RESEARCH ARTICLE

Preparation and characterization of diltiazem nanocapsules: Influence of various polymers

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Nanocapsules are submicroscopic colloidal drug delivery system and are composed of an oily or an aqueous core surrounded by a thin polymeric membrane. Nanocapsules have recently generated lot of interest in the area of controlled release with availability of biocompatible and biodegradable polymers. Nanocapsules of diltiazem were prepared with an objective of achieving controlled release of the drug in order to reduce the frequency of administration of drug, to obtain more uniform plasma concentration, and to improve patient compliance. Diltiazem was chosen as the model drug, as it is widely used in the treatment of chronic conditions such as hypertension and angina which require prolonged therapy. Nanocapsules were prepared by the interfacial deposition technique by taking different concentrations of polymers and phospholipid mixture. Five best formulations were selected based on the encapsulation efficiency. The morphology of nanocapsules was assessed by scanning electron microscope and they were found to be smooth, spherical, and discrete. The particles followed normal size distribution with particle size in the range of 20 to 380 nm. *In vitro* release studies indicated prolonged release for all polymers for 48 hours, with polycaprolactone as the best polymer releasing about 95 to 98%. The formulations were stable at 4°C but unstable at 25°C, and hence recommended for storage in refrigeration. Thus, it can be concluded that nanocapsules are a useful technology for controlled release of diltiazem.

Key words: *Nanocapsule, polycaprolactones, polylactic acid, polylactic-co-glycolic acid*

INTRODUCTION

Nanotechnology is very much the flavour of science fiction and popular science speculation now days. Nanotechnology is about with unprecedented capabilities.[1] Nanoscience is still in its infancy, lot has been only said theoretically but if it happens, it has a potential of changing the course of medical history in the years to come. People in future may not suffer from many diseases today, because they will have in them tiny soldiers (nanoparticles) guarding, fighting, and thus evading most of the infections of the human body. Significant effort has been devoted to develop nanotechnology for controlled drug delivery, because it offers a suitable means of delivering bioactive agents in biocompatible nanosystems such as nanoparticles, nanocapsules, micellar systems, and conjugates. These systems have multifaceted advantages in drug delivery. They may be used to provide targeted (cellular/tissue) delivery of drugs, improve oral bioavailability, sustain drug/gene effect in target tissue, solubilise drugs for intravascular delivery, improve the stability of therapeutic agents (e.g., against pH and enzymatic degradation), control drug release rate in target tissue for required duration of treatment for optimal therapeutic efficacy.

Nanoparticles/nanocapsules are unique colloidal drug delivery systems because they are the only colloids that can be injected intravenously (IV). In addition, they may be taken intramuscularly, orally, ophthalmically, subcutaneously, and topically. Nanoparticles are solid colloidal particles ranging in size from 10 to 1000 nm (1μ). They consist of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, and/or to which the active principle is adsorbed or attached.[2] Depending on the process used for their preparation, two different types of nanoparticles can be obtained, namely nanospheres and nanocapsules. The difference between these two forms lies in the morphology and body architecture. Nanospheres have a matrix type structure in which a drug is dispersed or may be adsorbed at their surface, whereas nanocapsules exhibit a membrane wall structure with

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an oily core containing the drug, but may also be adsorbed at their surface.[3] Theoretically, high quality nanocapsule systems achieve a constant rate drug release (zero order drug release), whereas first order drug release is typically observed in nanospheres. Coating a nanocapsule with different surfactants in order to modify surface charge and hydrophobicity alters this distribution and results in increased blood concentrations and circulation times as well as in lower RES organ concentration (e.g., surface modifications with polyethylene-glycol or poloxamers). A frequently observed but ignored phenomenon following the IV injection is that although the RES uptake of nanoparticles is lower initially, it increases steadily with time. Another unique advantage of nanocapsules is their ability to overcome the blood-brain barrier—an insurmountable obstacle for a large number of drugs.[4] Compared with other colloidal carriers, polymeric nanocapsules present a higher stability when in contact with biological fluids, and their polymeric nature allows obtaining the desired controlled and sustained drug release.[5]

The different methods to prepare nanocapsules are Interfacial polymer deposition technique by Fessi *et al.*,[15] in-situ polymerization by Khoury Fallouh *et al*., modified W/O/W double emulsion technology by Ze Lu *et al*., and interfacial polycondensation in miniemulsions. Compared with liposomes (which are also nanovesicular drug delivery systems), nanocapsules are robust and stable in both liquid and dry forms. Polymeric nanocapsules show significant advantages over microcapsules. Some investigations show that nanocapsules containing insulin prepared by interfacial polymerization are able to cross the intestinal mucosa into the blood system by oral administration.[6]

DL-lactic and glycolic acid copolymers have been widely studied for this sort of application. These polymers are extremely interesting for sustained release, as they are completely biodegradable to nontoxic metabolites and well tolerated by tissues. Polylactic acid (PLA), polylactic-co-glycolic acid (PLGA), and polycaprolactone (PCL) is biocompatible, biodegradable, and bioresorbable.

In the present study, polymeric nanocapsules were prepared by using diltiazem as a model drug. Diltiazem is a calcium antagonist that has been shown to be effective in the treatment of chronic conditions, hypertension, and angina pectoris.[7] The recommended dosage interval is 6 to 8 hours with a maximum dose of 360 mg/day. It has been proved that sustained release of diltiazem is more beneficial to patients with stable angina with respect to reduced frequency of dosing, more uniform drug levels and improved activity, and reduced side effects.[8]

In view of all the above, the present study was taken up with an objective of preparing diltiazem-loaded nanocapsules using PLA, PLGA, and PCL by interfacial deposition technique and evaluate the same.

MATERIALS AND METHODS

Diltiazem HCl was obtained as gift sample from Micro Labs Ltd., Bangalore; PLA (mol.wt-2 50 000), PLGA (mol. wt-1 50 000), and PCL (mol.wt-44 000) were received as gift samples from Sigma Aldrich, Bangalore; Epikuron 100 H was a gift sample from Sun Pharmaceuticals Ltd, Baroda; Poloxamer 188 (F-68) was obtained as gift sample from Ranbaxy Pharmaceuticals, Ponta Saheb; benzyl benzoate, acetone AR grade, ethyl alcohol, and methanol were from S.D. Fine Chem. Ltd, Mumbai; and dichloromethane from Qualigen Fine Chemicals, Mumbai.

Conversion of diltiazem from HCl salt

The hydrophilic diltiazem HCl salt was converted to its insoluble base (diltiazem). IR spectrum of the diltiazem was taken to confirm the conversion of the salt to its base. Absence of HCl peak at 2400 per cm confirms the formation of the diltiazem base.

A saturated solution of diltiazem HCl in distilled water (50 ml) was prepared with continuous stirring, and a 5% of sodium hydroxide solution was poured slowly to the saturated solution of salt with continuous stirring till no more precipitate was formed. The precipitate was filtered through 1 micron Whatman filter paper. The residue was then collected and dried under normal room temperature. IR spectrum of the residue was taken to confirm the conversion of the salt to its base and is shown in Figure 1 for diltiazem HCl and Figure 2 for diltiazem base.

Preparation of nanocapsules

Nanocapsules were prepared by the interfacial deposition technique proposed by Fessi *et al*. The nanocapsules are essentially an oil-in-water emulsion type. Different ratios of the polymer shown in Table 1 were dissolved in 50 ml solution, containing equal quantity of acetone and ethyl alcohol at stirring speed of 4000 rpm for about 30 minutes that formed the organic phase. 100 mg of phospholipid mixture (Epikuron 100 H, it contains hydrogenated lecithin) was added to the

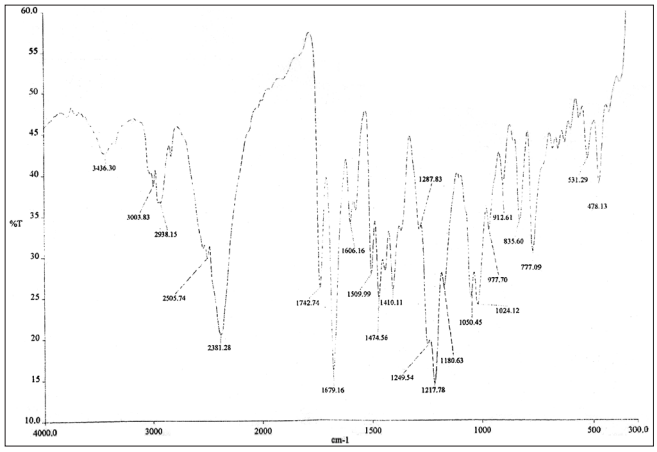


Figure 1: IR spectrum of diltiazem HCl

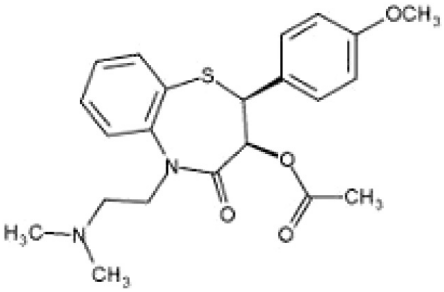
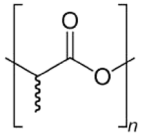
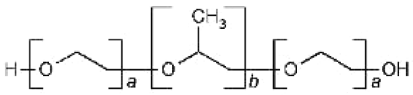
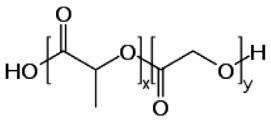
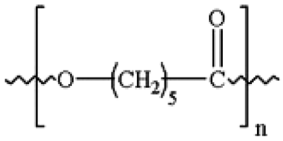
above organic phase maintaining the stirring speed for about an hour. 100 mg of the drug dissolved in 3 ml of benzyl benzoate was then added drop wise to the above organic mixture. The stirring was maintained for about an hour. This organic mixture was then progressively added drop wise (1 ml/min) through a syringe (0.22 mm) attached to a burette to distilled water (50 ml) containing poloxamer 188 (0.75%) as the stabilizer under constant stirring of about 4000 rpm. This was continued till a fine colloidal dispersion of nanocapsules was formed due to Tyndall effect. Organic solvent (acetone) was subsequently removed by heating under reduced pressure. The remaining colloidal mixture was then further concentrated to about 25 ml by centrifugation (4000 rpm). The supernatant obtained was analyzed for the amount of free drug. The preparation procedure is briefly shown in Figure 3, and the structures of each ingredient are shown in Table 1.

Different ratios of the drug and polymer were chosen as shown in Table 2. Biodegradable polymers such as PLA, PLC, and PLGA were chosen as the polymers to overcome the possibility of cross-reaction between the nanocapsules contents, particularly the drug molecules, which might otherwise limit the potential use of these nanocapsules.

Role of each ingredient used in the method

Polymers (PLA, PLGA, and PCL): Used for coating the encapsulated drug.[9]

Table 1: Structure of ingredients

Diltiazem	
Polylactic acid	
Poloxamer	
Polylactic-c-oglycolic acid	
Polycaprolactone	

Epikuron 100: A phospholipid mixture which consists of hydrogenated lecithin is used for encapsulating the drug.[9]

Poloxamer 188: A hydrophilic surfactant used as stabilizing agent.[9]

Benzyl benzoate: A solvent for dissolving the hydrophobic drug (oily core).[10]

Acetone – ethyl alcohol mixture: Solvents for dissolving drug and other ingredients.

Evaluation of nanocapsules

Encapsulation efficiency

Encapsulation sufficiency is a term used to represent the amount of drug encapsulated in the formulation. Drug content determination is a first step to calculate the encapsulation efficiency of a formulation. The method of choice for drug content determination is separation of free drug by centrifugation, followed by quantitative analysis of the drug from the pelleted formulation. Alternatively, the drug content can be determined in the supernatant. The amount of drug bound then can be calculated by subtraction of this amount from the total amount of drug present in the colloidal dispersion.

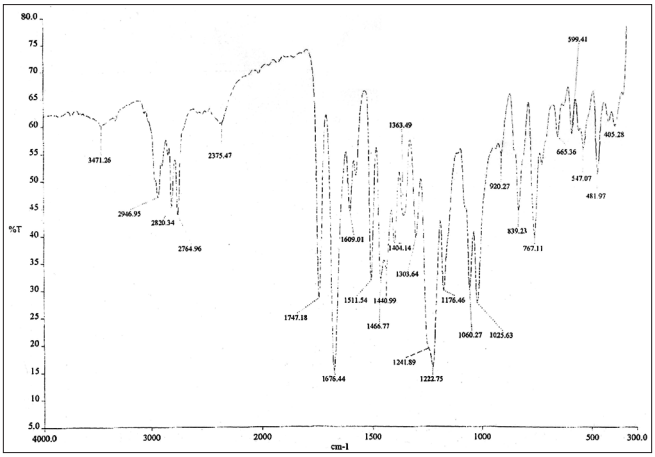


Figure 2: IR spectrum of diltiazem base

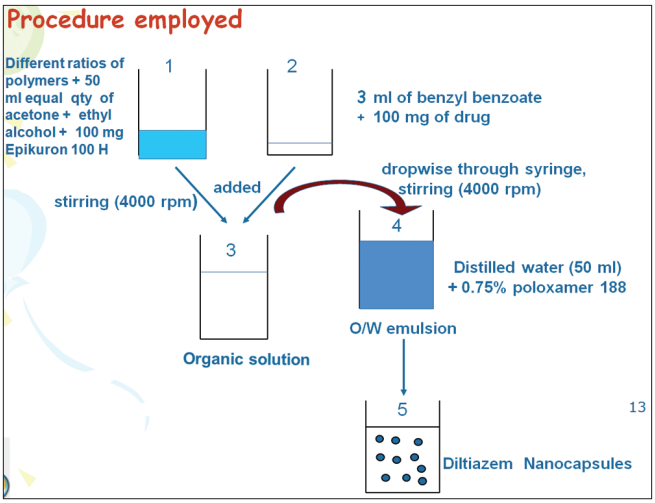


Figure 3: Preparation of nanocapsules

Percentage encapsulation efficiency may be calculated from the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of drug encapsulated in the formulation}}{\text{Total amount of drug in the formulation}} \times 100$$

Determination of drug content

Free drug of the formulations was first determined in the supernatant by choosing a solvent in which only the free drug gets dissolved and not the other ingredients.

Ten millilitre of the supernatant was made up to 100 ml with 0.1 N HCl and filtered through Whatman filter (size 44); 1 ml of the filtrate was taken then made up to volume with 10 ml of 0.1 N HCl and analyzed for free drug present.

Determination of encapsulated drug

Ten millilitre of the concentrate obtained after the centrifugation of colloidal dispersion was taken; 5 ml of ethanol was added to it in case of PCL nanocapsules, and 5 ml combination of ethanol and acetone (1: 1) was added in case of other formulations to completely dissolve the drug, polymer, and the other ingredients. It was then filtered through Whatman filter paper (size 44) and the amount of the drug in the filtrate was estimated after suitable dilution by ultraviolet (UV) spectrophotometer.

Selection of best formulations

The selection of best formulations was made on the basis of encapsulation efficiency. For PCL and PLGA, the drug: polymer ratio being 1: 2 was utilized as the constant, and effect of phospholipid mixture concentration was seen as the second process variable.

Formulation of nanocapsules with changing concentration of phospholipid

Different formulations of nanocapsules were prepared taking the ratios of drug: polymer as 1: 2 for PCL and PLGA and 1: 1 for PLA. The concentration of phospholipid mixture was used as shown in Table 3.

Selection of best formulations

On the basis of the best encapsulation efficiency, five formulations shown in Table 4 were selected for further characterization and *in vitro* studies.

Characterization of nanocapsules

Drug polymer interaction studies

The nanocapsules prepared were to be tested for intactness of the drug with polymer. This is to establish that the therapeutically active drug has not undergone any change after it has been subjected to processing steps during formulation. This can be confirmed by carrying out infrared light absorption and scanning spectroscopy studies. Diltiazem exhibits principle peaks at wave numbers of 2400 cm^{-1}, 1750 cm^{-1}, 1500 cm^{-1}, 1225 cm^{-1}, 1028 cm^{-1}.[11]

In the present study, potassium bromide pellet method was employed. The samples were thoroughly mixed with dry, powdered potassium bromide. The mixture was compressed

Table 2: Formulations of nanocapsules with changing concentration of polymer

Formulation code	Drug: Polymer ratio	Amount of epikuron 100H	Amount of benzyl benzoate	Speed of rotation
F1 (Drug: PCL)	1:0.5	100 mg	2 ml	4000 rpm
	1:1	100 mg	2 ml	4000 rpm
	1:2	100 mg	2 ml	4000 rpm
F2 (Drug: PLGA)	1:0.5	100 mg	2 ml	4000 rpm
	1:1	100 mg	2 ml	4000 rpm
	1:2	100 mg	2 ml	4000 rpm
F3 (Drug: PLA)	1:0.5	100 mg	2 ml	4000 rpm
	1:1	100 mg	2 ml	4000 rpm
	1:2	100 mg	2 ml	4000 rpm

Table 3: Formulations of nanocapsules with changing concentration of phospholipid

Formulation code	Drug: Polymer ratio	Amount of epikuron 100H	Amount of benzyl benzoate	Speed of rotation
F1 (Drug: PCL)	1:2	50 mg	2 ml	4000 rpm
		75 mg	2 ml	4000 rpm
		100 mg	2 ml	4000 rpm
F2 (Drug: PLGA)	1:2	50 mg	2 ml	4000 rpm
		75 mg	2 ml	4000 rpm
		100 mg	2 ml	4000 rpm
F3 (Drug: PLA)	1:1	50 mg	2 ml	4000 rpm
		75 mg	2 ml	4000 rpm
		100 mg	2 ml	4000 rpm

to form a disc using dies. The disc was placed in the spectrophotometer and the spectrum was recorded.

Scanning electron microscopy

The scanning electron microscope (SEM) used for the studies was model JEOL JSM-840. Cleaned brass specimen studs were used for mounting the samples. Wet solvent paint was applied on these brass specimen studs and while the paint was wet, the samples were placed on each stud and allowed to dry.

Particle size analysis and distribution

Any dispersion of particles is usually poly-dispersed in nature containing different particle size. It is necessary to know not only the size of a certain particle, but also how many particles of the same size exist in the sample. Thus, we need an estimation of the size range present and the number of weight of fraction of each particle size. This is called the particle size distribution and from this we may calculate an average particle size for the sample.

In vitro drug release studies

In the present study, the *in vitro* release studies of the formulations were carried out in pH 7.4 phosphate buffer at 37°C and the cumulative percent release of drug at different time intervals was determined.[12-14] Quantity of nanocapsules equivalent to 25 mg of the drug was taken in the dialysis bag.

Table 4: Selected best formulations on the basis of encapsulation efficiency

Formulation code	Drug: Polymer ratio	Amount of epikuron 100H	Encapsulation efficiency (%)
N1 (Drug: PCL)	1:2	125 mg	64
N2 (Drug: PLGA)	1:2	125 mg	58
N3 (Drug: PCL)	1:2	100 mg	57
N4 (Drug: PLA)	1:1	125 mg	56
N5 (Drug: PLGA)	1:2	100 mg	54

The dialysis bag was then suspended in a flask containing 100 ml of pH 7.4 phosphate buffer on a magnetic stirrer at 37°C. Required quantity (10 ml) of the medium was withdrawn at specific time periods (1, 2, 4, 6, 8, 10, 12, 24, 32, 48 hours) and the same volume of dissolution medium was replaced to the flask to maintain a constant volume. The withdrawn samples were filtered and then 5 ml filtrate was taken in a 100 ml volumetric flask and made up to volume with 0.1 N HCl and estimated by UV spectrophotometer at 235 nm for the drug content.

Stability studies

The use of exaggerated conditions of temperature, humidity, light, and others to test the stability of drug formulation is termed as accelerated stability testing. Accelerated temperature stability studies are generally conducted at 37, 50, and 60°C, as well as at room temperature and freezing temperatures. Short-term stability studies were carried out for the formulations for a period of 2 months.

The nanocapsule formulations were stored at 4 and 25°C for a period of 60 days. At weekly intervals, 5 ml of sample was withdrawn and analyzed for the drug content. They were also observed for physical changes during the period of storage.

RESULTS

Encapsulation efficiency of nanocapsules with varying concentration of polymers

Encapsulation efficiency

The free drug estimated in the supernatant is as shown in Table 5. The percentage free drug in F1 is in the range of 21 to 26%, for F2 it is in the range of 24 to 26%, and for F3 the range is 25 to 31%.

Estimation of encapsulated drug

The encapsulation of diltiazem in terms of percent total drug added during preparation for formulations with varying polymer concentration are shown in Table 6. Percentage encapsulation of the drug in F1 was found to be in the range of 50 to 58%, for F2 the encapsulation in the range of 42 to 54%, and for F3 the range is 42 to 48%.

Table 5: Estimation of free drug for formulations with varying concentration of polymers

Formulation code	Drug:Polymer ratio	Absorbance*	Concentration* µg/ml	Free drug concentration mg/100 ml	% of free drug*
F1 (Drug: PCL)	1:0.5	0.106	2.1	21	21
	1:1	0.108	2.2	22	22
	1:2	0.126	2.6	26	26
F2 (Drug: PLGA)	1: 0.5	0.123	2.4	24	24
	1:1	0.127	2.6	26	26
	1:2	0.127	2.6	26	26
F3 (Drug: PLA)	1: 0.5	0.149	3.1	31	31
	1:1	0.105	2.1	21	21
	1:2	0.125	2.5	25	25

*Average of three determinations

Encapsulation efficiency of nanocapsules with changing phospholipid concentration

Estimation of free drug

The free drug estimated in the supernatant is shown in Table 7. The percentage free drug in F4 is in range 11 to 20%, for F5 are in the range 12 to 29%, and for F6 are in the range 21 to 28%.

Estimation of encapsulated drug

The encapsulation of diltiazem in terms of percent total drug added during preparation for formulations with varying drug: polymer ratios are shown in Table 8. Percentage encapsulation of the drug in F4 was found in the range between 50 to 64%, for F5 the encapsulation was found in the range of 38 to 58%, and for F6 was found in the range 38 to 56%.

Drug polymer interaction studies

Identification of diltiazem sample was carried out by infrared spectrophotometer (FTIR 8700) and was compared with that of reference standard.

The IR spectrums of diltiazem sample were chosen for five best formulations based on encapsulation efficiency and are shown in Figures 4 to 8. Principal peaks of diltiazem are intact in the formulations.

Scanning electron microscopy

The photographs of the diltiazem nanocapsules prepared were acquired by scanning electron microscopy to compare the morphology of different formulations. The photographs are shown in Figures 9 to 13.

Table 6: Estimation of encapsulated drug for formulations with varying concentration of polymers

Formulation code	Drug: Polymer ratio	Absorbance*	Concentration* µg/ml	Free drug concentration mg/100 ml	% of free drug*
F1 (Drug: PCL)	1:0.5	0.118	2.50	50.0	50.0
	1:1	0.125	2.60	52.0	52.0
	1:2	0.149	2.90	58.0	58.0
F2 (Drug: PLGA)	1: 0.5	0.101	2.10	42.0	42.0
	1:1	0.113	2.10	48.0	48.0
	1:2	0.134	2.70	54.0	54.0
F3 (Drug: PLA)	1: 0.5	0.100	1.80	36.0	36.0
	1:1	0.115	2.40	48.0	48.0
	1:2	0.111	2.10	42.0	42.0

*Average of three determinations

Table 7: Estimation of free drug for formulations with changing phospholipid concentration

Formulation code	Drug: Polymer ratio	Amt. of phospholipid	Absorbance*	Concentration* µg/ml	Free drug concentration mg/100 ml	% of free drug*
F4 (Drug: PCL)	1:2	75 mg	0.103	2.0	20	20
		100 mg	0.071	1.6	16	16
		125 mg	0.051	1.1	11	11
F5 (Drug: PLGA)	1:2	75 mg	0.142	2.9	29	29
		100 mg	0.076	1.7	17	17
		125 mg	0.053	1.2	12	12
F6 (Drug: PLA)	1:1	75 mg	0.140	2.8	28	28
		100 mg	0.105	2.1	21	21
		125 mg	0.106	2.1	21	21

*Average of three determinations

Table 8: Estimation of encapsulated drug for formulations with changing phospholipid concentration

Formulation code	Drug: Polymer ratio	Amt. of phospholipid	Absorbance*	Concentration* µg/ml	Free drug concentration mg/100 ml	% of encapsulated drug*
F4 (Drug: PCL)	1:2	75 mg	0.124	2.50	50.0	50.0
		100 mg	0145	2.85	57.0	57.0
		125 mg	0.155	3.20	64.0	64.0
F5 (Drug: PLGA)	1:2	75 mg	0.093	1.90	38.0	38.0
		100 mg	0.138	2.70	54.0	54.0
		125 mg	0.147	2.90	58.0	28.0
F6 (Drug: PLA)	1:1	75 mg	0.091	1.90	38.0	38.0
		100 mg	0.115	2.40	48.0	48.0
		125 mg	0.142	2.80	56.0	56.0

*Average of three determinations

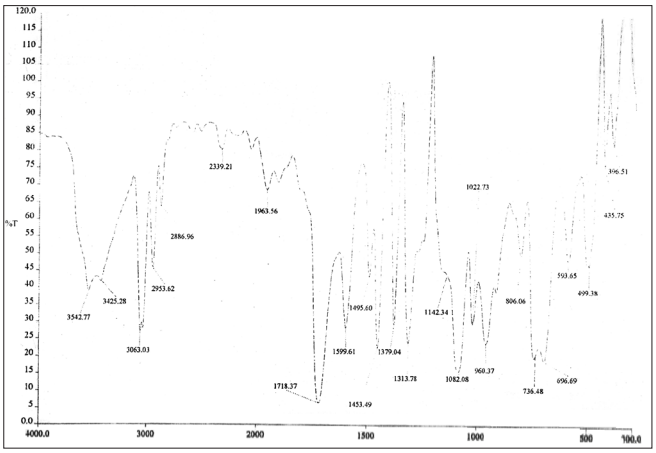


Figure 4: IR spectrum of formulation N1

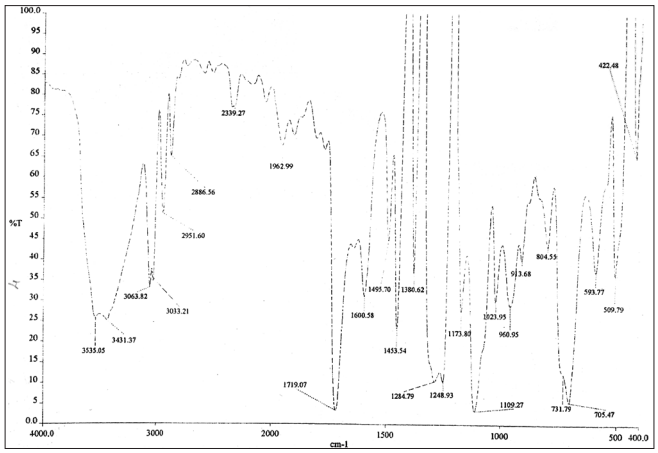


Figure 5: IR spectrum of formulation N2

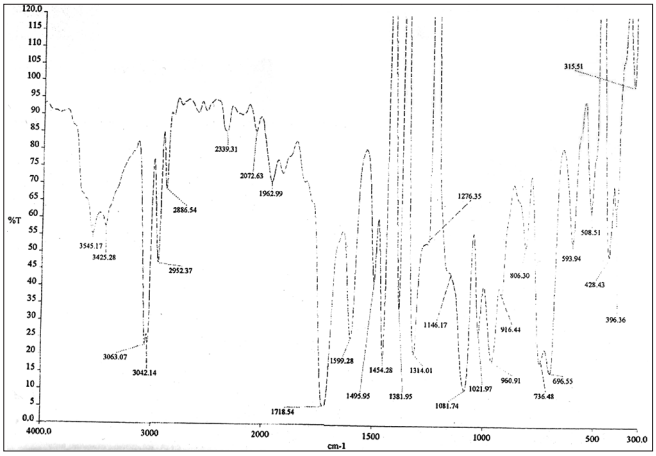


Figure 6: IR spectrum of formulation N3

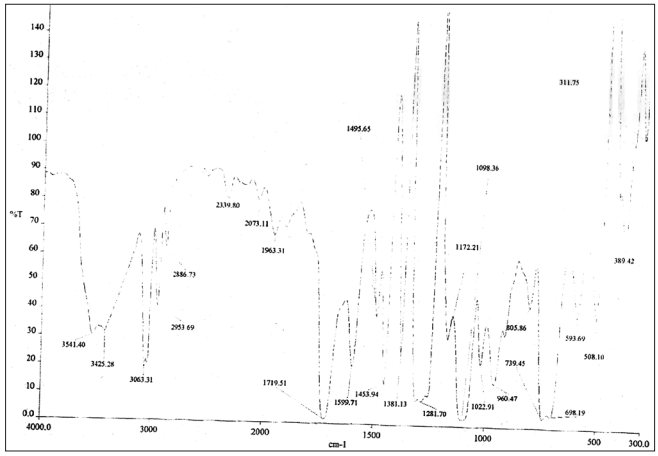


Figure 7: IR spectrum of formulation N4

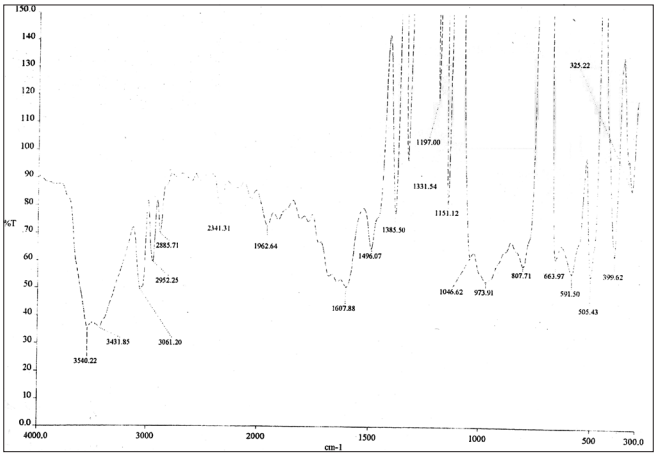


Figure 8: IR spectrum of formulation N5

Particle size analysis

The particle size analysis was carried out using Malvern mastersizer. The distributions of particles were as per the 'normal distribution.' The d (0.9) at obscuration of 3 to 5% was found to be in the range of 200 to 600 nm for all the five formulations. N1 showed the smallest particle size with d (0.9) of 0.228 mm, indicating that 90% of particles have a size of 228 nm. The particle size analysis of five best formulations based on encapsulation efficiency was done using Malvern mastersizer and is shown in Figures 14 to 18. The range of the particles was observed between 228 to 531 nm. The average particle size of the formulations is summarized in Table 9.

***In vitro** release studies*

In vitro release studies of the formulation were carried out in pH 7.4 medium for a period of 48 hours. Figure 19 and Table 10 shows the release pattern of diltiazem from the formulations N1-N5. The formulation N1 with Epikuron 100 H concentration of 100 mg showed 80.00% release in 12 hours, 93.28% release in 24 hours, and 96.88% release in 32 hours. The formulation N2 with Epikuron 100 H concentration of 100 mg showed 26.98% release in 12 hours, 32.40% release in 24 hours, 37.20% release in 32 hours, and 43.20% in 48 hours. The formulation N3 with Epikuron 100 H concentration of 100 mg showed 79.36% release in 12 hours, 92.40% release in 24 hours, and 98.16% release in 32 hours. The formulation N4 with Epikuron 100 H concentration of 125 mg showed 20.72% release in 12 hours, 26.66% release in 24 hours, 30% release in 32 hours, and 35.33% release in 48 hours. The formulation N5 with Epikuron 100 H concentration of 100 mg showed 26.40% release in 12 hours, 30.80% release in 24 hours, and 38.00% release in 32 hours.

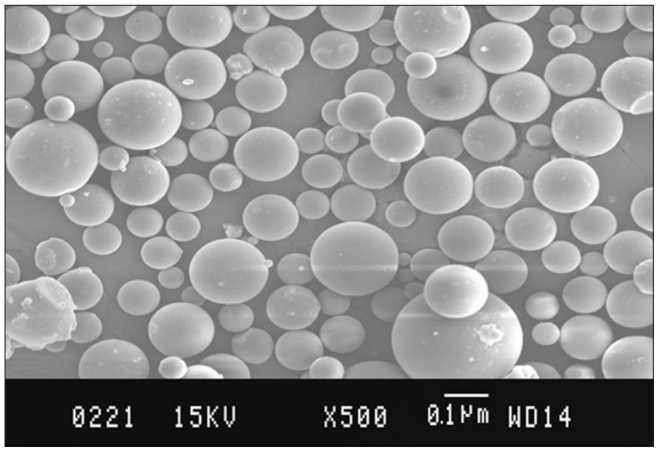


Figure 9: SEM photograph of formulation N1

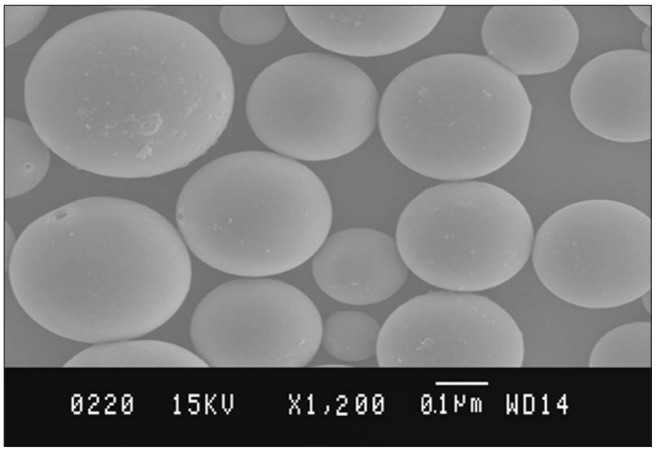


Figure 10: SEM photograph of formulation N2

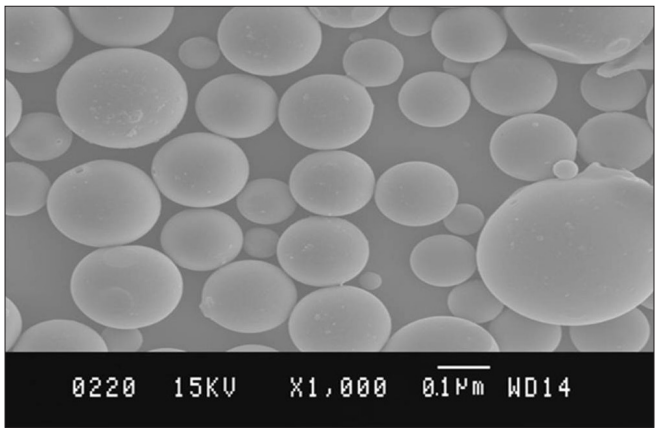


Figure 11: SEM photograph of formulation N3

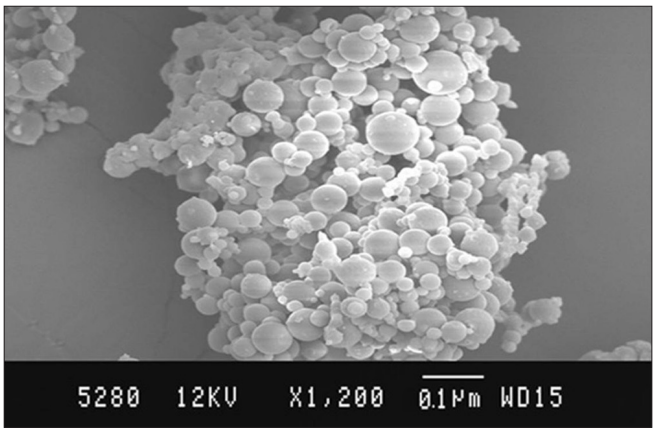


Figure 12: SEM photograph of formulation N4

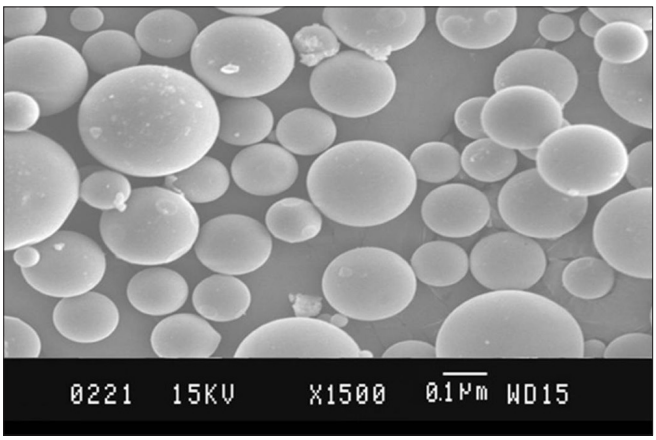


Figure 13: SEM photograph of formulation N5

Stability studies

Short-term stability studies

Short-term stability studies were carried out for a period of 2 months at refrigerated temperature (4°C) and at 25°C. The selected five best formulations were chosen on the basis of best encapsulation efficiency. The drug content was estimated at weekly intervals and is reported in Tables 11 and 12.

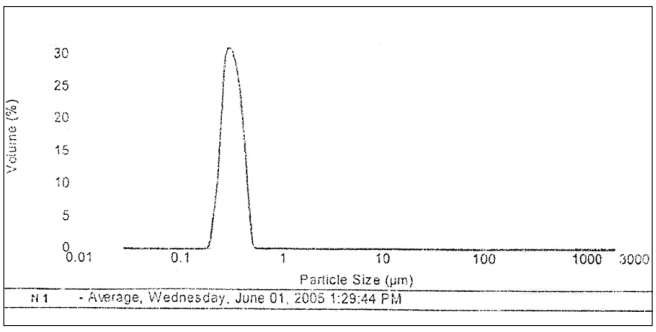


Figure 14: Particle size analysis of formulation N1

Table 9: Average particle size

Formulation code	Average particle size (nm)
N1	228
N2	562
N3	389
N4	311
N5	531

DISCUSSION

In the present study, nanocapsules were prepared by interfacial deposition technique proposed by Fessi *et al*.

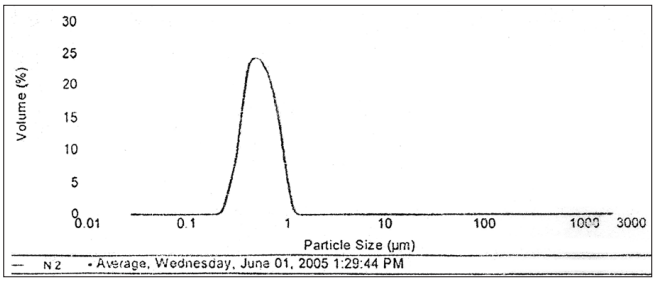


Figure 15: Particle size analysis of formulation N2

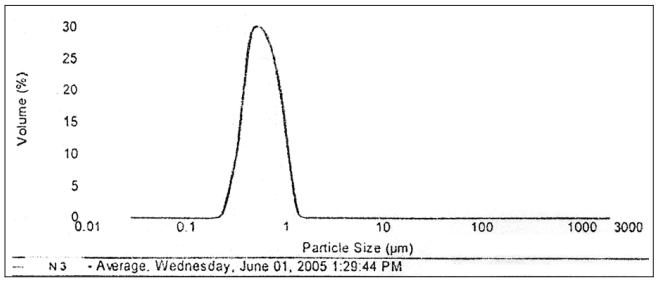


Figure 16: Particle size analysis of formulation N3

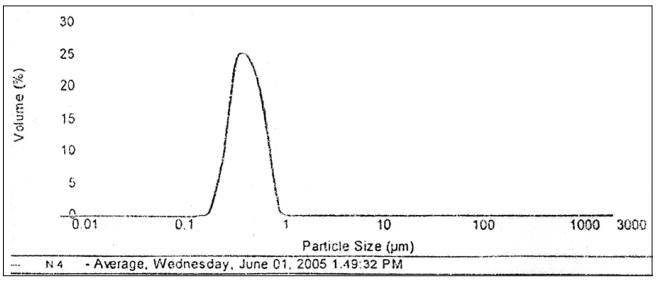


Figure 17: Particle size analysis of formulation N4

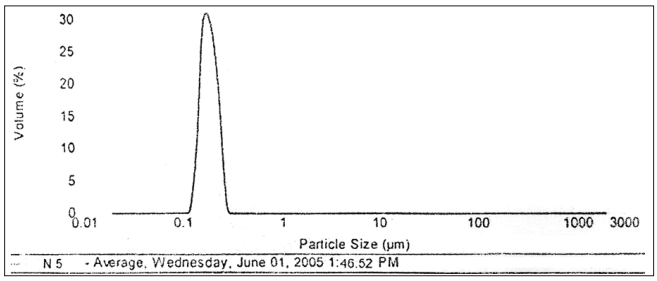


Figure 18: Particle size analysis of formulation N5

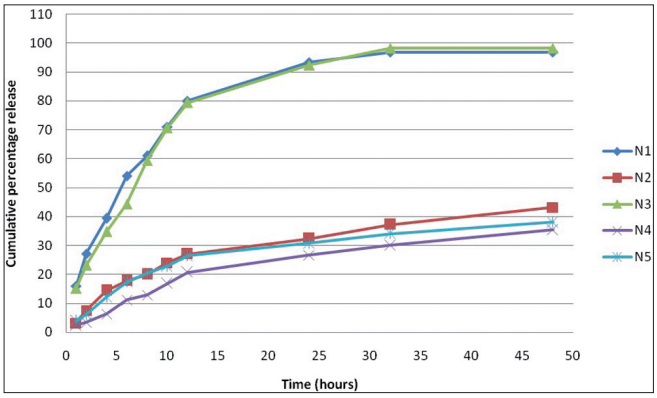


Figure 19: Comparative dissolution profiles of formulations N1-N5

Table 10: *In-vitro* release study of formulations N1-N5

Time (hrs)	Cumulative percentage release				
	N1	N2	N3	N4	N5
1	16.0	3.20	15.21	2.40	4.00
2	27.2	7.52	23.12	3.36	6.00
4	39.36	14.40	34.82	6.32	12.16
6	54.08	17.76	44.40	11.20	17.28
8	60.96	20.00	59.37	12.80	20.40
10	71.04	24.00	70.64	16.80	22.80
12	80.00	26.98	79.36	20.72	26.40
24	93.28	32.40	92.40	26.66	30.80
32	96.88	37.20	98.16	30.00	34.00
48	-	43.20	-	35.33	38.00

Among the various methods reported for the preparation of nanocapsules, this method was chosen as it is a simple and novel method which is feasible in industry. As this method is applicable to only hydrophobic molecules, diltiazem hydrochloride, a hydrophilic drug was converted to diltiazem, the base. The conversion of the salt to the base was confirmed from the IR spectra which showed the absence of prominent HCl peak at 2400 per cm. The method of estimation of diltiazem by UV spectrophotometer at 235 nm was standardized, and the drug was found to obey Beer Lamberts law in the concentration range of 2 to 12 µg/ml. Different polymers namely PCL, PLGA, and PLA were chosen as they are biodegradable, biocompatible, and successfully used in the preparation of nanocapsules.

Initially, three different ratios of the drug: polymer (1: 0.5, 1: 1, 1: 2) were taken and the phospholipids concentration was fixed as 100 mg. The concentration of the free drug in the formulations was estimated from the supernatant obtained after centrifugation of the colloidal dispersion. The concentration of free drug in all the formulation ranged from 21 to 30%. The amount of drug encapsulated in the nanocapsule gives an estimate of encapsulation efficiency which is an important parameter in the selection of nanocapsules for further characterization; it was determined by taking the concentrate obtained after centrifugation, adding suitable solvent, and estimating the drug content. The encapsulation efficiency in all the formulations ranged from 42 to 58%. It was observed that the encapsulation efficiency increased as the polymer concentration was increased. Thus, the greater the amount of polymer available, greater will be its ability to encapsulate. Maximum encapsulation of 58% was observed in F1 (1: 2) with PCL as the polymer followed by 54% as in case of F2 (1: 2) with PLGA as the polymer and 48% for F3 (1: 1) with PLA as the polymer. It was observed that as the molecular weight of the polymer increased, the encapsulation efficiency reduced. Among the different formulations, one best of each formulation was selected on the basis of encapsulation efficiency (F1 [1: 2], F2 [1: 2], F3 [1: 1]) and the concentration of the phospholipids was further

Table 11: Stability studies at refrigerated conditions (4°C)

Formulation code	Temperature	Physical stability					Drug content (%)				
		No. of weeks					No. of weeks				
		0	2	4	6	8	0	2	4	6	8
N1	4°C	No change in physical appearance					100	98.2	89.1	81.3	77.2
N2	4°C	No change in physical appearance					100	98.5	89.2	81.7	74.2
N3	4°C	No change in physical appearance					100	98.1	89.7	81.5	76.5
N4	4°C	No change in physical appearance					100	98.5	89.5	81.3	76.3
N5	4°C	No change in physical appearance					100	98.8	89.3	81.7	74.7

Table 12: Stability study at 25°C

Formulation code	Temperature	Physical stability					Drug content (%)				
		No. of weeks					No. of weeks				
		0	2	4	6	8	0	2	4	6	8
N1	25°C	No change in physical appearance		Phase separation seen			100	62.3	33.2	26.1	22.1
N2	25°C	No change in physical appearance		Phase separation seen			100	67.8	38.3	33.2	26.8
N3	25°C	No change in physical appearance		Phase separation seen			100	55.2	37.6	24.3	21.3
N4	25°C	No change in physical appearance		Phase separation seen			100	54.3	46.9	34.8	28.9
N5	25°C	No change in physical appearance		Phase separation seen			100	61.7	42.1	28.4	19.7

changed (75 mg, 100 mg) to see the effect on encapsulation. It was found that in case of all the three selected formulations, there was an increase in the encapsulation efficiency by 2 to 6% when the phospholipids concentration was increased from 100 to 125 mg. The encapsulation efficiency was seen to reduce by a marginal amount when the phospholipid concentration was reduced to 75 mg. Five formulations with highest encapsulation efficiency were selected for further characterization. The encapsulation efficiency was in the order of N1 >N2 >N3 >N4 >N5.

The intactness of the drug in the formulation was confirmed by the absence of any extra peaks and the intactness of the prominent peaks of the pure drug as studied by infrared spectroscopy. The surface morphological studies as determined by SEM revealed that the nanocapsules obtained have a smooth surface and were spherical in nature in all the formulations. The particles were discrete in all the formulations except N3, where they were aggregated. The particle sizes in all the formulations were in the nano range from 20 to 380 nm as seen by the SEM photographs. The particle size analysis was carried out using Malvern mastersizer. The distributions of particles were as per the 'normal distribution.' The d (0.9) at obscuration of 3 to 5% was found to be in the range of 200 to 600 nm for all the five formulations. N1 showed the smallest particle size with d (0.9) of 0.228 μm, indicating that 90% of particles have a size of 228 nm. Hence, the method adapted and the polymer and phospholipid concentration employed in the preparation were suitable to get formulation in nano range.

*In vitr*o release studies of the formulation were carried out in pH 7.4 medium for a period of 48 hours. It was observed that in all the five formulations, the release was prolonged. The drug release was slowest in case of PLA with a release of 35.33% and in case of PLGA, it was 38 to 43.20%. Because our aim was to prolong the release for 24 to 48 hours and not beyond, PCL was considered as the best polymer for the purpose of controlled release study in the form of nanocapsules with a release range of 95 to 98%.

Short-term stability studies carried out at refrigerated products indicated that the formulations did not show any physical changes or change at 4°C. However, there was phase separation and decrease in the drug content at the end of 4 weeks when stored at 25°C at 60% RH. Hence, it is recommended that the formulation be refrigerated.

CONCLUSION

Nanocapsules of diltiazem could be successfully prepared by interfacial deposition technique as proposed by Fessi *et al*.,[15] using biodegradable and biocompatible polymers such as PCL, PLGA, and PLA. The encapsulation efficiency in all the formulations was in the range of 42 to 64%. PCL nanocapsules showed the best encapsulation of 64% in the drug: polymer ratio of 1: 2 and phospholipid concentration of 125 mg. As seen by the SEM, the nanocapsules are spherical having smooth surface with size range of 20 to 380 nm. The particles exhibited normal size distribution, with 91% of the particles lying in the range of 200 to 600 nm. All the formulations showed prolonged release with PLA and PLGA, exhibiting only about 35 to 45% release at the end of 48 hours, while PCL was found to be the best polymer for controlled release of diltiazem from nanocapsules. The formulations were stable at 4°C and exhibited slight physical changes and reduction in the drug content at 25°C. Therefore, it is recommended to store the formulations at 4°C.

Therefore, it can be concluded that the formulation of nanocapsules is a useful technology for the controlled release of drugs and can be applied for other drug molecules which require controlled release.

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