



## Biodegradable polymers: emerging excipients for the pharmaceutical and medical device industries.

Bhavesh Patel\*, Subhashis Chakraborty

Technical Service-Pharma Polymer & Services, Evonik India Pvt Ltd, Research Centre India (RCI), Krislon house-first floor, Opp. Marwah Centre, Sakivihar road, Sakinaka, Andheri (E), Mumbai 400 072, Maharashtra, India

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Review Article

### ABSTRACT

Worldwide many researchers are exploring the potential use of biodegradable polymeric as carriers for a wide range of therapeutic applications. In the past two decades, considerable progress has been made in the development of biodegradable polymeric materials, mainly in the biomedical and pharmaceutical industries due to their versatility, biocompatibility and biodegradability properties. The present review focuses on the use of biodegradable polymers in various therapeutic areas like orthopedic and contraceptive device, surgical sutures, implants, depot parenteral injections, etc. Biodegradable polymers have also contributed significantly to the development of drug-eluting stents (DES) used for the treatment of obstructive coronary artery disease, such as angioplasty. Biodegradable synthetic polymers have potential applications in orthopedic device fixation due to properties that impact bone healing, formation, regeneration or substitution in the human body. The present review also emphasizes areas such as the chemistry of polymer synthesis, factors affecting the biodegradation, methods for the production of biodegradable polymer based formulations, the application of biodegradable polymers in dental implants, nasal drug deliveries, contraceptive devices, immunology, gene, transdermal, ophthalmic and veterinary applications, as well as, the sterilization of biodegradable based formulations and regulatory considerations for product filing.

**KEY WORDS:** Biodegradable polymers, degradation, orthopedic implants, sterilization, drug eluting stents, tissue engineering

### INTRODUCTION

The term “Biodegradable polymers” is quite fascinating in the pharmaceutical and medical device community. In fact, this class of polymers has been known for more than a 100 years in the chemical industry but unfortunately

has not received much attention because of their tendency to degrade under normal environmental conditions. Interestingly, it was later realized that this was an advantage for designing a biodegradable implant which would avoid the necessity of a second surgical procedure required to remove the remnants of a previous implant, as was the case for metallic implants. The first FDA approved biodegradable product, Dexon<sup>®</sup> was a suture introduced in 1970 by Davis and Geck (1).

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\*Corresponding author: Technical Service-Pharma Polymer & Services, Evonik India Pvt Ltd, Research Centre India (RCI), Krislon house-first floor, Opp. Marwah Centre, Sakivihar road, Sakinaka, Andheri (E), Mumbai 400 072, Maharashtra, India, Tel: +91 98337 46909; Fax: +91 22 6691 6996, E-Mail: [bhavesh.patel@evonik.com](mailto:bhavesh.patel@evonik.com)

Since then, there has been a revolution in the medical device industry which has introduced several orthopaedic implants such as pins, screws, cranio-maxilla-facial plates and so on. After about two decades, in the 1990s, the pharmaceutical industry entered in to this new era of biodegradable polymers introducing depot injections. One of the most popular and commercially successful depot injection is Lupron<sup>®</sup>, manufactured by Takeda primarily used for the treatment of prostate cancer (2,3). Although there has been significant scientific, as well as, commercial progress in this area, the understanding of this technology remains limited to a few global companies. This is primarily because of the complexities involved in the polymer chemistry, depot injection formulation technology and high precision molding. There is tremendous scope for the adoption of these technologies by a greater number of pharmaceutical and medical device companies. This will help in extending the benefits of these novel technologies to larger patient populations world-wide, offering better patient compliance, especially in the case of chronic disease management.

Through understanding polymer degradation in the presence of hydrolyzing agents available in the biological systems, various novel drug delivery systems for sustained and targeted release have been formulated as depot injections for weekly or monthly administration as implants, microspheres, microcapsules, nanoparticles, *in situ* gels etc., (4). Properties such as flexibility, durability and biocompatibility have made these polymers the preferred vehicles to administer safely *in vivo* for human use in the form of medical devices such dental and orthopedic implants, inserts, sutures, drug eluting stents, and contraceptive devices (4). Considering the sincere approach of environment protection, future outlook in the area of biodegradable polymers seems to be quite promising.

The objective of this review is to present a consolidated view on the basic aspects

pertaining to polymer chemistry, polymer synthesis and optimal polymer selection for pharmaceutical and medical applications. This review also attempts to discuss factors influencing the biodegradation process, together with recent developments in the area of novel drug delivery systems such gene and cancer therapy, the application in dental and orthopedic devices during surgery, drug eluting stents, orthopedic devices, sutures for tissue and scaffold engineering, veterinary and nasal drug delivery using biodegradable polymers.

## CLASSIFICATION

Broadly, biodegradable polymers can be classified into natural or synthetic. Natural biodegradable polymers are proteins or polysaccharides e.g., alginate, chitosan, agarose and collagen or starch based derivatives which undergo enzymatic degradation in the body. Synthetic polymers contain hydrolysable linkages such as esters, amide, peptide, urea, urethane or anhydride in the polymeric backbone that easily hydrolyzes in the human body. Examples of such synthetic polymers include (i) polyesters such as poly lactic acid (PLA), poly glycolic acid (PGA), poly l-lactide co-glycolide (PLGA) co-polymer, poly  $\epsilon$ -caprolactone (PCL) (ii) poly anhydrides (iii) poly ethylene glycol and their derivatives (iv) poly vinyl alcohol (v) poly peptide and poly amide (vi) polyhydroxy alkonates (PHA) derivatives, etc., (5, 6). A broad classification of biodegradable polymers is shown in Table 1.

Polyester PLGA is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). It is currently considered the best biomaterial available for drug delivery with respect to design and performance. Poly lactic acid contains an asymmetric carbon which is typically described as the D or L form and sometimes as R and S form, in classical stereo chemical terms. The enantiomeric forms of the polymer PLA are poly D-lactic acid (PDLA) and poly L-lactic acid (PLLA).

**Table 1** Classification of biodegradable polymers (5-7)

Sr. No	SYNTHETIC BIODEGRADABLE POLYMER	NATURAL BIODEGRADABLE POLYMER
1	Polyesters (Polyhydroxy alkanooates, polycaprolactone, poly L-lactide, poly lactic acid, poly p-dioxanones, poly urethane, poly vinyl alcohol, nylon, Poly phosphor esters, Poly glycolic acid, Poly lactic-co-glycolic acid)	Proteins based (collagen, gelatin and albumin)
2	Polyethers (Poly ethylene, polytetra methylene and poly propylene glycols)	Polysaccharides (starch, dextran, hyaluronic acid, and chitosan)
3	Polyethylene (PE)	
4	Polycarbonates (PC)	
5	Polyphosphazenes	
6	Polyamide and Polyimide	
7	Polyacrylamide	
8	Polytetra fluoro ethylene (PTFE)	
9	Poly ortho esters (POE)	
10	Poly anhydrides poly [bis(p-carboxyphenoxy) propane-co-sebacic acid]	

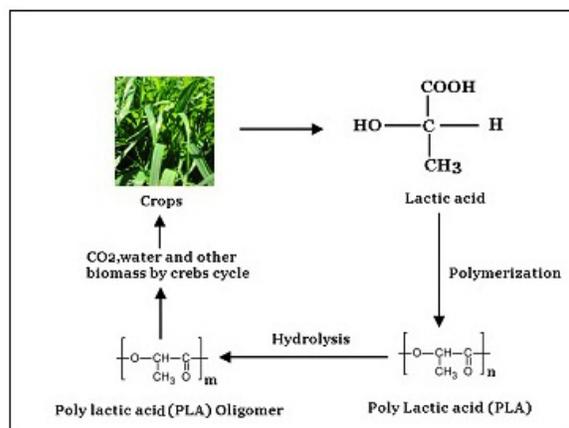
PLGA is generally an acronym for poly D, L-lactic-co-glycolic acid where D- and L- lactic acid forms are in equal ratio (7). Trade name and major global manufacturers of biodegradable polymer are shown in Table 2.

**Table 2** Trade name and some of global manufacturers of biodegradable polymers (8, 9)

Sr. No	COMPANY NAME	TRADE NAME
1	Galactic	Galacid®
2	Chronopol	Hepion®
3	Treofan	Treofan®
4	Mitsubishi Corporation	Ecoloju®
5	Biomer	Biomer® L
6	Evonik Industries	Resomer® and Lakeshore®
7	Dow Cargill	NatureWorks®
8	Toyota	Eco Plastic®
9	Mitsui Chemicals	Lacea®
10	Purac Biochem	L-PLA®
11	Shimadzu Corporation	Lacty®
12	Durect Corporation	Lactel®
13	BASF	Ecoflex®, Ecovio®
14	DuPont	Biomax®
15	Eastman Chemical company	Eastar Bio Ultra copolyester
16	Cereplast	Bioplastic resin
17	Procter and Gamble	Nodax®
18	PolyScience Inc	PLA-PGA and PCL co-polymer
19	MetaboliX Inc	Poly hydroxyl alkanooates (PHA)
20	Wako speciality chemical	PLA and PLGA

## Chemistry and synthesis

PLGA polymers are polyester based biodegradable polymers, manufactured synthetically from monomers like lactic acid, glycolic acid, dioxanone, tri-methyl carbonate

**Figure 1** Synthesis and biodegradation cycle of PLGA based biodegradable polymer

and  $\epsilon$ -caprolactone (see Figure 1).

When polymers are made of only one type of monomer, they are termed “homo-polymers” e.g., poly glycolic acid (PGA), poly lactic acid (PLA) and poly-dioxanone. If polymers are generated using a combination of monomers, they are termed as “co-polymers” e.g., poly (D, L-lactide co-glycolide) (PLGA), poly-(l-lactide-co-trimethylene carbonate), poly-(glycolide-co-trimethylene carbonate), poly-(glycolide-co-trimethylene carbonate-co-caprolactone) and so on.

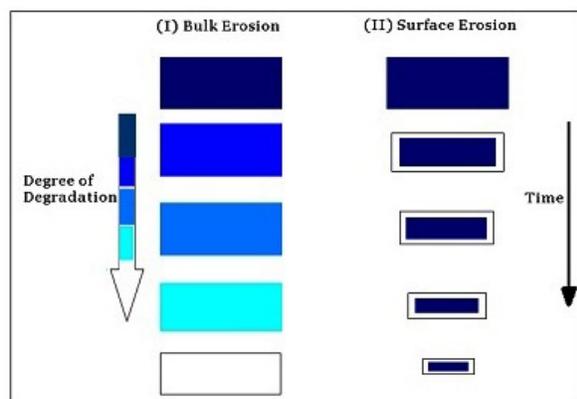
The basic monomer unit of PLA is lactic acid and is primarily produced in large quantities through bacterial fermentation of carbohydrates such as corn, sugar cane, whey, and so on, i.e., rich carbon sources. The majority of the fermentation processes are carried out using *Lactobacilli* species which offer high yields of lactic acid. After obtaining a monomer such as lactic acid, PLA can be further synthesized through a polycondensation reaction, or via ring-opening polymerization of cyclic diesters. The monomer’s concentration, ratio and sequence in the polymer and end group chemistry dictate their mechanical properties, degradation kinetics, solubility, thermal and rheological properties, and water uptake. Furthermore, the polymers can be tailored for controlled degradation through the addition of numerous functional groups including

carboxylic acids, esters, amides, anhydrides, and others. Synthetically, the polymers are prepared through ring-opening polymerization of the monomers in the presence of a catalyst. Post synthesis, the polymers are purified using supercritical carbon dioxide (for crystalline polymers) or aqueous precipitation from acetone (for amorphous polymers) in order to reduce the residual monomer content. The overall low residual monomer content and minimal impurities imparts high quality and stability to the final product (8).

### MECHANISM OF DEGRADATION

The term degradation designates the process of polymer chain cleavage which leads to a loss in molecular weight. Degradation induces the subsequent erosion of the material which is defined as mass loss of material. For biodegradable polymer, mainly two different processes of polymeric degradation can be proposed (1) Bulk erosion and (2) Surface erosion. The difference between the two degradation mechanisms is shown in Figure 2.

Biodegradable polymers contain a hydrolysable backbone which is susceptible to hydrolysis or enzymatic degradation in the cell environment by bulk erosion and surface erosion. The former involves degradation all over the cross section because of water penetration followed by slow scissions of long polymer chains, while the latter is a surface phenomenon. The rate of



**Figure 2** Degradation mechanism of biodegradable polymer

surface erosion depends on the area exposed to the hydrolytic environment and the rate of bulk erosion depends on the crystalline nature and porosity of the polymer matrix. The drug release pattern from the polymer matrix of microspheres and implants follow the following three step process, that is an initial burst release due to dissolution of surface drug, followed by slow release due to degradation-dependent network relaxation that creates sufficient free volume for drug dissolution, and finally an accelerated drug release. The accelerated drug release is triggered by the acidic micro-environment within the particle generated by the autocatalytic degradation of the polymers into lactic and glycolic acids. Overall, the release profile is dependent on the nature of the drug, polymer degradation rate, water permeability and drug-polymer matrix interaction. Once degraded, the solubilized monomers/oligomers such as lactic acid and glycolic acid are excreted by the kidney and finally metabolized into carbon dioxide and water through the tricarboxylic acid (Kreb's) cycle (10-12).

Various advanced analytical techniques are generally useful to understand and illustrate the polymer degradation mechanisms. A simple but relatively effective technique to characterize the degradation of a polymeric matrix is by recording the loss of mass during the degradation process. In most cases the main parameter used for monitoring degradation are changes in molecular weight, crystallinity, pH and thermal changes inside the core. Qualitative evaluation of polymer degradation can be performed using scanning electron microscopy (SEM) and atomic force microscopy (AFM) which offer insight into the external and internal morphology of degradable polymer systems. Other methods determine the molecular weight reduction of the polymer by means of gel permeation chromatography (GPC) or by intrinsic viscosity. For the changes that degradable polymers undergo during degradation, thermal analysis such as

differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA) are somewhat beneficial. Some of the more advanced techniques that are useful for the studying of polymer degradation are the determination of the molar fraction of the monomer using nuclear magnetic resonance (<sup>1</sup>H NMR), changes in crystallinity using wide angle x-ray diffraction (XRD), determination of monomer release using HPLC, end-group analysis using FT-IR and the study of polymer structure and degradation using raman scattering (12).

### **MARKET ANALYSIS FOR PLGA BASED FORMULATION**

The worldwide manufacturing capacity of biodegradable polymers (natural and synthetic) has grown dramatically since the mid-1990s. In 1995, they were primarily produced on pilot plant scale with total worldwide capacity accounting for not more than 25,000 to 30,000 tonnes. In 2005, the global capacity for biodegradable polymer had increased to around 360,000 tonnes and continues to show an upward trend (9).

In March 2012 Global Industry Analyst, Inc released a comprehensive global report on the market for biodegradable polymers forecasting that it is bound to reach 2.44 billion pounds by year 2017. The ever increasing demand in packaging, the largest end-use market, growing environmental concerns, soaring petroleum costs, launch of new types of biodegradable polymers, concerns over depleting landfills, and a shift towards renewable sources from traditional sources of fossil fuel are the key factors driving the demand for biodegradable polymers. Markets could further grow through technological innovations, emerging applications, increased regulations to prohibit packaging waste and disposal at landfills and improvements in infrastructure (13).

The major global players using biodegradable polymers in the pharmaceutical industry are mainly multinational companies such as Abbott, Astra-Zeneca, Takeda, Novartis, Astellas and Sanofi Aventis. A review of the sales data of biodegradable formulations (such as depot injections or medical devices) manufactured by the above key pharmaceutical companies indicates revenues of approximately 4.2 billion Euros generated to the year 2010. Out of the total revenue of 4.2 billion Euros, Janssen-Cilag has 72% of the market and the rest is split between other customers. There is an increasing trend in the growth of dosage forms and the total volume of dosage forms was 316 kilograms in 2007 increasing to 374 kilograms in 2009, i.e., at a rate of 9% increase per annum. Fragmenting the volume of each individual dosage forms shows that Risperidone has the highest market share at 73% followed by Leuprolide acetate with a 14% market share. The remaining dosage forms were formulations of Goserelin, Buserelin and Octreotide (14).

### **EMERGING TRENDS IN FORMULATION DEVELOPMENT USING BIODEGRADABLE POLYMERS**

There is a continuing increase in the number of drugs, drug therapies and diseases which require different kinds of formulations using novel manufacturing technologies and modified release kinetics. There is no single polymer that can satisfy all these requirements. Therefore the last 30 years has seen tremendous advances in area of biodegradable polymer. The use of biodegradable polymers in a formulation can aid in extending the release of the drug for a considerable period of time (weeks to months) and removes the need to remove device from the patient at the end of the treatment period. In addition, they also offer advantages in terms of targeted delivery of the drug and stabilization of the drug molecules in polymeric matrix (15-18). A list of some commercial formulations manufactured using PLA/PLGA polymers are shown in Table 3.

**Table 3** PLA/PLGA based commercial formulation (19-21)

PRODUCT NAME	DOSAGE FORM	MANUFACTURER	ACTIVE	DURATION MONTHS
Decapeptyl®	Microparticle	Ferring	Triptorelin acetate	1
Decapeptyl® SR	Microparticle	Ipsen-Beaufour	Triptorelin acetate	1, 3, 6 (PLGA)
Zoladex®	Implant	AstraZeneca	Goserelin acetate	1, 3 (PLGA)
Lupron Depot®	Microparticle	Takeda-Abbott	Leuprolide acetate	1, 3, 4 (PLGA)
Sandostatin LAR®	Microparticle	Novartis	Octreotide acetate	1 (PLGA)
Nutropin® Depot	Microparticle	Genentech	Somatropin (hrGH)	1,2 (PLGA)
Profact® Depot	Implant	Sanofi-Aventis	Buserelin acetate	2, 3 (PLGA)
Suprecur® MP	Microparticle	Sanofi-Aventis	Buserelin acetate	1
Eligard®	Implant	Sanofi-Aventis	Leuprolide acetate	1, 3 (PLGA)
Luproge®	Liquid	MediGene AG	Leuprolide acetate	1
Trelstar® Depot	Microparticle	Watson	Triptorelin pamoate	1 (PLGA)
Trelstar® LA	Microparticle	Watson-Debio	Triptorelin pamoate	1, 3 (PLGA)
Arestin®	Microparticle	OraPharma	Minocycline HCl	0.5
Atridox®	Implants	CollaGenex	Doxycycline	0.25 (PLA)
Risperdal® Consta	Microparticle	Johnson & Johnson	Risperidone	0.5 (PLGA)
SMARTShot B12	Microparticle	Stockguard	Vitamin B12	4, 8
Vivitrol®	Microparticle	Alkermes	Naltrexone	1 (PLGA)
Revalor®-XS	Implant	Intervet	Trenbolone/estra diol	6
Ozurdex®	Implant	Allergan	Dexamethasone	1
Gliadel®	Implants	MGI Pharma	Carmustine	20

## VARIOUS APPROACHES TO DEVELOP BIODEGRADABLE POLYMER BASED FORMULATIONS

### Single emulsion (w/o or o/w) technique

Lakshmana *et al.*, proposed a simple emulsification process followed by solvent evaporation for the preparation of sustained release microspheres containing Aceclofenac. Using this method, microspheres were prepared by dissolving the polymer and the drug into a common solvent such as dichloromethane followed by adding this oil phase into the higher volume of aqueous phase containing emulsifying agent, PVA (0.1 to 1% w/v) under continuous stirring for a specified period of time and revolutions per minute in a propeller stirrer. This mixture was then immediately transferred to a magnetic stirrer for complete solvent evaporation. The microspheres were collected through filtration, washed with de-ionized water and dried in vacuum desiccators and then further characterized and the

microsphere morphology analysed (22). Although this is a simple procedure it may not be suitable for proteins and peptides which may be denatured in the presence of organic solvents. It is also important to note that the encapsulation efficiency achieved by this process is quite low and therefore may not be a practical approach for manufacturing (23-24).

### Multiple (double) emulsification (w/o/w or o/o/w) technique

The microencapsulation process in which emulsification (single or multiple) is followed by the removal of the solvent has been widely used for the preparation of PLGA based microspheres and microcapsules.

### w/o/w multiple emulsification

In general, this method is used for the hydrophilic drug moieties which are easily soluble in aqueous media. First, an aqueous phase containing dissolved drug (~ 0.5 to 1 ml) is emulsified into an oil phase (containing a PLGA polymer) (~ 5 to 10 ml) using a high speed homogenizer. Suitable emulsifiers or surfactants such as Poloxamer 407 or 188 (~2% w/v) or Span 20 (1% to 20% v/v) can be incorporated during the initial emulsification process. Then the initial emulsion is added to a greater volume of different concentrations of poly vinyl alcohol (0.1 to 1% w/w) (~ 200 to 300 ml) continuously stirring using a mechanical stirrer to form a w/o/w multiple emulsions. Homogenization is continued for a few hours at room temperature until the solvent has completely evaporated. The microparticles that are generated are collected through suitable size filtration and are further washed with purified water and dried overnight at room temperature (25-27). Giovagnoli *et al.*, used a similar method to prepare microspheres of Capreomycin molecules using PLGA (50:50 ratio, Mw: 10 kDa) for pulmonary delivery (28), as did Herman and Bodmeier who prepared microspheres of Somatostatin using PLA (Mw = 6,000 Da) using this method.

The above studies showed that using various salts such as NaCl or CaCl<sub>2</sub> in the internal or external aqueous phases affected directly the drug release pattern because of the formation of micro pore like structures in the microsphere (29). Crotts and Park prepared microspheres of bovine serum albumin (BSA) using PLGA (75:25 ratio, Mw: 82,000 D) and studied the effect of the inner aqueous phase volume on the morphology and porosity of microspheres, under the assumption that the volume of the inner phase is directly related to the rate of solvent removal. It was found that at 5.6% inner aqueous volume fraction, dense and nonporous microspheres were formed while at 22.7% more porous microspheres were generated (30). Pandey *et. al.*, prepared nanoparticles encapsulating the anti-tuberculosis drugs Isoniazid and Rifampicin incorporating PLGA (50:50 ratio). They observed effective encapsulation of both drugs (~ 60%) and a prolonged blood circulation and anti-tubercular (TB) effect up to 9-11 days suggests that drug therapy based on PLGA nanoparticles can be capable of reducing dosing frequency (31).

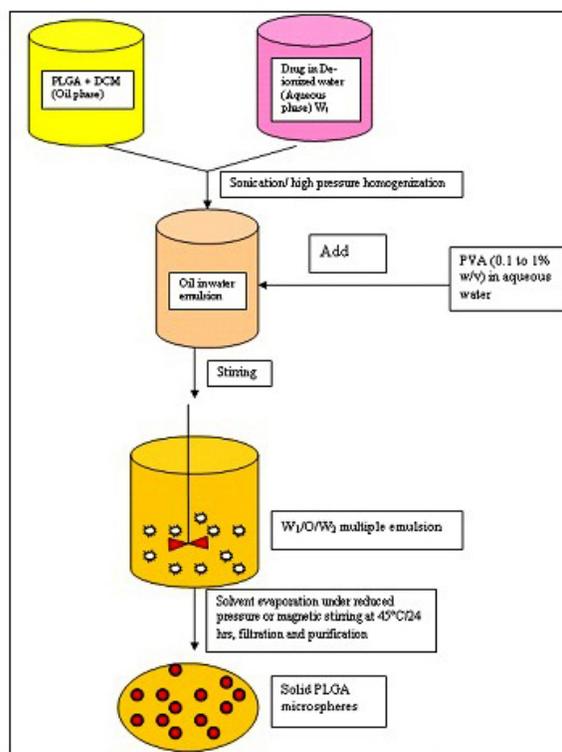
### ***o/o/w* multiple emulsification**

In general, the method of *o/o/w* multiple emulsification is suitable and, has been widely used, for lipophilic drugs. PLA or PLGA is dissolved in an oil phase (O<sub>2</sub>) such as dichloromethane (~ 5 ml) together with an emulsifier e.g. Labrafil as a lipid vehicle. The primary *o/o* emulsion is prepared by adding olive oil (O<sub>1</sub>) (~ 0.2 to 1 ml) into a second oil phase such as dichloromethane and then the mixture is sonicated using a probe type sonicator for ~30 seconds. Finally, the primary emulsion is re-emulsified into distilled deionized water containing PVA (0.1 to 1% w/v; ~ 100 ml) using a vigorous homogenization process for 3 minutes. After complete solvent evaporation the microspheres are collected by centrifugation and washed with distilled de-ionized water (32). A schematic diagram of multiple emulsification process is shown in Figure 3. A list of drugs manufactured

using emulsification based solvent evaporation are shown in Table 4.

### **Phase separation (co-acervation) method**

Phase separation, also known as a co-acervation technique, is a process focused on the preparation of microspheres through non-ionic liquid-liquid phase separation techniques. Phase separation methods consist of decreasing the solubility of the encapsulating polymer by adding a third immiscible component to the polymer solution. Similar to a solvent evaporation technique, this method is also used for both hydrophilic and hydrophobic drugs. Water-soluble drugs such as peptides and proteins are dissolved in water and dispersed in the polymer solution to make a *w/o* emulsion. Hydrophobic drugs, such as steroids, are either solubilized or dispersed in the polymer solution.



**Figure 3** Schematic illustration of multiple emulsifications followed by solvent evaporation method

**Table 4** List of drugs encapsulated using emulsification based solvent evaporation techniques

No	NAME OF DRUG	POLYMER USED	PREPARATION METHOD	REF
1	Capreomycin Sulfate	PLGA 50:50 (Mw = 10 kd)	w/o/w multiple emulsion	(28)
2	Bovine serum albumin	PLGA 75:25 (Mw = 82,000D)	w/o/w multiple emulsion	(30)
3	Isoniazid + Rifampicin	PLGA 50:50 (Mw = 25,000D)	w/o/w multiple emulsion	(31)
4	Labragfil® M 1944	PLA Mw = 2kDa	o/o/w multiple emulsion	(32)
5	Triptoreline	PLGA 50:50	w/o/w multiple emulsion	(33)
6	Bovine Superoxide Dismutase	PEG 6000	s/o/w multiple emulsion	(34)
7	Ganciclovir	PLGA 65:35 Mw = 70,000D	o/w emulsion	(36)
8	Somatostatin	PLA Mw = 6,000 D	w/o/w emulsion	(37)
9	Insulin	PLGA 50:50	o/o emulsion	(38)
10	Bovine serum albumin	PLGA 50:50 Mw = 13,600D	w/o/w multiple emulsion	(39)
11	Diclofenac Sodium	PLGA 50:50 Mw = 34,000D	o/w emulsion	(40)
12	5- Fluoro uracil	PLA Mw = 3,400 D	o/o emulsion	(41)
13	ABT 627 (NCE)	PLGA 50:50 Mw = 34,000D	o/w emulsion	(42)
14	Risperidone	PLGA 75:25	o/w emulsion	(43)
15	Dexamethasone	PLGA 50:50 Mw = 60,000D	o/w emulsion	(44)
16	Leuprolide Acetate	PLA and PLGA	o/o/w multiple emulsion	(45)
17	Doxorubicin	PLGA 50:50 Mw = 13,000D	o/w emulsion	(46)
18	Campothecin	PLGA 50:50 iv: 0.32-0.44 dl/g	o/w emulsion	(47)
19	Octreotide Acetate	PLGA 50:50 Mw = 53,600D	w/o/w multiple emulsion	(48)
20	Insulin	PLA Mw = 17,000D and PLGA 50:50 Mw = 14,100D	w/o/w multiple emulsion	(49)
21	Isoniazid+ Rifampicin	PLGA 50:50 Mw = 2,500D	Double emulsification	(50)

Organic non-solvents such as liquid paraffin, silicone, coconut or sunflower oil is then added to the solvent system (containing both the drug and polymer) under continuous stirring to extract the polymer solvent from the mixture. As a result, the polymer is subjected to phase separation forming very soft coacervate droplets (size controlled by stirring) which entrap the drug. This system is then added to a large quantity of another organic non-solvent such as formaldehyde or glutaraldehyde to harden the micro-droplets and form the final microspheres which are collected by washing, sieving, filtrating/ centrifuging, and finally drying (51-56). Thus, the co-acervation process includes the following three steps: (1) phase separation of the coating polymer solution by adding a third component (2) removal of the polymer solvents and adsorption of the coacervate around the drug particles and (3) solidification of microparticles and collection of the microspheres by washing, filtrating/ centrifuging and freeze drying. In a phase separation method, the rate of adding the first non-solvent must be controlled to ensure that the polymer solvent is extracted slowly so that

the polymer has sufficient time to precipitate and coat evenly onto the drug particle surface during the co-acervation (57).

Jayan *et. al.*, developed gelatin microspheres containing salbutamol sulphate using a co-acervation phase separation method utilizing temperature change. Gelatin was selected as a water-soluble naturally occurring biodegradable polypeptide which is easily hydrolyzed by proteolytic enzymes present in the body. The microspheres obtained were uniform and spherical in shape with mean particle size around 12.58  $\mu\text{m}$  providing sustained drug release over a period of 8 1/2 hours (58). Surendiran *et. al.*, developed microspheres from a gelatin-carbopol polymeric mixture using ibuprofen as the model drug using a similar method where microspheres with a high amount of carbopol showed more sustained drug release when compared to microspheres that contained a high amount of gelatin (59). These studies provide a basis for selecting suitable excipients for the design of sustained release biodegradable microspheres.

### Supercritical CO<sub>2</sub> anti-solvent precipitation method

Submicrometer-sized and nano-sized particles can be designed using various supercritical fluid (SCF) methods. A supercritical fluid can either be a liquid or gas and is used above its thermodynamic critical point of temperature and pressure. Most commonly used SCFs are carbon dioxide (CO<sub>2</sub>) and water (60). In this process, the hydrophilic drug is dissolved in a common solvent and injected in supercritical CO<sub>2</sub> with an ultrasonic field for enhanced molecular mixing. Supercritical CO<sub>2</sub> rapidly extracts methanol leading to the instantaneous precipitation of drug nanoparticles which are encapsulated in PLGA polymeric microspheres using a non-aqueous anhydrous solid-oil-oil-oil (s/o/o/o) technique. In this technique, PLGA is dissolved in dichloromethane (DCM) solvent into which drug nanoparticles are added continuously whilst stirring. In the next step, a small amount of silicone oil is added to the mixture resulting in the precipitation of PLGA in the form of the drug encapsulated in microspheres. Finally, the suspension is added to a large quantity of precooled hexane to extract the DCM and silicone oil.

Thote *et. al.*, developed PLGA (50:50 ratio, inherent viscosity: 0.39 dl/g) nanoparticles (150 - 200 nm) containing dexamethasone as the model drug using a supercritical CO<sub>2</sub> method. Interestingly, *in vitro* dissolution of the microencapsulated nanoparticles showed sustained release over a period of 700 hours without an initial burst release (61-64). Jordan *et. al.*, developed PLGA (50:50 ratio, inherent viscosity: 0.16-0.24 dl/g) based sustained release microspheres of human growth hormone using the SCF method for subcutaneous injections. The sustained release effect was observed for 14 days with minimal burst release. The formulation was well tolerated by both rats and monkeys without any evidence of subcutaneous inflammation or chronic inflammatory response (65). Salmaso *et. al.*, prepared PLA (Mw: 102 kDa) nanoparticles (200-400 nm) of Nisin as the model drug using

semi-continuous compressed CO<sub>2</sub> anti-solvent precipitation wherein the drug was released in the active form and the antibacterial activity was maintained up to 45 days (66).

Kluge *et. al.*, prepared PLGA micro- and nanocomposites (50:50 ratio) using supercritical fluid extraction of emulsions (SFEE) for the delivery of lysozyme. Particle sizes of around 100 nm and encapsulation efficiency of 48.5% was observed (67). Similarly, Lee *et. al.*, prepared PLA based microparticles using supercritical anti-solvent using an enhanced mass transfer (SAS-EM) process wherein drug encapsulation efficiency achieved was 83.5% and the drug release was controlled for more than 30 days. As compared to conventional SAS processes, SAS-EM process has major advantage of effective residual organic solvents removal (68).

### Spray/freeze drying

When freeze drying a solution of PLA or PLGA it is first dissolved in organic solvents which are further emulsified into an aqueous PVA solution using a homogenization process. This emulsion is then immediately added drop by drop to liquid nitrogen. The water and organic solvent is removed from the frozen sample under vacuum at 0°C, leaving behind the dry hollow PLA or PLGA particles. Similarly, when spray drying an organic solution that contains the polymer and a suitable surfactant such as dimethyl carbonate it is atomized using a coaxial spray nozzle under pressure. Then the sprayed particles are collected in liquid nitrogen and dried under vacuum at room temperature. In order to encapsulate the drug, these hollow PLA particles are dispersed into an aqueous solution of the drug by placing the solution in an ultrasonic bath for several seconds. Micro capsules are finally formed after stirring with the plasticizing solution (DCM/CO<sub>2</sub> in excess). Later the solution is washed through centrifugation/dispersion with water and then dried under vacuum (69). Christopher *et. al.*,

prepared spray dried nanoparticles of ovalbumin using a PLGA (50:50 ratio) containing an antigen carrier device for vaccine delivery. Electron microscopy confirmed that the cross-penetration of the microencapsulated antigen occurred quite easily wherein lysosomes were retained within the endosome for up to 3 days (70).

### The laser ablation method

The laser ablation method involves the production of microparticles by cutting a stream of drug-polymer-solvent solution using a pulsating laser, powerful enough to vaporize at intervals, thus producing continuous beads of micron-size particles. In this process, the PLGA polymer is dissolved in an organic solvent such as DCM and the solution is continuously pumped through a glass capillary with an opening of 10-100  $\mu\text{m}$ . A modulated laser constantly fires at the ejected stream to cut it into discrete droplets which are then collected in a large quantity of a PVA solution. This is then left overnight to harden the microparticles by extraction of DCM. The extracted particles are then collected by ultracentrifuge and dried or lyophilized to obtain the final product (71). There are only a few studies demonstrating this approach, probably because of the lack of access to the necessary equipment.

### Micro and nano fibrous scaffolds

In tissue engineering, an ideal scaffold should stimulate the growth of a natural extracellular matrix (ECM) in order to support cell attachment and guide three-dimensional (3D) tissue formation. PLGAs work as superior biodegradable candidates satisfying the needs of various tissue repairs. Pores should also be large enough to facilitate the growth of tissue cells (72).

Jifu *et al.*, prepared PLGA based macroporous and nanofibrous scaffolds together with salt particles using a phase separation technique.

The scaffold was prepared using a PLGA polymer (50/50) casted into a screw-cap. Sodium chloride particles with diameters of 200–450  $\mu\text{m}$  as porogens were inserted into the nanofibrous matrixes (72). After 7 days the cells were observed to show good morphology indicating their viability for tissue repair.

## IMPLANT PREPARATION TECHNIQUES

### Solvent casting and compression molding

Biodegradable implants, particularly PLGA- or PLA- based implants can be prepared using solvents. A PLA or PLGA based polymer and drug mixture is dissolved in an appropriate solvent (e.g., dichloromethane, trichloromethane or acetone) in the desired proportion, and the solvent is cast over a teflon mold at room temperature or slightly higher temperature and finally vacuum dried to remove the residual solvent. The resultant structure is a composite material of the drug together with the polymer. The solvent cast material is then compressed into the desired form at around 80°C and 25,000 psi obtaining a final density of 1 g/cc. Such an implant can be subcutaneously delivered into the body. A drawback of the above method is the presence of an organic solvent in the formula. Therefore stability of the incorporated drug becomes an issue, particularly for therapeutic proteins. It is necessary to test the stability of a drug in the presence of an organic solvent (73-75).

### Extrusion

Solvent casting techniques are not ideal for industrial scale-up for several reasons. First, the process requires large amounts of organic solvents increasing the risk of denaturation of the drug and/or protein during the encapsulation. Such inactive entities can cause unpredictable side effects, e.g., immunogenicity or other toxicity. Second, this process requires a very long time for complete solvent removal from the final product. It is a batch process which may result in increased occurrences of batch-to-batch variation in the composition of

the implants. Unlike solvent casting, extrusion is a continuous process of pressing the polymer-drug mixture through a die to create implants of fixed cross-sectional profile without any use of solvent (73).

Felt-Baeyens *et al.*, prepared triamcinolone acetonide loaded biodegradable scleral implants using a modified compression molding method for ocular drug delivery. *In vivo* studies indicated that stearic acid acted as an excellent plasticizer together with the polymer resulting in stable implants that had good ocular biocompatibility in rabbit eyes showing no inflammatory reaction and provided drug release for up to 5 weeks (74). Witt *et al.*, developed biodegradable implants for parenteral and controlled drug delivery systems using ABA triblock copolymers, consisting of poly (lactide-co-glycolide) A-blocks and poly (oxyethylene) B-blocks, and PLG, poly (lactide-co-glycolide) polymer by compressing them using a ram extruder. An electron paramagnetic resonance study showed that the implants remains intact and swells during erosion due to presence of PEO blocks as well as the pH inside the ABA triblock copolymers remains in the neutral range which indicates that ABA triblock copolymers are promising biomaterials for the parenteral delivery of pH-sensitive drugs (75).

The extrusion process is the most convenient route for manufacturing implants. Screw extruders are basically inside a stationary cylindrical barrel with either a single or twin helical rotating screws. The extruder is mainly divided into three subdivisions: feeding zone, transition zone, and mixing zone. The process constitutes an extruder and polymer-drug mixture with required micron size feed material. During the process, the polymer-drug mixture is heated to a semi-liquid state achieved by the combination of heating elements and shear stress from the extrusion screw. The screw pushes the mixture through the die. The resulting extrudate is then cooled and solidified before cutting into desired lengths for implants or other applications. Exposure of drug to high temperature can be disadvantageous as

denaturation can take place. Therefore, the extrusion process poses a limitation for drugs that cannot be used because of their melting point, polymorph stability and chemical interactions with PLGA (76-79).

Atrix Laboratories, USA developed an injectable *in situ* implant where a PLGA polymer together with the drug was dissolved into a biocompatible solvent (N-methyl pyrrolidone (NMP) and Dimethyl sulfoxide (DMSO). This manufacturing technology is also known as the "Atrigel Delivery System". Once injected, the polymer precipitates out or coagulates immediately in the body fluid due to its water insolubility. The key factor in the above system is the precipitation threshold (i.e., the limit above which the polymer may precipitate out from the aqueous media) and the polymer solubility in different solvents which can be calculated using the Flory Huggins solubility equation. When the formulation was subcutaneously administered into rats, the formulation with a polymer content below the precipitation threshold had twice the initial release compared to the formulation where the polymer content was above the precipitation threshold (80).

Rothen-Weinhold *et al.*, studied the influence of extrusion and injection molding on *in vitro* degradation and release profile. PLA (Mw: 6000 D) implants were prepared using a Somatostatin analogue such as Vapreotide as a model drug. In the extrusion method, implants were prepared by extruding a mixture of PLA and Vapreotide using a laboratory scale ram extruder. In this method, a powder mixture of the drug and the polymer was put into a barrel which had a 10 mm inside diameter at 80°C as the extrusion temperature. The die diameter was 4.6 mm die and the rods were cut into 2.75 cm long implants (81). Although both methods proved to be feasible for manufacturing the implants, the most important difference observed between these two methods was the *in vitro* release rate of Vapreotide which was higher in the extruded implants. The possible reason may be the significant porosity observed

in extrusion molded implants (81).

Kunou *et al.*, developed controlled release implants of Ganciclovir using PLGA (75:25 Mw: 20,000 D) based on lyophilisation compression molding. The drug, together with a PLGA polymer was first dissolved in acetic acid and lyophilized to obtain a homogeneous cake. The cake was then further compressed on a hot plate in the temperature range of 80 to 100°C. *In vivo* drug release studies in rabbits showed that the fragments of implants disappeared from the subconjunctival space after 5 months (82).

Orloff *et al.*, prepared biodegradable implants for repairing vascular thrombosis which showed good results in a broad variety of vascular disorders (83). Baro *et al.*, developed PLA (Mw:30 kDa) based bone implants containing gentamicin sulfate as a model drug using a compression molding technique using a hydraulic press and further coated with PLA (Mw: 200 kDa). Mixtures of hydroxyapatite and tricalcium phosphate powder were used in the manufacturing of PLA based ceramic implants, because it has similar chemical composition to bone minerals acting as a good carriers for treating bone infection. *In vitro* gentamicin release was delayed due to an additional PLA coating but when tested in the femur of rabbits, it showed faster release which was probably due to a greater degree of PLA degradation, changes in the concentration of phosphate blend along with PLA polymer and *in vivo* invading of the implant by the bone tissue (84).

#### **FACTORS INFLUENCING THE DEGRADATION OF PLGA BASED FORMULATIONS**

In general, the polymer degradation/ hydrolysis rate is accelerated by greater hydrophilicity in the backbone or end groups, amorphous nature, lower molecular weight, and smaller size of the finished device. In order to understand the release rate of a drug entrapped in the biodegradable polymeric matrix the erosion rate of the polymer must be understood. In general,

drug release rate from PLGA microspheres follows a triphasic profile: (1) an initial burst release of surface and pore associated drug, (2) a lag phase until sufficient polymer erosion has taken place and (3) a secondary burst with approximately zero order release kinetics (85, 86). In addition, the PLGA polymer is insoluble in water, but it is unstable in hydrolytic media and is degraded through hydrolytic attacks on its ester bonds. Due to this hydrolytic attack, random chain degradation occurs, resulting in the generation of smaller monomers such as lactic acids and glycolic acids.

Various factors can influence the biodegradation of a PLGA based formulation and simultaneously affect the release profile of microspheres, e.g., (1) polymeric properties such as molecular weight, the lactide:glycolide ratio and the end group and viscosity of the polymer, (2) the solubility of the drug, (3) the type of solvent, (4) the rate of agitation, (5) solvent evaporation, (6) the temperature (7) the drug loading (8) sterilization (9) residual solvents (10) porosity and (11) the pH of the biodegradation media (86).

#### **The effect of polymer composition**

Higher glycolic acid content in the polymeric composition can increase the intensity of the loss of molecular weight and simultaneous degradation of the polymer. For example, the PLGA 50:50 (PLA/PGA) has a faster degradation rate than the PLGA 65:35 due to the higher hydrophilicity of the glycolic acid in the PLGA 50:50. Subsequently PLGA 65:35 has a faster degradation rate than the PLGA 75:25 and PLGA 75:25 than PLGA 90:10. The reason for the lower degradation rate of lactic acid compared to glycolic acid is the presence of an additional methyl group in the structure of lactic acid, which acts as hydrophobic moieties and minimizes the attack by the water molecules. The amount of glycolic acid is a critical parameter in controlling the hydrophilicity of the polymer and, thus the degradation and drug-release rate (87). Janoria *et al.*, studied the effect of different lactic acid

and glycolic acid on drug release. They used PLGA 50:50 and PLGA 65:35 to prepare microspheres of Ganciclovir (GCV) as a model drug using an o/o emulsification followed by solvent evaporation. The T<sub>g</sub> of the PLGA 65:35 was higher than the PLGA 50:50 due to the higher lactide content. Although the lactide content in the polymer composition did not affect the Ganciclovir release, the higher lactide content of the polymer provided better entrapment properties due to its lipophilic nature (88).

### **The effect of crystallinity (or T<sub>g</sub>) of polymer**

The degree of crystallinity and melting point of a polymer is directly related to its molecular weight. The glass transition temperature (T<sub>g</sub>) of PLGA copolymers are reported to be above the physiological temperature of 37°C, hence are glassy in nature and thus exhibiting a fairly rigid chain structure. The T<sub>g</sub> of the PLGA decreases with decreasing lactide in the copolymer composition and molecular weight (88). Copolymer compositions also affect important properties such as the T<sub>g</sub> and the crystallinity which have an indirect effect on the degradation rate. At the moment, there are conflicting opinions on the effect of crystallinity on the degradation rate. In addition, crystallinity of lactic acid (PLA) increases the degradation rate as a result of an increase in the hydrophilicity of polymer (89).

### **The effect of molecular weight**

PLGA polymers are usually characterized in terms of intrinsic viscosity which is directly related to their molecular weight (M<sub>w</sub>) and degradation rate. In general, a polymer with a higher molecular weight contains long polymeric chains resulting in lower degradation rates. As the M<sub>w</sub> of the polymer decreases, the degradation rate increases due to reduced T<sub>g</sub>. Polymers with higher molecular weight polymer exhibiting high T<sub>g</sub> value are glassy in nature and have slow degradation kinetics (87, 90). Graves *et. al.*, studied the effect of M<sub>w</sub> on a PLGA polymer on Pendamidine microspheres

prepared by a double emulsification solvent evaporation method. They used two different PLGA 50:50 polymer grades (M<sub>w</sub> = 1,00,000 daltons and i.v. = 0.8 dl/g and M<sub>w</sub> = 14,000, i.v. = 0.2 dl/g). The formulation with the higher mol/wt polymer took 0.6 days to release 50% of the drug together with a burst release compared to the other which took about 6.3 days to release 50% of the drug without any burst release. This experiment demonstrates the influence of M<sub>w</sub> and i.v. on the rate of drug release (91).

### **Physicochemical properties of incorporated drugs**

In general, polymer-drug matrix degradation and the drug release rate vary as a function of a drug's physicochemical properties. The solubility of encapsulated drug(s) may significantly influence the degradation rate of the polymeric matrix. For example, the presence of a high amount of hydrophilic water soluble groups (-OH or -COOH group) increases the uptake of water molecules from the surrounding environment into the polymeric matrix. This process may result in the leaching of a water soluble drug from the polymer matrix and create a highly porous membrane-like structure very quickly. In contrast, a hydrophobic drug can retard the water diffusion into the polymeric matrix and simultaneously slow down the polymeric degradation. In addition, highly acidic or basic drug(s) may also influence the polymeric degradation kinetics. Studies indicate that such drug moieties auto catalyze the ester backbone hydrolysis resulting in an enhanced polymeric degradation rate (91, 92). The presence of specific functional groups such as amines in the drug molecule can induce polymeric chain degradation through nucleophilic reaction wherein nitrogen atom works similar to oxygen atom in water (93).

### **The effect of size and shape of the matrix**

The ratio of surface area to volume has been found to be a significant factor for degradation

of large devices in general. Greater surface area to volume ratio leads to a greater degradation of the matrix (94). Witt *et. al.*, studied the erosion of parenteral delivery systems such as rods, tablets, films and microspheres generated using PLGA (50:50 lactide to glycolide ratio) polymers. Upon evaluation of the onset time for bulk erosion ( $t_{\text{onset}}$ ) and the apparent rate of mass loss ( $k_{\text{app}}$ ) parameters for each device, it was found that in the case of PLGA, the  $t_{\text{onset}}$  was 16.2 days for microspheres, 19.2 days for films and 30.1 days for cylindrical implants and tablets. The  $k_{\text{app}}$  was  $0.04 \text{ days}^{-1}$  for microspheres,  $0.09 \text{ days}^{-1}$  for films,  $0.11 \text{ days}^{-1}$  for implants and  $0.10 \text{ days}^{-1}$  for tablets. The results indicate that the rate of erosion decreased in the following order: rods and tablets > film > microspheres and was dependent on the size of device (95).

#### The effect of pH and salts concentration

The *in vitro* biodegradation/hydrolysis of PLGA showed that both alkaline and strongly acidic media accelerates the polymer degradation process. The degradation was also found to be affected by the salt concentration in buffered solutions, suggesting that the cleavage reaction of the polymer ester bonds is accelerated through the conversion of the acidic degradation product into neutral salts. Studies by Li *et. al.*, showed that at alkaline pH (10.08) microparticles exhibit rapid weight loss but slower molecular weight decrease and the degradation pattern was close to surface degradation. However, in acidic pH (1.2) it showed faster reduction in molecular weight while slower weight loss and homogeneous degradation (96). Recently, Zolnik and Burgess have studied the effect of acidic (pH 2.4) and neutral (pH 7.4) condition on the degradation of a PLGA polymer and drug release kinetics. *In vitro* dissolution studies indicated that the initial burst and lag phases were similar for both pH values, but the secondary drug release kinetics was substantially higher in acidic pH as

compared to neutral pH. Thermal and morphological analysis showed that in an acidic environment the oligomeric units of the polymer accumulate within the microspheres due to their low solubility in acidic pH compared to the neutral pH (pKa of lactic acid: 3.8). These are crystallized inside the matrix and impart a brittleness to polymer due to which they become more susceptible to fracturing and rapid release (97).

#### The effect of monomer content and purity on degradation

Synthesis of PLGA based biodegradable polymer involves a continuous chain copolymerization process between the lactic acid and the glycolic acid molecules. There may be a chance of free traces of monomer remaining in the final product even after purification with different solvents. This free monomer content in the final product may get converted reversibly into the polymer during the storage of the product which may affect the drug release. During the manufacturing of any PLGA based formulation the monomer content of the PLGA product must be considered. PLGA polymers with monomer content as low as 0.5% are considered highly purified (98). Several purification steps are carried out in the final stage of the polymer manufacturing e.g., ultrafiltration, crystallization, distillation or passing of supercritical  $\text{CO}_2$ , etc., to remove free residual monomers from the amorphous polymer blend and to provide a better purity of the final product. Unbound monomers contain higher free acid contents and acts as an impurity and source for faster degradation of the product.

#### PHARMACEUTICAL APPLICATION OF PLGA BASED MEDICAL DEVICES

Biodegradable polymers have become an important group of materials with an increasing diversity in biomedical devices. Major

applications of PLGA polymers in some novel areas are shown in Table 5.

**Table 5** Novel areas of PLGA application

BIOMEDICAL APPLICATION	AREA OF APPLICATION
Biodegradable surgical suture	Wound closure
Biodegradable medical device	Screws, plates & pins for hard tissue fixation and tissue regeneration, contraceptive device, dental implants
Long active parenteral drug delivery system	Solid rods, injectable microparticles (as suspensions), <i>in situ</i> forming systems, depot controlled release injection, oncological application, gene therapy and proteomics, xenobiotics, immunology and veterinary application
Nasal and ocular delivery	Nasal and ocular drug application
Third generation fully biodegradable stents	Very innovative therapeutic approach for recovery after heart attack or stroke

### Drug eluting stents

In order to minimize the formation of fibrosis, thrombosis or clots, a metallic stent can be inserted within the peripheral or coronary artery by a cardiologist or radiologist performing angioplasty surgery. These bare metallic stents (BMS) made of cobalt or stainless steel are the 1<sup>st</sup> generation stents. They comprise of an elaborate mesh-like design to allow expansion, flexibility and, in some cases, the ability to enlarge constricted blood vessels. Cobalt chrome alloy is stronger and more radio-opaque than the usual 316L stainless steel (L605 CoCr alloy has less nickel than 316L stainless steel and so may be less allergenic). In order to avoid graft rejection, immunosuppressants are administered orally usually minimizing adverse effects (99).

2<sup>nd</sup> generation stents are drug-eluting stents (DES) which were approved by the FDA after being clinically proven to be better than BMS. DES contain a coating, typically of a biodegradable polymer such as PLGA on the metallic stents which holds and releases (elutes) the drug into the arterial wall by contact transfer. The stents are typically spray or dip coated. One to three, or more, layers can be coated e.g. a base layer for adhesion, a main layer for holding the drug, and sometimes a top coat to slow down the release of the drug and to extend its effect. DES contains a drug which

is mainly useful to inhibit neointimal growth (due to the proliferation of smooth muscle cells), arterial restenosis and neointimal hyperplasia. Generally combinations of immuno-suppressive and anti-proliferative drugs (Sirolimus, Paclitaxel and Everolimus) are used for medical applications in coronary stents. Because metal is a foreign substance, it can induce inflammation, scarring, and thrombosis (clotting). DES coated with polymers of biocompatible nature largely prevent some of these effects. However, neointimal hyperplasia occurring within the stent leading to in-stent restenosis is a main obstacle in the long-term success of percutaneous coronary intervention (PCI). Many large randomized clinical trials using DES have shown a remarkable reduction in angiographic restenosis and target vessel revascularization when compared with bare metal stents. DES has revolutionized the field of interventional cardiology by proving its safety and efficacy to prevent restenosis of coronary arteries using local drug delivery in many clinical trials (100-116).

Recently, 3<sup>rd</sup> generation stents have been developed and clinically approved. Here the metallic components have been completely replaced with a biodegradable polymer framework. The benefit of 3<sup>rd</sup> generation stents is that they prevent problems such as thrombosis and at the same reduces the necessity of taking lifelong medication of anti-platelets. The development of these types of stents has been a breakthrough innovation in the treatment of obstructive coronary artery disease since the introduction of balloon angioplasty. Ma *et. al.*, prepared Paclitaxel/Sirolimus loaded DES using a PLGA (65:35 lactide:glycolide ratio) polymer for the treatment of coronary artery disease. The stent provided controlled release of Paclitaxel and Sirolimus for 21 days both *in vitro* and *in vivo* with minimal burst release. This study showed that the combination of two drugs in a DES will not affect the individual drug release kinetic and, therefore, can be developed for the treatment of coronary arterial diseases (117).

Similarly, Raval *et. al.*, successfully developed a Dexamethasone loaded DES using poly L-lactide-co-caprolactone as the biodegradable polymer for intravascular drug administration for the treatment of tissue hyperplasia. The *in vitro* dissolution profile showed that the release of Dexamethasone can be modulated up to 3 weeks by optimizing the concentration of the polymeric blend. SEM data also showed a smooth surface of DES without any irregularities which suggests that the coating process parameters were optimum and efficient for a multiple layer coating (118).

### Contraceptive devices

Biodegradable polymers have recently been applied for the controlled release delivery of hormones and fertility regulating agents. Biodegradable or erodible delivery systems are represented by contraceptive devices where the matrix dissolves to release the drug and other components into the systemic circulation. An implantable contraceptive is likely to be a promising new option for fertility control, as well as, for the long term treatment of sexually transmitted diseases such as AIDS. These biodegradable systems have primarily two advantages: (1) no need for surgical removal of the system after the completion of the drug delivery period and, (2) the device reservoirs show zero order release kinetics which is necessary for efficient therapeutic treatment. Contraceptive systems that provide sustained release of low doses of synthetic hormones are of special interest because they have important clinical advantages over conventional oral contraceptive pills (119-127).

Alaee *et. al.*, developed PLA (Mw = 680,000, i.v. = 3.3 to 4.3 dl/g) based reservoir type of implantable contraceptive device for the controlled delivery of levonorgestrol (300 days) using a simple dip casting method. Dissolution studies showed that the drug was released from the reservoir for at least 9 months. Moreover, it was found that various formulation and manufacturing processes affected the release of the drug from implants such as implant weight,

presence or absence of polyethylene glycol (PEG) in the formulation, molecular weight and amount of PEG, presence of osmotically active agents, etc., (128). Similarly, Nonomura *et. al.*, developed a LH-RH antagonist loaded intrauterine device (IUD) to release the drug for a prolonged period of time using a PLGA block co-polymer (75:25 lactide: glycolide ratio). Once inserted into the uterus, the drug gradually released from the IUD during a period of several months (129).

### Dental implants

Though useful, the potential of biodegradable polymers have unfortunately not been explored in the field of medicated dental implants. Dental or orthodontic braces are devices used in orthodontics that align and straighten teeth, help to position them with regard to a person's bite and also improves dental health. Braces can be either cosmetic or structural. A biodegradable polymer could be good replacement for metallic dental braces because subsequent operative procedures are not required and it aligns the teeth in a particular period of time. In the past, dental implants made of tinidazole were formulated using poly ( $\epsilon$ -caprolactone) which showed good clinical efficacy to control acute periodontitis (130-138).

### Nasal delivery

Intranasal (IN) administration has attracted considerable interest because it provides a non-invasive method for bypassing the blood brain barrier (BBB) delivering drugs directly to the brain. The nasal cavity is a promising site for the delivery of vaccines because (1) its reduced enzymatic activity compared to other possible administration routes (e.g. oral route), (2) its moderately permeable epithelium, and (3) the high availability of immune-reactive sites (139-141).

Vila *et. al.*, developed PLA-PEG based nanoparticles to enhance the absorption of protein molecules across the nasal mucosa

using, for example, tetanus toxoid (TT) as a model drug. They showed that the efficacy in *in vivo* studies of the vaccine developed using nano particles showed approximately 70-80% bioavailability compared to intravenous administration (142). Yildiz *et. al.*, developed heparin loaded microspheres using poly lactic acid. A SEM study showed a uniform particle size of heparin loaded microparticles with smooth surface at around 1-5 micron. *In vitro* and *in vivo* analysis showed a sustained release profile for heparin of up to 8 hours and good absorption confirmed by a 143.63 % AUC level (143). Recently, Seju *et. al.* developed a PLGA (50:50 as lactide to glycolide content) loaded Olanzapine nanoparticulate system for direct nose-to-brain delivery. *In vivo* studies indicated an increased drug uptake at ~10 times which was more efficient for the treatment of central nervous system disorders (144). Li *et. al.*, developed microspheres using  $\alpha$ -Cobrotoxin as a model drug and a mixture of biodegradable polymers comprised of PLGA (50:50, i.v. = 0.8 dl/g) and poly [1,3-bis(p-carboxy-phenoxy) propane-co-p-(carboxyethylformamido) benzoic anhydride CPP:CEFB] using a w/o/o emulsion solvent evaporation method for intranasal delivery. The microspheres showed high entrapment efficiency (80%) with a 25  $\mu$ m mean particle size and effective sustained release profile (145). Tail flick assay indicated that compared to free  $\alpha$ -cobrotoxin and PLGA microspheres, PLGA/P(CPP:CEFB) microspheres showed an apparent increase in the strength and duration of antinociceptive effect at the same dose of  $\alpha$ -cobrotoxin (80 Ag/kg body weight) (145).

### Tissue engineering

Tissue engineering uses a combination of engineering and biologic materials to improve biological functions, as well as, for successful tissue and bone replacement. The requirements of a scaffold material for tissue engineering are important to support cell growth and proliferation as follows: (a) the material must not induce any unresolved inflammatory reaction or any foreign body reaction, (b) the

polymer should be absorbed completely into the body after fulfilling its function, (c) the mechanical properties of the scaffold materials must not collapse during handling, nor during a patient's routine activities and (d) the material must be easily sterilizable to prevent unwanted infections (146-158).

Suture materials are classified into two broad categories, biodegradable and non-biodegradable. Biodegradable sutures lose their entire tensile strength within 2-3 months, while non-biodegradable materials retain their strength longer than 2-3 months. Biodegradable or bioresorbable sutures are made from either collagen derived from sheep intestinal sub mucosa or synthetic polymers such as PGA. Surgical suture is a term used for medical devices which are used to close body tissue after an injury or surgery. Suture threads comes in very specific sizes and can be classified as mainly absorbable (made from biodegradable polymers such as PGA, PLA or poly dioxanones) or non-absorbable (made from polypropylene, polyesters or nylon) depending on whether or not, the body will naturally degrade and absorb the suture material over period of time. Absorbable surgical sutures are sometimes also coated with an antimicrobial substance to reduce the chance of infection of the wound.

Park *et. al.*, developed biodegradable polymeric micro needles using PLA and PLGA based synthetic polymers for transdermal drug delivery. They concluded that the new arthroscopic fixation device utilized knotless suture-based anchors eliminating the need for knot tying during arthroscopic surgery (159). Casalini *et. al.*, successfully developed a transient 1-dimensional model to show the release rate from a bioresorbable drug eluting suture together with its pharmacologic behavior and biodegradation rate (160). The model primarily highlights the degradation kinetics of a drug loaded resorbable suture in the tissue, as well

as, the behavior of a drug in the tissue. A model can provide predictive data without expensive and time consuming experimental activity based on the principal laws of conservation, mass transfer and hydrolysis (160). Morizumi *et al.*, developed a novel drug eluting suture coated with Tacrolimus for the treatment of neointimal hyperplasia. *In vivo* results from a porcine model confirmed that the DE-sutures can successfully inhibit neointimal hyperplasia at the anastomotic suture site without any inflammatory response indicating usefulness of this novel suture in both coronary artery bypass graft surgery and peripheral vascular bypass surgery (161).

### Orthopedic devices

During orthopedic surgery, bone fragments or filaments are usually fixed with metallic plates and screws. When the fracture has healed these metallic plates are usually removed from the body through a second surgery requiring general anesthesia to minimize pain. In order to avoid the second surgery, resorbable polymers can be used as alternative materials in a range of devices in trauma and fracture surgery satisfying various operational and technical requirements (162, 163). This is because they can overcome various issues such as stress protection, potential for corrosion, wear and debris formation, as well as, the necessity of implant removal (164).

These devices also maintain adequate mechanical properties *in vivo* for the time required for a bone fracture to heal and decompose gradually wherein the stresses are transferred gradually to the healing bone or tissues so that no stress shielding occurs (165, 166). Rangdal *et al.*, performed clinical studies to see how PLGA based biodegradable plates and screws affect the tissues of patients suffering of bimalleolar fractures. They found that only one patient had tissue reaction at 14 weeks post-surgery which settled down by debridement without the need of implant removal while the rest of the patients did not

show any signs of tissue reaction at the end of 18 months (167). Enislidis *et al.*, investigated zygomatic fracture fixation using a biodegradable osteosynthesis system (BioSorbFX<sup>®</sup>) to evaluate its stability, as well as, complications observed during the first postoperative year. They found that fixation of fractures of the zygoma using the BioSorbFX<sup>®</sup> system was simple and safe without showing any post-operative complication (168). Tasca *et al.*, successfully performed thyroid cartilage fracture surgery on a 29-year old man (injured while playing rugby) using Inion biodegradable plates that had been made with lactic and glycolic acid based polymers and specifically designed with the consideration of the thyroid cartilage structure and recommended in the treatment of laryngeal fractures (169). Kyriakou *et al.*, performed clinical studies on 20 patients between the ages of 18 to 40 years (both male and female) with facial skeleton maxilla fractures treated with resorbable plates. They found that none of the patient reported any infection or secondary mobility indicating excellent biocompatibility for resorbable plates (170).

### Ocular drug delivery

Currently, the most common way of administering therapeutic agents to the eye is using topical applications placed into the conjunctival *cul-de-sac*. Eye drops or ointments are, however, not very effective as they are rapidly cleared from the eye by continuous eyelid movements and drainage through lachrymal fluids. In order to increase the contact time between ophthalmic formulations and the eye corneal, more invasive procedures must be used such subconjunctival injections, retro-ocular injections containing biodegradable polymers to minimize clinical complication and side effects (171). Choonara *et al.*, developed a novel doughnut-shaped mini tablet (DSMT) containing different PLGA (50:50, i.v. = 0.16 - 8.2 dl/g) grades loaded with two anti-retroviral drugs such as foscarnet and ganciclovir for intraocular drug delivery. They used a special set of punches fitted with a central-rod in a

tableting press to manufacture the DSMT device providing a first-order release of both anti-retrovirals. The novel geometric design and veracity of the DSMT device was retained even after 24 weeks of use (172).

### Transdermal drug delivery

Biodegradable polymer based transdermal patches usually do not require removal post-implantation and as a result these systems have become quite common (173). Colloidal nanoparticles developed from poly (alkyl cyanoacrylate) (PACA) based biodegradable polymers have opened up new and exciting avenues in the field of transdermal drug delivery due to their biocompatibility and small size which permits increased transport across the epithelium and intracellular penetration. Miyazaki *et al.*, developed poly n-butylcyanoacrylate (PNBCA) polymer based biodegradable nanocapsules loaded with indomethacin to evaluate the possibility of delivering the drug systemically after its topical application. Nanocapsules prepared by an interfacial polymerization process showed 188 nm as average particle size with 76.6% drug loading. Conventional gel formulations containing indomethacin made from Pluronic F-127 was used as a reference formulation for *in vivo* comparison which showed a higher drug plasma concentration for the nanocapsule formulation than the conventional gel formulation containing Pluronic F-127. Due to the ultra-fine particle size and their oily vesicular nature, alkylcyanoacrylate nanocapsules can easily cross the stratum corneum layer of the skin and act as a micro-reservoir to provide sustained drug release (174).

### Cancer treatment

Various biodegradable polymer-based novel formulations such as depot injections containing microparticles or nanoparticles can be useful in chemotherapy treatment. Recently, Kun *et al.*, prepared nanoparticles using poly (ethylene glycol)/ poly (L-lactic acid) alternating

multi-block copolymers and investigated them for use as anti-cancer drug carriers. The thermo-sensitive nanoparticles responded well to local hypothermic temperatures and the release of doxorubicin was also dependent on the temperature at the tumor site (175). Bhuske *et al.*, developed PLGA based nanoparticles containing Curcumin which is a poorly soluble anti-cancer drug. They demonstrated an improvement in the aqueous solubility and anticancer activity of the drug (176). PLGA polymers have also been used for tumor imaging, for example Diou *et al.*, reported the use of PEGylated PLGA nanocapsules containing per-fluorooctyle bromide by <sup>19</sup>F MRI (177). There are numerous other reports of using PLGA based applications for targeting increased drug delivery at target site and cancer treatment (178-181).

Recently, Jin *et al.*, developed doxorubicin–poly (d,l lactic- co-glycolic acid)–poly(ethylene glycol) (DOX–PLGA–PEG) micelles decorated with the bivalent fragment HAb18 F(ab')<sub>2</sub> for treatment of hepatocellular carcinoma (HCC). They found that the developed micelles had an acceptable morphology and high drug loading efficiency. Cellular uptake and accumulation in the tumor was observed to be dependent on the dual effects of passive and active targeting. The drug-loaded micelles showed cytotoxicity on tumor cells *in vitro* and *in vivo* considered responsible for the improvement in the therapeutic response (178). Similarly, Li *et al.*, prepared polymer-coated magnetic nanoparticles as carriers for doxorubicin using a double emulsification method (179). Current research also shows the usefulness of multiblocks such as dicarboxylated poly (ethylene glycol) (PEG; Mw 2000) with poly (l-lactic acid) (PLLA)/PEG/PLLA triblock copolymers for targeting cancer cells.

### Gene therapy and proteomic applications

Luten *et al.*, demonstrated the use of PLGA and other biodegradable polymers in plasmid gene delivery by developing DNA loaded micro and nanoparticles (182). Recently, Ahn *et al.*,

developed a PLA and PEG based non-viral gene carrier by synthesizing a multi-block copolymer (183). Benfer and Kissel prepared PLGA based nanoparticles using poly [vinyl-3-(dialkylamino) alkylcarbamate-co-vinylacetate-co-vinylalcohol]-graft-poly (D,L-lactide-co-glycolide) or DEAPA-PVA-g-PLGA loaded with siRNA to improve the cellular uptake and thus increase therapeutic activity (184). Lewis *et. al.*, demonstrated that oligonucleotides can be entrapped into a PLGA matrix to improve nuclease stability in serum, and thus achieve an acceptable release profile of antisense oligopeptides such as very potent anti-HIV phosphorothioates (185).

Much attention has been focused on proteins (186-188), oligopeptide (189) and RNA (190) delivery using PLGA devices. It has been shown that specific genes such as synthetic short interfering RNA (siRNA) is a promising route for specific and efficient therapy of disease-related genes. However, *in vivo* application of siRNA requires an effective delivery system. Commonly used siRNA carriers are based on polycations which form electrostatic complexes with siRNA and such poly- or lipoplexes are of restricted use *in vivo* due to severe problems associated with toxicity, serum instability and non-specific immune-responses. In order to minimize some complications associated with non-biodegradable polymers, Cun *et. al.*, developed nanoparticles (NPs) loaded with siRNA using biodegradable PLGA polymers without including polycations. The NPs were found to be spherical in shape with an average particle size of 300 nm, poly dispersibility index < 0.2, encapsulation efficiency of 57% and zeta potential value of  $\sim 40$ mV. The integrity of siRNA was preserved during the preparation indicating that siRNA-NPs based on PLGA polymers without any cationic excipient can work as a potential and promising carrier for the delivery of siRNA (190). Recently, Tang *et. al.*, developed calcium phosphate embedded PLGA nanoparticles for plasmid DNA

(pDNA) delivery. It is known that DNA has negatively charged macromolecules which hinders its entry into the tumor cells through the negatively charged lipid bilayer. Therefore, the calcium phosphate was used to neutralize the DNA before entry and to achieve increased efficiency with improved release kinetics. Based on promising *in vitro* transfection efficiency formulations, calcium phosphate embedded PLGA nanoparticles can be considered a suitable vector for pDNA based gene delivery (191). Much work has been carried out in recent years to improve the delivery of DNA (192-196), gene (197), growth hormone (198), peptide (199) and bovine serum albumin (200) using PLGA devices or formulations.

### Immunological applications

A considerable amount of research has been carried out in the field of immunology using PLGA devices. Slutter *et. al.*, worked on particle engineering for nasal vaccine delivery and demonstrated good absorption of N-trimethylated chitosan along with PLGA via nasal mucosa (201). Sivakumar *et. al.*, developed an alternative adjuvant for Hepatitis B vaccine (HBsAg) that provides a long-lasting immune response after a single administration. The suitability of poly (D, L)-lactide-co-glycolic acid (PLGA), poly lactic acid (PLA) and chitosan polymers as adjuvants for HBsAg (202) were studied. Salvador *et. al.*, studied combinations of different immune stimulating adjuvants using PLGA microspheres to improve the immune response of a model vaccine. In this study, PLGA microspheres containing BSA was co-encapsulated with different adjuvants such as monophosphoryl lipid A (MPLA), polyinosinic-polycytidylic acid,  $\alpha$ -galactosyl ceramide and alginate. It was observed that a mixture of MPLA and  $\alpha$ -galactosyl ceramide within the microspheres provided a higher cellular response to increase vaccines immunogenicity as compared to other adjuvants (203). Other studies in this area include imaging (204), auto booster vaccines (205) and immunogenic

properties of antigens delivered through PLGA microparticles (206).

### Veterinary applications

In the recent years there has been an increasing interest in biodegradable polymers for veterinary use because they could provide significant benefits such as a reduction in stress resulting from reduced animal handling, no need to physically remove the delivery system and reduced cost in terms of the time spent by animal owner or caretaker. Presently, only a few biodegradable formulations are commercially available for veterinary use. The possible reason behind it is the cost of the biodegradable device, followed by regulatory considerations and challenges in formulation stability (207). Walduck *et. al.*, developed biodegradable implants using a cholesterol and lecithin polymeric mixture for recombinant antigen delivery to sheep. They also investigated the release profiles of antigen *in vitro* as well as *in vivo* and found that sheep produced significant levels of antibodies when immunized with implants showing promising tissue compatibility (208). Khan *et. al.*, developed biodegradable implantable matrix systems (pellets) from a cholesterol and lecithin mixture containing bovine serum albumin (BSA) as a model antigen for parenteral delivery in mice. For *in vivo* studies, pellets were subcutaneously injected into the mice which provided sustained drug release until 40 days with significant antibodies generation (209).

### Packaging and sterilization

As discussed previously, these polymers are very sensitive to moisture which cause degradation during manufacturing, as well as, during product storage. Therefore to minimize the effects of moisture uptake, biodegradable polymers are typically stored under refrigerated conditions. The solution for hydrolysis instability is simple in theory, eliminate moisture and prevent degradation. Therefore, precautions should be taken during the final

packaging of a product by placing the device or formulation in an airtight moisture-proof container containing a desiccant.

For example, sutures are wrapped around a specially dried paper holder that acts as a desiccant. The final devices should not be sterilized by autoclaving or dry heat because this may degrade the device since even dry heat can contain a lot of moisture (210). Gamma radiation, particularly at doses above 2 Mrad, can result in significant degradation of the polymer chain, resulting in a reduced molecular weight and altered mechanical properties and degradation times. Poly (glycolide), poly (lactide) and poly (dioxanone) are especially sensitive to gamma radiation and are usually sterilized by exposure to EtO (210). However, residual EtO is very toxic and takes a long time for degassing which adds to the final cost. Typically, a device is sterilized using gamma radiation (211-213), ethylene oxide (EtO) gas (214, 215), or other less-known techniques such as synchrotron or electron beam irradiation (216).

The effect of gamma radiation in either vacuum or in air at a dose of 25 kGy on the stability of PLGA 50:50 ( $M_w = 34000$  D and  $i.v. = 0.39$  dl/g) based microsphere was studied. Microspheres irradiated under vacuum were found to be stable over a period of 6 months and microsphere irradiated in presence of air shows showed that release rate were increased by 10% but did not change further during the storage time. Electronic paramagnetic resonance analysis confirmed the free radicals generated from both the polymeric matrix and the active ingredient during gamma radiation. In addition, they also confirmed the radio-stabilizing effect of the drug (clonazepam) by polymer/clonazepam spin transfer reactions. Furthermore, due to the same radio stabilizing effect, microspheres showed a 54% decrease in overall radiation yield as compared to placebo microspheres because the radiation was absorbed by the clonazepam during the sterilization process. It can be concluded that the type of API, as well as, the sterilization

conditions must be considered carefully on case to case basis (217). Mohr *et. al.*, studied the effect of gamma radiation on the stability of PLGA microspheres. Interestingly, on exposing microspheres to 5.1 to 26.6 kGy gamma radiations it was found that the drug loaded microspheres were converted to conjugation products with PLGA. The average molecular weight decreased with an increasing irradiation dose (218). Another study showed that gamma irradiation exposure has no apparent effect on the integrity and formulation properties such as morphology, size and peptide loading. Upon subcutaneous administration of irradiated and non-irradiated PLGA microspheres into mice induced a similar immune response (219).

### Regulatory consideration

For example, the maximum daily intake or IIG limit of lactic acid is known (220). If the quantity of the polymer is higher than the IIG limit, toxicity data from the manufacturers of the polymer can be submitted as a supportive documents to the regulatory agencies for product approval. To obtain FDA approval for formulations based on microspheres it is necessary to submit various clinical data, e.g., preclinical and toxicity data of biodegradable polymers, monomer content in the final formulation, the molecular structure of the polymer, residual solvent levels in the final formulation, composite formulations including number of laminates, etc. In addition, it is also mandatory to state possible clinical adverse information in detail on the product label (221, 222). Tegnander *et. al.*, reported swelling at the site of administration and sinus formulation when a patient with a fracture was treated with PGA or PLA based pins (223). *In vivo* and *in vitro* testing of polymeric materials should be designed to investigate polymer-skin interface reactions, effects on the subsurface tissue and systemic effects. *In vivo* procedures usually involve a subcutaneous and/or intramuscular implant test and a surface patch test for acute and chronic toxicity study. Preclinical and toxicological studies must be performed in accordance with guidelines set by FDA to eliminate formulations that are too toxic for human use. Similarly, for dossier submission,

roughness, porosity, surface area of device, coating layer or thickness as well as the effect of sterilization on properties of formulation and shelf life of product with specific recommendation storage guidelines should be clearly mentioned (224, 225).

### CONCLUSION

Biodegradable polymers are used extensively in pharmaceutical formulations, from drug delivery to implants and stents. To this effect, PLGA devices can be said to have become widely utilized in the pharmaceutical industry because of their many useful properties and compatibility with *in vivo* conditions. As evident, these polymers have found usage within various categories of drugs such as anti-cancer, antibiotics, contraceptive devices, orthopedic devices and biotechnology products such as antibodies and vaccines. Their flexibility is also defined through their various routes of administration such as parental, subcutaneous, nasal or ocular and, therefore, has the potential to be used widely in pharmaceutical formulations.

An in-depth knowledge and thorough understanding of the factors influencing polymeric degradation and release rate are very important to achieve a successful product. At any stage, drug delivery using PLGA based copolymers is an attractive area with countless opportunities for further research and developmental work. However, the achievement in these areas depends on the deep efforts and extensive research of scientists from diverse disciplines such as microbiology, pharmacy and polymer science to ensure much better health service to mankind as well as nature.

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