°C under continual magnetic stirring, and the nanoparticles prepared was dropped into the solution through dialysis membrane into the beaker holding 200ml of the medium. Every hour, a 1ml sample of the diffusion medium was obtained and replaced with 1ml

of fresh media. It took 24 hours to complete this procedure [16]. At a wavelength of 210 nm, the sample was subjected to an ultraviolet spectrophotometric measurement.

Scanning Electron Microscopy (SEM)

SEM was used to examine the morphology of the improved formulation (SEM). Adhesive tiny sample wads were attached directly in scotsch double adhesive tape for SEM investigation of the specimen. A snapshot was obtained using a 15Kv hitachi scanning electron microscope to examine the material [17].

Surface charge (zeta potential) determination

The zeta potential of a colloidal or dispersed system is an essential metric to analyse and develop an optimal environment for stability. Zeta potential was measured using a zeta potential analyzer on the produced nanoparticle suspension (Malvern Zeta Seizer) [18]. Electrical charges on the surface of a particle produce an electrical barrier, which is crucial to the stability of a medication. The surface characterization of the nanoparticle was examined to see how Eudragit RL 100 affected it [18].

pH and physical appearance

A pH metre was used to determine the formulation's pH. For stability and formulation action, it's a critical component. Examine the formulation's colour and any extraneous particles that may have been dissolved in it [19].

Stability studies of nanoparticles

At 45°C/70% RH, nanoparticles are tested for stability in an accelerated environment, and at 4°C on the refrigerator, as well as at ambient temperature. To conduct the following experiments, the formulations were stored at both temperatures for three months, and a suitable quantity of sample was obtained at regular intervals [20].

Results and Discussion
Development of methylphenidate hydrochloride nanoparticles

The Eudragit RL 100 solvent evaporation technique was used to make Methylphenidate Hydrochloride Nanoparticles in this work. Ethanol was used to dissolve the drug (Methylphenidate hydrochloride) and the polymer (Eudragit). 0.50mg sodium dodecyl sulphate dissolved in water was combined with 5ml of the other solution. For one minute, the mixture was homogenised in a vortex, and then sonicated with a probe. A flash rotator evaporator was then used to evaporate this mixture for 20 minutes.
Polymer formulations of various proportions were produced. Nanoparticle shape, particle size determination, drug release profile, and stability of the optimised formulation at different temperatures were all studied in this study.

Drug & polymer compatibility studies by FTIR

The material was handled in the FTIR spectrometer using a pressed pellet approach. It was possible to use this method to make a clear pellet from a little amount of sample and potassium bromide, and then place the pellet into a custom-made holding chamber for an infrared spectrometer using specific discs and high pressure.

It is possible to get IR spectra for both pure drug and physical mixtures of the drug and polymers. In this study, the spectra of physical mixtures and pure drugs were compared. Consequently, there was a strong interaction between drug and excipients because of the bands seen in the pure drug.

Entrapment efficiency of nanoparticle

Dialysis was used to evaluate the entrapment efficiency of Methylphenidate hydrochloride-loaded nanoparticles. The polymer (Eudragir RL100) formulation F1, F2, and F3 is taken in 10mg, 20mg, and 30mg concentrations. The entrapment efficiencies were 50%±0.16, 57%±0.13, and 62%±0.14 correspondingly. This suggests that the medication and polymer have less of a repulsive force.

Table 3
The ability of nanoparticles to be encapsulated

S. no.	Formulation code	Drug (mg)	Eudragit RL 100 (mg)	Entrapment efficiency (%)
1	F1	20	10	50±0.16
2	F2	20	20	57±0.13
3	F3	20	30	62±0.14
4	F4	20	40	68±0.11
5	F5	20	50	72±0.19
6	F6	20	60	78±0.14
7	F7	20	70	84±0.13
8	F8	20	80	89±0.10
9	F9	20	90	95±0.07
10	F10	20	100	46±0.06

The polymer (Eudragit RL100) concentration varies to 40mg, 50mg, and 60mg in subsequent formulations F4, F5, and F6. Also, the medication and polymer were less repellent to each other, with an efficiency rate of 68%±0.14, 72%±0.19, 78%±0.14%.

As a result, the polymer content of formulations F7, F8, and F9 (Eudragit RL100) is further increased to 70mg, 80mg, and 90mg. There was an entrapment efficiency of 84%±0.13, 89%±0.10 and 95%±0.07. An entrapment efficiency of 95% may be seen in the formulation F9, which shows a consistent rise in efficiency over time

F10 variations in polymer concentration of 100mg Eudragit RL100 were studied further. Increased polymer concentration reduced entrapment efficiency to 46%±0.06. Using the aforementioned results, formulation F9 was chosen for future tests since it had the maximum entrapment efficiency of 95 percent.

In vitro drug release profile of nanoparticles:

- There was a 24-hour in vitro drug release investigation of Methylphenidate hydrochloride nanoparticles using membrane diffusion technique.
- Nanoparticles containing Methylphenidate hydrochloride loaded with Eudragit RL 100 polymer were tested for in vitro drug release.
- Formulation F1's in vitro release of medication (Methylphenidate hydrochloride 20mg, Eudragit RL100 10mg). In only six hours, 99.45% of the medication had been released. The medication is released within six hours after being formulated.
- So, subsequent formulation F2, F3 with varied concentration of polymer (Methylphenidate hydrochloride 20mg with Eudragit RL 100 20mg, 30mg) the percentage of drug release was accordingly 98.46 percent , 97.45 percent in 8 hours. Which formulations were showed fast release (8hours) (8hours). Because of the low polymer content.
- The drug release percentage was 99.48 percent after 13 hours, 99.49 percent after 15 hours, 97.47 percent after 19 hours, and 99.49 percent after 20 hours with the formulation F4, F5, F6, and F7, which had a higher polymer concentration and reduced repulsive force.
- The formulation F8, F9 with increasing the concentration of polymer concentration the percentage of drug release was 93.45 percent in 24 hours, 99.49 in hours.
- The percentage of drug release in 24 hours for formulation F10 (Methylphenidate hydrochloride 20 with Eudragit RL 100 200mg) was 56.29 percent. An increase in polymer concentration results in a 56.29 percent increase in medication release.

For further analysis, formulation F9 was chosen as the best one of the aforementioned formulations (F1-F10) because of its high percentage of drug release (99.49 percent) in comparison to the other formulations (F1 to F10).

Table 4
In vitro drug release for F9

Time (h)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	0.3	0.3	3
2	0.8	0.80	8.03
3	1.3	1.30	13.05
4	1.7	1.70	17.08
5	2.2	2.21	22.10
6	2.8	2.81	28.12
7	3.3	3.31	33.15
8	3.9	3.91	39.18
9	4.4	4.42	44.21

10	4.7	4.72	47.23
11	5.1	5.12	51.25
12	5.5	5.52	55.27
13	5.9	5.92	59.29
14	6.2	6.23	62.31
15	6.6	6.63	66.32
16	7.0	7.03	70.34
17	7.4	7.43	74.36
18	7.9	7.93	79.38
19	8.2	8.24	82.41
20	8.6	8.64	86.42
21	8.9	8.94	89.44
22	9.2	9.24	92.46
23	9.5	9.54	95.47
24	9.9	9.94	99.49

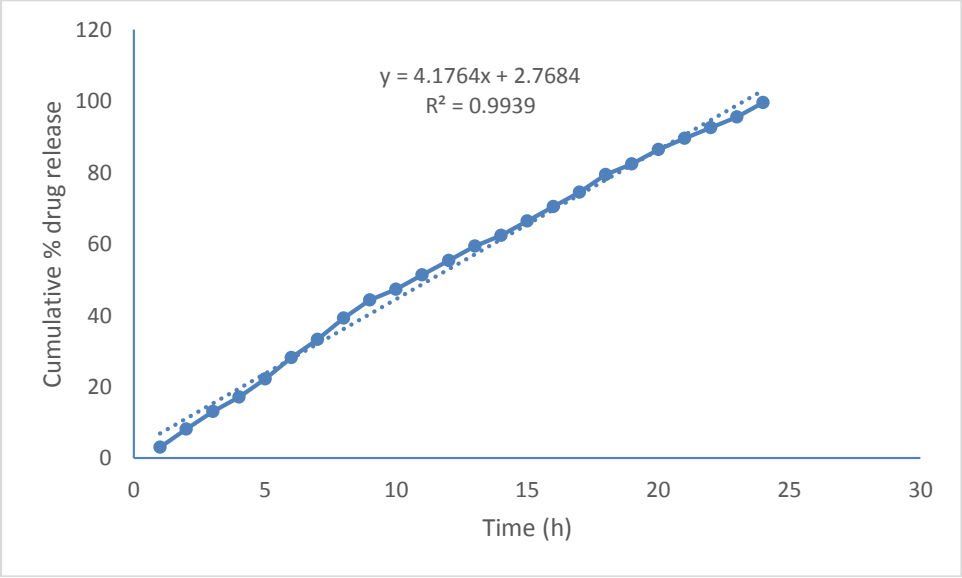

Fig. 1: *In vitro* drug release for formulation F9

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to investigate the surface features of the best formulation (F9) particle size. Using SEM imaging, we can see the polymer coating on the drug particle. An even and thin coating over the medication is shown by the granule-like appearance of nanoparticles under scanning electron microscopy (SEM).

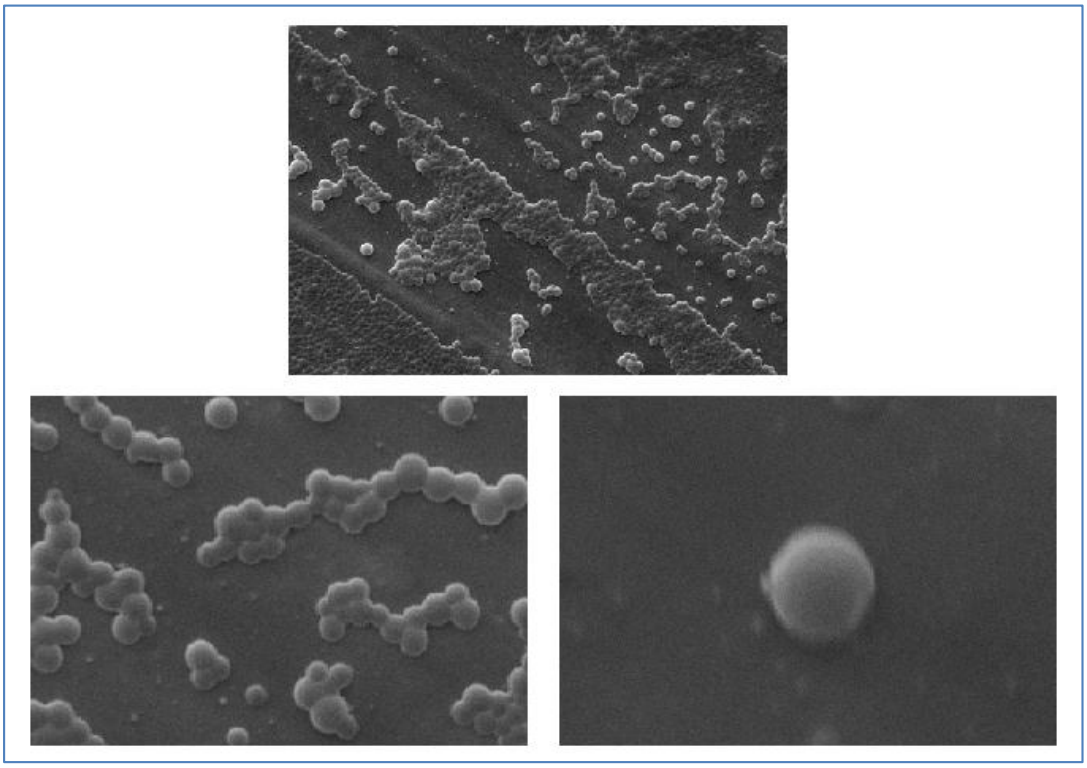

Fig. 2: SEM FOR F9

Surface charge (Zeta potential)

The surface charge attribute of a nanoparticle is often described using the potential of a nanoparticle. In this way, the electrical potential of particles is impacted by the content of the particles and the manner in which they are disseminated in the environment. When administered intravenously, nanoparticle formulations are readily recognised and detected by phagocytes. The nanoparticle's particle size and hydrophobicity surface affect the opsonin adsorption of blood components. The destiny of the nanoparticles is ultimately decided by these opsonins. These opsonins are referred to be opsonized when they are attached to the surface. Non-modified nanoparticles were quickly opsonized and are readily excreted from human systems. It is thus required to reduce the opsonization and extend the circulating time of the nanoparticles in vivo in order to maximise the likelihood of successful drug targeting by nanoparticles.

zeta potential (mV) is 59.0, zeta deviation (Mv) is 5.29, and conductivity (Ms/CM) is 0.086 for the formulation containing Eudragit RL 100, which is de-aggregated and more stable in the suspension. To make nanoparticles, polymers are more suited since they have a flat surface that repels opsonization.

Stability studies of methylphenidate hydrochloride nanoparticles

For a period of three months, the stability of the improved nanoparticle formulation F9 was studied. Temperatures ranged from 4 °C to 45 °C/70 % RH

during the experiment. Nanoparticle formulations were assessed for entrapment efficiency every month for one year. Compared to ambient temperature and (45 °C/70 % RH), nanoparticles formulation was more stable in the refrigerator (4°C).

Table 5
Methylphenidate hydrochloride nanoparticle stability studies

S. no.	Storage Condition	Test parameters	1st month	2nd month	3rd month
1	4 °C	pH	7.4	7.4	7.4
		colour	Clear & colourless	Clear & colourless	Clear & colourless
		Cumulative % drug release	99.49	98.27	97.90
2	Room Temperature	pH	7.4	7.4	7.3
		colour	Clear & colourless	Clear & colourless	Clear & colourless
		Cumulative % drug release	99.49	94.38	92.87
3	Acceleration condition at 45°C/70°% RH	pH	7.4	7.3	7.3
		colour	Clear & colourless	Clear & colourless	Clear & colourless
		Cumulative % drug release	96.12	92.23	90.26

Table 6
Stability analysis at 4 degrees Celsius of the improved formulation F9 *in vitro* release

Time (h)	Cumulative % drug release		
	1st month	2nd month	3rd month
1	3	3	2.8
2	8.03	8.03	7.00
3	13.05	13.02	12.98
4	17.08	17.04	16.00
5	22.10	22.06	22.02
6	28.12	28.06	28.02
7	33.15	33.10	33.00
8	39.18	39.04	38.98
9	44.21	44.08	43.94
10	47.23	46.98	46.90
11	51.25	50.92	50.77
12	55.27	54.93	54.65
13	59.29	58.56	57.24
14	62.31	61.90	61.16
15	66.32	65.52	64.98
16	70.34	70.04	69.88
17	74.36	73.16	73.04

18	79.38	79.05	78.97
19	82.41	81.70	81.71
20	86.42	85.68	85.24
21	89.44	88.23	88.03
22	92.46	92.19	92.17
23	95.47	95.07	94.59
24	99.49	98.27	97.90

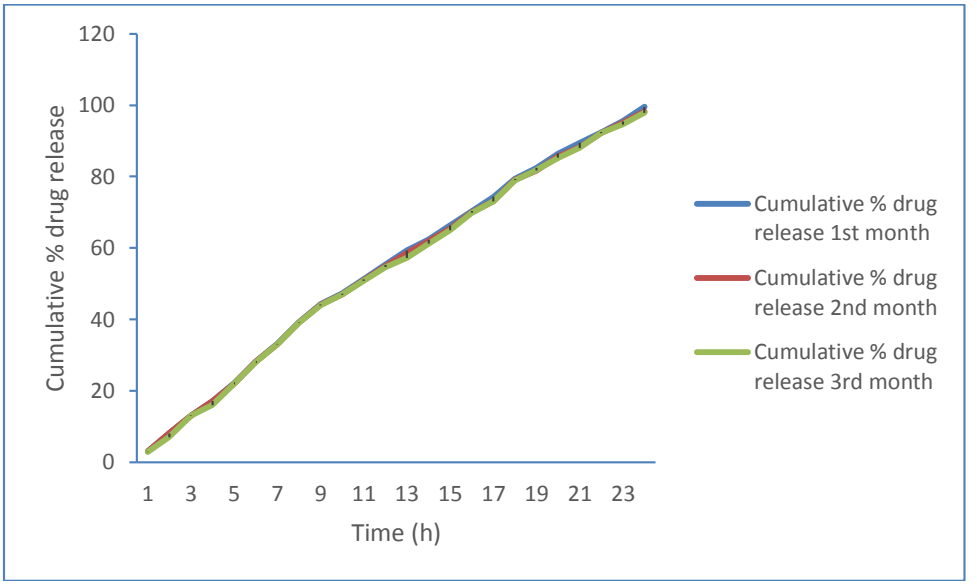

Fig. 3: Stability Study Results for Formulation F9 after 3 Months at 4 °C

Table 7
At room temperature, in vitro data for an improved formulation of F9 was collected

Time (h)	Cumulative % drug release		
	1st month	2nd month	3rd month
1	3	2.8	2.7
2	8.03	7.90	6.14
3	13.05	10.94	9.23
4	17.08	15.00	13.12
5	22.10	20.14	17.16
6	28.12	24.18	22.50
7	33.15	29.21	26.54
8	39.18	35.30	33.60
9	44.21	41.34	38.68
10	47.23	45.44	42.74
11	51.25	49.52	44.80
12	55.27	52.58	48.89
13	59.29	55.65	51.97

14	62.31	58.71	55.04
15	66.32	63.77	60.10
16	70.34	67.57	64.18
17	74.36	72.64	69.24
18	79.38	76.69	73.34
19	82.41	79.78	77.40
20	86.42	81.87	79.50
21	89.44	85.98	82.58
22	92.46	88.08	86.68
23	95.47	91.19	89.74
24	99.49	94.38	92.87

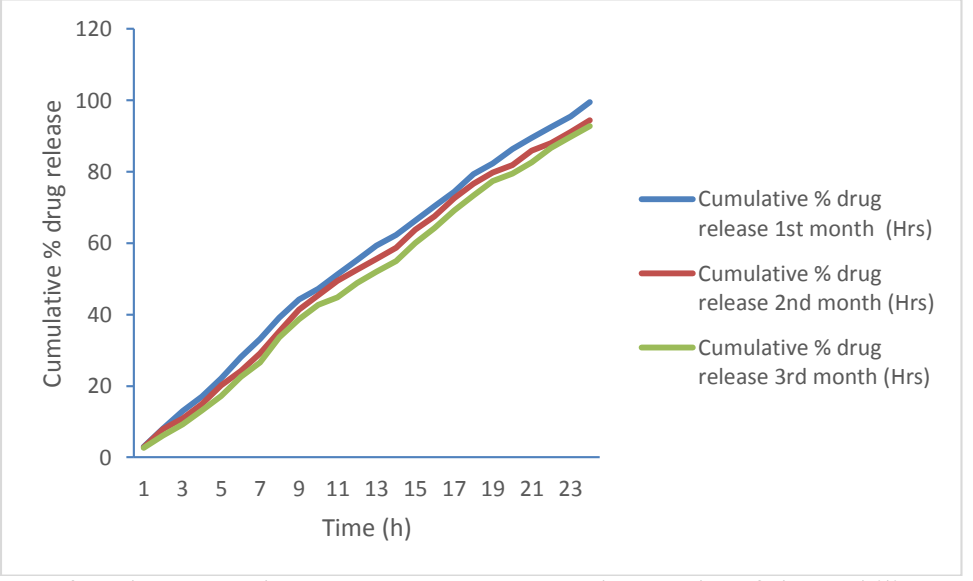

Fig. 4: After three months at room temperature, the results of the stability study were released for Formulation F9

Table 8
At 45 °C and 75% RH, we collected in vitro data for an improved formulation F9 study

Time (h)	Cumulative % drug release		
	1st month	2nd month	3rd month
1	3	2.8	2.4
2	8.54	7.46	6.36
3	12.08	11.50	9.41
4	16.16	14.58	12.47
5	20.22	17.64	15.53
6	24.30	20.70	17.60
7	27.38	24.80	20.74
8	31.46	28.88	25.80

9	34.52	30.98	28.85
10	37.58	32.10	30.91
11	41.68	35.18	33.98
12	46.74	39.27	36.23
13	50.23	44.17	37.89
14	55.30	47.08	41.63
15	60.87	60.18	55.33
16	64.23	64.28	59.00
17	69.26	68.08	62.05
18	73.61	72.14	66.15
19	77.31	75.34	70.38
20	81.60	80.23	75.19
21	85.86	83.15	78.18
22	88.23	87.30	82.38
23	92.64	90.28	86.23
24	96.12	92.23	90.26

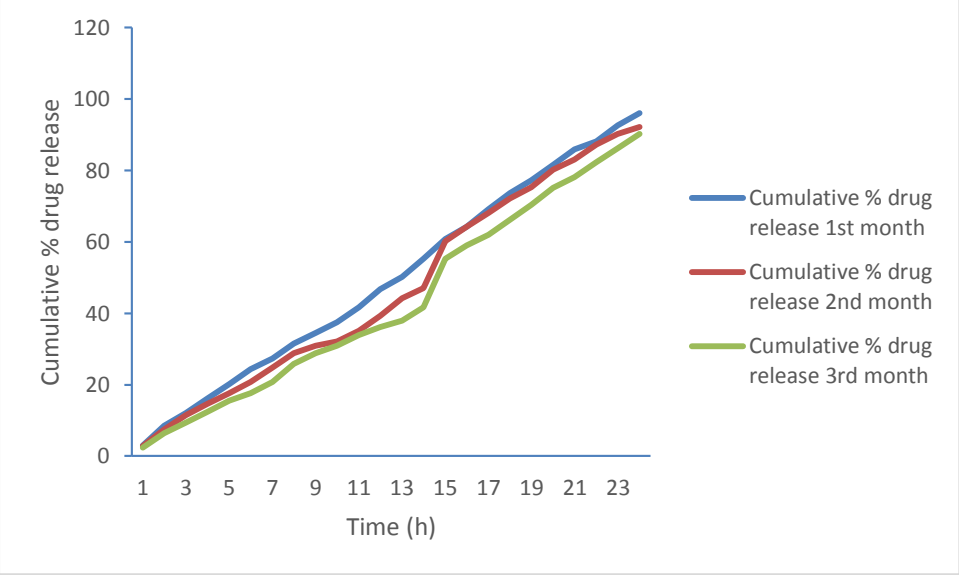

Fig. 5: A 45°/75 percent RH STUDY TO DEVELOP THE OPTIMIZED F9 formulation

Stability Discussion

Three months of stability testing were conducted in a variety of settings. A stable formulation was observed throughout the study period.

Conclusion

The current investigation, this medication delivery method uses the biodegradable polymer Eudragit RL100 to distribute methylphenidate hydrochloride nanoparticles. The solvent evaporation technique was used to create each batch

of nanoparticles (F1-F10). *In vitro* drug release was 98.16 after 24 hours of incubation with the improved formulation's entrapment efficiency of 940.04. Following the zero order, this follows as well. As determined by scanning electron microscopy, the optimal formulation has an average particle size of around 200 nm. During the stability test, the formulation was found to be satisfactory. In order to determine zeta potential, the optimal formulation was tested. Because of its greatest deviation of -59mV, the formulation F9 shows that the particles are separated and extremely repelling. In membrane filtration, this repelling feature was shown to be more beneficial in lowering opsonization. Three months of stability testing were conducted in a variety of settings. A stable formulation was observed during the trial period. The zero-order release pattern of the improved formulation F9 was discovered. The linearity of time vs. concentration was illustrated by the graph. Bioequivalence studies may be performed on F9 in the future, and its suitability for commercialization can then be determined. Optimizing formulation parameters was the project's primary goal.

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