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Research Article

DESIGN AND DEVELOPMENT OF RILUZOLE LOADED CHITOSAN NANOPARTICLES BY EMULSIFICATION CROSSLINKING

YATEENDRA SHANMUKHAPUVVADA*[1], SAIKISHORE VANKAYALAPATI[2]

[1]M.pharm Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, [2]Associate professor Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India.

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ABSTRACT

The major difficulty in treating most of the central nervous disorder was designing a formulation that overcoming the blood brain barrier and reaching the intended targeted site. In that case nanopariculate drug delivery is a successful dosage form to break the impasse In present study nanoparticles with hydrophilic polymers Chitosan nanoparticles were prepared using emulsification crossliking method. The formulation is evaluated for its particle size, zeta potential, drug entrapment efficiency, and invitro drug release profiles. The particle average particles size of all formulation were found to be **348, 492, 418, 382, 608 nm** respectively. The zeta potential of different formulations gave **17.3±0.5, 18.4±0.4, 22.6±0.4, 23.9±0.8, 27.6±0.9.** The practical evaluation of these formulation conclude formulation having 3:1.5 ratio was the best formulation based up on its release kinetics having 86.5% and 55% of entrapment efficiency. This formulation was exceptionally have prolonged period of drug releasing capability thus providing a sustained release drug delivery of riluzole.

Keywords: Riluzole, Chitosan, Nano particles, Emulsification, Characterization.

INTRODUCTION

Amyotropic lateral sclerosis is a neurological disorder which majorly affects the motor neurons of the upper and lower limbs. The disease is characterized by wasting of muscle and loss of muscle. Pathology mechanisms drawn in advancement of ALS have been inter correlated to the glutamatergic neurotransmitter system, with wastage of motor neurons triggered in the course of superfluous opening of glutamate receptors at the synaptic cleft [1].

Riluzole is the only drug licensed for symptomatic ALS treatment and is proposed to delay disease progression.Riluzole is a potent neuroprotective agent that intervenes at several sites in the process of signal transmission by the messenger glutamate. It is known that it reduces the release of glutamate into the synaptic gap and thus glutamate mediated activation of glutamate receptors and protects dopamine neurons [2]. In most of the cases the drug reaches the targeted site at very low concentrations of the total concentration. Bioavailability of the drug is affected by the physiochemical properties of the drug. In case of riluzole 60 % of the drug is metabolized in the liver giving inactive metabolites.

The moderate efficacy of Riluzole may be due to low bioavailability, lack of multifunctionality, Riluzole is approximately 90% absorbed following an oral dose but only 30-60% reaches the target site. This can be explained by the fact that this agent primarily undergoes rapid chemical degradation into its inactive metabolites (e.g. Riluzole-glucuronide) in the liver[3].

Hence, developing and designing a drug delivery system which enhances the therapeutic outcome of a drug at the same time reducing the unwanted toxic effects in-vivo is indeed purposeful task.

Drug delivery to central nervous system is a foremosthurdle in treating multiple cerebral diseases like, Amyotropic lateral sclerosis (Motor Neuron Disease) Alzheimer's, brain tumours, Parkinsons diseases. The blood brain barrier (BBB) is an obstinatehindrance for very good number of drugs including antibiotics, antineoplastic, antiepileptics, agents and a variety of central nervous system (CNS) active drugs.

Nanoparticulate drug delivery is specifically chosen to deliver such drugs to brain by penetrating BBB and these may provide a momentousstratagem to make way through thetough barrier [4].

In addition, due to the exceptionalphysiochemical properties chitosan a cationic hydrophilic polymer could reach overwhelming the blood–brain barrier (BBB) by endocytosis or transcytosis mechanism that occurs in the endothelial cells. When these nanoparticles reach the bloodstream, they may avoid the macrophage uptake of the mononuclear phagocyte system owing to their small size[5].

Chitosan is the second most abundant natural polymer after cellulose obtained by deacetylation of chitin. Chitosan possess some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable,nontoxic and inexpensive. These properties make chitosan a very attractive material as drug delivery carriers.

Chitosan nanoparticles are prepared by the emulsificationbased on the interaction between the negative groups of sodium tripolyphosphate (TPP) and Rendering positively charged amino (-NH2) and hydroxyl (-OH) groups, CS enables a high degree of chemical modification.

MATERIALS AND METHODS

Chitosan gratis sample was obtained from India Sea foods, Chochi.Viscosity was apparently 304mpaswith 89.79%degree of deacetylation, and molecular weight was about 1500 k Daltons. Trypolyphosphate was obtained from S.D.fines Mumbai. Gratis sample of Riluzole was obtained from the Hetero drugs, Hyderabad. Buffer chemicals were all reagent grade obtained from Sigma Aldrich Hyderabad.

Preparation of nanoparticles

chitosan nanoparticles were prepared by emulsification crosslinking method. gels were prepared by dissolving various concentrations of Chitosan(1-4mg/ml) 2%(W/V)containing 200 mg of Riluzole using ethanol as a cosolventglacial acetic acid with magnetic stirrer until homogenous gel like solution is obtained. Half the quantity Acetone equivalent to gel of was added into 15 ml of arachis oil and emulsified with magnetic stirrer. This emulsion is continuously stirred for half an hour for rapid formation of nanoparticles in the oil phase due to evaporation of acetone. To crosslink and separate the nanoparticles trypolyphosphate is added to the system. This is done by slowly adding with a micropipette [6].

The stirring is continued for about 1hr. The resultant nanoparticles suspensions are centrifuged at 20000x g for 30 min using REMI C24 centrifuge[7]. Excess of water is added to draw the nanoparticles into the aqueous phase. Out of which 5 formulations are found to be the better ones to investigate further and to reproduce a consistent formulation as depicted in the table 1.

Table 1: Selected ratios of chitosan- trypolyphosphate formulations

S. No.	Batch code	Amount of drug (mg/ml)	Conc.of chitosan (mg/ml)	Conc. of cross linking agent.(mg/ml)
1	F1	10	2	1.0
2	F2	10	2	1.5
3	F3	10	3	1.5
4	F4	10	4	2
5	F5	10	5	1

Characterization of prepared Chitosan Nanoparticles

Determination of particle size and morphology

The chitosan nanoparticles were observed with JOEL model JSM-6610LV (Detector- Everhart thornley). It was photographed using scanning electron gun operated with accelerating voltage of 0.3-30KV with a pre-centered tungsten hairpin filamentshape and size are characterized simultaneously[8].

Surface charge determination

The zeta potential of the chitosan nanoparticles are measured by using a Zetasizer® 3000 (Malvern Instruments,) at 90° scattering angle recorded for 90 seconds. The sample is distributed in the proper suspending medium, specifically an aqueous solution of NaCl (0.9% w/v), filtered (0.2 µm) double-distilled water [9].

Percentage yield

The nanoparticles production yield is calculated by gravimetric analysis. Fixed volumes of nanoparticles suspensions are centrifuged (16,000×g, 30 min, 15 ºC) and sediments are dried. The process yield is calculated as follows [10].

$$\text{percentage yeild} = \frac{\text{nanoparticles weight}}{\text{total solidweight}} \times 100$$

Percentage entrapment efficiency:

The entrapment efficiency of the nanoparticles is analyzedgravimetric analysis (mass balance). The drug trapped in chitosan nanoparticles and the free drug, unentrapped drug is estimated to know total amount of the drug entrapped.

The drug present in the formulated is extracted from the formulation and then analyzed for the drug content. A known volume of the nanosuspension is filtered through 0.22µm whatmann filter paper. To the sediment obtained 2% of sodium citrate is added and centrifuged at 1000rpm for 15 min, to damage the chitosan crosslinks. Add 4% of acetic acid solution and till the sediment forms a clear solution. 5ml of methanol is taken into chitosan solution and vortexed for 10 min, colloidal solution is estimated under UV spectrophotometer at 265 nm.

The supernatant is also estimated as well to know the amount of the drug unentrapped.1ml of the methanol is added to 1ml of supernatant solution and filtered through 0.22µm filter and absorbance at 265nm is noted under UV spectrophotometer.

In vitro release study

A franz diffusion cell is used to monitor Riluzole release from the nanoparticles. The receptor phase is 2:10 ratios of phosphate buffered saline (PBS, pH 7.4) and methanol respectively thermostatically maintained at 37°C, with each release experiment run in triplicate. Dialysis membrane) with molecular weight cut off 12,000 to 14000 Daltons is used to separate receptor and donor phases. The latter consisted suspension of nanoparticles containing Riluzole100 mg mixed for 5 seconds to aid re-suspension in Phosphate Buffer Solution. Samples (1ml) from the receptor phase are taken at time intervals and an equivalent volume of Phosphate Buffer Solution replaced into the receiver compartment. Diffusion of Riluzole into the receptor phase is evaluated spectrophotometrically.

RESULTS AND DISCUSSION

Determination of particle size and morphology

Morphology study of the nanoparticlesprepared by emulsification crosslinking technique were found be spherical with good structural composition having a definite boundary as shown in the **fig 1**. The average particle sizes were found to 348, 492, 418, 382, 608 nm for the formulations F1, F2, F3, F4, F5 respectively.

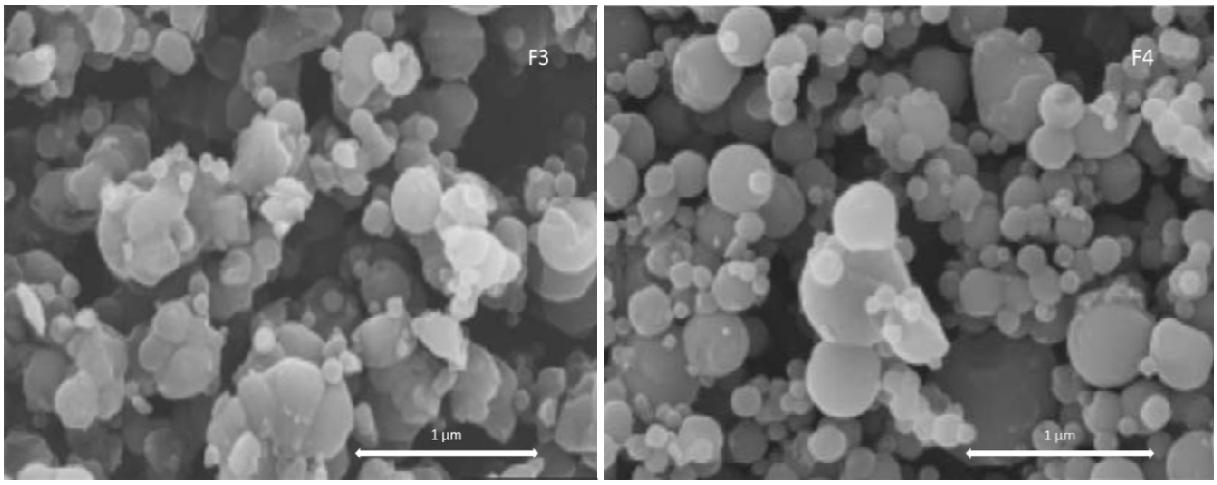


Fig. 1, 2: Chitosan nanoparticles prepared by emulsification crosslinking technique.

It was observed that the formulation with lower polymer and crosslinking agent no formation of particulate system was found. The formulations at particular ratio a proportional increase in the concentration increased size of the nanoparticles. It was found that the concentration at very higher ratio large particle size is observed due to high viscosity of the gel and no distributing room in the emulsion, above these concentrations resulted in formation of aggregates.

Determination of surface charge

The nanoparticles prepared are maintained at ambient pH and temperature to prevent the degradation of the formulation. However the crosslinking agent being proton rich have influence on the zeta potential[11]. (Table 2)

It was clear from the data at pH range of 4.8-5.5 increase in TPP concentration has equivalenteffect on zeta potential. The

responsible molecule for this effect is tripolyphosphate which is proton rich. The more the ions are exhausted to neutralize the more the amino groups present in the chitosan and these free ions on the surface are responsible for the surface charge.

Table 2: Corresponding zeta potentials of various formulations

Formulation	Zeta potential mV
F1	**17.3±0.5**
F2	**18.4±0.4**
F3	**22.6±0.4**
F4	**23.9±0.8**
F5	**27.6±0.9**

Percentage yield

The entrapment efficacy of the drug in to the formulations is analysed by the gravemetric (mass balance analysis) which shows the results of each formulations. Formulation with higher chitosan and Tpp concentrations was found to have more entrapment efficacy entrapping 55% of the drug for F3. While F4 is next best formulation with 48% entrapment efficacy.Where F1, F2 and F5 are not much efficient in entrapping not more than 36%, 39%, and42 % respectively.

Chitosan unique molecular geometry plays an important role in the drug entrapment efficacy. Most of the drug having positive charge is more suitable for the loading of drug into the chitosan network. The cationic drug like riluzole is barely difficult task to have good entrapment. Interestingly this major hurdle is solved by the method of preparation. Where the chitosan gel solution, is presented into immiscible oil phase consequently cross-linked by a crosslinking agent.

In vitro release study

In vitro studies of optimized chitosan nano formulations were carried out for release pattern across cellophane membrane. The release patterns of F1, F2, F3,F4, and F5 are 78.79,81.61,86.20,83.99,84.35 and 84.35 respectively (table 3)(graph 2). It was quite evident from the release profile that show extended the drug release through nanoparticles based upon which F3 was found to be the best formulation.

Table 3: Drug release profiles, first order kinetics, peppas plot, *n* values of selected formulations

Formulations	Percentage of drug released (%)	first order kinetics	Peppas plot	*n* values
F1	**78.79**	**0.9787**	**0.9777**	**0.9058**
F2	**81.61**	**0.9750**	**0.9772**	**0.8896**
F3	**86.20**	**0.9841**	**0.9282**	**0.8641**
F4	**83.99**	**0.9781**	**0.9786**	**0.8606**
F5	**84.35**	**0.9756**	**0.9746**	**0.8695**

Study of Release kinetics

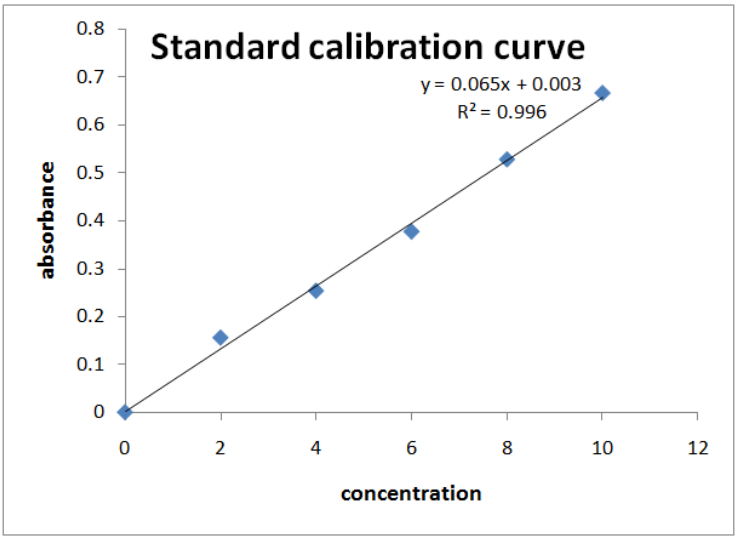
In order to define and correlate the release kinetics of all five formulations the release kinetics were done. The corresponding dissolution data were fitted in suited kinetic dissolution models (Table 3) (fig 3&4)(graph 3&4). The equation, which is used to describe drug release mechanism, is:

$$m_t/m_8 = kt^n$$

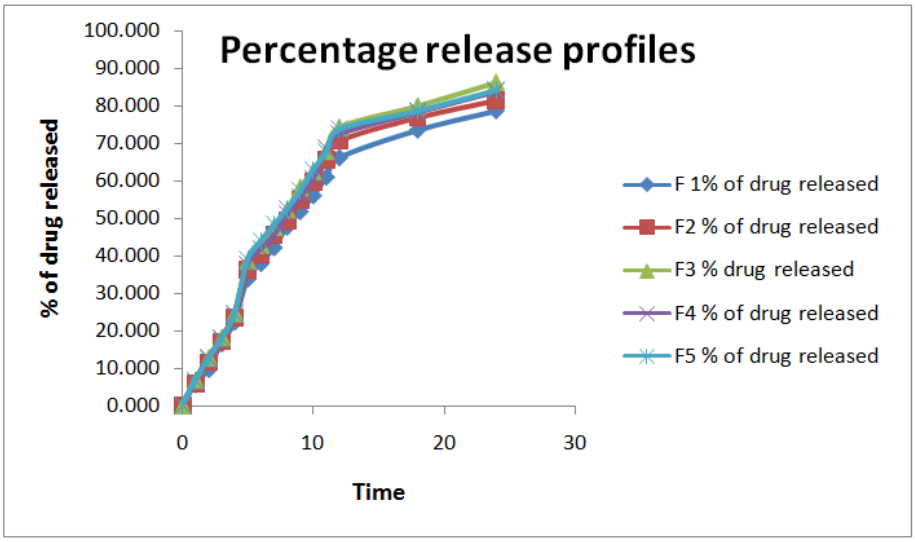
Where, m t / m 8 is the portion of drug release‘t’ is the release time ‘k’ is the constant. K dictates the properties of the macromolecular polymer system. ‘n’ is the release exponent indicative of the mechanism of release. The values of n and r^2 for coated batch was 0.521and 0.680.Since the values of slope (n) lies in between 0.5 and 1 it was concluded that the mechanism by which drug is being released is a non-Fickian (anomalous) solute diffusion mechanism, that is, drug release during dissolution test may be controlled by all diffusion, erosion and swelling mechanism.[12]

CONCLUSION

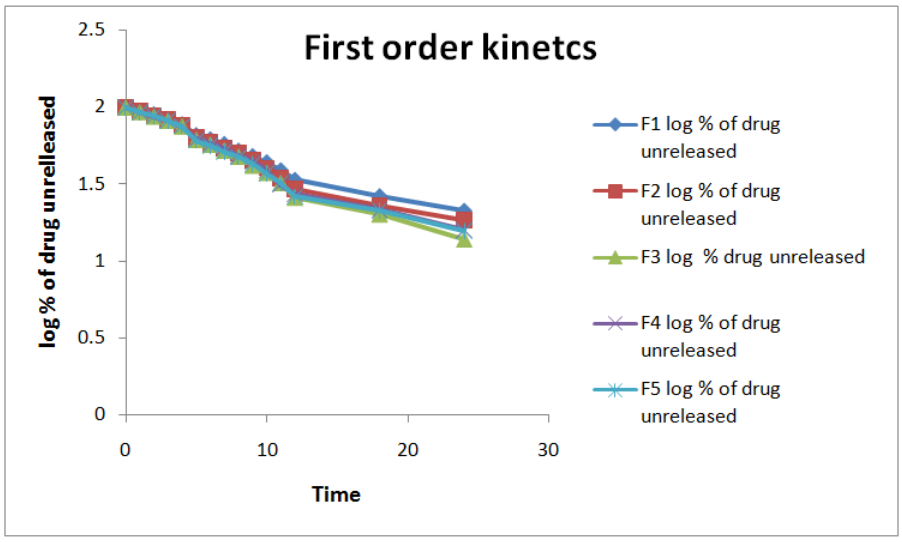
Emulsification cross linking technique was employed to formulate the nanoparticles using chitosan as polymer and trypolyphosphate as cross linking agent. The nanoparticles produce were found to be nano range with acceptable physical chemical nature. Based on percentage yield, drug entrapment efficiency, particle size morphology, zeta potential and *in vitro* release, formulation F3 was found to be the optimal formulation. Thus nanoparticles of Riluzole F3 with polymer crosslinking agent ratio 3:1.5 were found to be spherical, discrete and able to sustain the drug release effectively.



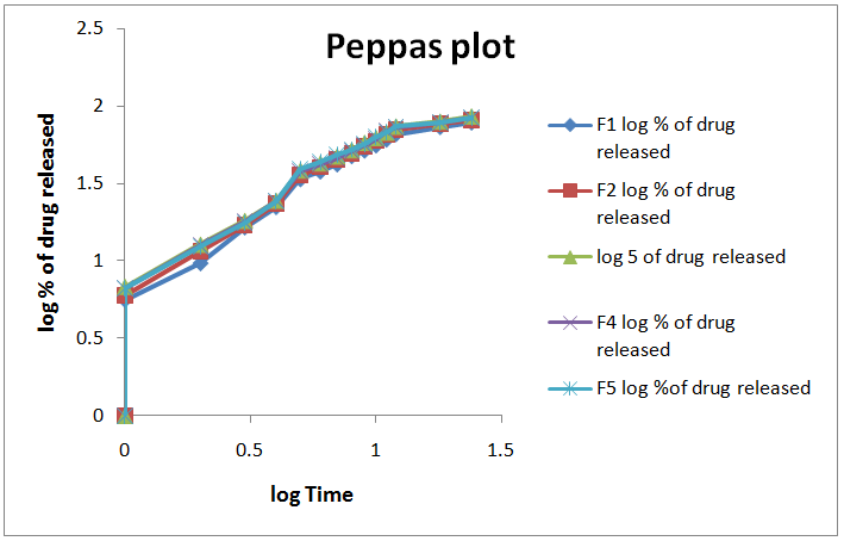
Graph 1: Standard calibration curve for riluzole



Graph 2: Drug release profile of some selected formulations prepared by emulsification crosslinking method.



Graph 3: First oder release kinetics of some selected formulations



Graph 4: Peppas plot of some slected formulations

REFERENCES

1. Cheah B.C, Vucic S, Krishnan A.V, Kiernan M.C: Riluzole, Neuroprotection and Amyotrophic Lateral Sclerosis. Current Medicinal Chemistry 2010; (1)7: 1942-1959.
2. Cifra A, Nani F, Nistri A: Riluzole is a potent drug to protect neonatal rat hypoglossal motoneurons in vitro from excitotoxicity due to glutamate uptake block. European Journal of Neuroscience 2011; 33(5): 899- 913.
3. Bensimon G, Lacomblez L, Meininger V: A Controlled Trial of Riluzole in Amyotrophic Lateral Sclerosis. N Engl J Med 1994; 330:585-591.
4. Chien-Liang GL, Lynn Bristol A, Lin Jin, Dykes-Hoberg M, Thomas Crawford, Lora Clawson, and Jeffrey D : Rothstein Aberrant RNA Processing in a Neurodegenerative Disease- the Cause for Absent EAAT2, a Glutamate Transporter, in Amyotrophic Lateral Sclerosis. Neuron1998; Vol. 20: 589–602.
5. Bondì ML, Craparo EF, Giammona G, Drago F: Brain-targeted solid lipid nanoparticles containing Riluzole - preparation, characterization and biodistribution. Nanomedicine 2010; 5(1):25–32.
6. Dhanya KP, santhi K, Dhanaraj SA and Sajeeth CI: Formulation and evaluation of chitosan nanospheres as a carrier for the targeted delivery of lamivudine to the brain. International Journal of Comprehensive Pharmacy 2011; 5 (13).
7. Simar PK, Rekha Rao, Afzal Hussain, Sarita Khatkar: Preparation and Characterization of rivastigmine Loaded Chitosan Nanoparticles. J. Pharm Sci. & Res 2011; 3(5): 1227-1232.
8. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ: Novel hydrophilic Chitosan–Polyethylene Oxide nanoparticles as protein carriers. J. Appl. Polym. Sci 1997; 63: 125–132.
9. Sangeetha S, Harish G, Malay Samanta K: Chitosan-Based nanospheres as Drug Delivery Systems for Cytarabine. International Journal of pharma and Bio Sciences 2010; 1(2).
10. Adlin Jino Nesalin J, Gowthamarajan K, Somashekhara C N: Formulation and Evaluation of Nanoparticles Containing Flutamide. International Journal of Chem tech Research 2009; 1(4): 1331-1334.
11. Liu H, Changyou Gao: Preparation and properties of ionically cross-linked chitosan nanoparticles. Polym. Adv. Technol 2009; 20: 613–619.
12. Sijumon K, Sajan J,Twan L: Understanding the mechanism of ionic gelation for synthesis of chitosan nanoparticles using qualitative techniques. Asian Journal of Pharmaceutics 2010; 4(2): 148-153.