Chapter III

Drug & Polymer Profile

3.1 PIOGLITAZONE HCl

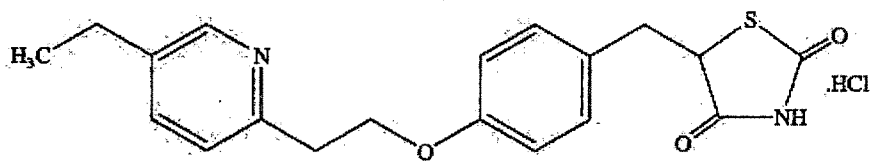
Pioglitazone hydrochloride is an oral antidiabetic agent that improves glycemic control by decreasing insulin resistance and reducing circulating insulin levels. It is used in the management of Type 2 diabetes mellitus (T2DM) (also known as non-insulin-dependent diabetes mellitus [NIDDM] or adult-onset diabetes). Pioglitazone received US-FDA approval on July 15, 1999.

IUPAC name : 5-[[4-[2-(5-ethylpyridin-2-l)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione

Molecular formula : $C_{19}H_{20}N_2O_3S \cdot HCl$

Molecular weight : 392.90 (Salt form), 356.44 (Base)

Structure :



Category : Antidiabetic

Appearance : Odorless, white, crystalline powder

Melting point : 183° C - 184° C

Solubility :

S.No.	Solvents	Solubility level#
1.	Water	Practically insoluble
2.	N,N-dimethylformamide	Soluble
3.	Ethanol (95%)	Slightly soluble
4.	Ether	Insoluble
5.	Acetone, Acetonitrile	Very slightly soluble
6.	Aq solutions of alkali hydroxides/carbonates	Soluble

#Descriptive terms for solubility

Descriptive terms	Parts of solvent for 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble, or insoluble	More than 10,000

3.1.1 MECHANISM OF ACTION

- Pioglitazone is a thiazolidinedione antidiabetic agent that depends on the presence of insulin for its mechanism of action. It selectively stimulates nuclear receptor peroxisone proliferator-activated receptor gamma (PPARγ) found in tissues important for insulin action such as adipose tissue, skeletal muscle, and liver. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the same tissues. As a result, pioglitazone reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose, insulin and glycated haemoglobin in the bloodstream.
- Although not clinically significant, pioglitazone decreases the level of triglycerides and increases that of high-density lipoproteins (HDL) without changing low-density lipoproteins (LDL) and total cholesterol in patients with disorders of the lipid metabolism.

- More recently, pioglitazone and other active TZDs have been shown to bind to the outer mitochondrial membrane protein mitoNEET with affinity comparable to that of pioglitazone for PPARγ (Colca et al., 2004; Paddock et al., 2007).

3.1.2 PHARMACOKINETICS

- Absorption is rapid after oral administration and in the fasting state, pioglitazone is first measurable in serum within 30 minutes, with peak concentrations observed within 2 hours (T_{max}). Food slightly delays the time to peak serum concentration to 3 to 4 hours, but does not alter the extent of absorption. Serum concentrations of total pioglitazone (pioglitazone plus active metabolites) remain elevated 24 hours after once daily dosing. Steady-state serum concentrations of both pioglitazone and total pioglitazone are achieved within 7 days. At steady-state, two of the pharmacologically active metabolites of pioglitazone, Metabolites III (M-III) and IV (M-IV), reach serum concentrations equal to or greater than pioglitazone. In both healthy volunteers and in patients with T2DM, pioglitazone comprises approximately 30% to 50% of the peak total pioglitazone serum concentrations and 20% to 25% of the total area under the serum concentration-time curve (AUC).
- Metabolism: Pioglitazone is extensively metabolized by hydroxylation and oxidation; the metabolites also partly converted to glucuronide or sulfate conjugates. Metabolites M-II and M-IV (hydroxy derivatives of pioglitazone) and M-III (keto derivative of pioglitazone) are pharmaco-logically active in animal models of T2DM. *In vitro* data demonstrate that multiple CYP isoforms (cytochrome P450 isoforms including CYP2C8, CYP3A4 and CYP1A1) are involved in the metabolism of pioglitazone.

3.1.3 PHARMACOKINETIC PARAMETERS

Oral availability	:	80%
Urinary Excretion	:	15 - 30%
Protein Binding	:	> 99%
Volume of Distribution	:	30.19 ± 13.06 L
Plasma half life	:	3 – 7 h

3.1.4 ADVERSE EFFECTS

- Upper respiratory tract infection, headache, sinusitis, myalgia, tooth disorder, may aggravate diabetes mellitus, pharyngitis, edema.
- A press release by GlaxoSmithKline in February 2007 noted that there is a greater incidence of fractures of the upper arms, hands and feet in female diabetics given rosiglitazone compared with those given metformin or glyburide (Belfort et al., 2006). The information was based on data from the ADOPT trial. Following release of this statement, Takeda also admitted that pioglitazone has similar implications for female patients (Reply letter to USFDA, 2007).

3.1.5 PRECAUTIONS

- Hypoglycemia, cardiovascular such as congestive heart failure, edema, weight gain, ovulation (in premenopausal anovulatory women), hematologic (cause decreases in hemoglobin and hematocrit).

3.1.6 THERAPEUTIC USES

- Pioglitazone is indicated as an adjunct to diet and exercise to improve glycemic control in patients with Type 2 diabetes. It is indicated for monotherapy. Pioglitazone can also be indicated for use in combination with

a sulfonylurea, metformin, or insulin when diet and exercise plus the single agent do not result in adequate glycemic control.

3.1.7 DOSE

- The dose of pioglitazone for oral administration is 15 mg, 30 mg, or 45 mg. Pioglitazone should be taken once daily without regard to meals. The management of antidiabetic therapy should be individualized by monitoring the response to therapy by evaluating HbA1c which is a better indicator of long-term glycemic control than FPG alone.

3.1.8 MARKETED PRODUCTS

The marketed dosage forms of Pioglitazone HCl are given in **Table 3.1.**

S.No.	Product	Manufacturer	Dosage Form
1.	Actos	Takeda	15 mg, 30 mg, 45 mg/Tablet
2.	P-Glitz	Cipla	15 mg, 30 mg/Tablet
3.	Opam	Wockhardt	15 mg, 30 mg/Tablet
4.	Path	Lupin	15 mg, 30 mg/Tablet
5.	Diavista	Dr. Reddy's	15 mg, 30 mg/Tablet
6.	Oglo	Panacea	15 mg, 30 mg, 45 mg/Tablet
7.	Glizone	Zydus	15 mg, 30 mg/Tablet
8.	Pioglar	Ranbaxy	15 mg, 30 mg/Tablet
9.	Pioglit	Sun	15 mg, 30 mg/Tablet
10.	Pioped	Torrent	1 mg, 2 mg/Tablet

Table 3.1: Proprietary medications of Pioglitazone HCl

3.1.9 METHODS OF ANALYSIS

Several methods are reported in the literature for estimation of pioglitazone in various working solutions and biological fluids. Some of them are described below:

(A) HPLC Methods:

1. Yamashita et al., 1996 developed a high-performance liquid chromatographic (HPLC) method for the simultaneous determination of pioglitazone and its metabolites (M-I to M-V) in human serum and urine for the pharmacokinetic studies of pioglitazone in clinical trials. The method involved the solid-phase and liquid-liquid extraction. The drug and its metabolites from urine with and without enzymatic hydrolysis using β-glucuronidase were extracted by liquid-liquid extraction. The compounds in the extract were analyzed using HPLC with UV detection at 269 nm. The detection limits of pioglitazone, M-I, M-II, M-III, M-IV and M-V in serum were 0.01-0.05 μg/ml, those in urine were 0.1-0.5 μg/ml, and those in urine after enzymatic hydrolysis were 0.3-0.5 μg/ml, respectively.
2. Zhong and Williams, 1996 developed a HPLC method for simultaneous determination of pioglitazone (U-72107) and its potential metabolites (M-1 to M-6) in human serum out of which M-3, M-4 & M-5 were pharmacologically active. The method involved a solid phase extraction (SPE) of pioglitazone, its metabolites, and the internal standard (U-92573) from serum using C18 SPE columns with an elution solvent of 0.5ml of acetonitrile-water (35:65, v/v). Separation of the eight analytes was achieved within 20 min using a reversed-phase Zorbax RX-C8 analytical column (250mm x 4.6mm i.d., 5μm particle size) with a mobile phase of acetonitrile-water (40:60, v/v) containing 3 ml acetic acid

per liter mobile phase (apparent pH 5.5). An UV detector operated at 269 nm was used when a linear response was observed from 0.02 to 2μg/ml for these analytes except for M-4 which was best fitted with a polynomial regression. Limit of quantitation was found to be 0.02μg/ml for pioglitazone, M-3, M-5 and M-6; 0.04 μg/ml for M-2 and M-4; and 0.5 μg/ml for M-1 when using a 0.5 ml serum sample for extraction. Obtained from the method validation, intra- and inter-assay precision was ≤ 9% and accuracy ranged from - 8.2 to 13.4% for all analytes.

3. Lin et at., 2003 developed a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method and validated for the simultaneous determination of pioglitazone (PIO) and its two pharmacologically active metabolites: M-III (keto-derivative) and M-IV (hydroxyl-derivative) in human plasma. Human plasma samples of 0.2 ml were extracted by a single step liquid-liquid extraction procedure and analyzed using a HPLC-electrospray tandem mass spectrometer system. The compounds were eluted isocratically on a C-18 column, ionized using a positive ion atmospheric pressure electrospray ionization source and analyzed using multiple reactions monitoring mode. The ion transitions monitored were m/z 357→134 for PIO, m/z 371→148 for M-III, m/z 373→150 for M-IV and m/z 413→178 for the internal standard. The chromatographic run time was 2.5 min per injection, with retention times of 1.45, 1.02 and 0.95 min for PIO, M-III and M-IV, respectively. The calibration curves of PIO, M-III and M-IV were well fit over the range of 0.5-2000 ng/ml (r^2>0.998759) by using a weighted ($1/x^2$) quadratic regression. The inter-day precisions of the quality control samples were ≤10.5% (N=15), coefficient of variation and the inter-day accuracy (%Nominal) ranged

from 84.6 to 103.5% for PIO, 94.4 to 104.0% for M-III, and 96.8 to 101.0% for M-IV. All three analytes demonstrated acceptable short-term, long-term, and freeze/thaw stability.

4. Xue et al., 2003 developed a simple, high throughput, direct-injection HPLC-tandem mass spectrometry method (LC/MS/MS) and validated it for the quantitation of pioglitazone in human serum. After mixing the internal standard with a sample, a 10 μL portion of the mixture was directly injected into a high-flow LC/MS/MS system, which included an extraction column, an analytical column and a six-port switching valve. The on-line extraction was achieved on an Oasis® HLB column (1 mm × 50 mm, 30μm) with a 100% aqueous loading mobile phase containing 5mM ammonium acetate (pH 4.0) at a flow rate of 4 ml/ min. The extracted analyte was eluted by a mobile phase which conta-ined 5mM ammonium acetate and acetonitrile. The analytical column was a Luna C18 column (4.6mm × 50 mm, 5μm). Detection was achieved by positive ion electrospray tandem mass spectrometry. The lower limit of quantitation of the method was 9 ng/ml. The standard curve, which ranged from 9 to 1350 ng/ml, was fitted by a weighted ($1/x^2$) quadratic regression model. The validation results demonstrated that this method had satisfactory precision and accuracy across the calibration range. There was no evidence of instability of the analyte in human serum following three freeze-thaw cycles, and samples could be stored for at least 2 weeks at −30°C.

5. Shrivastava et al., 2006 developed a simple, accurate, precise and economical HPLC method for estimation of pioglitazone HCl in tablet dosage form. To achieve the separation, the mixture of Acetonitrile : 10mM potassium dihydrogen phosphate buffer (pH 6), (50:50, v/v), pH

adjusted by potassium hydroxide was selected as mobile phase. This mixture was found to be appropriate allowing good separation of the pioglitazone HCl with a retention time 7.0±0.2 minute at flow rate of 1.5 ml/min and detection wavelength 226.0 nm. The linearity was found in the concentration range 10-70.0 g/ml. The correlation coefficient was found to be 0.999 indicating good linearity. The concentration of pioglitazone HCl was estimated in tablet dosage form was found to be in the range 99.06-101.32 %.

6. Sripalakit et al., 2006 developed an analytical method based on HPLC with ultraviolet detection at 269 nm for the determination of pioglitazone in human plasma. Rosiglitazone was used as an internal standard. Chromatographic separation was achieved with a reversed-phase Apollo C18 column and a mobile phase of methanol–acetonitrile-mixed phosphate buffer (pH 2.6; 10 mM) (40:12:48, v/v/v) with a flow rate of 1.2 ml/min. The calibration curve was linear over the range of 50–2000 ng/ml ($r^2 > 0.9987$) and the lower limit of quantification was 50ng/ml. The assay has been applied successfully to a pharmacokinetic study with human volunteers.

(B) UV-Vis Spectrophotometric Methods:

1. Mehta et al., 2005 developed two simple, accurate and economical spectrophotometric methods in ultraviolet and visible region for the determination of pioglitazone hydrochloride in bulk drug and in pharmaceutical formulation. In method A, pioglitazone hydrochloride showed λ_{max} at 269 nm in 0.2 N sulphuric acid, showing linearity in the concentration range of 10-60 µg/ml whereas in method B, pioglitazone hydrochloride was reacted with diazotized sulphanilic acid in an alkaline

medium. Yellowish orange coloured chromogen showed λ_{max} at 420 nm, showing linearity in the concentration range of 10-50 µg/ml.

2. Basniwal et al., 2008 developed and validated two simple, rapid and precise methods-linear regression equation (LRE) and standard absorptivity for the estimation of pioglitazone hydrochloride in tablet dosage form. The maximum absorbance (λ_{max}) of pioglitazone hydrochloride was found to be 269.8 nm in methanol : water : hydrochloric acid (250:250:1). Beer-Lambert law was obeyed in the concentration range of 10-50 µg/ml and the standard absorptivity was found to be 253.97 dl/g/cm. Pioglitazone hydrochloride was estimated in the range of 99.58-99.97% by LRE method and 100.25-100.75% by standard absop-tivity method.

3. Seedher and Kanojia, 2008 estimated few drugs using UV absorption spectroscopic technique. Due to the poor solubility of drugs in water/aqueous buffer, 0.1 M NaOH and 0.1 M HCl were used as solvents for drug analysis in the case of sulfonylureas (gliclazide, glyburide, glimepiride, glipizide) and glitazones (pioglitazone, rosiglitazone), respectively. Standard drug solutions in the appropriate solvent were prepared in the concentration range 10–100 µM and the ultraviolet absorption spectra were measured against solvent blank. Extinction coefficients in the relevant solvent, determined from the absorbance at wavelength corresponding to absorption maxima (λ_{max}) versus drug concentration plots, were used to calculate drug concentrations using Beer Lambert law. λ_{max} values used for drug analysis were 226, 228, 275, 280, 269, 317 nm in the case of giclazide and glyburide, glimepiride, glipizide, repaglinide, pioglitazone and rosiglitazone, respectively.

3.2 ROSIGLITAZONE MALEATE

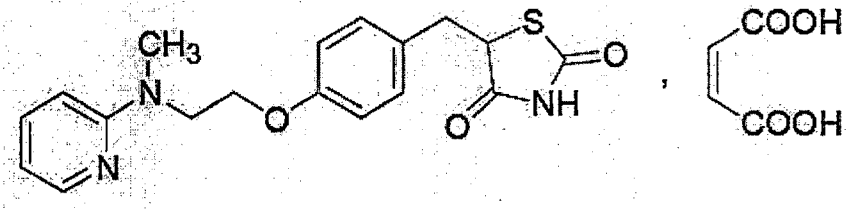
Rosiglitazone Maleate is a member of the thiazolidinedione class of antidiabetic agents and it improves glycemic control by improving insulin sensitivity. It is used in the management of type 2 diabetes mellitus (also known as non-insulin-dependent diabetes mellitus [NIDDM] or adult-onset diabetes). Rosiglitazone received US-FDA approval on May 25, 1999.

IUPAC name : 5-[[4-[2-(Methyl-2-pyridinylamino)ethoxy]phenyl] methyl]-2,4-thiazolidinedione

Molecular formula : $C_{18}H_{19}N_3O_3S.C_4H_4O_4$

Molecular weight : 473.50 (Maleate salt form), 357.43 (free base)

Structure :



Category : Antidiabetic.

Appearance : Odorless, white to off-white, crystalline powder.

Melting point : 122° C - 123° C (Salt form), 153° C - 155° C (Base)

Solubility :

S.No.	Solvents	Solubility level
1.	Water	Soluble
2.	Ethanol (95%)	Readily soluble
3.	Ether	Insoluble
4.	Acetone, Acetonitrile	Very slightly soluble
5.	Aq buffered solutions with pH of 2.3	Readily soluble

3.2.1 MECHANISM OF ACTION

- Rosiglitazone is also a thiazolidinedione antidiabetic agent which selectively stimulates nuclear receptor PPARγ found in adipose tissue, skeletal muscle and liver. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose production, transport & utilization and also lipid metabolism in the same tissues. As a result, rosiglitazone also reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose, insulin and glycated haemoglobin in the bloodstream.
- Pharmacological studies in animal models indicate that rosiglitazone inhibits hepatic gluconeogenesis. The expression of the insulin-regulated glucose transporter GLUT-4 was increased in adipose tissue. Rosiglitazone did not induce hypoglycemia in animal models of Type 2 diabetes and/or impaired glucose tolerance.

3.2.2 PHARMACOKINETICS

- **Absorption**: The absolute bioavailability of rosiglitazone is 99%. Peak plasma concentrations are observed about 1 hour after dosing. Plasma concentrations are approximately dose proportional over the therapeutic dose range. Administration of rosiglitazone with food results in no change in AUC, but the C_{max} decreases by approximately 28% and T_{max} delays by 1.75 hours compared to dosing in the fasting state. As these changes are not likely to be clinically significant; therefore, rosiglitazone may be administered with or without food. The absorption of rosiglitazone is not affected by increases in gastric pH.

- **Distribution:** The volume of distribution of rosiglitazone is approximately 14 litres in healthy volunteers. Plasma protein binding of rosiglitazone is high (approx 99.8%). Similarly the protein binding of its major metabolite (para-hydroxy-sulphate) is very high (>99.99%).
- **Metabolism:** Rosiglitazone is extensively metabolized with no unchanged drug excreted in the urine. The major routes of metabolism are N-demethylation and hydroxylation, followed by conjugation with sulfate and glucuronic acid. All the circulating metabolites are considerably less potent than parent drug and, therefore, are not expected to contribute to the insulin-sensitizing activity of rosiglitazone. *In vitro* data demonstrated that rosiglitazone is predominantly metabolized by Cytochrome P450 (CYP) isoenzyme 2C8, with CYP2C9 contributing as a minor pathway.
- **Elimination:** Total plasma clearance of rosiglitazone is around 3 L/h and the terminal elimination half-life of rosiglitazone is approximately 3 to 4 hours. The major route of excretion is the urine with approximately 64% of the dose being eliminated by this route, whereas faecal elimination accounts for approximately 25% of dose. No intact drug is excreted in urine or faeces. The terminal half-life for radioactivity was about 130 hours indicating that elimination of metabolites is very slow.

3.2.3 PHARMACOKINETIC PARAMETERS

Oral availability	:	99%
Urinary Excretion	:	15 - 30%
Protein Binding	:	99.8%
Volume of Distribution	:	17.6 L
Plasma half life	:	3 – 4 h

3.2.4 ADVERSE EFFECTS

- The adverse effects are similar to those of pioglitazone.
- A press release by GlaxoSmithKline in February 2007 noted that there is a greater incidence of fractures of the upper arms, hands and feet in female diabetics given rosiglitazone compared with those given metformin or glyburide (Belfort et al., 2006). The information was based on data from the ADOPT trial (Reply letter to USFDA, 2007).

3.2.5 PRECAUTIONS

- Hypoglycemia, cardiovascular such as congestive heart failure, edema, weight gain.
- The coadministration of rosiglitazone and insulin is not recommended.
- The total daily dose of rosiglitazone should not exceed 8 mg.

3.2.6 THERAPEUTIC USES

- Rosiglitazone is indicated as an adjunct to diet and exercise to improve glycemic control in patients with Type 2 diabetes mellitus. It is indicated for monotherapy in patients (particularly overweight patients) inadequately controlled by diet and exercise for whom metformin is inappropriate because of contraindications or intolerance.
- Rosiglitazone can be also indicated for use in combination with a sulfonylurea or metformin when diet and exercise plus the single agent do not result in adequate glycemic control.

3.2.7 DOSE

- Rosiglitazone therapy is usually initiated at 4 mg/day. This dose can be increased to 8 mg/day after eight weeks if greater glycaemic control is

required. In patients administered rosiglitazone in combination with a sulphonylurea, an increase in rosiglitazone to 8 mg/day should be undertaken cautiously following appropriate clinical evaluation to assess the patient's risk of developing adverse reactions relating to fluid retention. Rosiglitazone may be given once or twice a day and may be taken with or without food.

3.2.8 MARKETED PRODUCTS

The marketed dosage forms of Rosiglitazone Maleate are given in **Table 3.2.**

S. No.	Product	Manufacturer	Dosage Form
1.	Avandia	GlaxoSmithKline	2, 4, 8 mg/Tablet
2.	Enselin	Torrent	4, 8 mg/Tablet
3.	Reglit	Dr. Reddy's	2, 4, 8 mg/Tablet
4.	Rezult	Sun Pharma	2, 4, 8 mg/Tablet
5.	Roglin	Aristo	2, 8 mg/Tablet
6.	Rosicon	Glenmark	1, 2, 4 mg/Tablet
7.	Ross	Orchid	2, 4, 8 mg/Tablet

Table 3.2: Proprietary medications of Rosiglitazone Maleate

3.2.9 METHODS OF ANALYSIS

Several methods are reported in the literature for the estimation of rosiglitazone in various working solutions and biological fluids. Some of them are as follows:

(A) High performance liquid chromatography (HPLC)

1. Kolte et al., 2003 developed an HPLC method for the determination of rosiglitazone (I) in human plasma. Pioglitazone (II) was used as internal

standard. Both I and II were extracted from plasma using a liquid-liquid extraction procedure. Isocratic separation of I and II was carried out using a reversed-phase Zorbax SB C_{18}, 15-cm column with mobile phase consisting of methanol and a mixed phosphate buffer (10 mM monobasic sodium phosphate and dibasic sodium phosphate, pH adjusted to 2.6 with *ortho*-phosphoric acid) in the ratio 30:70 (v/v) and quantified by UV detection at 245 nm. Linearity was established over the range 5–1250 ng/ml using 1 ml human plasma. The method was specific as the endogenous components in plasma did not interfere with I and II. The recoveries of I and II from human plasma were about 79 and 60%, respectively.

2. Ho et al., 2004 described a convenient method for the separation and simultaneous detection of 10 anti-diabetic drugs at 10 ng/mL (namely glipizide, glibenclamide, glimepiride, gliclazide, tolazamide, tolbutamide, nateglinide, repaglinide, rosiglitazone and pioglitazone) in equine plasma and urine by LC–MS-MS. The anti-diabetics were isolated from equine plasma and urine by liquid–liquid extraction with 1,2-dichloroethane at acidic pH, and analysed by LC–MS-MS in the positive electrospray ionisation mode. Separation of 10 anti-diabetic drugs was achieved with a reversed phase C8 column using a mixture of aqueous ammonium formate (pH 3.0, 10 mM) and methanol as the mobile phase.

3. Pedersen et al., 2005 developed a fast and sensitive HPLC method for quantitative determination of rosiglitazone in human plasma. The extraction from plasma was performed using solid-phase extraction (SPE) on C4 silica (100 mg) disposable extraction cartridges (DEC). The separation of rosiglitazone and two metabolites was achieved on a Phenomenex® Synergi 4 µm MAX-RP (150 x 4.6 mm) column, protected by a guard column. The mobile

phase was 0.01 M ammonium acetate, pH 7.0 - acetonitrile (65:35, v/v). (3S)-3-OH-quinidine was used as internal standard. The analytes were detected using fluorescence detection. The limit of quantitation was 1 ng/mL and the detection limit was 0.25 ng/mL for rosiglitazone in human plasma. The recovery was 90% for rosiglitazone. Linearity was observed over a range of 1-1000 ng/mL (r^2=0.9959).

4. Walode et al., 2010 also developed a HPLC method for determination of rosiglitazone in its tablet dosage form using caffeine as an internal standard. It was performed on silica gel 60 GF_{254} thin layer chromatographic plates as a stationary phase using mobile phase methanol : toluene : chloroform : triethanolamine (1:8:0.5:0.5 v/v/v/v) and the detection was carried out in the absorbance mode at 264 nm showing Rf value 0.31 for rosiglitazone and 0.52 for caffeine. The linear regression data curve showed good linear relationship in the concentration range 1.0-7.0 µg/µl. The percent drug estimated of rosiglitazone from two different marketed formulations were found to be in the range 99.83-100.21. The recovery of drugs carried out by standard addition method was found to be 100.21±1.06 and 100.04±0.30 by height and area respectively.

(B) UV-Vis Spectrophotometric Methods:

1. Walash et al., 2009 developed two simple, sensitive and specific methods for the determination of rosiglitazone maleate (ROS) in pure form, pharmaceutical preparations, and biological fluids. Method I (spectrophotometry) was based on complex formation of (ROS) with both copper (II) chloride, and aluminum (III) chloride in borate buffer (pH 6.5). The absorbance of the formed complexes was measured at 318 nm. The absorbance-concentration plots were rectilinear over the concentration range

of 8-80 and 5-70 µg/ml, with detection limits of 1.98 and 1.32 µg/ml for Cu(II) and Al(III), respectively. Method II is based on the spectrofluorimetric determination of ROS through complex formation with Al^{+3} in acetate buffer of pH 5. The relative fluorescence intensity of the formed complex was twice that of the native fluorescence of the drug and was measured at 376 nm after excitation at 318 nm. The fluorescence intensity-concentration plot was rectilinear over the concentration range of 0.03-2.0 µg/ml with minimum quantification limit (LOQ) of 0.02 µg/ml and minimum limit of detection (LOD) of 0.01 µg/ml. Both methods were applied for the determination of ROS in its tablets. The results obtained were in good agreement with those obtained by the comparison method. Furthermore, method II was applied for the determination of ROS in spiked and real human plasma, and the mean % recoveries (n = 4) were 97.54 ±0.56, and 97.38±0.93 respectively. The stability of the formed complexes in both methods was studied, and the proposed methods were found to be stability indicating ones.

2. Dhakar et al., 2010 estimated rosiglitazone maleate using an UV spectrophotometric method. Solutions of rosiglitazone maleate were prepared in 0.1 N HCl and absorbance was measured on Shimadzu UV spectrophotometer at 228 nm. The method obeyed Beer's law in the range of 1-14 µg/ml and the regression coefficient was found to be 0.998.

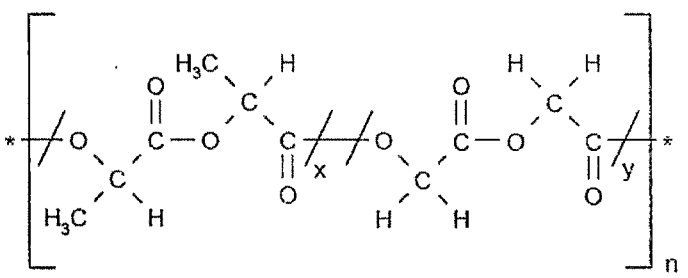
3. Jagathi et al., 2010 developed two simple and sensitive extractive spectrophotometric methods for the estimation of rosiglitazone in pure and pharmaceutical dosage forms. These methods were based on the formation of ion-pair complexes of the drug with basic dyes 3-Methyl-2-benzothiazolinone hydrazone (MBTH: λ_{max} 630 nm) and Bromocresol

green (BCG: λ_{max} 420 nm). The absorbance of the chloroform extracts was measured against the corresponding reagent blanks.

4. Jagathi et al., 2010 developed another two simple and sensitive extractive spectrophotometric methods for the estimation of rosiglitazone in pure and pharmaceutical dosage forms. These methods were based on the formation of ion-pair complexes of the drug with acidic dyes Solochrome black T (SBT: λ_{max} 510 nm) and Bromothymol Blue (BTB : λ_{max} 420 nm). The absorbance of the chloroform extracts was measured against the corresponding reagent blanks.

3.3 POLY (LACTIC-CO-GLYCOLIC ACID) (PLGA)

Nonproprietary Names	:	Poly(lactic-co-glycolic acid)
Functional Category	:	Bioabsorbable, Biocompatible, Biodegradable material
Trade Names	:	Purasorb PLG (PURAC, USA.) Medisorb 5050 DL (Alkermes Inc.) Resomer RG 503 H (Boehringer Ingelheim, Germany) Lactel 5050 DL PLGA (Birmingham Polymers Inc.)
Regulatory Status	:	GRAS listed by FDA.
Chemical Name	:	Poly (D,L-lactide-co-glycolide)
CAS Registry Number	:	26780-50-7
Material Number	:	60640801
Structural Formula	:	



$$-[(C_6H_8O_4)_x(C_4H_4O_4)_y]_n-$$

3.3.1 METHOD OF MANUFACTURE:

Generally, aliphatic polyesters like PLGA can be synthesized via polycondensation of hydrocarboxylic acids and catalytic ring-opening

polymerization of lactones. Ring opening polymerization is preferred because polyesters with high molecular weights can be produced. Moreover, the dehydration of hydrocarboxylic acids to form lactones does not have to be carried to a high degree of completion. Lactones can easily be purified owing to the differences of their physical and chemical properties from those of the corresponding hydrocarboxylic acid.

3.3.2 DESCRIPTION:

PLGA polymers are a group of synthesized copolymers of lactic and glycolic acid. They are nontoxic, and can be easily fabricated into variety of novel devices such as rods, screws, nails and cylinders. The polymers are available commercially in varying molecular weight and molecular ratio of lactic and glycolic acid. Co-monomer ratio of lactic acid and glycolic acid for PLGA ranges from 85:15 to 50:50.

3.3.3 TYPICAL PROPERTIES OF RESOMER® RG 503 H:

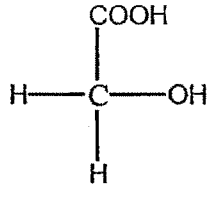
Description	:	White or nearly white amorphous powder.
Odor	:	Nearly odorless.
Inherent Viscosity	:	0.32-0.44 dl/g, 0.1% in chloroform, 25°C.
Molecular weight	:	33,000 g/mol
Glass Transition Temp	:	44 - 48 °C.
Solubility	:	Methylene chloride, ethyl acetate, acetone, tetrahydrofuran, chloroform.
Polymer composition	:	48:52 to 52:48 molar ratio of D, L lactide : glycolide.
Acid Number	:	3 mg KOH/g

Specific application : Resomer RG® 503 H is a non-end capped polymer and utilized for targeting via ligand conjugation.

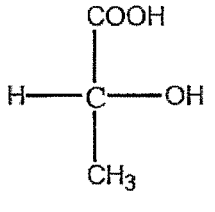
Polymer composition and crystallinity play important roles in the solubility of these aliphatic polyesters. The crystalline homopolymers of glycolic acid are soluble only in strong solvents like hexafluoroisopropanol. The crystalline homopolymer of lactic acid also do not have good solubility in most organic solvents. However, amorphous polymers of D, L-lactic acid and co-polymers of lactic acid and glycolic acid are soluble in many organic solvents.

3.3.4 PHYSICAL AND CHEMICAL PROPERTIES OF PLGA POLYMERS

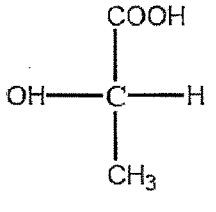
The structures of PLGA monomers, lactic and glycolic acids, are as follows:



Glycolic acid



D-lactic acid



L-lactic acid

Due to the presence of a chiral center in lactic acid, the following polymers can be produced: poly (glycolic acid) (PGA), poly (L-lactic acid) (P(L)LA), poly (D-lactic acid) (P(D)LA), poly (D,L-lactic acid) (P(D,L)LA), poly (D.L-lactic acid-co-glycolic acid) (P(D,L)LGA). In general, PLGA, PLA and PGA are block copolymers of lactic and/or glycolic acid (structures shown above), with the monomers linked by ester bonds. The aliphatic polyesters degrade in the fashion of bulk erosion (Shive et al., 1997). The most important feature of these polymers,

biodegradability, comes from its chemical structure-the ester linkage of polymer backbone, which can be hydrolyzed in the aqueous environment of body. The final hydrolytic products are monomers: glycolic acid and lactic acid. Both monomers enter the tricarboxylic acid cycle and can be eliminated from the body as carbon dioxide and water. Glycolic acid can also be excreted from the kidney in its original form (Wu, 1995). Another desirable feature of the class of PLGA polymers is that their physical and chemical properties can be manipulated by several key variables in polymer manufacturing: monomer composition (glycolic or D/L lactic acid), comonomer ratio, and molecular weight (M_w). The alteration in these variables directly causes the differences in the performance of these polymer devices. Lactic acid contains one more side methyl group and is more hydrophobic than glycolic acid. Therefore, the higher the content of lactide, the more hydrophobic is the polymer, the lower the water uptake and the slower the degradation rate. In addition, lactic acid in the polymer can be either in its optically active form (L) or as a racemate (D, L), which affects the crystallinity of the polymer. For example, poly (L-lactic acid) (PLLA) is semicrystalline. PGA is highly crystalline due to its highly stereoregular structure. In contrast, PLGA and poly (D, L-lactic acid) (PDLLA) are mostly amorphous (Cohen et al., 1994; Tice and Cowsar 1984). Besides hydrophobicity and crystallinity, M_w and polydispersity are also important molecular properties affecting the polymer performance. Several important bulk properties, including glass transition temperature, solubility in organic solvents, water uptake rate and biodegradation rate are closely related to the molecular properties of PLGA polymers. The first two variables are more relevant to the fabrication of PLGA devices, whereas water uptake and biodegradation rates are important in the performance of the polymer devices, specifically, drug release rate and stability issues. It is generally

well accepted that there is little enzyme-catalyzed degradation. The rate of PLGA degradation strongly depends on monomer composition, M_w and end group composition **(Table 3.3)**.

Table 3.3: The specific end groups in the polymer series generated by different chain length controllers.

Type	Chain length controller	End group	Comment
"H" (e.g. RG 502H)	hydroxycarboxylic acid	free carboxylic acid	For end group attachments
"S" (e.g. RG 752S)	alcohol	alkyl	-
" " (e.g. RG 502)	hydroxycarboxylic acid ester	alkyl ester	For low molecular weight polymers
" " (e.g. LR 708)	oligomer	free carboxylic acid (not detectable)	For high molecular weight polymers

An important issue regarding the application of PLGA as drug delivery system is polymer degradation behavior. Degradation of PLGA microspheres has been described as a three-stage process: water uptake, polymer degradation and erosion (Gopferich, 1996). The water uptake into PLGA devices initiates the polymer hydrolysis, and results in a gradual decrease in M_w and production of monomers and oligomers. The polymer hydrolysis can be autocatalyzed by these carboxylic acid end chain hydrolysis products. Random chain scission has been the expected hydrolysis mechanism, although recently chain-end scission was suggested by some researchers to be 10-fold higher than random scission (Shih, 1995). Weight loss or erosion occurs when degradation causes formation of pores through which oligomers and monomers can be released. During PLGA degradation, monomers and oligomers will accumulate in the polymer device.

This often results in an acidic microclimate in PLGA large dimension specimens and in microspheres (pH<4) (Shenderova et al., 1999).

3.3.5 STABILITY AND STORAGE CONDITIONS:

Aliphatic polyesters like PLGA are easily susceptible to hydrolysis in the presence of moisture. Hence they should be properly stored preferably refrigerated at around 4°C or below. It is necessary to allow the polymers to reach RT before opening the container. After original package has been opened, it is recommended to re-purge the package with high-purity nitrogen prior to resealing.

3.3.6 SAFETY:

PLGA and homopolymers of D,L-lactic acid and glycolic acid are used in parenteral pharmaceutical formulations and are regarded as biodegradable, biocompatible and bioabsorbable materials. Their biodegradation products are nontoxic, non-carcinogenic and non-teratogenic.

3.3.7 BIODEGRADATION AND TOXICITY OF PLGA:

The PLGA copolymers degrade in the body by hydrolytic cleavage of ester linkage to lactic and glycolic acid. These monomers are easily metabolized in the body via Kreb's cycle and eliminated as carbon dioxide and water. The degradation process of polymers both *in vivo* and *in vitro* is affected by several factors, including preparation method; the presence of low molecular weight compounds, size, shape and morphology; intrinsic properties of the polymer (molecular weight, chemical structure, hydrophobicity, crystallinity and glass transition temperature of the polymer); physiochemical parameters (pH, temperature and ionic strength of the environment); site of implantation and mechanism of hydrolysis.

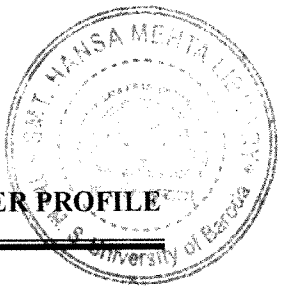
Bulk erosion is the main degradation pathway for PLGA copolymer. A three-phase mechanism for PLGA biodegradation has been proposed. Initially, a significant decrease in molecular weight of polymer is observed, with no appreciable weight loss and no soluble monomer products formed after random chain scission. This phase is followed by a decrease in molecular weight with rapid loss of mass and formation of soluble mono and oligomeric products. Finally, soluble monomer products are formed from soluble oligomer fragments, resulting in complete polymer degradation. PLGA copolymer biodegradation products are formed at a very slow rate and hence do not affect normal cell function.

3.3.8 HANDLING PRECAUTIONS:

Normal precautions appropriate to the circumstances and quantity of material handled should be observed. Contact with eyes, skin and clothing, and breathing the dust of the polymers should be avoided. PLGA produces acid materials such as hydroxyacetic acid 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.

3.3.9 APPLICATIONS IN PHARMACEUTICAL TECHNOLOGY:

Owing to their reputation as safe materials and their biodegradability, they are primarily used as biocompatible and biodegradable polymers for formulation of many types of implantable and injectable drug delivery systems for both human and veterinary use. Examples of implantable delivery system include rods, cyclinders, tubing, films, fibres, pellets and beads. Examples of injectable drug delivery systems include microcapsules, microspheres, nanoparticles and liquid injectable controlled release systems. These are currently being used in humans for resorbable sutures, bone implants and screws, and contraceptive implants.



REFERENCES:

- Basniwal, P.K., Srivastava, P.K., Jain, D. Spectrophotometric estimation of pioglitazone hydrochloride in tablet dasage form. *Asian J. Pharm.* 2008. 2(4), 225-227.
- Belfort, R., Harrison, S.A., Brown, K., Darland, C., Finch, J., Hardies, J., Balas, B., Gastaldelli, A., Tio, F., Pulcini, J., Berria, R., Ma, J.Z., Dwivedi, S., Havranek, R., Fincke, C., DeFronzo, R., Bannayan, G.A., Schenker, S., Cusi, K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N. Engl. J. Med.* 2006. 355(22), 2297–2307.
- Cohen, S., Alonso, M.J., Langer, R. Novel approaches to controlled-release antigen delivery. *Int. J. Tech. Assessment Health Care.* 1994. 10, 121-130.
- Colca, J.R., McDonald, W.G., Waldon, D.J., Leone, J.W., Lull, J.M., Bannow, C.A., Lund, E.T., Mathews, W.R. Identification of a novel mitochondrial protein ("mitoNEET") cross-linked specifically by a thiazolidinedione photoprobe. *Am. J. Physiol. Endocrinol. Metab.* 2004. 286(2), E252-60.
- Dhakar, R.C., Prajapati, S.K., Maurya, S.D., Gupta, A.K., Yadav, G.K., Dangi, G. Rosiglitazone maleate microspheres for extending drug release: Formulation and evaluation. *Int. J. Pharma. Res. & Dev.* 2010. 2(10), 56-65.
- Göpferich, A., Mechanisms of polymer degradation and erosion. *Biomaterials.* 1996. 17(2), 103-114.
- Ho, E.N.M., Yiu, K.C.H., Wan, T.S.M., Stewart, B.D., Watkins, K.L. Detection of anti-diabetics in equine plasma and urine by liquid chromatography–tandem mass spectrometry. *J. Chromatography B.* 2004. 811, 65–73.
- Jagathi, V., Rao, G.D., Praveen, P.S., Babu, C.H.M. Assay of rosiglitazone by extractive spectrophotometry. *Res. J. Pharm. Biol. Chem. Sci.* 2010. 1(4), 837-840.
- Jagathi, V., Rao, G.D., Praveen, P.S., Babu, C.H.M. Assay of Rosiglitazone by Visible Spectroscopy. *Int. J. Pharm. & Tech.* 2010. 2(3), 757-761.

- Kolte, B.L., Raut, B.B., Deo, A.A., Bagool, M.A., Shinde, D.B. Liquid chromatographic method for the determination of rosiglitazone in human plasma. *J. Chromatography B.* 2003. 788, 37-44.
- Lin, Z.J., Ji, W., Desai-Krieger, D., Shum, L. Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-MS/MS. *J. Pharm. & Biomed. Anal.* 2003. 33, 101-108.
- Mehta, R.S., Patel, D.M., Bhatt, K.K., Shankar, M.B. UV and Visible spectrophotometric Analysis of pioglitazone hydrochloride in bulk and tablets. *Ind. J. Pharm. Sci..* 2005. 67(4), 487-489.
- Paddock, M.L., Wiley, S.E., Axelrod, H.L., Cohen, A.E., Roy, M., Abresch, E.C., Capraro, D., Murphy, A.N., Nechushtai, R., Dixon, J.E., Jennings, P.A. MitoNEET is a uniquely folded 2Fe 2S outer mitochondrial membrane protein stabilized by pioglitazone. *Proc. Natl. Acad. Sci. U.S.A.* 2007. 104(36), 14342-7.
- Pedersen, R.S., Brøsen, K., Nielsen, F. HPLC Method for Determination of Rosiglitazone in Plasma. *Chromatographia.* 2005. 62, 197-201.
- Reply Letter to Healthcare Provider USFDA, from Robert Spanheimer, Senior Director, Diebetes, Metabolism, Medical and Scientific Affairs, Takeda Pharmaceuticals, North America, Inc. March 2007. [Cited 2011, May 31]. Available from :http://www.Drugs.com/fda/actos-pioglitazone-282.html
- Seedher, N. and Kanojia, M. Micellar Solubilization of Some Poorly Soluble Antidiabetic Drugs: A Technical Note. *AAPS Pharm. Sci. Tech.* 2008. 9(2), 431-6.
- Shenderova, A., Burke, T.G., Schwendeman, S.P. The acidic microclimate in poly (lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharm. Res.* 1999. 16, 241-248.
- Shih, C. Chain-end scission in acid catalyzed hydrolysis of poly (D,L-lactide) in solution. *J. Control. Rel.* 1995. 34, 9-15.
- Shive, M.S., Anderson, J.M. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv. Drug Del. Rev.* 1997. 28, 5-24.

- Shrivastava, P.K., Basniwal, P.K., Jain, D., Shrivastava, S.K. RP-HPLC method for estimation of pioglitazone hydrochloride in tablet formulation. *Asian J. Pharm.* 2006. 1(1), 55-59.
- Sripalakit, P., Neamhom, P., Saraphanchotiwitthaya, A. High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study. *J. Chromatography B.* 2006. 843, 164–169.
- Tice, T.R. and Cowsar, D.R. Biodegradable controlled-release parenteral systems. *Pharm. Tech.* 1984. 11, 26-35.
- Walash, M.I., El-Brashy, A., El-Enany, N., Kamel, M.K. Spectrofluorimetric and spectrophotometric determination of rosiglitazone maleate in pharmaceutical preparations and biological fluids. *Pharm. Chem. J.* 2009. 43(12), 697-709.
- Walode, S.G., Chaudhari, H.K., Saraswat, M.S., Kasture, A.V., Wadodkar, S.G. Validated high performance thin layer chromatographic determination and content uniformity test for rosiglitazone in tablets. *Ind. J. Pharm. Sci.* 2010. 72(2), 249-252.
- Wu, X.S. In: Encyclopedia Handbook of Biomaterials and Bioengineering. 1995. Eds. Wise. Marcel Dekker, New York, 1015-1054.
- www.drugbank.com accessed on 25-02-2008.
- www.drugs.com accessed on 26-05-2008.
- www.pioglitazone.com accessed on 22-02-2008.
- www.RxList.com accessed on 27-02-2008.
- www.wikipedia.com accessed on 22-02-2008.
- Xue, Y.J., Turner, K.C., Meeker, J.B., Pursley, J., Arnold, M., Unger, S. Quantitative determination of pioglitazone in human serum by direct-injection high-performance liquid chromatography mass spectrometry and its application to a bioequivalence study. *J. Chromatography B.* 2003. 795, 215–226.
- Yamashita, K., Murakami, H., Okuda, T., Motohashi, M. High-performance liquid chromatographic determination of pioglitazone and

its metabolites in human serum and urine. *J. Chromatography B.* 1996. 677, 141-146.

- Zhong, W.Z. and Williams, M.G. Simultaneous quantitation of pioglitazone and its metabolites in human serum by liquid chromatography and solid phase extraction. *J. Pharm. and Biomed. Anal.* 1996. 14, 465-473.