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Optimization of Nanoparticle Formulation of Neostigmine Bromide

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ABSTRACT

In the recent days targeted drug delivery has gained more prominence for various advantages like site specific delivery and controlled release from the formulations. Amongst the plethora of avenues explored for targeted drug delivery, polymeric nanoparticles backed foremost attention offering local drug delivery and controlled drug release solving problems like tissue damage and drug wastage. Formulating nanoparticles with polymers may provide a significant increase in the gastrointestinal residence time. Neostigmine bromide is a cholinesterase inhibitor used for the treatment of Myasthenia Gravis and is given by conventional routes like oral and intra venous. Polymeric nanoparticles of Neostigmine Bromide using synthetic and semi synthetic polymers like Chitosan and ethyl cellulose were prepared by emulsification solvent evaporation method. The nanoparticles were characterized for their preformulation and post formulation parameters like particle size determination, drug entrapment efficiency, particle percentage yield, in vitro drug release and stability studies.

Keywords : Nanoparticles, Nanotechnology, Myasthenia Gravis, Neostigmine Bromide

I. INTRODUCTION

Myasthenia gravis is an autoimmune disorder affecting about 1 in 10,000 population, due to development of antibodies direct to nicotinic receptors at the muscle endplate reduction in number of free Nm cholinoceptors to 1/3 of normal or less and structural damaged to the neuromuscular junction weakness and easy fatigability on repeated activity, with recovery after rest. Neostigmine and its congeners improve muscle contraction by allowing acetylcholinesterase released from prejunctional endings to accumulate and act on receptors over a larger area, and by directly depolarizing the endplate.[1] This is associated with impaired neuromuscular transmission. Due to this, there is fluctuating weakness in the skeletal muscles and the patient finds difficult to contract the muscles. Women are more prone of getting diagnosed with myasthenia gravis at a younger age than males, who are mostly diagnosed at the age of 60 or more. Generally, it is seen in the age groups of over 40. Its symptoms are only manageable and the disease cannot be cured. To understand the pathogenesis of Myasthenia Gravis, it is essential to understand about the neuromuscular junction which is also known as the myo-neuronal junction. The junction or the synapse between the motor neuron and the muscle fiber is called as neuromuscular junction. The muscle fiber is surrounded by the thin plasma membrane which is called as 'Sarcolemma'. The portion of the sarcolemma which interacts with the nerve ending is called as the motor end plate. This motor end plate has several folds to increase the surface area.[2]

Nanotechnology has been introduced into several aspects of the food and pharmaceutical science, including encapsulations and delivery system, which has versatile advantages such as incorporation of

the bioactive compounds into the food matrices with high physiochemical stability and minimal impact on the properties of the product, as well as protection of the encapsulated bioactive compounds from the interaction with other food components and maximize the uptake of the encapsulated compounds upon intake and their transport to this sites of action.[3]

Nanotechnology is the science and technology at the nanoscale, which is about 1 to 100 nanometers and it can be used across the entire spectrum of scientific fields including life sciences and healthcare. The ideas and concepts behind nanoscience and nanotechnology were given by physicist Richard Feynman, also known as father of nanotechnology. He described the idea of creating things out of tiny pieces as an alternative to constructing things smaller in his lecture - "There's Plenty of Room at the Bottom" while delivering his talk at an annual American Physical Society meeting in Pasadena, California.[4] Nanomedicine is one of the most intensive areas of research in nanotechnology and is applied widely for the prevention, diagnosis and treatment of diseases. It is utilized in pharmaceutical sciences with the objectives of reducing toxicity and minimizing side effects of drugs by targeting them to the specific site of action, reducing their dose through improved bioavailability; reducing dosing frequency by controlling drug release into the human body; and improving shelf life by enhancing their stability. This ultimately contributes to increased safety, efficacy, patient compliance, and extended shelf life of drug and finally reduced healthcare costs. [5,6]

There are many thrust areas where drug delivery systems can be developed using nanotechnology such as, depot preparations, transdermal drug delivery systems (TDDS) particularly for cancer, enhanced bioavailability through improved dissolution and absorption and more. application of nanotechnology to conventional biphasic liquid dosage forms such as suspensions, emulsions and micelles in improving their performance in drug delivery.

Nanoparticles exhibit attractive properties like high stability and the ability to modify their surface characteristics easily. Nanoparticle drug delivery, utilizing degradable and absorbable polymer, provides a more efficient, less risky solution to many drug delivery challenges.[7] Nanoparticles is a broad class comprised of both vesicular systems (nanocapsules) or a matrix system (nanospheres). Nanocapsules and nanospheres differs in their release profiles due to the nature of the containment of the active agent. Nanospheres encapsulate the drug molecules within the matrix of polymer in a uniform distribution. The release of the drug from the matrix occurs through diffusion as well as erosion of the matrix itself. if diffusion occurs more quickly than degradation, then the process is diffusion dependent, otherwise the process of degradation is highly influential. Conversely, nanocapsules have reservoir like morphology and exhibit release profile as such. The drug is contained in the core and must diffuse through the polymer shell in order to be released. This morphology theoretically leads to zero order kinetics of release.[8]

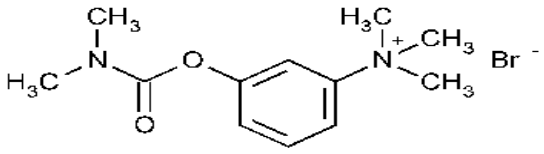
AIM & OBJECTIVES

Aim of this study is to improve the absorption and bioavailability of drug neostigmine bromide. The present research work has been proposed in order to formulate and evaluate the polymeric nanoparticles of Neostigmine Bromide. The objectives of this work includes-

- To improve the absorption and bioavailability of drug.
- To reduce the required dose so as to make it more cost effective.
- To reduce the side effect by targeting the drug at the site of action.

MATERIALS

Neostigmine Bromide is an official drug in Indian Pharmacopoeia. It is characterized as anticholinergic drug. Neostigmine Bromide was patented in 1931. It is on the World Health Organization's List of Essential Medicines. The term is from Greek neos, meaning "new", and "-stigmine", in reference to its parent molecule, physostigmine, on which it is based.[9]



Drug (API) and various chemicals used to formulate and evaluate the polymeric nanoparticles during this work are as follows: -

Table 1. Chemicals Used in the Formulation

Sr	CATEGORY	MATERIAL	SOURCE
1	DRUG (API)	Neostigmine Bromide	Yarrow Chem Products, Mumbai
2	POLYMER	Chitosan	Himedia
3	SOLVENT	Chloroform	Qualigens Fine Chem
4	SOLVENT	Distilled Water	
5	EMULSIFIER	Span 80	Merck Spec.Pvt.Ltd

Neostigmine Bromide was purchased from 'Yarrow chem. Products, Mumbai'. Polymers such as chitosan was also purchased from Yarrow chem. Products and other material like chloroform, span 80, and distilled water was procured from our own university's pharmacy department. Store and laboratories.

METHOD

Polymeric nanoparticles were prepared by emulsion solvent evaporation method.

FORMULATION OF POLYMERIC NANOPARTICLES OF NEOSTIGMINE BROMIDE

Organic Phase:

Stipulated amount of, Chitosan was dissolved in 15 ml of chloroform as organic phase, then the appropriate amount of drug was dispersed in this solution at room temperature using magnetic stirrer.

Aqueous phase:

0.4 ml of span 80 was dissolved in 19.6 ml of distilled water to make 2% solution of span 80, then this aqueous phase was added into the organic phase drop wise by using plastic syringe with 22 mm gauge needle and solution was sonicated with the help of ultra sonicator for 15 mins. After that organic solvent in emulsion was removed by overnight evaporation at room temperature under continuous stirring to obtain nanoparticles.

Survey of available literature shows that the combination of drug and polymer used (neostigmine bromide and chitosan) were not used previously for the preparation of neostigmine bromide nanoparticles. However, the ratio of drug and polymer were selected on the basis of literature review of other similar formulations of other drugs. For finding the effect of chitosan on formulation, four different concentrations were selected so as to prepare four types of formulations.

For developing different formulations of nanoparticles of neostigmine bromide, processing medium were taken in different quantities according to below matrix:

Formulation code	Drug (mg)	Polymer (mg)	Drug polymer ratio	% aq. Solution of Span 80
F1	100	200	1:2	2%
F2	100	400	1:4	2%
F3	100	600	1:6	2%
F4	100	800	1:8	2%

Table 2. Quantities of components used in the Formulations

EVALUATION OF POLYMERIC NANOPARTICLES

The evaluation of nanoparticles was done for the following parameters:

1. Particle size determination
2. Drug entrapment efficiency
3. Percentage yield

4. In-Vitro drug release study

Particle Size Determination [11]

Particle size and shape of prepared nanoparticles was studied using high resolution scanning electron microscopy (SEM). The samples on conductive carbon paint were placed in specimen holder, vaccum-dried, and sputter-coated using accelerating voltage of 5kV for 90 sec.

Drug entrapment efficiency [12]
Drug entrapment efficiency of prepared nanoparticles was determined by centrifuging the nanoparticles using ultracentrifuge at 10000 rpm for 30 min. The amount free neostigmine bromide is in supernatant was measured by analyzing suitable diluted supernatant in UV Spectrophotometer at 294 nm. The neostigmine bnromide entrapped in the nanoparticles was calculated as :

Entrapment efficiency (%) = (Tp-Tf) 100/Tp…….. (1)

Where, Tp is the total neostigmine bromide used to prepare the nanoparticles and Tf is the total free neostigmine bromide in the supernatant.

Percentage yield

Yield variance is the difference between actual output and standard output of a prepared nanoparticles. The percentage yield of neostigmine bromide nanoparticles was calculated using following formula:
Percentage yield = practical yield/ theoretical yield × 100

In-Vitro Drug release study [13]

Drug release from nanoparticles in-vitro was carried out by dialysis method. The donor chamber was filled with 100 mg of nanoparticles, whereas reservoir chamber was filled with 100 ml of phosphate buffer pH 7.4. This total setup was placed on magnetic stirrer at 50 rpm at 37 $\pm$ 1°C. At pre-determined time intervals the sample of from receiver chamber were withdrawn and replaced with equal volume of fresh phosphate buffer. The amount of Neostigmine bromide that diffused into the receiver chamber was quantified by UV Spectrophotometer at 294 nm.
FT-IR of pure drug: -

The drug shows peak at 2994.62 cm^{-1}, 1700 cm^{-1} 1740 cm^{-1} of C=O group, peak 1606.77 cm^{-1}, 1557.59 cm^{-1} shows the presence of CN group, and peak 668.36 cm^{-1}, 1027 cm^{-1} and 1069.57 cm^{-1} shows presence of Ar-Br group.

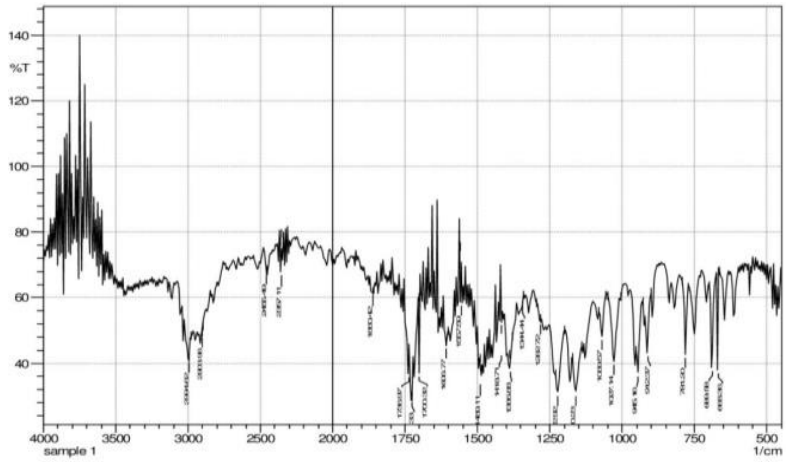


Fig 1. FT-IR Scan of Neostigmine bromide

DETERMINATION OF λ_{max} for estimation of drug in formulations by UV spectroscopic method:-

λ_{max} was determined by scanning of microgram solutions of drug in UV spectrophotometer in between the ultra-violet range of spectrum 200to 400 micrometers. Spectra shown in fig 2 and data given in table 3 was obtained:

S. No	Absorbance	Wavelength
1	265	0.348
2	280	0.488
3	**294**	**0.560**
4	314	0.460

Table 3. Maximum Wavelength of Neostigmine Bromide

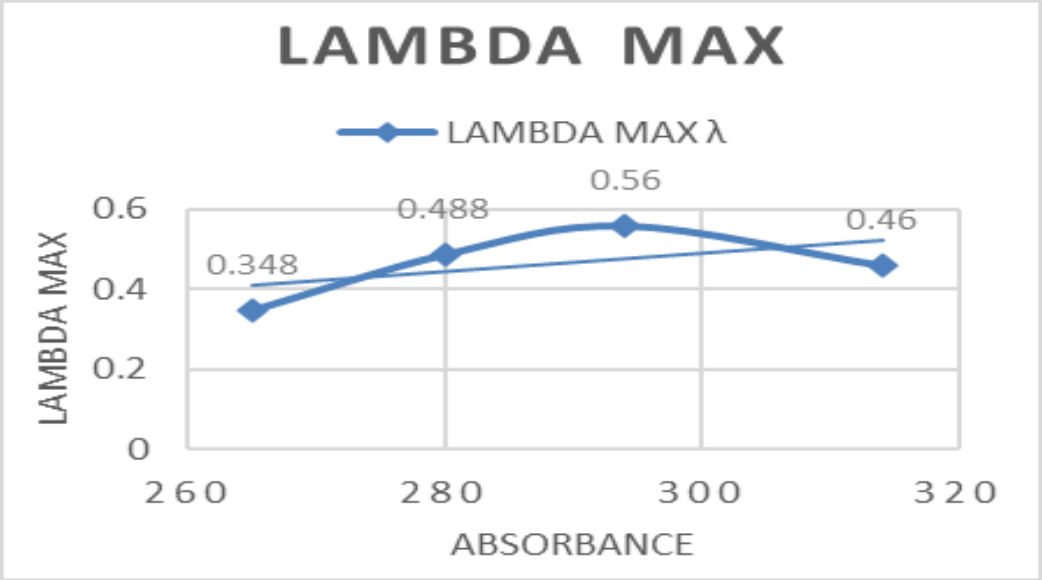


Fig 2. Representation of Wavelength of Neostigmine bromide

The λ_{max} was selected as 294 nm, since maximum absorbance was found at this wavelength.

Preparation of Standard Curve of Neostigmine Bromide:

Various concentration of drug solution in microgram/ml were prepared from the stock solution by suitable dilutions and then samples were examined for absorption spectra by UV Spectrophotometer at 294nm. The experiment was performed three time to calculate the average absorbances. Obtained results are given in table 4:

Sr.	Concentration (µg/ml)	Absorbance
1	1	0.049
2	2	0.097
3	3	0.145
4	4	0.198
5	5	0.246
6	6	0.298
7	7	0.349
8	8	0.400
9	9	0.445
10	10	0.522

Table 4. Absorbance of Neostigmine Bromide in Distilled Water

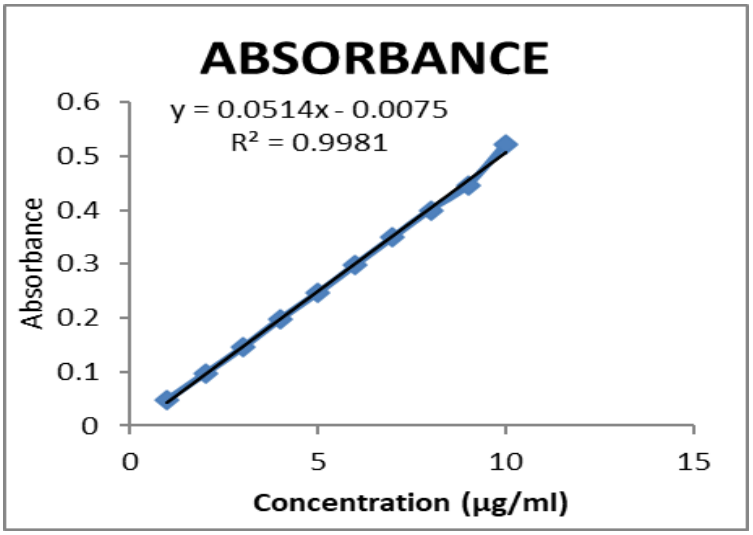


Fig 3. Representation of Calibration Curve of Neostigmine Bromide

RESULT & DISCUSSION

Practical yield:

The prepared nanoparticles were weighed and practical yield was calculated. The result of calculated practical yield is given in table 5:

Formulation code	% Practical Yield
F1	69.06
F2	71.25
F3	72.56
F4	73.33

Table 5. Percentage Practical Yield of Formulations

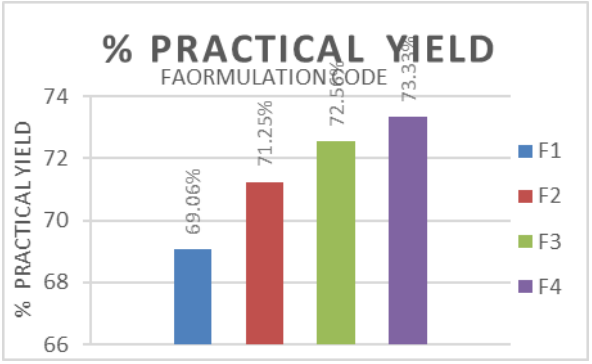


Fig 4. Representation of Percentage Practical Yield of Polymeric Nanoparticles of Neostigmine Bromide.

Particle size | Formulatio | Amount of drug in 100 | *Determination:*

The shape and size of prepared nanoparticles was studied using scanning electron microscopy (SEM). The photographs of examined nanoparticles are given below:

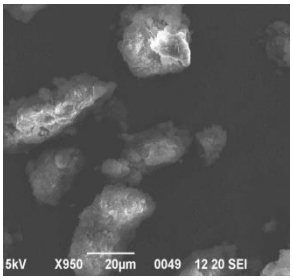


Fig 5. SEM Image of Neostigmine Bromide Nanoparticles (F1)

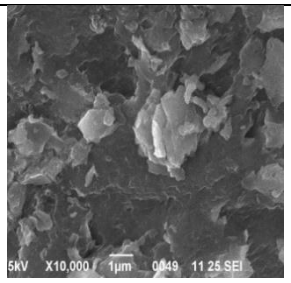


Fig 6. SEM Image of Neostigmine Bromide Nanoparticles (F2)

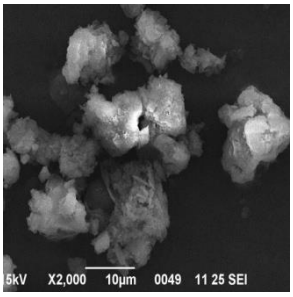


Fig 7. SEM Image of Neostigmine Bromide Nanoparticles (F3)

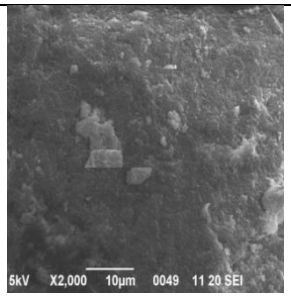


Fig 8. SEM Image of Neostigmine Bromide Nanoparticles (F4)

Drug entrapment efficiency:

Drug entrapment efficiency of nanoparticles was determined by placing 100 mg neostigmine nanoparticles dissolved in 100 ml of distilled water and kept for 24 hrs, then the solution was centrifuge by using ultracentrifuge at 10000 rpm for 30 min and analysed on UV Spectrophotometer at 294 nm. The result of drug content is shown in table:

n code	mg nanoparticles (mg)
F1	58.44±2.92
F2	53.06±2.65
F3	65.39±3.26
F4	49.83±2.49

Table 6. Amount of Neostigmine Bromide Present in Nanoparticles (n=5)

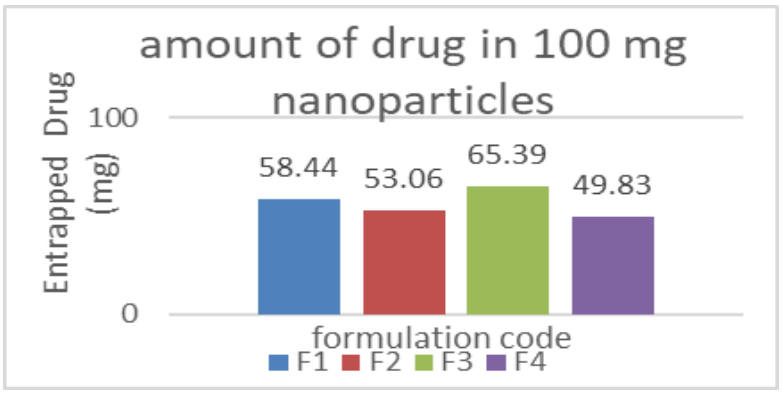


Fig 9. Representation of Amount of Drug Present In The Polymeric Nanoparticles

In-vitro drug release study:

In-vitro drug release was determined by placing 100 mg neostigmine bromide nanoparticles in 500 ml phosphate buffer of pH 7.4 and temperature was maintained at 37±2 ºC. The sample was taken at different time intervals and analyzed by UV spectrophotometer at 294 nm.

Sr.	Time (hrs)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
1	0.5	9.80±0.49	8.36±0.41	9.13±0.45	8.24±1.65
2	1	18.01±0.90	16.65±0.83	17.39±0.86	15.63±0.78
3	1.5	23.92±1.19	23.15±1.15	24.79±1.23	22.45±1.12
4	2	29.80±1.49	27.93±1.39	31.56±1.57	27.88±1.39
5	2.5	34.30±1.75	33.27±1.66	36.94±1.84	34.89±1.74
6	3	40.76±2.03	39.29±1.96	40.67±2.03	41.76±2.08
8	3.5	46.56±2.32	43.34±2.16	43.89±2.19	44.98±2.24
9	4	51.02±2.55	49.68±2.48	48.28±2.41	49.16±2.45
10	4.5	58.20±2.91	57.66±2.88	53.34±2.66	54.49±2.72
11	5	63.12±3.15	62.93±3.14	58.68±2.93	61.20±3.06
12	5.5	68.44±3.42	66.73±3.33	64.16±3.20	65.94±3.29

13	6	72.76±3.63	70.28±3.51	69.76±3.48	71.94±3.59
14	6.5	76.51±3.82	74.39±3.71	73.56±3.67	75.92±3.79
15	7	79.56±3.97	77.61±3.88	78.33±3.91	78.35±3.91
16	24	81.32±4.06	80.16±4.00	86.92±4.34	79.64±3.98

Table 7. Percent Drug Release of Polymeric Nanoparticles (n=5)

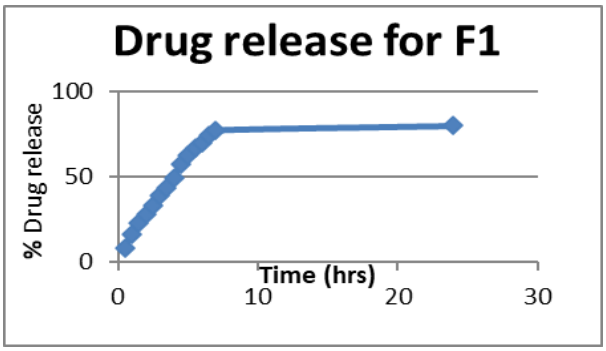


Fig 10. Representation of in-vitro drug release study of formulation 1.

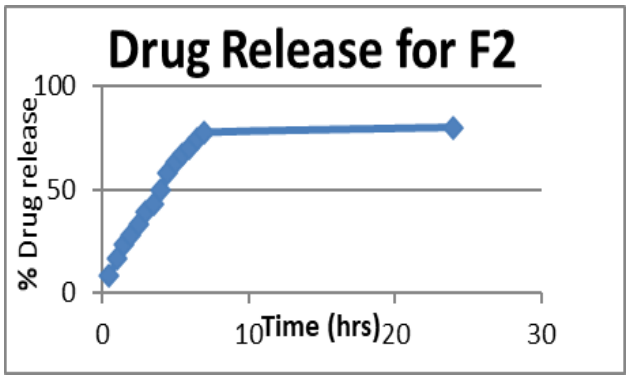


Fig 11. Represent The *in-vitro* Drug Release Study of Formulation 2.

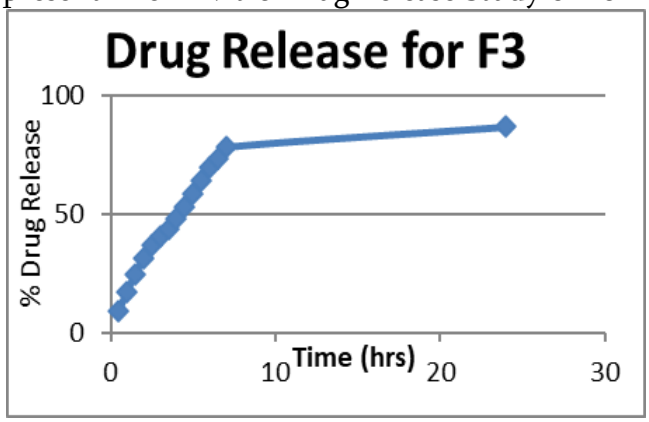


Fig 12. Representation of *in-vitro* Drug Release Study of Formulation 3.

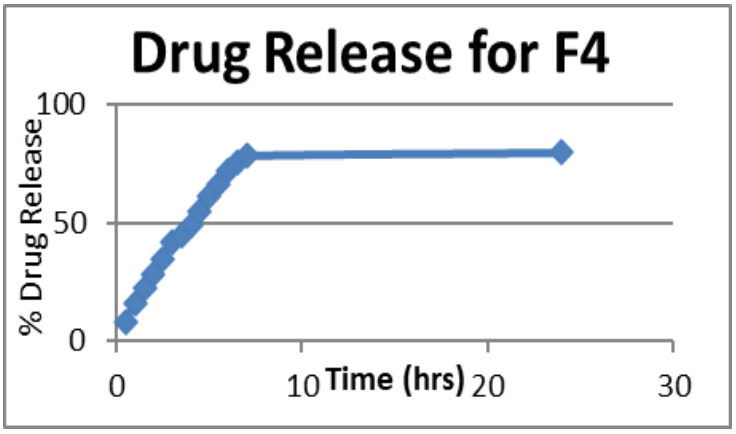


Fig 13. Representation of *in-vitro* Drug Release Study of Formulation 4.

Preformulation studies were done to verify drug and to assess the suitability of the drug neostigmine bromide to be formulated as polymeric nanoparticles. The physical appearance of the drug was found to be white in color and odorless in nature which was similar to as reported in Indian Pharmacopoeia. The average melting point of neostigmine bromide was found to be 185ºC which was similar the reported range of 184-186ºC. The FT-IR scan of drug also confirmed that the drug was authentic and there were all solvents peaks of functional group as that reported. Hence the drug was authenticated for its physiochemical property.

Through scanning, the λ_{max} was found to be 294 nm in distilled water.

The standard curve of drug was determined in distilled water by using UV spectrophotometer at λ_{max} 294 nm. The absorbance against concentration graph were plotted. The curve was found to be linear and shows the value of R^2 is 0.998, hence the drug obeyed lambart's law and equation was found to be:

Y=0.0514x-0.0075……..…….(2)

Four different formulations of neostigmine bromide nanoparticles were prepared and evaluated.

All the formulation were evaluated for percent practical yield. F4 formulation shows the highest practical yield which was 73.33%, and other formulations also show yield in between 69.06-72.56%. The order of percent practical yield was found to be: **F4>F3>F2>F1**

The drug entrapment efficiency of all formulations was performed to determine the percentage of drug present in formulation. The result shows that amount of drug varied from 49.83% to 65.39% and the highest amount of drug was present in F3 formulation. The order of amount of drug present in the formulation was found to be: **F3>F1>F2>F4**

According to the drug entrapment efficiency report, the 1:6 ratio of drug and polymer shows highest amount of drug entrapped in the polymeric nanoparticles and 1:2 ratio fairly entrapped the drug, whereas 1:4 and 1:8 ratio could entrap less amount of drug in the formulations.

The evaluation of particles through SEM shows that surface characteristics of polymeric nanoparticles. The particles were not in the spherical shape and the size was sub-micron to nano.

The polymeric nanoparticles were evaluated for the in-vitro drug release study in phosphate buffer of pH 7.4. the results show that the release of the drug increases with time. Formulation F3 shows the best release profile as it could release 86.92±4.34 % drug in 24 hrs. Formulation F1 was somewhat legging to F3 in terms of drug release characteristics. It released the 81.32±4.06% of drug in 24 hrs. The formulation F2 released 80.16±4.00 % in 24 hrs and formulation F4 released 79.67±3.98 % of drug in 24 hrs. The order of drug release of formulations found to be:

F3>F1>F2>F4

According to the drug release profile of the formulation it is clear that the nanoparticles prepared by using the 1:6 ratio of drug and polymer shows the highest drug release as compared to nanoparticles prepared using other ratios with the polymer chitosan.

CONCLUSION

From the present work, it was concluded that there are fair possibilities of formulating polymeric nanoparticles of neostigmine bromide using chitosan as polymer and span 80 as emulsifier by emulsion solvent evaporation method, which was found to be simple, reproducible and rapid. Formulation factor like drug: polymer ratio is an important factor for the preparation of nanoparticle formulations. Polymeric nanoparticles of neostigmine bromide were white in color and free flowing in nature and show controlled release upto 24 hrs. Considering all other parameters like percent practical yield, particle size determination by SEM and drug entrapment efficiency for all four formulations, F3 formulation was found to be most reliable since it shows optimum results for particle size, drug entrapment efficiency and in-vitro drug release.

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