INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-3, July-Sept-2012 Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Received: 29th May-2012 *Revised: 02nd June-2012* *Accepted: 04th June-2012*

Review article

PLGA/PLA MICROPARTICULATE SYSTEM: A BOON FOR HYDROPHOBIC DRUG DELIVERY

Thakor Namita M[1].*,Varde Neha M.[1], C.Sini Surendran[1], Shah Viral H.[1], Upadhyay U. M.[1]

[1]Dept. of pharmaceutics, Sigma Institute of Pharmacy, Vadodara, Gujarat, India

ABSTRACT : Controlling In-vitro drug release profiles for a system of PLGA/PLA microparticles encapsulating a hydrophobic drug. Challenges with the diversity of drug properties, microencapsulation methods, are evaluated with a focus on decreasing the time to lab-scale encapsulation of water-insoluble drug candidates in the drug development stage. The development of biodegradable microparticles systems that combined the beneficial properties of polymeric microparticles for hydrophobic drug delivery were reviewed here. Injectable biodegradable and biocompatible copolymers of lactic and glycolic acid are important advanced delivery system for week too month controlled release of hydrophobic drug (e.g., from biopharmaceutical classification system class IV), which often display poor oral bioavailability. Finally, three important properties affecting release behavior were identified as: polymer hydrophobicity, particle size and particle coating, . This review focuses on the microencapsulation of hydrophobic drugs, describes a variety of techniques for their preparation and analytics.

Key words- Controlled release microparticles ,drug release mechanism, drug delivery, PLGA end groups, Microencapsulation, hydrophobic drug.

INTRODUCTION

Poly(lactic-co-glycolic acid) (PLGA) is one of the most successfully developed biodegradable polymers because its hydrolysis leads to metabolite monomers, lactic acid and glycolic acid, these two monomers are endogenous and easily metabolized by the body via the Krebs cycle. Here we reviewed drug release profiles for a system of PLGA/PLA microparticles encapsulating a hydrophobic drug, The three important properties affecting release behavior were identified as: polymer hydrophobicity, particle size and particle coating. Increasing the polymer hydrophobicity & particle size, coating the particles, reduces the initial burst and increases the rate of release. Various combinations of the above three properties were used to achieve in-vitro release of drug over a period of 8 days, 25 days, and >40 days and steady release rate over the entire period of release. Drug properties relevant for microencapsulation and Release, Microencapsulation techniques for hydrophobic drugs, Controlling the polymer microparticle size, Encapsulation efficiency, Drug release from the microparticles, all were reviwed and studied (A. Kumari et.al.,2010).

Hydrophobic drug" roughly describes a heterogeneous group of molecule that exhibit poor solubility in water. Particularly with the development of BCS class IV drugs with a low solubility and a low permeability, which exhibit low oral bioavailability so companies are frequently faced with the choice to either develop or discard the early stage compound. In order to expedite this decision, the question of alternative delivery technologies needs to be discussed in the early stages of drug development. Therefore this review focuses on hydrophobic drug and seeks to develop some guiding principles to examine and solve issues related to encapsulation and release from injectable PLA and PLGA microparticle (Schwendeman, S.P.at.al,1996).

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

REASONS FOR SELECTING PLGA- POLYMER FOR DRUG DELIVERY(Fabienne Danhier at.al.,2012)

(i) Biodegradability and biocompatibility.
(ii) FDA and European Medicine Agency approval in drug delivery systems for parenteral administration.
(iii) Well described formulations and methods of production adapted to various types of drugs e.g. hydrophilic or hydrophobic small molecules or macromolecules.
(iv) Protection of drug from degradation.
(v) Possibility of sustained release.
(vi) Possibility to modify surface properties to provide stealthness and/or better interaction with biological materials.

MATERIALS AND METHODS

DRUG PROPERTIES RELEVANT FOR MICROENCAPSULATION AND RELEASE

Drug solubility in aqueous and organic media

The term "hydrophobic drugs" roughly describes a heterogeneous group of molecules that exhibit poor solubility in water but that are soluble in various organic solvents. Often, the terms slightly soluble (1–10 mg/ml),very slightly soluble (0.1–1 mg/ml), and practically insoluble(<0.1mg/ml) are used to categorize such substances(Martin, 1993;BP, 2001). Since insufficient solubility commonly accompanies undesired pharmacokinetic properties, the screening of kinetic and thermodynamic solubility as well as the prediction of solubility are of major importance in discovery (lead identification and optimization) and development (Alsenz, J.,at.al.,2007) As microparticles are most often prepared by emulsion techniques that include aqueous phases, the solubility of the drug in these media is an important value that needs to be determined in the initial phase of every microencapsulation study. Such external phases are commonly aqueous solutions containing polyvinyl alcohol (PVA), Most of the microencapsulation techniques for hydrophobic drugs employ volatile organic solvent to dissolve the matrix polymer and, if applicable, the drug as well so it is essential to determine the drug solubility in common organic solvents like methylene chloride and ethyl acetate, The results of the solubility studies will form the basis of most considerations of choosing the appropriate encapsulation technique. The octanol–water partition coefficient *K*p, calculated or experimentally determined for new molecules to describe their lipophilic/hydrophilic nature and to make predictions on their behaviour in biological systems, can suggest how the drug will distribute in two-phase solvent systems (Avdeef, 2007).

Drug stability

The most commonly used microencapsulation methods include organic phase emulsification, subjecting drug crystals or dissolved molecules to high local temperatures, shear forces, and the presence of the respective solvent(Basak, A.K.at al.2007).Drug sensitivity to temperature-induced degradation can be determined easily by stress-tests at different temperatures in relevant solvents including release media from room temperature to accelerated storage conditions. Stability studies of new compounds should address the sensitivity of dissolved drug to acids, bases, and oxidation as well as solid-state humidity-related, thermal, and photo-degradation(Alsante, K.M.,200.2007) Use of ultrasound for emulsification might result in degradation of drugs, especially those that contain hydrolyzable bonds such as esters. Accumulation of PLGA degradation products inside the microparticles under release conditions results in an acidic microclimate that also may affect hydrolyzable bonds in the drug molecule. Amine groups in the drug, especially primary amines, may undergo acylation by PLGA degradation products. samples of forced drug degradation should be included when establishing drug determination assays, typically utilizing reverse phase HPLC, to ensure that degradation products will be distinguished from the intact molecule.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Drug–polymer interactions

If weak bases or acids are to be encapsulated, the presence of any drug-induced polymer degradation should be evaluated(Li et al., 1996; Frank et al., 2005). It is well established that amine drugs can catalyze degradation of the PLA/PLGA polyester. For significant drug-induced polymer hydrolysis to occur the drug is presumed to partition into the polymer phase. Potential interaction of drugs with the matrix polymer should also be considered and may result in incomplete drug release. certain basic compounds (and likely those that do not partition into the polymer phase or have restricted nucleophilicity), there have been reports of a reduced polymer degradation via ionic interaction of the drug with cationic PLGA end-groups (Miyajima et al., 1998; Klose et al., 2008).

Drug solid-state properties

Before microencapsulation, the drug is in the solid state, It can be amorphous, crystalline, or combinations thereof. During microencapsulation, the drug will be dissolved or dispersed in a solvent and may be present in the microparticles as a solid solution, metastable molecularly dispersion, or may form amorphous or crystalline regions. If not already dissolved inside the polymer matrix, the drug needs to be dissolved during the last step before drug release, i.e., exposure to an aqueous medium after microparticle administration. This step is critical for hydrophobic drugs because of their typically low drug solubility, and therefore, slow dissolution rate. This dissolution rate may be reduced even further because poor mixing inside the polymer matrix where the drug is dissolving, giving rise to a substantial unstirred boundary layer for diffusion out of the microparticle. Drug properties that may affect its dissolution in aqueous media from the crystalline state include the wettability of a crystal, the stability of the crystal structure (heat of fusion), or the surface area. The initial characteristics of the employed drug material may be subjected to alterations if the drug is dissolved, at least partially, and precipitates during the encapsulation procedure due to solvent removal. Drug polymorphism may become a serious problem if the polymorphs show strong differences in, e.g., solubility, and conversion to another form occurs during microencapsulation, storage, or under release conditions. Therefore, if solid drug is going to be encapsulated, classically the thermodynamically most stable polymorph is preferred for pharmaceutical development although efforts have been made to engineer crystals by forming co-crystals or metastable polymorphs with altered dissolution behaviour (Blagden et al., 2007).

MICROENCAPSULATION TECHNIQUES FOR HYDROPHOBIC DRUGS: (Christian Wischke at.al.,2008)

1. o/w emulsion technique
2. s/o/w technique
3. o/o method
4. w/o/w method.
5. In situ forming microparticles
6. Salting out/phase separation
7. Melting techniques
8. Methods using supercritical fluids (SCF)
9. Spraying techniques
10. Ammonolysis

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

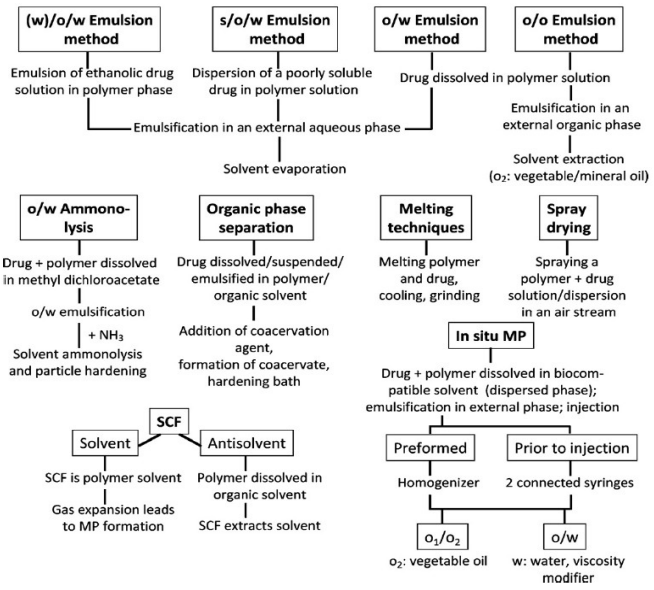

Figure1: Principle of microencapsulation techniques used for the incorporation of hydrophobic drugs into biodegradable microparticle.

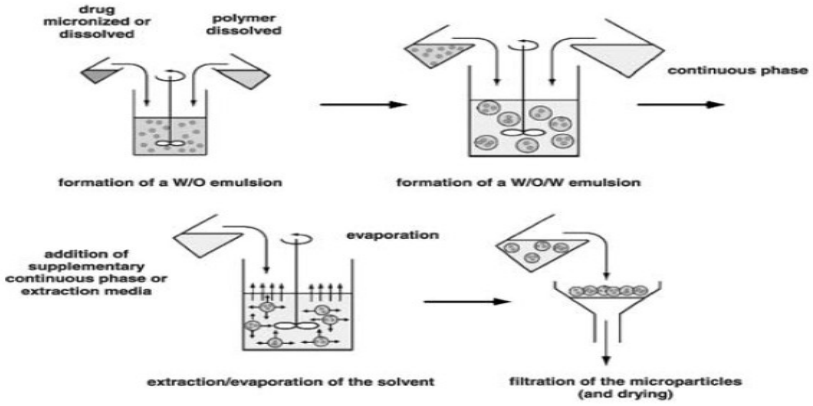

Figure2: Preparation of microparticles using a water-in-oil-in-water (W/O/W) technique (Freitas S, Merkle HP, Gander B 2005)

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

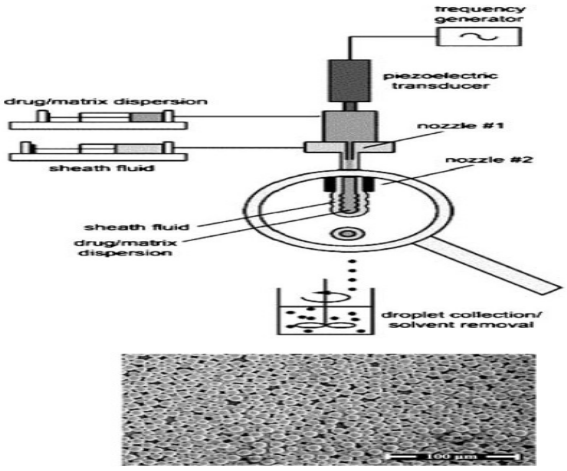

Figure3: "Jet Excitation method" to prepare drug-loaded microparticles with a very narrow size distribution using an oil-in-water (O/W) extraction/evaporation.

RATIONALE TO SELECT AN ENCAPSULATION TECHNIQUE

There are a variety of methods that already have been used for the encapsulation of hydrophobic drugs in PLA/PLGA microparticles. For a pharmaceutical company the approval of the formulation for the market might be faster, easier, and cheaper, when techniques similar to those of already available commercial products are used. PLGA-based microparticle for the hydrophobic drugs, risperidone and naltrexone, o/w solvent evaporation methods were described. Other substances, like water-soluble drug salts, peptides, or proteins, were encapsulated by coacervation, double emulsion, or spraying techniques. As the o/w and s/o/w methods are most commonly used for small-scale microencapsulation studies(Christian Wischke at.al.,2008).

CONTROLLING THE POLYMER MICROPARTICLE SIZE

Emulsification procedure

A large variety of o/w emulsification methods have been described ranging from simple set-ups with a beaker and stirrer to, for instance, methods based on static micromixers(Schalper et al., 2005; Wischke et al., 2006), where the particle size can be controlled by the flow rates of the o- and w-phase in the micromixer, or surface liquid spraying, where the o-phase is sprayed on the surface of the stirred water phase(Tang et al., 2007).. Also, a "jet excitation method" has been described to achieve size-uniform microparticles. mechanism of droplet formation is superimposed by ultrasonic high-frequency oscillation of the nozzle in the "jet excitation method", which allows prediction and control of the droplet size by the applied frequency and flow rate. In order to obtain injectable microparticles for long-acting depot applications, polydisperse PARTICLE size range of 20–100µm is usually desired. Smaller particles, <5–10µm are necessary, if the whole microparticles are passively targeted to phagocytic cells (Jeffery et al., 1991; Johanson et al., 2000; O'Hagan and Singh, 2004).Ultrasound (Muranishi et al., 1991) and high pressure homogenization provide a high energy density in the emulsification zone and are expected to produce a high fraction of nanoparticles rather than microparticles. However, high-speed rotor–stator homogenizers such as Ultra-Turrax® can produce submicron droplets only at a high continuous phase viscosity.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Formulation parameters

Particle size and ultrastructure, e.g., the O-phase volume and solvent, the concentration and type of polymer, the volume of the continuous phase and the type and concentration of stabilizer, the temperature, the stirring speed, and the stirrer type and geometry among others(Wu, 1995b; Jain et al., 1998). All are the formulation parameter which affects the particle size. In order to separate the o-phase into individual droplets, shear forces are commonly applied to the system. By increasing the intensity of these forces, e.g., by increasing the stirring speed of a rotor–stator homogenizer, the particle size can be reduced.

ENCAPSULATION EFFICIENCY

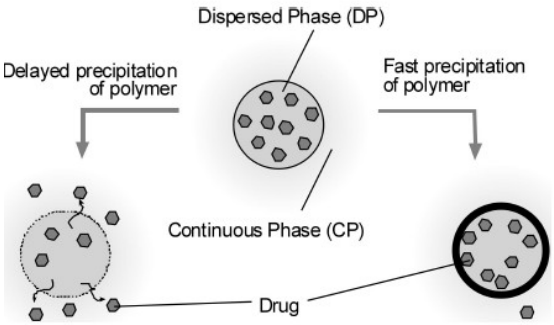

Figure4: Schematic description of the rationale for encapsulation efficiency (Mehta et al.1996).

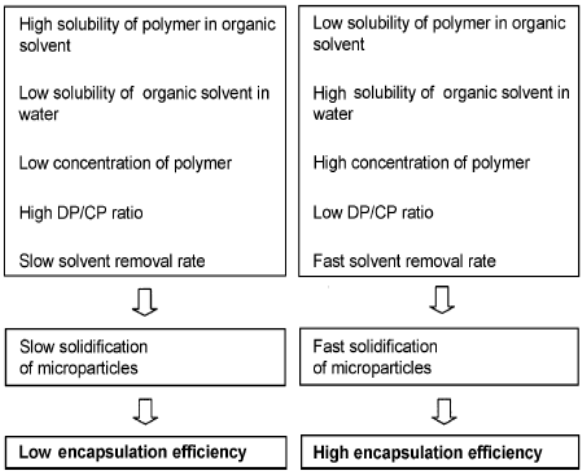

Figure5: Factors influencing encapsulation efficiency (Mehta et al.,1996).

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

MICROPARTICLES OFFER VARIOUS SIGNIFICANT ADVANTAGES AS DRUG DELIVERY SYSTEMS (Juergen Siepmann at.al.,2006)

1)An effective protection of the encapsulated active agent against (e.g. enzymatic) degradation.
2)The possibility to accurately control the release rate of the incorporated drug over periods of hours to months.
3)The possibility to avoid the gastrointestinal tract (certain drugs loose their activity upon oral administration) by intramuscular or subcutaneous injection.
4) Easy administration using standard needles (in contrast to alternative controlled release parenteral dosage forms, such as macrosized implants).
5)The possibility to directly administer the drug into the target tissue (thus, reducing the drug concentrations in the rest of the human body and the risk of related undesired side effects, the possibility to reach target tissues, which are normally not accessible for the drug (e.g., the Central Nervous System).
6)No need of surgical removal of empty remnants, if biodegradable matrix formers are used. Poly(lactic-co-glycolic acid) (PLGA) is a frequently used biodegradable matrix former, because it is biocompatible and degraded into lactic and glycolic acid, two naturally occurring substances in the human body.

MICROPARTICULATE SYSTEM AS A CONTROLLED DRUG DELIVERY

Controlled drug delivery systems can be extremely helpful to optimize the effects of pharmacotherapies. Each drug has a so-called "minimal effective concentration", below which no therapeutic effects occur and a characteristic "minimal toxic concentration", above which undesired toxic side effects occur(Tanquary AC,at.al.,1974). The range in-between is the so-called "therapeutic range", or "therapeutic window". To optimize the therapeutic effects of a medical treatment it is of major importance to maintain the drug concentration within the therapeutic range over prolonged periods of time. If the entire drug dose is administered at once using conventional pharmaceutical dosage forms, e.g. standard tablets, the whole amount is rapidly released into the stomach, absorbed into the blood stream and distributed throughout the human body. The rate at which the drug reaches its site of action is often high. The risk of toxic side effects can be considerable. Subsequently, as no continuous drug supply is provided and as the human body eliminates the active agent, the concentration of the latter decreases again. In some cases, the therapeutic range is attainted during only very short time periods.

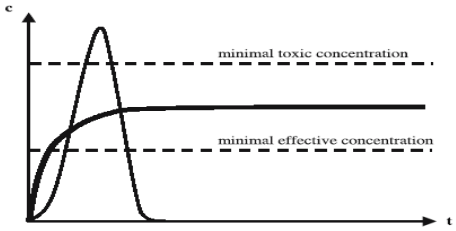

Figure6: Schematic presentation of the "therapeutic window" of a drug and possible drug concentration time profiles upon administration of oral immediate (*thin curve*) and parenteral controlled release dosage forms (*thick curve*) (*c* denotes the drug concentration at the site of action in the human body, *t* the time after administration)

To overcome these restrictions and control the resulting drug concentration-time-profiles at the site of action, controlled drug delivery systems can be used. The idea is to incorporate the drug within a matrix former (very often polymer are used), which controls the resulting release rate. Various processes, such as diffusion, erosion and swelling can be involved in the control of the overall drug release rate, resulting in a broad spectrum of possible release patterns and constant drug concentrations at the site of action over prolonged periods of time.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Table1: Examples for Pharmaceutical Products Based on Drug Loaded, Biodegradable Microparticles Available on the Market(Okada H,at.al., 1995)(Sinha VR,at. Al., 2003)

DRUG	TRADE NAME	COMPANY	APPLICATION
Leuprorelin acetate	Lupron Depot	Takeda	Prostate cancer
Leuprorelin acetate	Trenantone	Takeda	Prostate cancer
Recombinant human growth hormone	Nutropin depot	Genentech-Alkermes	Gowth hormone deficiency
Goserelin acetate	zoladex	I.C.I	prostatecancer
Octreotide acetate	Sandostatin LAR depot	novartis	GH suppression anticancer

PLGA-MICROPARTICLE AND ITS BIOLOGICAL APPLICATION.

Table gives examples for products, which are commercially available on the market. Since 1989, Lupron® Depot containing the anticancer drug leuprorelin acetate embedded within a poly(lactic-co-glycolic acid) (PLGA) matrix] is used for the treatment of prostate cancer. Leuprorelin acetate is a superactive luteinizing hormone-releasing hormone(LH-RH) agonist. Its biological activity is tenfold that of LH-RH. When administered chronically at a higher dose, it paradoxically produces antagonistic inhibitory effects on pituitary gonadotropin secretion and testicular or ovarian steroidogenises ("chemical castration"). These effects, attributable to a down-regulation of the receptors, are temporary and reversible. Importantly, they can be used for the treatment of hormone-sensitive tumors, such as prostate and breast cancer and endometriosis with minimized side effects and avoiding surgical castration.

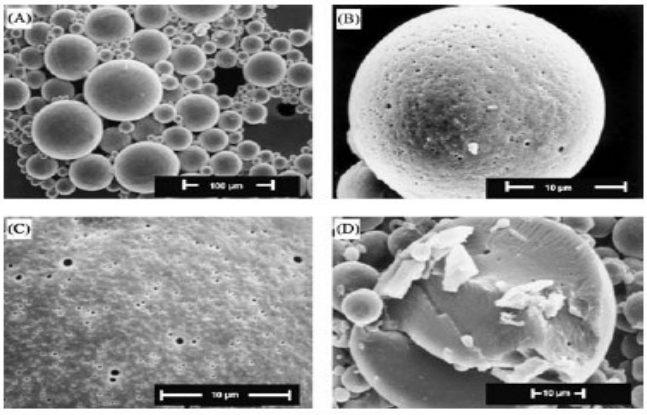

Figure7: Scanning electron micrographs of leuprorelin-loaded,poly(lactic-*co*-glycolic acid) (PLGA)-based microparticles (Lupron Depot®) used for the treatment of prostate cancer.

A) overview on an ensemble of microparticles,
B) surface of a single (*smaller*) microparticle,
C) surface of a single (*larger*) microparticle,
D) partial cross-section of a single microparticle

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

The Blood-Brain-Barrier (BBB) very well protects the Central Nervous System (CNS) against potential toxins and, thus, renders the treatment of brain diseases often extremely difficult. Only low molecular weight lipid-soluble molecules and a few peptides and nutrients can cross this barrier to a significant extent, either by passive diffusion or using specific transport mechanisms. Thus, for most drugs it is difficult to achieve therapeutic levels within the brain tissue. highly potent drugs (e.g., anticancer drugs and neurotrophic factors) that may be necessary to be delivered to specific areas in the CNS, often cause serious toxic side effects in other parts of the human body (especially if high systematic concentrations are required to assure sufficient drug levels in the target tissue).

The stereotaxic injection of drug-loaded, biodegradable microparticles directly into the brain tissue (intracranial administration) offers a very promising possibility to overcome this restriction. Optimized drug concentrations at the site of action can be provided over prolonged periods of time, improving the efficiency of the pharmacotherapy(Menei P,at.al.,2004)

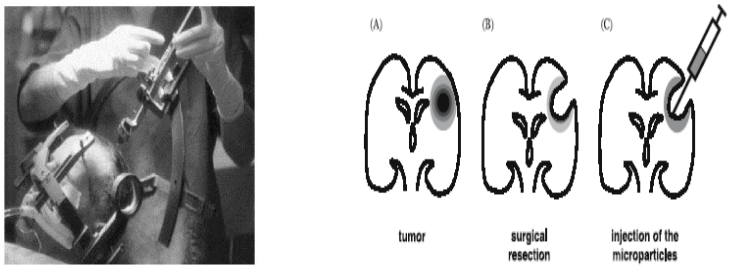

Figure8: Stereotaxic implantation of drug-loaded, biodegradable microparticles into human brain tissue.

This procedure allows an accurate and well-controlled injection into the targeted brain regions. Incorporated nerve growth factor (NGF) in PLGA-based microparticles and obtained promising results. Figure shows a schematic representation of this treatment method: Cells adhere to the microparticles, which release the growth factor in a time-controlled manner. This leads to improved cell survival and differentiation. Figure B,C shows optical and scanning electron microscopy pictures of PLGA-based microparticles containing NGF, with PC12 cells adhering to their surfaces. These systems are intended to be implanted into human brains: The differentiated cells can produce dopamine, which is needed to treat Parkinson's disease(Tatard VM.at.al.,2005).

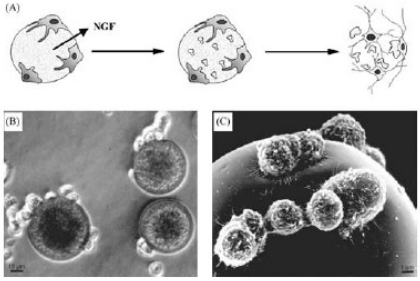

Figure9: Drug-loaded microparticles used to optimize cell growth and differentiation in cell therapies: A schematic illustration of the concept; B optical microscopy picture; and C scanning electron microscopy picture of cells adhering to the surfaces of the microparticles.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Drug Release Mechanisms from PLGA-based Microparticles

(1) Initial burst of drug release is related to drug type, drug concentration and polymer hydrophobicity. Drug on the surface, in contact with the medium, is released as a function of solubility as well as penetration of water into polymer matrix. Random scission of PLGA decreases molecular weight of polymer significantly, but no appreciable weight loss and no soluble monomer product are formed in this phase.

(2) In the second phase, drug is released progressively through the thicker drug depleted layer. The water inside the matrix hydrolyzes the polymer into soluble oligomeric and monomeric products. This creates a passage for drug to be released by diffusion and erosion until complete polymer solubilization. Drug type also plays an important role here in attracting the aqueous phase into the matrix.

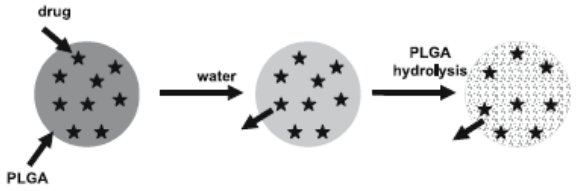

Figure10: Schematic illustration of a bulk-eroding, poly (lactic-*co*glycolic acid) (PLGA)-based microparticle. Water penetration into the system is much faster than polymer hydrolysis.

Three-phasic drug release patterns are observed with PLGA-based microparticles. The release of 5-FU-loaded systems in phosphate buffer pH 7.4. The three phases can essentially be attributed to: (i) pure diffusion at early time points (the very short diffusion pathway lengths lead to high initial drug release rates, so-called "burst effects"); (ii) a combination of drug diffusion, polymer chain cleavage and the limited solubility of 5-FU, leading to approximately constant drug release rates (the increase in the diffusion pathway lengths is compensated by an increase in drug diffusivity); and (iii) to the breakdown of the polymeric network as soon as a critical threshold value is reached, resulting in the disintegration of the microparticles(Siepmann J, Göpferich A 2001).

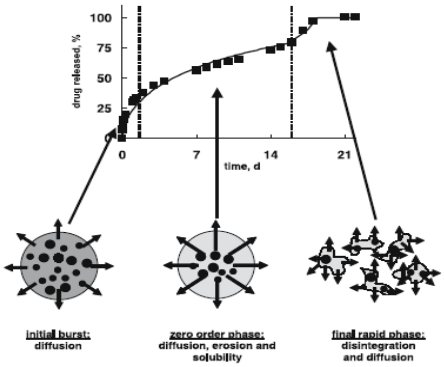

Figure11: Drug release from and drug release mechanisms in 5-fluorouracil (5-FU)-loaded, poly(lactic-*co*-glycolic acid) (PLGA)-based microparticles (Siepmann J, Faisant N, Benoit JP2002).

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

CONCLUSION

This review focuses on the microencapsulation of hydrophobic drugs, describes a variety of techniques for their preparation and analytics, Moreover, it has been pointed out how formulation parameters can be used to control the microparticle characteristics and, based on a mechanistic approach, how undesired microparticle properties can be overcome. Easily injectable, biodegradable formulations of hydrophobic drugs provide a unique and powerful method to treat chronic diseases. Both patients and health care professionals benefit, as slow-release microparticles require a lower administration frequency and thus, generally increase compliance of drug therapy. Moreover, such sustained release formulations might enable a class of drugs with good therapeutic and safety profile but poor solubility and oral bioavailability to provide their medicinal benefit to the patients. Therefore, the consideration of PLGA biodegradable microparticles – the most commonly studied biodegradable carrier for controlled release – for long-term delivery of their drug candidate.

REFERENCES

A. Kumari, S.K. Yadav, S.C. Yadav, (2010). Biodegradable polymeric nanoparticles based drug delivery systems, Colloids Surf. B Biointerfaces 75, 1–18.

Alsante, K.M., Ando, A., Brown, R., Ensing, J., Hatajik, T.D., Kong, W., Tsuda, Y.(2007).The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv. Drug Del. Rev. 59, 29–37.

Alsenz, J., Kansy, M., (2007). High throughput solubility measurement in drug discovery and development: Adv. Drug Del. Rev. 59, 546–567.

Anderson, J.M., Shive, M.S., (1997). Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv. Drug Del. Rev. 28, 5–24.

Aubert-Pouëssel, A., Bibby, D.C., Venier-Julienne, M.-C., Hindré, F., Benoît, J.P., (2002).A novel in vitro delivery system for assessing the biological integrity of protein upon release from PLGA microspheres. Pharm. Res. 19, 1046–1051.

Avdeef, A., 2007. Solubility of sparingly-soluble drugs. Adv. Drug Del. Rev. 59,568–590.

Baker R (1987) Controlled Release of Biologically Active Agents. Wiley, New York.

Basak, A.K., Raw, A.S., Yu, L.X. (Eds.), (2007). Pharmaceutical impurities: analytical, toxicological, and regulatory aspects. Adv. Drug Del. Rev. 59(Theme issue).

Berkland C, King M, Cox A, Kim K,Pack DW (2002) J Controlled Release 82:137

Carrio, A., Schwach, G., Coudane, J., Vert, M., (1995). Preparation and degradation of surfactant-free PLGA microspheres. J. Controlled Release 37,113–121.

Chen, J.-L., Yeh, M.-K., Chiang, C.-H., (2004). The mechanism of surface-indented protein-loaded PLGA microparticle formation: the effect of salt (NaCl) on the solidification process. J. Microencapsul. 21, 877–888.

Christian Wischke1, Steven P. Schwendeman,(2008). Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. International Journal of Pharmaceutics 364 (2008) 298–327.

Fan LT, Singh SK (1989) Controlled release. A Quantitative Treatment.Springer, Berlin

Fu, K., Pack, D.W., Klibanov, A.M., Langer, R., (2000). Visual evidence of acidic environment twithin degrading poly(lactic-co-glycolic acid (PLGA) microspheres. Pharm.Res. 17, 100–106.

Freitas S, Merkle HP, Gander B (2005) Controlled Release 102:313,

Herrero-Vanell, R., Ramirez, L., Fernandez-Carballido, A., Refolo, M.F., (2000).Biodegradable PLGA microspheres loaded with ganciclovir for intraocular administration. Encapsulation technique, in vitro release profiles, and sterilization process. Pharm. Res. 17, 1323–1328.Herrmann, W.O., Haehnel, W., 1927. Über den.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Jain, R.A., Rhodes, C.T., Railkar, A.M., Malick, A.W., Shah, N.H., (2000)a. Controlled release of drugs from injectable in situ formed biodegradable PLGA microspheres: effect of various formulation variables. Eur. J. Pharm. Biopharm. 50,257–262.

Jain, R.A., Rhodes, C.T., Railkar, A.M., Malick, A.W., Shah, N.H., (2000)b. Controlled delivery of drugs rom a novel injectable in situ formed biodegradable PLGA microsphere system. J. Microencapsul. 17, 343–362.

Jain, R.A., Rhodes, C.T., Railkar, A.M., Malick, A.W., Shah, N.H., (2000)c. Comparison of various injectable protein-loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices: in situ-formed implant versus in-situ-formed microspheres versus isolated microspheres. Pharm. Dev. Technol. 5, 201–207.

Jain, R.A., (2000)P. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21,2475–2490.

Jeong, J.H., Lim, D.W., Han, D.K., Park, T.G., (2000). Synthesis, characterization and protein adsorption behaviors of PLGA:PEG di-block co-polymer blend films. ColloidsSurf. B 18, 371–379.

Juergen Siepmann,Florence Siepmann: Microparticles Used as Drug Delivery Systems: Progr Colloid Polym Sci (2006) 133: 15–21

Johanson, P., Men, Y., Merkle, H.P., Gander, B., (2000). Revisiting PLA/PLGA microspheres: an analysis of their potential in parenteral vaccination. Eur. J. Pharm. Biopharm. 50, 129–146.

Jeffery, H., Davis, S.S., O'Hagan, D.T., 1991. The preparation and characterisation of poly(lactide-co-glycolide) microparticles: I: oil-in-water emulsion solvent evaporation.Int. J. Pharm. 77, 169–175.

Li, S., Girod-Holland, S., Vert, M., 1996. Hydrolytic degradation of poly(dl-lactic acid) in the presence of caffeine base. J. Controlled Release 40, 41–53.

Martin, A., 1993. Physical Pharmacy. Lea & Febiger, Philadelphia, p. 213.

Muranishi, S., Ikada, Y., Yoshikawa, H., Gen, S., 1991. Polylactic acid microspheres and process for producing the same. US Patent 4,994,281 (19 February).

Mehta, R. C., Thanoo, B. C., and DeLuca, P. P., Peptide containing microspheres from low molecular weight and hydrophilic poly(D,L-lactide-co-glycolide). J. Controlled Release, 41, 249-257 (1996).

Menei P, Jadaud E, Faisant N,Boisdron-Celle M, Michalak S,Fournier D, Delhaye M, Benoit JP(2004) Cancer 100:405

Okada H, Toguchi H (1995) Crit Rev Ther Drug Carrier Syst 12:1

Sinha VR, Trehan A (2003) J Controlled Release 90:261.

NewYork, pp. 1151–1200 (Chapter 32 Schalper, K., Harnisch, S., Müller, R.H., Hildebrandt, G.E., 2005. Preparation of microparticles by micromixers—characterisation of o/w process and prediction of particle size. Pharm. Res. 22, 276–284.

Siepmann J, Göpferich A (2001) Adv Drug Del Rev 48:229.

Siepmann J, Faisant N, Benoit JP(2002) Pharm Res 19:1885.

Schwendeman, S.P., Cardamone, M., Klibanov, A., Langer, R., Brandon, M.R., 1996. Stability of proteins and their delivery from biodegradable polymer microspheres.In: Cohen, S., Bernstein, H. (Eds.), Microparticulate Systems for the Delivery of Proteins and Vaccines. Marcel Decker, New York, pp. 1–49.

Tanquary AC, Lacey RE (1974),Controlled Release of Biologically Active Agents. Plenum Press, NewYork.

Tatard VM, Venier-Julienne MC,Saulnier P, Prechter A, Benoit JP,Menei P, Montero-Menei CN (2005)Biomaterials 26:3727.

Tang, H., Xu, N., Meng, J.,Wang, C., Nie, S.-F., Pan,W.-S., 2007. Application of a novel approach to prepare biodegradable polylactic-co-glycolic acid microspheres:surface liquid spraying. Yakugaku Zasshi 127, 1851–1862.

Wu, X.S., 1995b. Preparation, characterization, and drug delivery application of microspheres based biodegradable lactic/glycolic acid polymers. In: Wise, D., Trantolo, D.J., Altobelli, D.E., Yaszems, M.J. (Eds.), Encyclopedic Handbook of Biomaterials and Bioengineering:Materials/Applications.Marcel Dekker,).