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Development of New Controlled Release Formulation of Flurbiprofen: *In vitro – in vivo* Correlation

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A novel matrix system of flubiprofen as an oral controlled release formulation was prepared using gum karaya as release retardant. Lactose or dicalcium phosphate was incorporated to improve the drug release rate. Gum karaya matrices containing lactose showed satisfactory release characteristics. Flurbiprofen-gum karaya matrices showed first order release kinetics following super case II transport, where as matrices with both of the co-excipient followed first order kinetics with anomalous diffusion release. The selected experimental formulation showed comparable *in vitro* dissolution profile and *in vivo* blood level pattern with those of the commercial sustained release formulation. The data support a Level A correlation between *in vitro* release rate profile and *in vivo* absorption for flurbiprofen from both the formulations.

Flurbiprofen (FB) is a potent non-steroidal anti-inflammatory, analgesic and antipyretic drug. It is widely used in the treatment of periodontal diseases, rheumatoid arthritis, degenerative joint diseases, ankylosing spondylitis and allied conditions. It has been associated with different adverse effects that include dyspepsia, diarrhea, nausea, vomiting, rash, rhinitis, dizziness and tinnitus. It is given orally 150-200 mg daily in 3 or 4 divided doses and the elimination half-line is 3-4 h^{1-3}. The matrix system of polymer loaded with drug provides long duration of treatment and reduced adverse effects in patients. An attempt has been made here to achieve a better therapeutic profile through tablets with gum karaya (GK) alone and in combination with lactose or dicalcium phosphate (DCP). The bioavailability of experimental formulation, which showed dissolution profile similar to that of the innovator's product, was compared with that of a commercial sustained release formulation. The further purpose of the study was to establish an *in vitro* in *vivo* correlation between an *in vivo* parameter (percentage of dose absorbed) and *in vitro* release rate profile for prepared GK-FB matrix system and a commercial formulation.

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MATERIALS AND METHODS

Gum karaya (viscosity of 1% w/w in aqueous solution at 25° was 1800 cps) was bought from Girijan Co-operative Corporation Ltd., Visakhapatnam. Flurbiprofen used was a gift sample from Sun Pharmaceutical Industries Ltd., Mumbai. Ketoprofen used was a gift sample from Unichem Laboratories Limited, Mumbai. Lactose, dicalcium phosphate dihydrate, ethyl alcohol, sodium hydroxide, orthophosphoric acid, sodium dihydrogen phosphate and magnesium stearate are bought from S.D. Fine Chemicals Ltd., Mumbai. Commercial product used was Arflur SR (FDC Ltd. Mumbai).

Preparation and characterization of GK powder:

The crude tears of GK were taken and powdered using Kalweka ball mill (Cadmach Machinery Co. Pvt. Ltd., India). The powdered GK was sifted through #100 mesh. The powdered gum was stored in a closed container at 4° (in refrigerator) to prevent the deacetylation of gum during storage. This powder was used in further studies.

The SEM photographs of GK were obtained by Scanning Electron Microscope (Jeol, JSM – 840 A, Japan) with 20 kv accelerating voltage. The size distribution of the GK particles was measured using a calibrated eyepiece micrometer. True density of GK powder was determined by liquid displacement method. The bulk density of this polysaccharide was determined by the three tap method[4]. The percent compressibility index (I)[5] was also calculated. The parameter used to determine the flow properties of GK powder was the static angle of repose. This was determined by the fixed funnel and free standing cone method[6]. Moisture content was determined by method of loss on drying using IR moisture balance (Toshniwal Instruments and Engineering Co, Mumbai). Swelling and water retention capacity of GK were determined by using modified method reported by Gauthami and Bhat[7]. Volatile acidity of GK was determined by the method reported by Jacob[8].

Preparation and evaluation of GK-FB hydrophilic matrices:

The matrix systems were prepared by mixing of drug with GK alone or GK and lactose or GK and DCP and other excipients except magnesium stearate by following the formulae given in Table 1. Then sufficient quantity of 70% v/v ethanol was added to form wet powder mixture. The wet powder mixture was then granulated using a 1.40 mm mesh, and the granules obtained were dried in an oven (Tempo Instruments and Equipment Pvt. Ltd., Mumbai) at 60° for 2 h. After the granules were dried, they were again sieved using a 1 mesh, lubricated with magnesium stearate (0.5%w/w). Then the granules equivalent to 200 mg of FB were compressed into tablets using a single station tabletting machine (Cadmach Machinery Co. Pvt. Ltd., Mumbai) at an applied force of 500 kg/cm^2 and compression time of 10 sec using 11 mm round, flat and plane punches. IR spectra of GK powder, pure FB and mixture of GK and FB were obtained by using PERKIN-ELMER 841, IR Spectrophotometer. The samples were prepared with nujol as mulling agent.

The tablets were tested for hardness and FB content. Hardness was tested using a Monsanto hardness tester. FB content was estimated by the method as described below. Five tablets were weighed, powdered and mixed thoroughly. Sample of powder equivalent to 20 mg of drug was taken in a test tube and extracted with 5x10 ml quantities of methanol. The extracts were filtered and collected into 100 ml volumetric flask and made upto volume with methanol. Then, the solution was suitably diluted and assayed for FB at 247° nm using a double beam UV spectrophotometer (Shimadzu UV-205, Japan). The same procedure was used to estimate the FB content in commercial product.

Dissolution rate studies:

A USP dissolution rate test apparatus-2[9] (Paddle method) was used to determine *in vitro* release profiles of FB from the different tablets and the commercial product. A 900 ml volume of phosphate buffer (pH 7.2) at 37° was used as dissolution medium and 100 rpm rotation was maintained throughout the study. Five milliliter samples of dissolution fluid were withdrawn at different time interval throughout a period of 12 h and were filtered immediately.

TABLE 1: FORMULATION OF MATRICES PREPARED.

Ingredient (mg/tablet)	F1	F2	F3	F4	F5	F6	F7	F8
GK	50.5	100.0	150.0	200.0	200.00	200.0	200.00	200.0
FB	200.0	200.0	200.0	200.0	200.00	200.0	200.00	200.0
Lactose	-	-	-	-	25.00	50.0	-	-
DCP	-	-	-	-	-	-	25.00	50.0
Magnesium Stearate	2.5	3.0	3.5	4.0	4.25	4.5	4.25	4.5
Total weight (mg)	252.5	303.0	353.5	404.0	429.25	454.5	429.25	454.5

Granules equivalent to 200 mg of FB were compressed into tablets using a single station tabletting machine with 11 mm round, flat and plane punches.

The volume withdrawn at each time interval was again replaced with fresh quantity of dissolution fluid. The filtered samples were analyzed for their FB content at UV absorbance of 247 nm against blank. Three dissolutions were carried out in each case and the mean was presented.

The dissolution data obtained was fitted to zero order[10], first order[11] Higuchi[12] and Weibull[13] equations to understand the rate and mechanism of FB release from the prepared formulations and commercial product. In order to define a model, which will represent a better fit for the formulation, dissolution data was further analyzed by Peppas and Korsmeyer equation (Power law)[14-16].

$$M_t/M_\infty = K.T^n$$

where, M_t is the amount of drug released at time t and M_∞ is the amount released at time ∞, thus the M_t/M_∞ is the fraction of drug released at time t, k is the kinetic constant, and n is the diffusional exponent, a measure of the primary mechanism of drug release.

Human bioavailability studies:

The bioavailability studies were carried out on the experimental formulation (F6) and the commercial sustained release formulation (Arflur SR) represented as CF. The studies were carried out in five healthy male subjects with a mean age of 25.3 ± 1.8 y (ranging from 23 to 27 y) and a mean body weight of 72.3 ± 8.4 kg (ranging from 63 to 80 kg). All subjects were presented with full details of the investigation, both verbally and in written form, prior to providing written informed consent. An independent Institutional Ethics Committee of Andhra University, Visakhapatnam, approved this study.

The study was of a non-blinded, open-label and crossover design. Subjects were fasted for at least 10 h prior to timing of dose. Each volunteer received two different formulations on two different occasions to avoid variations due to time and human. The washout period between the two treatments had to be not less than 2w.

The assigned tablet was swallowed with 200 ml water. An indwelling cannula with heparin lock was applied in a suitable forearm vein and blood samples were drawn at 0 (before drug administration), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h following the drug administration. A standard lunch was given to all volunteers at 4 h after administration. Blood samples, 5 ml in volume, were collected in plain glass tubes and serum was separated by centrifugation at 3,000 rpm for 10 min, transferred to suitably labeled tubes, and stored at –20° until analysis.

Plasma concentration of the FB were measured by HPLC method reported by Mehendre *et al.*, 1991[17]. The chromatographic system consisted of a Model 2800 Bio Rad Solvent Delivery System, a reversed phase Bio Sil ODS-55 (catalog 125-0080, 250 mm x 4 mm) column and a detector (Bio Rad UV monitor model 1306). The mobile phase consisting of acetonitrile:water:acetic acid (122:100:0.35) was used at a flow rate of 1 ml/min. Ketoprofen was used as internal standard. A guard column (Bio Rad Model – 1250131) was used. The drug was quantified at 254 nm by measuring the peak-height ratio and the relative and absolute recoveries varied from 90 to 98%.

The maximum plasma concentration (C_{max}) and time of its occurrence (T_{max}) were directly computed from the plasma concentration vs time plot. The elimination rate constant (K_{el}) was determined from the terminal phase of the log plasma concentration vs. time profile by least square regression analysis. From this K_{el} was calculated as K_{el} = 2.303 x slope. The elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693 / K_{el}$. The area under curve from 0 to 24 h ($AUC_{0\ 24h}$) is calculated using trapezoidal rule. Area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the relation. $AUC_{0-\infty} = AUC_{0\ 24} + C_{24}/K_{el}$.

The Wagner-Nelson method[18] was used to determine the fractional oral absorption at each sampling time. % Absorbed = $[C(t)+K_{el}.AUC_{0-t}/K_{el}.AUC_{0-\infty}] \times 100$, where C (t) is the plasma concentration of FB at time 't' and AUC_{0-t} is the area under the FB plasma concentration curve zero to 't'.

Correlation between the *in vivo* and *in vitro* results was determined by plotting the mean percentage released *in vivo* at time, t versus the mean percentage released *in vitro* at time, t.

The pharmacokinetic parameters of the formulations were compared statistically by analysis of variance (ANOVA test). A P value of < 0.05 was considered statistically significant. Results are expressed as the mean±standard deviation.

RESULTS AND DISCUSSION

GK is a natural polysaccharide produced from trees of sterculia species belongs to the family *Streculiaceae* as

exudate. This polysaccharide contains 43% D-galactouronic acid, 13% D-galactose and 15% L-rhamnose. It has high acetyl content that attracts and immobilizes large amounts of water[19]. Many natural gums were evaluated, as release retardants because they are non-toxic, economical, biodegradable and biocompatible. Hence, GK was evaluated for its suitability in the preparation of FB controlled release matrix systems.

The SEM photograph of GK is showed in fig. 1 revealed that the shape of the GK granules was irregular and fragmented. When examined under compound microscope GK particles were also found to be irregular and 30-120 μm in size. True density of GK was found to be 1.51±0.59 g/cc. This value is comparable with that of reported value of starch[20] indicating GK may show similar handling properties with that of starch. The bulk density and compressibility index of GK were found to be 0.69±0.21g/cc and 13.68±0.27%, respectively. These values indicated that the GK powder has good flow properties and desirable packing characteristics. The static angle of repose value for GK was found to be 32.41±1.68° indicating good flow properties of GK powder. Moisture content of GK determined by the loss on drying method was found to be 8.47±0.59%. The swelling index of GK was found to be 3510±12.26%. The water absorption capacity of GK was found to be 36.66±2.67 ml. The volatile acidity of GK was found to be 18.23±1.87%. These findings further confirmed the results of Goldstein and Alter[21] as they reported that the GK has volatile acidity ranging from 13.4 to 23.6%.

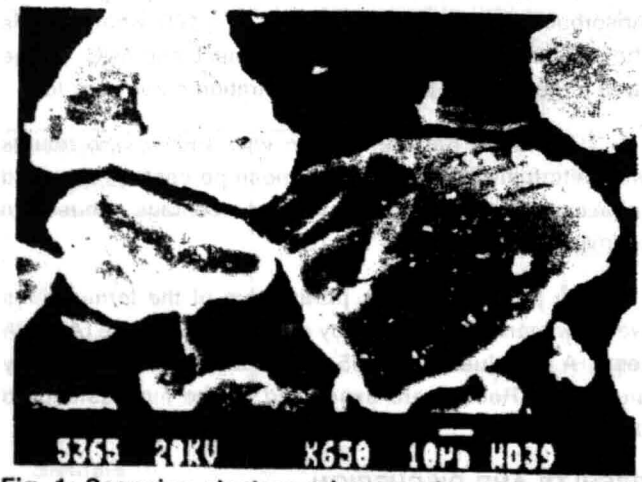

Fig. 1: Scanning electron microscopic photograph GK powder.

SEM photograph of GK powder was taken to characterize surface properties of the powder.

The IR spectrum of GK showed principle absorption peaks at 1247 and 1725 cm^{-1} (acetate group absorbance) and 3453 cm^{-1} (OH stretching of the carbohydrate) and the IR spectrum of pure FB has the carbonyl stretching band at 1703 cm^{-1}. The principle IR absorption peaks of FB and GK were also observed in the spectrum of FB-GK mixture indicated no interaction between the FB and GK. The hardness of all prepared formulations is found to be between 4-5 kg/cm^2. The FB content in all the formulations was found to fairly uniform. The low coefficient of variation values ensured consistency in the mean drug content of the batches prepared. Hence, the process of preparation of matrices was found to be reproducible.

The release pattern of the FB from the matrices made with 20, 25, 33.33 and 50 % w/w of GK as shown in fig. 2 indicated that the FB release was spread over extended period of time. The release of FB from GK matrices was decreased with increasing polymer content. The order of matrix systems in according to their release profiles is F1<F2<F3<F4. Therefore, changing the GK content can modify the drug release. The dissolution profiles of FB from F5 and F6 (lactose as co-excipient) and F7 and F8 (DCP as co-excipient) indicated that both the co-excipients enhanced the release rate of FB from matrices at studied concentrations compared to that of from matrix system with out co-excipient (F4). The release rate of drug from matrix systems containing lactose is higher than that of DCP as shown in fig. 2. The order of matrices with respect to release rate is F8>F7>F6>F5>F4.

The drug release from all the formulations followed first order kinetics which is indicated by the correlation coefficients (r) obtained for first order release model were found to slightly higher when compared to those of zero order release model (Table 2). Values of r given in Table 2 further indicated that dissolution data fitted to Weibull analysis Higuchi model. These results indicated that FB release from GK matrices might be operated through mixed order kinetics. The release kinetics of F1 is not important as complete drug release was observed with in 2 h. Values of n for F2, F3, F4, F7 given in Table 2 indicated that the release mechanism followed super case II transport i.e. swelling controlled erosion mechanism. However, F5, F6, F8 and CF followed non-Fickian diffusion i.e. drug release is mediated through erosion as well as diffusion.

Addition of co-excipient (lactose/DCP) to FB-GK matrix system showed marked influence on release kinetics Table 2. Erosion mediated FB release from F4 formulation

gradually changed to anomalous diffusion by the addition of lactose or DCP. Though the addition of DCP to formulation in low concentration not produced much difference in release mechanism, but the addition lactose at it concentration produced marked difference in release mechanism. The differences in n values of F6 and F8 clearly indicated that the drug release from formulation mediated through diffusion and erosion mechanism.

The *in vitro* dissolution profile of CF (Arflur SR) given in fig. 2 clearly indicated that the FB release from F6 is almost similar with that of from CF. This is further confirmed by the values of first order release rate constants (k) given in Table 2 as there is no marked difference between these values. The results of the study indicated that the release of FB from the CF as well as F6 followed first order kinetics via anomalous (non-Fickian) diffusion Table 2.

As the drug release from F6 is almost similar to CF, it is selected for comparative *in vivo* evaluation with commercial SR formulation (Arflur SR). The mean plasma concentrations versus time profiles of FB following administation of the two dosage forms in five healthy volunteers are presented in fig. 3. The results demonstrated that both the formulations behaved *in vivo* as sustained release systems as indicated by their plasma drug concentrations that could be traced in blood for longer

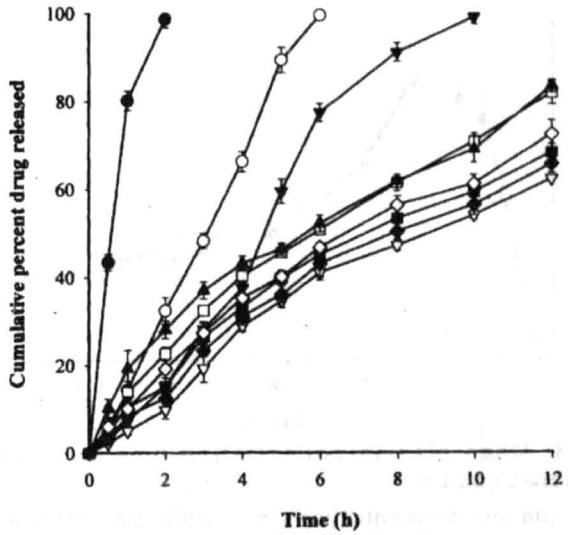

Fig. 2: Release profiles of flurbiprofen from prepared GK hydrophilic matrix systems and commercial sustained release formulation.

***In vitro* dissolution profiles of flurbiprofen from GK hydrophilic matrices F1 (-●-), F2 (-0-), F3 (-▼-), F4 (-▽-), F5 (-■-), F6 (-□-), F7 (-◆-), F8 (-◇-) and commercial formulation (-▲-) were studied in phosphate buffer (pH 7.4), samples drawn at regular time intervals and flurbiprofen content was measured spectrophotometrically at 247 nm.**

TABLE 2: RELEASE KINETICS FOR FLURBIPROFEN.

Product	Zero Order	First order	Higuchi equation	Weibull analysis	Peppas equation	n	k (h^{-1})
F1	0.9385	0.9867	0.9593	0.9981	0.9617	0.592	2.25
F2	0.9979	0.8495	0.9879	0.9003	0.9959	1.207	0.74
F3	0.9847	0.9225	0.9761	0.9473	0.9955	1.231	0.42
F4	0.9831	0.9962	0.9930	0.9983	0.9910	1.085	0.08
F5	0.9812	0.9978	0.9964	0.9989	0.9943	0.818	0.09
F6	0.9841	0.9929	0.9987	0.9969	0.9940	0.779	0.13
F7	0.9838	0.9974	0.9952	0.9989	0.9914	0.925	0.08
F8	0.9831	0.9969	0.9976	0.9982	0.9971	0.790	0.10
CF	0.9747	0.9830	0.9964	0.9971	0.9952	0.617	0.12

The dissolution profiles of FB from GK hydrophilic matrices and commercial product (CF) are fitted into various kinetic models and r values, diffusional exponent values derived from Peppas equation (n), First order release rate constant values (k) are given.

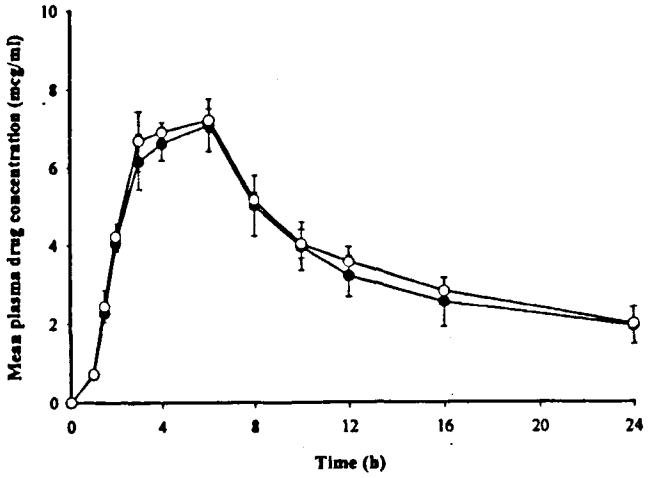

Fig. 3: Mean plasma concetration-time curves of diclofenac sodium.

Mean plasma concentrations of flurbiprofen following oral administration of experimental formulation (-●-) versus commercial SR formulation (-0-) to healthy human volunteers (n=5) were measured using a reported HPLC method.

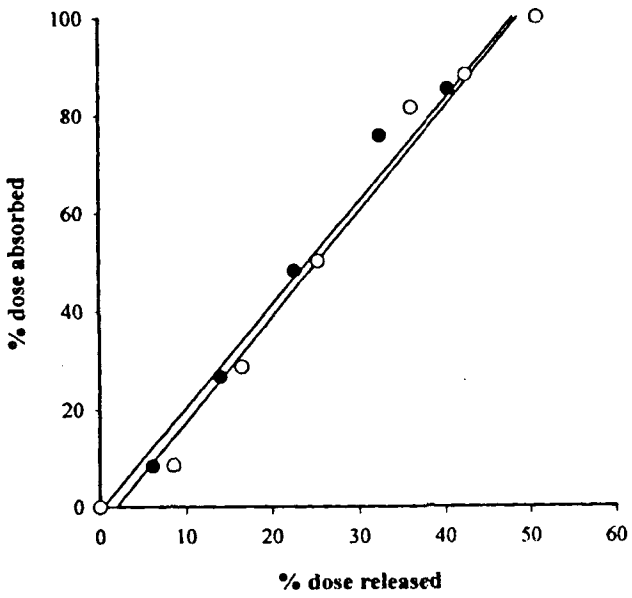

Fig. 4: Plots for *in vitro-in vivo* correlation.

Mean percentage of dose absorbed versus mean percentage of dose released from experimental formulation (-●-) and commercial SR formulation (-0-) used to assess the *in vitro-in vivo* correlation.

period of time. All the pharmacokinetic parameters (AUC_{0-24h}, C_{max}, T_{max}, K_a, $t_{1/2}$ and MRT) are listed in Table 3. The difference between F6 and CF was insignificant for AUC_{0-24h}, C_{max}, T_{max}, K_a, $t_{1/2}$ and MRT and were found to be comparable indicating that both the formulations exhibited comparable sustained release pattern.

The relationship between the percent dose absorbed *in vivo* and percent dose released *in vitro* for F6 and CF is shown in the fig. 4. The mean regression line was calculated to be y = -0.720 + 2.098x (r = 0.9929) for F6 and y = -4.067 + 2.143x (r = 0.9920) indicate a linear *in vitro-in vivo* relationship. This study suggests that the *in vivo* bioavailability of flurbiprofen SR tablets can be predicted by the *in vitro* dissolution profile of the dosage form.

In conclusion, GK can effectively act as release retardant. An inverse relationship is found between the

TABLE 3: PHARMACOKINETIC PRAMETERS OF FLURBIPROFEN.

Parameter	F6	CF
C_{max} (mcg mL^{-1})	7.19±0.482	7.22±0.294
T_{max} (h)	5.60±0.894	5.60±0.894
K_a (h^{-1})	0.47±0.128	0.46±0.135
AUC0-24 (mcg h mL^{-1})	85.60±9.125	90.39±4.152
$T_{1/2}$ (h)	14.55±3.295	13.91±2.035
MRT (h)	5.40±0.099	5.38±0.099

C_{max} is maximum plasma concentration and T_{max} is time of its occurrence, K_a is absorption rate constant, $t_{1/2}$ is elimination half-life, AUC_{0-24h} is area under curve from 0 to 24 h and MRT is mean residence time of flubriprofen following oral administration of experimental formulation (F6) and commercial formulation (CF) to human volunteers (n=5).

concentration of GK and FB release from matrices. The drug release from GK-FB matrix systems followed first order kinetics and erosion dominant release mechanism. The addition of co-excipients modified the release rate and mechanism. The *in vitro* drug release, particularly from F6 containing lactose is comparable with that of commercial SR product. Common pharmacokinetic parameters of FB from experimental formulation (F6) are statistically equal to those from a commercial SR formulation. The significant correlation between the *in vitro* and the *in vivo* data indicate that the *in vitro* release rate procedure reported here could be used as a development or quality control procedure for product development.

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