Available online on www.ijddt.com
International Journal of Drug Delivery Technology 2017; 7(1); 1-12
doi: 10.25258/ijddt.v7i1.8911
ISSN: 0975 4415

Research Article

Development of An Antidiabetic Phytocomposite Loaded Phytoceutical Formulation, Its Quality Control and Pharmacokinetic Studies and Establishing *In Vitro- In Vivo* Correlation

De Baishakhi[1], Bhandari Koushik[1], Katakam Prakash[2], Adiki K Shanta[3], Mitra Analava[1*]

[1]*School of Medical Science and Technology, IIT Kharagpur, 721302*
[2]*SSS Indira College of Pharmacy, Vishnupuri, Nanded-431606*
[3]*Nirmala College of Pharmacy, Guntur, South India*

Received: 11th Dec, 2016; Revised: 15th Jan, 2017; Accepted: 18th Jan, 2017; Available Online: 1st March, 2017

ABSTRACT
This study reports the development of solid oral phytoceutical formulations with Phytocomposite (PHC), an antidiabetic poly herbal preparation as the active core material. Spherical, monolithic PHC microspheres of size range (10 -100 µm) were obtained with Hausner ratio, Carr's index and angle of repose of 1.141± 0.010, 12.418±0.769 and 25.17±0.96 respectively. Encapsulation efficiency amongst different batches (F1-F5) ranged from 96.8- 100.7, with 99% release profile up to 12h. Conventional and sustained release tablets were prepared by direct compression and compatibility amongst polymers and the PHC checked by FTIR studies. Natural polymers viz. gum kondagogu, gum karaya, *Aegle marmelos* gum were used as release retardant. Optimized batch of conventional tablets (F6) showed 99.8 % release in 35 min and optimized batch of PHC-SR tablets (F12) showed 99.9% release at 12th hr, both followed zero order kinetics and non-Fickian diffusion. These optimized formulations were subjected to stability studies and the similarity factors (f2) of the conventional and SR tablets were 88.75 and 66.76 respectively. Pharmacokinetic parameters of three formulations in rat plasma were analyzed by PK Solver 2.0. *In vitro-in vivo* correlation (IVIVC) of three different formulations showed Level A correlation in all cases.

Keywords: phytocomposite, microspheres, conventional, sustained release, phytoceutical, Level A correlation.

INTRODUCTION

Considering the multiple etiology of Type 2 diabetes, therapeutic strategies in treating Type 2 diabetes have undergone a radical change and focuses on multi dimensional aspects viz. hormonal effects, oxidative stress, cell signaling defects, hyper or hypo activities of enzymes etc[1,2]. Enzymes like alpha amylase, alpha glucosidase, aldose reductase, dipeptidyl peptidase 4 are considered to play a role in the pathogenesis of Type 2 diabetes[2]. Currently there has been a great resurgence of interest in phytomedicine in the treatment of chronic ailments. Pharmacologically active molecules from natural sources inhibiting such enzymes can serve as effective therapeutic entities in the management of Type 2 DM. Indian subcontinent is bestowed with natural phytomedicinal hub with several pharmacologically active phytochemicals that can serve as Natural enzyme inhibitors (NEIs) as well as active pharmaceutical ingredients (API) which can be implemented in the control of this chronic disease[2].

Combination therapy with poly herbals or phytoceuticals has gained popularity in terms of providing multiple and synergistic health benefits[1]. Oleanolic acid is found to provide a synergistic effect with first line antidiabetic metformin[3]. Sesame oil forms a synergistic antidiabetic combination with glibenclamide[4]. Research works of Mitra et al. have shown that Fenugreek-tulsi composite or composite prepared from the Tulsi leaves (*Ocimum sanctum*), Amla (*Emblica officinalis*), Bitter Gourd (*Momordica charantia*), Gurmur leaves (*Gymnema sylvestre*) and Jamun (*Syzygium cumini*) fruit and its seed help in controlling the blood gluco-lipid profile of Type 2 diabetics and is accepted by the indigenous or tribal populace of Bengal as surveyed in Binpur and Jhargram area of rural Bengal[5-7].

Ficus benghalensis (Indian Banyan tree, family *Moraceae*), *Syzigium cumini* (Jamun or Black pulm, family *Myrtaceae*) and *Ocimum sanctum* (Holy Basil or Tulsi, family *Lamiaceae*) have documented anti-diabetic potentials. A poly herbal product, named as phytocomposite (PHC), prepared from the leaf powders of Banyan, Jamun and Tulsi in varying weight ratios is found to show synergistic antioxidant and anti-diabetic actions in various *in vitro* enzyme inhibitory assays that are found to play a role in the pathogenesis of Type 2 diabetes[2].

Despite immense potentialities of the phytomedicines, the preparation and delivery pattern being traditional (either as whole extracts or individual herbs) problem arises due to patient noncompliance owing to organoleptic issues,

**Author for Correspondence: analavamitra@gmail.com*

proper dosing, stability problems and microbial contamination on storage, side effects due to improper processing of herbal extracts, variant ADME profile of the phytochemicals resulting in bioavailability problems etc. The ultimate result is the suppression of immense potentiality of herbal medicines[8]. If such herbal preparations can be developed into definite formulations, the associated problems can be eliminated. This study aims to develop PHC solid dosage formulations, their quality evaluations, pharmacokinetic studies and establishing *in vitro- in vivo* correlation (IVIVC). IVIVC can serve the purpose of bio waivers that can waive the need for expensive human trials and hence a suitable substitute for bioequivalence studies[9].

MATERIALS AND INSTRUMENTS

Chemicals

Microcrystalline cellulose, Starch, Talc, Magnesium stearate, Polyvinyl pyrrolidone, Chitosan, Lactose, Colloidal silica, Mannitol; Hydroxyl Propyl Methyl Cellulose, Ethyl cellulose, Acetone, n-Hexane, Paraffin, Ethanol. All chemicals and reagents used were either of analytical or HPLC grade and were purchased from either Sigma (India) or Merck (India).

Instruments

Electronic balance (Shimadzu BL-220H, Japan); Bulk density apparatus (Indo lab VTAP/MATIC-II, India); Standard sieve 30# (Jayant scientific, India); Hot air oven (Chemi Equipments, India); Tablet compression machine (Cadmach, Ahmadabad, India); Friability apparatus (Veego scientific VFT-DV, India); Hardness tester (Monsanto, India); Vernier caliper (Indolab, MITUTOYO, Japan); USP Type I tablet dissolution apparatus (LABINDIA DS 8000); Infrared spectroscopy (Thermo Nicolet Nexus 870)

Preparation of PHC

Fresh leaves of *Ficus benghalensis* (Banyan, Voucher Specimen: IITKGP/HB/2014/J1), *Syzigium cumini* (Jamun, Voucher Specimen: IITKGP/HB/2014/J2) and *Ocimum sanctum* (Tulsi, Voucher Specimen: IITKGP/HB/2014/J3) were collected from natural and man-made forest areas of IIT Kharagpur and adjoining areas like Balarampur, Gopali and Prembazar and authenticated. After proper washing in tap water, sun drying, dried leaves were grinded in electrical grinder (Bajaj GX 11) and stored in air tight amber colored plastic containers with proper labeling until use. For the preparation of the PHC, leaf powders of Banyan, Jamun and Tulsi were mixed in weight ratios (1:1:2) and subjected to 6h dynamic followed by 18h static cold maceration at 20±5°C for consecutive three days using hydroethanolic solvent (50:50). The extraction process parameters (solvent ratio and temperature) and ratios of phytocomposite were optimized by Response Surface Methodology (RSM). PHC exhibited maximum synergism in terms of poly phenol content, antidiabetic and antioxidant effect in different *in vitro* enzyme inhibitory assays[2].

Chemo profiling and characterization of PHC

PHC is a poly herbal formulation with antidiabetic and antioxidant effect, indicating the presence of some pharmacologically active molecules. Basing on MALDI-TOF spectrum (Figure 1) of the PHC and quantitative HPLC analysis (Table 1) the identified molecules in PHC include the presence of Lupeol, Eugenol, Ursolic Acid and Rutin which have been identified by comparing the retention times with authenticated standards and further characterized spectroscopically. Research results have recognized Eugenol and Rutin as potent pharmacologically active phytochemical in PHC in terms of antidiabetic potentials. So, they are considered as marker compounds of PHC.

Pre formulation studies

Pre formulation is defined as the phase of research and development process where physical, chemical and mechanical properties of a new drug substance or chemical entities are characterized alone and when combined with excipients in order to develop stable, safe and effective dosage form. A thorough understanding of physicochemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compound development[10-12].

Polymer- PHC compatibility studies

Compatibility amongst PHC and the polymers to be used in the formulations were studied by FTIR analysis (Figure 2). An IR spectrum of PHC and properly blended mixtures of PHC with the polymers used were recorded in FTIR spectrophotometer in the scanning range of 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The basic purpose was to observe any changes in the spectrum pattern of the PHC due to polymers and thus identify the chances of any chemical interactions.

Preparation of PHC formulations

Three different types of PHC formulations have been prepared: microspheres, conventional and controlled release tablets.

PHC Microspheres

Microspheres containing PHC as the core material was prepared by non aqueous solvent evaporation method with slight modifications of literature methods[13]. PHC and the polymers (Ethyl cellulose- EC, Chitosan –CH and Hydroxyl Propyl Methyl Cellulose or HPMC) were mixed with acetone in varying ratios, added to liquid paraffin and subjected to mechanical stirring for 8hr at 1500 rpm. After evaporating the solvent, microspheres were filtered, washed repeatedly with n-hexane to remove all traces of oil and dried at room temperature and stored in sterile containers.

The percent yield of the prepared microspheres were calculated using the formula

% yield= [(wt. of microspheres)/(wt of polymer +PHC)] X 100

Conventional PHC tablets (C- PHC)

C- PHC tablets were prepared by direct compression using single punch tablet machines (Cad mach, Ahmadabad, India) with modifications of the literature methodologies[10-12,14,15]. In direct compression, steps involved include milling of PHC and the excipients,

mixing of PHC with the excipients and finally compression into tablets. Basing on the prepared formula (Table 2) all ingredients were accurately weighed and sieved through #60 standard sieve. In order to mix the ingredients thoroughly, the drug and all the excipients were mixed geometrically in a mortar and pestle for 15 min and blended again thoroughly in a polythene bag. Tablets were compressed on a single punch tablet machine (Cad mach, Ahmadabad, India) using 10 mm punches. Hardness of the tablets was maintained about 4-5 kg/cm^2.

Sustained release PHC tablets (SR- PHC)

Direct compression method was employed to prepare the tablets of natural polymers in different PHC to polymer ratios as per the composition given in Table 3. This method involves (i) Milling of drug and excipients, ii) Mixing of drug and excipients; and (iii) Tablet compression. All the ingredients were accurately weighed and sieved through #60 standard sieves. In order to mix the ingredients thoroughly, PHC and all the excipients were mixed geometrically in a mortar and pestle for 15 min and blended again thoroughly in a polythene bag. Tablets were compressed on a single punch tablet machine (Cad mach, Ahmadabad, India) using 8 mm punches. Hardness of the tablets was maintained about 4-5 kg/cm^2 [10-12,14,15].

Micro meritic properties of pre compression evaluation of powder blend

PHC and polymer powder blends of different combinations for the purpose of tabletting were evaluated for flow properties by measuring Angle of Repose (fixed funnel method); Bulk Density (BD) and Tapped Bulk Density (TBD) by Cylinder method; Carr's Compressibility Index using the equation: Carr's Compressibility Index (%) = [(TBD-BD)/ TBD] x100; and Hausner's ratio was determined by the equation**:** Hausner's Ratio = TBD/ LBD (Table 4)**.** Hausner's ratio less than 1.25 indicates good flow while greater than 1.5 indicates poor flow using standard procedures. The values obtained after testing are compared with the standard values and inferences were drawn[10-12].

Quality evaluations of PHC formulations

PHC microspheres

Morphological characterizations of the microspheres (both empty and PHC loaded) were done by Scanning Electron Microscopy (SEM, Merlin) (Figure 3). The samples for SEM analysis were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100Å) before microscopy under a reduced pressure of 0.001 torr.

Size distribution and Wall thickness of the microspheres

For size distribution of PHC microspheres, different batches prepared were separated by sieving method and the amounts retained on different sieves were weighed.

Theoretical mean wall thickness of the microspheres were determined by the equation below

$$h = \frac{\bar{r}(1-P)d_1}{3[Pd_2 + (1-P)d_1]}$$

where, h = wall thickness (µm); r = mean radius of microspheres (µm); d_1 = density of core material (g/cm^3); d_2 = density of coat material (g/cm^3) and P = proportion of medicament in the microspheres[16].

Microencapsulation efficiency

The loaded core material is PHC whose chemo profiling has shown the presence of some pharmacologically active compounds[2]. About 50 mg of accurately weighed PHC loaded microspheres were powdered and analyzed for rutin content (one amongst the pharmacologically active compounds in PHC) spectrophotometrically at 359 nm (Table 5)[17].

Post-compression physicochemical evaluation of PHC tablets

The C -PHC and SR- PHC tablets thus prepared were evaluated for hardness using Monsanto hardness tester; friability was determined using Roche Friabilator; the thickness and diameter of the tablets were determined using Vernier calipers; weight variation test was carried out as per official methods with the specification limit that not more than two of the individual weight deviates from the average weight by 10 % and none should deviate by more than twice that percentage (Table 6)[18,19].

In vitro PHC release studies from the formulations

In vitro releases of PHC from the formulations (microspheres, conventional and SR tablets) have been studied in USP Type I tablet dissolution apparatus (LABINDIA DS 8000) at 37ºC, 100 rpm, using phosphate buffer media. About 50 mg of PHC loaded formulations were placed in the dissolution medium, 5 ml of samples were withdrawn at definitive time intervals and replaced with fresh dissolution media and analyzed for Rutin content (one amongst the pharmacologically active compounds in PHC). Briefly, 5ml of samples were withdrawn at different time intervals and replaced with same volume of the disso media, filtered through Whatmann filter paper No.41; net 0.5 ml of this solution diluted to 10 ml with methanol and observed in UV at 359 nm (Figure 4)[17].

The concentrations of PHC in the samples were obtained from the regression equation of the calibration curve. Same procedure was followed for conventional and SR tablets.

From the drug release data, the permeability coefficient (Pm) for various microspheres was calculated using the equation as described below:

$$P_m = \frac{K_{app} \cdot V \cdot H}{A \cdot C_s}$$

where Pm = permeability coefficient (cm^2/min), K_{app} = apparent dissolution rate constant calculated as mg/min from, the slope of the early linear portion, V = volume of dissolution medium (cm^3), H = wall thickness of microspheres (cm), A = surface area of the microspheres (cm^2) and Cs = solubility of the core in the dissolution medium (mg) (Table 7)[20].

In order to understand the kinetics and mechanism of release of PHC from the formulations, the results of the *in vitro* release studies were fitted to various kinetic equations like zero order (cumulative percent drug release *vs.* time); first order (log cumulative percent drug retained *vs.* time); Higuchi (cumulative percent released *vs.* √time); Peppas (log of cumulative percent drug release

Table 1: HPLC method validation parameters of the active compounds.

	Lupeol	Rutin	Eugenol	Ursolic acid
Retention time	16 min	39 min	14.62 min	6.5 min
Method validation parameters				
Linearity	10-160µg/ml	0.1-0.5µg/ml	0.4-10µg/ml	20-100µg/ml
Specificity	No interferences [b]	No interferences [b]	No interferences [b]	No interferences [b]
Sensitivity				
Limit of detection (LOD, µg/ml)	0.39	0.49	0.05	0.41
Limit of quantitation (LOQ, µg/ml)	0.97	0.98	0.27	1.27
Precision[a]				
Inter day	0.2 [a]	1.6[a]	1.08[a]	0.25[a]
Intraday	0.3 [a]	1.1[a]	0.03[a]	0.31[a]
Repeatability[a]	0.31 [a]	0.21 [a]	0.59 [a]	0.29 [a]
Ruggedness[a]	0.31 [a]	0.27 [a]	0.33 [a]	0.33 [a]
Accuracy, (80,100,120)%	96-101 (0.3) [a]	101.1-103.4 (0.3) [a]	99-103 (0.3) [a]	100.2 (0.3) [a]
Robustness , (Flow rate, mL/min, mobile phase ratio)	0.18 [a] (1.4) 0.21 [a] (1.6)	1.7 [a] (1.4) 1.7 [a] (1.6)	0.2 [a] (1.4) 0.4 [a] (1.6)	0.2 [a] (1.4) 0.2 [a] (1.6)

[a]Percentage relative standard deviation (%RSD), [b]Specific nature of method where no interferences suggests the specificity of the method. The values of all parameters are found to lie within the recommended limits.

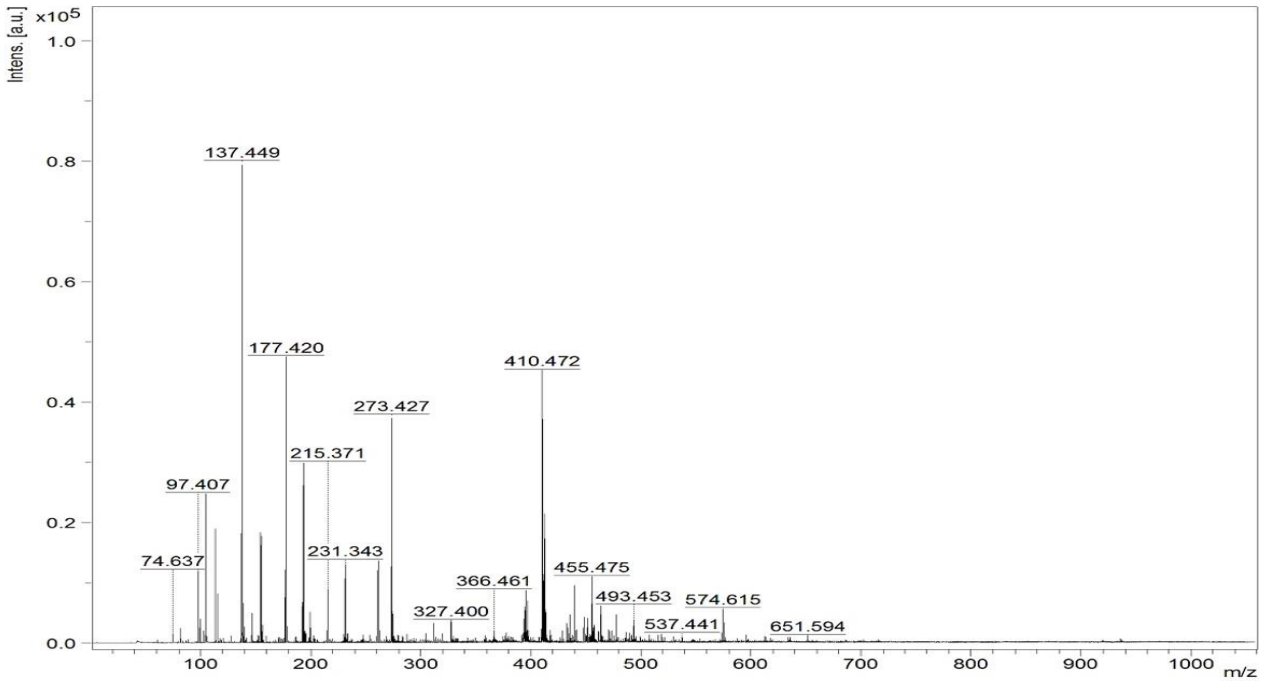

Figure 1: MALDI-ToF of the phytocomposite.

vs. log time). The kinetic model that best fits the dissolution data were evaluated by comparing the regression coefficient values (r) obtained in various models. The N values (release exponent) in Peppas model were used to characterize different release mechanisms, where values of n=0.5 indicates Fickian diffusion, values between 0.5–1.0 is for non-Fickian diffusion and n=1 indicates zero order (Table 8-9)[21-25].

Comparison of dissolution data

The dissolution profiles were further analyzed by difference factor (f1) and similarity factor (f2). Difference factor (f1) is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves[22,25]. The following equations were used to calculate f1 and f2 values:

$$f_1 = \frac{\Sigma_{t=1}^{n}|R_t - T_t|}{\Sigma_{t=1}^{n} R_t} X\ 100$$

$$f_2 = 50\ X \log\{[1 + (1/n)\Sigma_{t=1}^{n}(R_t - T_t)^2]^{-0.5}\ X\ 100\}$$

where 'n' is the number of points, R_t is the dissolution value of the reference product at time t and T_t is the dissolution value for the test product at time t. For

dissolution curves to be considered similar, f1 should be close to zero and f2 should be close to 100. Generally, f1 value ranges up to 15 (0–15) and f2 values greater than 50 (50–100) which ensures equivalence between two curves (Table 8-9).

Real time stability studies

product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. To observe the rate of product degradation at room temperature is time consuming. To avoid such undesirable delay, accelerated stability studies

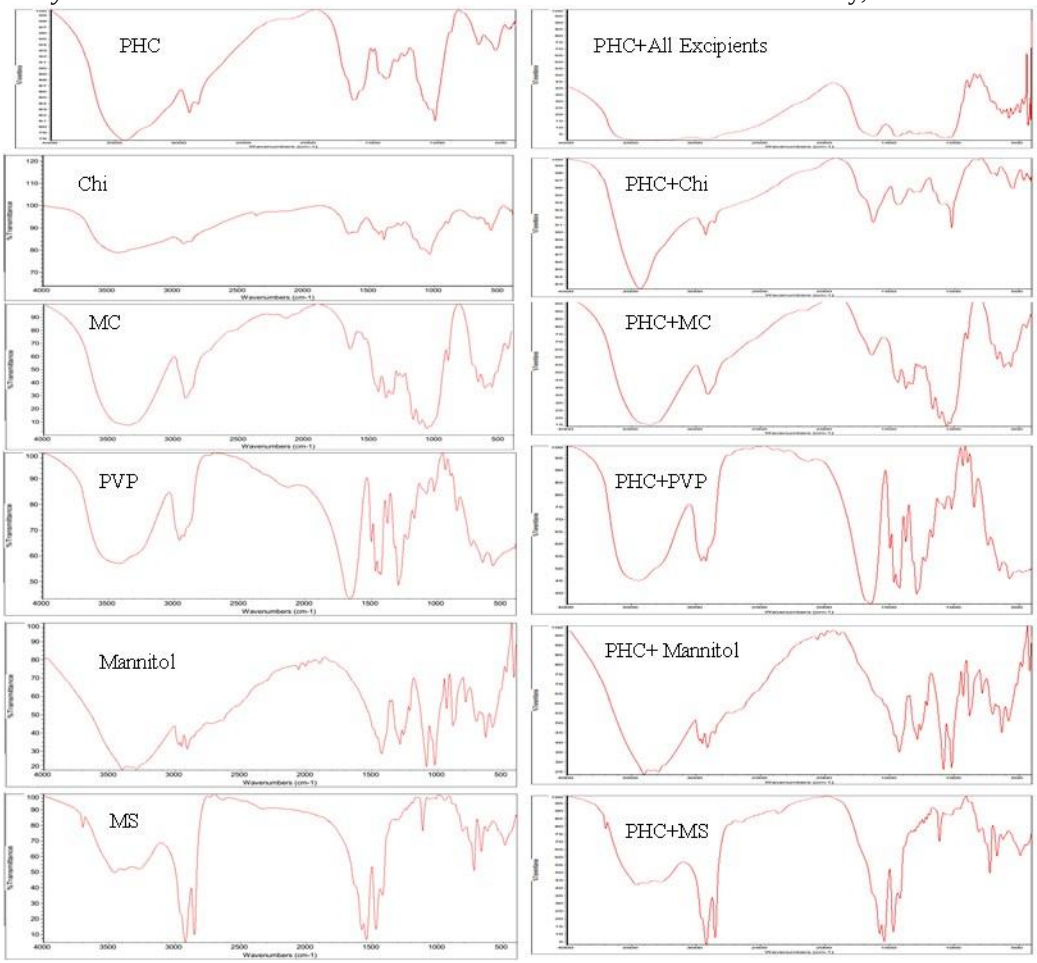

Figure 2: PHC-polymer compatibility studies by FTIR.

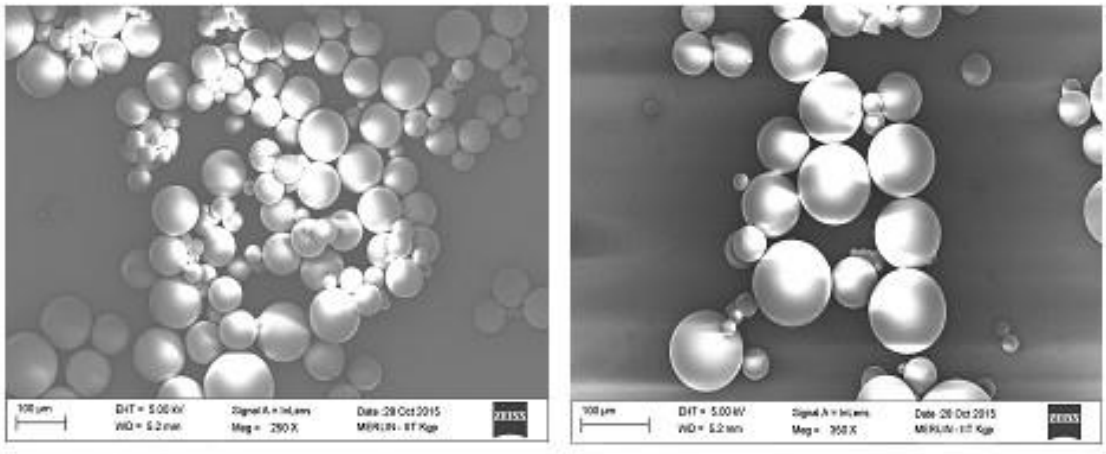

Figure 3: SEM images of empty (left) and PHC loaded microspheres (right).

The main purpose of stability testing is to provide evidence on how the quality of a drug substance or drug

have been adopted. Amongst different batches of formulations prepared, the one with most satisfactory *in*

vitro PHC release profile and high similarity factor was selected for stability studies. The optimized formulation batches were stored at 40°C, 75% RH in closed high density polyethylene bottles for 1.5 month (Table 10-11). The samples were withdrawn after periods of 1 and 2 month and were analyzed for its hardness, PHC content and *in vitro* release[10-12].

Pharmacokinetic profiling and in vitro- in vivo correlation

After obtaining permission from the animal ethical committee (Registration No: 1722/RO/ERe/S/13/CPCSEA, Approval No:

Table 2: Composition for PHC conventional tablets.

Ingredient Name	Use	F1	F2	F3	F4	F5	F6	F7	F8	F9
PHC	Active compound	500	500	500	500	500	500	500	500	500
Microcrystalline cellulose	Disintegrant	24	42	60	24	42	60	24	42	60
Lactose	Diluent	68.5	43	13						
Mannitol	Diluent				68.5	43	13			
Starch	Diluent							68.5	43	13
Talc	Lubricant	1.5	3	6	1.5	3	6	1.5	3	6
Magnesium Stearate	Lubricant	1.5	3	6	1.5	3	6	1.5	3	6
Colloidal Silica	Glidant	1.5	3	6	1.5	3	6	1.5	3	6
PVP	Binder	3	6	9	3	6	9	3	6	9

Table 3: Composition for PHC SR tablets.

Ingredients (mg)	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9	FN10	FN11	FN12
PHC	500	500	500	500	500	500	500	500	500	500	500	500
Gum kondagogu	50	100	150	200	-	-	-	-	-	-	-	-
Gum karaya	-	-	-	-	50	100	150	200	-	-	-	-
Aegle marmelos gum	-	-	-	-	-	-	-	-	50	100	150	200
Microcrystalline cellulose	220	170	120	70	220	170	120	70	220	170	120	70
Talc	10	10	10	10	10	10	10	10	10	10	10	10
Magnesium stearate	20	20	20	20	20	20	20	20	20	20	20	20
Total weight	800	800	800	800	800	800	800	800	800	800	800	800

Table 4: Pre compressional evaluation of Powder Blend for PHC conventional (C) & sustained release (SR) tablets.

Formulation Code	Formulation	Loose Bulk Density (gm/ml)*	Tapped Bulk Density (gm/ml)*	Hausner's ratio *	Carr's index (%)*	Angle of repose (θ°)*
F1	C	0.790±0.001	0.908±0.001	1.149±0.001	12.996±0.001	23.33±0.635
	SR	0.680±0.001	0.788±0.001	1.159±0.001	13.710±0.001	23.33±0.035
F2	C	0.683±0.006	0.792±0.007	1.160±0.003	13.763±0.004	24.29±0.028
	SR	0.613±0.006	0.732±0.007	1.194±0.003	16.257±0.004	21.29±0.028
F3	C	0.690±0.009	0.789±0.009	1.143±0.005	12.548±0.006	23.15±0.350
	SR	0.780±0.009	0.849±0.009	1.088±0.005	8.127±0.003	24.15±0.150
F4	C	0.796±0.001	0.899±0.001	1.129±0.001	11.457±0.001	25.48±0.330
	SR	0.796±0.001	0.809±0.001	1.016±0.001	1.607±0.001	22.48±0.330
F5	C	0.685±0.008	0.778±0.001	1.136±0.004	11.954±0.001	24.26±0.426
	SR	0.765±0.008	0.868±0.001	1.135±0.003	11.866±0.001	25.26±0.226
F6	C	0.780±0.001	0.895±0.006	1.147±0.001	12.849±0.002	25.78±0.203
	SR	0.630±0.001	0.855±0.006	1.357±0.001	26.316±0.003	24.78±0.203
F7	C	0.750±0.009	0.868±0.001	1.157±0.003	13.594±0.001	25.18±0.375
	SR	0.780±0.009	0.868±0.001	1.113±0.003	10.138±0.001	25.18±0.375
F8	C	0.743±0.001	0.899±0.001	1.210±0.001	17.353±0.001	23.56±0.201
	SR	0.653±0.001	0.799±0.001	1.224±0.001	18.273±0.001	21.56±0.101
F9	C	0.773±0.001	0.901±0.002	1.166±0.001	14.206±0.001	22.30±0.462
	SR	0.743±0.001	0.891±0.002	1.199±0.001	16.611±0.001	23.30±0.262
F10	SR	0.701±0.004	0.805±0.004	1.148±0.002	12.919±0.003	22.38±0.106
F11	SR	0.695±0.003	0.793±0.002	1.141±0.001	12.358±0.002	25.27±0.076
F12	SR	0.787±0.007	0.893±0.006	1.135±0.003	11.870±0.004	24.43±0.259

*All the values are expressed as mean± SE, n=3.

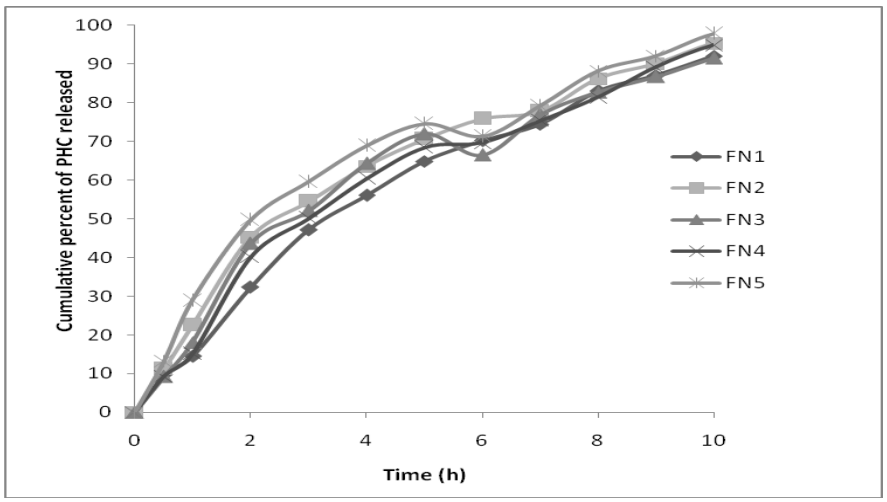

Figure 4: Release of PHC from the microspheres.

Table 5: Coat: Core ratio, PHC content, encapsulation efficiency and wall thickness of the microspheres prepare.

Microspheres	Coat:Core ratio	Percent PHC content		Encapsulation efficiency (%)	Wall thickness (µm)
		theoretical	practical		
F1	1:9	90	89.7 (1.58)	98.5	19.79
F2	2:8	80	79.6 (1.32)	96.8	31.81
F3	3:7	70	73.1 (4.12)	99.8	42.96
F4	4:6	60	62.3 (4.13)	100.3	51.15
F5	5:5	50	52.5 (3.51)	100.7	64.24

*figures in parentheses are coefficient of variation values.

ARTI/CPCSEA/2015/ARTI 09); healthy male Sprague-Dawley rats weighing between 200–250 g were purchased from local vendors and were kept in poly carbonated cages with bedding husk and maintained in lab feed and water *ad libitum*, as per CPCSEA guidelines[26,27].

Rutin (RUT) was found to be one of the pharmacologically active compound in the poly herbal product PHC and is considered as the marker compound. So, pharmacokinetic parameters were studied basing on the release of RUT. Twelve animals were divided into three equinumerous sub groups and mini tablets of PHC (microcapsules, conventional and sustained release tablets) designed particularly for the experimentation were placed firmly on the flat end of the gavage needle and inserted directly into stomach by intra gastric gavage[26]. Blood samples were withdrawn from the tail veins of each rats at 2,4,8, 10 and 12 h after administration of the tablets into heparinized tubes, subjected to immediate centrifugation at 5000 rpm for 15 min and the separated plasma stored at - 20^{o}C for future use for estimation of RUT.

HPLC analysis was done after proper pre-treatment of the plasma samples with some modifications of the literature methodologies[27,28]. Prior to HPLC analysis plasma samples were acidified with acetate buffer, centrifuged for 15 min at 5000 rpm, and the collected supernatants passed via Sep Pak C18 cartridges. The eluants were collected, diluted with methanol and injected in HPLC column with UV detection and chromatographic parameters set as per literature[27,28]. Pharmacokinetic parameters were determined with the help of PK Solver (Table 12).

Development of *in vitro- in vivo* correlation (IVIVC) has drawn the attention and being paid due consideration by the pharmaceutical industries, academics and regulatory sectors. IVIVC is a predictive mathematical tool establishing the relationship between *in vitro* drug release from dosage forms and a relevant *in vivo* response i.e. amount of drug absorbed or plasma drug concentrations. Correlations between *in vitro* testing's and *in vivo* performance are encouraged in terms of bio waivers. If *in vitro* tests can suitably reflect bioavailability data it is helpful to waive of bioequivalence studies on human volunteers. IVIVC can be developed at three main levels (A, B and C); level A (highest grade) relates the entire *in vitro* dissolution curve to *in vivo* absorption curve so that *in vitro* dissolution can serve as a surrogate marker for *in vivo* drug performances. By utilizing Statistical Moments Analysis at Level B of IVIVC, comparison can be made with the mean of any parameter of *in vitro* dissolution (e.g. mean dissolution time, $MDT_{in\ vitro}$) with the mean of any *in vivo* parameter (e.g. mean residence time, $MRT_{in\ vivo}$). Level C IVIVC establishes a single point correlation between an *in vitro* dissolution parameter (e.g. time to release 50% or 90% of the active drug, $T_{50\%}$ or $T_{90\%}$) and corresponding *in vivo* parameter (e.g. C_{max}, T_{max} or AUC)[9,29-39]. Non compartmental analysis of plasma data

Table 6: Post compressional evaluation of PHC conventional (C) & sustained release (SR) tablets.

Formulation Code	Formulation	Dimension		Hardness (kg/cm^2)**	Friability (%)*	Weight variation (%)***	Drug content (%w/w)*
		Diameter (mm)**	Thickness (mm)**				
F1	C	7.59±0.005	4.50±0.005	4.16±0.25	0.27±0.03	221.66±0.57	101.89±0.73
	SR	7.55±0.005	4.50±0.005	4.16±0.25	0.24±0.03	226.66±0.57	100.09±0.71
F2	C	7.58±0.005	4.55±0.005	4.33±0.25	0.35±0.07	226.16±0.28	98.67±0.26
	SR	7.64±0.005	4.62±0.005	4.33±0.25	0.34±0.07	226.16±0.28	99.17±0.16
F3	C	7.59±0.004	4.54±0.005	4.26±0.25	0.29±0.03	222.56±0.47	100.78±0.53
	SR	7.59±0.005	4.55±0.005	4.26±0.25	0.29±0.05	226.66±0.52	101.79±0.54
F4	C	7.59±0.004	4.55±0.005	4.31±0.25	0.31±0.05	224.23±0.44	99.59±0.29
	SR	7.62±0.005	4.57±0.005	4.25±0.25	0.31±0.05	225.52±0.54	98.47±0.71
F5	C	7.59±0.005	4.55±0.004	4.27±0.25	0.32±0.05	225.52±0.45	100.56±0.45
	SR	7.59±0.005	4.59±0.005	4.32±0.26	0.32±0.04	226.34±0.55	101.23±0.63
F6	C	7.59±0.005	4.52±0.004	4.31±0.25	0.35±0.06	226.49±0.41	100.67±0.71
	SR	7.61±0.005	4.61±0.005	4.31±0.25	0.27±0.06	225.29±0.51	99.25±0.52
F7	C	7.58±0.005	4.54±0.004	4.29±0.24	0.29±0.05	226.53±0.51	101.01±0.54
	SR	7.59±0.005	4.59±0.005	4.29±0.25	0.31±0.07	226.78±0.32	100.25±0.57
F8	C	7.58±0.005	4.55±0.005	4.33±0.25	0.33±0.07	224.61±0.32	99.87±0.65
	SR	7.62±0.005	4.57±0.005	4.30±0.25	0.31±0.07	226.61±0.28	98.54±0.54
F9	C	7.58±0.005	4.55±0.005	4.23±0.25	0.31±0.07	225.62±0.35	98.81±0.29
	SR	7.59±0.004	4.58±0.005	4.21±0.23	0.32±0.05	226.62±0.29	99.78±0.54
F10	SR	7.64±0.005	4.62±0.005	4.19±0.25	0.33±0.04	226.52±0.29	100.89±0.52
F11	SR	7.60±0.005	4.61±0.005	4.29±0.25	0.34±0.05	226.53±0.51	100.21±0.51
F12	SR	7.62±0.005	4.60±0.005	4.31±0.25	0.34±0.06	226.43±0.54	99.39±0.55

*All the values are expressed as mean± SE, n=3.**All the values are expressed as mean± SE, n=6. *** All the values are expressed as mean± SE, n=10.

Table 7: Permeability coefficient (Pm) values of the prepared microspheres.

Microspheres	Coat: Core ratio	Pm values (cm^2/min)
F1	1:9	8.12
F2	2:8	14.25
F3	3:7	15.11
F4	4:6	14.98
F5	5:5	16.17

was done using PK Solver 2.0.

In this study the IVIVC was determined by plotting the percentage of RUT released (RUT_{rel}) *in vitro* (from dissolution studies of the PHC formulations) against the cumulative percent of RUT excreted in urine for a sufficient period of time which is directly related to the total amount of RUT absorbed.

RESULTS

The FTIR spectrum (Figure 2) did not show any interactions amongst PHC and the polymers used in the formulations suggesting the compatibility amongst them. The dried microspheres were weighed and the percent yield was determined to be 86. The Coat: Core ratio, PHC content, encapsulation efficiency and wall thickness of the microspheres prepared is shown in Table 5. The SEM images (Figure 3) of empty as well as PHC loaded microspheres shows spherical shapes with the optimized procedure and size distributions have shown that most of the microspheres are within 40-70 µm and some were within 70-100 µm, very few where found between 10-40 µm. The release characteristics of active constituents from PHC microspheres and the permeability coefficient values are presented in Figure 4 and Table 7. The Hausner ratio, Carr's index and angle of repose of PHC microspheres thus prepared were found to be 1.141± 0.010, 12.418±0.769 and 25.17±0.96 respectively, which on comparison with the standard values were inferred to show good to excellent flow properties.

The formula adopted for the preparation of C -PHC and SR- PHC tablets are shown in Table 2-3 and the results of pre-compressional evaluation of the powder blends and post compressional evaluations of prepared tablets in Table 4 and Table 6 respectively. The kinetics of release of PHC from the conventional and SR formulations are presented in Table 8-9. The stability studies of the optimized batch in two different formulations are provided in Table 10-11. Table 12 shows the details of the pharmacokinetic values of the three formulations (microcapsules, conventional and SR tablets). IVIVC of three different formulations were in the order of sustained release (r^2= 0.99) > microcapsules (r^2= 0.97) > conventional (r^2= 0.90) and from the values it is evident that Level A correlation have been established amongst three different types of formulations.

DISCUSSIONS

The available SEM images of the PHC microspheres (Figure 3) were found to be smooth surfaced, spherical and free flowing. The low coefficient of variation in the percent PHC content (Table 5) indicated uniformity in the batch. The encapsulation efficiency ranged from 97.2 –

Table 8: Kinetics of the release of PHC from conventional tablets.

Code	Zero order		First order		Higuchi		Korsemeyer- Peppas		f2 values
	R^2	K_0 (mg/h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H (mg $h^{-1/2}$)	R^2	N	
F1	0.8753	4.9647	0.8511	0.2787	0.9817	23.947	0.9103	0.5107	84.87
F2	0.9451	4.3944	0.8159	0.2967	0.983	22.591	0.9088	0.3956	77.65
F3	0.9029	5.0994	0.8053	0.2951	0.9753	24.074	0.8908	0.5042	76.81
F4	0.9629	4.9178	0.8805	0.3754	0.984	24.339	0.8562	0.3924	78.75
F5	0.8784	4.862	0.8359	0.2167	0.983	23.091	0.9608	0.5142	81.79
F6	0.9947	5.102	0.8026	0.2354	0.9962	24.561	0.9942	0.5361	88.75
F7	0.9561	4.4105	0.8045	0.3075	0.9891	22.691	0.9503	0.424	83.24
F8	0.9457	4.875	0.8402	0.3416	0.9667	24.501	0.9426	0.4501	79.62
F9	0.9231	4.7517	0.8976	0.2591	0.9792	23.121	0.932	0.4431	72.91

K_0 = zero order rate constant, K_1 = first order rate constant, R^2 = correlation coefficient, K_H= Higuchi constant, N = Koresmeyer Peppas constant, f2 = similarity factor.

Table 9: Kinetics of the release of PHC from SR tablets.

Code	Zero order		First order		Higuchi		Korsemeyer-Peppas		f2 values
	R^2	K_0 (mg/h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H (mg $h^{-1/2}$)	R^2	N	
F1	0.8823	4.8547	0.9011	0.2687	0.9817	24.947	0.9003	0.5017	54.87
F2	0.9423	4.3944	0.8359	0.2167	0.983	22.09	0.9388	0.3956	57.65
F3	0.8929	5.0994	0.8923	0.2321	0.9753	23.674	0.8708	0.5542	62.31
F4	0.9519	4.5178	0.8585	0.3224	0.984	24.339	0.8432	0.3824	60.75
F5	0.8704	4.712	0.8359	0.2167	0.983	22.09	0.9708	0.5542	51.39
F6	0.878	5.7258	0.8997	0.2795	0.986	21.46	0.9307	0.4512	64.21
F7	0.8933	4.5309	0.8602	0.2273	0.9662	23.709	0.9265	0.3941	55.87
F8	0.8929	5.0994	0.8788	0.3238	0.9827	25.599	0.9104	0.4377	58.19
F9	0.9461	4.2135	0.8045	0.2975	0.9791	23.641	0.9473	0.324	63.74
F10	0.9357	4.805	0.8402	0.3896	0.9867	25.201	0.9536	0.4501	61.62
F11	0.9121	4.9417	0.8976	0.3591	0.9892	24.121	0.9207	0.4231	64.91
F12	0.9931	5.102	0.8156	0.2254	0.9912	24.561	0.9912	0.510	66.76

K_0 = zero order rate constant, K_1 = first order rate constant, R^2 = correlation coefficient, K_H= Higuchi constant, N = Koresmeyer Peppas constant, f2 = similarity factor.

Table 10: Stability studies of PHC conventional tablets.

Characteristics	Initial	1 month	2 month
Hardness (kg/cm^2)	5.00±0.07	5.00±0.04	5.00±0.04
PHC content (%mg/tablet)	99.08±0.12	98.98±0.37	98.91±0.12
In-vitro drug release at 40 min (%w/w)	100.83±1.38	100.54±01.53	100.53±1.90

All data is expressed as mean ±SD, m = month(s).

Table 11: Stability studies of PHC SR tablets.

Characteristics	Initial	1 month	2 month
Hardness (kg/cm^2)	5.03±0.07	5.01±0.04	5.00±0.04
PHC content (%mg/tablet)	99.68±0.12	98.87±0.37	98.81±0.12
In-vitro drug release at 16 hour(%w/w)	99.98±1.38	99.87±01.53	99.75±1.90

All data is expressed as mean ±SD, m = month(s).

100.6% and the PHC microspheres being spherical, their theoretical mean thickness of the wall calculated as per Luu et al. (1973) have shown that the wall thickness varied basing on the core: coat ratio (Table 5). The permeability values of the microspheres also varied basing on the core: coat ratios (Table 7). Basing on the optimized procedure the micromeritics of the prepared microspheres showed good free flowing granules. The release characteristics of PHC from the microspheres showed a controlled release profile with the formulations (F1- F5) where above 99% up to 12h. The microspheres prepared are of monolithic type where the active

Table 12: Pharmacokinetic parameters of three PHC formulations in rat plasma.

Formulation	Pharmacokinetic values								
	$[AUC]_{0-t}$ µg/mL/h	$[AUC]_{0-\alpha}$ µg/mL/h	$[AUC]_{o-t/0-\alpha}$ µg/mL/h	$t_{1/2}$ h	Cl/F (mg/µg/mL/h)	C_{max} µg/mL	T_{max} h	$AUMC_{0-\alpha}$ (µg/mL/h^2)	$MRT_{0-\alpha}$ h
CT	301.4±38.5	356.4±48.5	0.845±0.021	3.3±1.3	0.257±0.012	37.4±6.2	2.1±.84	2961.7±29.3	6.87±1.2
MC	355.7±27.8	380.7±45.6	0.934±0.035	5.5±2.9	0.019±0.005	39.7±5.9	5.9±2.2	3116.5±32.5	12.77±1.6
SR	321.9±24.7	344.4±41.4	0.933±0.102	6.4±2.3	0.016±0.003	38.2±6.5	8.5±3.1	2652.9±36.8	16.13±2.3

**CT- conventional tablets; SR- sustained release tablets; MC- microcapsules.

constituent PHC have been distributed throughout the matrix. The release of PHC from the microspheres followed first order kinetics and the mechanism of release was diffusion controlled. The microspheres being of monolithic type the path of travel of the core material (PHC) is not constant, as the material at the center has a longer path to travel than the material at the surface. From the results of BD, TBD, Hausner's ratio, Carr's Compressibility Index and Angle of repose it can be inferred that the powder blend exhibited good flow properties both for conventional and sustained release PHC tablets (Table 4). The PHC conventional and SR tablets formulated didn't show any visual defects like capping, chipping and lamination after punching. The results of physico-chemical evaluations of the conventional and SR tablets (Table 6) showed that the prepared tablets indicated good mechanical strength, the percentage friability of all the formulations in both cases were found to be less than 1%, percentage deviation from average tablet weight for all the formulations were within the Indian Pharmacoepial specified limits. Slight variances of PHC content among different batches of conventional and SR tablets suggest the uniformity amongst the prepared batches (Table 6).

After fitting the dissolution data of the PHC tablet formulations into different kinetic equations, in case of conventional tablets F6 showed 99.8 % release in 35 min and showed good linearity (R^2=0.99) indicating zero order release kinetics (Table 8) with non-Fickian diffusion the release mechanism controlled partly by diffusion and erosion. Thus, F6 is the optimized batch for PHC conventional tablets. In case of PHC-SR tablets F12 showed 99.9% release at 12th hr with good linearity (R^2=0.99) indicating zero order release kinetics (Table 9) with Fickian diffusion and is considered as the optimized SR formulation.

These optimized formulations were packed in aluminum foil and subjected to stability studies and the similarity factors (f2) of the conventional and PHC - SR formulations were 88.75 and 66.76 respectively which lies between 50 and 100. There exists a close similarity between the dissolution profiles of the tested formulation before and after stability studies. The results of real time stability studies under accelerated conditions showed appreciable stability of the PHC conventional and SR tablets (Table 10-11) showing that the adopted methodology was successful in developing the formulations.

Natural polymers are an attractive choice for drug release rate retardant owing to their non-toxicity, biocompatibility and biodegradability. Amongst the twelve trial formulations of PHC-SR tablets; gum karaya, gum gondagogu and *Aegle marmelos* gum were used for the purpose of controlling the release profile of PHC. However optimum release profile and desired flow kinetics were achieved with *Aegle marmelos* gum (AMG) as release rate controlling polymer in F12. Gums are polysaccharides which are polymeric in nature. AMG contains oligosaccharides viz. 3-0-β-D galactopyranosyl-L-arabinose, 5-0-β-D galactopyranosyl-L-arabinose, 3-0-β-D galactopyranosyl-D-galactose, uronic acids and finds use as a tablet binder and drug release is influenced by the binder concentration[40-42]. Literature evidences have also shown that AMG has high thermal stability and can be safely used in procedures subjected to thermal stress, having a high swelling index this natural polymer also finds use as disintegrating and matrixing agent[42]. Use of AMG as rate controlling polymer can serve as value addition to the antidiabetic actions of the active constituent PHC. In all the three PHC formulations correlation amongst *in vitro* release vs *in vivo* absorption being above 0.9 shows a strong relation exists.

Parameters C_{max}, T_{max}, $AUC^{0-\alpha}$ are considered as the main variables whereas $AUC^{o-t/0-\alpha}$, t1/2 are regarded as secondary pharmacokinetic parameters. Amongst the three PHC formulations, as evident from the T_{max} values, absorption rate from conventional tablets are much higher followed by the microspheres and SR tablets. Low T_{max} indicates faster absorption rate and high T_{max} values slower absorption rate; the delayed absorption is attributed to the sustained release action and release retardant effect of the polymers used. Not much variation was found in the C_{max} and AUC values of the three formulations (Table 12). From the AUC values it can be concluded that all the three formulations maintained the plasma concentrations of the PHC level (Table 12). The smaller half life (t1/2) of the CT indicates their rapid removal from plasma and supported by its clearance and the longer half life of MC and SR are indications of slower drug disposition and sustained release effect. The

sustained release effects of the MC and SR are also supported from the MRT values, showing prolonged retention in plasma (Table 12).

CONCLUSION

Previous experimentations have shown the antidiabetic potentialities of PHC, but PHC cant be consumed in its powdered form and for better acceptability, long term storage and providing the desired effective dose, its different solid dosage formulations have been prepared whose quality evaluations were compliant with the Pharmacoepial specifications with desirable release kinetics and stability profile. Developed formulations exhibited good pharmacokinetic profile basing on the desired requisites of the formulations and an attainment of Level A IVIVC.

ACKNOWLEDGEMENT

The SRF fellowship to first author has been provided by WBDST sponsored DTE project (Sanction Order No: 904(SANC)/ST/P/S and T/9G-14/2011) at IIT Kharagpur. The sponsor is deeply acknowledged. The lab facilities of Nirmala College of Pharmacy and Central Research Facilities of IIT Kharagpur are deeply acknowledged.

REFERENCES

1. De B, Bhandari K, Chakravorty N, Mukherjee R, Gundamaraju R, Singla RK, Katakam P, Adiki SK, Ghosh B, Mitra A. Computational pharmacokinetics and *in vitro-in vivo* correlation of antidiabetic synergistic phyto-composite blend. World Journal of Diabetes 2015; 6: 1179-1185.
2. De B, Bhandari K, R, Singla RK, Katakam P, Samanta T, Kushwaha DK, Gundamaraju R, Mitra A. Chemometrics optimized extraction procedures, phytosynergistic blending and *in vitro* screening of natural enzyme inhibitors amongst leaves of Tulsi, Banyan and Jamun. Pharmacognosy Magazine 2015; 11: 522-532.
3. Wang X, Cheng Y, Abdelkader D, Hassan W, Sun H, Liu J. Combination therapy with oleanolic acid and metformin as a synergistic treatment for diabetes. Journal of Diabetes Research 2015; 2015: 1-13.
4. Sankar D, Ali A, Sambandam G, Rao R. Sesame oil exhibits synergistic effect with anti-diabetic medication in patients with type 2 diabetes mellitus. Clinical Nutrition 2011; 30: 351-358.
5. Mitra A, Bhattacharya D. Dose-dependent effects of Fenugreek composite in Diabetes with dislipidaemia, Internet. Journal of Food Safety 2006; 8: 49-55.
6. Mitra A. Effects of a Composite of Tulsi Leaves, Amla, Bitter Gourd, Gurmur Leaves, Jamun Fruit and Seed in Type 2 Diabetic Patients. Journal of Clinical and Diagnostic Research 2007; 6: 511-520.
7. Mitra A. Some Salient Points in Dietary and Life-Style Survey of Rural Bengal Particularly Tribal Populace in Relation to Rural Diabetes Prevalence. Ethno-Medicine 2008; 2: 51-56.
8. Dey B, Katakam P, Mitra A, Chandu BR. Comparative Evaluation of Hypoglycemic Potentials of Eucalyptus Spp. Leaf Extracts and their Encapsulations for Controlled Delivery. Journal of Pharmaceutics and Drug Delivery Research 2014; 3(2): 1-10.
9. Dey B, Katakam P, Assaleh FH, Chandu BR, Adiki SK, Mitra A. *In vitro–in vivo* studies of the quantitative effect of calcium, multivitamins and milk on single dose ciprofloxacin bioavailability. Journal of Pharmaceutical Analysis 2015; 5: 389-395.
10. Lachman L, Liberman HA. The Theory and Practice of Industrial Pharmacy, 7th Edn. 2009, 297, 300.
11. Radhika PR, Karanam D, Sivakumar T. Fabrication of sustained release matrix tablets of venlafaxine hydrochloride using eudragits. International Journal *of* Research *in* Ayurveda *and* Pharmacy 2011; 2: 1386-1389.
12. Atul AB, Prashant Z, Chandewar AV, Jaiswal SB. Designing and Evaluation of Extended Release Tablet of Venlafaxine Hydrochloride Using Hydrophobic Matrix. Der Pharmacia Lettre 2010; 2: 329-335.
13. Garud N, Garud A. Preparation and *in vitro* evaluation of metformin microspheres using non-aqueous solvent evaporation technique. Tropical Journal of Pharmaceutical Research 2012; 11: 577-583.
14. Shivanand P, Viral D. Development and *In vitro* Evaluation of Propranolol Hydrochloride Based Gastro–Retentive Floating Tablet. Der Pharmacia Lettre 2010; 2: 75–86.
15. Swapna V, Sashmitha SB, Konde A. Formulation and evaluation of floating ranitidine hydrochloride tablets by using moringa gum as a functionality carrier. International Journal of Pharmaceutical Sciences Review and Research 2012; 16: 116–120.
16. Luu SN, Patrick F, Pierre CD, Jean G, Didier L. Determination of coating thickness of microcapsules and influence upon diffusion. Journal of Pharmaceutical Sciences 1973; 62: 452–455.
17. Pawar NP, Salunkhe VR. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Rutin and Gallic Acid in Hydroalcoholic Extract of Triphala churna. International Journal of PharmTech Research 2013; 5: 724-729.
18. Phoke SV, Sakarkar DM, Mitkare SS. Formulation and characterization of sustain release matrix tablets of venlafaxine hydrochloride by using natural polymers. International journal of pharmaceutical frontier research 2012; 2: 14-26.
19. Raju K, Deepika B, Prasanna L, Gouthami M, Anusha G. Formulation and *in vitro* evaluation of nizatidine floating tablets: effect of natural and synthetic polymers on floating properties. International Journal of Ayurveda and Pharma Research 2013; 4: 1358–1367.
20. Koida Y, Kobayashi M, Samejima M. Studies on microcapsules. IV. Influence of properties of drugs on microencapsulation and dissolution behavior. Chemical and Pharmaceutical Bulletin 1986; 34: 3354 -3361.
21. Demirturk E, Oner L. Evaluation of *in vitro*

dissolution profile comparison methods of immediate release Gliclazide tablet formulations. journal of Faculty of Pharmacy 2005; 25: 1–10.
22. Costa P, Lobo MS. Modelling and Comparison of Dissolution Profiles. Euro. Journal of Pharmaceutical Sciences 2001; 13: 123–33.
23. Colombo P, Bettini R, Santi P, Peppas NA. Swellable Matrices for Controlled Drug Delivery, Gel Layer Behaviour, Mechanisms and Optimal Performance. Pharmaceutical Science & Technology Today 2000; 3: 1-12.
24. Ritger PL, Peppas NA. A Simple Equation for Description of Solute Release II. Fickian and Anomalous Release from swellable Devices. Journal of Controlled Release 1987; 5: 37-42.
25. Ma MC, Lin RP, Liu JP. Statistical evaluations of dissolution similarity. Statistica Sinica 1999; 9: 1011–1027.
26. Resztak M, Hermann TW, Sawicki W, Danielak DZ. Pharmacokinetics and Pharmacodynamics of Gliclazide from Immediate and Modified Release Formulation Tablets in Rats. Iranian Journal of Pharmaceutical Research 2014; 13: 29-37.
27. Kayoko S, Kazuma Y, Taketoshi K, Yumiko U, Takashi Y. Absorption and Urinary Excretion of Quercetin, Rutin, and αG-Rutin, a water soluble Flavonoid, in Rats. Journal of Agricultural and Food Chemistry 2003; 51: 2785−2789.
28. Ecker A, Vieira FA, DeSouza PA, DosSantos MM, Ramos A, Ferreira RD, Teixeira GDM, Klimaczewski CV, Seeger RL, daRocha JBT, Barbosa dV NB. Effect of *Syzygium Cumini* and *Bauhinia forficata* aqueous-leaf extracts on oxidative and mitochondrial parameters *in vitro*. EXCLI Journal 2015; 14: 1219-1231.
29. Shohin IE, Ramenskaya GV, Savchenko AY. Developing *in vitro-in vivo* correlation for Trimetazidine, Indapamide and Ciprofloxacin extended release solid oral dosage forms. International Journal of pharma and bio sciences 2011; 2: 573–580.
30. Faiyazuddin MD, Ahmad S, Mustafa G, Ahmad FJ, Shakeel F. Bio-analytical approaches, bioavailability assessment, and bioequivalence studies for waiver drugs: *In vivo* and *In vitro* perspective. Clinical Research and Regulatory Affairs 2010; 27: 1–10.
31. Bamigbola E.A. Correlation of *in vitro* dissolution profiles with *in vivo* pharmacokinetic parameters of some commercial brands of aspirin tablets marketed in Nigeria. Readings in advanced pharmacokinetics-theory, methods and applications. Noreddin A (Ed.) InTech, 2012, 251–266.
32. Yeh KC, Kwan KC. A comparison of numerical integrating algorithms by trapezoidal, lag range, and Spline approximation. Journal of Pharmacokinetics and Biopharmaceutics 1978; 6: 79–98.
33. Cheng H, Gillespie WR, Jusko WJ. Mean residence time concepts for non-linear pharmacokinetic systems. Biopharmaceutics & Drug Disposition 1994; 15: 627–641.
34. Landaw EM, Katz D. Comments on mean residence time determination. Journal of Pharmacokinetics and Biopharmaceutics 1985; 13: 543–547.
35. Oishi TS, Nimmi I, Islam SMA. Comparative *in vitro* bioequivalence analysis of some generic tablets of Atorvastatin, a BCS class II compound. Bangladesh Pharmaceutical Journal 2011; 14: 61–66.
36. Ma MC Lin, RP, Liu JP. Statistical evaluations of dissolution similarity. Statistica Sinica 1999; 9: 1011–1027.
37. Chanter DO. The determination of mean residence time using statistical moments: Is it correct? Journal of Pharmacokinetics and Biopharmaceutics 1985; 13: 93–100.
38. Weiss M. The relevance of residence time theory to pharmacokinetics. European Journal of Clinical Pharmacology 1992; 43: 571–579.
39. Cheng H, Gillespie WR. Volumes of distribution and mean residence time of drugs with linear tissue distribution and binding and non linear protein binding. Journal of Pharmacokinetics and Biopharmaceutics 1996; 24: 389–402.
40. Sangramsinh LG, Sachinkumar VP, Sachin SN, Nigar KM. *Aegle marmeloes* Gum as Tablet Binder and its Evaluation. International Journal of PharmTech Research 2014; 6: 951-953.
41. Shabnam A, Qurratul A, Babita K. Investigations on Novel Bio-Binder Isolated from *Aegle marmelos* used in Ibuprofen Tablets. International Journal of Pharmacy and Pharmaceutical Sciences 2015; 7: 414-415.
42. Jashabir A, Suvakanta D. Optimization and Characterization of purified gummy polysaccharide isolated from *Aegle marmelos* fruit pulp as a novel pharmaceutical excipient. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6: 777-783.