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PRECLINICAL STUDIES TO DEVELOP A NOVEL SOFT GELATIN CAPSULE FOR PARACETAMOL WITH FASTER ONSET TIME OF ACTION

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ABSTRACT
Objectives: The present research study was carried out to develop a novel soft gelatin capsule of paracetamol (PRT), an antipyretic and analgesic agent in which the pain relief onset time is improved to less than 4 minutes. The role of liquid fill formulation of PRT (PRT-LF) in a soft gelatin capsule SGC (commercial oblong empty SGC of size 20) in increasing the *in vitro* and preclinical *in-vivo* bioavailability of PRT in Sprague Dawley rats was studied. Experimental: PRT-LF were formulated and optimized using permeation enhancer/permeability glyco-protein (p-gp) inhibitor polyethylene glycol (PEG-400). The optimized PRT-LF formulation was then incorporated into the empty commercial SGC and tested for the increased drug percent released at 2 minutes, 4 minutes (DP_2 and DP_4) by the assistance of PEG-400. *In vitro* permeation studies were carried out using Franz diffusion cells for a period of 24 h. Results and Discussion: The optimized PRT-LF SGC had good physicochemical properties. The formulations were stable up to 6 months without undergoing any degradation.From the Franz diffusion cell apparatus permeation studies it was evident that the delivery of PRT into the dialysis membrane by liquid fill formulations for soft gels was significantly higher than that from marketed formulation paracip-650, with apparent permeability coefficient of 0.815±0.006 cm/h for F3 formulation. Conclusion: when compared to existing oral onset action time of 37 minutes and intravenous pain relief onset time of 8 minutes, F3 PRT-LF SGC could be used to show the faster onset of action evident from *in-vivo* studies in rats.

KEYWORDS: Paracetamol, soft gelatine capsules, Bioavailability.

INTRODUCTION
Soft gelatin capsules are commonly known as soft gels. Soft gelatin capsules are becoming a popular dosage form for the administration of liquids, suspensions, pastes, and dry powders in the dietary supplement industry. Soft gels can be effective delivery system for oral drugs, especially poorly soluble drugs.[1] This is because the fill contain liquid ingredients that help increase solubility or permeability of the drug across the membranes in the body. The absorption of poorly soluble compounds encapsulated in soft gels may also be higher compared to that from other conventional dosage forms not only due to the solubilization of the compounds in the fill formulation but also due to the fill excipients induced inhibition of P-glycoprotein-mediated drug efflux and reduced enzyme-catalysed degradation of the compound in the lumen of the GIT.[2]

Soft gels are easy to swallow, once swallowed, release their contents quickly. Another advantage that derives from the liquid nature of fill is rapid release of the contents with enhanced bioavailability. The proper choice of vehicle may promote rapid dispersion of capsule contents and drug dissolution. Soft gelatin capsules are available in a wide variety of sizes and shapes. Specialty packages in tube form (ophthalmic, ointments) or bead forms.[3] The pH of the liquid can be between 2.5 and 7.5. Liquids with more acidic pH would tend to leakage by hydrolysis of gelatin. Both liquids with pH > 7.5 and aldehydes decrease the shell solubility by tanning the gelatin.[4]

Paracetamol (PRT) was chemically known as N-(4-hydroxyphenyl) acetamide ($C_8H_9NO_2$).[5-7] The melting point of Paracetamol is 169 °C. It has analgesic and antipyretic properties. It is a class-III drug. It is readily soluble in water, methanol, ethylenedichloride, dimethylformide slightly soluble in ether. Although the

exact site and mechanism of analgesic action is not clearly defined, acetaminophen appears to produce analgesic by elevation of the threshold.[8] The potential mechanism may involve inhibition of the nitric oxide pathway mediated by a variety of neurotransmitter receptors incuding N-methyl-D-aspartate and substance P.[9] Vellani V *et al.*, studied on Effects of NSAIDs and Paracetamol (acetaminophen) on protein kinase C epsilon translocation and on substance P synthesis and release in cultured sensory neurons. Roger dobson *et al* studied on how Paracetamol can reduce stress and sharpen your memory. Pranati srivastava rishabha *et al* studied on that formulation and evaluation of Paracetamol tablets to assess binding property of orange peel pectin. David A. Perrott *et al* studied on efficacy and safety of acetaminophen vs ibuprofen for treating children's pain or fever meta- analysis free, but there were no reported works found on the liquid fill formulations of Paracetamol.

Hence in the present research work, PRT was formulated into liquid fill formulations for soft gels and delivered through oral soft gelatin capsules with an aim of faster onset of action. Overall, soft gels may very well bridge the need for patient friendly faster onset of action and clinically efficient PRT soft gels. This work is an extension and scale up of the work done by Jyothirmayee Devineni etal published in International journal of pharmaceutical development and technology, 2015.[10] No reports were published so far on the invivo studies of soft gels of PRT liquid fill formulations.

MATERIALS AND METHODS

Materials

Paracetamol of analytical grade (purity, > 98%) was a gift sample from Parkinson Pharma, Mohali, India. Polyvinylpyrrolidone k-30(LOBA CHEMI Laboratories-Mumbai), Polyethylene glycol-400 (gift sample from S.D Fine chemicals Ltd, Mumbai), Propylene glycol (gift sample from Central drug house, Bombay), and Ethyl alcohol of HPLC grade (gift sample from Changshu Yang chemicals, China), Butylated Hydroxy toluene, (Merck Specialities Pvt. Ltd, Mumbai, India) were used. Distilled de-ionized water was used. Empty soft gelatine capsules (gift sample from Kahira pharm.chem.co, Cairo, Egypt), were bought from Sigma Aldrich, India. All the materials used were of pharmacopoeial and analytical grades.

Analytical methods

An UV-VIS Spectrophotometric method based on the measurement of absorbance at 249 nm in methanol stock solution was used in the present research work for the estimation of PRT *in vitro* studies.For the estimation of PRT in different aqueous fluids the stock solution was subsequently diluted to get a series of dilutions 2, 4, 6, 8 and 10 µg/mL of solution and the absorbance was measured at 249 nm (UV- VIS spectrophotometer, SL-150, Elico) against the same dilution as blank.

HPLC method for estimation of PRT in rat plasma

A new reverse phase HPLC method with UV detection was developed for the estimation of PRT in plasma samples. For this purpose a calibration curve was constructed by analyzing plasma samples containing different amounts of PRT. The experiment was conducted to develop a liquid chromatographic method for the determination of NLF using Waters Alliance 2695, HPLC system with Auto Sampler and 2487 UV-Visible detector. The chromatographic studies were performed using Hypersil ODS C_{18} column (4.6 ID X150 mm, 5µm) at ambient temperature. Data acquisition was done by using Empower 2 software.

Paracetamol 10 mg was weighed accurately and transferred into a 10 ml volumetric flask containing 5 ml of methanol. The contents were sonicated for 5 minutes and then the volume was made up with a further quantity of methanol to get a free base concentration of 1 mg/ml. This stock solution of the drug was stored in a refrigerator at a temperature below 10^0C. One ml of the drug stock solution was diluted up to 10 ml in a volumetric flask using mobile phase [Phosphate buffer (pH 3.0) and ACN in a ratio of 40:60 v/v] as a diluent to get a concentration of 100µg/ml. The secondary stock solution of the drug was stored in a refrigerator at a temperature below 10^0C.

Stability of PRT in pH 1.2 buffer

The stability studies of PRT were performed in pH 1.2. The samples (20 µg/ml) were placed at 37°C in an orbital shaker for a period of 48 hr and were withdrawn at different time points that were analyzed by UV-VIS spectrophotometric method.

Preparation of PRT Liquid fill formulations for soft gels

Some of the parameters that affect the final properties of PRT liquid fill formulations for soft gels are PEG-400, PG, PVP K-30 amounts. Three variables at two levels of formulations were investigated in full factorial design (2^3) and finally, eight different formulations were prepared and also by varying the ratios of PEG 400:PG (1:1, 5:1, 1:5). (Table 1)

Preparation of liquid fill formulations of PRT

Liquid fill formulations were prepared as per the formulae given to a batch size of 6g. Initially propylene glycol, and PEG-400 were taken into a small beaker and stirred to dissolve well. PVP K-30 was then added and dissolved. Accurate amount of PRT was then weighed and transferred into this beaker and mixed thoroughly. It was followed by the addition of ethyl alcohol to dissolve the drug completely (evaporation of ethyl alchohol is avoided by covering the beaker during stirring). Paracetamol 650 mg was weighed accurately. BHT was then added and the system was mixed thoroughly. The prepared formulation was sonicated for 3 minutes in order to remove any entrapped air. The weight of liquid ingredients like ethyl alchohol, propylene glycol (PG),

polyethylene glycol – 400, was converted to volume from density values and taken accordingly. The volume of the above liquid ingredients was derived from the available values of density reported in standard literature (density of ethyl alchohol is 1 gm/cm^3, propylene glycol is 1.038 gm/cm^3, PEG -400 is 1.12 gm/cm^3). Empty soft gelatine capsules were incubated at 40° C for 10 minutes with an objective of removing moisture taken up by the capsules during storage. Each oblong shaped soft gelatin capsule of size 20 equivalent to 1.232 ml was taken for filling. Each capsule was filled by injection with 1.0 ml of each of the formulation. Each capsule should be filled up to 75 percent of its total volume. Using a glass syringe the liquid fill was injected into the capsule, which was then sealed by heat. The soft gelatin capsules filled with liquid fill formulations of PRT were then subjected to different tests to evaluate for various parameters.

Table 1. Formulae of all the liquid fill formulation for soft gels of Paracetamol

Ingredients (mg/cap)	F1	F2	F3	F4	F5	F6	F7	F8
Paracetamol	650	650	650	650	650	650	650	650
PVP k-30	50	-	50	50	150	150	150	150
Ethyl alcohol	-	150	-	50	-	-	-	-
PG	150	150	150	150	250	243	50	50
PEG-400	150	150	150	150	43	50	250	250
BHT	-	-	1	-	1	1	-	-
DMSO	-	-	6	-	6	6	-	-
Water	100	-	93	50	-	-	-	-
Total wt	1100	1100	1100	1100	1100	1100	1100	1100

Table 2: Formulae of all the liquid fill formulation for soft gels of F3 optimized formulation with fill volume.

Name of the Ingredient	Composition(mg) per capsule	Percent w/w	Fill volume(micro litres) for liquid ingradients using density values
Paracetamol	650	59.09	
Polyethylene Glycol-400	150	13.63	133(density=1.12g/cm^3)
Propylene Glycol	150	13.63	144(density=1.038g/cm^3)
Dimethyl sulfoxide	6	0.5	6(density=1g/cm^3)
Butylated hydroxytoluene(BHT)	1	0.1	
Polyvinylpyrrolidone(PVPK-30)	50	4.54	
Water	93	4.54	93(density=1g/cm^3)
Total	1100	100	

Table 3: Formulae of shell composition for F3 formulation.

Name of the Ingredient	Composition(mg) per capsule	Percent w/w
Gelatin	293	40
Glycerine	110	15
Methyl paraben	1.46	0.2
Propyl paraben	0.4	0.05
Purified water QS	328	44.75
Total	733	100

Table 4: Formulae of fill weight and shell weight for F3 formulation.

Name of the Ingredient	Composition(mg) per capsule	Percent w/w
FILL WEIGHT	1100	60
GEL/SHELL WEIGHT	733	40
Total	1833	100
Dieroll used	Dierolls are 3 inches long and 4 inches in diametre with encapsulation speed 2-5 rpm ARBES-SOFTGEL-CATALOGUE	
SOFT GELATIN CAPSULE SHAPE AND DIMENSIONS	20 OBLONG with 1150-1400 micro litres fill volume Upto 75 % of the capsule is filled with liquid fill	

Evaluation parameters for PRT liquid fill formulations for soft gels

Appearance

Appearance is one of the most important parameter of liquid fill formulations. All the formulations were evaluated for clarity by visual observation against a black background. Clarity is the most important characteristic feature of liquid fill formulations.

pH

The developed PRT liquid fill formulations were evaluated for pH by using Elico LI 120 pH meter and estimations were carried out in triplicate. Soft gel formulations should have a pH range between 2.5 and 7.5.

Drug content uniformity

Drug content was estimated in the liquid fill formulations by weighing approximately 25mg of the fill formulation into a 5 ml volumetric flask. A small volume of methanol was then added, the flask was stirred well, and the volume was made up to 5 ml with remaining methanol. Samples were suitably diluted with 0.1N HCl and the samples were analysed for PRT content by measuring absorbance at 249 nm. The estimations were carried out in triplicate.

Moisture absorption studies

In order to study the effect of liquid fill composition on the water sorption behavior of the softgels, they were subjected to water migration studies. Three capsules from each formula were weighed, transferred to a small dry pre-weighed beaker and kept in a sealed glass humidity chamber containing 100 ml of a saturated aqueous solution of sodium chloride (to provide an atmosphere of 75% relative humidity). The weight of the beaker, with its contents, was recorded every day until it became constant indicating that equilibrated moisture absorption had been achieved. The water content of the prepared softgels was determined at the beginning of the water migration study and after equilibrium to calculate the weight of water gained by each formula at equilibrium, and this was expressed as a percentage of the initial capsule weight. A Karl Fischer titrator (Veego, Matic-MD, Veego Instruments Corporation, India) was used to determine the moisture content of the softgels and to do this the capsules were cut, inserted into the titration vessel containing dried methanol (Karl-Fisher grade) and titrated with Hydranal Composite 5 reagent (Riedel-de-Haën, Seelze, Germany) after stirring for 2 minutes. Three capsules were analyzed from each formula and the results were presented as a mean value ± SD. For comparision, the water sorption behaviour of empty capsule shells and filled soft gelatin capsules was studied.

Rheological studies

Viscosity of all the formulations was measured using a Brookfield DV-II + PRO viscometer. The formulations were taken in the cup of the Brookfield DV-II + PRO viscometer and it was rotated with CP52 spindle. The angular velocity was fixed at 10-100 rpm. The viscosity measurements were made in triplicate using fresh samples each time at room temperature.

FTIR studies

Samples were analyzed using an ATR-FTIR spectrometer (Bruker, Germany). ATR spectra were measured over the wave number range of 4000–500 cm−1 at a resolution of 1.0 cm^{-1}. The powder or film sample is simply placed onto the ATR crystal and the sample spectrum is collected. The sample is then cleaned from the crystal surface and the accessory is ready to collect additional spectra. ATR analysis is less complicated than using KBr pellets. It is a fast process and a very small amount of the sample is needed.

***In-vitro* drug release studies for PRT liquid fill formulations**

Vertical type Franz diffusion cells (area 1.44 cm^2) with a dialysis membrane were used for *in vitro* drug release studies to determine the release rate of PRT from different formulations. Hydration of the Dialysis (molecular weight G12000) membrane was performed in distilled water at 25ºC for 24 hr. The membrane was clamped between the donor and receptor compartments of the cell. The receptor chamber contained 14 ml of buffer pH 1.2 and was continually stirred using a magnet stirrer (300 rpm) at 37ºC. Two ml of the sample was withdrawn from each batch at definite time intervals (30 sec, 1, 2, 3, 4, 5, 6, 30 min) and replaced with the same amount of buffer to maintain sink conditions. Single beamUV/Visible Spectrophotometer (Elico SL-150) at 249 nm was used to determine the release concentrations of PRT. The results were plotted as cumulative release drug percent versus time. Various kinetic models such as zero order, first order[24] were employed to explain drug release from formulations. The formulation with higher r^2 was selected. *In vitro* drug release studies were conducted in triplicate.

***In vitro* dissolution studies for PRT liquid fill formulations incorporated in soft gelatin capsules**

The dissolution studies were conducted using 900mL of 0.1N HCl, as dissolution medium using USP XXI type I/II (paddle method) dissolution apparatus (DISSO 8000, LAB INDIA). A temperature of 37 ± 0.5°C and a rotation speed of 100/50rpm were maintained. Dissolution studies were performed. As the capsule tends to float in the dissolution medium, sinkers were used. Samples of 5ml were withdrawn at predetermined time intervals over a period 1hr, passed through a 0.45µm nylon membrane. Then the sample removed was replaced with the same volume of fresh dissolution medium in order to maintain constant dissolution medium. The filtered samples were suitably diluted and analyzed at 249 nm using UV-Visible Elico SL150 spectrophotometer. Dissolution experiments were conducted in triplicate.

Drug release kinetics & mechanisms

There are a number of kinetic models, to describe the overall release of drug from the dosage form. Because qualitative and quantitative changes in a formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. The rate of release of PRT from prepared dosage form was analyzed

by fitting drug release data into first order release kinetics equation:
Log C = log C_O – k t/2.303.

Where, C_O is the initial concentration of the drug and k is the first order constant. By plotting log cumulative of % drug unreleased against time a line was obtained and from its slope, k was calculated.

Stability studies on PRT liquid fill formulations for soft gels

Liquid fill formulations, apart from their other requirements, should be stable with regard to their properties, especially their dissolution characteristics. The stability of PRT liquid fill formulations developed in the present study was evaluated as per ICH guidelines. The capsules were packed in amber coloured bottles and stored at 40^0 C and 75% RH for 6 months. During storage, the products were monitored for viscosity, pH of the formulation, drug content, appearance, precipitation, and dissolution profile studies. These studies were carried out at 3rd and 6th months.

Bioavailability and Pharmacokinetic Evaluation of optimized formulations of Paracetamol

***In vivo* studies**

In vivo pharmacokinetic studies were carried out on the following groups

- First group containing six (3 male and 3 female) (n=06) was treated with Paracetamol liquid fill for soft gels designated as group-A (PRT-SGS) - **TEST**
- Second group containing six (3 male and 3 female) (n=06) was treated with Paracetamol i.v.injection designated as group-B (PRT-IV) - **STANDARD**
- Third group containing six (3 male and 3 female) (n=06) was untreated - **CONTROL**
- Fourth group containing six (3 male and 3 female) (n=06) was treated with Paracetamol marketed formulation designated as group-B (PRT-MRK) - **COMPARISION**

Experimental design

Dose regimen and administration

Food was withdrawn from the rats 12 hrs before drug administration and until 24 hrs post dosing. All rats had free access to water throughout the study. Animal housing and handling were in accordance with the CPCSEA guide lines. This study on animals was carried out in the pharmacology laboratories of the KVSR College of Pharmaceutical Sciences, Vijayawada, is approved by the institutional ethics committee, for experimentation on animals and has a registration number CPCSEA (Regd.No.993/A/ 06/CPCSEA) for experimentation on animals.The test is optimized liquid fill formulation of paracetamol 650 mg, the standard is iv injection of paracetamol 650 mg, the comparision is marketed formulation of paracetamol 650 mg. The dosage is administered as liquid (tablet powder suspension) suspended in 0.9 % sodium carboxy methyl cellulose to yield a concentration of 650mg of Paracetamol. All the formulations were prepared 30 minutes before administration at room temperature. Suspension was prepared with a magnetic stirrer.The dose administered was 650mg/kg. Oral LD 50 of Paracetamol in albino rats=0.77±0.02 gm/kg body weight. Rats of about 250 mg are selected for the study. Hence the lethal dose should not exceed 0.192 gm = 192 mg.

***In vivo* study protocol**

After collecting the zero hour blood sample (blank), The products involved in the study were administered orally at a dose equivalent to 650 mg of Paracetamol/kg of body weight of the rat. Blood samples of 0.2 ml (200 µl) volume were collected from the tail vein at 0, 60 sec, 75 sec, 5 min, 15 min, 30 min, 45 min, 1.0 hr, 1.5, 2.0 hr, 3.0hr, 4.0hr, 8.0hr, 10hr and 12.0 hrs after administration of the product. The blood samples were centrifuged at 5,000 rpm for 10 min and the separated plasma samples were stored at -20°C until analysis.

Extraction procedure of PRT from rat plasma

To 0.5 ml of plasma, 1 ml of ACN was added in 1.5 ml of poly propylene tubes. The tubes were vortex-mixed for 5 minutes in cyclomixer and then centrifuged for 10 minutes at 4000 rpm. The upper organic layers of the tubes were collected, filtered and injected into the column and then analyzed by using a sensitive high performance liquid chromatography (HPLC) assay method.

Paracetamol concentrations in plasma following the administration of experimental products are From time versus plasma concentration data, various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak concentration occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half - life ($t_{1/2}$) and mean residence time (MRT) were calculated in each case.

Statistical Analysis of the data

All the data was given as mean ± SD. Statistical analysis of the data and the interpretation of results was carried out with a one-way ANOVA analysis (using Fisher's Least-Significant-Difference post hoc test) using SYSTAT 13 software (Systat Software Inc., CA, USA). Statistical significance was checked at a threshold of $p < 0.05$. Results with p value less than 0.05 were considered to be statistically significant variance.

RESULTS AND DISCUSSION

Stability of PRT in pH 1.2 buffer

The samples were investigated for a period of 48 hr to assess the stability of PRT and analyzed using UV-VIS spectrophotometric method. No significant degradation of PRT was observed in 1.2 pH buffer and therefore pH 1.2 buffer was selected.

Preparation of PRT liquid fill formulations for soft gels

Major variables that influence the liquid fill properties include PVP K-30, DMSO amounts and ratio of PG:PEG in the preparation. The successful was F3 formulation with optimum properties.

The HPLC method was developed, validated and the total run time was set at 08 minutes. Paracetamol was appeared on the chromatogram at 5.916 minutes. The retention time of the drug was the same for all the six injection samples. The regression of PRT concentration (250 - 1250 ng/ml) over its peak area of drug was found to be Y= 32.094X- 0.2 with a high correlation coefficient (r =0.999), where Y is peak area and X is concentration of PRT. This regression equation was used to estimate the amount of PRT in rat plasma.

The developed HPLC method was validated for intra-day and inter-day variation. When the solutions containing 500, 750 and 1000 ng/ml of nelfinavir were injected repeatedly on the same day and on other day, the coefficient of variation in amounts estimated was less than 0.1709%. The results indicated that the HPLC method is highly reproducible. In the accuracy assessment, the recovery was found to be 98.93 - 99.62%. Thus, the developed HPLC method is simple, sensitive, precise and highly accurate and required only a small quantity of plasma sample. This method is applicable to the estimation of PRT in rat plasma obtained in pharmacokinetic evaluation of liquid fill formulations for soft gels.

Evaluation parameters for PRT liquid fill formulations for soft gels

The formulations prepared were evaluated for various parameters like viscosity, pH, appearance, drug content, water absorption, drug-excipient compatibility and *in vitro* drug release studies and stability studies, BA/BE studies in rats.

Appearance: All liquid filling formulations of PRT were visually tested for clarity, colour and precipitation of drug if any. The formulations were clear, homogenous and free of precipitation.

pH

pH is another important parameter for liquid filling formulations. The two areas of critical importance are the effect of pH on solubility and stability. Liquid fill formulations for soft gels should have their pH in the range of 2.5 to 7.5. At pH values below 2.5, gelatin is hydrolyzed causing leakage of the soft gel, whereas at pH values above 7.5, gelatin may be either hydrolyzed or tanned (i.e., cross-linked) resulting in decreased solubility of the gelatin shell. The pH of all the formulations was close to 7.2. The pH of the soft gelatin fill formulation without drug was found to be 5.4 (placebo). Therefore, all the batches of the formulations are suitable for capsule filling.

Drug content estimation

The drug content was found to be in acceptable range for all formulations indicating uniform distribution of drug. Percent dug content was found to be in the range of 98.89 ± 0.05 - 99.68 ± 0.31.

Moisture absorption studies

The moisture absorption was found to be in the acceptable range for all formulations indicating their stability. Percent moisture absorption was found to be in the range of 1.17 ± 0.95 – 4.83 ± 0.64.

Rheological studies

Viscosity is one of the important parameters, which provides vital information during the optimization of the liquid filling formulation for soft gels. In general, the viscosity of liquid filling formulations for soft gels is in the range of 0.222-3000 cps.

Rheological studies were carried out for all the liquid filling formulations in Brookfield DV-II PRO viscometer. The viscosity was measured by plotting the shear stress on x- axis, and shear rate on y-axis. The resultant rheograms were straight lines. From the slope values viscosity was calculated for each formulation. The consistency and viscosity of the liquid fill formulations were related to each other because both are dependent on the concentration of PVP K-30.

Table 5: Viscosity data for Paracetamol formulations (0-6Months).

Formulae	Viscosity cps		
	0-M	3-M	6-M
F1	-	-	-
F2	79.46 ± 3.15	79.66 ± 3.05	73.26 ± 3.05
F3	37.95± 2.01	34.95± 2.61	31.95± 2.31
F4	59.24± 1.07	61.24± 1.37	58.24± 1.27
F5	1056.46 ± 3.42	1016.6 ± 3.12	1026.6 ± 3.02
F6 Placebo	219.78± 3.56	209.78± 2.56	229.78± 2.36
F7	216.92± 2.76	205.92± 2.16	215.92± 2.36
F8	138.65± 2.14	128.65± 2.34	124.65± 2.04

All the formulations showed Newtonian type of fluid behaviour. A significant increase in viscosity was observed with increase in PVP K-30 concentration, as, because of PVP K-30, the system offers more resistance

to flow. The decrease in shear viscosity with increasing shear rate is due to tendency of PVP K-30 molecules to orient more in the direction of shear. It is clearly evident that changes in the viscosity and consistency of liquid fill formulations for soft gels were because of change in concentration of PVP K-30. All the liquid ingredients in the formulations are co- solvents to ethanol. Therefore, solution form of dosage form, that shows Newtonian type of fluid behaviour, results in the liquid fill formulations for soft gels of PRT, in the present investigation suspension forms show pseudoplastic type of fluid behaviour when PVP K-30, PEG 400, are used. The viscosity of all the formulations was studied.

FTIR spectra for liquid fill formulations

The IR spectra of Paracetamol pure drug and all other formulations were obtained by KBr pellet method by ATR-FTIR spectrometer (Bruker, Germany). The characteristic spectrums were observed for all the formulations within specified I.R. ranges, indicates that there was no interaction between drug and excipients. From the overlay it was observed that there was no interaction between the PRT and other excipients. The presence of all major functional groups of PRT confirmed that there were no chemical interactions between the pure PRT and any of the excipients used.

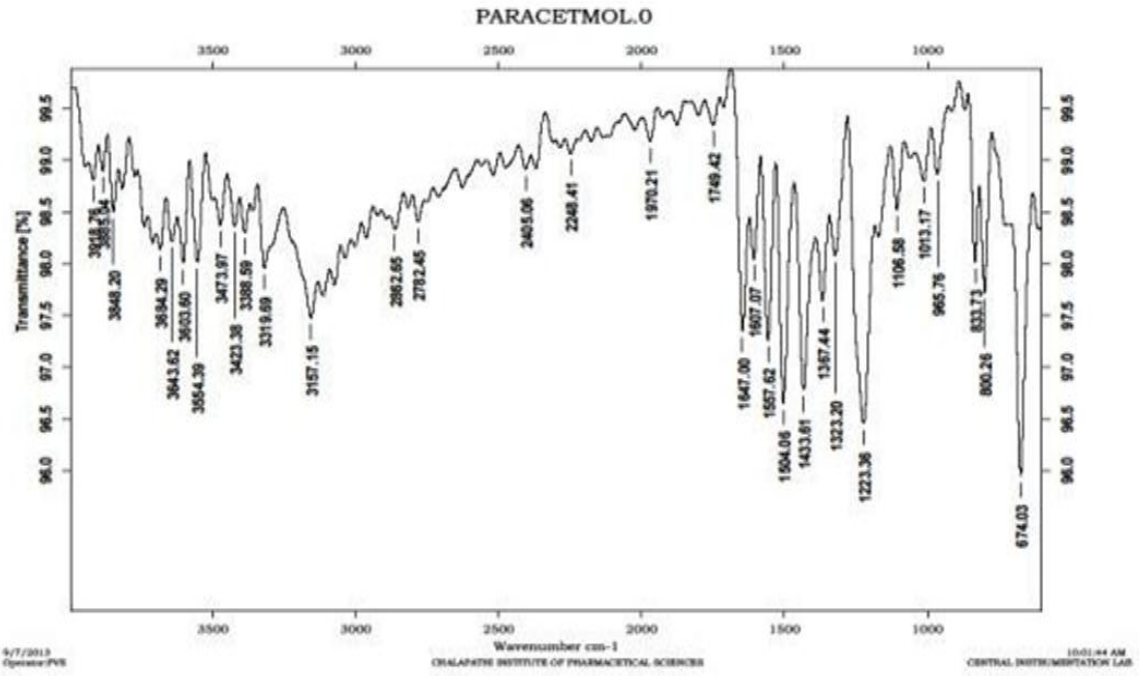

Fig. 1: FTIR spectrum of Paracetamol.

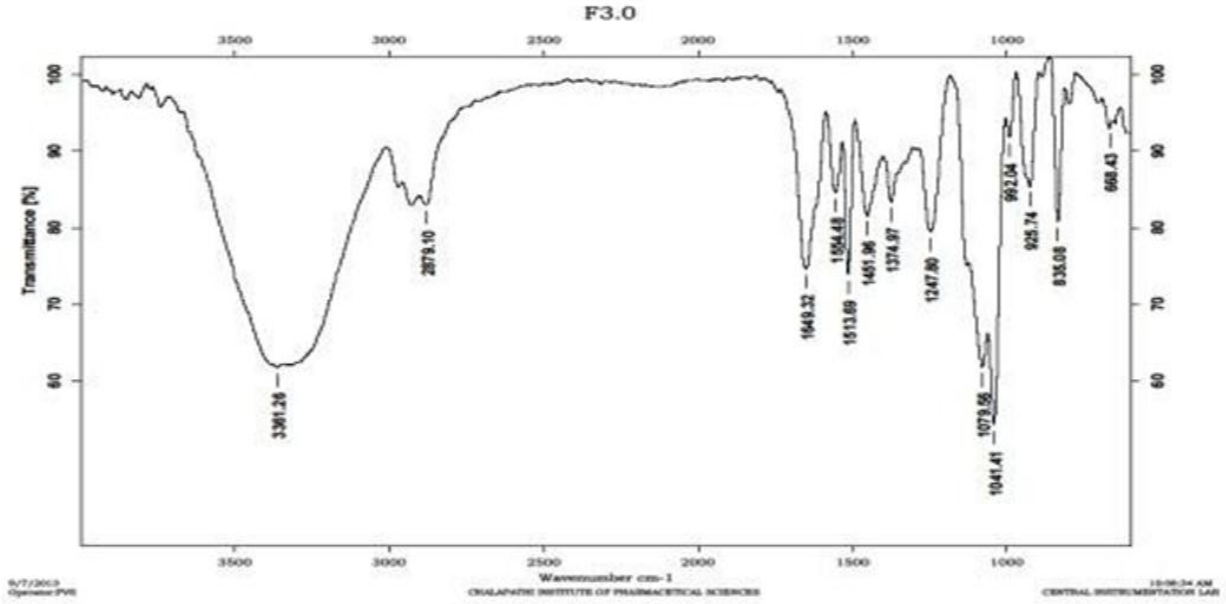

Fig. 2: FTIR spectrum of F3 paracetamol formulation.

***In-vitro* drug release studies for PRT liquid fill formulations**

Formulation F3 is having superior release properties, hence F3 was selected as optimized formulation. This confirms that water/PEG/PG system is better for Paracetamol release than Ethanol/PEG/PG and PVP/PEG/PG systems.

Table 5: Permeation parameters of F3 formulation and marketed Paracip-650.

Permeation parameter/variable	Dialysis membrane, molecular weight G12000	
	Paracip-650	F3 Formulation
Apparent Permeability Coefficient (cm/h)	0.025±0.012	0.815±0.006
Diffusion Coefficient (10^{-6}) (cm^2/s)	1.72±0.12	28.12±1.58

In vitro release studies were performed to assess the dissolution parameters like drug percent released and first order release kinetic data for the prepared PRT liquid fill formulations.

Significant increment in PRT permeation was observed (p<0.05) when compared to marketed formulation, paracip-650. The PRT flux values were also found to be more with F3 formulation. Significant higher amounts of PRT were found to be distributed in dialysis membrane at the end of 24 h with f3 formulation which is an indication of potential PRT deposition.

***In vitro* dissolution studies for PRT liquid fill formulations incorporated in soft gelatin capsules**

In vitro dissolution studies were carried out to evaluate the drug release from liquid fill formulations & tablets. Dissolution studies were conducted in 900mL of 0.1 N HCl at 37 ± 0.5^0 c. Totally eight different formulations of Paracetamol Liquid filled capsules were prepared from all liquid fill formulations. The comparative *in-vitro* dissolution profiles for all the formulations F1-F8 were shown in figure 3.The dissolution properties showed that in F1 formulation PVP K-30 was used and percent drug release was found to be 65% in 60min.Even though PEG-400 and PG were used the drug release was less. This may be due to incompatible co-solvents present.

In F2 formulation ethyl alcohol, PG, PEG were used instead of PVP K-30 then the percent drug release was found to be 100% in 15min.This may be due to compatibility of ethyl alcohol with PEG and PG. In F3 formulation water at 9% along with PEG 14% and PG 14% were used instead of PVP K-30 and ethyl alcohol. The percent drug release was found to be100% in 4min. Hence water is the best co-solvent for PEG and PG to Paracetamol drug release.

From the dissolution profiles it was observed that F3 containing water/PEG/PG system showed better drug release (100% in 4min) when compared to formulation F8 containing PVPK-30(100% in 6min). This may be due to increase in viscosity of the liquid fill formulation. Similarly when formulations F5 and F6 were compared, the difference is due to the ratio of PG:PEG.

Formulation F3 is having superior release properties, hence F3 was selected as optimized formulation. This confirms that water/PEG/PG system is better for Paracetamol release than Ethanol/PEG/PG and PVP/PEG/PG systems.

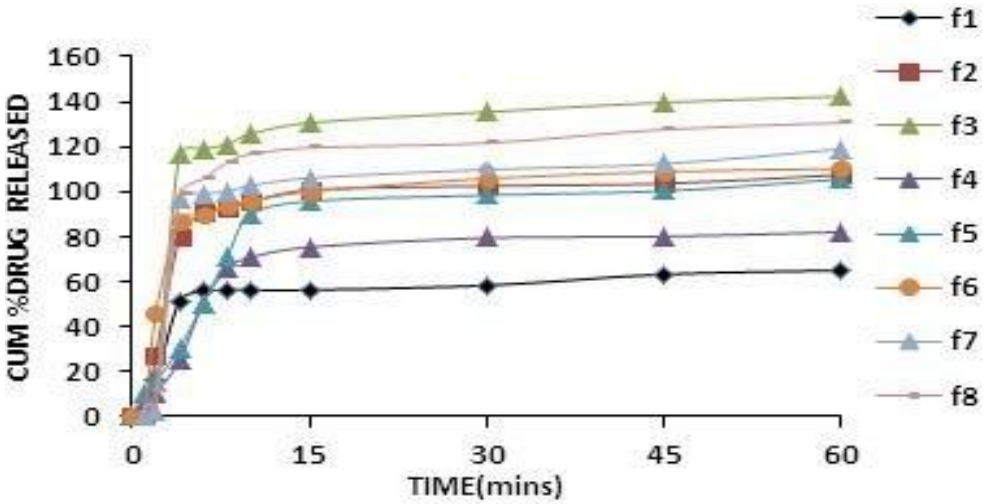

Fig. 3: Comparitive dissolution profile of Paracetamol Formulations.

Drug release kinetics & mechanisms

To analyze the *in-vitro* drug release data, various kinetic models were used to describe the release kinetics for both liquid filling and tablet formulations. Both liquid filling and tablet formulations followed the First order release kinetics. The 'k' values were significantly higher

for PVP K-30 containing liquid filling formulation when compared to other formulations.

The order of regression coefficient (R^2) values for all the formulations were F8>F7>F6>F2>F5>F3 >F4>F1. In F8 formulation higher the r^2 value and it indicates that F7 has superior release characteristics at 50 rpm speed among all the liquid fill formulations. Finally, the release kinetics was studied and showed that F3 better fits the first order release kinetics among all formulations. The viscosity modifier, PVP k-30 was added in this formulation as per the specified limits and better release of Paracetamol compared to remaining formulations.

Table 6: First order kinetics data for Paracetamol and liquid fill formulations for soft gels.

Formula	K value	R^2
F1	0.011	0.442
F2	0.340	0.955
F3	0.06	0.971
F4	0.027	0.659
F5	0.154	0.914
F6	0.340	0.936
F7	0.879	0.9
F8	1.077	0.79

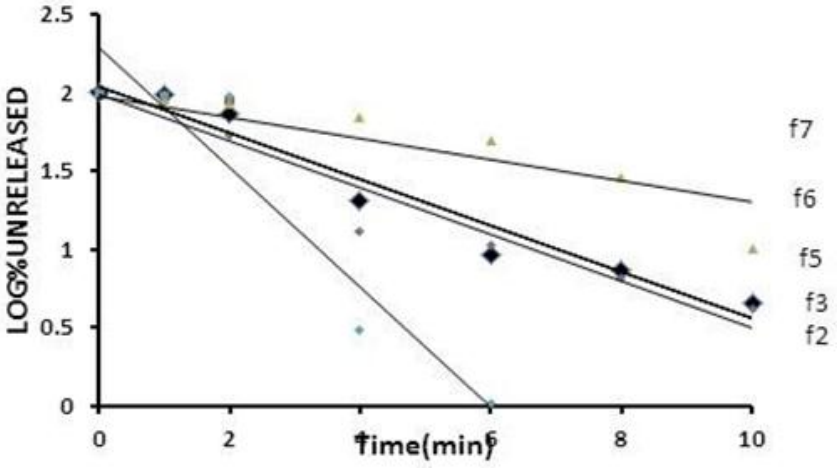

Fig. 4: Comparative first order plots of f2 , f3, f5, f6, f7.

Table 7: Comparitive stability studies for PRT pure drug and liquid fill formulations for soft gels (0-6 M).

Formulations	Appearance			Precipitation			Viscosity			pH			Drug content		
	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6
F1	*X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F6	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F7	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F8	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

X - No change

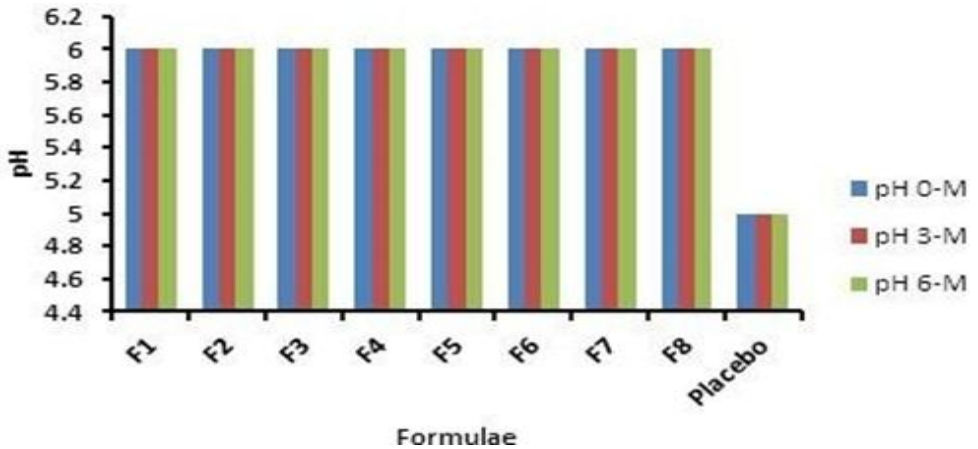

Fig. 5: Comparative profiles of pH at 0, 3 and 6 months.

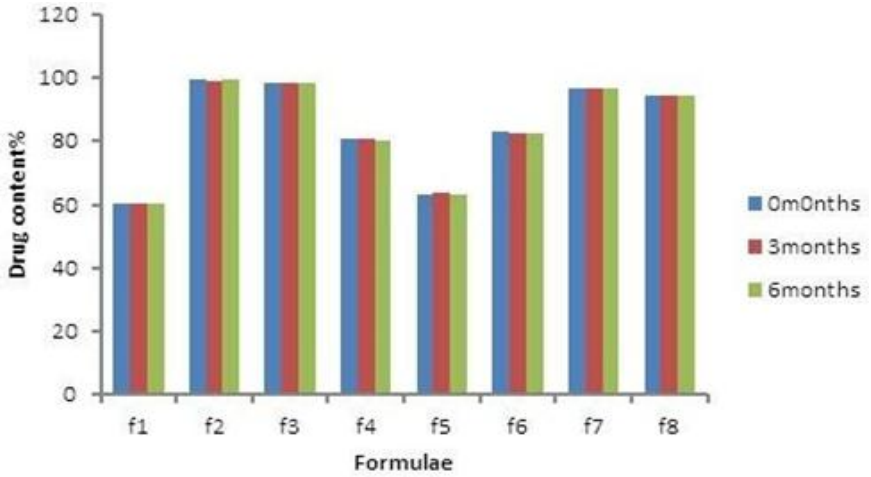

Fig. 6: Comparative profiles of drug content at 0, 3 and 6 months.

Fig. 7: Appearance at 0 months.

Fig. 8: Appearance at 3 months.

Fig. 9: Appearance at 6 months.

Bioavailability and Pharmacokinetic Evaluation of optimized formulations of Paracetamol

As per *in vivo* study protocol, PRT products were administered per orally to healthy rats and the plasma concentrations were determined by HPLC method. Oral LD 50 of Paracetamol in albino rats=0.77±0.02 gm/kg body weight. The test is liquid fill formulation of paracetamol 650 mg/kg. The standard is intravenous injection of paracetamol 650 mg/kg.

The compared marketed product is Paracip-650 of paracetamol 650 mg, administered as liquid (tablet powder suspension) suspended in 0.9 % sodium carboxy methyl cellulose to yield a concentration of 650mg of Paracetamol. All the formulations were prepared 30 minutes before administration at room temperature. Suspension was prepared with a magnetic stirrer. The dose administered was 650mg/kg. Rats of about 250 mg are selected for the study. Hence the lethal dose should not exceed 0.192 gm = 192 mg.

Pharmacokinetic parameters were determined, after the oral administration of test F3 formulation elimination rate constant k_{el} was found to be 0.109 hr^{-1} and the corresponding biological half-life ($t_{1/2}$) was found to be 1.514 hours. The MRT was found to be 3.99 hours. A peak plasma concentration of 310 ng/ml was observed at 1.5 hours after administration paracip-650. When the test F3 formulation was administered orally, peak concentration of 690 ng/ml was observed at 1.5 hours. The elimination rate constant k_{el} for test F3 formulation was found to be 0.1098 hr^{-1} and the corresponding biological half life ($t_{1/2}$) was found to be 1.28 hours. The MRT was found to be 4.205 hr. Very high C_{max}, AUC0-∞ (extent of absorption) and $AUMC_{0-\infty}$ were observed with F3 test formulation, when compared to paracip-650. So, it may be concluded, that the test formulation F3 is having higher absorption and bioavailability in comparison with the paracip-650.

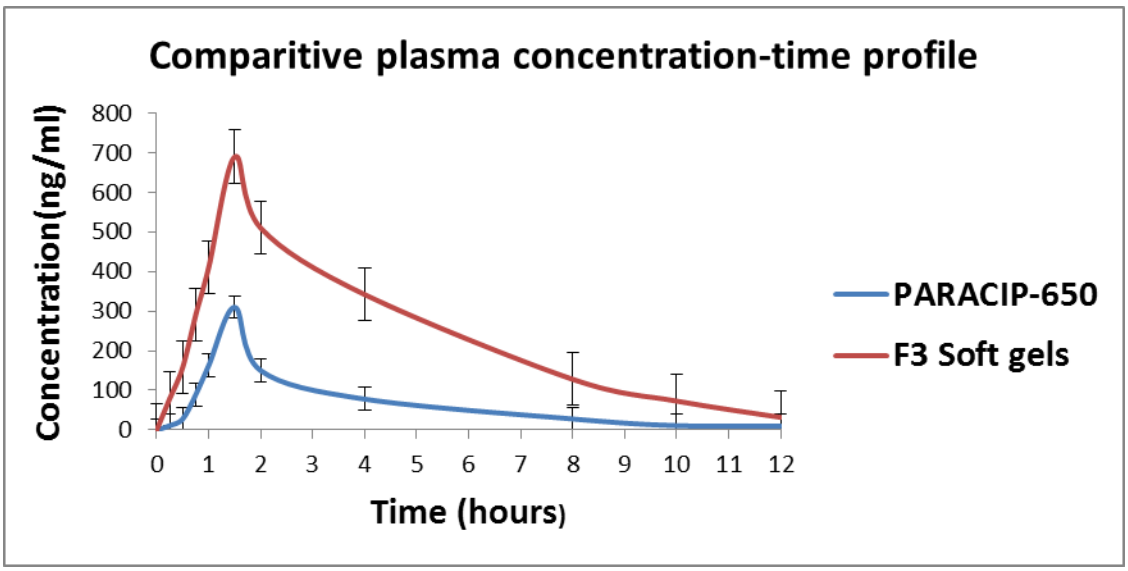

Fig. 10: Comparitive *in-vivo* studies of F3 formulation and paracip-650.

CONCLUSION

Paracetamol can be solubilised by the use of a co solvent system (PEG/PG/water, PEG/PG/ethanol) in liquid fill formulations and showed improved dissolution properties when compared to the Paracetamol marketed formulation. Liquid fill formulations with PEG/PG/water gave the superior results when compared to formulations containing PEG/PG/water/ethanol. All the liquid fill formulations showed good physicochemical properties. The formulations were stable up to 6 months without undergoing any degradation. When compared to existing conventional oral paracip-650 tablet having onset action time of 37 minutes and PRT intravenous injection (IV) pain relief onset time of 8 minutes (trained person is necessary for the IV administration and also the patient experiences pain at the site of action), F3 PRT-LF administered through soft gelatine capsules could be used for the faster onset of action which is evident from *in-vivo* studies in rats with good patient compliance.

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