DEVELOPMENT AND CHARACTERISATION OF CLINDAMYCIN HYDROCHLORIDE LOADED PLA/PLGA NANOPARTICLES

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CERTIFICATE

This is to certify that the dissertation entitled " ***DEVELOPMENT AND CHARACTERISATION OF CLINDAMYCIN HYDROCHLORIDE LOADED PLA/PLGA NANOPARTICLES*** **" submitted to the NIT, Rourkela, in partial fulfillment of the requirements for the one year project in M. Sc. Life science .This bonafide research work carried out by Niladri Mohan Das at National institute of technology, Rourkela under the supervision of Dr. B .Nayak. It is further testified that no part of this work has been submitted for any other degree or diploma.**

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DECLARATION

I hereby declare that the thesis entitled "**DEVELOPMENT AND CHARACTERISATION OF CLINDAMYCIN HYDROCHLORIDE LOADED PLA/PLGA NANOPARTICLES**", submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfillment of the Master Degree in Life Science is a faithful record of bonafied and original research work carried out by me under the guidance and supervision of Dr. (Miss) Bismita Nayak, Assistant Professor, Department of Life Science , National Institute of Technology, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

Date:
Place: NIT, Rourkela NILADRI MOHAN DAS

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Place: NIT, Rourkela NILADRI MOHAN DAS

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ABSTRACT

Clindamycin hydrochloride drug most commonly used as antibiotics in dental and oral infection acts upon various bacterial infections also. In this experiment PLA and PLGA nanoparticle prepared and conjugated with Clindamycin hydrochloride drug. From these two nanoparticles PLGA shows good result**.** It is found through SEM and Zeta sizer study that these polymers after conjugation with clindamycin hydrochloride gaining mean particular size of 178.6 nm with zeta potential -17.5 mv. Due to very less zeta potential nanoparticles are remain far apart so no clumping found among the drugs. In DSC study it is shown that the T_g (glass transition temperature) of Clindamycin hydrochloride is about 150^0C so it take time to disperse inside the body but after conjugation with PLGA its $T_{g\ getting}$ reduced to about 48^0C.Due to this low glass transition temperature it can easily disperse inside the body. After conjugation with Clindamycin hydrochloride there also an investigation done through FTIR studies from which we got that there must be a good conjugation of clindamycin hydrochloride with PLGA nanoparticle. This is because the abundance of OH, C=O, group both are common in PLGA and Clindamycin hydrochloride drug. The stretching band in PLGA-clindamycin hydrochloride is 3644.32 cm^{-1} refers to the absence of hydrogen bond among the Clindamycin hydrochloride and PLGA which may stands for hydrophobic bond due to much abundance of OH group. At the end it can be tell that after conjugation there is no serious alteration of Clindamycin hydrochloride structure but due to very easily dispersible nature it can shows its bactericidal effect more rapidly in comparison to the conventional medicine which generally takes two to three days.

INTRODUCTION

There is emergent interest in developing biodegradable nanoparticle because of their application with small molecular drug, proteins or genes by either localized or targeted delivery to the tissue of interest. Nanoparticles are uniform colloidal system of size range typically from 10 to 100 nm. These biodegradable polymer based nanoparticles are conjugated with curative agents those are either entrapped or adhered chemically onto the polymer matrix. Although there are number of polymers that have been used for formulating biodegradable nanoparticles but among them poly (D,L- lactide-co-glycolide) (PLGA), poly lactic acid (PLA), are FDA approved and considered as biodegradable and biocompatible polymer for its application in human use(Parket *et al.*, 1995; Vert *et al*., 1998). PLGA and PLA polymers have the advantage of being well characterized and commercially used for microparticulate and nanoparticulate drug delivery systems (Allemann and Leroux, 1999).

Drug delivery research is clearly moving from the micro to nano size scale. Nanotechnology is therefore evolving as a promising field in medicine that elicits significant therapeutic benefits. The development of effective nano delivery systems has capable of carrying the drug specifically to a desired site of action is one of the most challenging tasks of pharmaceutical formulators. Attempts have been made to reformulate these formulations for better use and add new advantages to the existing drugs for positive scientific outcomes and therapeutic breakthroughs. The nanodelivery systems mainly include nanoemulsions, liposomes and lipid or polymeric nanoparticles. Nanoemulsions are mainly used as vehicles for lipophilic drugs in subsequent intravenous administration. Where as, the ultimate objective of the other nanodelivery system is to alter the normal biofate of potent drug molecules in the body following their intravenous administration to markedly improve their efficacy and reduce their severe adverse effects.

The lactide/glycolide polymers chains are cleaved by hydrolysis into natural metabolites (lactic and glycolic acids), which are eliminated by normal metabolic pathway (citric acid cycle). PLGA provides a wide range of degradation rates, ranges from months to years. It depends on its composition and molecular weight (Brannon *et al*., 1995; Anderson et al., 1997)

Poly (lactic acid) (PLA):

Fully biodegradable synthetic polymers have been available since many years, such as poly (lactic acids) (PLA). Among all biopolymers, PLA was broadly studied in medical implants, suture, and mostly in drug delivery systems since 80s due to its extensive biodegradability nature.

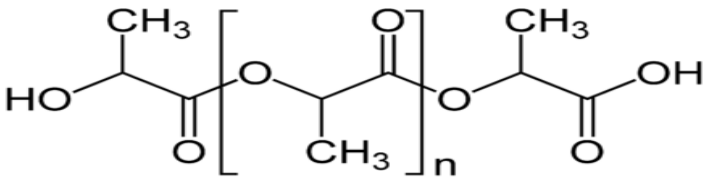


Figure.1: Structure of Poly lactic-acid; n- no of chains

Polylactic acid or polylactide (PLA) thermoplastic aliphatic polyester derived from renewable resources (corn starch), tapioca products (roots, chips or starch mostly in Asia) or sugarcanes (in the rest of world). The biodegradability depends upon certain conditions, such as the presence of oxygen that is difficult to recycle. The name "polylactic acid" is to be used with caution, not complying with standard nomenclatures (such as IUPAC) and potentially leading to ambiguity. But PLA is not a polyacid (polyelectrolyte), but rather polyester.

Poly (lactic-co-glycolic acid) (PLGA):

Over the past few decades, poly (lactic-co-glycolic acid) (PLGA), have been broadly studied for a wide variety of pharmaceutical and biomedical applications. This biodegradable polyester has been regarded as one of the few synthetic biodegradable polymers with excellent biocompatibility, convenient biodegradability, and high safety. Among these polyesters PLGA plays an important role in drug delivery system.

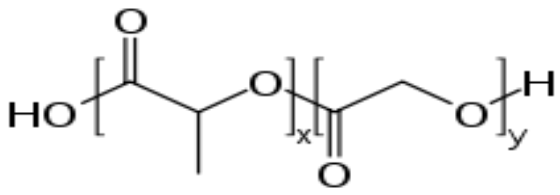


FIG.2: Structure of poly (lactic-*co*-glycolic acid). *X*: number of units of lactic acid; *y*: number of units of glycolic acid

Poly (lactic-*co*-glycolic acid) **(**PLGA) copolymer is an attractive delivery vehicle because of its superb biocompatibility, high safety profile. Whereas Inject able PLGA delivery systems involve encapsulation of a growth factor, as the PLGA capsule is hydrolytically degraded over time *in vivo* or *in vitro*, growth factors are released into the surrounding region. In addition, the release kinetics of this system can be easily adjusted by altering the ratio of PLA: PGA. Recently, focus has been shifted to encapsulation of bioactive substances of nanometer scale and their delivery to targeted site. Nanoparticle encapsulation confers several advantages over micro particle encapsulation, including a lower risk of mobilization.

Clindamycin hydrochloride (CLH)

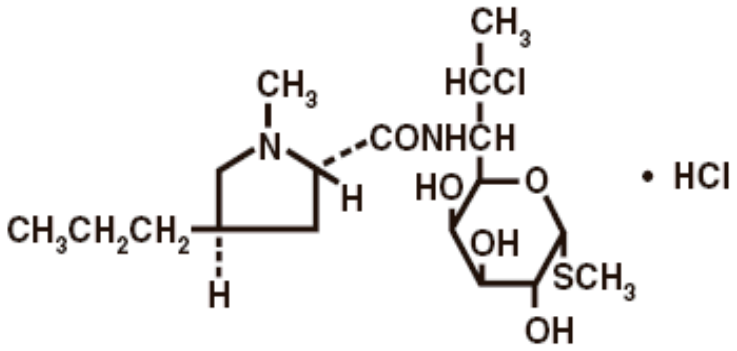


Figure.3: Structure of Clindamycin hydrochloride

Clindamycin hydrochloride antibiotics have the chemical name 7-chloro-7-deoxylincomycin hydrochloride which is a semi synthetic analogue of natural antibiotic lincomycin. It is shaped by a 7(S)-chloro-substitution of the 7(R)-hydroxyl group of lincomycin. Antibacterial and antiprotozoal antibiotic of the licosamide class inhibits protein synthesis in bacterial by binding to 50s ribosomal subunit. It is active mainly towards gram-positive microorganisms. It is used mostly in the treatment of penicillin-resistant infections and in patients allergic to penicillin. It may be used alone or with combined medicines. Chemical name of clindamycin is Methyl 7-chloro-6,7,8-trideoxy- 6-(1 -methyl-trans-4-propyl-L-2-pyrrolidine-carboxamido)-1 -thio-L-threo-a-o-galacto- octopyranoside. If we take into consideration of clindamycin antibiotic then it is divisible into three types of antibiotics these are Clindamycin Hydrochloride which is the hydrochloride salt of clindamycin it is white crystalline powder in nature and administered orally. Next one is the Clindamycin Palmitate Hydrochloride which is the palmitic ester of clindamycin in hydrochloride form it is also a white crystalline powder and it soluble in water for the preparation of solutions. Clindamycin Phosphate is the phosphoric acid ester of clindamycin having properties of white crystalline powder, soluble in water and administered intramuscularly or intravenously. Clindamycin is used to treat a wide variety of bacterial infections that works by stopping the growth of bacteria. This antibiotic treatment is only done for bacterial infections. It will not work for virus infections (e.g.,common cold, flu). Unnecessary use or misuse of any antibiotic can lead to its decreased efficiency. This drug may

also be used before dental procedures in patients with certain heart conditions (e.g., artificial heart valves) to help prevent serious infection of the heart (bacterial endocarditis).

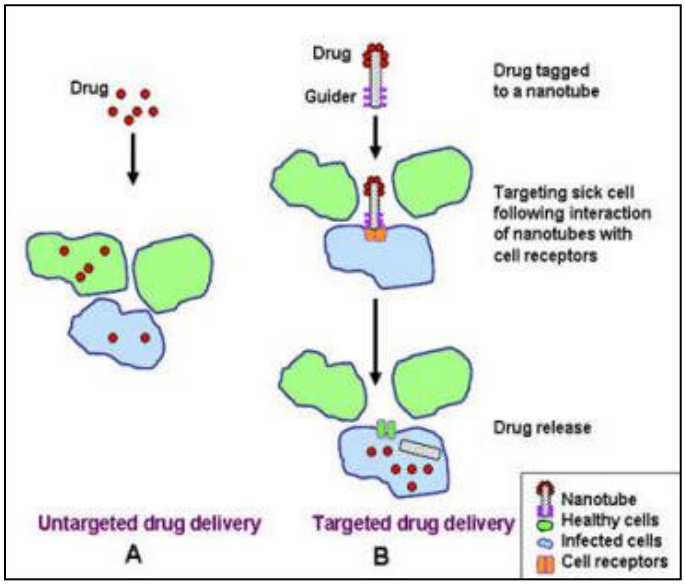


Figure.4: NTS (Nanotech systems inc.) utilizes polymer based nano particles that have been formulated to encapsulate a drug, which allow for an intracellular site of action. The drug binds to the cytoplasmic receptors and the succeeding drug-receptor complex is transported to the nucleus resulting in the manifestation of the drug product.

MOTIVATION TO DO THIS WORK:

As Clindamycin hydrochloride antibiotics most profoundly prescribed by physician to use during oral infection, dental infection, immunological irresponse after using penicillin and other bactericidal infections as for its very successful bactericidal effect. But generally this antibiotic takes two/three days for the medication (www.ehow.com/how). In dental infection it most profoundly prescribed by doctors to use it within two days but as its bactericidal effect takes two to three days so patient bound to suffer more two/three days with pain of this disease .In this experiment Clindamycin hydrochloride drug conjugated with PLA/PLGA nanoparticle and its characters after conjugation is studied.

2. Review of Literature

Conventional preparations like solution, suspension or emulsion for drug delivery purpose has various boundaries like high dose and low availability, faster reach effect, intolerance, instability. They exhibit some changes in blood plasma drug levels and do not provide sustained effect. Due to presence of various acidic and basic medium inside our body it is necessary that every drug should reach to its target site without any alteration in its physical and chemical properties. Therefore, there is a need for some novel carriers which could reach to its target side without making any adverse effect to body and can carry the drug easily and safely to its destination. Recently nanoparticles delivery system has been proposed as colloidal drug carriers. Nanoparticles (NP) are a type of colloidal drug delivery system comprising particles with a size range from 10 to 1000 nm (diameter). Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials (Buzea *et al.,* 2007). The major advantages of nanoparticles are improved bioavailability by enhancing aqueous solubility, increasing resistance time in the body (increasing half life for clearance/increasing specificity for its associated receptors and targeting drug to specific location in the body .This is why nanoparticle are increasingly used in variety of applications that includes drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane etc (Abhilash, 2010). They are based on biocompatible lipid that provide sustained effect by either diffusion or dissolution (Cavalli *et al.,* 1995; Muller *et al.,* 2000). Moreover it can be said that nanoparticles are now a day's acting as very prolific device for drug delivery system. The first nanoparticulate drug delivery systems was liposomes which proposed by Dr. Gregory Gregoriadis in 1974 leading to several breakthrough discoveries by using nanoparticles as drug carriers resulting from cutting-edge researches based on multidisciplinary approaches and many more applications have developed since then.

NANOPARTICLE DRUG DELIVERY SYSTEMS FOR BLOOD BRAIN BARRIER:

Effectiveness of the chemotherapy of brain pathologies is often impeded by inadequate drug delivery across the blood–brain barrier (BBB). Galeperina(2006) from Russia has patented poly(butyl cyanoacrylate) nanoparticles coated with polysorbate 80,which showing the

proficient brain-targeting drug delivery system by crossing the BBB . Doxorubicin in free form cannot pass the BBB but after employment of poly butyl cyanoacrylate nanoparticles showed high effectiveness of nanoparticle-bound doxorubicin in intracranial glioblastoma in rats. Another research review, covering various techniques used for crossing the BBB, discusses the application of nanoparticulate drug delivery systems for this purpose (Juillerat JL 2008). Kreuter *et al.*,(2007) have described the application of covalently bonded apolipoprotein A-I and apolipoprotien B-100 to albumin nanoparticles, enabling these to deliver the drug into the brain . A fascinating review on the application of nanotechnology in breast cancer therapy is exposed by Takemi *et al.,* (2009). According to him a PEGylated form of liposomally encapsulated doxorubicin is routinely used for the treatment of metastatic cancer, and albumin nanoparticulate chaperones of paclitaxel are standard for the locally recurrent and metastatic cancer tumors. Above 150 clinical trials are being conducted through worldwide for the treatment of breast cancer by using nanotechnology based yield. This review covers different generations of nanotechnology tools used for drug delivery, especially in breast cancer.Generally injectable drug delivery nano vectors are used for cancer therapy, especially while multiple-drug therapy is used. These vectors need to be large enough to equivocate the body defense but it should be sufficiently small to avoid blockages in even the capillaries. The nano size plays an important and supportive role in such capillary blockages. As these vectors are smaller than the diameters of the capillaries, the blockages can be successfully prevented. The anticancer drugs can be integrated in such nanovectors. These nanovectors can functionalize in order to actively bind to specific sites and cells after extravasations thorough ligand–receptor interactions. Nano sized vectors incorporate fusion proteins and immunotoxins/polymers, dendrimers, polymer–drug conjugates, polymeric micelles, polymerosomes and liposomes, and metal nanoparticles like gold nanoparticles or nanoshells. The major alarm of nanovectors based on polymers is their biodegradability, biocompatibility, and release of drug from the polymer nanosystem in to the body at the site of action.

ANTIBODY TARGETING OF NANOPARTICLES

Many studies have been reported about the antibody mediation of the nanoparticles to build up targeted drug delivery systems, particularly in the application of cancer treatment. Antibody targeting of drugs can improve the therapeutic efficacy of the drug substance, as well

as it can progress the circulation and absorption of the drug at the targeted site of drug action McCarron *et al.*,(2008). Two novel approaches to create immune nanoparticles with improved therapeutic effect against colorectal tumor cells were studied by using poly (lactide) polymers and CD95/ APO-1 antibody to target nanoparticles. Pan *et al.*, in 2007 used dendrimer–magnetic nanoparticles for proficient delivery of gene-targeted systems for cancer treatment. Later the use of nanostructures calcium nanophosphates for non viral gene delivery were described and they studied the influence of various synthesis and formulation parameters on transfection efficiency (Olton D, Li J;Wilson ME, *et al*., 2007).

HYDROGEL NANOPARTICLES IN DRUG DELIVERY

Hydrogel nanoparticles and their applications in drug delivery as well as therapeutic applications in various disease conditions is more fruitful (Hamidi M, *et al* 2008). Scientists have used polymeric group of poly (lactide)- tocopheryl polyethylene glycol succinate (PLA-TPGS) copolymers, were used to convey protein and peptide drugs (Lee SH *et al.*,2007) through double-emulsion technique for protein drug formulation, with BSA as the model drug. They have used confocal laser scanning microscopy observations to demonstrate the intracellular uptake of the PLA-TPGS nanoparticles by fibroblast cells and Caco-2 cells, having great potential of these polymeric carriers for protein and peptide drugs.

Nano vehicular intracellular delivery systems (Prokop A *et al.*, 2008.) recently developed system by which various aspects of nanodrug delivery systems and their uptake was studied in biological environment at different cellular levels. (Devapally *et al.*, 2007). With many examples, they have shown that nanoparticulate drug delivery systems show a gifted approach to attain desirable delivery properties by altering the biopharmaceutic and pharmacokinetic properties of the molecule.

The industrial scene of nanotechnology developments is very promising. Its application to drug delivery is broadly expected to create novel therapeutics, capable of enhancing the landscape of pharmaceutical and biotechnology industries. Various nanotechnology platforms were investigated, either in development or in clinical stages, and many areas of interest where there will be effectual and safer targeted therapeutics for a numerous of clinical applications were used. It will be evolving out very soon for the detriment of humanity at large scale. Biodegradable polymers are valuable in many ways over other materials for use in drug delivery

systems such as nanoparticles. They can be fabricated into various shapes and sizes, with modified pore morphologies, mechanical properties, and degradation kinetics for variety of applications. By selecting the appropriate polymer type, molecular weight, and copolymer blend ratio, the degradation erosion rate of the nanoparticles can be controlled to accomplish the much preferred type and rate of release of the encapsulated drug. The common biodegradable polymers used in drug delivery include (*i*) polyesters, such as lactide and glycolide copolymers, polycaprolactones, poly(hydroxybutyrates), (*ii*) polyamides, which includes natural polymers such as collagen, gelatin, and albumin, and semisynthetic pseudo-poly(amino acids) such as poly(*N*-palmitoyl hydroxyproline ester), (*iii*) polyurethanes, (*iv*) polyphosphazenes, (*v*) polyorthoesters, (*vi*) polyanhydrides, and (*vii*) poly(alkyl cyanoacrylates) by D'Mello *et al*.,2005 One of the most popular biodegradable polymers used in drug delivery are aliphatic polyester copolymers based on lactic and glycolic acids. Poly (d,l-lacticco- glycolic acid) (PLGA) is used for the manufacture of implants and internal sutures. As PLGA is degrading to produce the natural products lactic acid and glycolic acid it is known as biocompatible material. PLGA nanoparticles undergo homogenous hydrolytic degradation, which is modulated by various factors such as chemical composition, porosity, hydrophilicity/hydrophobicity, morphology (crystalline/ amorphous), and molecular weight and molecular weight distribution. Owing to the presence of methyl groups in the lactide polymers, these are more hydrophobic than the glycolide polymers. As glycolide ratio in the copolymer increases the water uptake also increases. The homopolymers, PLA is highly crystalline compared with PGA and erode slowly since it is more resistant to hydrolysis, whereas the PLGA copolymers with an increasing ratio of PGA tend to be less crystalline and thus have a faster rate of biodegradation. The transition glass temperatures of the copolymers range from 36^0C to about 67^0C. PLGA polymers undergo bulk hydrolysis/erosion of the ester bonds and metabolized to monomeric acids due to molecular weight decreases whereas the mass remains unchanged and undergoes elimination through Krebs cycle. Pitt et *al.*, demonstrated that molecular weight of the polymer decreases in first stage of degradation owing to the random hydrolytic cleavage of the ester linkage, followed by the onset of weight loss and a change in the rate of chain scission in the second stage (Pitt CG *et al.,* 1981). Furthermore, hydrolysis is enhanced by the accumulation of acidic products and the reduction of pH facilitated by the carboxylic acid end groups, which is an autocatalytic degradation process (Pistner H *et al.,* 1993). The degradation of these polymers differs in vivo

and in vitro conditions, mainly because, although in vivo there is no major influence of enzymes during the glassy state of the polymer, these enzymes can play a significant role when the polymer becomes rubbery (Amidon GL *et al*., 1995). Normally, 50:50 lactide/glycolide copolymers have the fastest half-life of degradation, around 50 to 60 days, whereas 65:35, 75:25, and 85:15 lactide/glycolide copolymers have progressively longer degradation half-lives *in vivo* condition (Jalil R *et a.,l* 1990) demonstrated that although physical properties of the microparticles were not seriously affected by the molecular weight of poly(d,l-lactide), swelling properties (which are a function of hydrophilicity of the polymer) could be affected due to the core loading and the variations in the molecular weight. The half-life of these linear polyesters can be increased by co blending with more hydrophobic co monomers such as polycaprolactone. The complete breakdown of the poly(d,l-lactide) nanoparticles was achieved within 480 days, whereas the PLGA nanoparticles degraded in 63 days, this is due to hydrophilic and semicrystalline nature of the glycolide part.

The nanoparticle is coated by polymer, which releases the drug by controlled diffusion or erosion from the core across the polymeric membrane or matrix .But the membrane coating acts as a barrier to release the drugs from core of nanoparticle, therefore, the solubility and diffusivity of drug in polymer membrane becomes the influential factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of supporting ingredients. When the drug is involved in interaction with these supporting ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect (Chen *et al*., 1994). To develop a successful nanoparticulate system, both drug release and polymer biodegradation are two important consideration factors. Moreover, drug release rate depends on solubility of drug, desorption of the surface bound/ adsorbed drug, drug diffusion through the nanoparticle matrix, nanoparticle matrix erosion/degradation and combination of erosion/diffusion process (Mohanraj and Chen, 2006). Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. There is a wide range of nanoparticulate materials and structures have been developed for the delivery of therapeutic compound and each has its own scrupulous advantages, but as these nanoparticles become optimized for their unambiguous application, the outcome will be better-controlled therapy. As a result of targeted delivery of smaller amounts of effective drugs to the required sites in the body, various types of nanoparticles those are on focus due to their use in drug delivery and various

biomedical approaches are recognized as Fullerenes, Solid lipid nanoparticles (SLNs), Liposomes, Nanostructured lipid carriers (NLC), Quantum dots (QD), Super paramagnetic nanoparticles and dendrimers.

PLGA generally used for the manufacture of implants and internal sutures and is known to be biocompatible, degrading to produce the natural products lactic acid and glycolic acid (Visscher GE *et al*.,1985) and these acids are eliminated from body through lactic acid cycle . PLGA nanoparticles undergo homogenous hydrolytic degradation, which is modulated by different factors such as chemical composition, porosity, hydrophilicity/hydrophobicity, morphology (crystalline/ amorphous), and molecular weight and molecular weight distribution (Anderson JM *et al.*,1997). Due to the presence of methyl groups in the lactide polymers, these are more hydrophobic than the glycolide polymers. Also, the water uptake increases as the glycolide ratio in the copolymer increases (Gilding DK *et al.*, 1979). As PLA is more resistant to hydrolysis it slowly erodes and it is highly crystalline on compared with PGA whereas the PLGA copolymers with an increasing ratio of PGA tend to be less crystalline and thus have a faster rate of biodegradation. The transition glass temperatures of the copolymers range from 36◦C to about 67◦C.PLGA polymers undergo bulk hydrolysis and erosion of the ester bonds, due to which the molecular weight decreases and the mass remains unchanged. Furthermore, hydrolysis is enhanced by the accumulation of acidic products and the reduction of pH facilitated by the carboxylic acid end groups. The degradation of these polymers differs in vivo and in vitro, although *in vivo* there is no major influence of enzymes during the glassy state of the polymer, these enzymes can play a significant role when the polymer becomes hard (Li SM *et al.*, 1990). It was verified that although physical properties of the microparticles were not seriously affected by the molecular weight of poly(d,l-lactide), swelling properties (which are a function of hydrophilicity of the polymer) could be affected due to the variations in the molecular weight and the core loading. The half-life of these linear polyesters can be increased by co-blending with more hydrophobic co-monomers such as polycaprolactone. Visscher *et al.,* performed the biodegradation studies of poly(d,l-lactide) and 50:50 poly(d,l-lactide-coglycolide) in the rat gastrocnemius muscles (Visscher GE *et al.*, 1985). The 50:50 ratio of PLGA is thus advantageous as polymeric nanoparticles for small-molecule drugs **(**Jalil R *et al* ., 1990**)** compared with other polymers due to its fastest degradation rate, and as a result, fastest drug release from the nanoparticles.

For encapsulating peptide or protein generally PLGA nanoparticls/microparticless are used followed by mainly three methods: water–oil–water (w/o/w) emulsion technique, phase separation methods and spray drying (Freitas, 2005). Generally, peptides or proteins are dispersed in an organic solution of PLGA and processed in an aqueous solution of water-in-oil (w/o) emulsion. The dispersion step is carried out by means of high speed sonicator. Raghavendra *et al.*, (2008), reported that, micro particles are produced by either extracting organic solvent or by adding a non-solvent i.e., silicone oil, thereby inducing coacervation. The first process is frequently referred as w/o/w method,which also known as the phase separation technique. In both of the cases, the particle formation occurs in the liquid phase. But In spray drying technique, particle formation is achieved by atomizing the emulsion into a stream of hot air under vigorous solvent evaporation. Different methods are schematically displayed in Fig: 5.

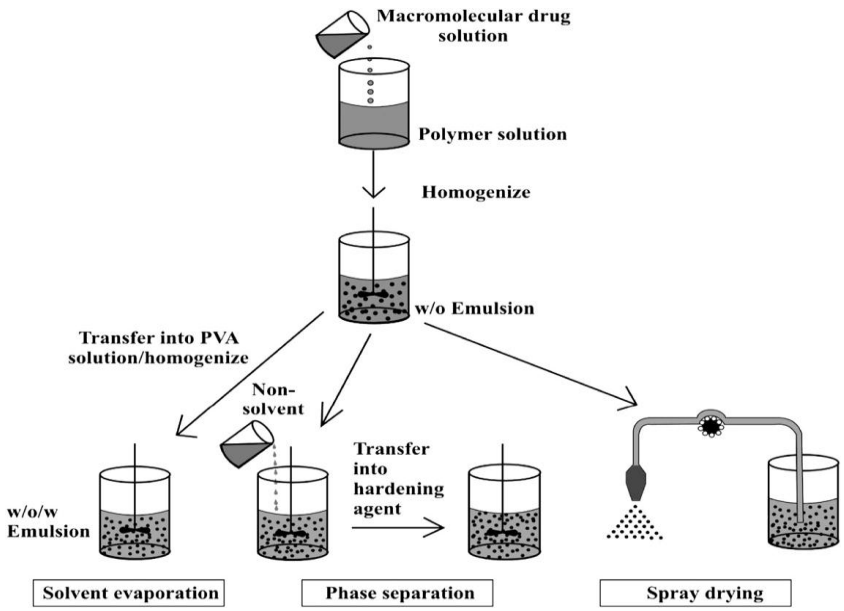


Figure.5: correlation of microencapsulation methods: (i) solvent evaporation, (ii) polymer phase separation and (iii) spray drying. Aqueous solution is dispersed in the organic polymer solution by ultrasonication (w/o) emulsion; the w/o emulsion is processed further by specific methods to prepare the drug-loaded microparticles.

According to Raghavendra *et al.*, (2008), proteins encapsulated by w/o or w/o/w techniques into nanoparticles or microparticles are susceptible to denaturation, aggregation, oxidation and cleavage, particularly at the aqueous phase-solvent interface. Protein denaturation

may also result in a loss of biological activity. Improved protein integrity has been achieved by the addition of stabilizers such as carrier proteins (e.g., albumin), surfactants during the primary emulsion phase or molecules such as trehalose and mannitol to the protein phase. Protein stability may also be improved if the protein is encapsulated as a solid rather than in solution. It should be noted that all the nano/micro-encapsulation techniques create mechanical, thermal and chemical stresses on the system under investigation.

At present PLA is one of the most promising biodegradable polymers (biopolymers) and has been the subject of profuse of literature over the last decade. PLA can be processed with a large number of techniques and is commercially available (large-scale production) in a wide variety of grades. It is relatively economical and has some remarkable properties, which make it suitable for different applications. Its biodegradability is modified to short-term packaging, and its biocompatibility in contact with living tissues is exploited for biomedical applications like implants, sutures, drug encapsulation. PLA belongs to the family of aliphatic polyesters commonly made from hydroxy acids (D'Mello *et al*), for example, polyglycolic acid (PGA). It is one of among the few polymers in which the stereo chemical structure can easily be modified by polymerizing a controlled mixture of l and d isomers to yield high molecular weight and amorphous or semi-crystalline polymers. Properties can be modified through the variation in the relative contents isomers (l / d ratio) and the homo and (d ,l)copolymers. Besides these PLA can be adapted by formulation involving adding plasticizers, other biopolymers and fillers. PLA is considered both as biodegradable and as biocompatible in contact with living tissues as it is widely used for biomedical applications. PLA can be degraded by a biotic degradation which represents simple hydrolysis of the ester bond without requiring the presence of enzymes to catalyze it. During the biodegradation process, the enzymes degrade the residual oligomers till final mineralization (biotic degradation).As long as the basic monomers (lactic acid) are produced from renewable resources (carbohydrates) by fermentation; PLA complies with the rising worldwide concept of sustainable development and is classified as an environmental friendly material.

Antibiotics Clindamycin hydrochloride is the hydrated hydrochloride salt of clindamycin. Clindamycin hydrochloride is a semi synthetic antibiotic shaped by a 7(S)-chloro substitution of the 7(R) hydroxyl group of the parent compound linomycin. Cleocin HCl Capsules contain clindamycin hydrochloride equivalent to 75 mg, 150mg, or 300 mg of clindamycin. Serum level

studies with a 150 mg oral dose of clindamycin hydrochloride in 24 normal adult volunteers showed that the clindamycin drug was rapidly absorbed after oral administration. An average peak serum level of 2.50 mcg/mL was reached within 45 minutes; serum levels averaged 1.51 mcg/mL at 3 hours and 0.70 mcg/mL at 6 hours. Absorption of an oral dose is virtually complete (90%), and the affiliated administration of food does not significantly modify the serum concentrations; serum levels have been uniform and predictable from person to person and dose to dose. Serum level studies following multiple doses of Cleocin HCl for up to 14 days .But there is no evidence of accumulation or altered metabolism of drug found. Next it is found that healthy volunteers were well tolerated to the doses of up to 2 grams of clindamycin per day for 14 days, except that incidence of gastrointestinal side effects is greater with the higher doses. Concentrations of clindamycin in the serum increased linearly with increased doses .Serum levels surpass the MIC (minimum inhibitory concentration) for most indicated organisms for at least six hours following the administration of recommended doses. Clindamycin is widely circulated in body fluids and tissues (including bones). No significant levels of clindamycin are found to attained in the cerebrospinal fluid, even in the existence of inflamed meninges. Clindamycin inhibits bacterial protein synthesis by binding to the 50S subunit of the ribosome (Wayne, PA: Clinical and Laboratory Standards Institute; 2010). It has activity against Gram-positive aerobes and anaerobes as well as –some Gram-negative anaerobes. As Clindamycin is also bacteriostatic so cross-resistance between clindamycin and lincomycin is complete. Antagonism *in vitro* has been demonstrated between clindamycin and erythromycin. Clindamycin has inducible resistance which has been identified in macrolide-resistant staphylococci and beta-hemolytic streptococci. Macrolide-resistant isolates of these organisms should be screened for clindamycin by using the D-zone test. Clindamycin can also be treated in case of serious infections due to susceptible strains of streptococci, pneumocooci and styphalocooci. Its uses should be reserve for penicillin allergic patients and this antibiotics is also used for the treatment of infected wounds, abscesses, and dental infections in dogs and cats and osteomyelitis in dogs (Bioequivalence Guideline, October 9, 2002) .

OBJECTIVES:

1. To prepare PLA and PLGA conjugated clindamycin hydrochloride drug nanoparticles.
2. Characterization study of PLA/PLGA conjugated Clindamycin hydrochloride drug like its efficiencies, surface morphology, particle size, surface charge, chemical composition study by FTIR, dispersion rate was studied.

PLAN OF WORK:

Loading of Clindamycin hydrochloride drugs into PLA and PLGA microparticles/nanoparticles

↓↓

Measurement of particle size and zeta potential using Zeta sizer

↓↓

Morphological characterization by using scanning electron microscope (SEM)

↓↓

Differential scanning calorimetric (DSC) measurements

↓↓

Fourier transform infra-red (FTIR) measurements

3. MATERIALS AND METHODS

3.1 Materials

The biodegradable polymers Polylactic acid (PLA) and Poly (d,l-lacticco- glycolic acid) (PLGA) 50:50 H (sigma Aldrich, USA), with an average molecular weight of 40000-65000 Da and 40000-75000 Da respectively were studied. As surface active agent polyvinyl alcohol (PVA,) from Sigma-Aldrich, USA was used. Antibiotic Clindamycin hydrochloride powder usp (manufactured by Pfizer pgm, France) those are commercially available were used to analyze and to prepare drug containing nanoparticle. Ultrapure water from Milli-Q water system used.

EQUIPMENTS

- Stratos low-temperature high-speed centrifuge(Thermo, Germany)
- Cooling centrifuge (REMI)
- Magnetic stirrer
- Sonicator
- Zeta sizer (Malvern)
- Scanning electron microscope (Jeol Jsm-6480 LV)
- Fourier transform infra-red (FTIR)
- Differential scanning calorimetry (DSC) [NETZCH]

3.2. Methods

3.2.1 Preparation of drug loaded nanoparticle by (w_1/o) w_2 solvent evaporation method

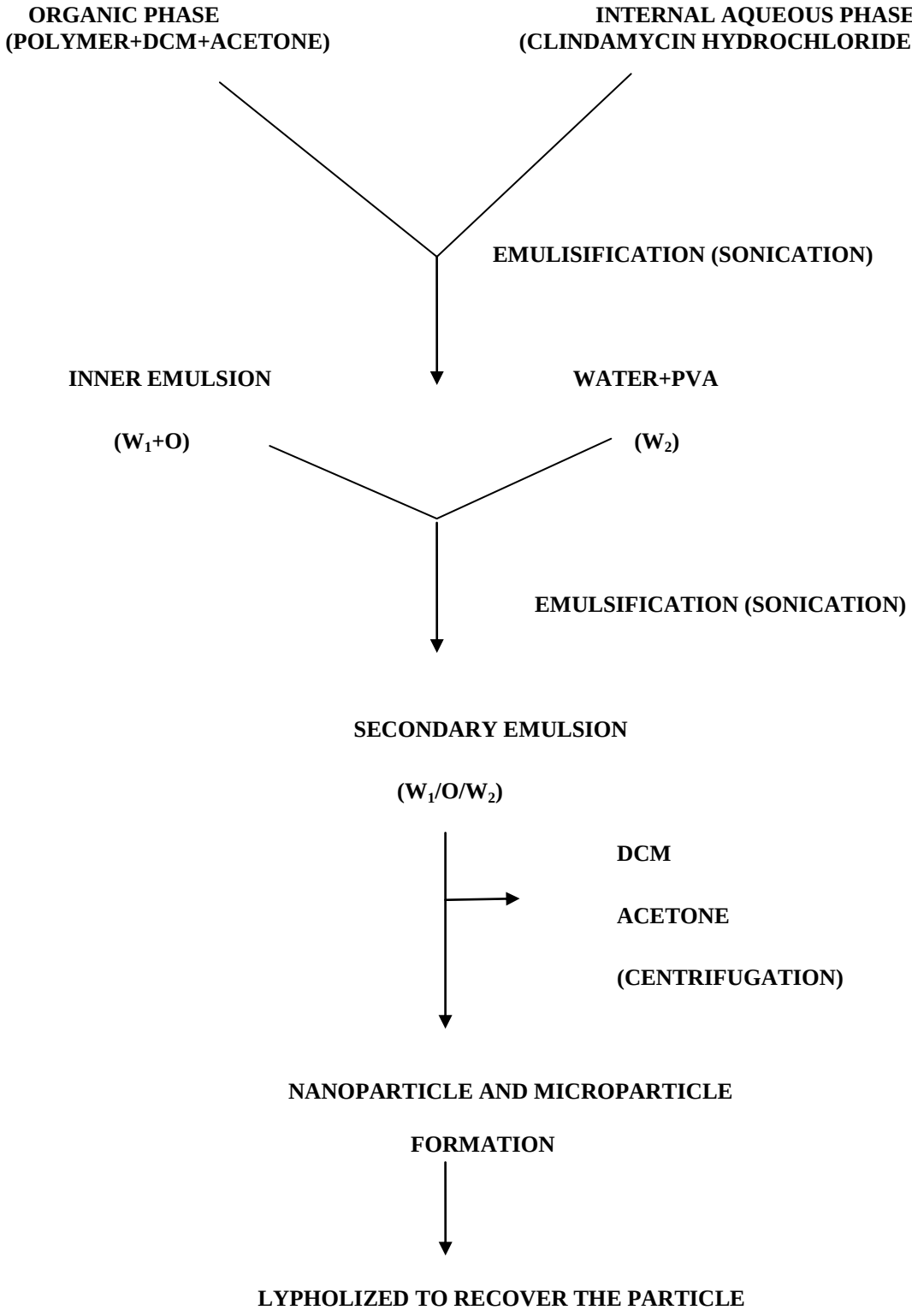


Fig-6: Preparation of Nanoparticle by Emulsion Solvent Evaporation Method

In organic phase 0.4 gm of PLGA and PLA polymers were taken separately each in 8ml of mixture of DCM and acetone (85:15,v/v) to prepare nanoparticle and in internal aqueous phase 40 mg Clindamycin hydrochloride antibiotic (10% dry weight of polymer) dissolve in PBS(67mM,pH 6.0).The two solutions were mixed by ultrasonication for 30 sec under cooling (output 4,40% duty cycle) to form W_1/O emulsion. This is so called inner emulsion was slowly added to 100 ml of 1% (w/v) aqueous PVA solution which was homogenized with a high speed mixture for 8 min at 8500 rpm. The resulting $w_1/o/w_2$ emulsion was stirred at 300 rpm over night to maximum evaporation of organic solvent. Then the samples were washed 3 times with milli-Q water at 12000 rpm for 15 min then nanoparticles/microparticles were formed. These nanoparticles/microparticles were taken for lyphophilization where as supernatants were preserved for further analysis.

3.2.2 Morphology

The morphology of the nanoparticle was investigated by Scanning electron microscopy (Jeol JSM microscope). The nanoparticles were fixed on adequate supports and coated with platinum using platinum sputter module in a higher vacuum evaporator. Observations under different magnifications were performed at 20kv.

3.2.3 Particle size and zeta potential

The size, size distribution and zeta potential of the nanoparticles were analyzed by Zeta sizer(ZS 90 malvrn). The lyophilized samples were made a dilution with PBS of 67 mm and ph 6.0 on mg/ml and analyzed. During analysis of size these samples were first kept in an another clean cubet and put it on to the zeta size analysis chamber to get various peak and next to find its average zeta size. On for analysis surface charge potential or zeta potential samples were kept into the zeta sizer analysis chamber observe for its peak to get an data of zeta potential. During analysis of these data monodisperse nature are always took in to consideration rather than polydisperse character.

3.2.4 DSC analysis

The physical state of Clindamycin hydrochloride entrapped in the nanoparticles as well as the polymers and the blank nanoparticles of PLA and PLGA were characterized by differential scanning calorimetry thermogram analysis (Netzch DSC 200 F$^{s)}$. The samples (~12 mg) were weighed and sealed in Aluminum pans and heated under nitrogen by heating rate of 10^0 C/min, the heat flow being recorded from 30^0C to 200^0C. Indium was used as standard reference material to calibrate the temperature and energy scales of the DSC instruments. After getting data through Microsoft exel we got the DSC thermograph.

3.2.5 FTIR analysis

PLA and PLGA nanoparticles were conjugated with Clindamycin hydrochloride. Due to this conjugation there may be chances of adsorption of some functional groups to the newly formed conjugated nanoparticles. Hence, FTIR analysis was done to study the chemical properties of nanoparticles conjugated clindamycin hydrochloride and after knowing the functional groups its bonding nature with nanoparticle was also characterized.

4. RESULT AND DISCUSSION

4.1 Zeta size and Zeta potential

Clindamycin hydrochloride loaded PLA nanoparticles:

Blank –PLA, code L in table II was the normal sample without any conjugation called as blank PLA. The particle size observed was 43 nm taken comparison between PLA conjugated Clindamycin hydrochloride and blank PLA. But after conjugation of clindamycin hydrochloride with PLA nanoparticles, the size observed was 323.5 nm.

Clindamycin hydrochloride loaded PLGA nanoparticles:

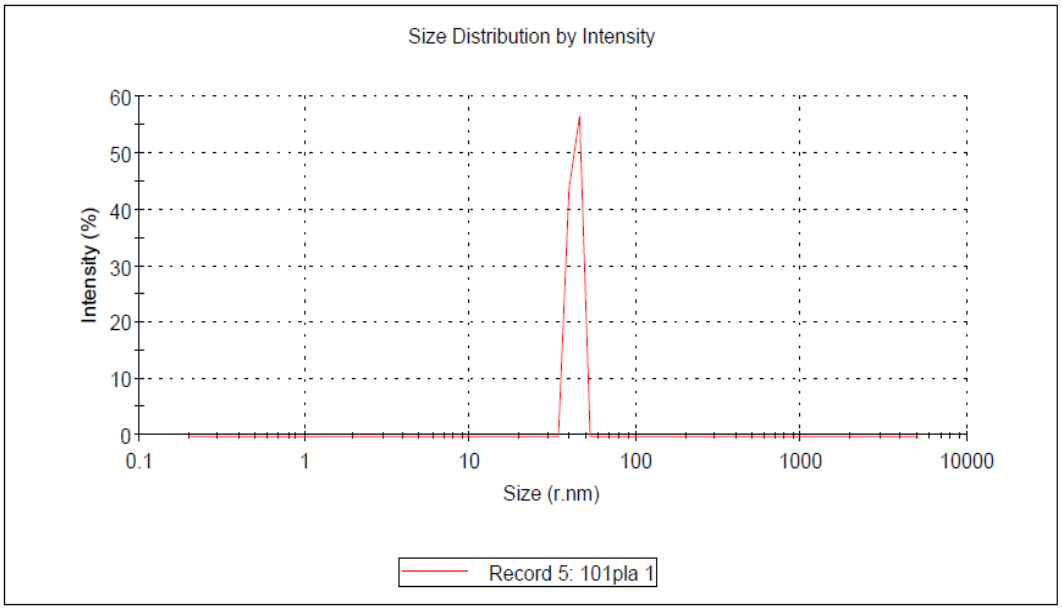
Blank- PLGA was the normal sample with particle size analyzed 178.6nm taken for comparison between PLGA conjugated Clindamycin hydrochloride and blank PLGA. When Clindamycin hydrochloride was conjugated with PLGA nanoparticle, the observed size was 45.64nm.

Table I: PLA/PLGA blank nanoparticles and PLA/PLGA nanoparticle with Clindamycin hydrochloride , their mean particle size, zeta potential.

Sl.No	Sample name	Code name	Mean Particle Size	Zeta Potential
1	Blank -PLA	L	42.93nm	-24.8mv
2	Blank- PLGA	G	258.3nm	-32.7mv
3	CLH+PLA	2	323.5 nm	-11.5 mv
4	CLH+PLGA	3	178.6nm	-17.5 mv

In this study Blank-PLA and Blank-PLGA were taken as standard for comparison of particle size and zeta potential of CLH conjugated PLA and CLH conjugated PLGA. From blank-PLA we found that its mean particle size was 42.93 nm but when we took consideration about CLH conjugated PLA its size increases to 323.5 nm from 42.93 nm. In case of blank-PLGA we

found that its mean particle size was 258.3 nm but it was interesting to see that when PLGA conjugated with CLH drug its size reduces to 178.6 nm. As such as particle size, zeta potential also plays a vital role by preventing drugs to aggregate. More -/+ the zeta potential more is the repulsion of nanoparticle so they can remain far apart without aggregation. It can be deduced from the table- II that blank-PLA and blank-PLGA having zeta potential -24.8 mv and -32.7 mv but after conjugation of CLH-PLA and CLH-PLGA these zeta potential value shifts to -11.5 mv and -17.5 mv respectively. Due to very high zeta potential nanoparticles will gain the characteristic to remain separated without aggregation that is very essential for drug delivery.



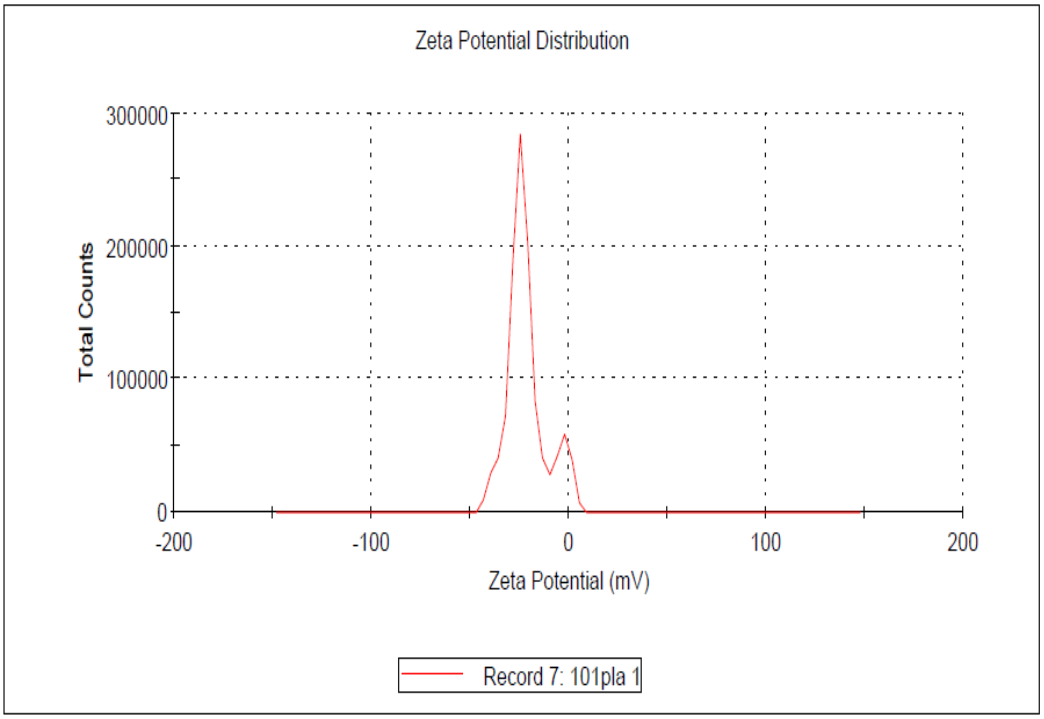
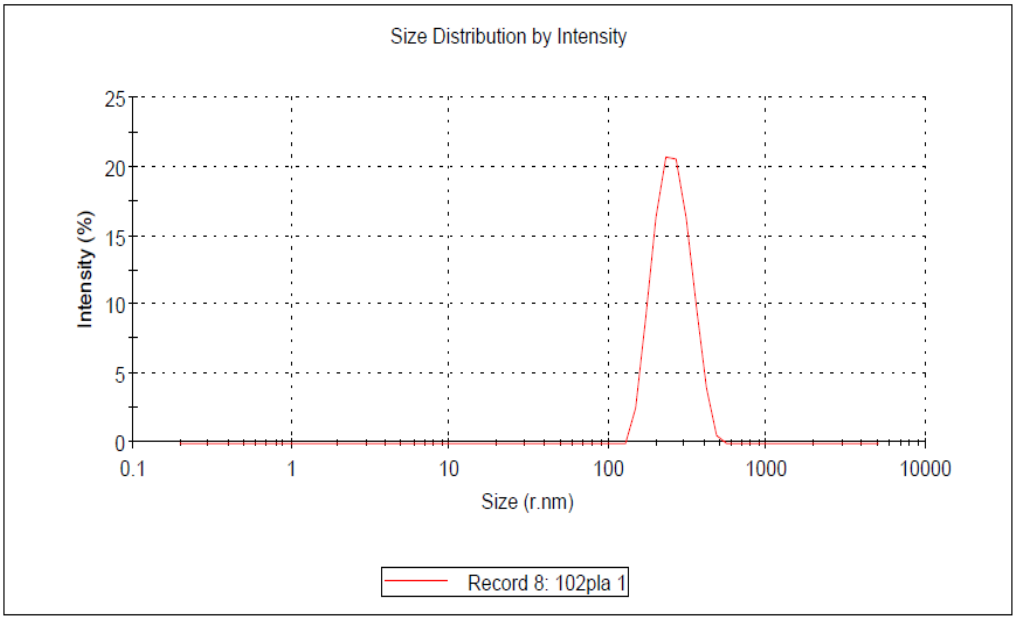


Fig.7.1: Sample code L showing the size (42.93nm) and potential (-24.8mv).



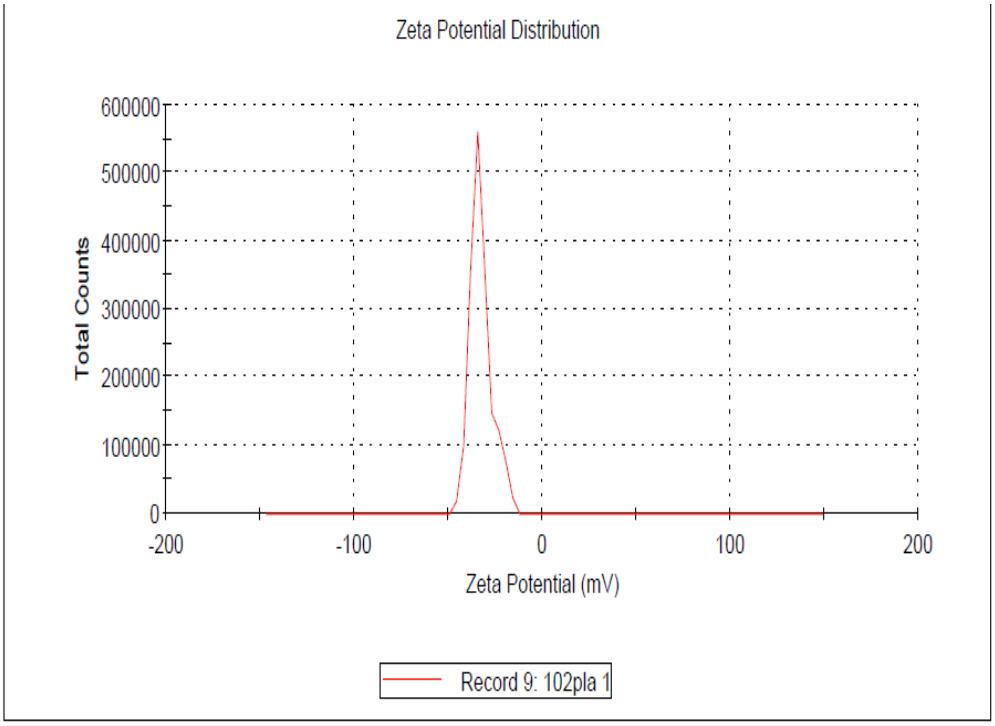
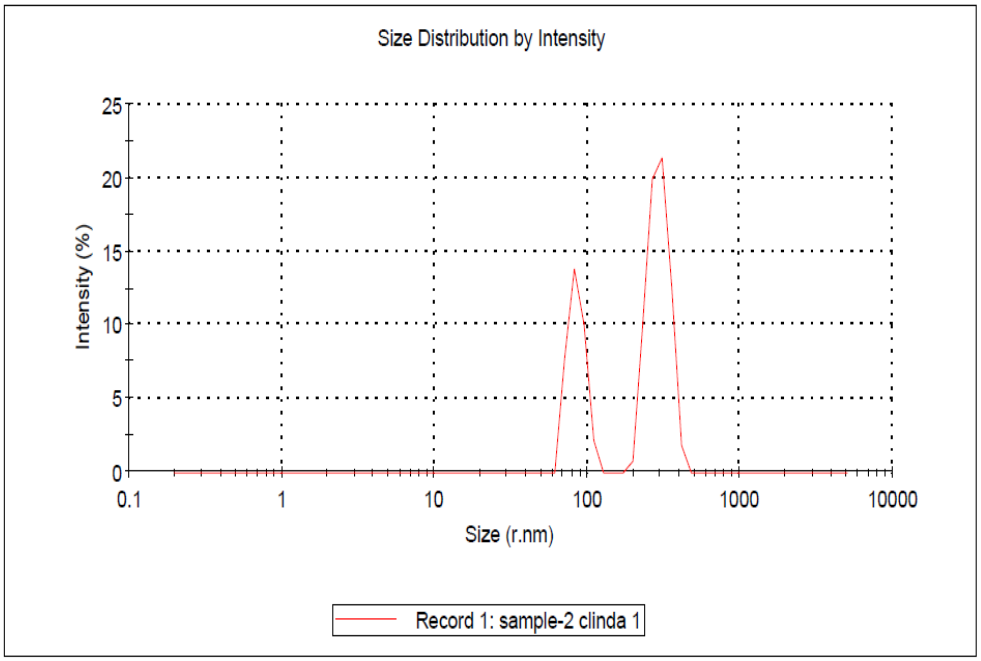


Fig.7.2: Sample code G showing the size (258.3nm) and potential (-32.7mv)



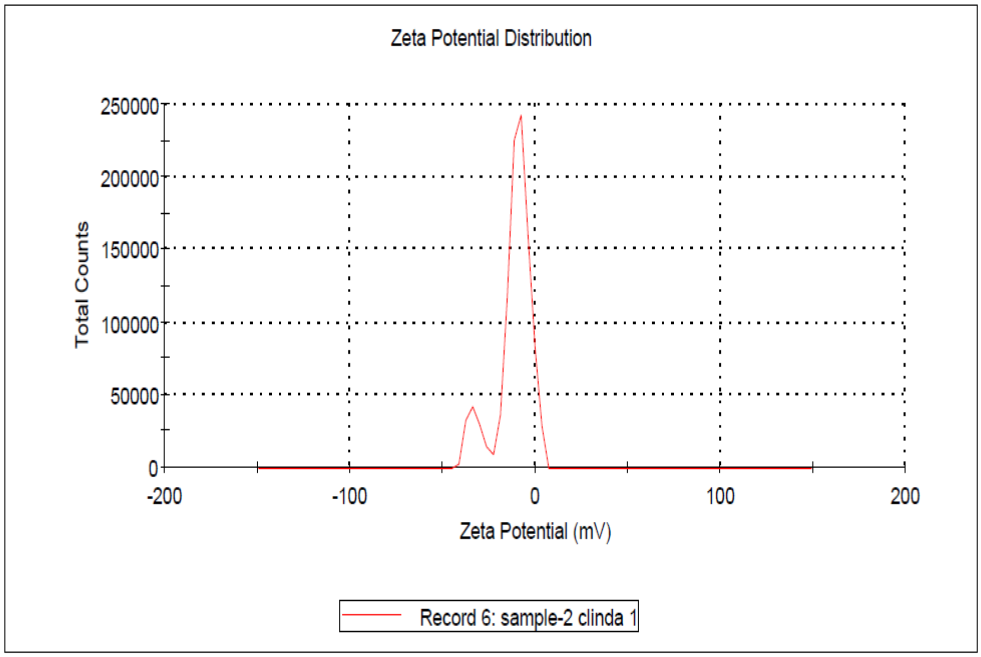
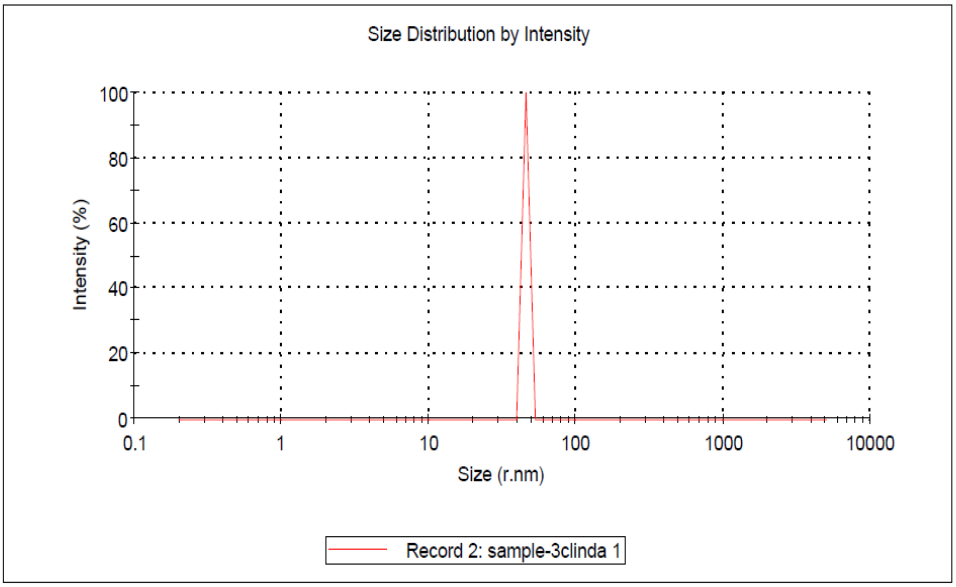


FIG 7.3: Sample code 2 showing the size (323.5 nm) and potential (-11.5 mv)



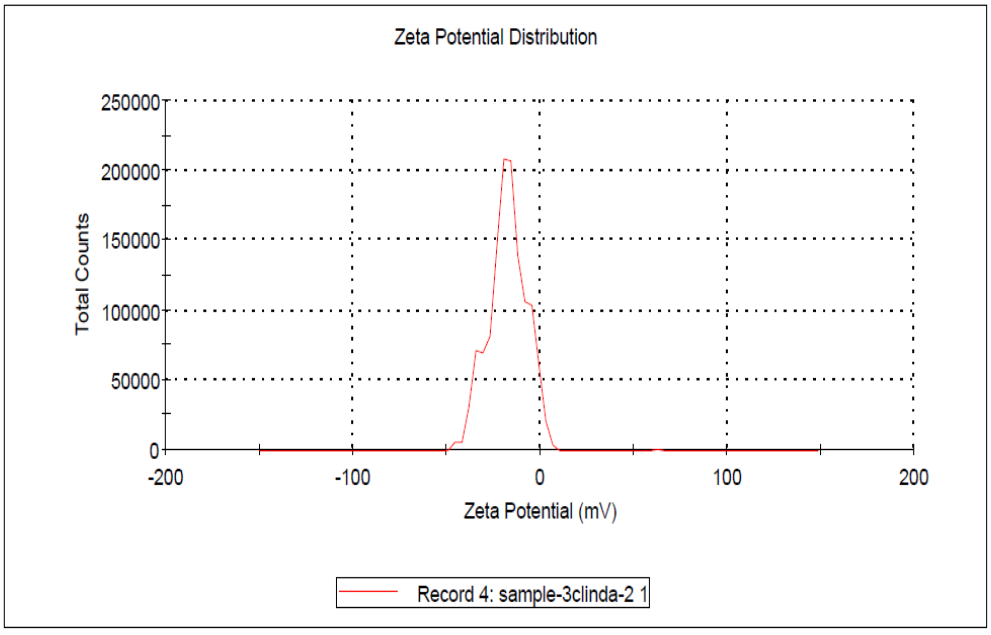


FIG 7.4: Sample code 3 showing the size (45.64nm) and potential (-17.5 mv)

4.2 Morphology

The morphology of these PLA and PLGA particles were spherical structures as resolute by using scanning electron microscope (SEM). Figure 8(A) is the structure of PLGA-CLH particles where as Figure 8(B) are structure of PLA-CLH particles. The surfaces of the particles were rough and rounded. It was reported that, when the ratio of the IAP to EAP was increased, the relative sizes of the pores also lean to increase (Nayak *et al.*, 2009).

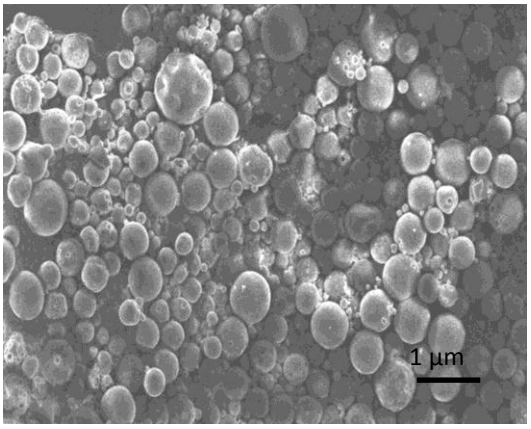


Fig 8 (A): Surface structure of PLGA-CLH

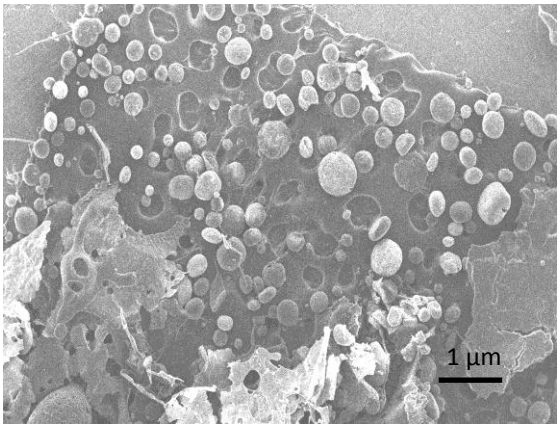
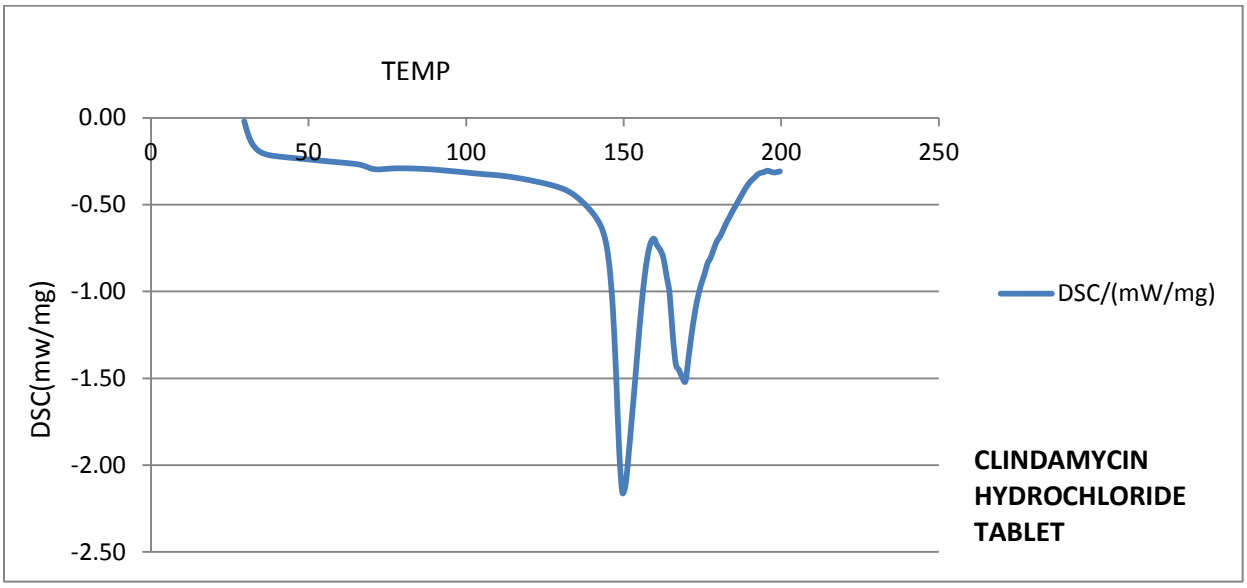
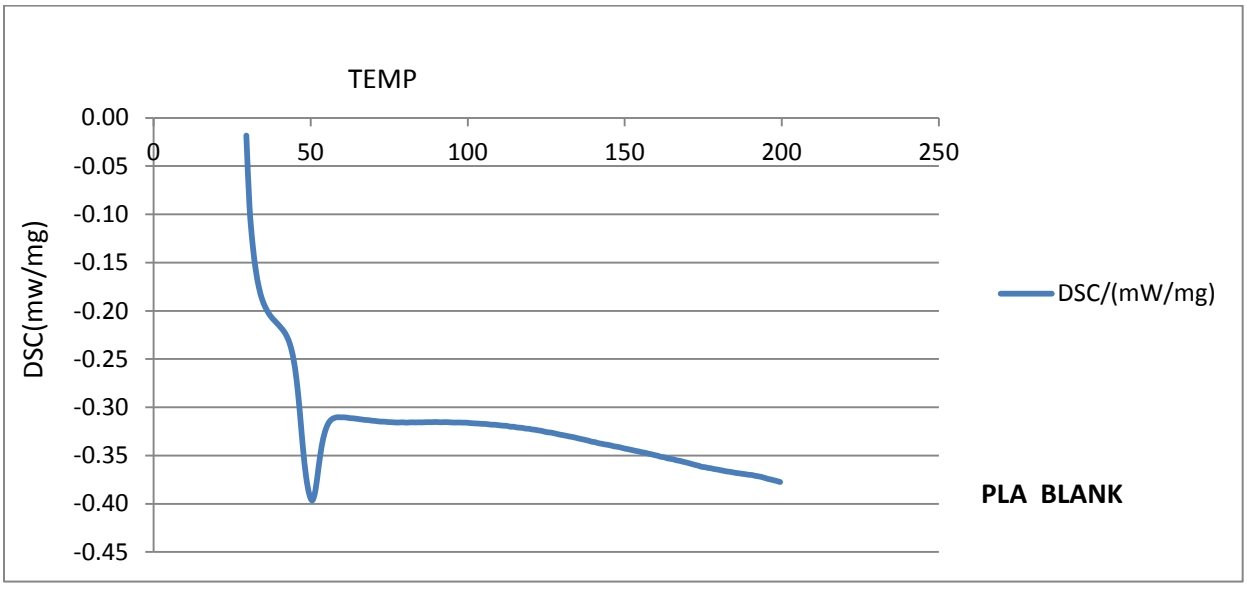


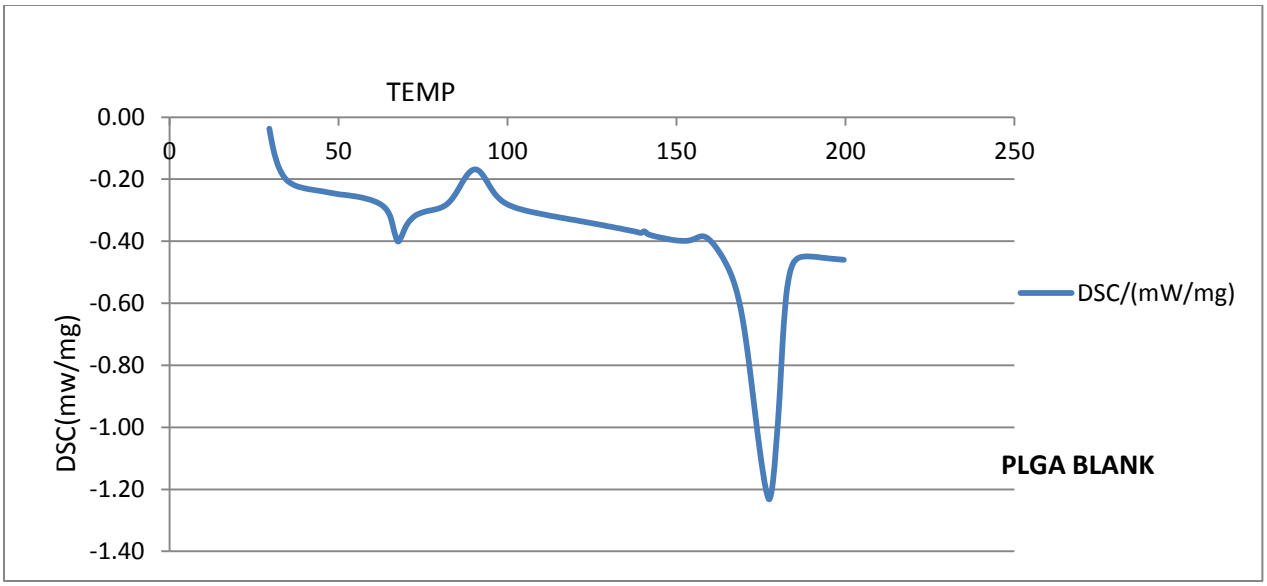
Fig 8 (B): Surface structure of PLA-CLH

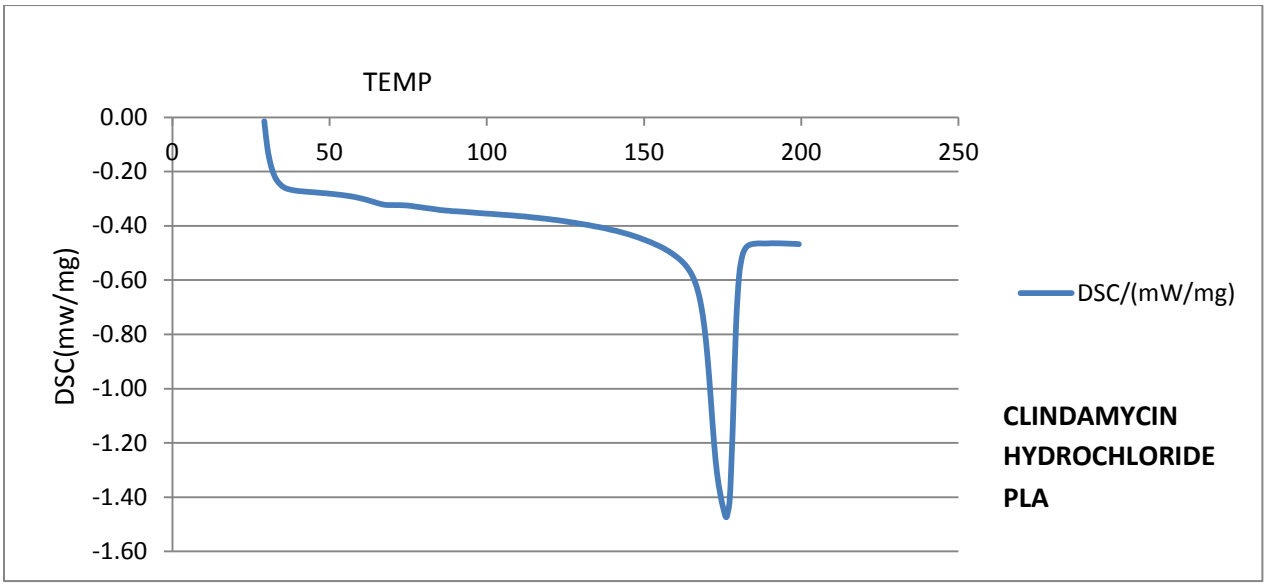
4.3 DSC studies

Figure 9 and Table III shows the values for the DSC data of blank PLA, blank PLGA, Clindamycin hydrochloride conjugated with PLA, Clindamycin hydrochloride conjugated with PLGA and the melting point of Clindamycin hydrochloride alone.






TEMP
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
-1.20
-1.40
0
50
100
150
200
250
DSC(mw/mg)
DSC/(mW/mg)
PLGA BLANK


TEMP
0.00
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-1.00
-1.20
-1.40
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200
250
DSC(mw/mg)
DSC/(mW/mg)
CLINDAMYCIN HYDROCHLORIDE PLA

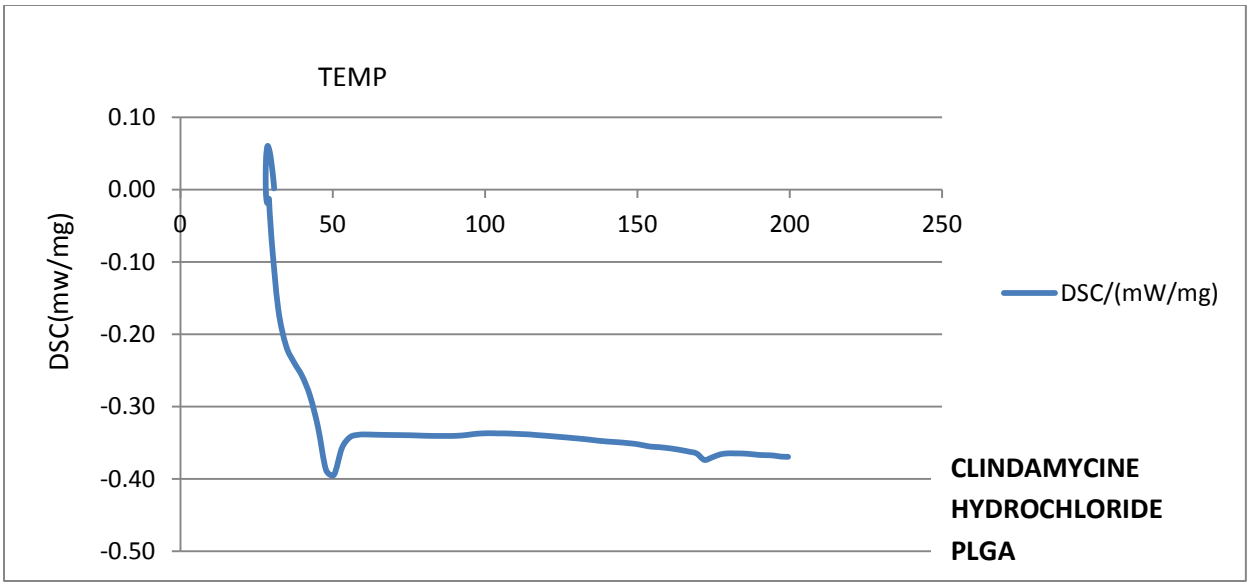


Fig 9: DSC data of Clindamycin hydrochloride tablet, blank PLA, blank PLGA, Clindamycin hydrochloride conjugated with PLA and Clindamycin hydrochloride conjugated with PLGA.

Table II: The glass transition temperature (T_g) obtained from DSC analysis of Clindamycin hydrochloride tablet, blank PLA, blank PLGA , Clindamycin hydrochloride conjugated with PLA nanoparticleand Clindamycin hydrochloride conjugated with PLGA nanoparticle.

Materials	Temperature(^{0}c)
Clindamycin hydrochloride tablet	**150**
Blank PLA	**50**
Blank PLGA	**170**
Clindamycin hydrochloride-PLA	**160**
Clindamycin hydrochloride-PLGA	**48**

This is a comparable study for blank PLA/PLGA nanoparticle and Clindamycin hydrochloride conjugated with nanoparticle. On DSC result it was found that Clindamycin Hydrochloride having glass transition temperature of 150^{0}C whereas blank PLA and PLGA polymer showed glass transition temperature at 50^{0}C and 170^{0}C respectively. But interestingly

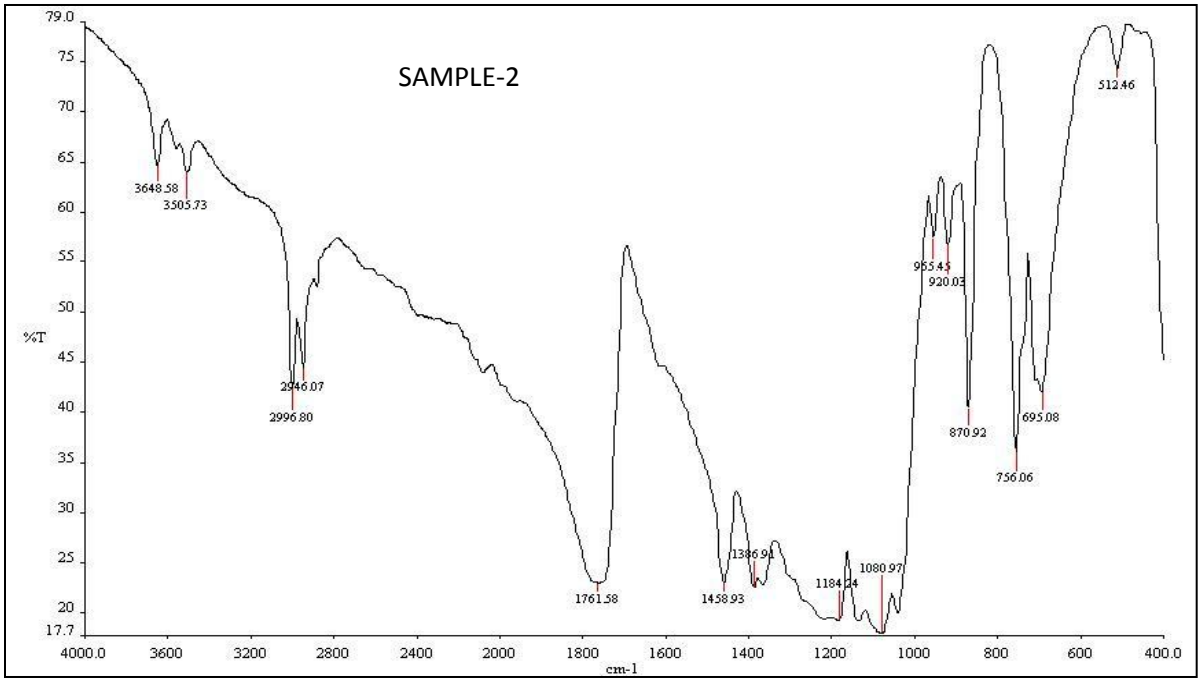
on conjugation of PLA to the Clindamycin hydrochloride its glass transition increases from 50^0c to 160^0c but it was observed that PLGA on conjugation with Clindamycin hydrochloride its glass transition temperature decreased from 170^0C to 48^0 C.

Thermal analytical studies of polymeric drug delivery system are significant since the processes used to their preparation are able to modify the organization of the polymer chains (Dubernet, 1995). Thermal analysis data (fig and table) showed a Tg for blank PLA around 50^0C, of 170^0C for blank PLGA, around 160^0C for clindamycin hydrochloride-containing PLA and 48^0c for clindamycin hydrochloride-containing PLGA. For lactic acid polymer, the Tg represents a measure of the polymer chain flexibility and indicates how the hydrolysis of the ester bonds will occur (Ford and Timmins, 1998).

The thermo gram of the drugs alone shows an endotherm corresponding to the clindamycin hydrochloride melting at 150^0 C. However such a peak is not visible in the thermo gram of PLGA nanoparticle containing drug. In this way it can be suggested that the drug can easily dispersed throughout the system (Ford and Timmins, 1989). It is a well known fact that through the determination of Tg it is possible to assess the drug dispersion within the carrier system and that the disappearance of the peak in thermograph is referred to the crystalline melting of drug. It indicates that the drug is homogeneously dispersed throughout the polymer matrix at an equal molecular level (Hariharan and price, 2002).

4.4 FTIR analysis

FTIR studies of Clindamycin hydrochloride conjugated PLA/PLGA nanoparticle were performed to characterize the chemical structure of drug conjugated nanoparticle. FTIR spectra of Clindamycin hydrochloride conjugated PLA /PLGA nanoparticle shown in figure10. A sharp peak band found at 1761.31 cm $^{-1\ for}$ PLA-CLH and another sharp shift at 1761.58 cm^{-1} was found for PLGA-CLH. These values lies between range of 1700-1800 cm $^{-1}$ so that this peak is attributed to C=O group. The shift at 3505.73 cm^{-1} is a sharp peak for PLA-CLH and a shallow peak found on the same region on for PLGA-CLH attributed to secondary amine stretching frequency (2^0 N-H group) as the value lies between 3475-3150 cm^{-1} range.



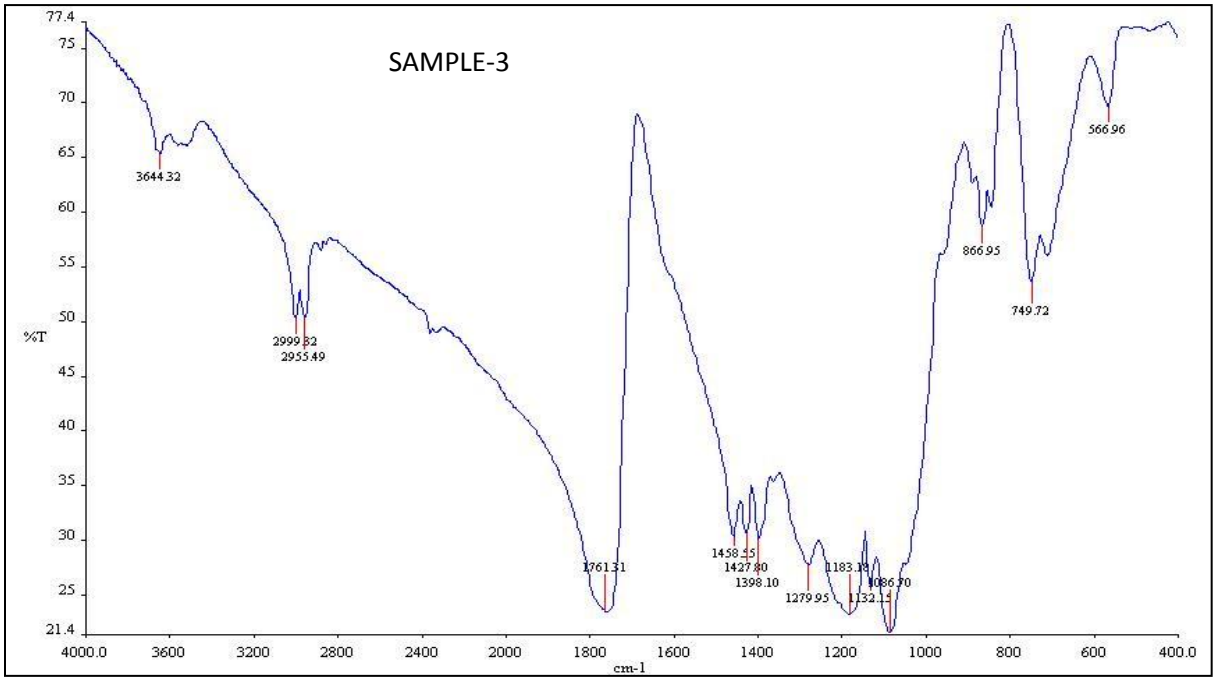


Fig 10: FTIR data analysis of PLA-CLH(sampl-2)and PLGA-CLH(sample-3) .

A sharp peak and low intense spectrum found for PLA-CLH which gives band at 3648.58 cm^{-1} and on for PLGA-CLH the band found at 3644.32 cm^{-1} as its range lies above the 3600 cm^{-1} there is no hydrogen bonding found and which stands for free OH group present. Another two peaks of 2946.07 cm^{-1} and 2996.80 cm^{-1} for PLA-CLH and for PLGA-CLH 2999.32 cm^{-1} and 2955.49 cm^{-1} giving two low intense peak, as the result lies nearby 3000 cm^{-1} so this is attributed to sp^3 hybridized CH stretching frequency. The strong intense peak of 1080.97 cm^{-1} for PLA-CLH and 1086.30 cm^{-1} stretching frequency for PLGA-CLH which ranges lies between 1260 cm^{-1} to 1000 cm^{-1} so it is attributed to C-O group.

As drug has secondary amine group on its general structure so it is found in FTIR studies that there is no alteration of secondary amine after conjugation with PLA/PLGA nanoparticle. But after conjugation PLA/PLGA some groups like C=O, OH those are abundant in PLGA/PLA nanoparticle and this functional group present in CLH drug is very less. It was also found by FTIR studies, that there is not much alteration in general structure of CLH drug because of OH group and it is present in free form without forming any hydrogen bond with any other group. CH group stretching band was also found through FTIR studies. As CH group is also present in CLH drug, there is no alteration of CH group in the conjugated drug. C-O bond is common in both the polymers and CLH drug so it has also no effect on to the drug's general structure. For further structural details of the compound investigation on various functional groups can be carried by NMR studies.

CONCLUSION

From all the above studies we concluded that PLGA nanoparticle shows maximum efficiency towards Clindamycin hydrochloride drug in comparison to PLA nanoparticle. From size analysis we concluded that the prepared PLGA-CLH samples were of nanoparticle range with a mean diameter of 178.6 nm and having zeta potential of -17.5 mv. From DSC analysis we concluded that PLGA nanoparticle on conjugation with CLH drug showed very low glass transition temperature of about 48^0C which is very close to our normal body temperature. Therefore, it can easily disperse in our body in comparison to present available drug in market without PLGA conjugated nanoparticle. Due to easy disperse in our body its bactericidal and therapeutic effect may be appear early within the same day compared to the conventional medicine which generally takes two to three days. For more convincing data regarding this *in vitro* drug release study through HPLC will be a necessary step. Due to lack of time scope this part could not be included as the part of the thesis. From FTIR data analysis we concluded that after conjugation with nanoparticle CLH has not shown very serious alteration in its general structure. For more appropriate structural analysis study on various functional groups by NMR studies could be beneficial. In conclusion, we have seen that the solubility, dispersion and properties of PLGA nanoparticle conjugated Clindamycin drug increases to be better used for drug delivery purpose.

ACKNOWLEDGEMENT

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