Chapter-2

Review of Literature

2.1. Nanotechnology

A large number of scientists are working in the development of nanotechnology for the benefit of human kind. Nanotechnology has started to revolutionize the modern technology not only in electronics and machinery but also in drug delivery, drug targeting, medical devices and diagnosis of medical complications. It also precisely combines the knowledge of Physics, Chemistry, Biology, Medicine, Informatics, and Engineering fields (Logothetidis, 2012). In 2015, American government and private sector altogether spent quarter of trillion dollar in projects related with nanotechnology (Dong et al., 2016). Traditionally, nanotechnology originates from Feynman speech entitled "There's plenty of room at the bottom" about miniaturization principle upto atomic level precision without violating any physics law (Emerich & Thanos, 2003). The "magic bullet" concept of Paul Ehrlich, Nobel Prize for medicine in 1908, has become true with the use of nanotechnology in drug delivery and active targeting (Black & Gregoriadis, 1974). There is a plethora of nanoparticle systems which have emerged or in developmental phase. Nanomedicine is a branch of science and technology which uses nano-sized tools for the diagnosis, prevention and treatment of disease and to gain increased understanding of the complex underlying patho-physiology of disease. The ultimate goal is improved quality-of-life. The particle size of nanoparticles for theranostic (combination of therapy and diagnosis of a disease) application is within 1-1000nm range which is a common concept in pharmaceutical science. Polymeric nanoparticles, inorganic nanoparticles (metal or non-metal nanoparticles), carbohydrate nanoparticles, solid lipid nanoparticles, nano-structured lipid carriers, dendrimers, liposomes, polysomes, neosomes, ethosomes, virosomes, carbon nanotubes, quantum dots and many more were reported nanomedicines for delivering pharmacologically active agents more efficiently and selectively to

pathological site and keeping away from potentially endangered healthy tissue, subsequently improves the balance between efficacy and toxicity of the administered theranostic agents (Lammer et al., 2011; Lammers et al., 2008; Peer et al., 2007; Sanhai et al., 2008; Davis et al., 2008;). They carry the therapeutic or diagnostic agent from site of administration to the site of action in controlled manner in body.

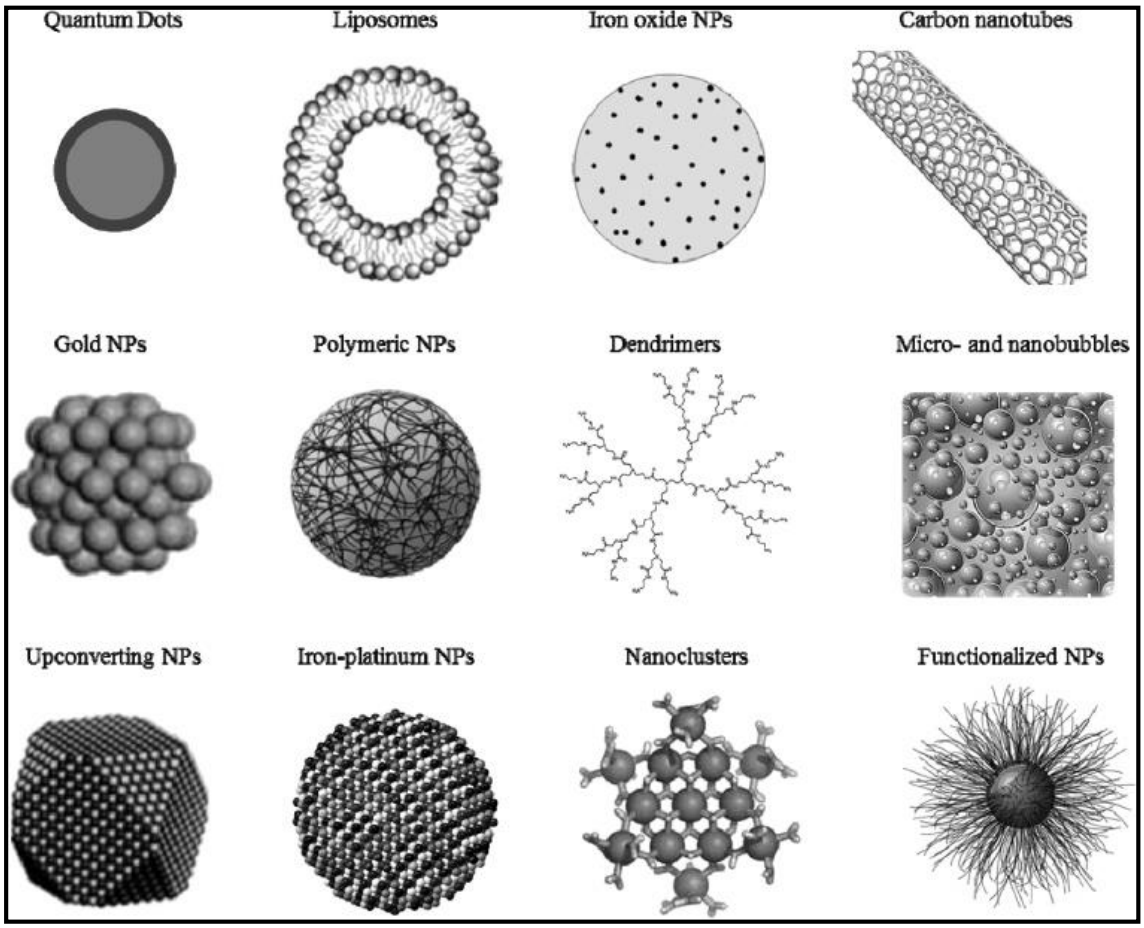


Fig. 2.1 Various nanosystems used for delivery and targeting of theranostic agents (Sharma et al., 2015)

2.2. Polymeric nanoparticles

Polymeric nanoparticles (PNs) are submicron-sized colloidal system comprising of natural (gelatine, chitosan, chitin and albumin) or synthetic [polylactic acid (PLA), poly (lactic-co-glycolic acid) (PLGA), poly-ϵ-caprolactone (PCL), Eudragit etc.] polymers. Polymeric nanoparticles have been explored for not only in nanomedicine (to enhance theranostic efficacy of bioactives and

keeping toxicity at minimum level) but also in electronics, material science, machinery, pollution control sensor and many innumerable field which can be observed by ever increasing number of publication during past decades (**Fig. 2.2**). Oral route has been preferred over other routes of administration due to patient compliance, cost effectiveness and convenience but several barriers of gastrointestinal tract (GIT) limit the oral bioavailability of drugs. There are a number of barriers of GIT that must be overcome to obtain better bioavailability of drugs. Foremost barrier is the harsh pH environment of GIT (from pH 1 in stomach to pH 8 in intestines which can cause oxidation and hydrolysis of drugs thereafter, loss of activity) as well as digestive and other cytochrome enzymes secreted locally. Enzymatic degradation happens with protease, lipase and nuclease enzyme whereas biotransformation/inactivation due to cytochrome enzymes. Epithelial cell monolayer also possessed crucial barrier to drug absorption. Drug loaded PNs overcome most of these barriers of GIT. PNs are stable in harsh GIT surrounding and can protect encapsulated drug from acidic/basic pH and enzymatic degradation. Generally, drug loaded PNs transport from GIT lumen to blood streams happen via crossing epithelium layer by transcellular, paracellular and transcytosis routes (**Fig. 2.3**) (Pridgen et al., 2014). PNs also follow lymphatic pathway via M Cells of payer patches associated in gut associated lymph tissue and bypass first pass metabolism (Jain et al., 2011).

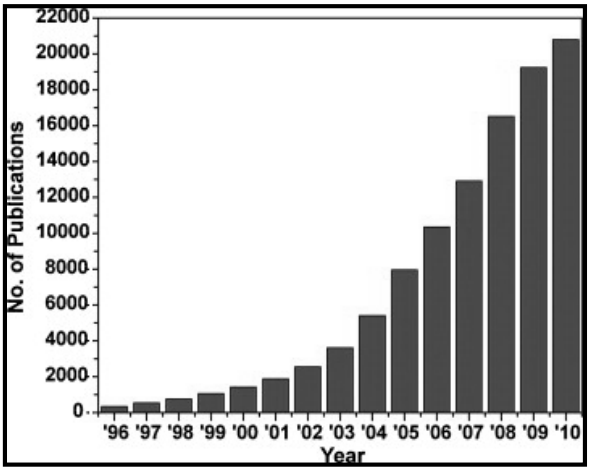


Fig. 2.2 Graphical representation of the number of publications cited in Scopus database on polymeric nanoparticles during the period of 1996–2010. (Rao and Geckeler, 2011)

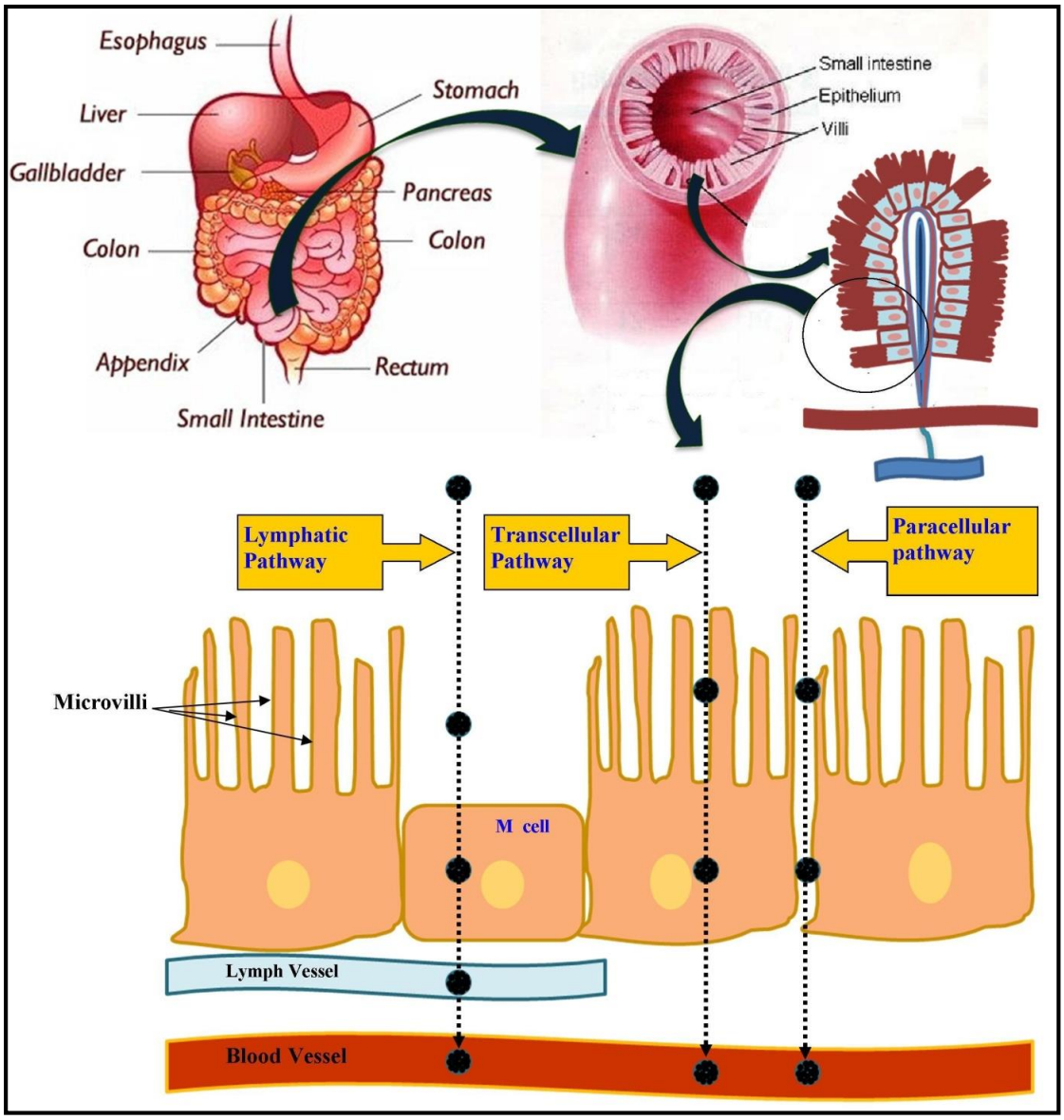


Fig. 2.3 Schematic representation of systemic drug absorption from intestinal lumen

2.3. Preparation of polymeric nanoparticles

PNs can be conventionally prepared by either dispersion of preformed polymer (solvent evaporation, nanoprecipitation, salting out, dialysis and supercritical fluid technology) or by direct polymerization of monomers using polymerization technique (micro-emulsion, mini-emulsion, surfactant-free emulsion, interfacial polymerization and controlled/living radical polymerization (C/LRP)) as shown in **Fig. 2.4**.

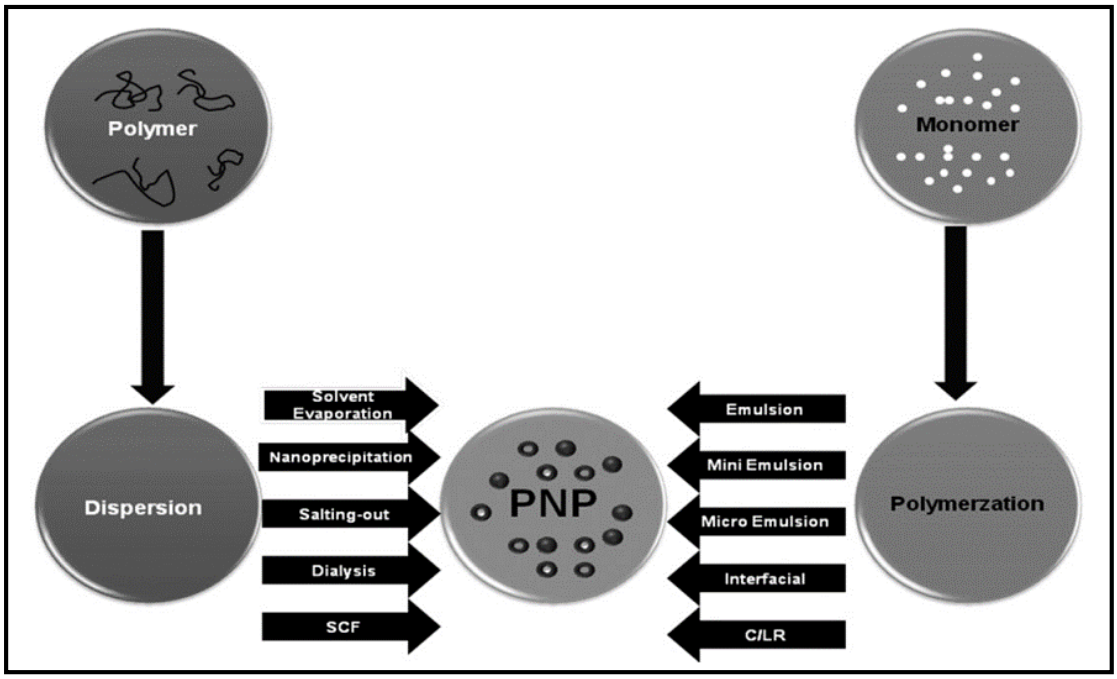


Fig. 2.4 Schematic representation of various methods of polymeric nanoparticle preparation. SCF: supercritical fluid technology, C/LR: controlled/living radical (adapted from Rao & Geckler, 2011)

2.3.1. Dispersion of preformed polymer

Various type of dispersion techniques are discussed here to prepare PNs with preformed polymers.

2.3.1.1. Solvent evaporation method

It is the first method developed for the preparation of PNs. It is frequently used technique to prepare PNs. In this technique the polymer solutions are prepared in organic solvent and dispersed in aqueous media containing stabilizer to form emulsion by using high speed/pressure homogenizer or ultra sonicator and thereafter subjected for solvent evaporation under normal/ reduced pressure condition to form dispersion containing PNs. In other term the method is also called emulsification solvent evaporation or emulsification-solvent diffusion-evaporation technique as per solvent used. Generally used solvents are water immiscible chloroform, dichloromethane and slightly water miscible ethyl acetate. Ethyl acetate is mostly used due to less toxic over other solvents and it follows emulsification-solvent diffusion-evaporation method. In solvent evaporation method the concentration of polymer, molecular weight of

polymer, emulsification method, volume of organic solvent, concentration of stabilizer and evaporation method apparently influences the property (particle size, entrapment efficiency and polydispersity index) of prepared PNs. There are several reported PNs preparations based on solvent evaporation method (Anton et al., 2008; Julienne & Benoit, 1996; Zambaux et al., 1998; Quellec et al., 1999; Zambaux et al., 1999; Musyanovych et al., 2008; Bilati et al., 2003).

2.3.1.2. Salting-out method

Salting-out method is the modified emulsification method where surfactants and chlorinated solvents are not used. Emulsions are prepared by pouring polymer solution in water miscible solvent (acetone, tetra hydrofuran, etc.) in electrolyte saturated aqueous solution and then addition of excess water results in diffusion of solvents from the polymer solution thereafter PNs formation. In this method, first polymer solution in water miscible solvent is prepared and emulsification is carried out using saturated electrolyte aqueous solution. In this emulsion, organic solvent does not diffuse out from emulsion as it is immiscible in aqueous phase containing concentrated electrolyte. After dilution with excess of pure water, the electrolyte concentration is lowered and organic solvent diffuse out from emulsion globules, and subsequently PNs are formed (Allemann et al., 1992). This is also called ouzo effect where no surfactants are used while preparing PNs (Ganachaud et al., 2005). Commonly used electrolytes are magnesium chloride, calcium chloride, sodium chloride and magnesium acetate. There are several reported polymeric nanoparticles prepared by this method (De Jaeghere et al., 1999; Nguyen et al., 2003; Zweers et al., 2004; Galindo-Rodriguez et al., 2005; Zweers et al, 2006; Fan et al., 2006). Influence of polymer concentration and stirring speed are significant on particle size, entrapment efficiency and polydispersity index of PNs.

2.3.1.3. Nanoprecipitation method

Nanoprecipitation method is simple, fast and reproducible which is widely used for preparation of PNs and also called solvent displacement method. It was first discovered by Fessi et al. to prepare nanoparticles (Fessi et al., 1989).

The fundamental rationale of this method is based on the interfacial deposition of a polymer after displacement of a water miscible semipolar solvent, from a lipophilic solution. Rapid movement of the solvent (organic water miscible solvent) into non-solvent phase (aqueous) results in the decrease of interfacial tension between the two phases, which enhances the surface area and resulted into the formation of small droplets of organic solvent. The polymer, the polymer solvent and the non-solvent are the basic components of nanoprecipitation system. Frequently utilized polymers are synthetic poly(ε-caprolactone) (PCL), polylactide (PLA), poly(lactide-co-glycolide) (PLGA), Eudragit, polyalkylcyanoacrylate (PACA), allylic starch, dextran easter (Chang et al., 2009; Limayem et al., 2006; Zili et al., 2005; Kim et al., 2009; Moinard-Chécot et al., 2008; Ferranti et al., 1999; Seyler et al., 1999; Legrand et al., 2007; Nehilla et al., 2008; Yallapu et al., 2010; Yordanov & Duskin, 2010; Stella et al., 2007; Deepak et al., 2009; tan et al., 2009; Hornig et al., 2009). The commonly used polymer solvents are acetone, ethyl alcohol, methanol and acetonitrile. The non-solvent consists of aqueous solution supplemented with stabilizer. In this method when organic solvent containing polymer and drug is slowly added to the non-solvent under moderate stirring, PNs are produced (**Fig. 2.5**). Nanoparticle formation by nanoprecipitation method occurred in three stages: nucleation, growth and aggregation. Uniform particle size has been achieved when a clear separation is there between nucleation and growth process (Lince et al., 2008). Perfectly, operating condition requires high nucleation rate to obtain uniform distribution of particle size which depend upon degree of supersaturation and low growth rate. Particle size, polydispersity index and entrapment efficiency of nanoparticles are governed by organic phase to aqueous ratio, polymer concentration, surfactant concentration, stirring speed and rate of addition of polymer solvent to aqueous phase.

2.3.1.4. Dialysis method

Dialysis is simple and efficient method to prepare uniformly sized PNs. Polymer solution in organic solvent is kept inside a dialysis tube with appropriate molecular weight cut-off and dialysis is carried out against a non-

solvent (aqueous media) (Jeong et al., 2001; kostog et al., 2010; Jeon et al., 2000). The replacement of the solvent inside the membrane by non-solvent is followed by the progressive aggregation leading formation of mono disperse suspensions of nanoparticles (**Fig. 2.5**). The mechanism can be considered very similar to nanoprecipitation method. Several block copolymer nanoparticles are reported using dimethyl formamide, dimethyl sulfoxide and dimethyl acetate as organic solvent (Jung et al., 2004; Liu et al., 2007; Hornig et al., 2007; Heinze et al., 2007; Park et al., 2007; Choi et al., 2007; He et al., 2008).

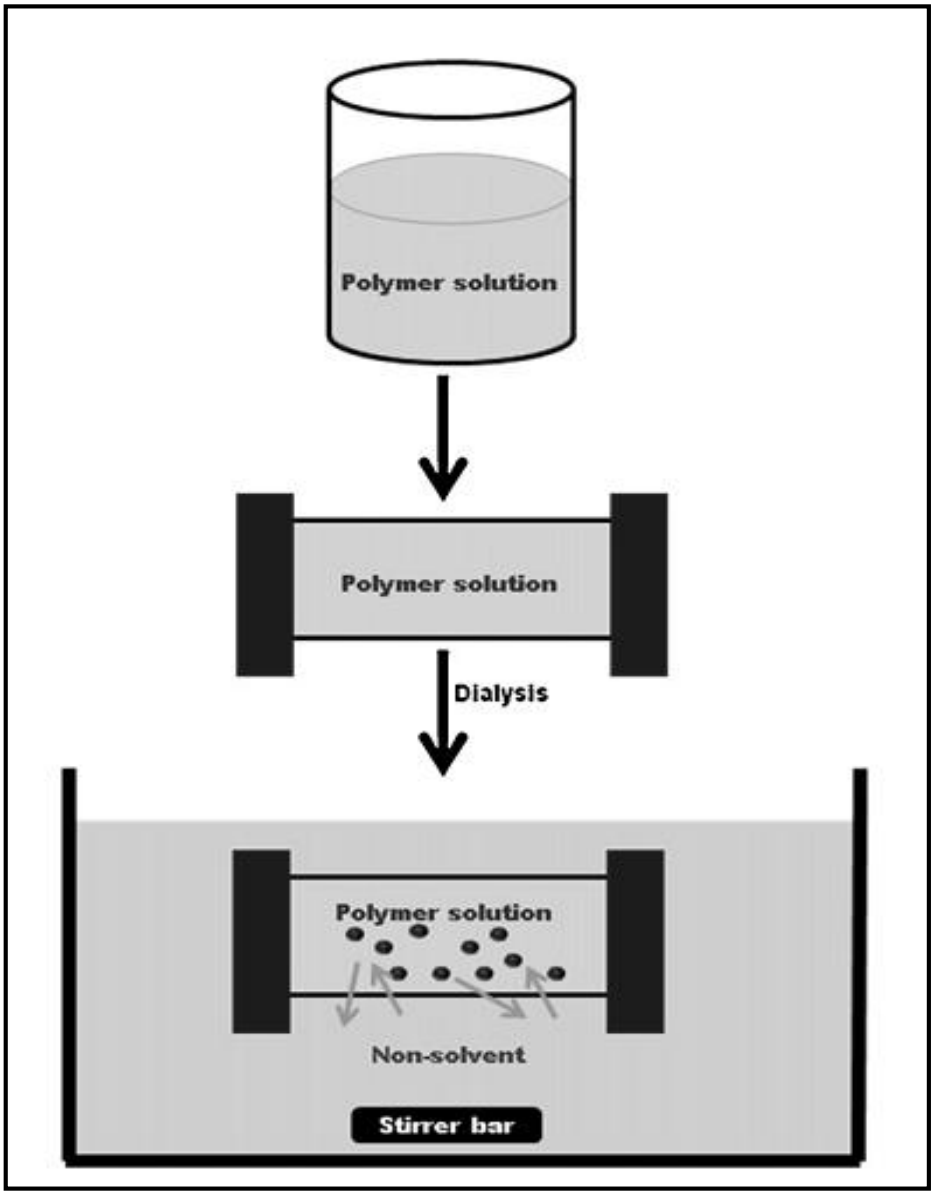


Fig. 2.5 Schematic depiction of dialysis method for nanoparticle preparation (adapted from Rao & Geckler, 2011)

2.3.1.5 Supercritical fluid technology

In supercritical fluid technology carbon dioxide is used as supercritical fluid and produce homogeneous nanoparticles without any use of organic solvent (York, 1999). Polymer is dissolved in supercritical fluid at high pressure and then rapid expansion of solution through capillary nozzle into air/aqueous solvent resulted into homogeneous nucleation and henceforth formation of uniformly dispersed particles (Reverchon, 1999, Jung & Perrut, 2001). The supercritical fluid technology apparatus is shown in **Fig. 2.6**. It consists of three major parts: high pressure mixing cell, syringe pump and a pre-expansion unit. Polymer and drug dissolved in liquid carbon dioxide are pumped through syringe in pre-expansion unit and subjected to heating isobaric to the pre-expansion temperature and passed through nozzle into ambient air or solvent to obtain nanoparticles. The concentration and degree of saturation significantly influences the particle size as well as morphology (Shariati & Peters, 2003). The poor solubility of polymer in supercritical fluid limits usage of this technique.

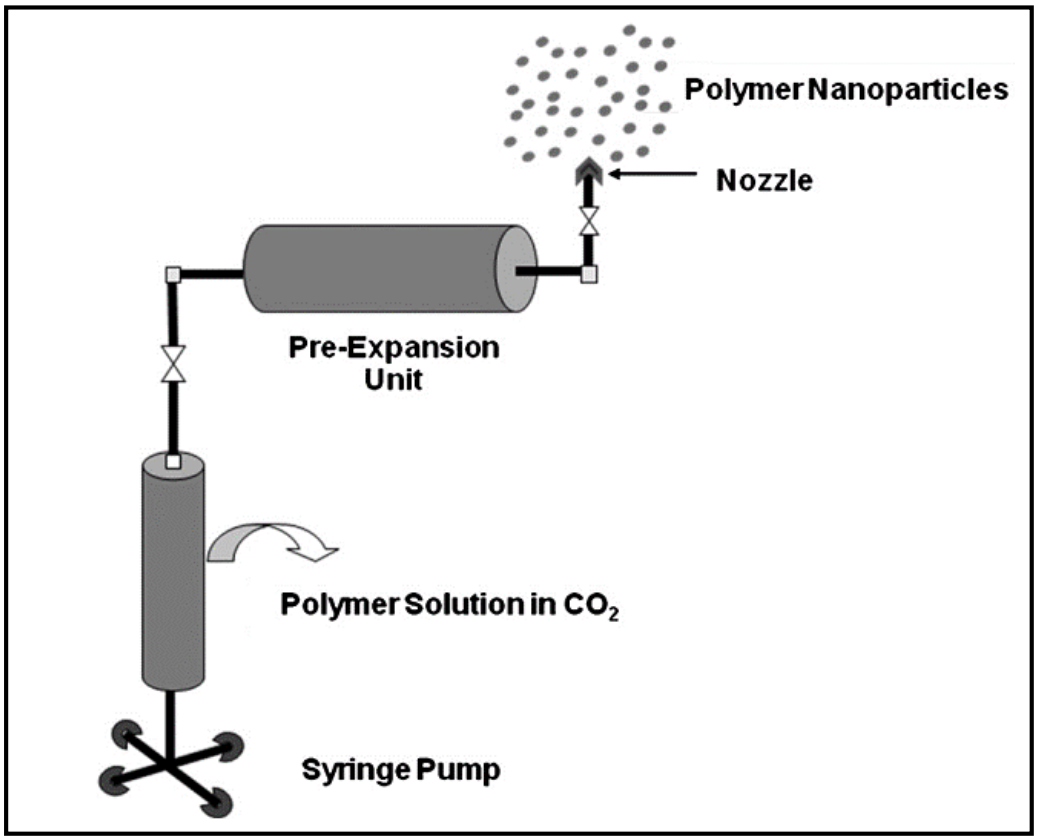


Fig. 2.6 Showing the empirical design of supercritical fluid technique methodology of nanoparticle preparation (adapted from Rao & Geckler, 2011)

2.3.2. Polymerization of monomers

Suitable polymeric nanoparticles are prepared by polymerization of monomers. There are several reported methods as shown in **Fig. 2.4** are discussed below.

2.3.2.1 Emulsion polymerization

Ingredients of emulsion polymerization consist of water, a monomer of low water solubility, water-soluble initiator and a surfactant. Initiation of polymerization starts when a monomer molecule dissolved in the continuous phase collides with an initiator molecule that may be an ion or a free radical. Instead of an initiator molecule, one can transform monomer into an initiating molecule by high-energy radiation, including ϒ-radiation, ultraviolet or strong visible light. Formation of nanoparticles takes place just before or after termination of polymerization reaction. Several polymeric nanoparticles like polystyrene, poly(methylmethacrylate), poly(vinylcarbazol), poly(ethylcyano-acrylate) and poly (butylcyanoacrylate) were prepared by this technique (Kreuter, 1982; Munoz-Bonilla et al., 2010; Costa et al., 2009; Su-Jung et al., 2009). Surfactants, concentration of monomer and ionic initiator were predominantly influenced the nanoparticles property.

2.3.2.2. Mini-emulsion polymerization

The ingredients used in mini-emulsion polymerization are water, monomer mixture, co-stabilizer, surfactant and initiator. The difference between emulsification and mini emulsification polymerization is the usage of low molecular mass of compound as co-stabilizer and the high shear device (ultrasound, etc.) utilization. This system is stabilized by providing high shear to counter high interfacial tension. Various PNs were developed with several co-stabilizer and initiator combination (Wang et al., 2007; Bardajee et al., 2007; Rotureau et al., 2008; Yildiz & Landfester, 2008; Ethirajan et al., 2009; Crespy & Landfester, 2009; Wu et al., 2009; Baruch-Sharon & Margel, 2010; Jiang et al., 2010). Core-shell polymer nanoparticles with a magnetic core (magnetite) and a

biodegradable polymeric shell were prepared by using this method (Arias et al., 2001).

2.3.2.3. Micro-emulsion polymerization

Micro-emulsion polymerization method is appeared to be similar in respect of high molar mass of produced nanoparticles but differ in kinetic aspects. There are three reaction rate intervals in emulsion polymerization but two in micro-emulsion polymerization. Both particle size and average chain of polymer are significantly smaller in micro-emulsion polymerization (Puig, 1996). Water soluble initiator is added to the aqueous phase of a thermodynamically stable micro-emulsion containing swollen micelles due to excess of surfactants. The high quantity of surfactants lead to nearly zero interfacial tension at the oil/water interface and this is desired condition for initiation of polymerization with the help of initiator (Macias et al., 1995). The initiator concentration, type, surfactant, monomer and reaction temperature are important factors influencing the micro-emulsion polymerization kinetics and the properties of PNs (Sosa et al., 2000).

2.3.2.4. Interfacial polymerization

As name indicates, polymerization occurs at the interface of disperse phase and continuous phase of an emulsion. It involves step polymerization of two reactive monomers dissolved in respective continuous and dispersed phase, and the reaction takes place at the interface of the two phases (Karode et al., 1998). Hollow PNs and nanocapsule were prepared using this method (Crespy et al., 2007; Danicher et al., 2000; Torini et al., 2005; Khoury-Fallouh, 1986).

2.3.2.5. Controlled/living radical polymerization (C/LRP)

C/LRP has advantage over other conventional polymerization technique which produces nearly uniform PNs molar mass, the molar mass distribution, the end-functionalities and the macro-molecular architecture (Zetterlund et al., 2008). The particle size of PNs is governed by nature and concentration of control agent, monomer, initiator and emulsion type (Braunecker &

Matyjaszewski, 2007). Three approaches are generally used in C/LRP: (1) nitroxide-mediated polymerization (NMP), (2) atom transfer radical polymerization (ATRP) and (3) reversible addition and fragmentation transfer chain polymerization (RAFT) (Cunningham, 2008; Nicolas et al., 2005; Zetterlund et al., 2007; Matyjaszewski & Xia, 2001).

2.4. Use of polymeric nanoparticles or nanostructures in atherosclerosis

A plethora of PNs based drug delivery systems has been reported for efficient treatment/diagnosis of cardiovascular disease (Wang et al., 2016). Polymeric nanoparticle based various drug eluting stents (DES) are reported recently to overcome limitation of conventional DES. Polymeric nanocarriers deliver the loaded drug to target site by tailoring the surface functionality or passive targeting as depicted in **Fig. 2.7**. Occluded vessels offer flow derived shear stress which helps in PNs adhesion and internalization by endothelial cells (Han et al., 2012; Korin et al., 2012).

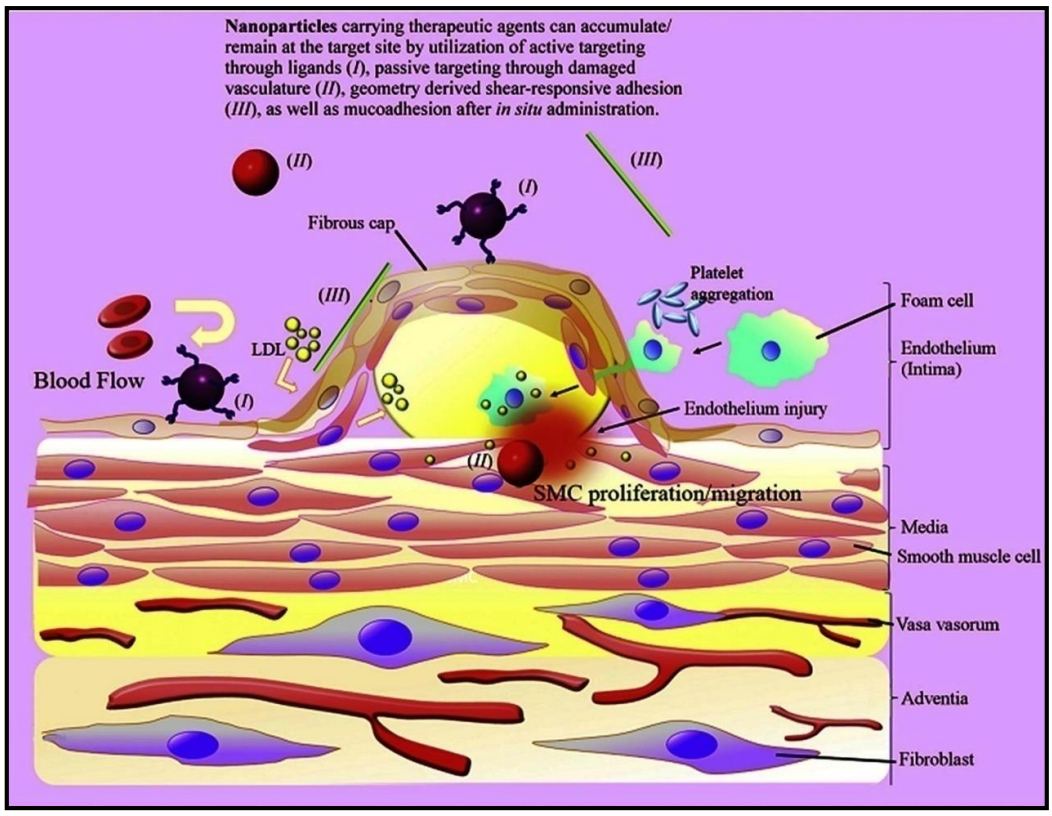
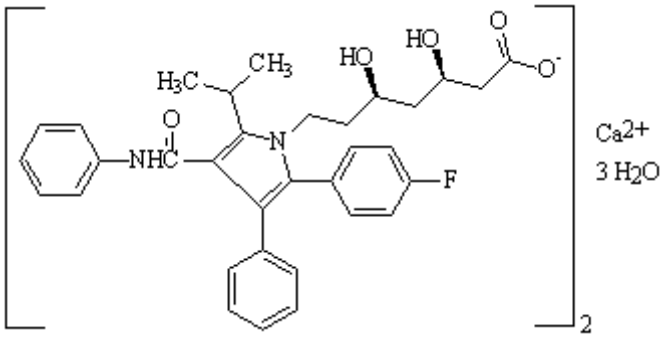


Fig. 2.7 Conceptual drawing of atherosclerotic/damaged vessels and polymeric nanocarriers with various targeting mechanisms (Adapted from Wang et al., 2016)

Pitavastatin loaded PNs exhibited significant reduction in infarct size, amelioration in left ventricle dysfunction and inhibition of inflammation as well as cardiomyocyte death in the infracted myocardium (Nagaoka et al., 2015). Besides low toxicity and a good therapeutic index (better safety and efficacy), polymeric nanosystems should improve risk stratification when translated into the clinical practice, and allow personalized therapeutic regimen in cardiovascular diseases (Cicha et al., 2016). A better understanding and collaborative research project among material scientist, biomedical engineers, cardiologists, heart and vascular surgeons as well as biologists is the need of hour to encourage the production of new generation bio-polymeric grafts, stents, patches or drug carriers that can be translated into the clinical trials.

2.5. Atorvastatin calcium (ATR) specific review



IUPAC Name: bis[(3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid], calcium trihydrate

Molecular Formula: $(C_{33}H_{34}FN_2O_5)_2Ca \bullet 3H_2O$

Molecular Weight: 1209.42

ATR the leading drug among statins, inhibits the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase resulting in reduced cholesterol synthesis. The salt form of the drug, ATR is most widely used for the treatment of hyperlipidemia. The statin market is increasing globally because of the associated risk of cardiovascular disorders with elevated lipid profiles. It is used in certain patients to reduce the risk of heart attack, stroke, chest pain caused by angina, or blood vessel blockage. It is also used in certain patients to reduce the

risk of hospitalization for congestive heart failure, or the need for medical procedures to open blocked heart blood vessels.

Physical Properties (Drug bank):

Identification: Infrared spectrum, Specific Rotation and Chromatography (USP 2009 review).

Description: white to off-white crystalline powder .

Solubility: insoluble in aqueous solutions of pH 4 and below. ATR is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile; slightly soluble in ethanol; and freely soluble in methanol.

Melting point: 178-180°C

Acidic in nature

Log P: 5.7

Storage: Preserve in tight container and store in cool and dark place.

Pharmacokinetic: The absolute bioavailability (parent drug) of ATR is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and/or first-pass metabolism in the liver. Although food decreases the rate and extent of drug absorption by approximately 25% and 9%, as assessed by C_{max} and AUC respectively, LDL_C reduction and HDL_C elevation are similar when ATR is given with and without food.

Bioavailability: 14% (p.o.)

T_{max}: 1 to 2 hrs

Elimination half-life: 14 hrs.

Apparent volume of distribution: 381 liters.

Total body clearance: 625 mL/min

Protein bound: ≥ 98%

Metabolism: ATR is extensively metabolized to ortho- and para-hydroxylated derivatives by cytochrome P-450 3A4 (CYP 3A4) and to various beta-oxidation products. *In vitro*, inhibition of HMG-CoA reductase by ortho- and para-hydroxylated metabolites is equivalent to that of ATR. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active

metabolites. In animals, the ortho-hydroxy metabolite undergoes further glucuronidation. ATR and its metabolites are eliminated by biliary excretion.

Mechanism of Action: ATR lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of Low Density Lipoprotein (LDL). It also reduces LDL-Cholesterol (LDL_C) and the number of LDL particles. ATR also reduces Very Low Density Lipoprotein-Cholesterol ($VLDL_C$), serum triglycerides (TG) and Intermediate Density Lipoproteins (IDL), as well as the number of apolipoprotein B (apo B) containing particles, but increases High Density Lipoprotein-Cholesterol (HDL_C). Elevated serum cholesterol due to elevated LDL_C is a major risk factor for the development of cardiovascular disease. Low serum concentration of HDL_C is an independent risk factor. Elevated plasma TG is also a risk factor for cardiovascular disease, particularly due to increased IDL, or associated with decreased HDL_C or increased LDL_C.

Indication: ATR is used as primary prevention in individuals with multiple risk factors for coronary heart disease (CHD) and as secondary prevention in individuals with CHD to reduce the risk of myocardial infarction (MI), stroke, angina, and revascularization procedures. It is used to reduce the risk of cardiovascular events in patients with acute coronary syndrome (ACS). May be used in the treatment of primary hypercholesterolemia and mixed dyslipidemia, homozygous familial hypercholesterolemia, primary dysbetalipoproteinemia, and/or hypertriglyeridemia as an adjunct to dietary therapy to decrease serum total and low-density lipoprotein cholesterol (LDL_C), apolipoprotein B (apoB), and triglyceride concentrations, while increasing high-density lipoprotein cholesterol (HDL_C) levels.

Dose and administration: Usual oral dosage 10, 20, 40 and 80mg.

Table 2.1 Reported development carried out by various research groups to improve bioavailability of ATR

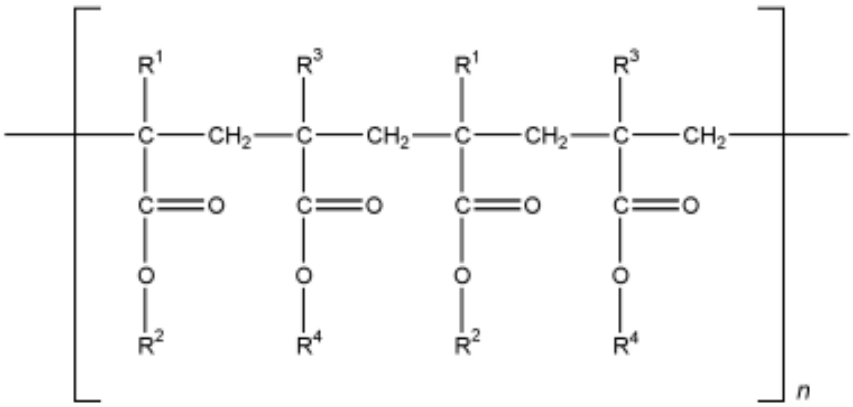
S. No.	Title of Research	References
1.	Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin.	Shen & Zhong, 2006
2.	Physicochemical properties and oral bioavailability of amorphous atorvastatin hemi-calcium using spray-drying and SAS process	Kim et al., 2008
3.	Preparation, characterization and *in vivo* evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process.	Kim et al., 2008
4.	Micronization of atorvastatin calcium by antisolvent precipitation process	Zhang et al., 2009
5.	A Quadruped Study on Chitosan Microspheres Containing Atorvastatin Calcium: Preparation, Characterization, Quantification and in-Vivo Application	Eroglu et al., 2010
6.	Microwave induced solubility enhancement of poorly water soluble atorvastatin calcium.	Maurya et al., 2010
7.	Enhanced Bioavailability of Atorvastatin Calcium from Stabilized Gastric Resident Formulation.	Khan & Dehghan, 2011
8.	Development and characterization of an atorvastatin solid dispersion formulation using skimmed milk for improved oral bioavailability.	Choudhari et al., 2012
9.	Effect of solvent type on the nanoparticle formation of atorvastatin calcium by the supercritical antisolvent process.	Kim et al., 2012
10.	Preparation of candesartan and atorvastatin nanoparticles by solvent evaporation.	Vaculikova et al., 2012
11.	Formulation development and *in vitro* evaluation of solidified self-microemulsion in the form of tablet containing atorvastatin calcium.	Ali et al., 2013
12.	Atorvastatin-loaded hydrogel affects the smooth muscle cells of human veins.	Dubuis et al., 2013
13.	Oral absorption of atorvastatin solid dispersion based on cellulose or pyrrolidone derivative polymers	Kim et al., 2013
14.	Coamorphous atorvastatin calcium to improve its physicochemical and pharmacokinetic properties.	Shayanfar et al., 2013
15.	Preparation and Evaluation of Solid Dispersion of Atorvastatin Calcium with Soluplus by Spray Drying Technique.	Ha et al., 2014

S. No.	Title of Research	References
16.	BSA nanoparticle loaded atorvastatin calcium - a new facet for an old drug	Sripriyalakshmi et al., 2014
17.	Formulation of solid self-nanoemulsifying drug delivery systems using N-methyl pyrrolidone as cosolvent.	Agrawal et al., 2015
18.	Effects of carbohydrate polymers in self-microemulsified tablets on the bioavailability of atorvastatin: *In vitro–in vivo* study.	Biswal et al., 2015
19.	Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin.	Czajkowska-Ko´snik et al., 2015
20.	Custom fractional factorial designs to develop atorvastatin self-nanoemulsifying and nanosuspension delivery systems – enhancement of oral bioavailability.	Hashem et al., 2015a
21.	Optimized zein nanospheres for improved oral bioavailability of atorvastatin.	Hashem et al., 2015
22.	Lecithin/TPGS-based spray-dried self microemulsifying drug delivery systems: In vitro pulmonary deposition and cytotoxicity.	Ishak & Osman, 2015
23.	Dissolution enhancement of atorvastatin calcium by co - grinding technique.	Prabhu & Patravale, 2015
24.	Development, optimization and characterization of glycyrrhetinic acid–chitosan nanoparticles of atorvastatin for liver targeting.	Rohilla et al., 2015
25.	Development and characterization of floating spheroids of atorvastatin calcium loaded NLC for enhancement of oral bioavailability.	Sharma et al., 2015
26.	Development and optimization of a self microemulsifying drug delivery system for atorvastatin calcium by using D-optimal mixture design.	Yeom et al., 2015
27.	Depot injectable atorvastatin biodegradable in situ gel: development, optimization, *in vitro*, and *in vivo* evaluation.	Ahmed et al., 2016
28.	Evaluation of physicochemical properties and *in vivo* efficiency of atorvastatin calcium/ezetimibe solid dispersions.	Jahangiri et al., 2016
29.	Chlorogenic acid stabilized nanostructured lipid carriers (NLC) of atorvastatin: formulation, design and *in vivo* evaluation	Khan et al., 2016
30.	Perivascular sustained release of atorvastatin from a hydrogel-microparticle delivery system decreases intimal hyperplasia.	Mylonaki et al., 2016

S. No.	Title of Research	References
31.	Development of a solidified self-microemulsifying drug delivery system (S-SMEDDS) for atorvastatin calcium with improved dissolution and bioavailability	Yeom et al., 2016

2.6. Excipients specific review

(a) Eudragit RSPO



For *Eudragit RL* and *Eudragit RS*:

R^1 = H, CH_3; R^2 = CH_3, C_2H_5; R^3 = CH_3; R^4 = $CH_2CH_2N(CH_3)^{3+}Cl^-$

Chemical name: Poly(ethyl acrylate, methyl methacrylate, trimethylammonio-ethyl methacrylate chloride)1 : 2 : 0.2

Functional Category: Film former, tablet binder and tablet diluents.

Applications: Polymethacrylates are primarily used in oral capsule and tablet formulations as film-coating agents. *Eudragit RL*, *RS*, *RD 100*, *NE 30 D* and *NE 40 D* are used to form water-insoluble film coats for sustained-release products. *Eudragit RL* films are more permeable than those of *Eudragit RS*, and films of varying permeability can be obtained by mixing the two types together. *Eudragit RLPO* is also used as high permeable sustained release tablet matrix and is independent of pH of medium.

Polymethacrylates are also used as binders in both aqueous and organic wet-granulation processes. Larger quantities (5–20%) of dry polymer are used to control the release of an active substance from a tablet matrix. Solid polymers may be used in direct-compression processes in quantities of 10–50%. Polymethacrylate polymers may additionally be used to form the matrix layers of

transdermal delivery systems and have also been used to prepare novel gel formulations for rectal administration

Description: *Eudragit RL PO* and *Eudragit RS PO* are fine, white powders with a slight amine-like odour. They are characteristically the same polymers as *Eudragit RL* and *RS*. They contain 97% of dry polymer.

Solubility: Practically insoluble in water and ether; soluble in polar organic solvent.

Melting point: Charred at 270°C.

Density (bulk): 0.390 g/cm^3

Density (tapped): 0.424 g/cm^3

Stability and Storage Conditions: Dry powder polymer forms are stable at temperatures less than 30°C. Above this temperature, powders tend to form clumps, although this does not affect the quality of the substance and the clumps can readily be broken up. Dry powders are stable for at least 3 years if stored in a tightly closed container at less than 30°C.

Dispersions are sensitive to extreme temperatures and phase separation occurs below 0°C. Dispersions should therefore be stored at temperatures between 5 and 25°C and are stable for at least 18 months after shipping from the manufacturer's warehouse if stored in a tightly closed container at the above conditions.

Incompatibilities: Incompatibilities occur with certain polymethacrylate dispersions depending upon the ionic and physical properties of the polymer and solvent. For example, coagulation may be caused by soluble electrolytes, pH changes, some organic solvents, and extremes of temperature. For example, dispersions of *Eudragit L 30 D*, *RL 30 D*, *L 100-55*, and *RS 30 D* are incompatible with magnesium stearate. *Eastacryl 30D*, *Kollicoat MAE 30 D*, and *Kollicoat MAE 30 DP* are also incompatible with magnesium stearate. Interactions between polymethacrylates and some drugs can occur, although solid polymethacrylates and organic solutions are generally more compatible than aqueous dispersions.

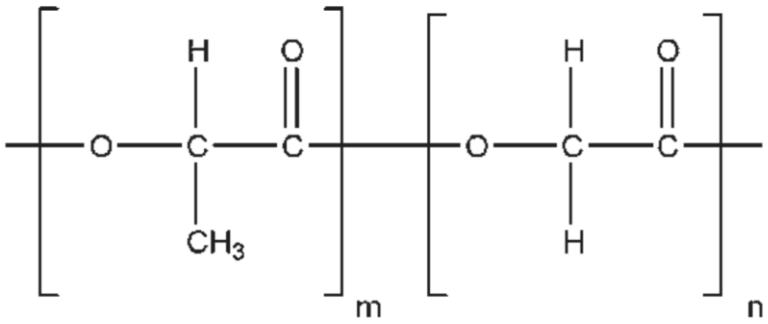
Safety: Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical formulations. They are also used in topical formulations and are generally regarded as nontoxic and non-irritant materials. A daily intake

of 2 mg/kg body-weight of *Eudragit* (equivalent to approximately 150 mg for an average adult) may be regarded as essentially safe in humans.

Handling Precautions: Observe normal precautions appropriate to the circumstances and quantity of material handled. Additional measures should be taken when handling organic solutions of polymethacrylates. Eye protection, gloves, and a dust mask or respirator are recommended. Polymethacrylates should be handled in well-ventilated environment and measures should be taken to prevent dust formation.

In the UK, the occupational exposure limit for methyl methacrylate has been set at 208 mg/m^3 (50 ppm) long-term (8-hour TWA), and 416 mg/m^3 (100 ppm) short-term (Handbook of Pharmaceutical Excipients).

(b) Poly (dl-lactide-co-glycolide) (PLGA)



Chemical name: 1,4-Dioxane-2,5-dione, 3,6-dimethyl-, polymer with 1,4-dioxane-2,5-dione.

Empirical Formula: It is copolymers of lactide and glycolide.

Functional Category: Bioabsorbable biocompatible and biodegradable material.

Applications in Pharmaceutical Formulation: Owing to its reputation as safe materials and biodegradability, PLGA is primarily used as biocompatible and biodegradable carriers in many types of implantable or injectable drug-delivery systems for both human and veterinary use. Examples of implantable drug delivery systems include rods, cylinders, tubing, films, fibers, pellets, and beads. Examples of injectable drug-delivery systems include microcapsules, microspheres, nanoparticles, and liquid injectable controlled-release systems such as gel formulations.

Description: PLGA is a synthetic copolymer of lactide and glycolide. It is nontoxic and can easily be fabricated into a variety of novel devices, such as rods, screws, nails, and cylinders. The polymer is commercially available in varying molecular weights as copolymers.

Molecular weights: 40000 Da to 100 000 Da.

Solubility: Practically insoluble in water; soluble in organic solvent.

Melting point: Amorphous

Glass Transition temperature: 45-55°C

Specific gravity: 1.27 to 1.34

Color: White to yellow color

Inherent Viscosity (mpas): 0.5 – 0.8

Co-monomer ratios of lactic acid and glycolic acid (or lactide and glycolide) for poly(DL -lactide- co -glycolide) range from 85 : 15 to 50 : 50.

Stability and Storage Conditions: Polymer is easily susceptible to hydrolysis in the presence of moisture. Hence, it should be packaged under high-purity dry nitrogen and properly stored in airtight containers, preferably refrigerated at below 8°C. It is necessary to allow the polymers to reach room temperature in a dry environment before opening the container. After the original package has been opened, it is recommended to re-purge the package with high-purity dry nitrogen prior to resealing.

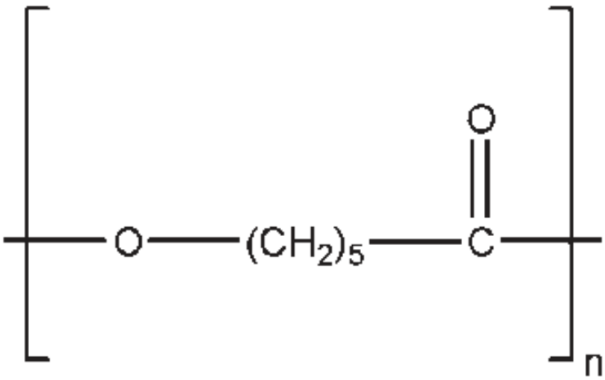
Safety: Poly (lactic- co-glycolic acid) or poly (lactide- co -glycolide) is used in parenteral pharmaceutical formulations and is regarded as biodegradable, biocompatible, and bioabsorbable materials. Its biodegradation products are nontoxic, non-carcinogenic, and non-teratogenic. In general, PLGA exhibits very little hazard.

Handling Precautions: Observe normal precautions appropriate to the circumstances and quantity of material handled. Contact with eyes, skin, and clothing, and breathing the dust of the polymers should be avoided. Aliphatic polyesters produce acid materials such as hydroxyacetic and/or lactic acid in the presence of moisture; thus, contact with materials that will react with acids, especially in moist conditions, should be avoided.

Regulator Status: GRAS listed and included in the Canadian List of Acceptable Non-medicinal Ingredients. Poly(lactide) and poly(lactide-co-glycolide) have been used in medical products and medical devices approved by the FDA.

Comments: PLGA is a synthetic, nontoxic and biodegradable polymers. In an aqueous environment, the polymer backbone undergoes hydrolytic degradation, through cleavage of the ester linkages, into nontoxic hydroxycarboxylic acids. Aliphatic polyesters are eventually metabolized to carbon dioxide and water, via the citric acid cycle. The rate of biodegradation and drug-release characteristics from injectable drug-delivery systems formulated with the aliphatic polyesters can be controlled by changing the physicochemical properties of the polymers, such as crystallinity, hydrophobicity, monomer stereochemistry, copolymer ratio, end group, and polymer molecular weight or by changing the porosity and geometry of the formulation. Due to their ability to form complexes with heavy metal ions, aliphatic polyesters are added to skin-protective ointments.

(c) Poly (ε-caprolactone) (PCL)



Chemical name: 2-Oxepanone, homopolymer

Empirical Formula: It is homopolymer of ε-caprolactone.

Functional Category: Bioabsorbable biocompatible and biodegradable material.

Applications in Pharmaceutical Formulation: PCL is as biocompatible and biodegradable carriers in many types of implantable or injectable drug-delivery systems for both human and veterinary use. Examples of implantable drug delivery systems include rods, cylinders, tubing, films, fibers, pellets, and beads. Examples of injectable drug-delivery systems include microcapsules, microspheres and nanoparticles.

Description: PCL is a synthetic homopolymer of ε-caprolactone. It is nontoxic and can easily be fabricated into a variety of novel devices, such as rods, screws, nails, and cylinders. The polymer is commercially available in varying molecular weights as copolymers.

Molecular weights: 20000 Da to 80000 Da.

Solubility: Practically insoluble in water; soluble in chloroform, acetone, methanol, tetrahydrofuran, ethyl acetate, dichloromethane and hexafluoroisopropanol.

Melting point: 58-63°C

Glass Transition temperature: -65 to -60°C

Specific gravity: 1.11

Color: White

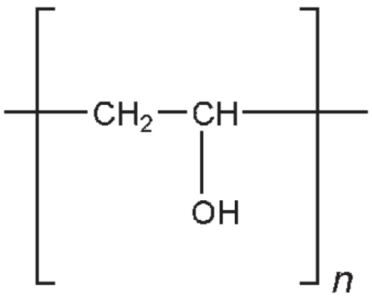
Inherent Viscosity (mpas): 1.0 – 1.3

Stability and Storage Conditions: Same as PLGA but it is more stable than PLGA.

Safety & Handling precaution: Same as PLGA.

Regulatory Status: Listed as GRAS, and included in Canadian list of Non-medicinal ingredients. Its medicinal devices are FDA approved.

(d) Polyvinyl alcohol (PVA)



Chemical name: Ethenol, homopolymer

Empirical Formula: It is homopolymer of Vinyl alcohol $(C_2H_4O)_n$. Commercially available n value is 500-5000.

Functional Category: It is used as coating agent, lubricant, stabilizing agent and viscosity-increasing agent.

Applications in Pharmaceutical Formulation: Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulations. It is used as a

stabilizing agent for emulsions (0.25–3.0% w/v). Polyvinyl alcohol is also used as a viscosity-increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solutions for lubrication purposes, in sustained-release formulations for oral administration and in transdermal patches. Polyvinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution.

Description: Polyvinyl alcohol occurs as an odourless, white to cream-colored granular powder.

Molecular weights: 20000 Da to 200000 Da.

Solubility: Soluble in water; slightly soluble in ethanol (95%); insoluble in organic solvents. Dissolution requires dispersion (wetting) of the solid in water at room temperature followed by heating the mixture to about 90°C for approximately 5 minutes. Mixing should be continued while the heated solution is cooled to room temperature.

Melting point: 228°C for fully hydrolyzed grades and 180–190°C for partially hydrolyzed grades.

Refractive index: 1.49-1.53

Specific gravity: 1.19–1.31 for solid and 1.02 for 10% w/v aqueous solution at 25°C

Color: White

Viscosity: 4 – 65 mpas (as per grade)

Stability and Storage Conditions: Polyvinyl alcohol is stable when stored in a tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion-resistant sealed containers. Preservatives may be added to the solution if extended storage is required. Polyvinyl alcohol undergoes slow degradation at 100°C and rapid degradation at 200°C; it is stable on exposure to light.

Incompatibilities: Polyvinyl alcohol undergoes reactions typical of a compound with secondary hydroxyl groups, such as esterification. It decomposes in strong acids, and softens or dissolves in weak acids and alkalis. It is incompatible at high concentration with inorganic salts, especially sulphates and phosphates;

precipitation of polyvinyl alcohol 5% w/v can be caused by phosphates. Gelling of polyvinyl alcohol solution may occur if borax is present.

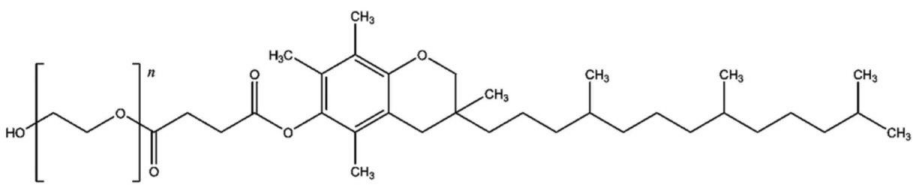
Safety: Polyvinyl alcohol is generally considered a nontoxic material. It is non-irritant to the skin and eyes at concentrations up to 10%; concentrations up to 7% are used in cosmetics. Studies in rats have shown that polyvinyl alcohol 5% w/v aqueous solution injected subcutaneously can cause anaemia and infiltrate various organs and tissues. (7) LD50 (mouse, oral): 14.7 g/kg LD50 (rat, oral): > 20 g/kg

Handling Precautions: Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended. Polyvinyl alcohol dust may be an irritant on inhalation. Handle in a well-ventilated environment.

Regulatory Status: Included in the FDA Inactive Ingredients Database (ophthalmic preparations and oral tablets), in nonparenteral medicines licensed in the UK and in the Canadian List of Acceptable Non-medicinal Ingredients.

Comments: Various grades of polyvinyl alcohol are commercially available. The degree of polymerization and the degree of hydrolysis are the two determinants of their physical properties. Pharmaceutical grades are partially hydrolyzed materials and are named according to a coding system. The first number following a trade name refers to the degree of hydrolysis and the second set of numbers indicates the approximate viscosity (dynamic), in mPas, of a 4% w/v aqueous solution at 20°C.

(e) D -a-tocopheryl polyethylene glycol 1000 succinate (TPGS)



n = 20-22

Chemical name: 4- O -(2-Hydroxyethyl)-1- O-[2,5,7,8-tetramethyl-2-(4,8,12-tri-methyltridecyl)-3,4-dihydrochromen-6-yl]butanedioate.

Empirical formula: $C_{33}O_5H_{54}(CH_2CH_2O)_{20\text{-}22}$

Functional Category: It is used as absorption enhancer, antioxidant, emulsifying agent, granulation aid, ointment base, solubilizing agent, surfactant, suspending agent and tablet binder.

Applications in Pharmaceutical Formulation: Vitamin E polyethylene glycol succinate is an esterified vitamin E (tocopherol) derivative primarily used as a solubilizer or emulsifying agent because of its surfactant properties. Structurally, it is amphipathic and hydrophilic, unlike the tocopherols, and therefore it is a water-soluble derivative that can be used in pharmaceutical formulations such as capsules, tablets, hot-melt extrusion, microemulsions, topical products and parenterals. One of the most important applications is its use as a vehicle for lipid-based drug delivery formulations. It can also be used as a source of vitamin E. Vitamin E polyethylene glycol succinate has been characterized with respect to its mechanism of action and studied as a P-glycoprotein inhibitor.

Description: TPGS is a synthetic product. It is available as a white to light-brown, waxy solid and is practically tasteless. Chemically, it is a mixture composed principally of monoesterified polyethylene glycol 1000, the diesterified polyethylene glycol 1000, free polyethylene glycol 1000 and free tocopherol.

Critical micelle concentration: 0.02% by weight (37°C)

HLB value: 13.2

Melting point**:** 37–41°C

Solubility: Miscible in water in all proportion

Specific gravity: 1.06 (at 45°C)

Stability and Storage Conditions: TPGS is stable at ambient room temperature for up to 4 years. It reacts with alkalis and acids. Aqueous solutions of TPGS are stable over a pH range of 4.5–7.5 and can be further stabilized with propylene glycol.

Incompatibilities: TPGS is incompatible with strong acids and strong alkalis.

Safety: TPGS has been used at levels of 280 mg/capsule in the product Agenerase (amprenavir), which was dosed at 8 capsules (2240 mg TPGS) per day. An additional assessment of the safety of TPGS has been published, which

includes a report showing no-observed-adverse-effect-level (NOAEL) in rats of 1000 mg/kg/day.

Handling Precautions: Observe normal precautions appropriate to the circumstances and quantity of the material handled. Gloves and eye protection are recommended.

Regulatory Status: Listed in GRAS, FDA Inactive Ingredients Database (ophthalmic solution or drops; oral capsules, solution, tablet; topical solution or drops) and Canadian List of Acceptable Non-medicinal Ingredients.

Comments: TPGS is most widely used in cancer therapy targeted novel drug delivery system.