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RESEARCH ARTICLE

Preparation and evaluation of Pramipexole dihydrochloride loaded chitosan nanoparticles for brain-targeting

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ABSTRACT:
The present investigation was undertaken to develop nanoparticles of a hydrophilic drug pramipexole dihydrochloride and improve the entrapment efficiency of the drug. Pramipexole dihydrochloride nanoparticles where prepared with two methods by using Chitosan and another method by utilizing Chitosan, sodium alginate and Pluronic F-127. Five different trials were prepared with different concentrations of Chitosan in both methods. In two methods variables were found to have significant effect on the particle size, entrapment efficiency of drug which is influenced by Chitosan and sodium alginate. The maximum entrapment efficiency and least particle size were obtained with 3% Chitosan along with sodium alginate PS3 of 220.7 nm with 32.4 mV zeta potential with 91.2 % entrapment efficiency but in PC3 with 3% Chitosan show least particle size of 252nm with 44.7 mV zeta potential but less entrapment efficiency 82.8 % of than PS3.The surface morphology of PC3 and PS3 shows smooth surface with the *in vitro* release of PC3 with 90.2 %, PS3 with 96.12% in 24 hrs. PS3 shows better entrapment efficiency than PC3, Hence preparation methods along with two polymers may influence in entrapment efficiency of pramipexole dihydrochloride nanoparticles which meet the treatment of Parkinson's disease which has a potential influence on brain-targeting.

KEYWORDS: pramipexole dihydrochloride; Chitosan sodium alginate; entrapment efficiency; Parkinson's disease.

INTRODUCTION:
The development of polymer based drug carriers has attracted increased attention over the last years. Polymeric nanoparticle is the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences (1). Due to their unique size-dependent properties, A polymer nanoparticle offers the possibility to develop new therapeutics. The development of new drug alone is not sufficient to provide the base for the progress in drug therapy and the various researches in this area evident that poor absorption, rapid metabolism and elimination lead to insufficient drug concentration at the specific site seen in their reports (2).

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To overcome this nanoparticle based drug delivery system may be helpful in the pharmaceutical field (3). The aim of the present study is to develop nanoparticles of pramipexole dihydrochloride using two different methods for the preparation of nanoparticles for the treatment of Parkinson's disease.

Pramipexole dihydrochloride is a well known antiparkinsonism drug. It has less bioavability and only a minimal amount of the drug is crossing the blood brain barrier. The polymer as a carrier plays an important role in transport the drug across the blood brain barrier which may be effective in producing the therapeutic effect (4,5). The use of biodegradable natural polymers controlled drug delivery has shown significant therapeutic potential suggested by many reports and most promising approaches for CNS drug delivery (6). Their drug loading efficiency may be limited of their conjugation sites in the polymer leads to target active

site. Depending upon the method of preparation of nanoparticles also influence in the penetration of drug across blood brain barrier which can be evidence by more entrapment efficiency of the drug by *in vitro* (7, 8). Due to this the drug can able to penetrate the blood brain barrier easily for targeting the brain disorder with increased bioavailability (9). Hence the present study is to develop nanoparticles of a hydrophilic drug pramipexole dihydrochloride and improve the entrapment efficiency for treating Parkinson's disease,

MATERIALS AND METHODS:

Fourier Transform Infra Red Spectroscopy (FTIR) studies:

The FTIR Spectroscopic studies were carried out for the pramipexole dihydrochloride, chitosan, sodium alginate, pluronic F-127, a mixture of pramipexole dihydrochloride-chitosan and a mixture of pramipexole dihydrochloride- chitosan - sodium alginate - pluronic F-127 by KBr pellet technique using Bomem FTIR MB II Spectrophotometer. Test samples were mixed with KBr, pressed into a pellet and scanned from 400 to 4000 cm^{-1}.

Differential Scanning Colorimetry (DSC):

Diffrential Scanning Colorimetry is a thermal analysis technique that looks at how a material's heat capacity (Cp) is changed by temperature. A sample of known mass is heated or cooled and the changes in its heat capacity are tracked as changes in the heat flow. DSC samples should be small enough to fit in one of the aluminium DSC pans. Sample weight should be between 0.5 and 100mg.Weigh 0.1mg of sample with the analytical balance and record the weight. Use forceps to place the aluminium pan lid on top of the sample. Use forceps to load the aluminium pan and sample into the encapsulating press. Align the sample pan in the encapsulating press and press down on the handle to seal the aluminium pan. Crimp an empty aluminium pan and lid as a reference sample. The sample pan and an empty reference pan are placed on small platforms within the DSC chamber. The DSC studies were carried out for pramipexole, chitosan, Sodium alginate, Pluronic F-127 and mixtures of pramipexole–chitosan, Pramipexole dihydrochloride-Chitosan-Sodium alginate-Pluronic F-127 by Differential Scanning Calorimeter.

Preparation of standard plot of pramipexole dihydrochloride:

Accurately weighed 100mg of pramipexole dihydrochloride dissolved in 100ml standard volumetric flask using distilled water to get the stock solution of 100 mg/ml. From this stock solution 10ml of solution was withdrawn and made to 100ml. From these aliquots of 1, 2, 3, 4, and 5ml were withdrawn and further diluted to 10ml with water to obtain a concentration range of 10 - 50 µg/ml. The absorbance of the solutions was measured at 263nm by using UV-spectrophotometer. A graph of Concentration vs Absorbance was plotted.

Preparation of nanoparticles:

Method 1:

Preparation of nanoparticles containing pramipexole dihydrochloride and Chitosan by ionotropic gelation process (10). Chitosan solution was prepared by dissolving in 100ml of 1% v/v acetic acid and the resulting solution was stirred at 1500rpm for 30min on magnetic stirrer (Different concentration of chitosan solution such as 1%, 2%, 3%, 4% and 5% were prepared). TPP solution (1%w/v) was prepared by dissolving 100mg of TPP in 100ml of deionized water. Add 100mg pramipexole dihydrochloride to the 1% TPP solution and mix to form a homogenous mixture by stirring with a glass rod. Add the above mixture of TPP and Pramipexole dihydrochloride solution drop by drop (10ml) to the chitosan solution and kept stirring at 2500rpm for 3hours on mechanical stirrer. Nanoparticles were obtained upon the addition of a TPP and pramipexole dihydrochloride aqueous solution to a chitosan solution. The NP suspension is then centrifuged at 15,000 rpm for 10 min using high-speed centrifuge (Sigma). Discard the sediment and the preserve the supernatant. The formation of nanoparticles results in interaction between the negative groups of TPP and the positively charged amino groups of chitosan.

Table 1: Formula of pramipexole dihydrochloride nanoparticles by method 1

S. NO	INGREDIENTS	PC1	PC2	PC3	PC4	PC5
1.	Pramipexole dihydrochloride	100mg	100mg	100mg	100mg	100mg
2.	Chitosan	1%	2%	3%	4%	5%
3.	Tripoly phosphate	100ml	100ml	100ml	100ml	100ml
4.	1% Acetic acid solution	1ml	1ml	1ml	1ml	1ml

Table 2: Formula of pramipexole dihydrochloride nanoparticles by method 2

S. NO	INGREDIENTS	PS1	PS2	PS3	PS4	PS5
1.	Pramipexole dihydrochloride	100mg	100mg	100mg	100mg	100mg
2.	Chitosan	0.1%	0.2%	0.3%	0.4%	0.5%
3.	Pluronic F-127	0.025%	0.025%	0.025%	0.025%	0.025%
4.	Sodium alginate	0.1%	0.1%	0.1%	0.1%	0.1%
5.	1% Acetic acid solution	1ml	1ml	1ml	1ml	1ml

Method 2:
Preparation of nanoparticles containing pramipexole dihydrochloride, Chitosan, sodium alginate and Pluronic F-127. Chitosan solution was prepared by dissolving in 50ml of 1%v/v acetic acid and the pH of the resulting solution was adjusted to 5 by adding 1M sodiumhydroxide (NaOH). Different concentration of chitosan solution such as 0.1%, 0.2%, 0.3%, 0.4% and 0.5% were prepared).The solution was stirred at 1500rpm for 30min on magnetic stirrer. Pluronic F-127 (0.025%w/v) and pramipexole dihydrochloride solution was prepared by dissolving in 50ml of deionised water. Add the above mixture of pluronic F-127 and pramipexole dihydrochloride solution drop by drop (10ml) to the chitosan solution and kept stirring at 2500rpm on mechanical stirrer. Simultaneously sodium alginate solution (0.1%) was prepared by dissolving 10mg in 50ml of deionised water and the pH of the solution was adjusted to 5 by adding 0.05M Hydrochloric acid (HCl).Spray the above mixture of solution and stir at 2500rpm for 1hour on mechanical stirrer.The nanoparticles produced are then centrifuged at 15,000 rpm for 10 min using high-speed centrifuge (Sigma). Discard the sediment and the preserve the supernatant.

Particle size and Zeta potential:
The size of the prepared nanoparticles was analyzed by using Photon Correlation Spectroscopy (PCS). All samples were diluted with ultra purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25^{o}C. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate. The Zeta measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted.

Surface morphology:
The surface morphology of the nanoparticle were studied using Scanning Electron Microscopy Quanta 200 FEG scanning electron microscope (FEI Quanta FEG 200} set at 200 kV by placing an air dried nanoparticle suspension on copper electron microscopy grids and the image was captured at desired magnification

Drug content:
The total drug amount in nanosuspension was determined spectrophotomertically. A 0.50-ml aliquot of nanosuspension was evaporated to dryness under reduced pressure at 35 ^{o} c. the residue was dissolved in water and filtered with a 0.45µm filter, and pramipexole dihydrochloride content was assayed spectrophotomertically at 263nm.

Drug entrapment efficiency:
The entrapment efficiency is also known as Association Efficiency. The drug loaded nanoparticles are centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer.

***In vitro* release studies:**
Invitro diffusion studies (drug release studies) were performed by using diffusion apparatus. A semi permeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was faced towards donor compartment. The glass beaker was filled with 100ml of phosphate buffer of (ph:6.8) at a temp 37 ^{o} c sample of 2ml was withdrawn at regular intervals from glass beaker for analysis.2ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100ml.The absorbance of sampling was measured at 263nm by using UV spectrophotometer.

RESULTS AND DISCUSSION:

Fourier transforms infrared spectroscopy (FTIR):
The FTIR Spectroscopic studies were carried out for the pramipexole dihydrochloride, chitosan, sodium alginate, pluronic F-127, a mixture of pramipexole dihydrochloride-chitosan and a mixture of pramipexole dihydrochloride- chitosan - sodium alginate - pluronic F-127 by KBr pellet technique using FTIR spectrophotometer. The peaks of bonds are seen separate peaks in the mixture of polymer and drug as the peaks appeared in individual spectra so no incompatibility and also no significant interaction between drug and polymer.

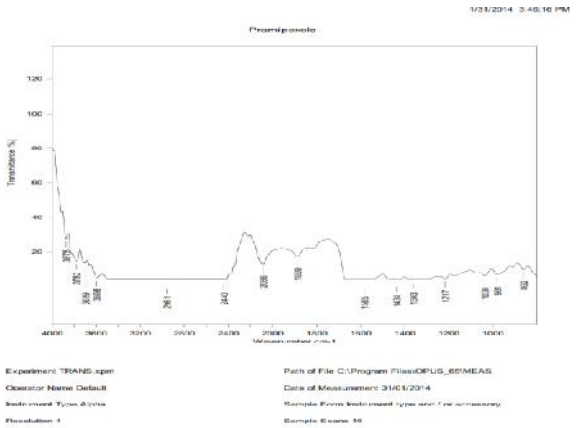

Figure 1: FTIR of Pramipexoledihydrochloride

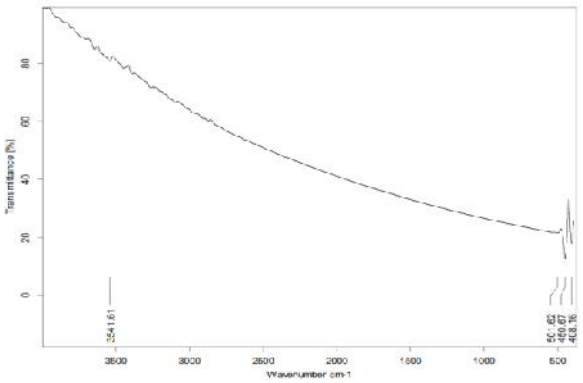

Figure 2: FTIR of Chitosan

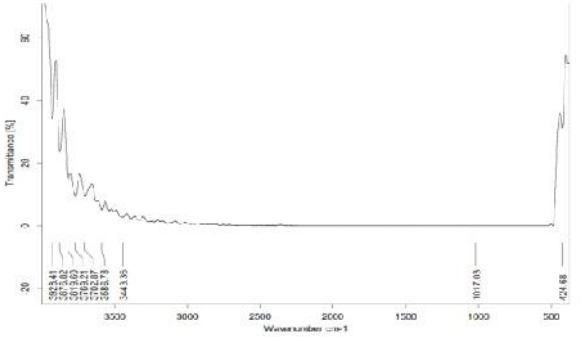

Figure 3: FTIR of Sodium alginate

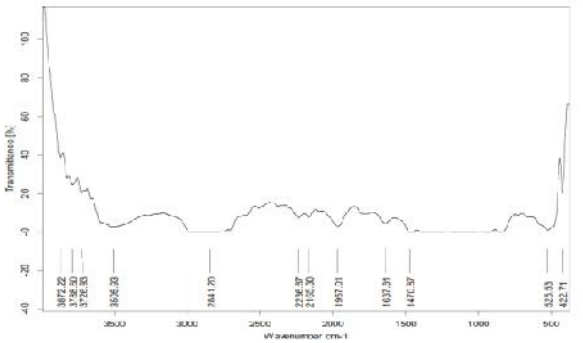

Figure 4: FTIR of Pluronic F-127

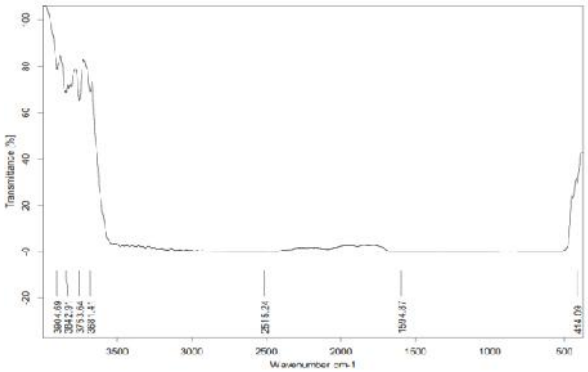

Figure 5; FTIR of Pramipexole dihydrochloride and Chitosan

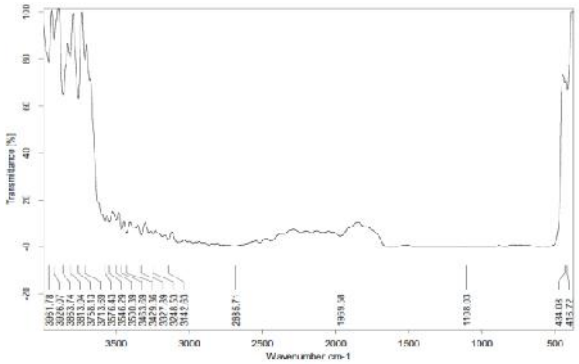

Figure 6: FTIR of Pramipexole dihydrochloride, Chitosan, Sodium alginate and Pluronic F-127

Differential Scanning Colorimetry (DSC):

The DSC studies were carried out for pramipexoledihydrochloride, chitosan, Sodium alginate, Pluronic F-127 and mixtures of pramipexoledihydrochloride –chitosan, Pramipexole dihydrochloride-Chitosan-Sodium alginate-Pluronic F-127 by Differential Scanning Calorimeter. The thermal peak was observed at 270.64^0 C in pramipexoledihydrochloride and the same peak was found in mixture of drug as well polymers, hence based on result it was found that there was no interaction between drug and polymers.

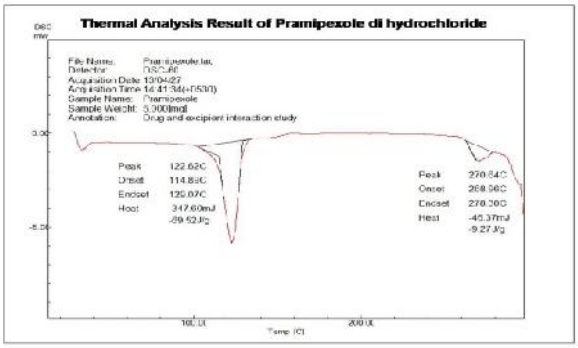

Figure 7: DSC of Pramipexole dihydrochloride

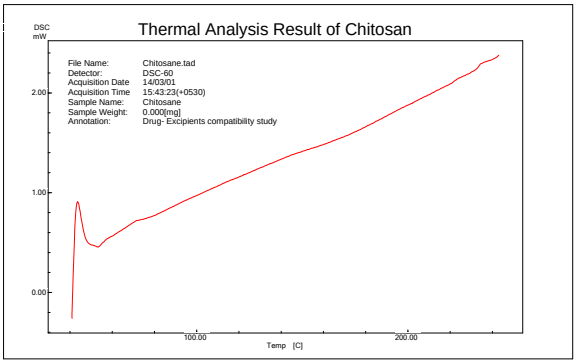

Figure 8: DSC of Chitosan

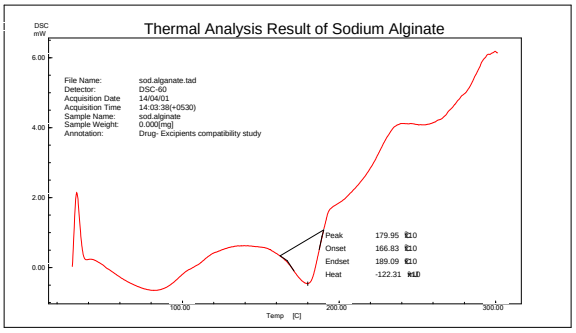

Figure 9: DSC of Sodium alginate

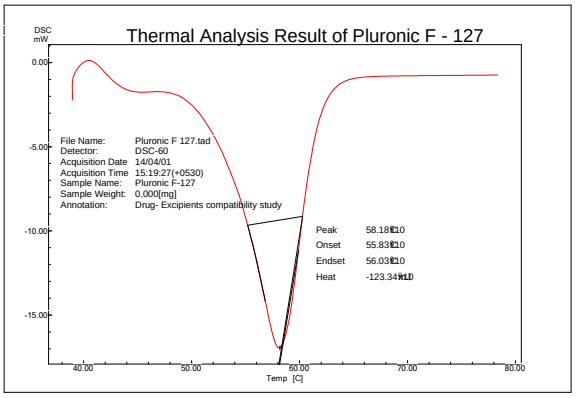

Figure 10: DSC of Pluronic F-127

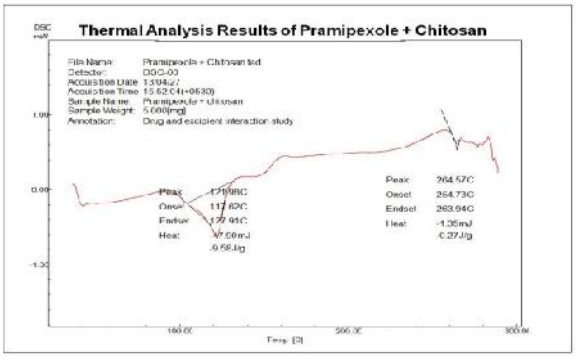

Figure 11: DSC of Pramipexole and Chitosan

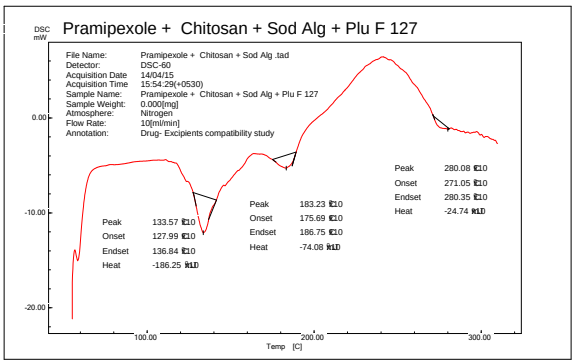

Figure 12: DSC of Pramipexole dihydrochloride, Chitosan, Sodium alginate and Pluronic F-127

Standard plot of Pramipexole dihydrochloride:

A standard plot of pramipexole dihydrochloride was plotted for concentration of 10, 20, 30, 40, 50 μg/ml with the absorbance measured at 263nm. Slope is found as R= 0.999

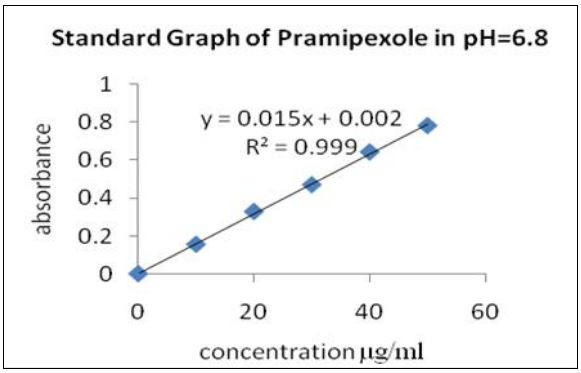

Figure 13: Standard graph of Pramipexole dihydrochloride

Preparation of pramipexole dihydrochloride nanoparticles:

Pramipexole dihydrochloride loaded chitosan nanoparticles were prepared by ionotropic gelation method by varying the concentrations of chitosan. Pramipexole dihydrochloride loaded chitosan and Sodium alginate nanoparticles also prepared according to the method show above. The prepared nanoparticle formulations were found to be turbid and stable, and they were packed in air tight containers and stored in a cool place and used for further studies.

Particle size and Zeta Potential analysis

The particle sizes of prepared nanoparticles were measured from the microphotograph of 100 particles. The particle size of the method 1 formulation ranged from 252nm to 432nm for various batches. The increase in the concentration of the polymer ratio caused an increase in particle size. The PC3 shows least particle size of 252nm with 44.7 mV zeta potential where selected as best formulation in this method where PC 1, PC 2 show more particle size may be due to less polymer concentration coating may be improper on the other hand PC 4, PC 5 with more amount of polymer concentration the particle size in more and also more zeta leads to unstable. Hence PC 3 may be considered as best formulation by method 1.

The particle sizes of prepared nanoparticles by method 2 particle size range from 220nm to 485 nm. This result may be influence of preparation method adopted because in this method sodium alginate an another polymer is used this may interfere in particle size along with zeta potential, In this method PS 3 shows least particle size of 220nm with 32.4. The particle size may be due to addition of another polymer sodium alginate and also the method used for preparation also be an impact on particle size along with zeta potential.

Table 3: Particle size for method 1 formulation

S. NO.	FORMULATION	RATIO	PARTICLE SIZE (nm) *	ZETA POTENTIAL (mV) *
1.	PC1	1:1	325 ± 4.5	49.2 ± 2.3
2.	PC2	1:2	356 ± 3.8	48.4 ± 2.8
3.	PC3	1:3	252 ± 2.6	44.7± 2.2
4.	PC4	1:4	376 ± 4.8	45.4± 2.9
5.	PC5	1:5	432 ± 5.2	44.7± 3.2

* Values indicated in the results of triplicate trials ± S.D

Table 4: Particle size for method 2 formulation

S. no.	Formul a-tion	Ratio	Particle Size (nm) *	Zeta Potential (mv) *
1.	PS1	1:1	282.2± 3.8	33.6 ± 2.4
2.	PS2	1:2	356.8 ± 4.2	34.7± 3.2
3.	PS3	1:3	220.7± 2.5	32.4± 2.4
4.	PS4	1:4	344.5± 3.2	31.4± 3.6
5.	PS5	1:5	485.6 ± 3.9	30.4± 4.6

* Values indicated in the results of triplicate trials ± S.D

In this formulation PS 3 show 220.7 nm of particle size with 32.4 mV may be an best formulation by the ;east particle size with zeta size this trial more stable, so it may selected as best formulation for the method 2 preparations.

Surface morphology:

SEM analysis of the prepared formulations was carried out to understand the morphology of nanoparticles. The nanoformulation shows the morphological characters of smooth surface with spherical shaped in appearance. The figure 10 & 15 shows the surface of the particle which describe the PC 3, PS3 are well spherical shape which make an evidence of good nnanoformulation.

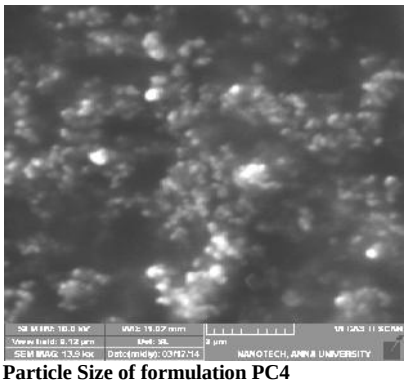

Figure 14: Particle Size of formulation PC4

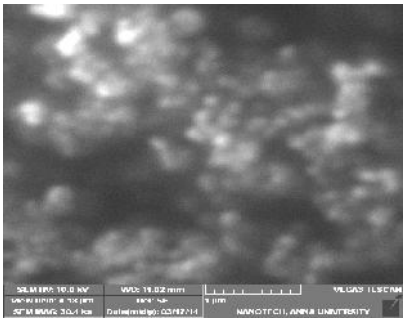

Figure 15: Particle Size of formulation PS3

Drug content and entrapment efficiency:

The total drug amount in nanosuspension was determined spectrophotomertically. The drug content analysis was done to determine percentage of the drug loaded in the nanoformulations. The drug content and entrapment efficiency of Pramipexole dihydrochloride loaded chitosan nanoparticles by method 1 shown in table- 7. From the results maximum amount of the drug was loaded in the PC 3 nanoformulation of 0.364 mg/ml with 82.8 % entrapment efficiency shows the maximum % of entrapment efficiency in the nanoformulation. Based on the results, PC 3 show maximum drug cont it may be due to polymer chitosan and the method used for the preparation of nanoparticles..

Table 5: Drug content and entrapment efficiency of pramipexole nanoparticle formulated by method 1

Formulation	Average drug content (mg/ml) *	Average entrapment efficiency (%)*
PC1	0.257± 0.03	51.5± 2.2
PC2	0.335± 0.05	67.1± 3.4
PC3	0.464± 0.02	82.8± 1.5
PC4	0.352± 0.07	78.5± 3.6
PC5	0.368± 0.11	80.6± 3.8

* Values indicated in the results of triplicate trials ± S.D

The drug content and entrapment efficiency of Pramipexole dihydrochloride loaded chitosan, sodium alginate nanoparticles by method 2 shown in table- 8.The report shows a maximum difference in drug content and % of entrapment efficiency may be due to the use of two polymer along with the method of preparation also influence in the % of entrapment efficiency which is conformed by the results. From the results maximum amount of the drug was loaded in the Ps 3 nanoformulation of 0.570mg/ml with 91.2 % entrapment efficiency shows the maximum % of entrapment efficiency in the nanoformulation.

Table 6: Drug content and entrapment efficiency of pramipexole nanoparticle formulated by method 2

Formulation	Average drug content (mg/ml) *	Average entrapment efficiency (%)*
PS1	0.389± 0.04	58.9± 3.3
PS2	0.435± 0.02	65.9± 3.4
PS3	0.570± 0.04	91.2± 1.4
PS4	0.462± 0.07	85.1± 2.3
PS5	0.411± 0.06	82.5± 3.7

* Values indicated in the results of triplicate trials ± S.D

***In vitro* release studies:**

The table- 7 shows *invitro* drug release of all formulations by method 1. In this PC1 shows the highest drug release followed by PC2 but they were rejected due to very high particle size. PC3, PC4 and PC5 have low particle size.

Table 7: *Invitro* Release Studies of Pramipexole dihydrochloride nanoparticles formulated by method 1

S. no.	Time (hours)	% cumulative drug release*				
		PC1	PC2	PC3	PC4	PC5
1.	0	0	0	0	0	0
2.	1	9.8 ± 2.3	8.1 ± 1.8	7.2 ± 2.1	6.7 ± 2.7	6.1 ± 2.4
3.	2	12.3 ± 2.1	11.2 ± 2.4	10.8 ± 3.3	11.1 ± 3.6	9.2 ± 3.2
4.	4	46.1 ± 3.4	33.7 ± 3.2	21.2 ± 3.5	22.7 ± 3.9	19.9 ± 2.1
5.	6	67.5 ± 2.2	56.1 ± 3.7	33.5 ± 4.3	35.4 ± 3.1	24.7 ± 4.2
6.	8	88.2 ± 2.1	77.5 ± 3.4	46.1 ± 2.7	42.1 ± 3.5	33.4 ± 1.2
7.	12	100 ± 0.1	89.1 ± 2.1	52.6 ± 3.8	50.3 ± 3.7	45.3 ± 1.5
8.	16	-	100 ± 0.2	67.3 ± 4.3	61.8 ± 4.1	54.5 ± 2.8
9.	20	-	-	85.7 ± 2.5	71.2 ± 2.4	62.7 ± 1.6
10.	24	-	-	90.2 ± 1.8	82.3 ± 1.3	72.1 ± 1.4

* Values indicated in the results of triplicate trials ± S.D

Table 8: Invitro Release Studies of Pramipexole dihydrochloride nanoparticles formulated by method 2

S. no.	Time (hours)	% cumulative drug release*				
		PS1	PS2	PS3	PS4	PS5
1.	0	0	0	0	0	0
2.	1	6.5 ± 1.2	5.1 ± 1.4	4.7 ± 1.5	4.3 ± 1.3	3.6 ± 1.8
3.	2	19.59 ± 2.4	8.21 ± 2.2	7.99 ± 3.4	7.16 ± 3.6	6.80 ± 2.2
4.	4	34.61 ± 3.4	23.93 ± 3.7	12.83 ± 2.9	10.98 ± 3.5	9.59 ± 2.8
5.	6	53.60 ± 3.6	32.87 ± 2.4	31.94 ± 3.1	20.10 ± 2.8	19.28 ± 2.6
6.	8	85.55 ± 2.8	66.48 ± 3.4	48.08 ± 3.4	30.64 ± 2.4	29.73 ± 3.6
7.	12	100 ± 0.3	89.63 ± 3.1	59.75 ± 2.6	43.58 ± 2.6	40.85 ± 3.7
8.	16	-	100 ± 0.2	71.33 ± 2.5	52.19 ± 3.6	50.16 ± 3.1
9.	20	-	-	82.82 ± 1.4	64.88 ± 2.6	62.45 ± 2.6
10.	24	-	-	96.12 ± 1.1	75.51 ± 2.2	69.13 ± 2.5

* Values indicated in the results of triplicate trials ± S.D

But PC4 and PC5 were rejected as they act as release retardants due to high concentration of polymer. So, PC3 was selected as best formulation due to its optimum particle size and controlled drug release.

The table- 8 shows *invitro* drug release of all formulations by method 2. In this PS1 shows the highest drug release followed by PS2 but they were rejected due to very high particle size. PS3, PS4 and PS5 have low particle size. But PS4 and PS5 were rejected as they act as release retardants due to high concentration of polymer. So, PS3 was selected as best formulation due to its optimum particle size and controlled drug release.

CONCLUSION:

Therefore, it can be concluded that Pramipexole dihydrochloride loaded mamoparticle influence in particle size and entrapment efficiency by using combination of two polymer. The preparation method has been a great impact on entrapment efficiency which is confirmed by the results, Hence this may establish its potential on increasing the brain-targeting efficiency of drugs and will be used as novel brain-targeting.

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REFERENCES:

1. Mohanty Sivasankar and Boga Pramod Kumar, Role of Nanoparticles in Drug Delivery System, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2010, Vol. 1 (2), p .41-66.
2. Swatantra Bahadur Singh, Novel Approaches for Brain Drug Delivery System-Review, International Journal of Pharma Research & Review, June 2013, 2(6), 36-44.
3. Celeste Roney, Padmakar Kulkarni, Veera Arora, Peter Antich, Frederick Bonte, Aimei Wu, N.N. Mallikarjuana, Sanjeev Manohar, Hsiang-Fa Liang, Anandrao R. Kulkarni, Hsing-Wen Sung, Malladi Sairam, Tejraj M. Aminabhavi .Targeted nanoparticles for drug delivery through the blood–brain barrier for Alzheimer's disease. Journal of Controlled Release, 2005, p. 193-214
4. Bhatt Neha, Bhatt Ganesh, Kothiyal Preeti, "Drug delivery to the brain using polymeric nanoparticles: a review, International Journal of Pharmaceutical and Life Sciences".2013, Volume 2, Issue 3, p.107-132
5. Mohammad Fazil, Shadab Md, Shadabul Haque, Manish Kumar, Sanjula Baboota, Jasjeet kaur Sahni, Javed Ali, Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting, European Journal of Pharmaceutical Sciences 2012, 47, 6–15.
6. Jiang Chang, Youssef Jallouli , Maya Kroubi, Xu-bo Yuan, Wei Feng, Chun-sheng Kang, Pei-yu Pu, Didier Betbeder, Characterization of endocytosis of transferrin-coated PLGA nanoparticles bythe blood–brain barrier International Journal of Pharmaceutics, 2009, 285–292.
7. Krishna Sailaja.A, Amareshwar.P, Chakravarty.P, Different techniques used for the preparation of nanoparticles using natural polymers and their application, International Journal of Pharmacy and Pharmaceutical Sciences.2011, Vol 3, Suppl 2, p .45-50.
8. Adriana Trapania, Elvira De Gigliob, Damiana Cafagnab, Nunzio Denoraa, Gennaro Agrimic, Tommaso Cassanod, Silvana Gaetanie, Vincenzo Cuomoe, Giuseppe trapania, Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery, International Journal of Pharmaceutics, 2011, 419, 296– 307
9. Huang R, Ma H, Guo Y, Liu S, Kuang Y, Shao K, Li J, Liu Y, Han L, Huang S, An S, Ye L, Lou J, Jiang C, Angiopep-Conjugated nanoparticles for targeted long-term gene therapy of Parkinson's disease. Pharm Res. 2013, 30(10):2549-59.
10. Emmanuel N. Koukaras, Sofia A. Papadimitriou, Dimitrios N. Bikiaris and George E. Froudakis. Insight on the Formation of Chitosan Nanoparticles through Ionotropic Gelation with Tripolyphosphate. Mol. Pharmaceutics 2012, 9, 2856−2862