DEVELOPMENT AND EVALUATION OF DOCETAXEL ENCAPSULATED NANOPARTICLES FROM POLY(3-HYDROXYBUTYRATE-*CO*-4-HYDROXYBUTYRATE) BIOPOLYMER

FAISALINA BT AHMAD FISOL

UNIVERSITI SAINS MALAYSIA

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by

FAISALINA BT AHMAD FISOL

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This thesis is dedicated to,

My dear husband, father, mother, my family, and friends

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LIST OF ABBREVIATIONS

3HB	3-hydroxybutyrate
4HB	4-hydroxybutyrate
ACN	Acetonitrile
AFM	Atomic Force Microscopy
AUC	Area under the concentration curve
Da	Dalton
DMSO	Dimethylsulfoxide
DCM	Dichloromethane
DLS	Dynamic Light Scattering
DCX	Docetaxel
i.e.	Example
EPR	Enhanced permeability and retention
EE	Encapsulation Efficiency
et al.	And others
FDA	Food and Drug Administration
FTIR-PAS	Fourier transform infra-red photoacoustic spectroscopy
g	gram
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation
IV	Intra Venous
LDV	Laser Doppler Velocimetry
kg	kilogram

Lecithin	L-α-phosphatidylcholine from soybean,Type II-S
mg	miligram
min	minute
ml	mililitre
Mw	Molecular weight
nm	nanometer
NCs	Nanocapsules
NPs	Nanoparticles
NSs	Nanospheres
O/W	Oil-in-water
P(3HB-*co*-4HB)	Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)
PCX	Paclitaxel
PALS	Phase Analysis Light Scattering
PDI	Polydispersity index
PEG	Polyethylene glycol
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PLA	Poly(lactic acid)
PVA	Polyvinyl alcohol
Pluronic® F-68	Polyoxyethylene-polyoxpropylene block polymer
RI	Refractive Index
rpm	Revolutions Per Minute
SEM	Scanning electron microscopy

THF	Tetrahydrofuran
Vitamin E	(+/-)α-tocopherol
Vitamin E TPGS	d-α-tocopheryl polyethylene gycol 1000 succinate
XPS	X-ray photoelectron spectroscopy
µm	Micrometer

LIST OF PUBLICATIONS

Amirah, M.G, **Faisalina, A.F**, Habibah, A.W, Majid, M.I.A and Amirul, A.A. Production of Polymeric Nanoparticles from Locally Produced Polymer Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate). International Conference and Exhibition on Pharmaceutical, Nutraceutical and cosmeceutical Technology, Kuala Lumpur, Malaysia, 2010.

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PEMBANGUNAN DAN PENILAIAN NANOPARTIKEL YANG MENGANDUNGI DOCETAXEL DARIPADA BIOPOLIMER POLI(3-HIDROKSIBUTIRAT-*KO*-4-HIDROKSIBUTIRAT)

ABSTRAK

Tiga jenis nanopartikel (NPs) yang mengandungi drug antikanser yang kurang larut air iaitu docetaxel (DCX), telah dibangunkan projek penyelidikan ini menggunakan polimer terbiodegradasi poli 3-hidroksibutirat-*ko*-4-hidroksibutirat [P (3HB-*ko*-4HB)]. Formulasi optimum P(3HB-*ko*-4HB) nanopartikel telah dibangunkan dengan menyaring tensioaktif berlainan (PVA, lesitin, pluronic F-68 dan vitamin E TPGS) menggunakan kaedah emulsi/penyejatan pelarut dan kaedah nanopemendakan. Hasil daripada semua eksperimen yang dijalankan, didapati bahawa nanopartikel P(3HB-*ko*-4HB) dengan ciri-ciri fizikokimia yang baik (saiz zarah <200 nm, PDI <0.15 dan keupayaan zeta -10 mV) telah dihasilkan dengan menggunakan kaedah emulsi / penyejatan pelarut tanpa berhadapan dengan masalah seperti penggumpalan zarah dan pemendakan polimer. Nanopartikel yang mengandungi DCX dan tidak mengandungi DCX kemudian dicirikan untuk saiz dan taburan saiz, cas permukaan, dan morfologi. Nanopartikel P(3HB-*ko*-70% 4HB) yang mengandungi DCX memberi keputusan yang paling baik iaitu saiz zarah 140-180 nm dengan nilai indeks polidispersiti (PDI) yang rendah iaitu 0.09. Caj permukaan bagi nanopartikel berkenaan adalah antara -10mV dan -22mV. Selain itu, hasil kecekapan memerangkap menunjukkan bahawa pada 30% nisbah drug kepada polimer, DCX-P(3HB-*ko*-16%4HB) NPs dapat memerangkap sehingga 42% DCX, DCX-P(3HB-*ko*-

30%4HB) NPs memerangkap sehingga 46% DCX dan DCX-P(3HB-*ko*-70%4HB) NPs memerangkap sehingga 50% daripada amaun DCX dalam sistem nanopartikel. Daripada keputusan ini DCX-P(3HB-*ko*-70% 4HB) NPs telah dipilih untuk ujikaji profil perlepasan drug dan keputusan kajian menunjukkan dua ciri pelepasan drug telah dihasilkan . P(3HB-*ko*-70% 4HB) NPs dengan nisbah drug kepada polimer yang tinggi membawa kepada profil pelepasan yang agak cepat, manakala nanopartikel dengan nisbah drug kepada polimer yang lebih rendah daripada 30% jumlah DCX di dalam P(3HB-*ko*-70%4HB) nanopartikel menunjukkan profil pelepasan drug yang berpanjangan. Sebanyak 60% DCX telah dilepaskan dari sistem DCX-P (3HB-*ko*-70%4HB) nanopartikel dalam tempoh 8 jam hingga 7 hari untuk 5%, 10% dan 20% daripada nisbah DCX / P(3HB-*ko*-70% 4HB) manakala bagi 30% dan 40% daripada nisbah DCX / P(3HB-*ko*-70%4HB), mereka mempunyai pelepasan yang hampir lengkap (98%) selepas 7 hari pengeraman.

DEVELOPMENT AND EVALUATION OF DOCETAXEL ENCAPSULATED NANOPARTICLES FROM POLY(3-HYDROXYBUTYRATE-*CO*-4-HYDROXYBUTYRATE) BIOPOLYMER

ABSTRACT

Three types of nanoparticles (NPs) loaded with poorly water soluble anticancer drug, docetaxel (DCX), were developed in this research using poly 3-hydroxybutyrate-*co*-4-hydroxybutyrate P(3HB-*co*-4HB) biodegradable polymers. The optimized P(3HB-*co*-4HB) nanoparticles formulation was developed by screening the use of different tensioactives (PVA, lecithin, pluronic F-68 and vitamin E TPGS) in modified emulsion/solvent evaporation and nanoprecipitation production methods. From all the experiments carried out, it was found that P(3HB-*co*-4HB) nanoparticles with good physicochemical characteristics (particle size <200 nm, PDI <0.15 and zeta potential -10 mV) were prepared with emulsion/solvent evaporation method without problems such as particle agglomeration and precipitation of the polymer. The blank and DCX loaded nanoparticles were then characterized for their size and size distribution, surface charge, and morphology. DCX loaded P(3HB-*co*-70% 4HB) nanoparticles gave the best results with particles size of 140-180 nm with low PDI of 0.09. The surface charge of the nanoparticles was between -10mV and -22mV. Furthermore the encapsulation efficiencies result showed that at 30% of drug to polymer ratio, DCX loaded P(3HB-*co*-16% 4HB) NPs were able to encapsulate up to 42% of DCX; DCX loaded P(3HB-*co*-30% 4HB) NPs encapsulated up to 46% of DCX and DCX loaded P(3HB-*co*-70% 4HB)

NPs encapsulated up to 50% of DCX in the nanoparticles system. From these results DCX loaded P(3HB-*co*-70% 4HB) NPs were chosen for the release experiments and two release behaviours were observed. P(3HB-*co*-70%4HB) nanoparticles with high drug/polymer ratio led to a relatively rapid release, while nanoparticles with drug to polymer ratio lower than 30% DCX amount in P(3HB-*co*-70%4HB) nanoparticles showed a prolonged drug release profile. 60% of DCX was released from the DCX-P(3HB-*co*-70% 4HB) NPs within 8 hr to 7 days for 5% , 10% and 20% of DCX/ P(3HB-*co*-70% 4HB) ratio while the 30% and 40% of DCX/ P(3HB-*co*-70% 4HB) ratio, NPs gave an almost complete drug release (98%) after 7 days of incubation.

CHAPTER ONE

INTRODUCTION

1.1 Nanoparticles in Drug Delivery

Nanoparticles were first developed about 35 years ago (Ravi Kumar, 2000). They were initially developed as carriers for vaccines and cancer chemotherapy agents because of their ability to affect drug pharmacokinetics and pharmacodynamics through modification of absorption, distribution, metabolism and elimination of the drug in the body (Rao et al., 2010).

Nanoparticles are stable, solid colloidal particles consisting of biodegradable polymer or lipid materials and range in size from 10 to 1000 nm. Drugs can be absorbed onto the particles surface, entrapped inside the polymer/lipid, or dissolved within the particle matrix (Kingsley et al., 2006). Depending upon the method of nanoparticles preparation, nanospheres or nanocapsules can be obtained (Soppimath et al., 2001). Nanocapsules are core-shell systems in which the drug is confined to a cavity surrounded by a unique polymer membrane (Figure 1.1), while nanospheres are matrix systems in which the drug is physically and uniformly dispersed (Figure 1.1).

Nanocapsules differ from nanospheres in that they are a reservoir form, in which a solid polymer shell surrounds a core which is liquid or semisolid at room temperature (15-25°C) (Vauthier and Bouchemal, 2009). In the first nanocapsules formulations, the core was composed of oil hence allowing a high payload of a liposoluble drug (Vauthier and Bouchemal, 2009). More recently, nanocapsules with an aqueous core able to encapsulate water-soluble compounds are developed. The content of the nanocapsules is determined by the nature of the dispersed phase of the formulation. Generally, the polymer shell surrounding the liquid core is formed due to polymerization taking place at the interface between the dispersed and continuous phase of the emulsion (Bouchemal et al., 2006; Hillaireau et al., 2006; Mayer, 2005; Bouchemal et al., 2004; Watnasirichaikul et al., 2002; Lambert et al., 2000; Wohlgemuth et al., 2000) or by precipitation of a preformed polymer at the surface of emulsion droplets (Moinard-Chécot et al., 2008; Quintanar-Guerrero et al., 1998; Zambaux et al., 1998;).

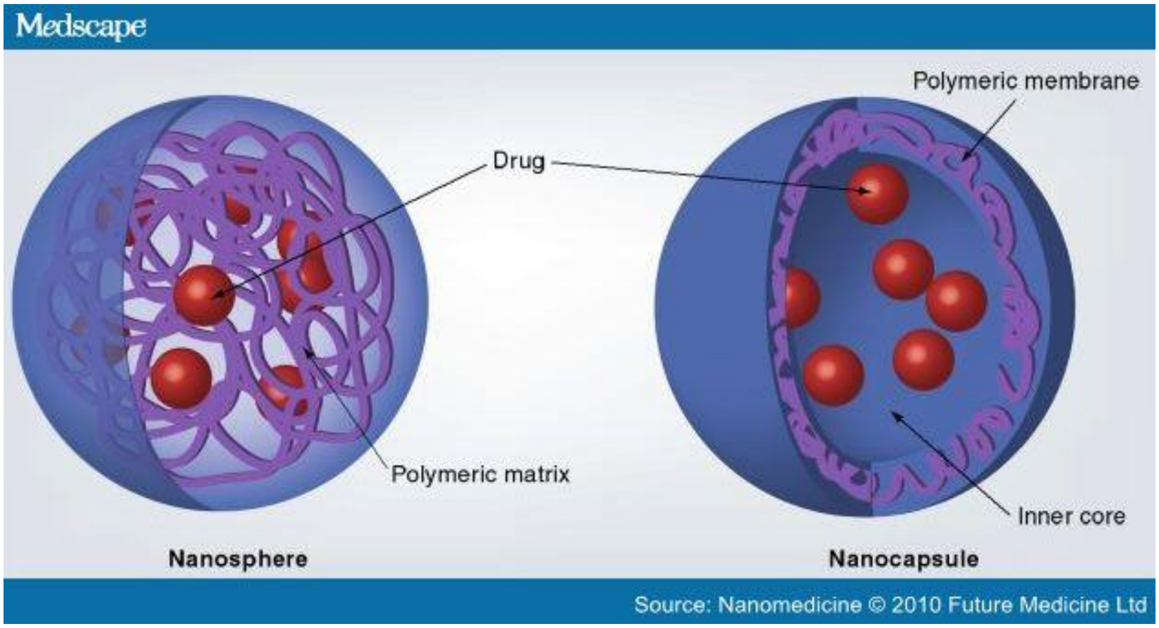

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Figure 1.1**:** Nanospheres and nanocapsules (adapted from Bei et al., 2010)

Nanospheres are matrix particles, i.e. particles whose entire mass is solid. These particulate systems are characterized by a size ranging from several tenths of nanometers to a few hundred of nanometers. In general, they are of spherical shape and to remain well dispersed in liquid dispersion, like all types of colloids, need to be stabilized using amphiphilic molecules or colloid protecting agents (Vauthier and Bouchemal, 2009).

Nanospheres and nanocapsules designed as drug carriers can be loaded by drug entrapment or sorption on particle surface (Vauthier and Bouchemal, 2009). In general, fragile molecules are better preserved from enzymatic degradation occurring in biological medium when they are entrapped in the nanocarriers (Toub et al., 2006; Lambert et al., 2000). In this case, their association with the drug carrier should be done during the preparation of the nanocapsules or the nanospheres (Nafee et al., 2007; Hillaireau et al., 2007; Vogel et al., 2005; Oster et al., 2004; Perez, et al., 2001; Lambert, et al., 2000). However, when the drug is highly susceptible to degradation which may occur during the preparation process of the drug carrier or when it does not associate during the preparation of the drug carrier, it can be loaded by adsorption on the surface of already prepared carriers (Zobel et al., 2000). Table 1.1 lists examples of FDA-approved colloidal drug delivery systems. These were collectively designed to be taken up and delivered by mononuclear phagocytes (MP).

Table 1.1 : FDA approved nanoparticles drug delivery system (adapted in part from Allen and Cullis, 2004)

Drug or therapeutic agent (trade name)	Indication	Reference
Liposomal amphotericin B (Ambisome, Ablecet, Amphotec)	Fungal infections, Leishmaniasis	Alder-Moore (1994)
PEG-adenosine deaminase (Pegademase)	Severe combined immunodeficiency disease	Bory et al. (1991)
PEG-stabilized liposomal doxorubicin (Doxil, Evacet)	Kaposi's sarcoma, refractory ovarian cancer	Muggia and Hamilton (2001), Northfelt et al. (1996)
Liposomal cytosine arabinoside (DepoCyt)	Lymphomatous miningtis, neoplastic meningtis	Glantz et al. (1999a) Glantz et al. (1999b)
Interleukin 2-diptheria toxin fusion protein (Denileikin Diffitox)	Cutaneous T cell lymphoma	Olsen et al. (2001)
Liposomal verteporfin (Visudyne)	Wet macular degeneration	Bressler (2001)
PEG-interferon α-2b (Pegasys)	Hepatitis C	Glue et al. (2000)
PEG-granulocycte colony stimulating factor (Neulasta)	Chemotherapy associated neutropenia	Siena et al. (2003)
Protein bound paclitaxel (Abraxane)	Metastatic breast cancer	Nyman et al. (2005)
PEG L-asparaginase (Oncaspar)	Acute lymphocytic leukemia	Rosen et al. 92003)
PEG aptanib (Macugen)	Wet macular degeneration	Lee et al. (2005a,b)

1.2 Preparation of Nanoparticles

Polymeric nanoparticles generally present as a core-shell structure consisting of a dense polymer core in which a hydrophobic drug can be encapsulated and a corona made of a hydrophilic polymer, such as PEG, PVP, or polysaccharides, which serves to confer steric stability and stealth properties to the particles upon IV administration (Gaucher et al., 2010). Conventionally, nanoparticles have been prepared mainly by two methods: (i) dispersion of the preformed polymers; and (ii) polymerization of monomers.

1.2.1 Dispersion of preformed polymers

Several methods have been suggested to prepare biodegradable nanoparticles from PLA, PLG,PLGA and poly(ε-caprolactone) by dispersing the preformed polymers (Soppimath et al., 2001).

1.2.1.1 Solvent Evaporation Method

In this method, the polymer is dissolved in an organic solvent like dichloromethane, chloroform or ethyl acetate. The drug is dissolved or dispersed into the preformed polymer solution, and this mixture is then emulsified into an aqueous solution to make an oil (O) in water (W) emulsion by using a surfactant/emulsifying agent like gelatin, poly(vinyl alcohol), polysorbate-80, poloxamer etc. After the formation of a stable emulsion, the organic solvent is evaporated by increasing the temperature/under pressure or by continuous stirring (Soppimath et al., 2001). The multiple emulsion method had also been used to prepare water-soluble drug loaded nanoparticles by forming W/O/W emulsions (Zambaux et al., 1998). Both the above methods use a high speed homogenization or sonication to obtain a sufficiently finely dispersed internal phase. However, these procedures are good for laboratory-scale operation, but for a large-scale pilot production, alternative methods using low-energy emulsification is required (Soppimath et al., 2001).

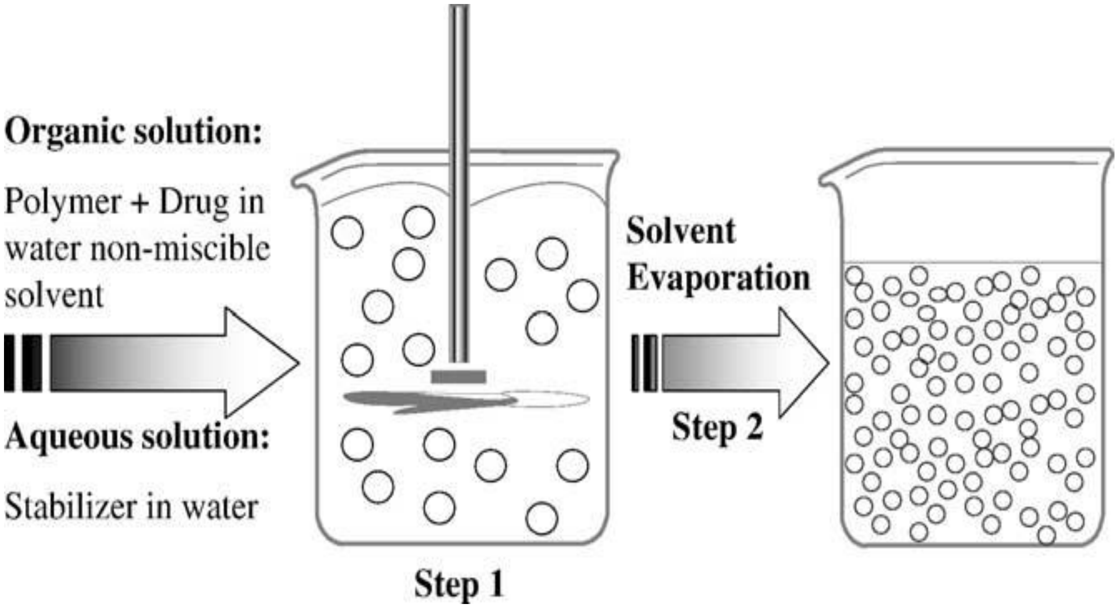


Figure 1.2: Schematic representation of solvent evaporation technique (adapted from Pinto Reis et al., 2005)

1.2.1.2 Spontaneous Emulsification/ Solvent Diffusion Method

In this method, an oil/water emulsion is formed between a well-accepted partially water-miscible solvent like acetone or methanol along with the water insoluble organic solvent like dichloromethane or chloroform were used as an oil phase containing the drug and polymer and an aqueous solution containing a stabilizer. Due to the spontaneous diffusion of water-soluble solvent (acetone or methanol), an interfacial turbulence is created between two phases leading to the formation of smaller dispersed droplets of the immiscible solvent, resulting in the formation of nanoparticles. As the concentration of water-soluble solvent (acetone) increases, a considerable decrease in particle size can be achieved (Soppimath et al., 2001). Depending on the boiling point of the solvent, this can be eliminated under reduced pressure or by cross-flow filtration. The technique has been reported to be efficient, versatile, of easy implementation and allows a high efficiency entrapment of lipophilic drugs in polymeric matrices (Colombo et al., 2001; Kwon et al., 2001).

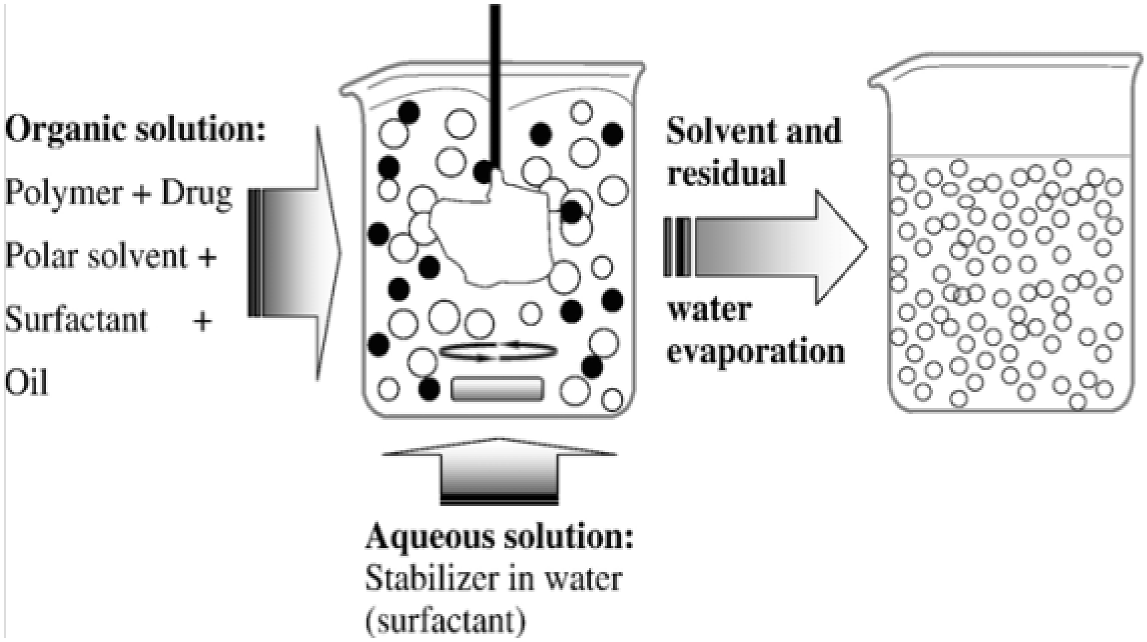


Figure 1.3: Schematic representation of emulsification/solvent diffusion technique (adapted from Pinto Reis et al., 2005)

1.2.1.3 Salting Out Method

Nanoparticles were prepared using the salting-out method in which acetone was chosen as the water-miscible organic solvent because of its pharmaceutical acceptance with regard to toxicity (Alle´mann et al., 1993). The method consists of the addition of a water soluble PVA in a highly concentrated salt solution in water (aqueous phase) to a polymer solution in acetone (organic phase). Although acetone is miscible with pure water in all ratios, the high salt concentration of the aqueous phase prevents mixing of the phases. After emulsification, the addition of pure water in a sufficient quantity causes acetone to diffuse into the aqueous phase, resulting in the formation of nanoparticles.

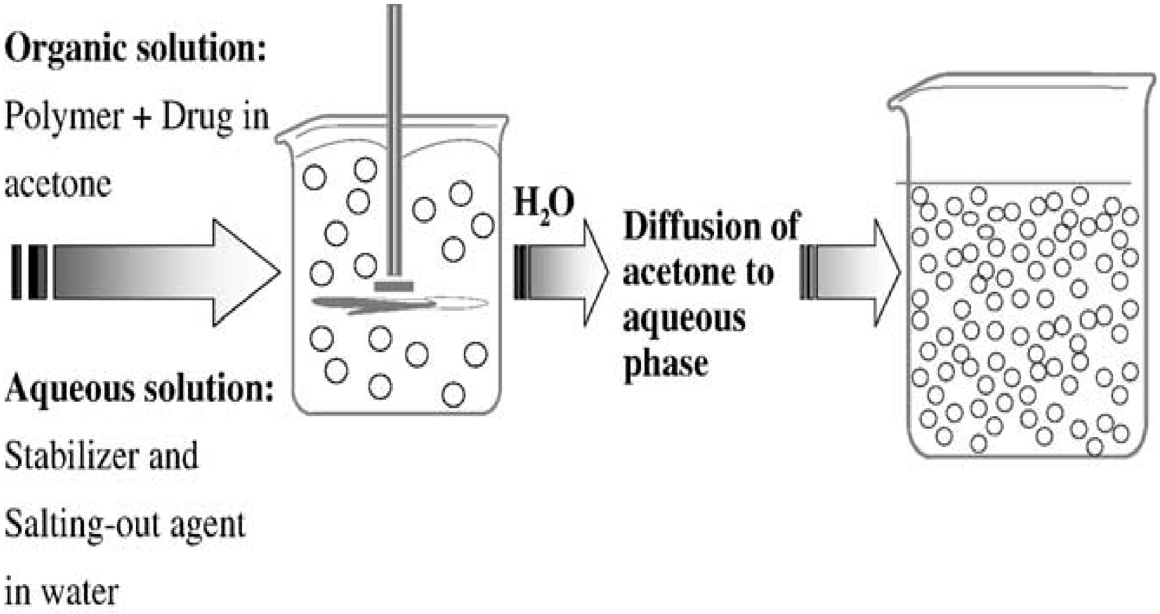


Figure 1.4: Schematic representation of salting out technique (adapted from Pinto Reis et al., 2005)

1.2.2 Polymerization of monomers

Nanoparticles can also be prepared by polymerization of monomers. Poly(alkylcyanoacrylate)s, PACA, being biodegradable, have been used as tissue adhesives in surgery since these are well tolerated *in vivo*. This had prompted intense research activity to study polymerization reactions leading to the formation of this class of polymers. The production of nanoparticles (~ 200 nm diameter) by mechanically polymerizing the dispersed methyl or ethyl cyanoacrylate in aqueous acidic medium in the presence of polysorbate-20 as a surfactant without irradiation or an initiator (Soppimath et al., 2001). Here, the cyanoacrylic monomer is added to an aqueous solution of a surface-active agent (polymerization medium) under vigorous mechanical stirring to polymerize alkylcyanoacrylate at ambient temperature. Drug is dissolved in the polymerization medium either before the addition of monomer or at the polymerization reaction (Soppimath et al., 2001). The nanoparticles suspension is then purified by ultracentrifugation or by resuspending the particles in an isotonic surfactant free medium. The mechanism of polymerization of PACA monomer is given below.

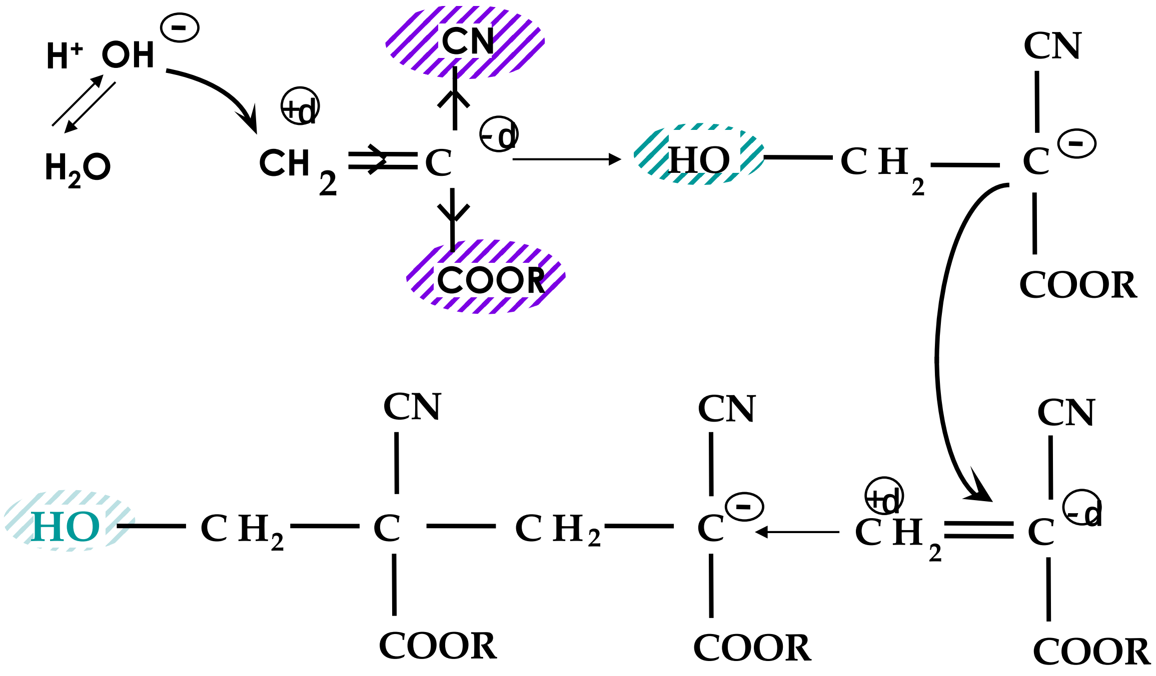


Figure 1.5: The mechanism of polymerization of PACA monomer (adapted from Soppimath et al., 2001)

Polymerization follows the anionic mechanism, since it is initiated in the presence of nucleophilic initiators like OH^- , CH_3O^- and CH_3COO^- leading to the formation of nanoparticles of low molecular mass due to rapid polymerization. Such nanoparticles are degraded very fast. In order to circumvent this problem and to produce higher molecular mass as well as stable nanoparticles, polymerization must be carried out in an acidic medium (pH 1.0-3.5). After dispersing the monomer in an aqueous acidic medium containing surfactant and stabilizer, polymerization is continued for 3-4 hours by increasing the pH of the medium to obtain the desired products (Soppimath et al., 2001).

During polymerization, various stabilizers like dextran-70, dextran-40, dextran-10, poloxamer-188, -184,-237 etc are added. In addition, some surfactants like polysorbate-20,-40 or -80 are also used. A schematic representation for the production of poly(alkylcyanoacrylate) nanoparticles is shown is Figure 1.6. Particle molecular mass of NPs depend upon the type and concentration of the stabilizer and/or surfactant used, upon the pH of the polymerization medium (Behan et al., 1999), as well as the concentration of monomer and the speed of stirring (Soppimath et al., 2001).

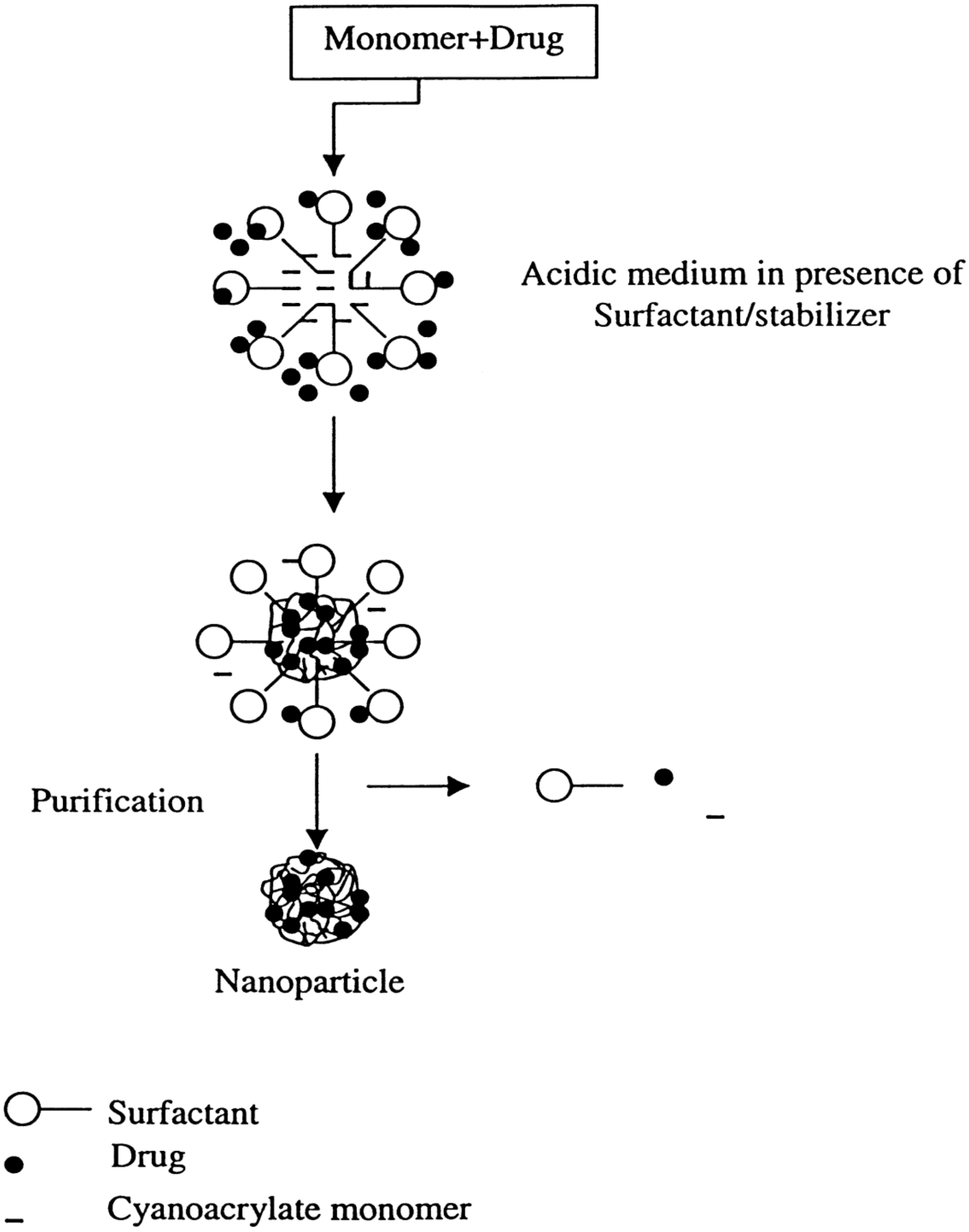


Figure 1.6: Schematic representation for the production of poly(alkylcyanoacrylananoparticles (adapted from Soppimath et al., 2001).

1.3 Chemotherapy with Nanoparticles

The majority of pharmaceutical research into the use of nanoparticles drug delivery system has been in the area of oncology. Nanocarriers can concentrate preferentially in tumor masses, inflammatory sites, and infectious sites by virtue of the enhanced permeability and retention (EPR) effect on the tumor vasculature (Shenoy et al., 2005). In fact among the characteristics of solid tumors: extensive angiogenesis, defective vascular architecture, impaired lymphatic drainage/recovery system, and increased production of a number of permeability mediators are common features (Kingsley et al., 2006).

According to the EPR concept, biocompatible macromolecules accumulate at much higher concentrations in tumor tissues than in normal tissues or organs, even higher than those in plasma (Matsumura and Maeda, 1986; Iwai et al., 1984; Maeda et al., 1984). This EPR effect can be observed with macromolecules having an apparent molecular size larger than 50 kDa which have long plasma half-lives (Maeda et al., 1986). It is also seen with large liposomes and some lipids (edible oils) in various experimental as well as human tumor systems (e.g., Noguchi et al., 1998 ; Iwai et al., 1987; Maeda et al., 1986; Matsumura and Maeda, 1986; Iwai et al., 1984; Maeda et al., 1984), such as sarcoma 180; colon 38; Walker 256 carcinoma; melanoma 16; AH (Yoshida hepatoma) 109B and 136B; VX-2, in mice, rats, and rabbits; and most human solid tumors.

In general, most polymeric drugs accumulate in tumor tissue at concentrations 5–10 times higher than the concentration in plasma 24 hour after intravenous injection, and frequently at levels more than 10 times higher than that in normal tissue such as noncancerous muscle (Maeda, 1991; Maeda and Matsumura, 1989; Maeda et al., 1986; Matsumura and Maeda, 1986). Most conventional drugs have a plasma half-life of less than 20 min in mouse or human. However, it takes at least 6 hour for drugs in circulation to exert the EPR effect. This means that any candidate drug must have a large molecular size, above the renal clearance threshold, to circulate for a long time. Indeed, the plasma AUC (area under the concentration curve) paralleled the accumulation of drug in tumor. Also, polymeric drugs should not be cationic but either neutral or anionic, because the luminal surface of blood vessels is highly negatively charged and thus cationic polymer drugs are adsorbed on the vascular surface and are expected to have short in vivo half-lives. In addition, it is obvious that these molecules should not exhibit antigenic or immunogenic characteristics (Maeda, 1994; Maeda, et al., 1992; Meada, 1991; Maeda and Matsumura, 1989).

More than 17 years ago, Maeda and his colleagues described the EPR effect for albumin, immunoglobulinG, and transferrin; however, other smaller proteins of less than 30 kDa do not exhibit the EPR effect. A synthetic polymer-conjugated anticancer agent, SMANCS (Maeda et al., 1986; Matsumura and Maeda, 1986; Maeda et al., 1984; Maeda et al., 1979), which is poly(styrene–co–maleic acid/half-n-butyl ester) (SMA) conjugated with neocarzinostatin (NCS), size of 16 kDa, (Maeda et al., 1985; Maeda et al., 1979) did show the EPR effect because it is bound to albumin, thus exhibiting a molecular size of about 80 kDa. Transferrin is of interest because radioimaging with radioactive g-emitting gallium scintigraphy, which is commonly used in clinical practice, depends on selective accumulation in tumor of 67Ga entrapped in transferrin, which has a molecular size of about 90 kDa (Larson, 1978). Thus, the size of this complex, 67Ga-transferrin, is ideal for capitalizing on the EPR effect.

In addition, the EPR phenomenon was also observed with various polymer conjugates such as poly(hydroxypropylmethacrylamide) (HPMA) copolymer, polyethylene glycol, polyvinyl alcohol, the lipid contrast agent Lipiodol and some vegetable oils, and liposomes (Maeda and Kojima, 1997; Duncan et al., 1996; Seymour et al., 1995; Maeda, 1991; Maeda and Matsumura, 1989; Maeda et al., 1986; Maeda et al., 1984), as well as to plasma proteins. Therefore, the EPR effect appears to be a key mechanism for tumor-selective drug delivery and is a gold standard in anticancer drug design.

As a consequence of the leaky neo-formed blood vessels in solid tumor, colloidal carriers are able to extravasate and accumulate at the site of tumor, where generally the will not able to pass the vascular endothelial barrier in healty organs. The EPR effect provides an opportunity for more selective targeting of anticancer drug loaded nanoparticles (lipid or polymer based) to the tumor (Vicent and Duncan, 2006; Sengupta, et al., 2005; Maeda et al., 2000).

Additionally, it is possible to fabricate nanoparticles with several different drugs and allow for the nanocarriers to selectively deliver the drugs to the malignant tissue, induce uptake by the malignant cells, and then release the intracellular anticancer drugs killing malignant cells (Kingsley et al., 2006). In one example, an anticancer drug incorporated within a biodegradable colloidal shell and another antiangionesis drug that was placed within a lipid layer that surrounds the colloidal shell has been produced. When the nanocarrier is administered intravenously, the malignant cells internalize the nanoparticle. Once inside the cell, the antiangionesis drug within the lipid layer of the nanocarriers is released, inhibiting the mediators for blood vessel production. Then, the core anticancer drug within the colloidal shell is released, effectively killing the malignant cell. All of this can be fabricated in a “nanocell” that is less than 250 nm (Sengupta et al., 2005). This is truly an optimized delivery of anticancer drugs to tumor site.

1.4 Taxanes nanoparticle preparation

Typically, the hydrophobic polymer and drug must first be dissolved in an appropriate organic solvent and then mixed into an aqueous phase which generally consists of a surfactant solution (Gaucher et al., 2010). If the solvent is water miscible such as acetone, DMF or dimethylsulfoxyde, diffusion of the solvent into the surrounding water phase will cause the polymer to precipitate, and drug-loaded nanoparticles stabilized by surfactant molecules, will form (Gaucher et al., 2010). This process termed nanoprecipitation has been applied to the formulation of DCX-loaded nanoparticles (Cheng et al., 2007; Musumeci et al., 2006). Alternatively, the organic solvent can be dialysed against water to obtain polyester-based nanoparticles incorporating PCX (Zhang et al., 2008; Kim et al., 2006; Xie and Wang, 2005; Kim and Lee 2001). When non-water-miscible solvents, such as dichloromethane and chloroform are employed, an oil-in-water (O/W) emulsion is formed upon mixing of the 2 liquid phases, and particles form upon solvent evaporation (Gaucher et al., 2010). PCX has been incorporated into polyester-based nanoparticles by this method using either probe (Gaucher et al., 2007; Win and Feng, 2006) or high-pressure homogenization (Dong and Feng, 2007).

1.5 Taxanes Loading and Release

A successful nanoparticle system may be the one, which has a high loading capacity to reduce the quantity of the carrier required for administration. Drug loading into the nanoparticle is achieved by two methods: one, by incorporating the drug at the time of nanoparticle production or secondly, by absorbing the drug after formulation of nanoparticles by incubating them in the drug solution (Soppimath et al., 2001). The method and experimental parameters employed for nanoparticle preparation can impact the physicochemical properties of formulations. Danhier *et al.*, 2009 have reported significantly higher encapsulation efficiencies for PCX loaded into PLGA nanoparticles with the nanoprecipitation method (70%) compared to the emulsion/solvent evaporation technique (40%). With the nanoprecipitation method of producing DCX-loaded PLGA nanoparticles it was suggested that limiting drug loading to 1% (*w/w*) minimized particle aggregation and yielded DCX-loaded poly(lactide-*co*-glycolide) (PLGA) nanoparticles with narrower size distributions (Cheng et al., 2007).

Elsewhere, it was shown that an increase in the oil-to-water phase ratio (Fonseca et al., 2002) and the polymer concentration of the organic phase (Dong and Feng, 2006) could enhance the encapsulation efficiency of PCX within polyester-based nanoparticles produced by the nanoprecipitation technique. It is established that the nature of the organic solvent could influence the particle size and PCX encapsulation efficiency of nanoparticles generated by the dialysis method, with more water-miscible solvents (DMF) yielding smaller particles diameters and reasonable PCX loading levels (Zhang et al., 2008). Moreover, the mean diameter of the nanoparticles was also shown to modulate drug incorporation and release, with smaller diameters giving lower encapsulation efficiencies and faster PCX release because of shorter diffusion paths and higher surface area (Dong and Feng, 2007; Dong and Feng, 2006). Alternatively, the nature of the core-forming polymer can impact drug loading and release kinetics. Upon incubation under sink conditions, the PCX released profiles were also very different. While PHA nanoparticles immediately released over 90% of their cargo, PCX release from PDLLA/PLGA nanoparticles varied according to polymer composition (Gaucher et al., 2010).

Drug release from nanoparticulate systems is governed by several factors, including drug location inside the vector and ease with which the drug molecules are able to diffuse through the core matrix. Burst effects are typically attributed to the release of drug molecules located near the particle surface, upon exposure to sink conditions. Alternatively, the nature of the core-forming polymer may affect drug diffusion from nanoparticles (Gaucher et al., 2010).

1.6 Polyhydroxyalkanoates (PHAs) Biodegradable Polymer

The rapid depletion of crude oil and the mounting apprehension about the environmental effects of synthetically produced materials has prompted much interest in biologically derived polymers, particularly of the biodegradable class (Philip et al., 2007). Among the various biodegradable polymers, a class that is drawing considerable attention is the polyhydroxyalkanoates.

Polyhydroxyalkanoates (PHAs) are a family of linear polyesters of 3, 4, 5 and 6-hydroxyacids, synthesized by a wide variety of bacteria through the fermentation of sugars, lipids, alkanes, alkenes, and alkanoic acids. PHAs can be classified according to the length of their alkyl side-chains as short- or medium-chain PHAs (Lenz and Marchessault, 2005). Their chemical structure is very similar to the highly popular synthetic biodegradable polymers, the polylactides (PLA) and their copolymers with glycolides, poly(lactide-co-glycolide) (PLGA), but they generally degrade at a much slower rate (Amass et al., 1998). Figure 1.7 shows general structure of PHA which had been synthesized by different microorganisms. They are found as discrete cytoplasmic inclusions in bacterial cells.

Once extracted from the cells, PHAs exhibit thermoplastic and elastomeric properties. PHAs are recyclable, natural materials that can be easily degraded to carbon dioxide and water. Hence they are excellent replacements for petroleum-derived plastics in terms of processibility, physical characteristics and biodegradability (Philip et al., 2007).

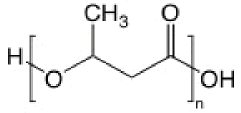
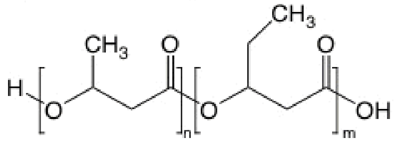
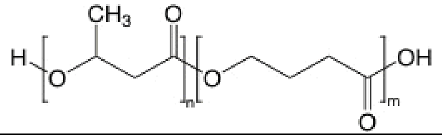
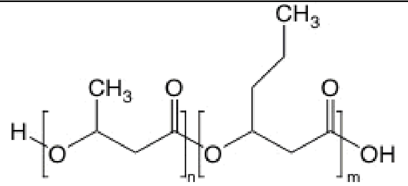
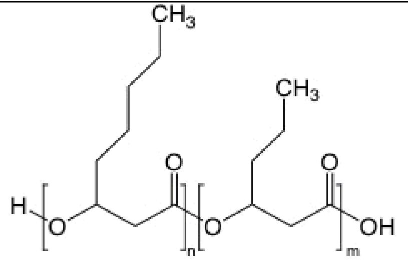
Microorganisms	PHA synthesized	PHA chemical structure
Ralstonia eutropha	PHB [poly(3-hydroxybutyrate)]	
	PHBV [poly(3-hydroxybutyrate-co-3-hydroxyvalerate)]	
	P3HB4HB [poly(3-hydroxybutyrate-co-4-hydroxybutyrate)]	
Aeromonas hydrophila	PHBHHx [poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)]	
Pseudomonas putida	PHOHHx [poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate)]	

Figure 1.7: General structure of Polyhydroxyalkanoates (adapted from Philip et al., 2007).

In addition, these polymers are biocompatible and hence have several medical applications (Steinbuchel, 2005; Mochizuka, 2002). All of the monomeric units of PHAs are enantiomerically pure and in the R-configuration (Philip et al., 2007). R-hydroxyalkanoic acids produced by the hydrolysis of PHAs can also be widely used as chiral starting materials in fine chemical, pharmaceutical and medical industries (Philip et al., 2007).

Polymers and copolymers of lactic and glycolic acids are the most commonly used to develop drug delivery systems due to their safe and approved use for applications in humans (Maia et al., 2004). However, although these synthetic biodegradable polymers are highly popular, the use of natural biodegradable polymers remains attractive because of their abundance in nature, good biocompatibility, and ability to be readily modified. Thus, natural biodegradable polymers have been studied to increase the number of biodegradable materials available for pharmaceutical and medical applications.

1.7 Biosynthesis of PHAs

A wide variety of bacteria both Gram negative and Gram positive such as *Pseudomonas, Bacillus, Ralstonia, Aeromonas, Rhodobacter* and certain Archea, especially members of the Halobactericeae, like *Haloferax sulfurifontis*, synthesis polyhydroxyalkanoates (Philip et al., 2007). They function as energy storage compounds and are present in the cells as insoluble granules in the cytoplasm. Marine prokaryotes, both bacteria and archae, also produce PHAs that have tremendous commercial potential. PHAs accumulate up to 80% dry cell weight in these organisms when present in a ‘high-nutrient econiche’ (Weiner, 1997). This demonstrates the widespread occurrence of PHA-producing microbes in the environment (Philip et al., 2007).

PHAs are divided into two groups based on the number of constituent carbon atoms in their monomer units –short-chain-length (SCL) PHAs and medium-chain-length (MCL) PHAs. The former consists of monomers with 3-5 carbon atoms and the latter contains monomers with 6-14 carbon atoms. There have been reports of several bacteria that are able to synthesize PHAs containing both SCL- and MCL-monomer units (Doi et al., 1995). PHA_{SCL} are stiff and brittle with a high degree of crystallinity whereas PHA_{MCL} are flexible with low crystallinity, tensile strength and melting point.

The supply of the substrate monomer and the polymerization of these monomers are the two main steps involved in the biosynthesis of PHAs (Philip et al., 2007). The PHA synthesized by a microbe is dependent on the carbon source used. Carbon sources have been classified as 'related' sources that give rise to monomers that are structurally identical to that particular carbon source and 'unrelated' sources that generate monomer that are completely different from the metabolic pathways operating in the microorganism. There are three well-known PHA biosynthetic pathways (Taguchi et al., 2002) (Figure 1.8).

Pathways I (Figure 1.8) used by *Cupriavidus necator* (previously known as *Wauterisia eutropha* or *Ralstonia eutrophus*) is the best known among the PHA biosynthetic pathways (Philip et al., 2007). In this pathway, 3HB monomers are generated by the condensation of two acetyl-CoA molecules, from the tricarboxylic acid (TCA) cycle to form acetoacetyl-CoA by the enzyme β-ketothiolase. Then acetoacetyl-

CoA reductase acts on acetoacetyl-CoA to form 3-hydroxybutyryl-CoA into poly(3-hydroxybutyrate) (P(3HB)).

Pathways involved in fatty acid metabolism generate different hydroxyalkanoate monomers utilized in PHA biosynthesis. The fatty acid β-oxidation pathway (Pathway II) generates substrates that can be polymerized by the PHA synthases of *Pseudomonads* belonging to the ribosomal RNA-homology group I such as *Pseudomonas aeruginosa*. These microbes can synthesis PHA_{MCL} from various alkanes, alkenes and alkanoates. The monomer composition is related to the carbon source used. In *Aeromonas caviae*, the β-oxidation intermediate, trans-2-enoyl-CoA is converted to (R)-hydroxyacyl-CoA by a (R)-specific enoyl-CoA hydratase (Philip et al., 2007).

Previous study by Huijberts et al., 1992, showed that PHA synthases (such as that of *Pseudomonas putida*) that catalyse PHA synthesis from fatty acids are also responsible for PHA synthesis from glucose. The intermediates for this channel of synthesis were obtained from the fatty acid *de novo* biosynthetic pathway (Pathway III) (Philip et al., 2007). Pathway III is of significant interest because it helps generate monomers for PHA synthesis from structurally unrelated and simple, inexpensive carbon sources such as glucose, sucrose and fructose. The (R)- 3-hydroxyacyl intermediates from the fatty acid biosynthetic pathway are converted from their acyl carrier protein (ACP) form to the CoA form by the enzyme acyl-ACP-CoA transacylase (encoded by *phaG*). This enzyme is the key link between fatty acid synthesis and PHA biosynthesis (Philip et al., 2007).

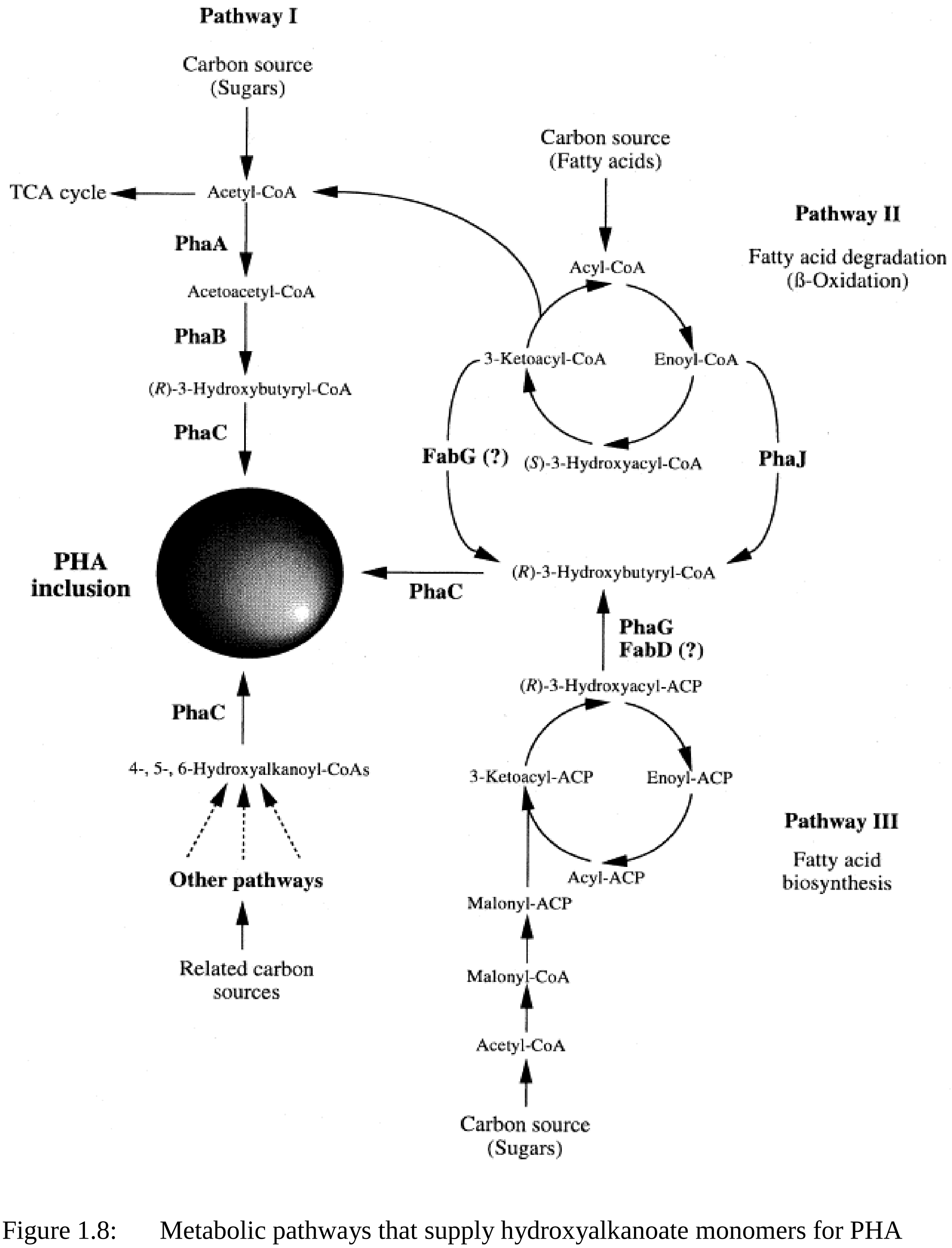


Figure 1.8: Metabolic pathways that supply hydroxyalkanoate monomers for PHA biosynthesis (adapted from Sudesh et al., 2000)