WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 6, 764-777 Research Article ISSN 2278 – 4357

EVALUATION OF EFFECT OF ORGANIC SOLVENT ON CLARITHROMYCIN LOADED PLGA NANOPARTICLES

Ashvini H. M.*, Shivanand K. Mutta and Anudeep Balla

Dept. of Pharmaceutics, Acharya and B M Reddy College of Pharmacy, Bangalore.

Article Received on
26 March 2018,

Revised on 16 April 2018,
Accepted on 06 May 2018,

DOI: 10.20959/wjpps20186-11680

***Corresponding Author**
Ashvini H. M.
Dept. of Pharmaceutics,
Acharya and B M Reddy
College of Pharmacy,
Bangalore.

ABSTRACT

Clarithromycin loaded Poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles were prepared by an emulsification-solvent evaporation method. PLGA was employed as a GRAS (Generally Regarded As Safe) polymer. Four different solvent system was used to determine the effect of organic phase solvent on the mean particle sizes of obtained PLGA nanoparticles, different organic solvents like acetone, acetonitrile, ethyl acetate and combination of acetone and ethanol were used with poly(vinyl alcohol) as stabilizers. Prepared nanoparticles characterised for physicochemical parameters in terms of particles' size, poly dispersity index (PDI), entrapment efficiency, release profile, thermal behaviour, and morphology. The particle size analysis results revealed that the smallest particle size of 333.5 nm was obtained with acetone as organic solvent, while large PLGA nanoparticles 875 nm was obtained for mixture of acetone and ethanol as organic phase. Thermal analysis demonstrated the entrapment of drug into the PLGA. Change in organic solvent phase also affected percentage encapsulation efficiency, highest encapsulation was with acetone i.e. 80% and *in vitro* releases profile consist of initial burst release followed by a slower release.

KEYWORDS: Clarithromycin, PLGA nanoparticles, emulsification solvent evaporation, Particle, organic solvents.

INTRODUCTION

Antibiotic therapy is the basis of pneumonia treatment.[1] *Streptococcus pneumoniae* stays to be a significant respiratory pathogen, and growing antimicrobial resistance negotiations the use of β-lactam and macrolide antibiotics in drug delivery.[2] System, nanotechnology is

emerging as boon for treatment of infectious diseases. Antibiotics entrapped in biodegradable polymeric nanoparticles have shown great possiblilty in replacing the intake of antibiotics in its original form. Tissue and cellular barriers can be overawed by polymeric nanoparticles and chance of delivering antibiotics into very dense tissues and unreachable target cells. In case of nanoparticles, due to the smaller particle size possessing shorter diffusional distance most of the active ingredient is released in the tissue.[3]

Clarithromycin (CLM) is a broad spectrum antibiotic[4], active *in vitro* and effective *in vivo* against the major pathogens responsible for respiratory tract infectious[5] bacteria such as *Pseudomonas aeruginosa, Chlamydophila pneumoniae, Klebsiella pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae* and *Haemophilus influenza*.

CLM is practically insoluble in water and its poor solubility is pH dependent.[6] Acid stability of CLM makes it suitable it for oral administration. Despite the well absorption of CLM from gastrointestinal tract, systemic bioavailability of drug is relatively poor (55%) and it is related to the rapid hepatic first pass metabolism. On the other hand, volume of distribution of CLM is relatively high and it rapidly diffuses from plasma to other tissues.[7] Consequently, the concentration of drug in the tissues will be higher than plasma. Clarithromycin exerts its antibacterial action by binding to the 50s ribosomal subunit of susceptible organisms and by inhibiting protein synthesis.[8]

Clarithromycin is known to exhibit anti-inflammatory properties, this could be a direct inhibition of a chloride channel. All above results could bring some benefits since bacterial colonisation is accompanied by inflammation in the respiratory tract infection.[9]

Among FDA approved polymer, Poly (D,L-lactic-co-glycolic acid) (PLGA) is an aliphatic polyester approved for human use.[10] It was employed in research and in clinic as a carrier for a wide range of compounds.[11] PLGA has created incredible interest due to its unique biocompatibility, biodegradability, mechanical strength, swelling behaviour, capacity to undergo hydrolysis, the biodegradation rate.[12] PLGA nanoparticles have been employed for the therapeutic delivery of a wide range of bioactives.[13] Many tactics are offered for the preparation of PLGA nanoparticles like emulsification-evaporation method, spontaneous emulsification- solvent diffusion method and nanoprecepitation method are all widely used for preparing various diameters of PLGA nanoparticles.[14]

Because of the scale similarities between nanoparticles and cell/tissue system, nanoparticles are able to cross biological membranes. By this nanoparticles setback access to cells, tissues and organs. These nanoparticles, have the ability to improve the pharmacokinetics and biodistribution of therapeutic agent to target tissues.

Nanoparticles as carrier vehicles for antimicrobial agents recommends a new and promising approach in the designing effective therapy counter to pathogenic bacteria.[15] Polymeric nanoparticles own several exclusive characteristics for antimicrobial drug delivery. This is due to structural stability with a monomodel size distribution, tuneable properties size, zeta potentials, and drug release profiles by selecting different polymer, surfactants, and organic solvents during the synthesis.

1. MATERIALS AND METHODS

2.1.Materials

Clarithromycin (CLM) powder was obtained from calyx pharmaceuticals. Poly (lactic-co-glycolic acid) was purchased from Lactel – Durect Corporation, USA. PVA (Cold), HPLC grade water, acetonitrile and methanol were purchase from SD Fine. Potassium dihydrogen phosphate from Finar reagents and o-phosphoric acid from Merck chemicals. All other chemicals used were of analytical grade and HPLC grade.

Thermal analysis

Thermogram of clarithromycin, nanoparticle, physical mixture of drug with polymer and nanoparticles were carried out using DSC-Shimadzu 60 with TDA trend line software to analyse CLM nanostructure. The analysis was performed at a rate of 10.0° C min^{-1} from 10° C to 300° C.

Fourier-transform infrared spectroscopy (FTIR)

FTIR of intact clarithromycin, nanoparticles and physical mixture were obtained from FT-IR spectrophotometer, model TENSOR 27, BRUKER. Spectra are obtained by ATR technique. The scanning range was 4000- 400 cm^{-1}.

Preparation of PLGA nanoparticles

The PLGA nanoparticles, loaded with clarithromycin, were prepared by an emulsion–solvent evaporation method.[16] Calculated amount of CLM and PLGA were dissolved in organic solvent as internal phase at room temperature. The organic phase was added drop wise to an

aqueous phase, containing PVA as stabilizing agent. As water solubility of CLM decreases in higher pH, in order to increase drug loading, the aqueous phase pH was adjusted at 8. Homogenisation was carried out under 15,000 RPM homogenization for 30 min in an ice water bath. The emulsion formed was magnetically stirred to evaporate organic solvent. The nanoparticles were recovered by ultracentrifugation (12,000 RPM using REMI Cold centrifuge at 4°C for 30 min) and the precipitated nanoparticles were washed twice by deionized water. Effect of organic solvent on formulation parameters on PLGA nanoparticles were investigated. The purified nanoparticles were freeze-dried.

Determination of Clarithromycin entrapment efficiency[17]

CLM content in nanoparticles were determined by a HPLC method[18-21] after appropriate dilution with HPLC grade Acetonitrile. 20 µl was injected into a HPLC system. (Shimadzu LC-20AT) equipped with a UV detector (at 205 nm) and Phenomenex Kinetex 5µ C18 (250 mm x 4.6 mm i.d., 5 µm particle size) column. The flow rate was1.0 ml/min, the detector wavelength was set at 205 nm. Three samples from each formulation were injected into HPLC column with appropriate dilution.

Entrapment efficiency (%EE) in the clarithromycin loaded PLGA nanoparticles were calculated according to the equations below.

$$EE\% = \frac{\text{Total amount of CLM loading - free CLM in the supernatant}}{\text{Total amount of CLM loading}} \text{ X } 100$$

Characterisation of nanoparticle- size, surface morphology and zeta potential

Mean particle size (Z average), particle size distribution (PDI) and zeta potential were measured using a Malvern Nano ZS 90 (Malvern Instruments, UK).Particle sizes were measured in aqueous medium at room temperature without any further dilution.[22] All measurement was taken in triplicates. The morphology of nanoparticles was investigated by scanning electron microscopy (EM-LEO435VP, Carl zeiss SMT Inc., NY).

***In vitro* dissolution studies**

Dissolution experiments were carried out on marketed suspension of CLM and prepared CLM PLGA nanoparticles using conventional dialysis technique. Briefly, suspension equivalent to 10 mg CLM was placed in dialysis bag and dialysed against 100 mL of phosphate buffer (pH 7.4) at 50 rpm and at 37±1°C, and the sink conditions were maintained throughout the course of study. Samples were withdrawn at defined intervals and filtered

through 0.45 µm filters. The amount of dissolved CLM was determined using HPLC (Shimadzu LC-20AT), according to the method described for encapsulation efficiency. The analysis was performed in triplicates.[23,24]

Stability studies

The stability of different formulations was evaluated after storing the formulations at room temperature and at 4° C in a freeze for a period of 3 months. Samples were collected at every 1 month interval and analysed for CLM nanosuspension particle size and polydispersity index (PDI) as the key parameters in evaluation of physical stability. Any changes in the encapsulation and in vitro release were assessed.[25, 26, 27]

RESULTS AND DISCUSSION

3.1. Fourier-transform infrared spectroscopy (FTIR)

The FITR spectra of nanoparticle, drug and physical mixture drug and polymer are shown in figure 1 and 2. CLM show characteristic peaks of OCH_3 Stretching at 2880 cm^{-1}, CH_2 Stretching at 2827 cm^{-1}, C-CH_3 Stretching at 2943 cm^{-1}, C=O Stretching at 1727 cm^{-1}, C-O Bending at 1366 cm^{-1}, C-N Bending at 1171 cm^{-1}. The spectra of both physical mixture and nanoparticles did not show any prominent changes in the peak position from clarithromycin spectra. The results revealed that there is absence of any chemical interaction between drug and chitosan.

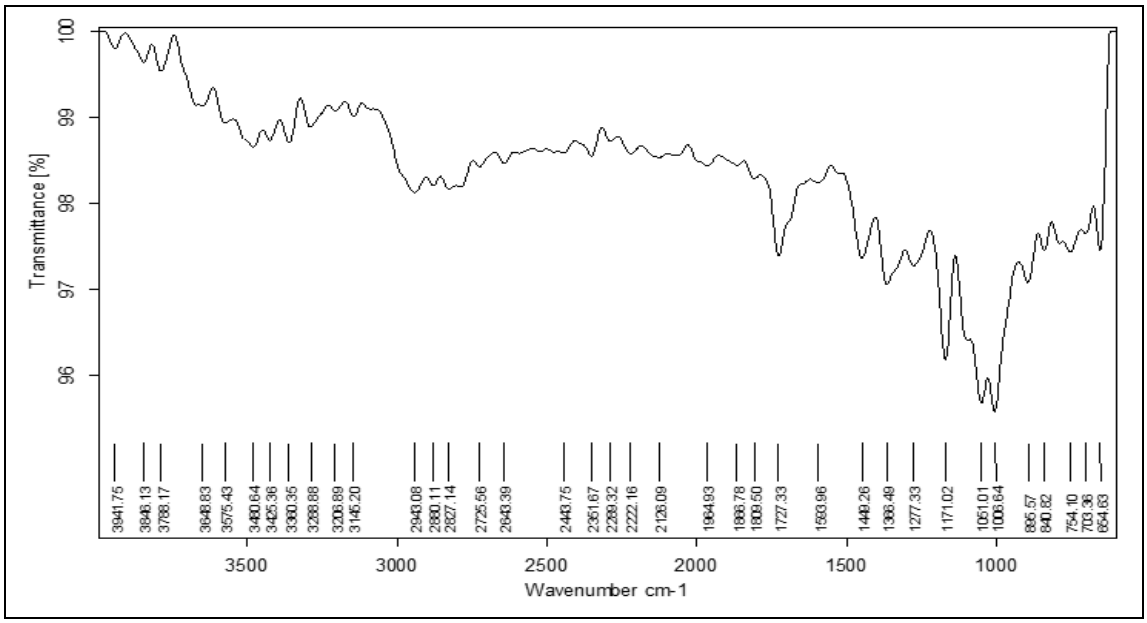

Figure 1: FTIR spectrum of Clarithromycin.

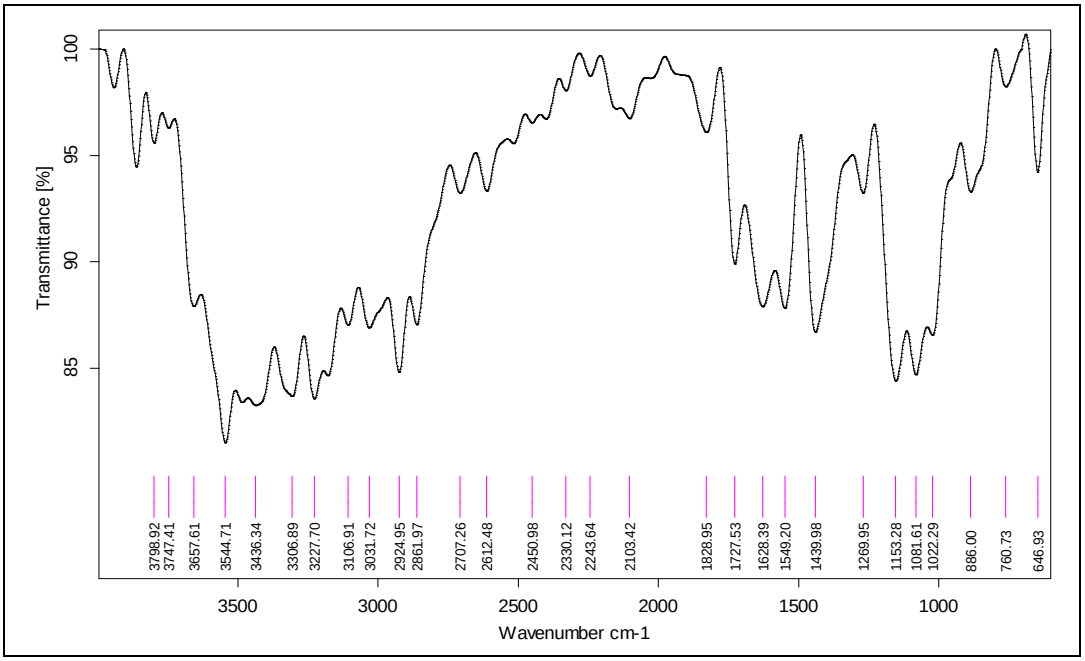

Figure 2: FTIR spectrum of physical mixture of Clarithromycin, PLGA and PVA.

Differential scanning calorimetry (DSC)

DSC was performed to check the thermal behaviour and crystallinity of clarithromycin before and after encapsulation. Figure 1 DSC thermogram of drug showed a sharp melting endotherm at 221.81° C whereas there was a distinct decrease in drug melting point in the thermogram of the physical mixture i.e. at 231.05° C. This indicated that the there is no interaction between drug and polymer.

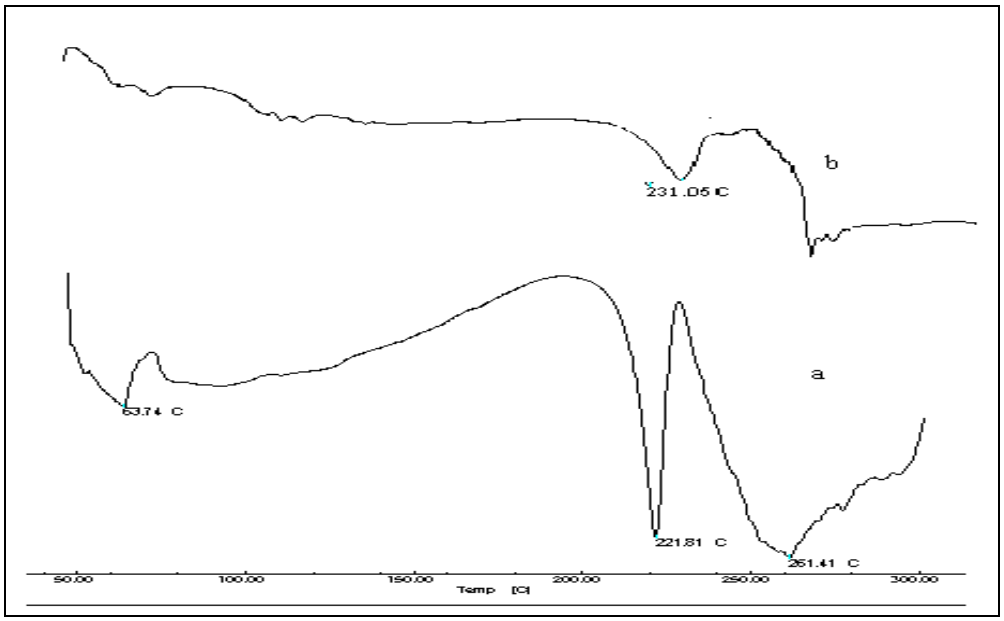

Figure 3: DSC thermogram of clarithromycin (a) and physical mixture of clarithromycin, PLGA and PVA (b).

Characterisation of Prepared nanoparticles

Since the size of the colloidal particles is a key factor for the biological destiny of the NPs. Small size nanoparticles, generally bypass the macrophages uptake, the goal of this study was to reduce the particle size of NPs.

PLGA nanoparticles were prepared by the emulsification-diffusion technique. When the equilibrium between organic and aqueous phase got disturbed, this results in formation of nanoparticles. Destabilizes the equilibrium occurs when excess of water was added. It leads to the diffusion of organic solvent to the external phase. During this diffusion, PLGA nanoparticles are produced.

PLGA nanoparticles were prepared by emulsification solvent evaporation technique using of PLGA and PVA. Concentration of PLGA and PVA were fixed by trial (results not mentioned).

Based on the previous trials 125 mg PLGA and 1.5% PVA was selected. The size of the formed nanoparticles may be dependent upon the type of organic phase solvents used. To evaluate the relationship between nanoparticles size and solvent miscibility with water, organic solvents (acetone, acetonitrile, ethyl acetate and ethanol + acetone) were used for the preparation of CLM-PLGA-NPs (table 1).

EA and ACN are partially water-soluble and are good solvents for PLGA. ACE and ethanol is completely miscible with water in all proportions and is a good solvent for PLGA. At constant concentration of PLGA and PVA, particle size was 333.5 nm (acetone), 378.8 nm (ACN), 556.2 nm (EA) and 875.6 nm (EtOH+ acetone). The particles size varied with change in organic system. Reason for getting smallest particle size for acetone, may be the interfacial turbulence caused by the rapid diffusion of acetone from organic phase to water. Which contributes to the spontaneous breakups of droplets into nano sized ones.

Table 1: Solubility properties of organic solvents in water at 25 °C and solubility properties of PLGA in organic solvents.

	Acetone (Ace)	**Acetonitrile (ACN)**	**Ethyl acetate (EA)**	**Acetone + ethanol (A+E)**
PLGA	Good solvent	Good solvent	Good solvent	Good solvent
Water	Very soluble	Miscible	Slightly soluble	Very soluble
Polarity index	5.1	5.8	4.4	-

Table 2: Evaluation parameters of clarithromycin loaded PLGA nanoparticles.

	Organic solvent	PLGA (MG)	PVA (%)	Particle size (nm)	PDI	Zeta Potential (mV)	%EE	*In vitro* Release
F1	Acetone	125	1.5	333.5	0.1	3.81	80	85 %
F2	Acetonitrile			437.5	0.17	3.45	71	70 %
F3	Ethyl acetate			641.7	0.34	3.97	63	-
F4	Acetone + ethanol			875.6	0.35	2.77	57	-

As shown in table 2 represents evaluation parameters of CLM-PLGA nanoparticles. The mean particle size distribution of CLM-PLGA-NPs decreased with an increase of water miscibility, while maintaining all other experimental parameters constant.

The impact of acetone on size reduction may be due to its diffusion to the aqueous phase and prevention of the particles aggregation. Increase in particle size for ethyl acetate may due to its slight water miscibility and low volatility.

However in this study combination of ethanol and acetone leads to the aggregation of the PLGA nanoparticles and it may be a due to higher affinity of acetone to PLGA than to PVA and ethanol has higher affinity to PVA than to PLGA. When the PLGA solution is added to the PVA solution, as we discussed earlier, the disturbance set by the combination solvent, spontaneously produces a larger interfacial area, which leads to nano-sized emulsion droplets of PLGA solution. Thus, the ethanol preferentially diffuses out of the droplets leaving acetone and PLGA resulting in the larger particles.

Polydispersity of all formulation were found to be unimodel (~0.1) except for ethyl acetate (~0.25) bimodal distribution of particles. Formulation showed encapsulation efficiency ranging from 57 % to 80 %, (Table 1) from results it was observed that as organic solvent affects the encapsulation efficiency. When compared to other individual solvents used, ethyl acetate was found to have lower values for encapsulation efficiency. Ethyl acetate have higher polarity, therefore higher miscibility with the external phase, resulting in the less stable emulation. In the EA/PVA system, due to higher polarity, smaller number of particles is produced, resulting in lower encapsulation efficiency.

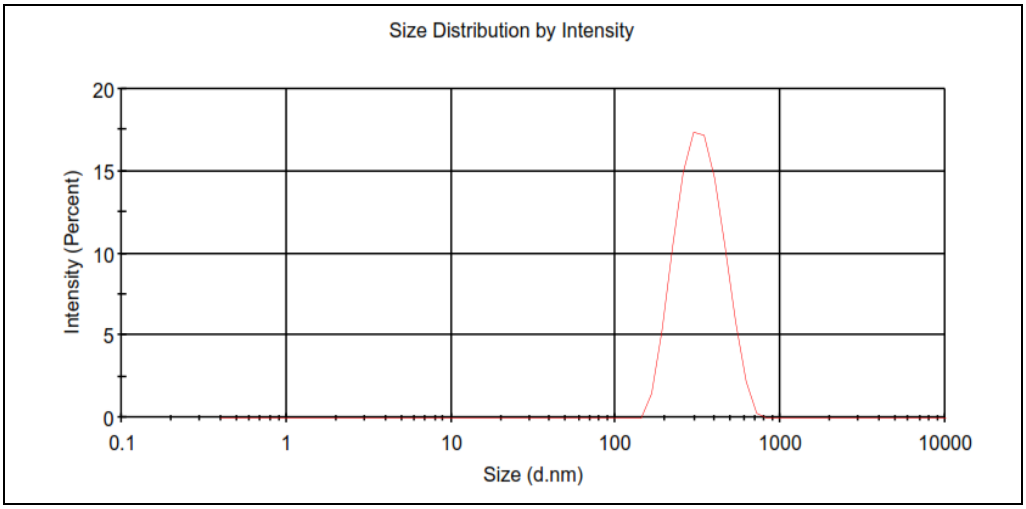

Figure 1: particle size distribution of F1 formulation.

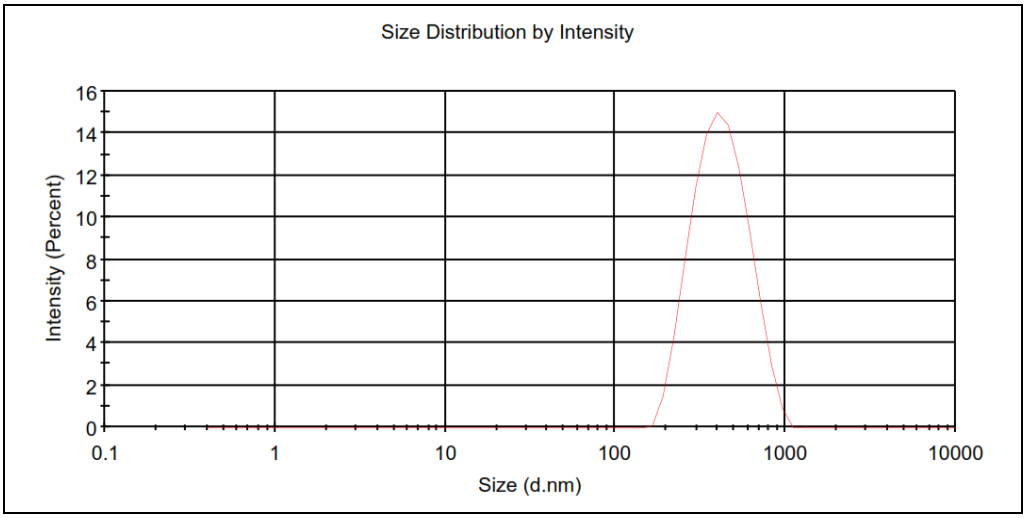

Figure 2: particle size distribution of F2 formulation.

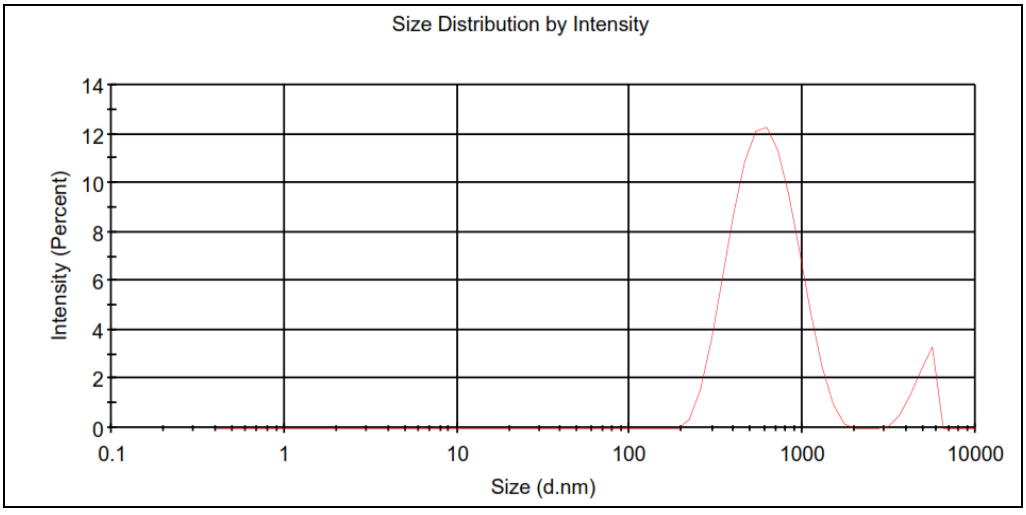

Figure 3: particle size distribution of F3 formulation.

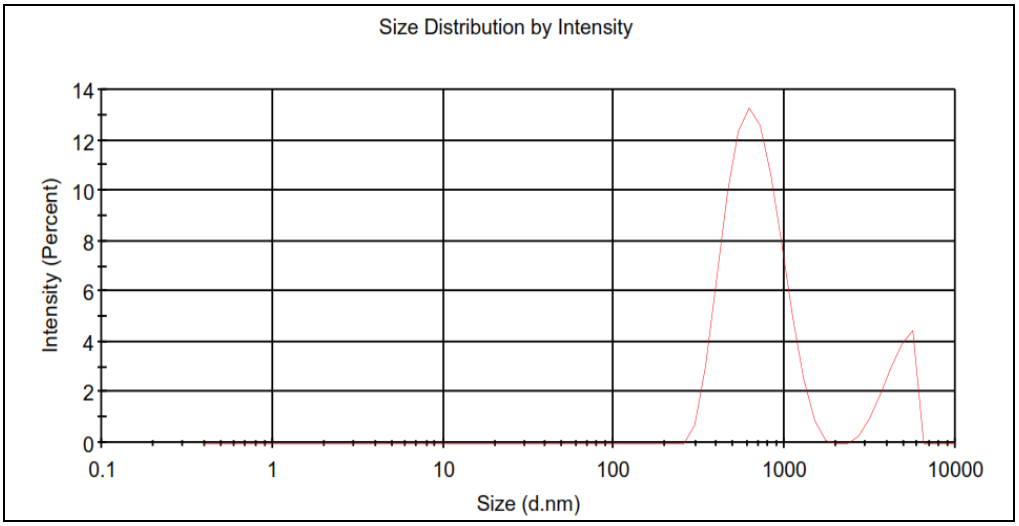

Figure 4: particle size distribution of F4 formulation.

***In vitro* release studies**

The amount of clarithromycin release from PLGA nanoparticles were studied using convention dialysis technique. Results are shown in figure 5 graphically as % cumulative drug release Vs. time. *In vitro* studies for F3 and F4 were not carried out due to particle size. When compared to F1 and F2, F1 formulation showed more release when compared to F2. This can be explained on the basis of particle size and entrapment efficiency. Increase in the particle size increases the surface area and surface area/volume ratio decreases, resulting in the decreased buffer penetration into nanoparticles and slowdowns the release.

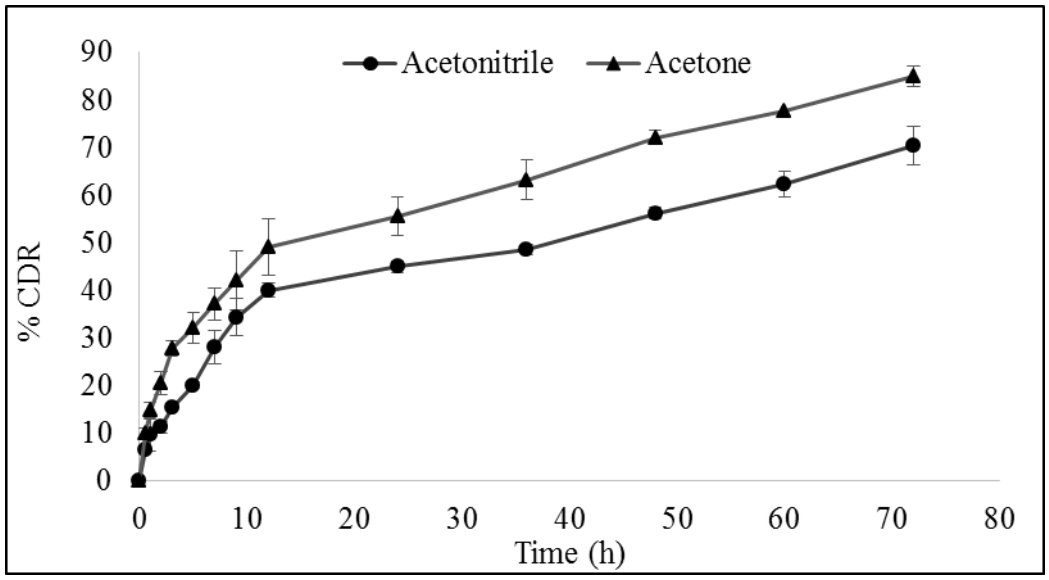

Figure 5: *In vitro* release profile of CLM loaded PLGA nanoparticles.

Stability studies

One of the major criteria for any rational design of a dosage form is its stability. The best formulation F1 was stored in sealed container in aluminium foil. Then the formulation was exposed to temperature at room temperature (15-20 °C) and refrigerator (3-5 °C). The results of stability studies of nanoparticles shown in the table 3. Based on the observation, there were increase in the particle size at room temperature. But there was no change in physical appearance at temperature 3-5 °C. Based on the observation, it was concluded that the developed CLM PLGA nanoparticles were stable and retain their pharmaceutical properties at 3-5 °C over period of 3 months.

Table 2: Stability study results of F3 formulation.

	Particle size (nm)	PDI	Zeta potential (mV)	% Cumulative drug release
F3 (refrigerator (3-5 °C)				
0 month	333.5± 6.45	0.12± 0.20	-3.81± 0.10	85.01 ±1.45
1 month	332.2± 10.2	0.12± 0.07	-3.79±1.26	83.21±2.56
2 month	330.6± 11.2	0.13± 0.04	-3.69±1.57	82.45±3.15
3 month	329.1± 9.2	0.15±0.10	-3.12±1.97	81.96±3.56

CONCLUSION

Clarithromycin loaded PLGA nanoparticles were successfully prepared by emulsification solvent evaporation method. The FTIR spectra and DSC thermogram revealed the new interactions between the drug and PLGA. The main intension of the present investigation was to study the effect of organic solvent on physicochemical parameter of PLGA nanoparticles.

From the results we conclude that as the change in the organic solvent had a significant effect on particle size, encapsulation of drug and it's *in vitro* release. The stability studies at different temperature and humidity condition reveals that formulation negligible change in physical appearance and *in vitro* release was observed at 3-5 °C. From the present study, it is concluded that acetone was found to be best solvent for preparation of PLGA nanoparticles and PLGA nanoparticles are effect carrier for the design of controlled drug delivery of poor water soluble drug, Clarithromycin.

ACKNOWLEDGEMENTS

The author thank to Dr. Divakar Goli, principal, Acharya & B M Reddy College of pharmacy, Bangalore for providing required facilities to carry out this research work.

REFERENCES

1. Pneumonia Diagnosis and management of community- and hospital-acquired pneumonia in adults. Clinical guideline 191 Methods, evidence and recommendations 3 December 2014. Commissioned by the National Institute for Health and Care Excellence.
2. Nisarga R, Premalatha R, Shivananda, Ravikumar Kl, Shivappa U, Gopi A, et al., Hospital-based Surveillance of Invasive Pneumococcal Disease and Pneumonia in South Bangalore, India. Indian Peadiatrics, 2015; 15: 205-11.
3. Wacker M. Nanocarriers for intravenous injection-The long hard road to the market. Int J Pharm, 2013; 457(1): 50-62.
4. Peters DH, Clissold SP. Clarithromycin. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic potential. Drugs, 1992; 44(1): 117-64.
5. Langtry HD, Brogden RN. Clarithromycin. A review of its efficacy in the treatment of respiratory tract infections in immunocompetent patients. Drugs, 1997; 53(6): 973-1004.
6. Salem I, Duzgunes N. Efficacies of cyclodextrin-complexed and liposome encapsulated clarithromycin against Mycobacterium avium complex infection in human macrophages. Int J Pharm, 2003; 250(2): 403-14.
7. Rodvold KA. Clinical pharmacokinetics of clarithromycin. Clin Pharmacokinet, 1999; 37(5): 385-98.
8. Whitman MS, Tunkel AR. Azithromycin and clarithromycin: overview and comparison with erythromycin. Infect Control Hosp Epidemiol, 1992; 13(6): 357-68.
9. Gabrielle P, Veronique D B, Karl T, Hamidou T, Francis V, Karim A et al., Carrier-free combination for dry powder inhalation of antibiotics in the treatment of lung infections in cystic fibrosis. Int J Pharm, 2013; 451(1-2): 112-20.
10. Jian-M L, Xinwen W, Christian M M, Hao W, Peter H L, Qizhi Y, et al., Current advances in research and clinical applications of PLGA-based nanotechnology. Expert Rev Mol Diagn, 2009; 9(4): 325–41.
11. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly(d,l-lactide-co-glycolide) and its derivatives. J Cont Rel, 2008; 125: 193-209.
12. Dinarvand R, Sepehri N, Manoochehri S, Rouhani H, Atyabi F. Polylactide-co-glycolide nanoparticles for controlled delivery of anticancer agents.Int J Nanomedicine, 2011; 6: 877– 95. doi: 10.2147/IJN.S18905.
13. Luo R, Neu B, Venkatraman SS. Surface functionalization of nanoparticles to control cell interactions and drug release. Small, 2012; 8(16): 2585-94.

14. Mehrotra A, Pandit JK. Preparation and Characterization and Biodistribution Studies of Lomustine Loaded PLGA Nanoparticles by Interfacial Deposition Method. J Nanomedine Biotherapeutic Discov, 2015; 5: 138. doi:10.4172/2155-983X.1000138.
15. Huh A J, Kwon Y J. "Nanoantibiotics": A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. Journal of Controlled Release, 2011; 156: 128–145.
16. Moghaddam H, Ramezani V, Esfandi E, Vatanara A, Nabi-Meibodi M, Gilani K, et al., Development of a nano–micro carrier system for sustained pulmonary delivery of clarithromycin. Powd techn, 2013; 239: 478-83. doi:10.1016/j.powtec.2013.02.025.
17. Xiaomei W, Na C, Xing T. Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. Eur J Pharm Biopharm, 2008; 70(3): 735–40.
18. Navneet S, Parshotam M, Senshang L. Effect of process and formulation variables on the preparation of parenteral paclitaxel-loaded biodegradable polymeric nanoparticles: A co-surfactant study. Asian J Pharma Sci, 2016; 11(3): 404-16.
19. Hong Jiang, Meimei Xiong, Qiuyan Bi, Ying Wang, Chong Li. Self-enhanced targeted delivery of a cell wall– and membrane-active antibiotics, daptomycin, against staphylococcal pneumonia. • Acta Pharm Sinica B, 2016. doi:10.1016/j.apsb.2016.05.010
20. Shadabul H, Michael R W, Michelle P M, Colin W P, Lisa M K. Disposition and safety of inhaled biodegradable nanomedicines: Opportunities and challenges. Nanomed: Nanotech, Bio Med, 2016; 12(6): 1703-1724.
21. Moghaddam H, Ramezani V, Esfandi E, Vatanara A, Nabi-Meibodi M, Gilani K, et al., Development of a nano–micro carrier system for sustained pulmonary delivery of clarithromycin. Powd techn, 2013; 239: 478-83. doi:10.1016/j.powtec.2013.02.025.
22. Xiaomei W, Na C, Xing T. Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. Eur J Pharm Biopharm, 2008; 70(3): 735–40.
23. Esmaeili F, Atyabi F, Dinarvand R. Preparation and characterization of Estradiol-loaded PLGA nanoparticles using homogenization-solvent diffusion method. Daru, 2008; 16(4): 196-202.
24. Avadi MR, Sadeghi AM, Mohammadpour N, Abedin S, Atyabi F, Dinarvand R et al., Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. Nanomedicine, 2010; 6(1): 58-63.
25. Surassmo S, Saengkrit N, Ruktanonchai UR, Suktham K, Woramongkolchai N, Wutikhun T, et al., Surface modification of PLGA nanoparticles by carbopol to enhance

mucoadhesion and cell internalization. Colloids Surf B Biointerfaces, 2015; 130: 229-36. doi: 10.1016/j.colsurfb.2015.04.015.

26. Harsha S, Chandramouli R, Shobha R. Ofloxacin targeting to lungs by way of microspheres. Int J Pharm, 2009; 380(1-2): 127–32.
27. Ashvini HM, Shivanad K, Anudeep B. Preparation and evaluation of clarithromycin loaded chitosan nanoparticles. World J Pharm Pharm Sci, 2018; 7(4).