Chapter 2

DRUG AND POLYMER PROFILE

[Chemical structures and diagrams]
2 PROFILE OF CYCLODEXTRINS, DRUGS AND POLYMERS

2.1 CYCLODEXTRINS

Cyclodextrins (CDs) are cyclic oligosaccharides discovered approximately 100 years ago. CDs have been used as solubilizers, stabilizers for biologically active substances, enzyme models, as separating agents in chromatography or batch processes, catalysts and additives (Szejtli, 1998). CDs can be used to reduce or prevent gastrointestinal (GI) or ocular irritation, reduce or eliminate unpleasant smell or taste, prevent drug-drug or drug-additive interactions, or even to convert oils and liquid drugs into microcrystalline or amorphous powder. Marketed CDs are well accepted because of their low oral and local toxicity, low eye, mucous irritability etc. (Shimpi et al, 2005)

2.1.1 Structure and physiochemical properties

Cyclodextrins (CD) are cyclic oligosaccharides of α-D-glucose joined through 1-4 bonds. The three major natural CDs are α-CD, β-CD and γ-CD, which are built up from 6, 7 and 8 glucopyranose units respectively (Fig 2F-1, Szejtli, 1988; Thompson, 1997). CDs with less than 6 glucopyranose units cannot be formed for steric reasons (too strained to exist), while those with greater than 8 are difficult to isolate in crystalline form due to their high solubility resulting from higher flexibility. They are also thought to have low complexing ability and therefore of little practical value (Szejtli, 1988; Connors, 1997). The general physiochemical properties of the CDs are presented in table (Table 2T-1, Szejtli, 1988; Connors, 1997; Lofsson et al, 2004).

Fig 2F-1. Chemical structure of CDs. Arrows indicate the 2-, 3-(Secondary) and 6- (primary) hydroxyls of a glucopyranose unit.
Table 2T-1 Characteristics of α-CD, β-CD and γ-CDs

<table>
<thead>
<tr>
<th>Property</th>
<th>α-CD</th>
<th>β-CD</th>
<th>γ-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of glucose</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C₃₆H₆₀O₃₀</td>
<td>C₄₂H₇₀O₃₅</td>
<td>C₄₈H₸₀O₄₀</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>972.85</td>
<td>1134.99</td>
<td>1297.14</td>
</tr>
<tr>
<td>Specific rotation[α]D²⁵</td>
<td>150.5 ± 0.5</td>
<td>162.5 ± 0.5</td>
<td>177.4 ± 0.5</td>
</tr>
<tr>
<td>Aqueous solubility</td>
<td>145</td>
<td>18.5</td>
<td>232</td>
</tr>
<tr>
<td>Cavity diameter</td>
<td>4.7-5.3 Å</td>
<td>6-6.5 Å</td>
<td>7.5-8.3 Å</td>
</tr>
<tr>
<td>Height of torus</td>
<td>7.9±0.1 Å</td>
<td>7.9±0.1 Å</td>
<td>7.9±0.1 Å</td>
</tr>
<tr>
<td>Periphery diameter</td>
<td>14.6±0.4 Å</td>
<td>15.4±0.4 Å</td>
<td>17.5±0.4 Å</td>
</tr>
<tr>
<td>Approx. cavity vol./ g CD (mL)</td>
<td>0.1</td>
<td>0.14</td>
<td>0.2</td>
</tr>
</tbody>
</table>

a-Solubility (mg/mL) in pure water at approximately 25°C.

**Fig 2F-2. Functional schematic of β-cyclodextrin**

[The secondary hydroxyls at the 2- and 3- positions exist on the secondary face of the structure, and the primary hydroxyls at the 6- position exist on the primary face]

CDs are not perfectly cylindrical, but are instead shaped like a truncated cone. All secondary hydroxyls line one end of ring, while the primary hydroxyls line the other end of ring (Fig 2F-2, Szejtli, 1988; Thompson, 1997). The free rotation of the primary hydroxyls at one end of the molecule reduces its effective diameter (Szejtli, 1988). The interior of the cavity is free of hydrogen bonding groups, and lined with non-polar methylene groups and non-polar ether oxygens (Yalkowsky, 1999). In addition, the C₂-OH of one glucopyranose unit can form a hydrogen bond with the C₃-OH of its adjacent glucopyranose unit, resulting in the formation of a secondary belt,
making it a rigid structure (Szejtli, 1988). This secondary hydrogen belt is incomplete in α-CD as one of the glucopyranose units is distorted. The CDs with exterior relatively hydrophilic and the interior lipophilic resembles with micelles. However, unlike micelles, whose size is fluid and can therefore adjust to a wide variety of sizes and shapes, the CD interior is fixed in size and shape (Yalkowsky, 1999).

The natural CDs, particularly β-CD, have limited aqueous solubility. The lower solubility of β-CD is attributed to the stable formation of the secondary hydrogen belt and its ability to form a more stable lattice, which limits its interaction with water (Szejtli, 1988; Connors, 1997). This secondary hydrogen belt is incomplete in the other natural CDs, and so allows more favorable interactions between the CD and water molecules. In addition, γ-CD has a more flexible structure and therefore the most soluble of the three. The aqueous solubility of the CDs increases with temperature, approximately doubling with every 20°C increase in temperature (Yalkowsky, 1999). CDs are susceptible to acid hydrolysis, particularly at high temperatures.

2.1.2 Regulatory status

The regulatory status of CDs continues to evolve (Loftsson et al, 2004; Davis et al, 2004), β-CD is listed in a number of pharmacopoeia sources including the US Pharmacopoeia/National Formulary (USP/NF), European Pharmacopoeia (Ph.Eur.) and Japanese Pharmaceutical Codex (JPC). While α-CD is similarly listed in the Ph.Eur, USP/NF and JPC and γ-CD is referenced in the JPC and will soon be included in the Ph.Eur. and USP/NF. A monograph for HP-β-CD is available in the Ph. Eur. (4th edition (suppl.4.6) and 5th edition) and a draft has been circulated for the USP28/NF23. Other derivatives are not yet compendial but efforts are underway for their inclusion. α-CD, β-CD and γ-CD were also introduced into the generally regarded as safe (GRAS) list of the FDA for use as a food additive in 2004, 2001 and 2000, respectively, and HP-β-CD is cited in the FDA’s list of Inactive Pharmaceutical Ingredients (IPI). SBE-β-CD (sulfobutylated beta cyclodextrin) is also available in various dosage forms and is also listed in the FDA’s compilation of inactive pharmaceutical ingredients. Consensus seems to be building among regulators that CDs are excipients and not part of the drug substance although various opinion have been given and interpretation related to this point can be division and product-
specific (Loftsson et al, 2004). The regulatory status of cyclodextrins is given in table (Table 2T-2, Loftsson et al, 2007; Loftsson et al, 2004; Davis et al, 2004).

Table 2T-2 Regulatory status of cyclodextrins

<table>
<thead>
<tr>
<th>CD Type</th>
<th>Food Approval</th>
<th>Pharmacopoeia Monographs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>US</td>
<td>Europe</td>
</tr>
<tr>
<td>α-CD</td>
<td>In preparation</td>
<td>Planned</td>
</tr>
<tr>
<td>β-CD</td>
<td>GRAS</td>
<td>Food Additive</td>
</tr>
<tr>
<td>γ-CD</td>
<td>GRAS</td>
<td>Pending</td>
</tr>
<tr>
<td>HP-β-CD</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2.1.3 Toxicological considerations

CDs are associated with molecular weight ranging from almost 1000 to over 2000 Da and are hydrophilic with a significant number of H-donors and acceptors and, thus, are not significantly absorbed from the gastrointestinal tract in their intact form. The natural α-CD and β-CD, unlike γ-CD, cannot be hydrolyzed by human salivary and pancreatic amylases (Irie et al, 1997; WHO, 2002). However, both α-CD and β-CD can be fermented by the intestinal microflora. α-CD can be found in one marketed parenteral solution and in tablet formulations. Oral administration of α-CD is, in general, well tolerated and is not associated with significant adverse effects. Only small fractions of α-CD are absorbed intact from the gastrointestinal tract and it is mainly excreted unchanged in the urine after i.v. injection. β-CD can be found in numerous marketed oral dosage forms as well as in topical, buccal and rectal drug formulations. β-CD cannot be given parenterally due to its low aqueous solubility and adverse effects (e.g. nephrotoxicity) but it is essentially non-toxic when given orally. After oral administration, the non-toxic effect level of β-CD was determined to be 0.7–0.8 g/kg/day in rats and about 2 g/kg/day in dogs (Bellringer et al, 1995). β-CD is a common food dose excreted intact in the faeces with the remainder mainly being metabolized by the intestinal microflora. Fewer published references on the toxicological potential of SBE-β-CD are available however this CD derivative can be found in several marketed product, including voriconazole parenteral solution (Vfend®). The available toxicological information on G2-β-CD and HP-γ-CD is even more limited. G2-β-CD is not yet available in a marketed product but HP-γ-CD is
found in two products, i.e. an eye drop solution formulation and a parenteral diagnostic product. Lipophilic CD derivatives, such as the methylated CDs, are absorbed to a somewhat greater extent from the gastrointestinal tract into the systemic circulation and have been shown to be toxic after parenteral administration (Irie et al, 1997). Presently, oral administration of methylated β-CD is dose limited because of its potential toxicity with higher dosages i.e. approximately above 70mg/kg body weight. The oral bioavailability of RM-β-CD is about 5% in rats and with more than 90% of the material excreted unchanged with faeces. More than 95% of RM-β-CD (randomly methylated β-cyclodextrin) is excreted unchanged with urine after intravenous injection to rats (Toxicological data, 1997). The haemolytic effect of CDs on human erythrocytes in phosphate buffered saline are in the order methylated β-CDs > β-CD > HP-β-CD > G2-β-CD > α-CD > γ-CD > HP-γ-CD > SBE-β-CD (Irie et al, 1997; Thompson, 1997). There appears to be a correlation between the hemolytic activity and the ability of the CDs to bind or extract cholesterol from the membranes (Irie et al, 1997). This in-vitro cellular lysis study, as well as other comparable in-vitro studies using intestinal cells, E. coli bacterial cells, human skin fibroblasts and liposomes, do not indicate in-vivo toxicity but rather provide a method to classify CDs according to their potential to destabilize or disrupt cellular membranes (Thompson, 1997). In humans, the acceptable daily oral intakes (ADI) of the natural CDs and RM-β-CD are 1.4 g for α-CD, 0.35 g for β-CD, 10 g for γ-CD and 0.07 g for RM-β-CD per kg body weight (Antlspenger et al, 1996). Various data on safety overview of selected cyclodextrins are presented in table (Table 2T-3, Brewster et al, 2007).
<table>
<thead>
<tr>
<th>Cyclodextrins</th>
<th>The pharmacokinetics in rats</th>
<th>Acute toxicity, ( LD_{50} ) rat (g/kg)</th>
<th>Acute toxicity, ( LD_{50} ) rat (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( t_{1/2} ) after i.v. injection (min)</td>
<td>Fraction excreted unchanged in urine</td>
<td>Oral absorption</td>
</tr>
<tr>
<td>( \alpha )-CD</td>
<td>25</td>
<td>( \sim 90% )</td>
<td>2–3%</td>
</tr>
<tr>
<td>( \beta )-CD</td>
<td>20</td>
<td>( \sim 90% )</td>
<td>1–2%</td>
</tr>
<tr>
<td>HP-( \beta )-CD</td>
<td>20</td>
<td>( \sim 90% )</td>
<td>( \leq 3% )</td>
</tr>
<tr>
<td>SBE-( \beta )-CD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM-( \beta )-CD</td>
<td>18</td>
<td>( &gt; 95% )</td>
<td>0.5–12%</td>
</tr>
<tr>
<td>G2-( \beta )-CD</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \gamma )-CD</td>
<td>20</td>
<td>90%</td>
<td>( \leq 0.02% )</td>
</tr>
<tr>
<td>HP-( \gamma )-CD</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
2.1.4 β-Cyclodextrin (β-CD)

*Formula* - $C_{42}H_{76}O_{35}$
*Mol. Wt.* - 1135

*Chemical Name* - 7 Cyclo-α (1, 4)-anhydroglucose units
*Synonyms* - β-Cycloamylose; β-Cycloheptaamylose; β-Dextrin; Cycloheptaamylose; Cycloheptaglucan; Cycloheptaglucosan; Schardinger β-dextrin; Cyclomaltoheptaose.

### 2.1.4.1 Physical and chemical properties

*Physical State* - White powder
*Melting Range* - 255-298 °C
*Solubility in Water* - Soluble (18.5 mg mL$^{-1}$ at 25 °C)
*Stability* - Stable under normal conditions.
*Specific Rotation* - $+160^\circ$ to $+165^\circ$ (c=1 in H2O on dry basis)
*Moisture* - 5.0% max

![Structure of β-cyclodextrin](image)

**Fig 2F-3. Structure of β-cyclodextrin**

### 2.1.4.2 Fate

The cyclic structure of β-CD resists enzymatic hydrolysis by β-amylases and salivary α-amylases and is poorly hydrolyzed in the human small intestine but is fermented by the colonic micro flora. Less than 1% of the ingested β-CD is absorbed intact by the small intestine and is excreted in the urine.

### 2.1.4.3 Safety

β-CD is currently considered to be an essential non toxic and non irritant ingredient, authorized for food application in many countries and used in oral pharmaceutical...
applications as well as cosmetic application. β-CD is not suitable for parenterals administration because of its nephrotoxicity (Vyas et al, 2008; Loftsson et al, 1996).

2.1.4.4 Regulatory status

Acceptable daily intake (ADI) - ADI of 5 mg-350 mg for β-CD /kg body weight
Pharmacopoeial References- European Pharmacopoeia; Betadex monograph- USP/NF; Betadex monograph (Szejtli, 1998; Vyas et al, 2008; Loftsson et al, 1996).

2.1.5 Hydroxypropyl-β-cyclodextrin (HP-β-CD)

Formula- \((C_6H_9O_2)7(C_3H_7O)_{4.5} [C_{42}(H)_{70-n}O_{35}(C_3H_7)_{n}]\)

Mol. Wt.- 1399

Chemical Name- Cyclodextrin 2-hydroxypropylether; Hydroxypropyl-beta cyclodextrin

Synonyms- β-Hydroxypropylcycloamylose; Hydroxypropylcycloheptaglucan; β-Hydroxypropylcyclodextrin; β-Cyclodextrin 2-hydroxypropyl ethers; HP-β CD; HPCD.

2.1.5.1 Physical and chemical properties

Physical State- White amorphous powder


Solubility in Water- Soluble (100g in100 ml) (Szejtli, 1998)

Stability- Stable under normal temperatures and pressures

Specific Rotation- +127° ~ +132° (c=1 in H2O on dry basis)

Moisture- 5.0% max

Odour- Odourless

Structure- [Diagram of HP-β-cyclodextrin]

Fig 2F-4. Structure of HP-β-cyclodextrin
2.1.5.2 Fate

Gerloczy et al. studied that the absorption, distribution and excretion of orally administered HP-β-CD, and found that less than 10% was absorbed from the gastrointestinal tract (Gerloczy et al, 1985). Hydrolysis of HP-β-CD by colon microflora occurs at a slower rate compared with the unsubstituted CDs, as the adducts interfere with enzymatic degradation (Müller et al, 1986; Monbaliu et al, 1990). Of the absorbed HP-β-CD, most is initially metabolized by the microflora in the large intestine. A maximum of 2-3% (possibly as low as 1%) of the absorbed HP-β-CD is intact, which is then removed by glomerular filtration (Frijlink et al, 1990). Up to 86% of the administered dose is unabsorbed and passed in the faeces within 72 hours, of which approximately 60-70% is intact HP-β-CD (Gerloczy et al, 1990; Monbaliu et al, 1990).

2.1.5.3 Safety

Number of clinical studies reported in literature has shown that HP-β-CD was well-tolerated and safe in the majority of patients receiving HP-β-CD at daily oral doses of the 4-8 g for at least two weeks (Irie et al, 1997). Higher oral daily doses of 16 to 24 g and use for 14 days to volunteers resulted in increased incidence is of sauce tools in diarrhoea. Therefore based on these clinical data, HP-β-CD was considered to be non-toxic for 14 days if a daily dose is less than 16 g.

In an intravenous dose and single doses study up to 3 g were found well tolerated by all volunteers and no measurable effect and kidney function was observed (Seiller et al, 1990). Following one week intravenous study and a single dose level of 1 g showed no adverse effects (Janssen technical bulletin, 1992).

2.1.5.4 Regulatory status

The American FDA has given market approval to oral and i.v. formulations containing HP-β-CD.

Pharmacopoeial Conformity-The USP and EP have published draft monographs for HP-β-CD. The EP monograph has gained official status in 2002.
2.1.6 Randomly methylated-β-CD (RM-β-CD)

**Formula** - C₅₄H₉₂O₃₅

**Mol. Wt.** - 1303.3

**Chemical Name** - β-Cyclodextrin methyl ethers

**Synonyms** - β-Cyclodextrin methylethers; Methyl-β-CD; MBC; β-W7M1.8; β-CYD; Dimethyl-β-Cyclodextrin; Methyl-β-cyclodextrin; methyl- β-cyclodextrin cell culture tested; methyl-β-cyclodextrin cyclomaltoheptaose, methylether; (RANDOM) methyl beta cyclodextrin, technical grade; Methyl-β-cyclodextrin non-toxic solubiliz ER; (RANDOM) methyl β-cyclodextrin, pharmaceutical G; Methyl-β-cyclodextrin= Cyclodextrinmethyle; Methyl-SS-cyclodextrin; β-Cyclodextrin methyl ether; Methyl- β-cyclodextrin.

2.1.6.1 Physical and chemical properties

**Physical State** - Anhydrous white powder

**Melting Range** - 280-282 °C

**Solubility in Water (Mg/Ml)** - Soluble (>500)

**Stability** - Stable under normal conditions

**Moisture** - 5.0% max

**Structure** -

![Structure of RM-β-cyclodextrin](image)

Fig 2F-5. Structure of RM-β-cyclodextrin

2.1.6.2 Fate

The cyclic structure of RM-β-CD resists enzymatic hydrolysis by β-amylases and salivary-α-amylases. It is poorly hydrolyzed in the human small intestine but is fermented by the colonic micro flora less than 1% of the ingested RM-β-CD is absorbed intact by the small intestine. RM-β-CD is excreted through the urine (Duchêne et al, 1990; Uckama et al, 1987; Szente et al, 1999).
2.1.6.3 Safety

Preliminarly toxicological results on RM-β-CD have shown it to have good potential for high biological tolerance. It does not penetrate barriers which protect organism, tissues, cells, but, when used at very high concentrations, can damage these barriers by solubilizing and, thus extracting their components. RM-β-CD if administeres per os, it remain mainly in G.I. tract until excreted. At a different level parenterally administered CD derivatives are eventually excreted in urine with a small fraction being able to remain in the kidneys. As with other common products, it is expected that such an excretion pathway could provoke renal toxicity when very high doses are used (Duchêne et al, 1990; Uekama et al, 1987; Szente et al, 1999).

2.1.6.4 Regulatory status

Not available in reference to pharmacopeial consideration.

2.2 DRUGS

2.2.1 Simvastatin

Simvastatin is a lipid-lowering agent which is derived synthetically from a fermentation product of *Aspergillus terreus* and widely used to treat hypercholesterolemia (Florey, 2008; Vanheek et al, 1997; Neil, 2006). Simvastatin an inactive lactone is converted to corresponding β, delta-dihydroxy acid in liver by cytochrome P450 (CYP) 3A after oral administration. SV is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol (McClelland et al, 1991). However, it is practically insoluble in water and poorly absorbed from the gastrointestinal (GI) tract (Ambike et al, 2005; Kang et al, 2004). Simvastatin is official in B.P, European pharmacopoeia and USP.

2.2.1.1 Proprietary names/Brand Names/Synonyms

Cholestat; Coledis; Colemin; Corolin; Denan; Labistatin; Lipex; Liponorm; Lodales Medipo; Nivelipol; Pantok; Rendapid; Simovil; Simvastatin [Usan:Ban:Inn]; Simvastatina [Spanish]; Simvastatine [French]; Simvastatinum [Latin]; Sinvacor;
Sivastin; Synvinolin; Vasotenal; Vytorin; Zocor; Zocord (Moffat et al, 2004; Florey, 2008).

2.2.1.2 Drug Category
Anticholesteremic agents; Antilipemic agents; Hydroxymethylglutaryl-CoA Reductase inhibitors (Moffat et al, 2004; Drug bank, 2008).

2.2.1.3 Physicochemical properties
Simvastatin is a white to off-white, non hygroscopic, crystalline powder that is practically insoluble in water, and freely soluble in chloroform, methanol and ethanol (Florey, 2008; Neil, 2006). At room temperature partition coefficient of simvastatin between octanol and either pH 4.0 or pH 7.2 acetate is > 1995 (Florey, 2008; Sweetman, 2005). The drug is officially listed in the United States Pharmacopoeia and the official method of its determination is high-performance liquid chromatography (The United States Pharmacopoeia, 2004; Arayne et al, 2007). The Physicochemical properties of simvastatin are given in Table 2T-4 (Moffat et al, 2004; Sweetman, 2005; Florey, 2008; Robinson, 2007; Neil, 2006).

Table 2T-4 Physicochemical properties of simvastatin

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White powder</td>
</tr>
<tr>
<td>Odour</td>
<td>Odourless</td>
</tr>
<tr>
<td>Melting Point</td>
<td>138-141</td>
</tr>
<tr>
<td>Solubility</td>
<td>0.03 mg/mL</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C23H38O3</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>418.56</td>
</tr>
<tr>
<td>LogP/ Hydrophobicity</td>
<td>4.68</td>
</tr>
</tbody>
</table>

2.2.1.4 Chemistry
Simvastatin is butanoic acid, 2,2 - dimethyl - ,1,2,3,7,8a - hexahydro - 3,7 - dimethyl - 8 - [2 - (tetrahydro - 4 - hydroxy - 6 - oxo - 2H - pyran - 2 - yl) - ethyl] - 1 - naphthalenyl ester, [1S-[1\alpha,3\alpha,7\beta,8\beta(2\alpha^*,4\alpha^*)]-8a \beta]] (Florey, 2008; Neil, 2006). Its structural formula is (Fig 2F-6):
2.2.1.5 Pharmacology and Mechanism of Action

Simvastatin, the methylated form of lovastatin, is an oral antilipemic agent which inhibits HMG-CoA reductase. Simvastatin is used in the treatment of primary hypercholesterolemia and is effective in reducing total and LDL-cholesterol as well as plasma triglycerides and apolipoprotein B (Be Dell, 1996; Neil, 2006).

![Fig 2F-6. Structure of simvastatin](image)

The 6-membered lactone ring of simvastatin is hydrolyzed in-vivo to generate mevinolinic acid (mevalonate pathway), an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme. Interference
with the activity of this enzyme reduces the quantity of mevalonic acid, a precursor of cholesterol (Fig 2F-7, Corsini et al, 1999; Florey, 2008; Neil, 2006).

2.2.1.6 Pharmacokinetics

Simvastatin is pharmacologically inactive prodrug for several active metabolites which are HMG-CoA reductase inhibitors. The metabolites, of which the most potent with respect to HMG-CoA reductase inhibition is simvastatin \( \beta \)-hydroxyacid, are formed by hydrolysis of the lactone ring. The inhibitors may be referred to as active or total inhibitors (Florey, 2008).

2.2.1.7 Absorption and Distribution

After oral administration in humans, simvastatin is rapidly extracted by liver where it is metabolized. The systemic bioavailability of the \( \beta \)-hydroxyacid after administration of simvastatin is therefore low (less than 5% compared with an I.V. reference dose of the \( \beta \)-hydroxyacid).

Using inhibition of HMG-CoA reductase as basis for assay, studies have shown that the maximum concentration of inhibitors occurred between 1.3 and 2.4 hours after dosing. The areas under the plasma concentration-time curves (AUC) indicated that the relationship between circulating levels of inhibitors and dose is linear. The plasma profile is essentially unaffected by concomitant administration of food (Florey, 2008).

2.2.1.8 Metabolism and excretion

Simvastatin undergoes extensive first-pass metabolism in the liver. Studies with radiolabelled drug have shown that the levels of circulating total inhibitors accounted for 42% of the AUC, indicating the most of the metabolites were inactive or weak inhibitors. Recoveries from urine were 13% and from faeces were 60 %, the latter including both unabsorbed drug and drug excreted in bile. Less than 0.5% of the administered dose of drug was present as active inhibitors in urine. In humans main metabolite is the \( \beta \)-hydroxyacid (Moffat et al, 2004; Florey, 2008). The pharmacokinetic profile of simvastatin is given in table (Table 2T-5, Schachter, 2004; Haider et al, 2004).
Table 2T-5 Pharmacokinetic properties of simvastatin

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>Lipophilic</td>
</tr>
<tr>
<td>Protein binding</td>
<td>95-98%</td>
</tr>
<tr>
<td>Active metabolites</td>
<td>yes</td>
</tr>
<tr>
<td>Half-life</td>
<td>2-3 h</td>
</tr>
<tr>
<td>Metabolism cytochrome &amp; isoform</td>
<td>by 3A4</td>
</tr>
<tr>
<td>Renal excretion</td>
<td>13%</td>
</tr>
<tr>
<td>Optimal time of dosing</td>
<td>Evening</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>5 (%)</td>
</tr>
<tr>
<td>Effect of food</td>
<td>No effect</td>
</tr>
<tr>
<td>Renal excretion</td>
<td>13%</td>
</tr>
</tbody>
</table>

2.2.1.9 Indication

Simvastatin is used for the treatment of hypercholesterolemia.

2.2.1.10 Contraindications

Simvastatin is contraindicated in case of:

*Hypersensitivity:* Hypersensitivity to any component of this medication

*Liver Dysfunction:* Active liver disease or unexplained persistent elevations of serum transaminases (Harper et al, 2007; Jacobson, 2006).

*Pregnancy and lactation:* Simvastatin is contraindicated during pregnancy and in nursing mothers (Armitage, 2007; Jacobson, 2006).

*Myopathy/Rhabdomyolysis:* Simvastatin therapy should be discontinued immediately if myopathy is diagnosed or suspected. Simvastatin, like other inhibitors of HMG-CoA reductase, occasionally causes myopathy manifested as muscle pain, tenderness or weakness with Creatine Kinase (CK) above ten times the Upper Limit of Normal (ULN). Myopathy sometimes takes the form of rhabdomyolysis with or without acute renal failure secondary to myoglobinuria, and rare fatalities have occurred.

*Potent inhibitors of CYP3A4:* Simvastatin, like several other inhibitors of HMG-CoA reductase, is a substrate of cytochrome P450 3A4 (CYP3A4). When Simvastatin is
used with a potent inhibitor of CYP3A4, elevated plasma levels of HMG-CoA reductase inhibitory activity can increase the risk of myopathy and rhabdomyolysis, particularly with higher doses of simvastatin (Table 2T-6). The use of Simvastatin concomitantly with the potent CYP3A4 inhibitors should be avoided (Armitage, 2007; Harper et al, 2007; Jacobson, 2006).

2.2.1.11 Drug Interactions

Simvastatin is metabolized by CYP3A4 but has no CYP3A4 inhibitory activity; therefore it is not expected to affect the plasma concentrations of other drugs metabolized by CYP3A4. Potent inhibitors of CYP3A4 increase the risk of myopathy by reducing the elimination of Simvastatin. Below table shows drug interactions associated with increased risk of myopathy/ rhabdomyolysis, Table 2T-6, Drug information online, 2007; Drug bank, 2008; Neil, 2006.

Table 2T-6 Drug interactions associated with Increased Risk of Rhabdomyolysis

<table>
<thead>
<tr>
<th>Interacting Agents</th>
<th>Prescribing Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itraconazole, Ketoconazole, Erythromycin, Clarithromycin, Telithromycin, HIV protease inhibitors, Nefazodone</td>
<td>Avoid Simvastatin</td>
</tr>
<tr>
<td>Gemfibrozil, Cyclosporine, Danazol</td>
<td>Do not exceed 10 mg Simvastatin daily</td>
</tr>
<tr>
<td>Amiodarone, Verapamil</td>
<td>Do not exceed 20 mg Simvastatin daily</td>
</tr>
</tbody>
</table>

2.2.1.12 Other Drug Interactions

* Cyclosporine or Danazol: The risk of myopathy/rhabdomyolysis is increased by concomitant administration of cyclosporine or danazol particularly with higher doses of simvastatin (Drug information online, 2007; Neil, 2006).

* Amiodarone or Verapamil: The risk of myopathy/rhabdomyolysis is increased by concomitant administration of amiodarone or verapamil with higher doses of simvastatin.

* Propranolol: In healthy male volunteers there was a significant decrease in mean Cmax, but no change in AUC, for Simvastatin total and active inhibitors with concomitant administration of single doses of simvastatin and propranolol. The
clinical relevance of this finding is unclear. The pharmacokinetics of the enantiomers of propranolol was not affected (Drug information online, 2007; Neil, 2006).

**Digoxin:** Concomitant administration of a single dose of digoxin in healthy male volunteers receiving simvastatin resulted in a slight elevation (less than 0.3 ng/mL) in digoxin concentrations in plasma (as measured by a radioimmunoassay) compared to concomitant administration of placebo and digoxin. Patients taking digoxin should be monitored appropriately when simvastatin is initiated.

### 2.2.1.13 Therapeutic concentration

After administration of a single 40 mg dose of simvastatin the peak plasma concentration of active inhibitors was 10.3 μg (equivalent)/L and total inhibitors 34.5 μg (equivalent)/L, reached in 2.5 and 2.3 hrs, respectively (Pentikainen et al, 1992; Moffat et al, 2004).

### 2.2.1.14 Therapeutic dosage

The usual daily oral dose is 5-80 mg/day. The recommended usual starting dose is 20 to 40 mg once a day in the evening. For patients at high risk for a CHD event due to existing coronary heart disease, diabetes, peripheral vessel disease, history of stroke or other cerebrovascular disease, the recommended starting dose is 40 mg/day. Lipid determinations should be performed after 4 weeks of therapy and periodically thereafter (Moffat et al, 2004; Zocor, 2006; Neil, 2006).

### 2.2.1.15 Side/Adverse Effects

**Common Side Effects:** The most common side effects of simvastatin are headache, constipation, muscle aches and pains, diarrhoea, upper respiratory infection, indigestion, nausea bloating, stomach upset, gas, heartburn (Sweetman, 2005; Brown et al, 1980).

**Serious Side Effects:** Memory loss, depletion of CoQ10, allergic reaction: hives; difficulty breathing; swelling of your face, lips, tongue, or throat, muscle pain, tenderness, or weakness with fever or flu symptoms and dark colored urine (Whitaker, 2002; Neil, 2006).
Less common: Body aches or pain, Chills, Cough, difficulty in moving, ear congestion, fever, joint pain, loss of voice, nasal congestion, runny nose (Sweetman, 2005).

2.2.1.16 Analytical profile

Literature survey revealed that HPLC (Ochiai et al, 1997; Zhang et al, 2004; The United States Pharmacopoeia, 2004) electrophoresis and U.V spectroscopic (Wang et al, 2000) methods have been reported for the analysis of simvastatin. Some of the official methods for analysis and identification of simvastatin are discussed below:

Elemental analysis: Analysis for carbon and hydrogen gives values as, carbon- 71.79 and hydrogen-8.88.

Thin layer chromatography: Chromatographic plates (silica gel 60 F254) with a mobile phase 5:2:1 cyclohexane: chloroform: isopropanol solution (0.05% w/v butylated hydroxyl toluene). Visualization by under UV light or by spraying the developed plate with dilute methanolic sulphuric acid solution and application of heat. The Rf value of simvastatin is approx. 0.4 (Florey, 2008).

High performance liquid chromatographic systems: A variety of gradient isocratic reverse phase HPLC systems has been used to chromatograph simvastatin (Table 2T-7, Florey, 2008).

Table 2T-7 High performance liquid chromatographic systems for simvastatin

<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>Detection λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherisorb ODS</td>
<td>Acetonitrile: 0.025 M NaH₂PO₄</td>
<td>238 nm</td>
</tr>
<tr>
<td>Perkin elmer C-18R</td>
<td>Acetonitrile: 0.01% (v/v %) H₃PO₄ (50:50)</td>
<td>238 nm</td>
</tr>
<tr>
<td>Jones apex 1, C-18</td>
<td>0.025 M sod. Acetate: acetonitrile</td>
<td>238 nm</td>
</tr>
<tr>
<td>PRP</td>
<td>Aqueous ammonium phosphate pH= 6.1: acetonitrile</td>
<td>260 nm</td>
</tr>
<tr>
<td>Hypersil OSD</td>
<td>Acetonitrile: water (0.025 M NaH₂PO₄ pH=4.5) 60:40</td>
<td>238 nm</td>
</tr>
</tbody>
</table>
Gas chromatography: A sensitive and selective method for monitoring simvastatin in human plasma using derivatization and GC/MS with selected ion monitoring was described by (Takano et al, 1995; Florey, 2008).

Infrared Spectrum: Principal peaks at wavenumbers 3546 (free O-H stretch), 3011 (olefinic C-H stretch), 2969 (methyl C-H symmetric stretch), 1718 (Lactone C=O stretch), 1701 (Ester C=O stretch, associated), 1459 (methylene C-H symmetric bend), 1389 (Gem-dimethyl C-H bend) etc.

Ultraviolet Spectrum: The ultraviolet absorption spectrum of simvastatin is characterized by absorption maxima at 231, 238 and 247 nm with A1% l cm values of 516, 604 and 408 respectively. The absorption maxima at 238 nm is typical for a substitute diene chromophore (Florey, 2008).

2.2.2 Rosuvastatin

Rosuvastatin is a super statin, which can lower LDL-cholesterol and triglycerides more effectively than first generation statin drugs. Rosuvastatin is used to treat hypercholesterolemia and related conditions, and to prevent cardiovascular disease (Osson et al, 2002). Rosuvastatin is developed by shionogi company in 1996 for the once daily oral treatment of hyperlipidemia. It inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme that converts HMG-CoA to mevalonate a precursor of cholesterol and thereby checks the synthesis of cholesterol (Schachter, 2004; Sweetman, 2005; Chapman et al, 2002). It is sparingly soluble in water. Rosuvastatin is official in I.P. B.P, European pharmacopoeia and USP. The absolute bioavailability of rosuvastatin is approximately 20% (Rosenson, 2003).

2.2.2.1 Proprietary names/ Brand Names/Synonyms

Arvast; Crestor; Novastat; Razell; Roseday; Rosupil; Rosuvas; Rozavel; Rozucor; Turbovas (Drug bank, 2007).

2.2.2.2 Drug Category

Lipid metabolism regulator; Anticholesteremic agents; Antilipemic agents; HMG-CoA Reductase Inhibitors (Drug bank, 2007; McTaggart et al, 2001).
2.2.2.3 Physicochemical properties

Rosuvastatin is a white amorphous powder that is sparingly soluble in water and methanol, and slightly soluble in ethanol. Rosuvastatin is a hydrophilic compound with a partition coefficient (octanol/water) of 0.13 at pH of 7.0 (Hojjati et al, 2007; Chapman et al, 2002; McTaggart et al, 2001). The drug is officially listed in the 2004 United States Pharmacopoeia and the official method of its determination is high-performance liquid chromatography. The Physicochemical properties of rosuvastatin are given in Table 2T-8 (Hojjati et al, 2007; Drug bank, 2007; McTaggart et al, 2001).

Table 2T-8 Physicochemical properties of rosuvastatin

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White powder</td>
</tr>
<tr>
<td>Odour</td>
<td>Odourless</td>
</tr>
<tr>
<td>Melting Point</td>
<td>126-132°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>6 mg/ml</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{22}H_{25}FN_{3}O_{6}S</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>481.539 g/mol</td>
</tr>
<tr>
<td>LogP/Hydrophobicity</td>
<td>3.135</td>
</tr>
</tbody>
</table>

2.2.2.4 Chemistry

Rosuvastatin (ROSU) is chemically 7-[[4-(4-fluorophenyl)-6-(1-methylethyl)-2-(methyl-methylsulfonylamino)-pyrimidin-5-yl]-3, 5-dihydroxy-hept-6-enoic acid (Drug bank, 2007). Its structural formula is (Schachter, 2004; Hojjati et al, 2007) (Fig 2F-8):

Fig 2F-8. Structure of Rosuvastatin
2.2.2.5 Pharmacology and Mechanism of Action

Rosuvastatin, similar to all other hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA RIs), reduces cholesterol by blocking the actions of HMG-CoA reductase, which is an early and rate-limiting step in the cholesterol synthesis process. It is used to treat primary hypercholesterolemia and mixed dyslipidemia (Fredrickson types IIa and IIb). By blocking the action of this enzyme, the conversion of HMG-CoA to mevalonic acid is inhibited (Fig 2F-7) and the subsequent result is a decrease in cholesterol production in the hepatocytes (Meister, 2003). Compared to other HMG-CoA reductase, rosuvastatin possesses the highest bonding interactions with HMG-CoA reductase, resulting in the most potent inhibition of cholesterol synthesis.

Rosuvastatin is a hydrophilic molecule that is selective for hepatic cells. Hydrophilicity results in reduced diffusion into non-hepatic cells, thereby decreasing the potential for adverse effects (McTaggart et al, 2001; Reijneveld et al, 1996). Pravastatin is the only other HMG-CoA reductase with similar properties.

2.2.2.6 Pharmacokinetics

Rosuvastatin is administered orally following which, the active moiety, is rapidly absorbed, reaching peak plasma concentration 3 to 5 hours after dosing. Both peak concentration (Cmax) and area under the plasma concentration-time curve (AUC) increase in proportion to rosuvastatin dose (Crestor, 2003; Crouse, 2008). The absolute bioavailability of rosuvastatin is approximately 20% and there is no accumulation on repeated dosing (Meister, 2003; Crestor, 2003). Rosuvastatin may be given with or without food. Administration in the morning or evening did not affect the rate and extent of absorption neither the ability of rosuvastatin to reduce LDL-C. Rosuvastatin undergoes first pass extraction in the liver, which is the primary site of cholesterol synthesis and LDL-C clearance. The mean volume of distribution at steady-state of rosuvastatin is approximately 134 liters. Rosuvastatin is approximately 90% bound to plasma proteins, mostly albumin (Martin et al, 2000; Chapman et al, 2002; Crestor, 2003). This binding is reversible and independent of plasma concentrations. Rosuvastatin is not extensively metabolized with approximately 90% of a radio labeled dose recovered as the parent compound (Martin et al, 2000; Chapman et al, 2002). The major metabolite is N-desmethyl rosuvastatin, which is formed principally by cytochrome P450 2C9 (McTaggart et al, 2001), and in-vitro
studies has demonstrated to have approximately one-half the HMG-CoA reductase inhibitory activity of rosuvastatin.

Following an oral dose, rosuvastatin and its metabolites are primarily excreted in the faeces (90%) with the remainder being excreted in the urine (Martin et al, 2000; Chapman et al, 2002; Chong et al, 2002). Fecal recovery represents absorbed drug, metabolites in the bile, and unabsorbed drug. The elimination half-life \( t_{1/2} \) of rosuvastatin is approximately 19 hours and does not increase with increasing doses (Warwick et al, 2000; Crouse, 2008).

A population pharmacokinetic analysis revealed no clinically relevant differences in pharmacokinetics among Caucasian, Hispanic and Black or Afro-Caribbean groups. However, pharmacokinetic studies with rosuvastatin, including one conducted in North America, have demonstrated an approximate 2-fold elevation in median exposure (AUC and Cmax) in Asian subjects when compared with a Caucasian control group (Crestor, 2003). The pharmacokinetic profile of rosuvastatin is given in Table 2T-9 (Rosenson, 2003; Fujino et al, 1999; Schachter, 2004).

Table 2T-9 Pharmacokinetic properties of rosuvastatin

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>Hydrophilic</td>
</tr>
<tr>
<td>Protein binding</td>
<td>90%</td>
</tr>
<tr>
<td>Active metabolites</td>
<td>Minor</td>
</tr>
<tr>
<td>Half-life</td>
<td>19 h</td>
</tr>
<tr>
<td>Metabolism cytochrome &amp; isoform</td>
<td>Limited cyp450</td>
</tr>
<tr>
<td>Renal excretion</td>
<td>10%</td>
</tr>
<tr>
<td>Optimal time of dosing</td>
<td>Any time of day</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>20%</td>
</tr>
<tr>
<td>Effect of food</td>
<td>No effect</td>
</tr>
<tr>
<td>Renal excretion (%)</td>
<td>10</td>
</tr>
</tbody>
</table>

2.2.2.7 Indication

Rosuvastatin is used for the treatment of pure hypercholesterolemia and mixed hyperlipidaemia.
**2.2.2.8 Contraindications**

Rosuvastatin is contraindicated in case of:

*Hypersensitivity:* Hypersensitivity to any component of this medication.

*Liver Dysfunction:* Rosuvastatin is contraindicated in patients with active liver disease or with unexplained persistent elevations of serum transaminases (Culhane et al, 2005).

*Pregnancy and lactation:* Cholesterol and other products of cholesterol biosynthesis are essential components for foetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they may cause foetal harm when administered to pregnant women. Therefore, HMG-CoA reductase inhibitors are contraindicated during pregnancy and in nursing mothers (Culhane et al, 2005; Galatti et al, 2006; Drug bank, 2007).

*Myopathy/Rhabdomyolysis:* Rare cases of rhabdomyolysis with acute renal failure secondary to myoglobinuria have been reported with rosuvastatin and with other drugs in this class (Crestor, 2003; García-rodriguez et al, 2008).

**2.2.2.9 Drug Interactions**

Since there is minimal metabolism via the CYP isoenzyme system, the potential for drug-drug interactions with rosuvastatin is minimal (Kostapanos et al, 2010; Crestor, 2003; Davidson, 2007).

The potential is much less than with the other medications in this class, except for pravastatin, which is metabolized through sulfation. Isoenzymes of the CYP system do not extensively metabolize rosuvastatin, and CYP isoenzyme inhibitors, including erythromycin, itraconazole, and ketoconazole, do not substantially affect it. Below table (Table 2T-10) shows a complete list of all known drug interactions for rosuvastatin (Culhane et al, 2005; Andrus, 2004; García-rodríguez et al, 2008).
Table 2T-10 Drug and food interactions associated with rosuvastatin

<table>
<thead>
<tr>
<th>Drug or Food</th>
<th>Rosuvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potent CYP3A4 inhibitors</td>
<td>No significant interaction</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Increased level, max. 5 mg</td>
</tr>
<tr>
<td>Verapamil, diltiazem</td>
<td>NR</td>
</tr>
<tr>
<td>Niacin</td>
<td>NR</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>Increased level, max. 10 mg</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>NR</td>
</tr>
<tr>
<td>Cholestyramine, Colestipol</td>
<td>NR</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>NR</td>
</tr>
<tr>
<td>Digoxin</td>
<td>No significant interaction</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>NR</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Increased INR</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>NR</td>
</tr>
<tr>
<td>Cimetidine, Omeprazole</td>
<td>NR</td>
</tr>
<tr>
<td>Antacids</td>
<td>Decreased level, give 2 hrs after</td>
</tr>
</tbody>
</table>

Max. = maximum dose; NR = not reported, INR = international normalized ratio.

2.2.2.10 Other Drug Interactions

Cyclosporine: When rosuvastatin 10 mg was co-administered with cyclosporine in cardiac transplant patients, rosuvastatin mean cmax and mean AUC were increased 11-fold and 7-fold, respectively, compared with healthy volunteers. These increases are considered to be clinically significant and require special consideration in the dosing of rosuvastatin to patients taking concomitant cyclosporine (García-rodríguez et al, 2008; Drug information online, 2007).

Warfarin: Coadministration of rosuvastatin to patients on stable warfarin therapy resulted in clinically significant rises in INR (>4, baseline 2-3). In patients taking coumarin anticoagulants and rosuvastatin concomitantly, INR should be determined before starting rosuvastatin and frequently enough during early therapy to ensure that no significant alteration of INR occurs. Once a stable INR time has been documented, INR can be monitored at the intervals usually recommended for patients on coumarin anticoagulants. If the dose of rosuvastatin is changed, the same procedure should be repeated. Rosuvastatin therapy has not been associated with bleeding or with changes in INR in patients not taking anticoagulants (Drug bank, 2007; Crestor, 2003; Meister, 2003).
Gemfibrozil: Coadministration of a single rosuvastatin dose to healthy volunteers on gemfibrozil (600 mg twice daily) resulted in 2.2- and 1.9-fold, respectively, increase in mean cmax and mean AUC of rosuvastatin (Sweetman, 2005; Lennernas et al, 1997).

2.2.2.11 Therapeutic dosage and Administration

The FDA-approved dosage range of rosuvastatin is 5 to 40 mg daily, however the 40 mg dose should only be used in patients who do not reach their LDL-cholesterol goal with the 20 mg dosage. The recommended starting dose of rosuvastatin should be individualized to each patient, with an initial dose of 10 mg daily in most patients, administered with or without food. A 5 mg daily dose is recommended in patients 1) requiring less aggressive cholesterol reduction, 2) with renal impairment, 3) with concurrent use of cyclosporine, or 4) with predisposing risk factors for myopathy. In patients with higher LDL-cholesterol (>190 mg/dl) and in patients with homozygous familial hypercholesterolemia, the recommended starting dose of rosuvastatin is 20 mg (Meister, 2003; Sweetman, 2005; Lennernas et al, 1997).

2.2.2.12 Side/Adverse Effects

Some side effects with rosuvastatin, while occurring infrequently, are potentially serious. These include but are not limited to:

**Signs of liver damage:** Yellow eyes or skin (jaundice), upper-right abdominal pain, dark urine, elevated liver enzymes (found using a simple blood test), muscle pain, tenderness, or weakness, significant, unexplained changes in the amount of urine.

**Signs of pancreatitis:** Severe upper abdominal pain (stomach pain) accompanied by nausea and vomiting, memory loss.

**Signs of an allergic reaction:** Rash, itching, hives, wheezing or difficulty breathing, swelling of the mouth, tongue, or throat. Other adverse effects include Rhabdomyolysis with myoglobinuria and acute renal failure and myopathy (including myositis).
2.2.2.13 **Analytical profile**

A survey of literature showed few solid phase extraction using tandem MS, few LC-MS methods (Singh et al, 2005; Ravi et al, 2005), few UV-Visible spectrophotometric method (Gupta et al, 2009; Hojjati et al, 2007), few HPLC and one HPTLC method (Sane et al, 2005) for the estimation of rosuvastatin in pharmaceutical preparations and in biological fluids. Rosuvastatin is reported to be identified by following methods:

**Thin Layer Chromatography (TLC):** Sane et al. established TLC method for the estimation of rosuvastatin calcium in its bulk drug and pharmaceutical formulations. Aceclofenac was used as internal standard. The optimized mobile phase was toluene-methanol-ethyl acetate-formic acid, $6.0 + 1.0 + 3.0 + 0.1$ (v/v). Quantization was performed densitometrically at $A = 265$ nm (Sane et al, 2005).

**HPLC:** A high-performance liquid chromatography (HPLC) method was developed by Kumar et al. for the estimation of rosuvastatin in rat plasma. The assay procedure involved simple liquid-liquid extraction of rosuvastatin and internal standard (IS, ketoprofen) from a small plasma volume directly into acetonitrile. Mobile phase consisting of $0.05$ m formic acid and acetonitrile (55:45, v/v) was used. The detection of the analyte peak was achieved by monitoring the eluate using a UV detector set at 240 nm (Kumar et al, 2006).

While Sultana et al. developed and validated a high-performance liquid chromatographic method for the simultaneous determination and quantification of atenolol, rosuvastatin, spirinolactone, glibenclamide and naproxen sodium in bulk drugs, pharmaceutical formulations and in human plasma in the presence of internal standard (flurbiprofen). Solvent system used was methanol and water (80:20, v/v), with 235 nm UV detection (Sultana et al, 2008).

**LC-MS/MS:** Several LC-MS methods have been reported for the estimation of rosuvastatin and its metabolite in biological matrices. Amongst them the LC-MS/MS method used for the analysis of human plasma samples derived from clinical trials of Crestor tablets is the most sensitive one, with a limit of quantitation of 0.1 ng mL$^{-1}$. The method employed deuterated rosuvastatin as an internal standard. The sample purification and pre concentration were performed by solid phase extraction (SPE).
Chromatographic separation was performed with a mobile phase (methanol: 0.2% formic acid in water 70:30 v/v).

**HPTLC:** A high performance thin layer chromatography (HPTLC) method was developed for the separation and quantification of simvastatin, pravastatin sodium and rosuvastatin calcium in pharmaceutical dosage forms by Chaudhari et al. (Chaudhari et al, 2007). The stationary phase used was precoated silica gel 60F 254. The mobile phase used was a mixture of chloroform: methanol: toluene (6:2:2, v/v/v). All the drugs were extracted from the respective tablets using methanol. The percentage recoveries ranged from 100 to 101 for simvastatin, 98 to 101 for pravastatin sodium and 98 to 102 for rosuvastatin calcium (Chaudhari et al, 2007).

**UV:** Gupta et al. developed a UV-Visible spectrophotometric method in ultraviolet region for the determination of rosuvastatin in bulk and in pharmaceutical formulations. Results revealed that rosuvastatin exhibits absorption maxima at 244 nm with apparent molar absorptivity of 7.2345 x104 L/mol.cm in methanol (Gupta et al, 2009). While Hojjati et al. also used UV-Vis spectrophotometer for determining solubility of rosuvastatin at wavelength (λ) 236 using methanol/acetic acid as solvent system (Hojjati et al, 2007).

### 2.3 POLYMERS

Polymers are macromolecules consisting of multiple repeating units or monomer residues linked together usually by covalent linkages. End-groups are the structural units that terminate polymer chains. Three types of polymers were selected for present study belonging to each class of natural and synthetic polymers (Fig 2F-9).

![Fig 2F-9. Polymers selected for study](image-url)
2.3.1 Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan is structurally similar to glycosaminoglycans with a chemical formula of \((C_6H_{11}O_4N)_n\) (Hejazi et al, 2003), schematically represented in Figure 2F-10.

![Chemical structure of chitosan](image)

**Fig 2F-10. Chemical structure of chitosan (Kurita, 2001)**

2.3.1.1 Source and synthesis

Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc) Fig 2F-11.

![Synthesis of chitosan](image)

**Fig 2F-11. Synthesis of chitosan**

2.3.1.2 Biophysicalchemical properties of chitosan

Chitosan is a weak base with a pKa of about 6.2-7.0, and it requires a certain amount of acid to become soluble (Hamman et al, 2000). The word ‘chitosan’ refers to a large number of polymers, which differ in their degree of N-deacetylation (40-98%) and molecular weight (50,000–2,000,000Da). These two characteristics are very important to its physicochemical properties and may have a major effect on the biological properties. Pharmaceutical grade chitosan is deacetylated between 90 and 95% and food grade between 75 and 80% (Paul et al, 2000).
Table 2T-11 Chemical and biological properties of chitosan

<table>
<thead>
<tr>
<th>Chemical properties of chitosan</th>
<th>Biological properties of chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic polyamine</td>
<td>Biocompatible</td>
</tr>
<tr>
<td>High charge density at pH &lt; 6.5</td>
<td>Natural polymer</td>
</tr>
<tr>
<td>Adheres to negatively charged</td>
<td>Biodegradable to normal body</td>
</tr>
<tr>
<td>surfaces</td>
<td>constituents</td>
</tr>
<tr>
<td>Forms gels with polyanions</td>
<td>Safe and non-toxic</td>
</tr>
<tr>
<td>High molecular weight, linear</td>
<td>Hemostatic, bacteriostatic and fungistatic</td>
</tr>
<tr>
<td>polyelectrolyte</td>
<td></td>
</tr>
<tr>
<td>Viscosity, high to low</td>
<td>Spermicidal</td>
</tr>
<tr>
<td>Chelates certain transitional</td>
<td>Anti-cancerogen</td>
</tr>
<tr>
<td>metals</td>
<td></td>
</tr>
<tr>
<td>Amiable to chemical modification</td>
<td>Anti-cholesteremic</td>
</tr>
<tr>
<td>Reactive amino/hydroxyl groups</td>
<td>Versatile</td>
</tr>
</tbody>
</table>

Chitosan salts are soluble in water; the solubility depends on the degree of deacetylation and the pH of the solution. The pharmaceutical requirements of chitosan are: particle size < 30 μm, density between 1.35 and 1.40 g/cm³, pH 6.5-7.5, insoluble in water, and partially soluble in acids (Hejazi et al., 2003). The chemical and biological properties of chitosan are summarized in table 2T-11.

2.3.1.3 Applications

The intriguing properties of chitosan have been known for many years and this polycationic polymer (in acidic environments) has been used in the fields of agriculture, industry and medicine (Hamman et al., 2000). It is widely used in the management of wounds and burns (Muzzarelli, 1997). Chitosan oligosaccharide stimulates fibroblasts production by means of affecting fibroblasts growth factor (FGF). Thus, collagen production is stimulated as well as other components of connective tissue. The preparation promotes acceleration of the wound-healing process, and connective tissue gets an ordered structure. Chitosan oligosaccharide application prevents rough scar formation (Dodane et al., 1998). Due to its unique polymeric cationic character, gel and film forming properties, non-toxicity, biocompatibility and biodegradability, chitosan has been extensively examined in the pharmaceutical industry for its potential in the development of drug delivery systems.
It is presently considered as a novel carrier material in drug delivery systems (Dodane et al., 1998). Medical and pharmaceutical applications of chitosan include drug delivery, wound healing ointments and dressings, artificial skin, homeostatic agents, enzyme immobilization, dialysis membranes, contact lenses or eye bandages, orthopaedics, surgical sutures and dentistry (Paul et al., 2000). The ability of chitosan to enhance the paracellular transport of several peptide drugs, both \textit{in-vivo} and \textit{in-vitro}, is considered to be one of the most important pharmaceutical application of chitosan (Hamman et al., 2000; Paul et al., 2000).

2.3.2 Eudragit RS100

Polymethacrylates are synthetic cationic and anionic polymers of dimethylaminoethyl methacrylates, methacrylic acid, and methacrylic acid esters in varying ratios (Fig 2F-12). Eudragit RS100 is one such polymer belonging to this class. These are also referred to as ammonio methacrylate copolymers, consisting of fully polymerized copolymers of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups (5%). The ammonium groups are present as salts and give rise to pH-independent permeability of the polymers. Polymethacrylates are primarily used as film-coating agents to form water-insoluble film coats for sustained-release products (Lehmann et al., 1973, Lehmann, 1973). Depending on the type of polymer used, films of different solubility characteristics can be produced.

\begin{center}
\textbf{Fig 2F-12. Structure of Eudragit RS100}
\end{center}

where,

\[ R^1 = H, \text{CH}_3; \quad R^2 = \text{CH}_3, \text{C}_2\text{H}_5; \quad R^3 = \text{CH}_3, \]

\[ R^4 = \text{CH}_2\text{CH}_2\text{N} (\text{CH}_3)_3^+ \text{Cl}^- \]

2.3.2.1 Synthesis

Eudragit RS100 is prepared by the polymerization of acrylic and methacrylic acids or their esters, e.g. butyl ester or dimethylaminoethyl ester.
2.3.2.2 Physicochemical properties

Polymer is water-insoluble and films prepared from Eudragit RS are only slightly permeable to water. Solutions are colorless or slightly yellow in color, and may be clear or slightly turbid; they have an odor characteristic of the solvents. Solvent-free granules contain ≥97% of the dried weight content of the polymer (Burchman et al, 1976).

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 (Rohm Pharma, 2005). 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 (Gurny et al, 1997). Some important general properties of eudragit RS100 is presented in Table 2T-12.

<table>
<thead>
<tr>
<th>Viscosity</th>
<th>Ammonio methacrylate units</th>
<th>Limit of methyl methacrylate</th>
<th>Limit of ethyl acrylate</th>
<th>Polymer dry wt content</th>
<th>Solubility/permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15 mPa s</td>
<td>4.48-6.77%</td>
<td>≤0.005%</td>
<td>≤0.025%</td>
<td>97%</td>
<td>Low</td>
</tr>
</tbody>
</table>

2.3.2.3 Applications

Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical formulations (Okor et al, 1990). They are also used in topical formulations and are generally regarded as nontoxic and nonirritant materials. Included in the FDA Inactive Ingredients Guide (oral capsules and tablets), nonparenteral medicines licensed in the UK, Canadian List of Acceptable Non-medicinal Ingredients.
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