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FORMULATION AND EVALUATION OF SUSTAINED RELEASE MUCOADHESIVE MICROPARTICLES OF PIOGLITAZONE HCl

R.Aravind*,K.Tharun Kumar[1], Dr.T.Mangilal[2]

*Department of Pharmaceutics,Vijaya College of Pharmacy,Thorrur,Himayath Nagar,Hyderabad,Telangana,India.

[1]Mother Teresa College of Pharmacy, NFC Nagar,Ghatkesar, Hyderabad,Telangana,India.

[2] Professor, Geethanjali College of Pharmacy, Cheeryal,Keesara, Rangareddy-501301,Telanga,India.

Abstract:

Mucoadhesive microcapsules of pioglitazone were prepared using sodium alginate as a shell forming polymer and Carbapol 974, HPMC, Sodium CMC as a mucoadhesive polymer for the potential use of treating acute and chronic diabetes mellitus. Large spherical microcapsules with a coat consisting of sodium alginate and a mucoadhesive polymer could be prepared by orifi ce-ionic gelation process. The microcapsules exhibited good mucoadhesive properties and drug release from these mucoadhesive microcapsules was slow and extended over longer periods of time, depending on the composition of the coat. These mucoadhesive microcapsules are, thus, suitable for oral controlled release of pioglitazone.

Key words: Controlled release, microcapsules, mucoadhesive, orifi ce-ionic gelation method, pioglitazone, sodium alginate.

Corresponding Author
R.Aravind,
Department of Pharmaceutics,
Vijaya College of Pharmacy,Thorrur,
Himayath Nagar,Hyderabad,Telangana,India.
Email- aravind.pharmacy333@ gmail.com,**Mobile**-+919177694889.

INTRODUCTION:

Glucose is a simple sugar that provides the body with its primary source of energy. This type of sugar comes from digesting carbohydrates into a chemical that the body can easily convert to energy. When glucose levels in the bloodstream aren't properly regulated, a person can develop a serious condition, such as diabetes.

Normal glucose haemostatis is tightly regulated by three interrelated processes

1) Glucose production in the liver
2) Glucose uptake & utilization by peripheral tissues (skeletal muscle)
3) Actions of insulin & counter regulatory hormones (glucagon)

Insulin & glucagon the counter regulatory hormones are secreted by the corresponding cell types in the pancreatic islets (or) islets of langerhans, the endocrine tissue in the pancreas respectively the beta & alpha cells. Low blood glucose levels stimulate the secretion of glucagon from alpha cells of pancreatic islets resulting in the rise of blood glucose levels by glycogenolysis & gluconeogenesis pathways.

If blood glucose levels continuous to rise, high blood glucose levels inhibits the release of glucagon & stimulates the secretion of insulin by beta cells facilitating the diffusion of glucose into cells.

The principle metabolic function of insulin is to increase the rate of glucose transport into cells in the body & mostly in to the striated muscle cells& to a lesser extent adipocytes. Defects in insulin secretion, insulin action, or most commonly the both which leads to a condition known as hyperglycaemia.

The American diabetes association(ADA) recognizes four clinical classifications of diabetes as follows

a) Type-I (insulin dependent diabetes mellitus)
b) Type-II (non-insulin dependent diabetes mellitus)
c) Gestational diabetes
d) Diabetes due to other causes (e.g genetic defects or medications)

Although Hyperglycaemia acts as a common feature of all forms of diabetes mellitus, the underlying causes of hyperglycaemia, very widely the majority of cases of diabetes fall in to one of the following categories.

There are two types of diabetes mellitus

a) Insulin dependent (type1)
b) Non-insulin dependent (type 2)

Type- I is a disease characterised by the absolute deficiency of insulin secretion by beta cells, usually resulting from an auto immune attack and it constitutes about 10% of all diabetic cases.

Type- II diabetes it constitutes about 80-90% of all diabetes cases, is caused by a combination of peripheral resistance to insulin action& an adequate compensatory response of insulin secretion by pancreatic beta cells (relative insulin deficiency).

The incidence of diabetes is growing rapidly worldwide, it is estimated that more than 250 million people worldwide are afflicted with diabetes, and its prevalence is expected to exceed 350 million by the year 2030.

Since it is becoming a major problem of morbidity and mortality, its treatment options are at its peak importance.

Classification

1) Insulin secretagoges
   a) Sulfonyl ureas
   Ex: Glimiperide, Gliclazide, Glipizide
   b) Glinides
   Ex: Repaglinide, Nateglinide
2) Insulin sensitizers
   a) Biguanides
   Ex: Metformin, Phenformin
   c) Thiazolidinediones (glitazone)
   Ex: Rosiglitazone, Pioglitazone, Troglitazone
3) α- Glucosidase inhibitors
   Ex: Acarbose, Miglitol
4) Dipeptidyl peptidase-IV inhibitors
   Ex: Sitagliptin, Saxagliptin
5) Incretin mimetics
   Ex: Exenatide, Liraglutide

Mechanism of action

Thiazolidinediones are also referred to as (TZDs or glitazones). TZDs work by binding to the peroxisome proliferator-activated receptor-$_{\gamma}$ (PPAR-$_{\gamma}$), which are primarily located on fat cells and vascular cells. They enhance insulin sensitivity at muscle, liver & fat tissues indirectly, causing preadipocytes to differentiate into mature fat cells, which are more sensitive to insulin.

Muscle intracellular fat products, which contribute to the insulin resistance also decline by TZDs. TZDs also affect adipokines (eg: angiotensinogen, tissue necrosis factor α, interleukin-6) that can positively affect insulin sensitivity, thereby decreasing the insulin resistance.

Oral Drug Delivery

More than 50% of the pharmaceutical preparations in the market are for the oral administration. One of the most feasible approaches for achieving desired plasma profile of drugs in the GI tract is possible by oral route. This can be achieved by controlling the gastric residence time (GRT) by various mechanism. Dosage forms with a prolonged GRT i.e., gastro retentive dosage forms(GRDFs) will provide us with new and important therapeutic options.

Gastroretentive systems remain in the gastric region for several hours & hence significantly prolong the gastric residence time of drugs. Prolonged gastric

retension improves bioavailability, reduces drug waste and improves solubility of drugs that are less soluble in a high pH environment. It also has applications for local drug delivery to the stomach & proximal small intestine.

In the delivery of sparingly soluble & insoluble drugs, it is known that as the solubility of a drug decreases the time available for drug dissolution becomes less adequate & thus the transit time becomes a significant factor affecting drug absorption.

To override the problem, erodible gastroretentive dosage forms have been developed that provide continuous administration of these drugs at the absorption site by delivering the drugs incorporated in to the carriers such as liposomes, nanoparticles, protenoid microspheres & pharmacosomes etc.

Bioadhesive / mucoadhesive Microparticles for oral drug delivery

The term mucoadhesion can be defined as adhesion of a synthetic or natural substrate generally the polymer substrate to the mucus membrane. When adhesion occurs in a biological setting it is often termed bioadhession, further more if this adhesion occurs on mucosal membrane it is termed as mucoadhesion.

Microparticles include microspheres & microcapsules (having a core on the drug) of 1- 1000nm in diameter respectively. Microparticles in general have the potential to be used for targeted & sustained release drug delivery generally due to a high surface to volume ratio. Coupling of mucoadhesive properties to microparticles has additional advantages e.g. efficient absorption & enhanced bioavailability of the drugs, also provides an intimate contact with the mucus layer.

Mucoadhesive microparticles can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity, urinary & gastro intestinal tract, thus offering the possibilities of localized as well as systemic controlled release of the drugs.

Mucoadhesion is a complex phenomenon which involves various mechanisms using wide variety of mucoadhesive polymers. These mechanisms include mechanical inter locking, electrostatic interactions, diffusion interpenetration, adsorption and fracture processes. The mucoadhesive polymers provide adhesion between the drug delivery system & mucus or the epithelial cell surface. They have the advantage of not being absorbed & therefore would not+ be expected to display systemic toxicity.

There are two broad classes of mucoadhesive polymers, hydrophilic polymers & hydrogels.

The hydrophilic polymers those containing carboxylic group, exhibit the best mucoadhesive properties, polyvinyl pyrrolidone (PVP) , methyl cellulose(MC) , sodium carboxy methyl cellulose(SCMC), hydroxy propyl cellulose(HPC) and other cellulose derivatives .

Hydrogels are the class of polymeric biomaterial that exhibit the basic characteristics of an hydrogel to swell by absorbing water, interacting by means of adhesion with the mucus that covers epithelia.

Anionic group – carbopol, sodium alginate, SCMC, tragacanth, polyacrylates

Cationic group – chitosan, gelatin

Neutral group – eudragit, HPMC

Mucoadhesive polymers when used as drug carriers, may achieve

a. An increased residence time with in the GI tract.
b. An intensified contact between the mucosa and the drug.
c. Increased drug concentration at the site of deposition.
d. Facilated drug permeation through the mucosa.
e. There is therefore the possibility of increased drug bioavailability

Utilising mucoadhesive polymers in the form of microspheres provides protection to the incorporated drugs and due to their sustained drug release, may also result in desirable blood concentration profiles.

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and having a short half life are eliminated quickly from the blood circulation. To avoid this problem, the oral sustained release formulations have been developed as this will release the drug slowly into the GIT and maintain a constant drug concentration in the serum for a longer period of time. Such oral drug delivery devices have a restriction due to the gastric retention time (GRT) a physiological limitation.

Need for Sustained Drug Delivery

The basic rationale for the development of controlled release (CR) drug delivery is to modulate the magnitude and duration of drug actions. While the pharmacokinetic consequences of controlled release dosage forms are generally well taken into consideration, the pharmacodynamic aspects (like the relationship between drug concentration and magnitude of pharmacologic effect) that relate the required drug input rate for the optimal therapeutic response are less considered. This has become a major limitation for the application of controlled drug delivery. In such cases the development of sustained release (SR) dosage forms become empirical.

The sustained release mode of drug delivery has certain features that have an important impact and sole objectives on the magnitude of the pharmacologic response, i.e.,-

i) It minimizes the fluctuations in the blood plasma concentrations of the drug, i.e., between peak and trough
ii) It produces a slow input rate which tends to minimize the body's counteraction to drug's intervening effect on the regulated physiological processes
iii) It provides a continuous mode of the drug administration.

The population of patients with chronic disease or complications of other disease has been recently increasing. These situations necessitate taking drug for long period and or multiple medicines simultaneously, which can lead to increase in non-compliance. The problem would be worse for drugs with short biological half life. One method to solve such problem is to find a dosage form capable of releasing the drug gradually. One such dosage forms that releases the drug in a gradual manner is the sustained release dosage forms.

Oral sustained release drug delivery system is an oral delivery system that provides continuous release of drugs with predictable and reproducible kinetics for a predetermined period of time. A sustained release drug delivery system is usually designed to deliver the drug at a particular rate for required time duration. The rate of drug release is based on the desired therapeutic concentration and pharmacokinetic profile of the selected drug.

Pioglitazone HCl is a thiazolidinediones, which belongining to category of insulin sensitizer act by binding to the peroxisome proliferator activated receptor- γ (PPAR γ), which are primarily located on fat cells and vascular cells, they enhance insulin sensitivity at muscle , liver and fat tissues

The drug has the half- life of 3- 4hours. The general dosing of Pioglitazone HCl is initially once daily 15 mg or 30mg, not more than 45mg/day. After 8 -12 weeks of monotherapy dose will be doubled and plasma level also doubles.

The absorption site of the drug is stomach and it has a bioavailability of about 80%, which can be increased by increasing the gastric residence time of the drug. One of the approaches for improving the gastric residence is formulation of the corresponding drug as bioadhesive or the mucoadhesive microparticles.

The purpose of the present study is to prepare sustained release Pioglitazone HCl loaded mucoadhesive microparticles for the gastric delivery, where these microparticles can entrap the drug in sufficient amounts and also can successfully deliver the drug in stomach for prolonged duration of time.

MATERIALS METHODS:

List of Materials

Materials	Source
Pioglitazone HCl	Phaarmasia ltd ,hyd
Chitosan	Himedia biosciences
HPMC 5cps	Yarrow chemicals, Mumbai
Eudragit RS100	Evonik industries, Mumbai
Eudragit RL100	Evonik industries, Mumbai
Gelatin	Yarrow chemicals, Mumbai
Glacial acetic acid	Phaarmasia ltd ,hyd
Acetone	Phaarmasia ltd ,hyd
Glutaraldehyde	Merck
Propylene glycol	Phaarmasia ltd ,hyd
Hydrochloric acid	Phaarmasia ltd ,hyd,
Sodium chloride	Phaarmasia ltd ,hyd

Equipment's

List of Equipment's

Equipment's	Model/ Company
UV-Visible spectrophotometer	Spectrophotometer UV-1700, Shimadzu
Electronic analytical balance	AUX-220, Shimadzu
Fourier transform infrared spectrophotometer	Tensor-27, Bruker
Magnetic stirrer	Servewell instruments
Digital pH meter	Eutech Instruments
USP Dissolution apparatus	TDT-08L, Electrolab; Lab India DS 8000.

Pre-Formulation Study

Pre-formulation study is one of the important prerequisite in development of any drug delivery system. Thus, a preformulation study was carried out to check the compatibility between drug and selected polymers and development of analytical method of drug.

Melting point Determination

Melting point of drug was determined by using capillary tube method. In which the pure drug is placed in a capillary tube which was fused at one end and placed in a digital melting point apparatus and noted down the temperature at which drug starts melting**.**

Identification λ max of Pioglitazone Hcl by UV Spectrum [44]

Pioglitazone HCl was dissolved in pH 1.2 and the solution was analyzed in the range of 200-400nm using shimadzu 1700 UV-Visible spectrophotometer, to fix the wavelength maxima.

Compatibility Studies

Compatibility studies were carried out using FTIR spectrophotometry.

FTIR has been used to quantify the interaction between the drug and the carrier used in formulation. Spectra were recorded for pure drug and for drug and polymer (1:1) physical mixture, on bruker tensor-27 spectrophotometer.

Preparation of Standard Curve of Pioglitazone Hcl [44]

An accurate known weight of Pioglitazone HCl was dissolved in 1.2 pH and the concentrations of 15, 20, 25, 30, 35, 40µg/ml were prepared. Absorbance of these solutions was measured against a blank solution of 1.2 pH at 269nm. Regression coefficient (r^2) value and slope value were determined.

Procedure [36, 37,38, 42,45]

Preparation of chitosan Solution

Weigh the given amount of chitosan and add it slowly into the 1% glacial acetic acid with continuous stirring at a slow speed by using magnetic stirrer and stirring continued for 3-5 h to get clear solution.

Preparation of sodium tripolyphosphate solution

Weighed amount of sodium tripolyphosphate added into the water.

Preparation of Beads

The bubble free chitosan solution slowly squeezed with the help of syringe and added drop wise to Tpp solution under continuous stirring at fixed speed. The height of syringe from the surface of Tpp solution maintained at 5cm beads were taken out after 30 min of cross linking procedure.

Drying Procedure

The resulting beads were filtered using whattman filter paper and then it is spread on petridish, dried at room temperature for 24hrs.

Chitosan Eudragit RL100 microparticles

Given quantity of RL100 dissolved in 5ml of acetone with continuous stirring and then this clear solution mixed with chitosan solution with stirring.

Preparation of Chitosan RL100 microparticles

The bubble free RL100 chitosan solution slowly squeezed with the help of syringe and added drop wise to Tpp solution under continuous stirring at fixed speed. The height of syringe from the surface Tpp solution maintained at 10 cm particles were taken out after 30 min of cross linking procedure.

Chitosan Eudragit RS100 microparticles

Given quantity of RS100 dissolved in 5ml of acetone with continuous stirring and then this clear solution mixed with chitosan solution (1%) with stirring.

Chitosan HPMC 5cps microparticles

Given quantity of HPMC 5cps dissolved in 5ml of water with continuous stirring and then this clear solution mixed with chitosan solution (1%) with stirring.

Chitosan Gelatin microparticles

Given quantity of gelatin dissolved in the chitosan solution (1%) with stirring.

Formulation chart of sustained release mucoadhesive microparticles

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7
DRUG	30	30	30	30	30	30	30
CHITOSAN	500	166	166	166	250	200	166
HPMC5cps	__	333	__	__	__	__	__
EUD RS 100	__	__	333	__	__	__	__
EUD RL 100	__	__	__	333	__	__	__
GELATIN	__	__	__	__	250	300	333
PROPYLENE GLYCOL	2ml	2ml	2ml	2ml	2ml	2ml	2ml
1% GAA	48ml	16ml	16ml	16ml	23ml	18ml	14ml
ACETONE	__	__	32ml	32ml	__	__	__
WATER	__	32ml	__	__	__	__	__

Evaluation of Formulation

Drug Entrapment efficiency (%) [25,30,36,42,45]

Drug entrapment efficiency (DEE) of sustained release mucoadhesive microparticles of Pioglitazone HCl was estimated by a UV- spectrophotometric method.

An accurate weight 100 mg of microparticles was suspended in 30ml of pH 1.2 buffer. The resulting solution was kept shaking for overnight in the mechanical shaker. The solution was filtered using Whattmann filter paper and the filtrate was analyzed for Pioglitazone HCl content at 269 nm using UV-Visible spectrophotometer. The amount of Pioglitazone HCl present in the microparticles was obtained and the drug entrapment efficiency was determined using following formula.

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Swelling Studies [38]

Microparticles were studied for swelling characteristics. Sample from drug loaded microparticles were taken, weighed and placed in basket of USP dissolution apparatus I. The basket containing particles was put in a beaker containing 900 ml of pH 1.2 buffer maintained at 37°C. The particles were periodically removed at predetermined intervals and weighed. Then the swelling index was calculated as per the following formula.

$$\text{Swelling Index} = \frac{\text{Final- Initial}}{\text{Final}} \times 100$$

In-Vitro Drug Release Study [25,30,37]

In vitro drug release studies were carried out in USP type I dissolution test apparatus. Microparticles equivalent to 30 mg of pure drug were used for dissolution study. Microparticles containing active ingredient was placed in 900 ml of dissolution medium maintained at 37 ± 0.5° C. Dissolution test was carried out in pH 1.2 buffer for 24h. The speed of the basket was maintained at 50 rpm. 5 ml of the aliquot was withdrawn at predetermined intervals. The solution was analyzed for the drug content spectrophotometrically at 269 nm against blank medium. Equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The percentage drug release was calculated and plotted against function of time to study the pattern of drug release.

In- Vitro Mucoadhesive Test [24,25,28,30]

The time taken for detachment of microparticles from the goat stomach mucosa was measured in buffer pH 1.2. This is measured by an *in vitro* adhesion testing method known as wash off method. The small rectangular piece of mucosa is mounted on to the glass slide using cyanoacrylate glue. Microparticles were spread on the mucosa and then it is hung to the USP tablet disintegration test apparatus at an angle of 45˚. The cell containing glass slide is attached to the outer assembly which is previously warmed to 37±0.5˚c, the apparatus is operated giving the tissue specimen regular up and down movement within a beaker of disintegration apparatus containing simulated gastric fluid. The number of microparticles still adhereing on to the tissue were counted at hourly interval up to 12h.

$$\% \text{ Mucoadhesion} = \frac{\text{number of microparticles remining}}{\text{number of microparticles applied}} * 100$$

Kinetic Modeling of drug Dissolution Profiles [46,47,48]

To analyze the drug release rate kinetics and mechanism of drug release from microparticles, the *in vitro* dissolution studies data were fitted into Zero order, First order, Higuchi and Korsemeyer-Peppas models. Kinetic studies involve comparing the regression (R) values obtained to select the best-fit model.

a. Zero order release kinetics

Zero order release equation is given as

$$Q = Q_0 + K_0 t$$

Where Q is the amount of drug released or dissolved, Q0 is the initial amount of drug

in solution and K0 is the zero order release constant. P lot was made between

cumulative percentage drug release and time.

First order release kinetics

First order equation is given as

$$\log C = \log C_o - kt/2.303$$

Where, Co is the initial concentration of drug and k is the order constant. Plot was made between log cumulative percentage drug release remaining and time.

c. Higuchi model

Higuchi model was developed on the basis of Fick‟s law and it describes the fraction of drug release from a matrix is proportional to square root of time as given below

$$Q_t = K_H \sqrt{t}$$

Where „K_H‟ is the Higuchi‟s rate constant, and „Q_t‟ is the amount of drug released at time„t‟. Plot was made between cumulative percentage drug release and square root of time.

d. Korsmeyer-Peppas model

It describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation.

$M_t/M_\infty = Kt^n$

where M_t/M_∞ corresponds to the amount of drug released at time „t" and after an infinite time, „K" is a constant comprising the structural and geometric characteristics of the tablet. Plot was made between log cumulative percentage drug release and log time. Peppas used „n" value in order to characterize different release mechanisms.

Release mechanisms based on "n" value

n value	Release mechanism
< 0.5	Fickian diffusion
0.5<n<1	Non Fickian (anamolous transport)
1	Zero order, case-II transport
>1	Super case- II transport

Stability Studies of the Most Satisfactory Formulation [49]

The most satisfactory formulation (optimized formulation) were subjected for stability studies. Capsule is packed in aluminum foil and kept in humidity chamber maintained at 30±2°C/65±5% RH for two months. Samples were withdrawn at the interval of one month and were analyzed for the drug entrapment efficiency (%) and *in vitro* dissolution. The shelf life of the product was analyzed by fitting into exponential form of first order equation.

First order kinetics[47]

First order equation is given by $C=C_0.e^{kt}$

Where C_0 is the initial concentration of the drug, C is the concentration of drug at time

"t".

In logarithm form it is given as

$\log C = \log C_0 - kt/2.303$

$k = \frac{2.303}{t} \log \frac{C_0}{C}$

Half life of drug ($t_{50\%}$) = 0.693/ k

Shelf life of the product ($t_{90\%}$) = 0.1052/k

Results

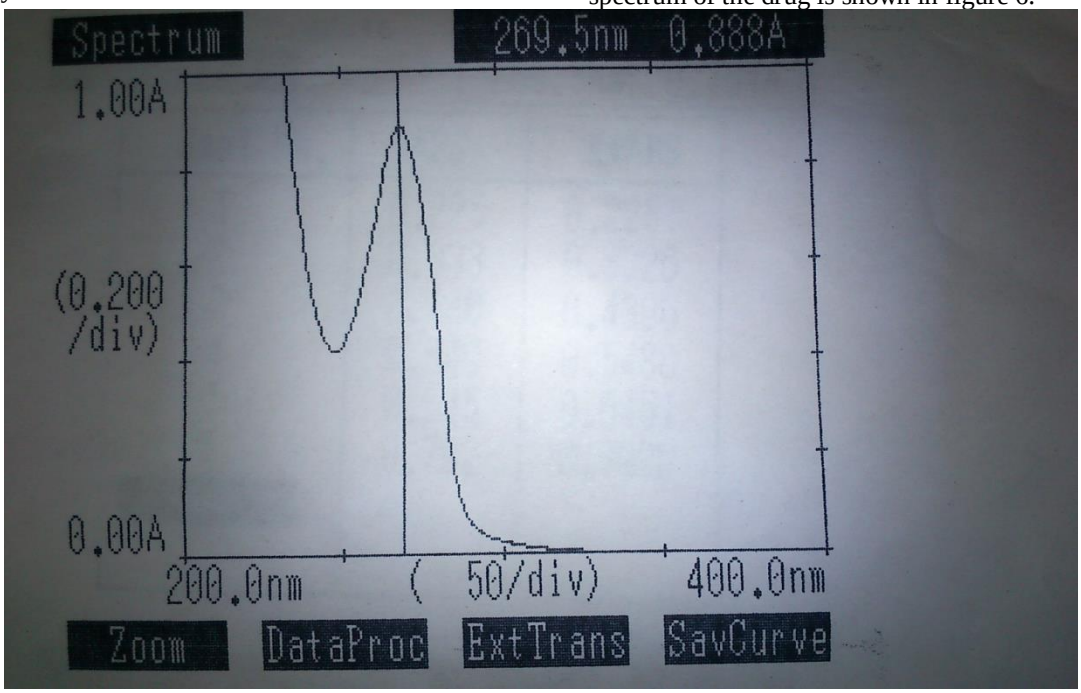
Preformulation Study

Melting Point

Melting point of Pioglitazone HCl was found to be 189 ±1°C (n=3) which is close to the actual melting point of 188- 192°C.

Identification of Pioglitazone HCl By UV Spectrum

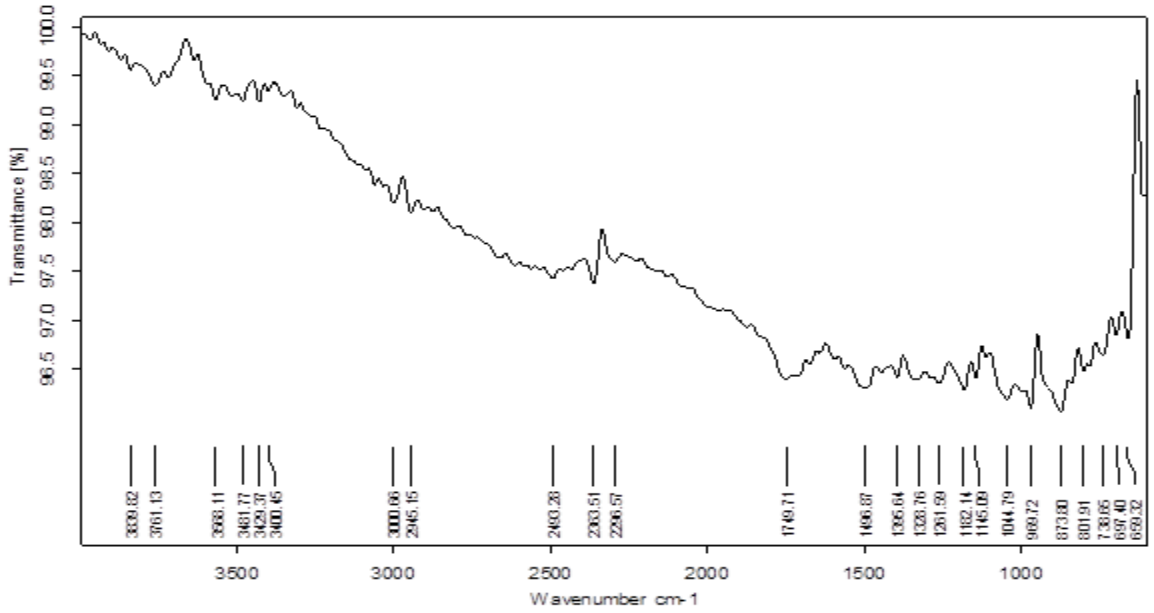
λmax of the drug was found to be at 269.5nm, UV spectrum of the drug is shown in figure 6.



UV Spectrum of Pioglitazone Hcl

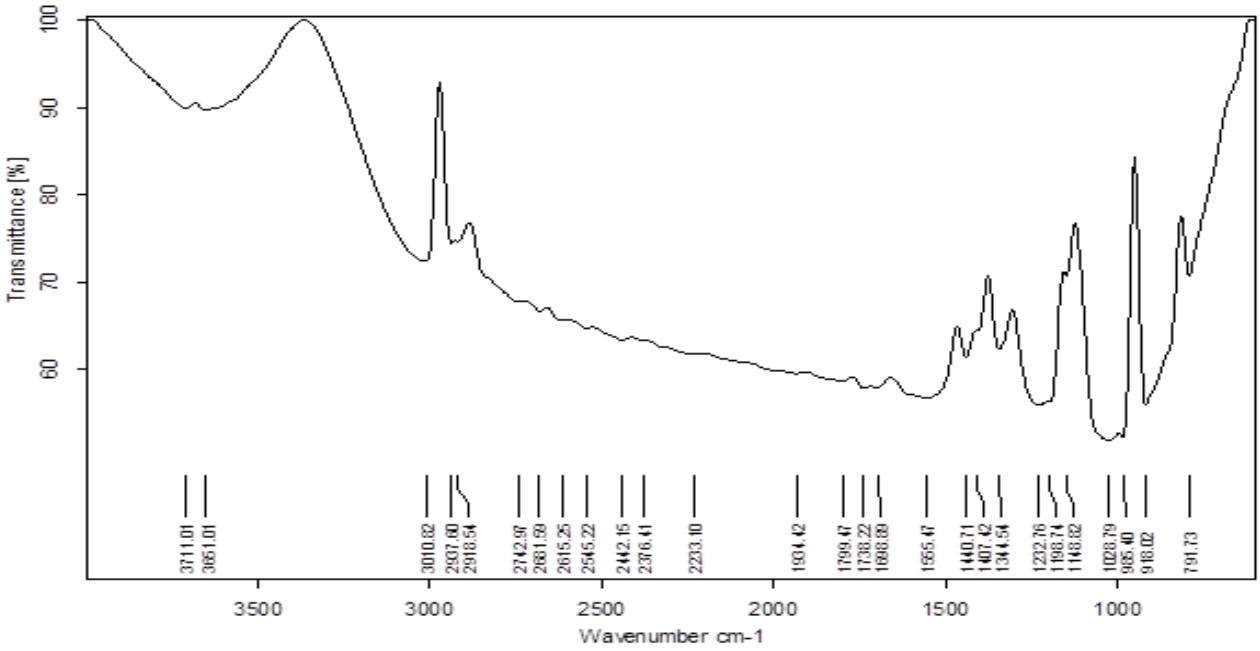
Compatibility Studies

FTIR spectrum of pioglitazone HCl is shown in figure 2



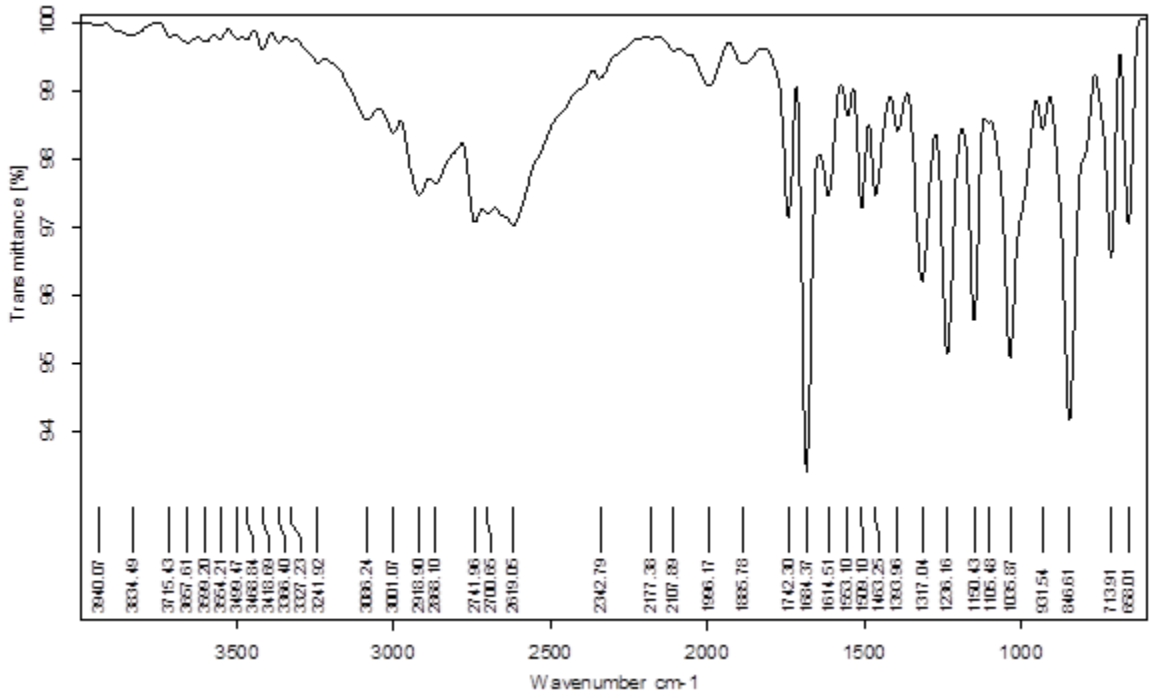
FTIR (ATR) spectrum of Pioglitazone HCl with chitosan and gelatin shown in the following figure

FTIR (ATR) spectrum of Pioglitazone Hcl

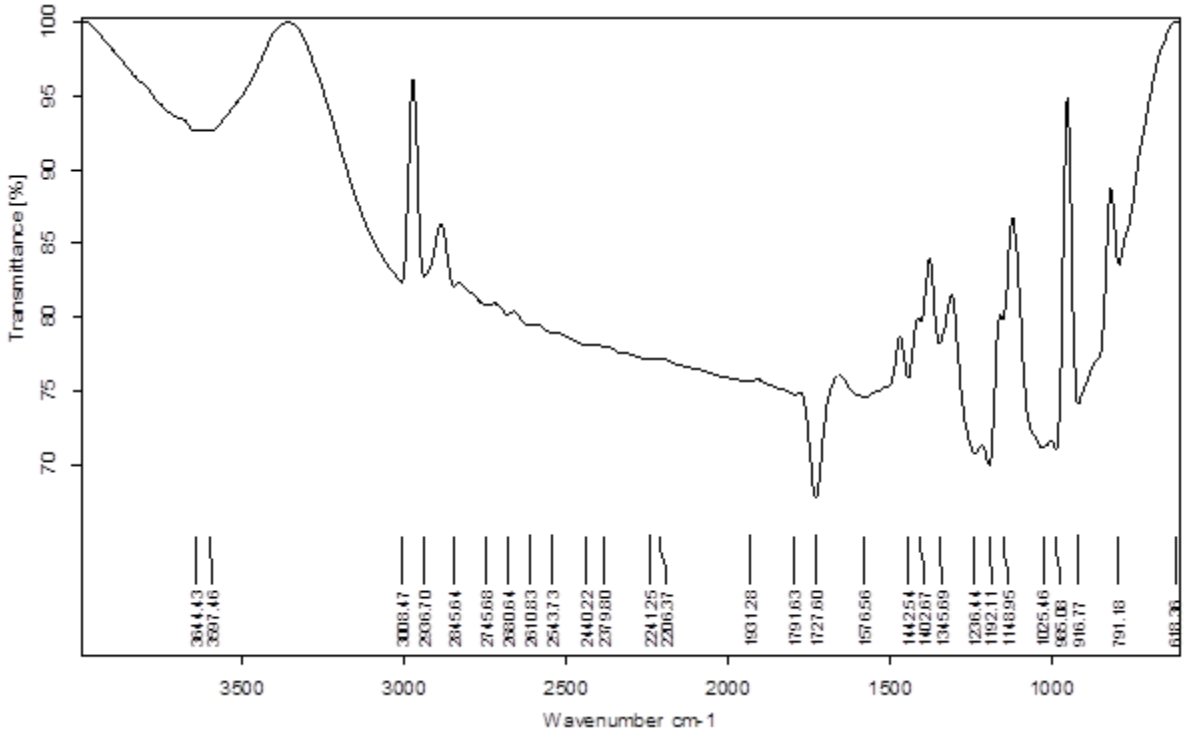


FTIR (ATR) spectrum of Pioglitazone HCl with chitosan, Eudragit RS 100 is shown in the following figure

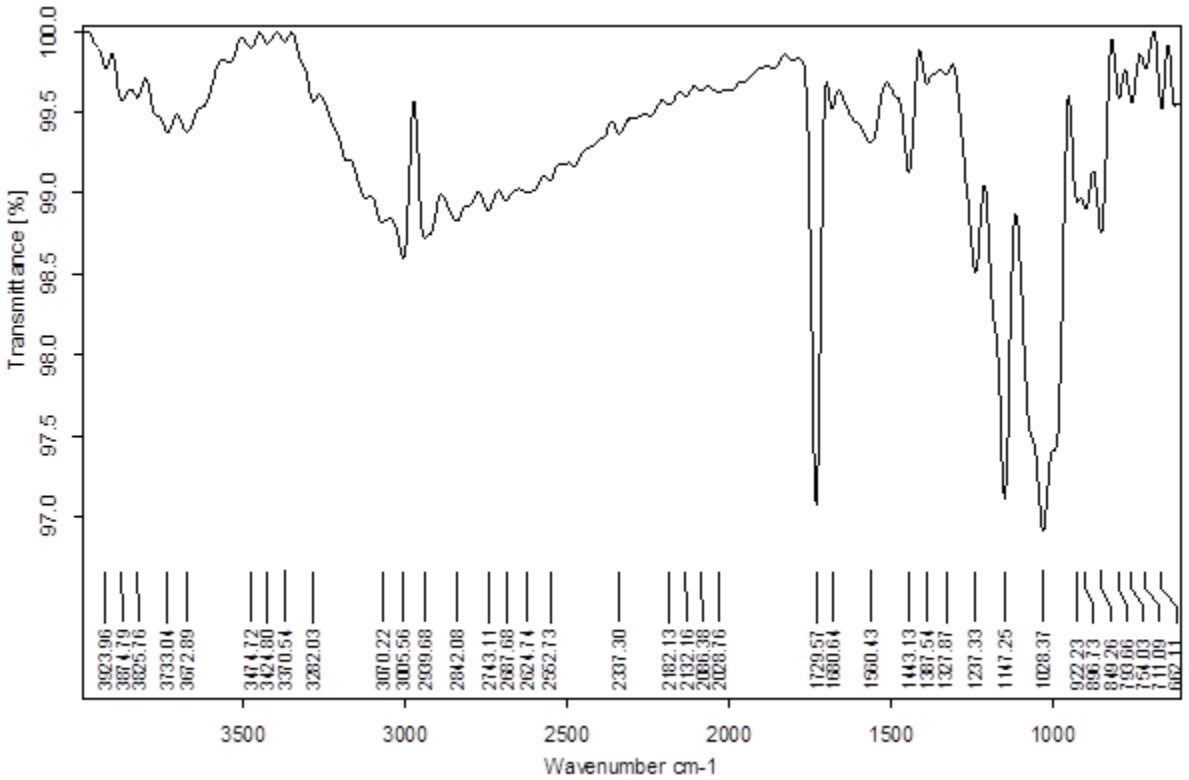
FTIR (ATR) spectrum of Pioglitazone Hcl with chitosan



FTIR (ATR) spectrum of Pioglitazone HCl with chitosan, Eudragit RS 100



FTIR (ATR) spectrum of Pioglitazone HCl with chitosan, Eudragit RL100



Characteristic peaks of Pioglitazone HCl and its physical mixture with polymers in FTIR (ATR) Spectrum

Description	Standard range	Pioglita-zone HCl	Pioglita-zone HCl + chitosan	Pioglita-zone HCl +chitosan + gelatin	Pioglita-zone HCl + chitosan +hpmc	Pioglita-zone HCl + eud RS100	Pioglita-zone HCl + eud RL100
C-H stretch alkene	3100-3000	3015	3001	3010	3012	3008	3005
C-H stretch alkane	3000-2850	2924	2918	2918	2932	2936	2939
O-H stretch	3400-2400	2617	2619	2615	2616	2610	2624
C=O	1725-1705	1742	1742	1738	1743	1727	1729

*All values are in cm^{-1}

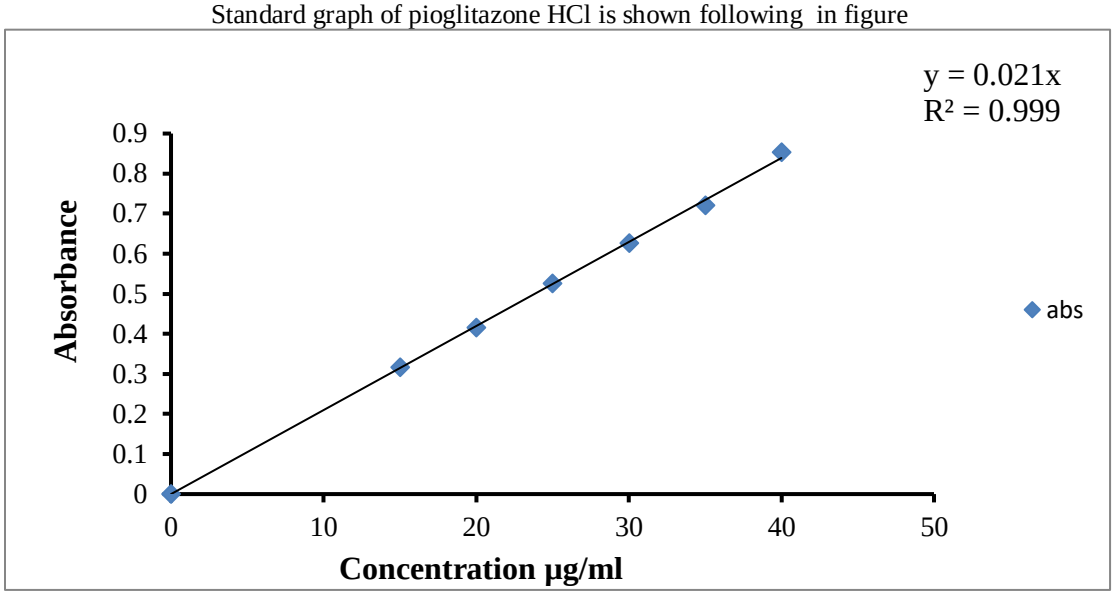
Standard Graph of Pioglitazone Hcl

Standard curve of Pioglitazone HCl at 269nm in HCl solution pH 1.2

Sl.NO	Concentration(µg/ml)	Absorbance
1	0	0
2	15	0.315±0.08
3	20	0.414±0.07
4	25	0.522±0.07
5	30	0.627±0.06
6	35	0.721±0.06
7	40	0.853±0.05

* All values are mean of 3 readings ± standard deviation

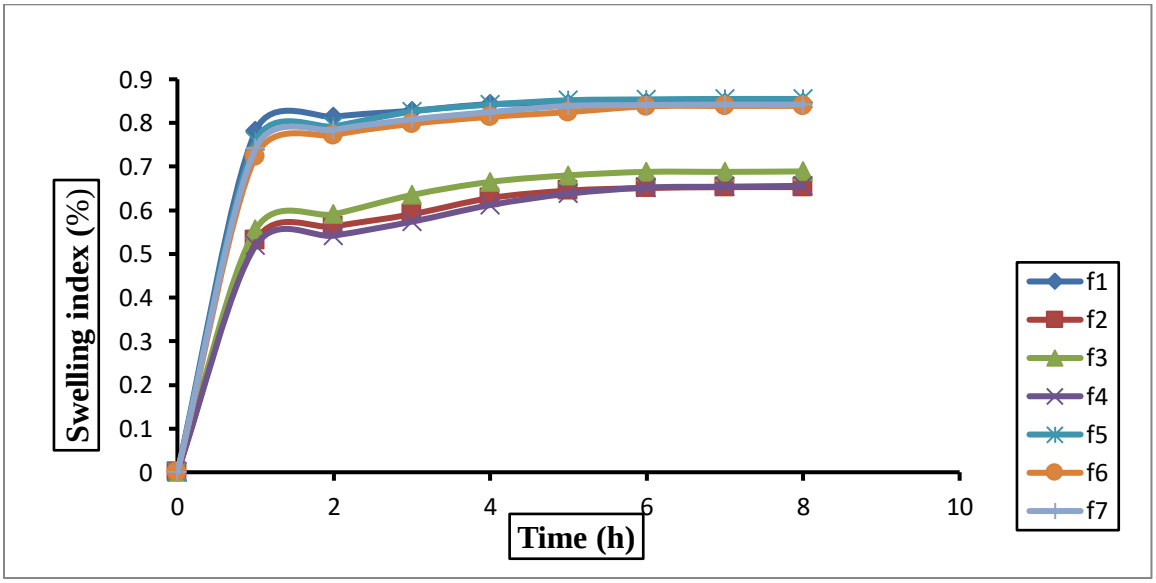
Standard graph of pioglitazone HCl is shown following in figure



Standard cure of Pioglitazone Hcl in 0.1N HCl(pH 1.2)

Drug entrapment efficiency (DEE), Swelling index, Mucoadhesive study results of sustained release mucoadhesive microparticles

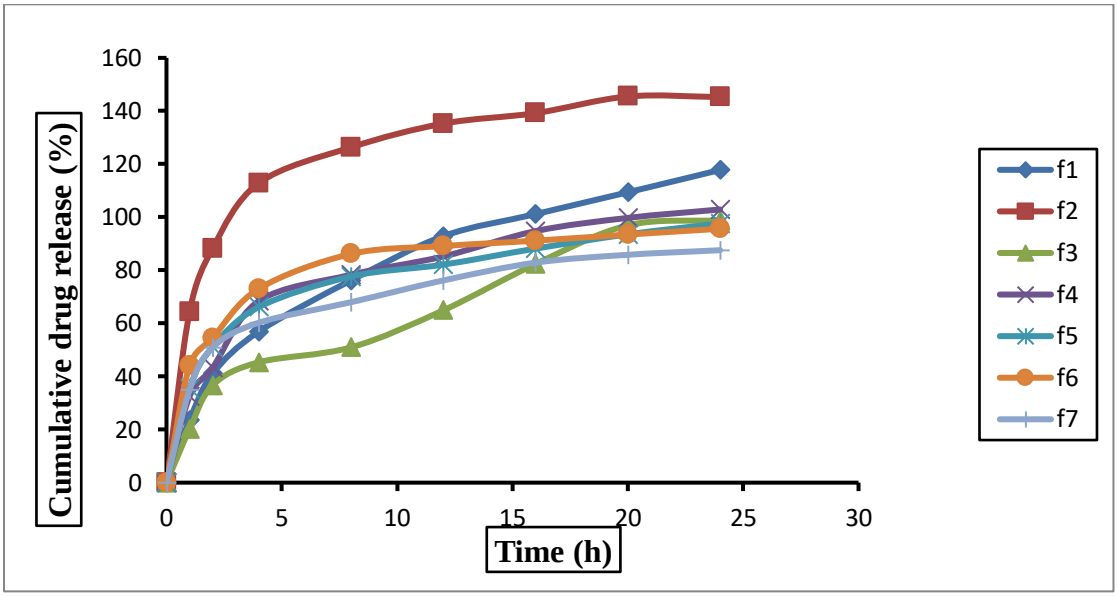
Formulation code	DEE (%)	Swelling index (%)	Morphological	Mucoadhesiveness (%)
F1	99.6	0.654	Spherical	54
F2	99.6	0.345	Hemispheical	__
F3	77.6	0.388	Hemispherical	__
F4	88.3	0.394	Hemispherical	__
F5	80.69	0.86	Spherical	64
F6	92.14	0.91	Spherical	__
F7	97.2	0.88	Spherical	62



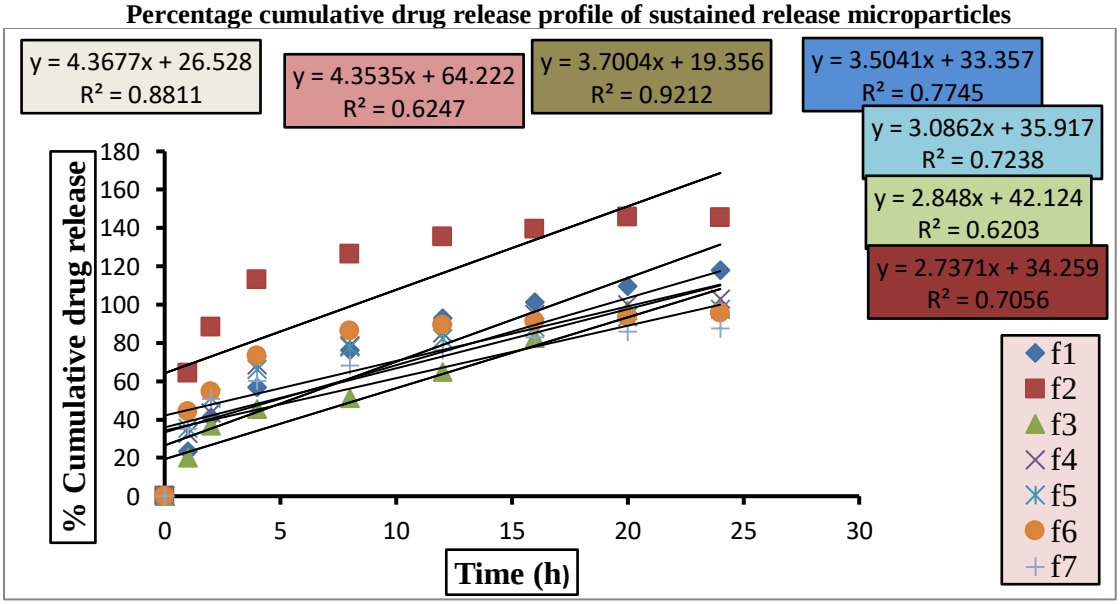
Swelling index of pioglitazone HCl formulations in 0.1N HCl solution (pH 1.2) *versus* time

Cumulative drug release (%) profile of sustained release mucoadhesive microparticles

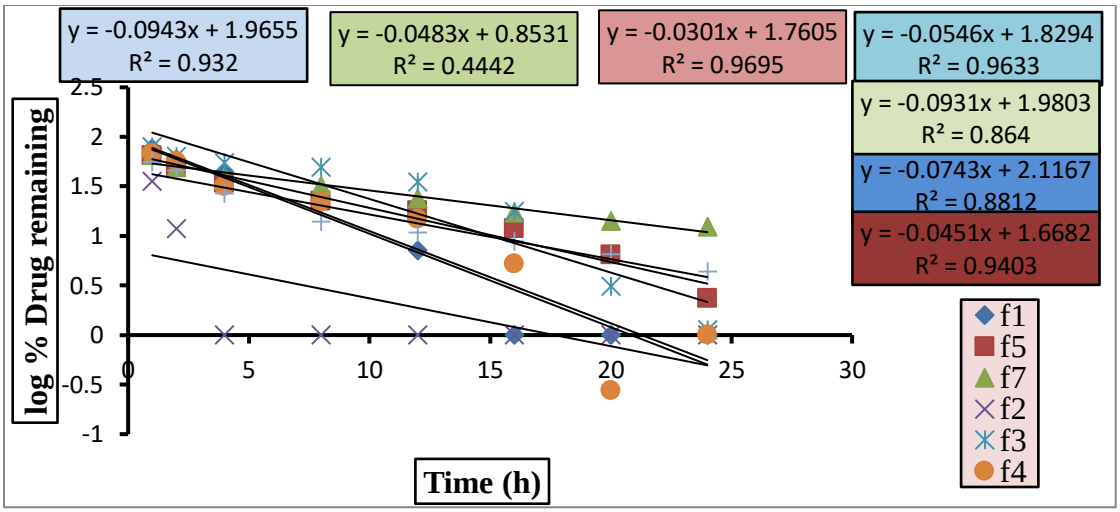
Time (h)	Cumulative drug release (%)						
	F1	F2	F3	F4	F5	F6	F7
1	23.46	64.24	20.11	32.54	35.47	43.96	34.91
2	41.05	88.20	36.67	43.28	51.03	54.43	50.88
4	56.90	112.84	45.34	68.35	66.11	73.02	60.24
8	76.18	126.31	50.95	78.30	77.65	86.13	67.97
12	92.84	135.24	64.91	85.20	82.12	89.12	76.17
16	101.15	139.19	82.36	94.75	88.16	91.14	82.87
20	109.37	145.25	96.91	99.72	93.52	93.44	85.83
24	117.17	145.45	98.86	102.89	97.66	95.62	87.55



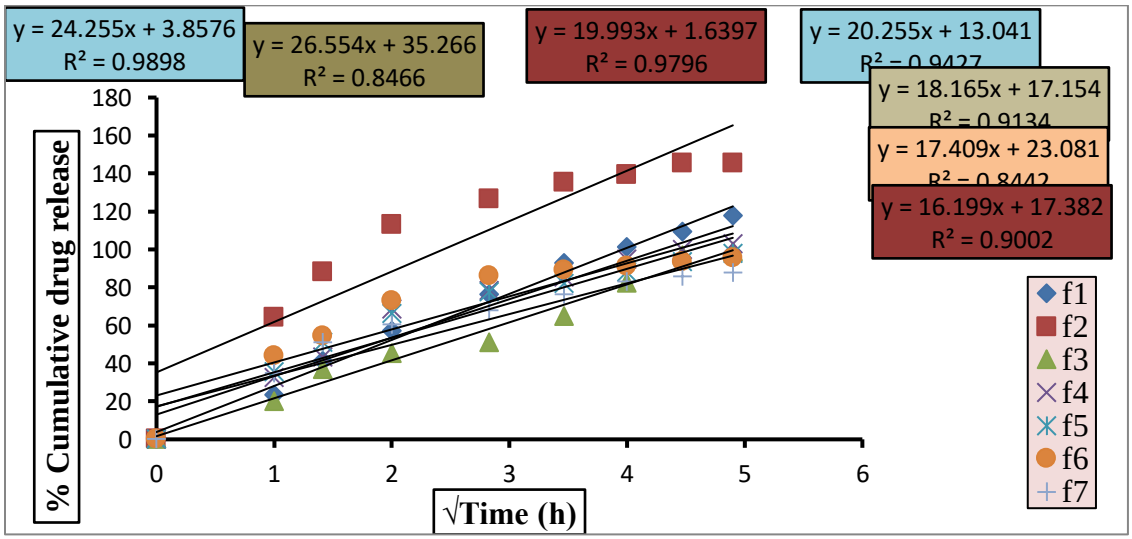
Percentage cumulative drug release profile of sustained release microparticles



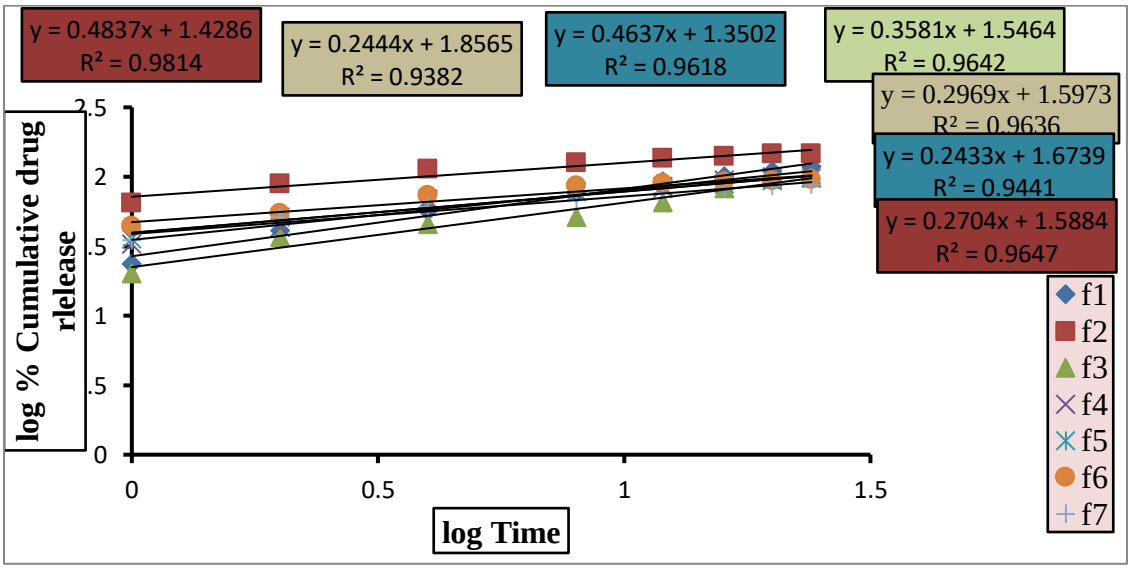
Zero order plots of sustained release mucoadhesive microparticles



First order plots of sustained release mucoadhesive microparticles



Higuchi model plots for sustained release mucoadhesive microparticles



Koyersmeyer-peppas model plots for sustained release mucoadhesive microparticles

Table 9: Correlation coefficients of drug release curves for sustained release mucoadhesive microparticles for various kinetic models

Formulation	Zero order		First order		Higuchi		Korsmeyer	
	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
F1	4.3670	0.8811	-0.0943	0.932	24.255	0.9898	0.4837	0.9814
F2	4.3535	0.6247	-0.0483	0.4442	26.554	0.8466	0.2444	0.9382
F3	3.7004	0.9212	-0.0301	0.9695	19.993	0.9796	0.4637	0.9618
F4	3.5041	0.7745	-0.0546	0.9633	20.255	0.9427	0.3581	0.9642
F5	3.0862	0.7238	-0.0931	0.864	18.165	0.9134	0.2969	0.9636
F6	2.848	0.6203	-0.0743	0.8812	17.409	0.8442	0.2433	0.9441
F7	2.737	0.7056	-0.0451	0.9403	16.199	0.9002	0.2704	0.9647

"n" value (Peppas constant) of drug release curves for sustained release mucoadhesive microparticles

"n" value	F1	F2	F3	F4	F5	F6	F7
	0.4837	0.2444	0.4637	0.3581	0.2969	0.2433	0.2704

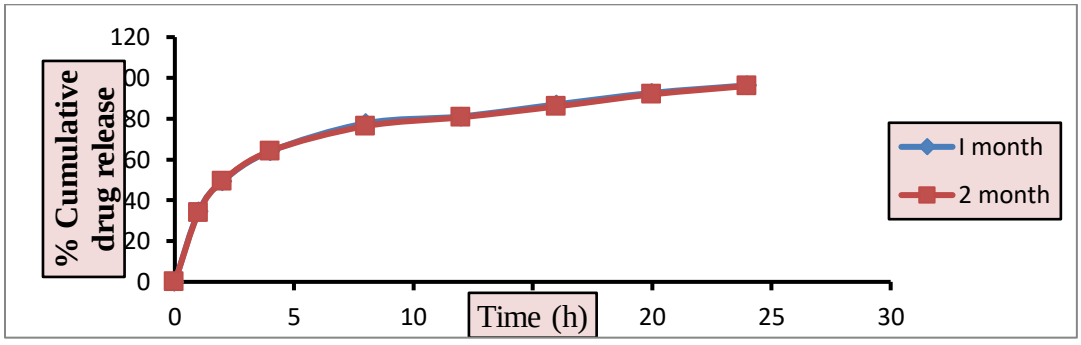
Stability Studies

Table 11: Drug entrapment efficiency of optimized formulation after stability studies

Time in days	Drug Entrapment Efficiency (%) 30±2˚C/65±5% RH		
	F1	F5	F7
0	99.6	80.69	97.2
30	98.9	79.5	96.5
60	98.71	79.2	95.96

Cumulative drug release (%) profile of optimized formulation after stability studies at

Time (hr)	Oth day			After 30 days			After 60 days		
	F1	F5	F7	F1	F5	F7	F1	F5	F7
	%CDR	%CDR	%CDR	% CDR	%CDR	%CDR	%CDR	%CDR	% CDR
1	23.46	35.47	34.91	23.46	34.49	34.35	22.9	34.07	33.65
2	41.05	51.03	50.88	40.21	49.35	51.72	40.62	49.48	49.76
4	56.90	66.11	60.24	54.81	64.00	59.27	55.79	64.14	58.97
8	76.18	77.65	67.97	74.39	77.7	64.48	73.83	76.36	66.70
12	92.84	82.12	76.17	89.32	81.12	75.45	89.18	80.69	74.74
16	101.15	88.16	82.87	98.47	87.01	81.58	97.49	86.02	82.13
20	109.37	93.52	85.83	111.8	92.65	85.80	111.57	91.93	85.09
24	117.17	97.66	87.55	114.41	96.36	87.38	114.13	96.06	86.39



Cumulative drug release (%) profile of 'F5' optimized formulation after stability studies

Discussion

Oral route is a safe, convenient and most adopted route for drug administration. Sustained release drug system provides continual release of drug to maintain therapeutic effect over a predetermined period of time.

Pioglitazone HCl is a thiazolidinediones, which belongs to category of insulin sensitizer, is used for

the treatment of Type II *diabetes mellitus.* Various conventional formulations of the drug currently available in the market like Actos (. However, they have disadvantages like multiple dose requirement, fluctuation in blood levels, less bioavailability etc. Development of a well defined sustained release dosage form is the requirement of the day to solve above problems and for the better treatment of type II *diabetes mellitus*. Hence, an attempt was made to formulate sustained release system of pioglitazone HCl, so that the drug is available for longer duration of time thereby resulting in a prolonged therapeutic effect.

A sustained release mucoadhesive microparticle was prepared using chitosan as the main mucoadhesive agent. HPMC 5cps cP, Eud RS100, RL100 are used as drug release modifiers.

Preformulation Study

rmulation studies such as identification, assay of drug, selection of polymers, drug polymer compatibility study and analytical investigation of drug were carried out using standard methods.

Melting Point Determination

Melting point of drug was determined by using Thiel`s tube apparatus. Melting point of pioglitazone HCl was found to be 189 ±1°C (n=3) which is close to the actual melting point.

Identification of Pioglitazone Hcl by UV Spectrum

Solution containing Pioglitazone HCl dissolved in 0.1N HCl (pH 1.2) was scanned in the wavelength range of 200-400nm, Peak was observed at a wavelength of 269nm, indicating wavelength maxima (λ_{max}) of the drug.

Compatibility studies

FTIR study showed that there is no interaction between drug and polymers. So, the drug and polymers are compatible. Drug–excipient interactions play a vital role with respect to biological performance and formulation stability. FTIR spectroscopy was used to study the physical and chemical interactions between drug and excipients. The characteristic absorption peaks obtained for drug alone and in the presence of polymers (1:1) are depicted in Figure 7,8,9,10,11,12. From the spectra, it is clear that the main drug peaks and the frequencies of peaks observed were within the standard range (Table 5). This indicates that the drug was compatible with the formulation components.

Preparation of Standard Curve of Pioglitazone HCl

The regression coefficient (r^2) value found for Pioglitazone HCl calibration curve developed in 0.1 N HCl solution was 0.999. The standard calibration curves are linear over the concentration range of 15–40 µg/ml and follow Beer's law with high r^2 values.

Procedure

Preparation of chitosan microparticles

Due to the advantageous biological properties of chitosan, such as relative non-toxicity, biocompatibility, biodegradability, cationic properties, bioadhesive characteristics and permeability-enhancing properties chitosan is selected as the main mucoadhesive agent

Chitosan Pioglitazone HCl entrapped chitosan microparticles were prepared using ionic gelation method. aqueous chitosan solution, chitosan concentration is fixed to 1.0%. Cross linking agent TPP was fixed to 2%w/v from the experiment.

Chitosan at the concentration of 1.0%w/v was found to give rigid particles with well defined morphological characteristics. Above this concentration chitosan solution was found to be highly viscous and unable to force the liquid through the syringe gauge #18. Below the 1.0%w/v concentration, formed microparticles were found to be hemispherical and microparticles were not rigid. Hence chitosan solution with 1.0%w/v was fixed as an ideal concentration for further studies. HPMC 5cP, Eudragit RS100, Eudragit RL100 at the concentration 3.0%w/v was found to give rigid particles with well defined morphological characteristics. Above this concentration the obtained particles were more rigid. Below the 3%w/v concentration, formed microparticles were found to be hemispherical and were sufficiently not rigid in nature. Hence HPMC 5cP, Eudragit RS100, Eudragit RL100 solution with 3.0%w/v was fixed as an ideal concentration for further studies. Gelatin at the concentration 1%w/v was found to give rigid particles with well defined morphological Above this concentration chitosan solution was found to be highly viscous and unable to force the liquid through the syringe gauge #18. Below the 1.0%w/v concentration, formed microparticles were found to be hemispherical and microparticles were not rigid. Hence chitosan solution with 1.0%w/v was fixed as an ideal concentration for further studies.

Among variety of methods developed to prepare chitosan microparticles, ionic gelation technique have attracted considerable attention due to this as this process is non-toxic, organic solvent free, convenient and controllable. Ionic gelation technique is based on the ionic interactions between the positively charged primary amino groups of chitosan and the negatively charged groups of polyanion, such as sodium tripolyphosphate (TPP), which is the most extensively used ion cross-linking agent due to its non-toxic and multivalent properties. This physical cross-linking process not only avoids the use of chemical cross-linking agents and emulsifying agents which are often toxic to organisms, but also prevents

the possibility of damage to drugs, particularly biological agents

Curing time

The curing time is the optimum time required for efficient cross linking of chitosan with sodium tripolyphosphate. The curing time was optimized to 30 min as it gives rigid and stiff microparticles, microparticles that were stirred less than 30 min results in non-stiff, irregular
shaped microparticles and found to be unstable after drying. If the curing time is more than 30 min results in smaller size microparticles.

Concentration of sodium tripolyphosphate

Concentration of sodium tripolyphosphate affects the size of microparticles, increase in concentration of sodium tripolyphosphate resulted in smaller size of microparticles so concentration of sodium tripolyphosphate was optimized to 1% to maintain the uniformity in size of microparticles.

Formulation contains sustained release mucoadhesive microparticles placed in a single capsule of size "0".

Morphological characteristics

Morphological studies of selected three formulations (F1, F5 and F7) were subjected for scanning electron microscopic (SEM) studies. Three formulations were selected based on the results of drug entrapment efficiency, mucoadhesive studies, swelling index and drug release pattern. The topographical studies shown in fig were correspond to chitosan(F1), chitosan-Eudragit(F3), chitosan-gelatin(F5)

Drug entrapment efficiency (%)

The drug entrapment efficiency (%) was carried out using single point standardization method. The encapsulation efficiency of drug increased with increasing polymer concentration. The reason may be that at a higher polymer solution viscosity (at the highest polymer concentration), the diffusion of the drug into the external phase is expected to decrease, which would result in higher encapsulation efficiency. Drug entrapment efficiency (%) of all the developed formulations was in the range of 77.6% to 99.6%. it was found in the limits specified by *IP*.

Swelling index

In the present study it was observed that in each batch there was a maximum swelling of microparticles followed by increase in weight in next observation. The polymer concentration has significant effect on swelling ratio of microparticles. As the amount of polymer was increased, the swelling ratio of microparticles increased. This result may be because of maximum crosslinking of polymers that yielded compact microparticles. The polymer mixture is also responsible for different swelling behaviour of microparticles. The swelling property increased from 0.345 to 0.88. Among all the batches F5 and F7 were found to show good swelling property.

Study of Mucoadhesive property

Mucoadhesive property of selected three formulations (F1, F5 and F7) was subjected for *in vitro* wash off studies. The *in vitro* wash off test, mucoadhesion increased from 54 to 64%. Mucoadhesion of chitosan-gelatin was found to be significantly high, this may be due to significant mucus gel strengthening, which results in formation of stable mucoadhesive joint. Mucoadhesion of only chitosan formulation (F1) was found to be poor when compared to chitosan-gelatin formulations, this is due to their low swelling capacity. F5, F7 has shown good mucoadhesive property.

Dissolution studies

The *in vitro* release profile of Pioglitazone HCl from microparticles is shown in Fig no.4. It was observed that with the increase in the concentration of chitosan the release of the Pioglitazone from the polymer matrix was retarded, the less is the concentration of chitosan in the formulation, the faster is drug released from microparticles, this may be due to the swelling property of chitosan. Hence all polymers show retardation of drug release upto 24 h. The drug release was good with formulation F1, F5 and F7 which were 112.27%, 97.66%, and 87.55% in 24h from among all the formulations.

Kinetic Modeling of Drug Dissolution Profiles

The *in vitro* release data of all the sustained release formulations were fitted into various kinetic models. Correlation coefficients (r^2) of formulation F5 showed higher correlation with first order plots than zero order plot. So, the predominant mechanism of drug release follows first order kinetics. Further in korsmeyer-peppas plot "n" value was found to be 0.296, indicating Fickian mechanism of drug release.

Stability Studies of the Most Satisfactory Formulation

The most satisfactory formulation (optimized formulation) was subjected for short term stability studies. Capsule (containing sustained release microparticles) is packed in aluminum foil and kept in humidity chamber maintained at 30±2°C /65±5% RH for two months. Samples were withdrawn at the interval of 1month and were analyzed for the drug content, and *in vitro* dissolution. There was no significant difference in drug content, and *in vitro* dissolution pattern at various sampling points

CONCLUSION:

Pioglitazone HCl is a thiazolidinediones, which belongs to category of insulin sensitizer, can be used for the treatment of type II *diabetes mellitus*. Conventional formulations of the drug have disadvantages like multiple dose requirement, fluctuation in blood levels, less bioavailability etc.

These problems may be solved by developing a well defined sustained release dosage form. Hence an attempt was made to formulate sustained release system of pioglitazone HCl, in order to make the drug available for longer duration of time thereby resulting in a prolonged therapeutic effect.

Batches of sustained release mucoadhesive microparticles were prepared by ionic gelatin method using polymers Chitosan, Hpmc 5cps, Eudragit RS100, Eudragit RL100 and gelatin. Developed sustained release mucoadhesive microparticles possessed ideal drug entrapment efficiency (%), mucoadhesive property, swelling property.

From all the developed sustained release microparticles, formulation F5 showed maximum drug release of 97.66% so, it was selected as satisfactory sustained release formulation.

Therefore, it was concluded that the developed sustained release formulation of pioglitazone could release the drug satisfactorily for the period of 24h. Also the microparticles properties found to be satisfactory even after the stability period of 2 months.

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