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Comparison of PLGA and PCL nanoparticles loaded with Rivastigmine by Double emulsion solvent evaporation technique for Alzheimer's disease

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ABSTRACT: Background: Rivastigmine is a drug of choice with the size of a small nanoparticle (>200) that is desirable to penetrate the blood-brain barrier for treating Alzheimer's disease. **Aim:** This study was aimed to assess various formulation parameters to enhance the incorporation of a hydrophilic drug (Rivastigmine) into Poly-d,l-lactide-co-glycolide (PLGA), and Poly-caprolactone (PCL) nanoparticles prepared by Double Emulsion Solvent Evaporation (DESE) technique. **Method:** Initial Preformulation studies reveal that there is no interaction between drugs and polymers. Twelve formulations were prepared using PLGA and PCL as polymers. The prepared formulation was evaluated for particle size, zeta potential, entrapment efficiency, and *in vitro* drug release. **Result:** The effect of organic solvents (Dichloromethane, Ethyl Acetate) and stabilizers (Pluronic F-127 and Polyvinyl Alcohol) on the particle size and zeta potential of the formulation was observed. In this, ethyl acetate facilitates the formation of nanoparticles with lesser size and higher amount of polymer showed good entrapment efficiency. Comparatively, PCL formulations showed better results in overall studies. The particle size of optimized formulation F9 showed 27.63 nm with 0.291 mV zeta potential and showed an entrapment efficiency of 92.25 %. *In vitro* drug release studies revealed 82.2 % of drug release for 24 h. **Conclusion:** Formulation F9 was considered to be the best preparation and was selected for further *in vivo* animal study. In sum, the DESE technique is one of the best methods to incorporate hydrophilic drugs into a nano particulate system.

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Keywords: Hydrophilic drug, Rivastigmine, Alzhemier's disease, DESE technique, PLGA polymer, PCL polymer.

INTRODUCTION:

Alzheimer's disease (AD) is a neurodegenerative disorder that progresses over time and causes memory loss, personality changes, and dementia in older people [1]. Alzheimer's disease treatment is a challenging topic because many hydrophilic drugs and neuropeptides cannot cross the blood-brain barrier (BBB), which is made up of three biological components, including endothelial cells, astrocyte end-feet, and pericytes. A

diffusion barrier created by tight junctions between the cerebral endothelial cells prevents the majority of blood-borne chemicals from selectively entering the brain [2,3]. Conventional drug delivery techniques release medications into the bloodstream, which does not adequately carry drugs to the brain for treating CNS disorders like Alzheimer's disease, dementia, and Parkinson's disease [4]. However, novel biodegradable polymeric nanoparticles (NPs) can deliver therapeutic and diagnostic drugs for neurological disorders beyond the BBB. Stabilizers, amyloid-affinity agents, and the physicochemical properties of the NPs at varied surfactant concentrations could all influence the transport mechanism.

Nanoparticles made of synthetic or natural polymers with a size range between 10 and 1000 nm, exhibit better stability and can achieve controlled & prolonged drug release and it is used as carriers to deliver a variety of drugs, including hydrophilic and hydrophobic pharmaceuticals, proteins, vaccines, and biological macromolecules [1,5]. Polymeric NP is a potential drug delivery system because of their small size and ability to accumulate pharmaceuticals precisely where they are required in the body by penetrating through even tiny capillaries and being taken up by cells [2]. Fig 1 presents an overview of the forms, causes, and symptoms of Alzheimer's disease.

Rivastigmine, an acetylcholinesterase (AChE) inhibitor used to treat Alzheimer's disease, considerably inhibits AChE more efficiently in the central nervous system (CNS) compartment than in the peripheral nervous system (PNS), according to preclinical and clinical studies. Rivastigmine absorbs fast and peak plasma concentrations of rivastigmine are obtained in about an hour, and its absolute bioavailability after a 3-mg dose is around 36 % [6]. PLGA (poly-d,l-lactide-co-glycolide) has been used most successfully for the development of nanomedicines that are hydrolyzed in the body to produce biodegradable metabolite monomers lactic acid and glycolic acid.

The processes of emulsification-diffusion, solvent emulsion-evaporation, interfacial deposition, and nanoprecipitation are frequently used to develop PLGA nanoparticles. PCL (poly-caprolactone) has received a great deal of attention for usage in the delivery of drugs successfully since it is hydrolyzed by its ester linkages under physiological conditions. PCL nanoparticles have usually been formulated using solvent displacement, solvent evaporation, and nanoprecipitation [7]. This study's goal is to develop, evaluate and compare the PLGA and PCL nanoparticles loaded with a hydrophilic drug (Rivastigmine) for treating Alzheimer's disease.

MATERIALS AND METHODS:

Rivastigmine Tartrate received as a gift sample from Alembic, Gujarat, Vadodara, Poly Caprolactone, Poly (Lactic – co-glycolic acid) PLGA obtained from Sigma Aldrich chemicals Pvt. Ltd., USA, Pluronic F68, Potassium Dihydrogen Orthophosphate, Dichloromethane, Ethyl acetate purchased from Himedia laboratories Pvt Ltd., Mumbai, Polyvinyl alcohol obtained from Qualigens fine Chemicals., Mumbai, All other chemicals and reagents used in this were of analytical grade.

Determination of λ_{max} of Rivastigmine using UV Spectrophotometer:

Rivastigmine 10 mg was dissolved in 100 ml of pH 7.4 phosphate buffer saline (100 µg/ml). About 10 ml of this solution was taken in a 100 ml volumetric flask and made up to the mark with pH 7.4 (10 µg/ml) buffers and the obtained solution was scanned on a UV Scanner between 200 to 400 nm. The graph's highest value was considered as λ_{max} for the pure drug.

Calibration curve of Rivastigmine:

Rivastigmine 100 mg was dissolved in a phosphate buffer of pH 7.4 in 100 ml of a volumetric flask and made up to the volume with phosphate buffer of pH 7.4 to give 1000 µg/ml. From the above solution 1, 2, 3, 4, 5, and 6 ml were diluted to 10 ml using a phosphate buffer to give the concentration of 100, 200, 300, 400, 500, 600 µg /ml. The absorbance of this solution was measured at 264 nm by using a UV-Visible spectrometer. The regression coefficient values were found to be 0.999.

FT- IR Drug - Polymer interaction study:

Spectral (FTIR) analysis of Rivastigmine and combination of the Rivastigmine with PCL and PLGA polymer were carried out by using a FTIR Spectrophotometer (Bruker, India) to investigate any changes in the chemical composition of the drug after mixing it with the excipients. From the obtained spectra it was observed that all the characteristic peaks of Rivastigmine were present in the combination spectra thus indicating the compatibility of the drug with the polymer used.

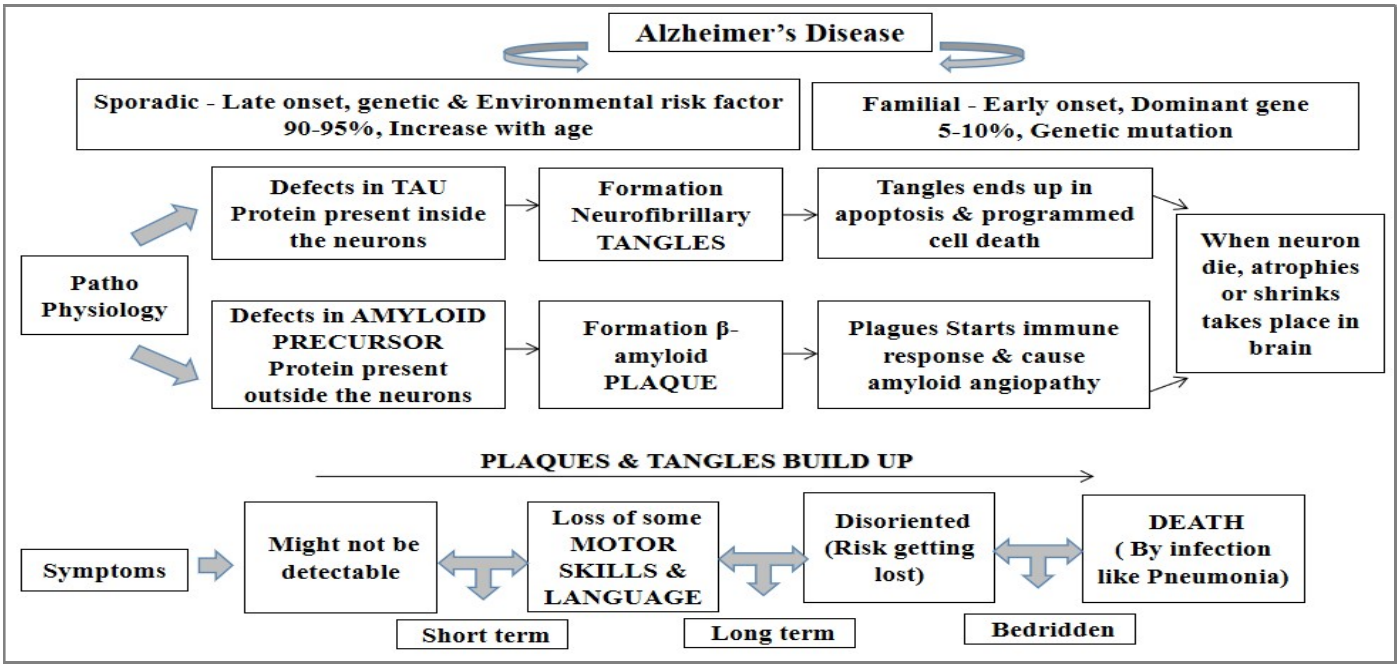

Fig 1. Overview of Alzheimer's disease types, causes and symptoms.

Table 1. List of formulation loaded with Rivastigmine using PLGA polymer.

FR	Drug (mg)	Water (ml)	Solvent (s)	Polymer (mg)	HG (rpm)	Secondary emulsion (rpm)
F1	10	1	DCM (6ml)	50	24,000	24ml water + 240mg F-127
F2	10	1	EA (6ml)	50	24,000	24ml water + 240mg F-127
F3	10	1	DCM (3ml) + EA (3ml)	50	24,000	24ml water + 240mg F-127
F4	10	1	DCM (6ml)	50	24,000	24ml water + 240mg PVA
F5	10	1	EA (6ml)	50	24,000	24ml water + 240mg PVA
F6	10	1	DCM (3ml) + EA (3 ml)	50	24,000	24ml water + 240mg PVA

FR – Formulations and HG – Homogenization.

Table 2. List of formulation loaded with Rivastigmine using PCL polymer.

FR	Drug (mg)	Polymer (mg)	Stabilizer	Vol. of Aqueous Phase W/O (ml)	Vol. of Aqueous Phase W/O/W (ml)	Organic solvent
F7	8	40	1% F127	6	10	EA
F8	8	80	1% F127	6	10	EA
F9	8	120	1% F127	6	10	EA
F10	8	40	1% F127	6	10	DCM
F11	8	80	1% F127	6	10	DCM
F12	8	120	1% F127	6	10	DCM

FR – Formulations.

Preparation of PLGA nanoparticles:

Rivastigmine nanoparticles were developed using the double emulsion solvent evaporation process [7]. Rivastigmine of 10 mg and 1 ml water were added to 50 mg of PLGA polymer, which had been dissolved in 6 ml solvent (either ethyl acetate or Dichloromethane (DCM), or a mixture of both). The solution was then homogenized at 24,000 rpm to prepare a primary emulsion. The developed primary emulsion was then added to 24 ml of water together with 240 mg of stabilizer, such as PVA and pluronic F-127. Repeating the homogenization process resulted in the formation of a secondary emulsion. A magnetic stirrer was used to evaporate the solvent present in the resulting solution [8,10]. List of formulations loaded with Rivastigmine using PLGA polymer are given in Table 1.

Preparation of PCL nanoparticles:

Rivastigmine-loaded PCL nanoparticles were prepared by the double emulsification solvent evaporation method. Initially, the drug and polymer were dissolved in the organic solvent and then added to the aqueous phase containing stabilizer, which is then emulsified using a high shear homogenizer at 24,000 rpm for 10 min. This Primary emulsion (w/o) was further added to another aqueous phase containing surfactant to form w/o/w emulsion and stirred at 100 rpm using a magnetic stirrer until the organic solvent was evaporated [9,10]. A schematic representation of the preparation of Rivastigmine-loaded nanoparticles is given in Fig 2 and 3. List of formulations loaded with Rivastigmine using PCL polymer are given in Table 2.

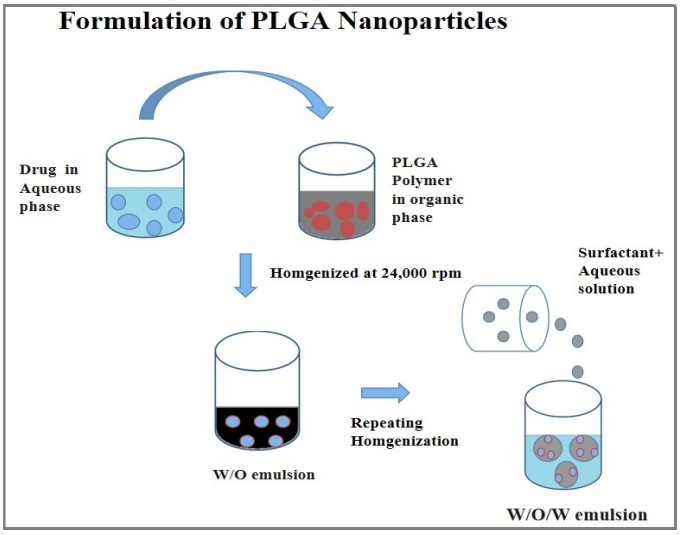

Fig 2. Schematic representation of Preparation of Rivastigmine PLGA Nanoparticles.

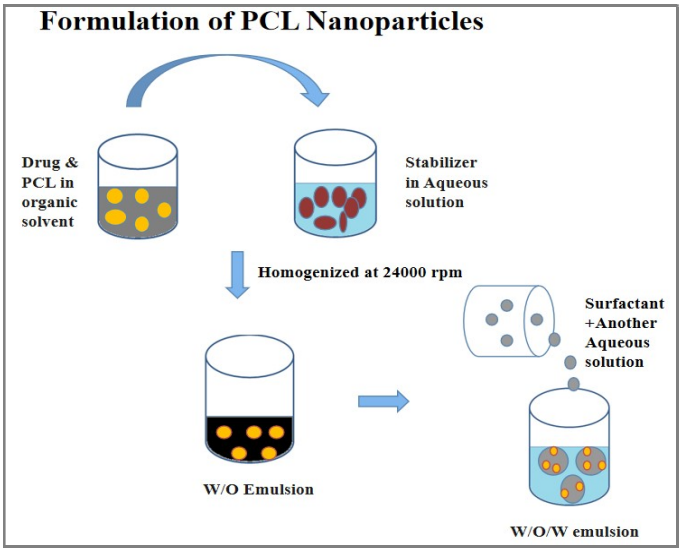

Fig 3. Schematic representation of Preparation of Rivastigmine loaded PCL nanoparticles.

Characterization of Rivastigmine Nanoparticles:

***Particle size and zeta potential*:**

The size analysis and polydispersity index of the nanoparticles were determined using a Malvern zeta sizer ZS (Malvern instruments). Each sample was suitably diluted with filtered distilled water (up to 2 ml) to avoid multi scattering phenomena and placed in a disposable sizing cuvette. The narrowness of the particle size distribution was examined using the polydispersity index.

Three measurements were taken for the size analysis of a sample, and the findings are expressed as mean size ±SD. Zeta potential distribution was measured using a Zeta sizer (nano ZS, Malvern Instruments). Each limit ranged from ±30 (mV). The average of three measurements of each sample was used to derive the average zeta potential [11,12].

***Determination of entrapment efficiency (EE)*:**

About 2 ml of the formulation is centrifuged at 13,000 rpm for 20 min and the supernatant solution is collected and analyzed for the drug concentration using UV visible spectroscopy.

The amount of drug entrapped is found using the equation 1.

$$EE\ (\%) = [(W1 - W2)/W1] \times 100 \quad \ldots.(1)$$

Where W is amount of drug added in the system and W2 is the amount of drug present in the supernatant.

***In vitro release study*:**

In vitro release of Rivastigmine from PLGA and PCL Nanoparticles was evaluated by the dialysis bag diffusion technique. The release study of Rivastigmine from nanoparticles was performed in 50 ml phosphate buffered saline (PBS) (pH 7.4) to create a perfect sink condition. About 2 ml of the formulation was placed in a dialysis bag (cut-off 12,000 Da; Himedia, Mumbai, India), which was previously soaked overnight in water and sealed at both ends.

The dialysis bag was immersed in the receptor compartment containing 50 ml PBS (pH 7.4), which was stirred at 100 rpm and maintained at 37 ± 2 °C. The receptor compartment was enclosed to stop the released liquid from evaporating. At regular intervals, samples were taken out and fresh release medium was added in the same volume. The samples were analyzed spectrophotometrically at 264 nm. The average results of each experiment were determined after it was completed in triplicate [13,14].

RESULTS AND DISCUSSION:

On a whole, 12 formulations were prepared by changing the polymer, organic solvent, and stabilizer. The first 6 formulations were prepared with PLGA and the next 6 with PCL. All the formulations were homogenized at 24,000 rpm. The size range of the PLGA nanoparticles from formulations F1 to F6 varied from 194 to 1022 nm and PCL nanoparticles F7 to F12 varied from 27.63 to 664.3 nm. Fig 4 shows particle size of optimized formulation F9.

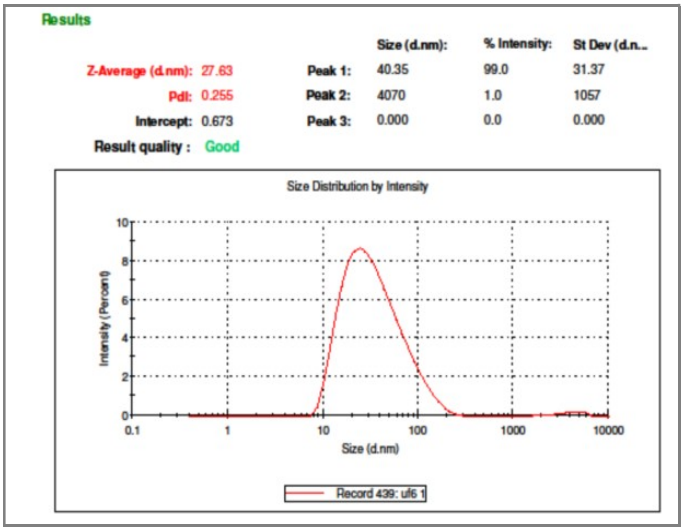

Fig 4. Particle size of optimized formulation F9.

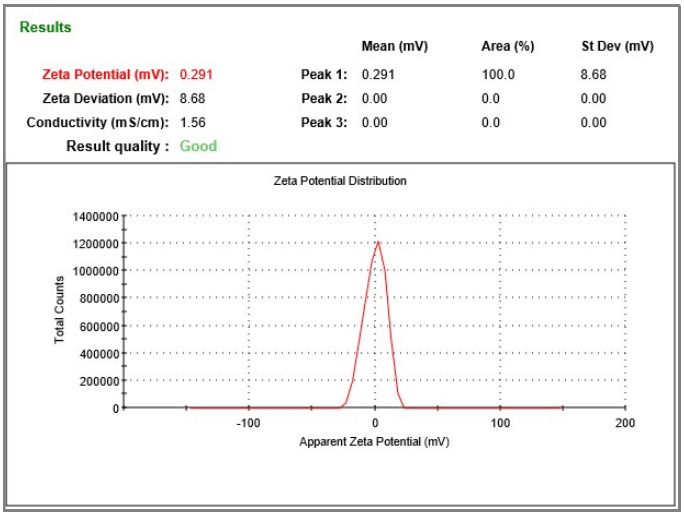

Fig 5. Zeta Potential of optimized formulation F9.

The type of organic solvent used also plays a major role in determining the size of the nanoparticles. Particle size was higher for the formulations where dichloro methane was used than the formulations where ethyl acetate was used. Hence it can be said that in order to get the least particle size ethyl acetate is favorable. The zeta potential of the PLGA formulations F1 to F6 varied from -4.30 mV to 0.475 mV and PCL formulation F7 to F12 varied from -2.43 mV to 1.45 mV. Fig 5 shows zeta Potential of optimized formulation F9.

The entrapment efficiencies of the formulations F1 to F6 varied between 20.48 to 45.78 % and formulations F7 to F12 varied between 24.45 to 92.25 %. Maximum entrapment efficiency was observed for formulation F9 which had the highest amount of PCL polymer. From the above observation, it is found that hydrophilic loaded PCL nanoparticles F9 show less particle size of 27.63 nm with maximum entrapment efficiency of 92.25 % than PLGA nanoparticles F6 show 194 nm with entrapment efficiency of 45.78 %. Table 3 shows the Particle size, Zeta potential, and Entrapment efficiency of formulated Rivastigmine nanoparticles.

The *in vitro* drug release studies revealed a maximum of 98.6 % for formulation F7 to a minimum of 15.20 % for formulation F1 release at the end of 24 h. *In vitro* release studies of prepared Rivastigmine nanoparticles are given in Table 4.

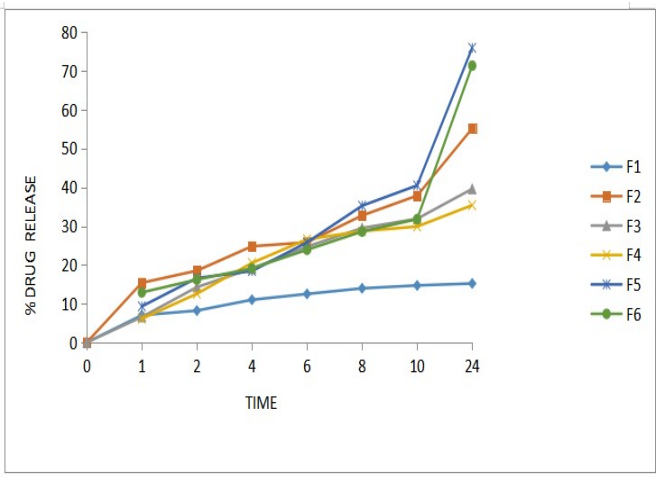

Fig 6. The cumulative Percentage drug release of PLGA nanoparticles.

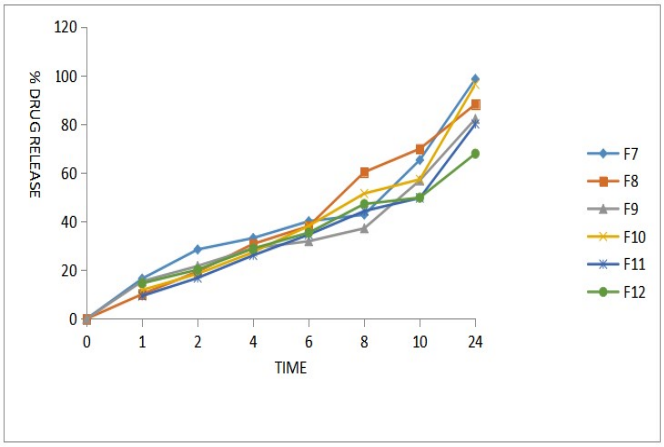

Fig 7. The cumulative Percentage drug release of PCL nanoparticles.

Table 3. Particle size, Zeta potential, Entrapment efficiency of Rivastigmine nanoparticles.

Formulation	Particle Size (nm)	Zeta potential (mV)	Drug Entrapment (%)
F1	1022	-4.30	20.5
F2	194	-0.676	37.34
F3	284	-2.57	31.32
F4	2134	-0.978	45.8
F5	906	-0.0698	38.55
F6	492	0.475	45.7
F7	105.3	-2.43	37.8
F8	65.67	1.45	41.4
F9	27.63	0.291	92.25
F10	440.1	-0.508	24.4
F11	448.1	0.722	53.4
F12	664.3	1.34	92

Table 4. *In vitro* release study of prepared Rivastigmine nanoparticles.

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
	Cumulative percentage drug release (%)											
1	7.0	15.3	6.5	6.1	9.3	12.9	16.5	10.1	15.4	11.8	9.4	14.6
2	8.2	18.5	14.2	12.5	16.6	16.2	28.5	19.4	21.7	18.5	16.8	20.2
4	11.0	24.8	19.0	20.5	18.4	19.1	33.2	30.8	29.0	27.4	26.1	28.9.
6	12.5	25.8	24.7	26.6	25.8	23.9	40.1	38.1	31.9	38.2	34.6	35.4
8	13.9	32.7	29.5	28.7	35.2	28.6	42.8	60.3	37.2	51.5	44.3	47.2
10	14.7	37.8	31.9	29.9	40.5	31.8	65.3	69.9	56.9	57.3	49.7	49.8
24	15.2	55.2	39.6	35.4	76.0	71.4	98.6	88.2	82.2	96.4	80.1	67.9

Overall observation shows PCL nanoparticle formulation shows better results than PLGA nanoparticle formulations. Fig 6 and 7 shows percentage drug release of PLGA and PCL nanoparticles.

CONCLUSION:

The present work was proposed to prepare and optimize Rivastigmine loaded PLGA and PCL nanoparticles prepared by double emulsification solvent evaporation with a size less than 200 nm in solvent on the particle size of the formulations was clearly observed where the use of ethyl acetate produced smaller particles when compared with dichloromethane. Hence for the production of smaller-sized particles ethyl acetate is the preferred solvent. It can be concluded that a higher amount of polymer is necessary for obtaining good entrapment efficiency. Rivastigmine, being a hydrophilic drug, has lower amounts of entrapment efficiency because most of the drug escapes into the aqueous phase from the partially soluble aqueous phase. While comparing PLGA and Poly-caprolactone nanoparticles, Poly-caprolactone nanoparticles show better entrapment efficiency, and drug release with less particle size. Formulation F9 prepared with Poly-caprolactone polymer was considered to be the best preparation and selected for further *in vivo* animal study. In sum, the Double Emulsion Solvent Evaporation technique is one of the best methods to incorporate hydrophilic drugs into a Nanoparticulate system.

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