RESEARCH PAPER

Accepted 27 July 2001
Revised 20 July 2001
Received 26 December 2000
Indian J. Pharm. Sci. 2001, 63(5) 408-412

Controlled Release of Diclofenac Sodium by Gum Karaya - Chitosan Complex Coacervate: *In Vivo* Evaluation

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In this study microcapsules prepared from gum karaya (GK) and chitosan (CH) using the principle of complex coacervation for the first time with a continuous oil-phase were evaluated for their *in vivo* performance. Diclofenac sodium (DFS) was used as the drug of choice.The dissolution profiles of DFS from prepared microcapsules as well as commercial product followed non-Fickian diffusion.The formulation displayed a sustained *in vivo* blood level pattern that is comparable to that of a commercial controlled release formulation.

The use of new natural hydrophilic polymers as drug carries has received considerable attention in the last few years, especially from the viewpoint of cost, environment concerns and safety. Gum karaya (GK) is the dried exudation obtained from trees of *Sterculia urens* family Sterculiaceae, a tree native to India. GK comes under the group of gums which contain basal chains of galactouronans or galacturonorhamnans with residue of both D-galacturonic acid (in interior chain) and D-glucuronic acid (or its 4-methyl) ether (as terminal units in side chain attached to a variety of different sugar residues). GK contains 43% D-galacturonic acid, 13% D-galactose and 15% L-rhamnose[1]. Chitosan (CH) is a hydrolyzed derivative of chitin, a biopolymer widely distributed in nature. CH has attracted attention as a matrix for controlled release system since it possesses reactive functionalities and easily degraded into nontoxic products by enzymes[2].

Complex coacervation involves the use of more than one colloid. This coacervation is accomplished mainly by charge neutralization of the colloids carrying opposite charges rather than dehydration[3]. This technique has been used in microencapsulation of different drugs for different purposes in pharmaceutical research such as controlling the drug release, improving the flow characteristics and preventing the decomposition of light sensitive drugs. As coacervation can be induced in systems containing both cationic and anionic hydrophilic colloids, complex coacervation is likely to occur between CH, a cationic polysaccharide and GK, an anionic polysaccharide.

Diclofenac sodium (DFS), the candidate under investigation is a potent anti-inflammatory, antipyretic and analgesic drug. It has high tolerability index with proven safety and efficacy. As it has short plasma half-life the drug is to be administered repeatedly for the maintenance of therapeutic drug levels. Repeated oral administration of the drug in the long-term therapy, however, causes gastrointestinal disturbances[4]. Hence it is an ideal candidate for controlled release dosage forms. Further, the formulation of a multiparticulate system is thought to be preferable to a single unit dosage form because the small particles spread out more uniformly in the gastrointestinal tract. This result in a more reproducible drug absorption and reduced the risk of local irritation.

In the present investigation, bioavailability of DFS from the GK-CH microcapsules (experimental formulation) in healthy human volunteers was investigated. The bioavailability of experimental formulation (EF) was compared with that of commercial sustained release formulation (CF).

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MATERIALS AND METHODS

Diclofenac sodium was supplied by NATCO Pharma Ltd., Hyderabad as a gift sample. Gum karaya (viscosity of 1% w/v in aqueous solution at 25° was 1800 cps) was bought from Girijan Co-operative Corporation Ltd., Visakhapatnam. Chitosan (viscosity of 1% w/v in aqueous solution at 25° was 180-200 cps and degree of deacetylation > 80% as per Suppliers specification) was supplied as a gift sample by Central Institute of Fisheries Technology, Kochhi. Difen® SR capsules of SOL Pharma Ltd (Batch no. J. 701) were purchased from local market. Formaldehyde, liquid paraffin, sodium hydroxide, hydrochloric acid, trisodium phosphate and acetic acid (BDH, Hyderabad) were of analytical grade.

Preparation of GK-CH microcapsules:

Preparation of microcapsules containing DFS as the core material and GK-CH as coat material was done by following the procedure reported by Murali Mohan Babu *et al.*[5] GK colloidal solution (1.5% w/v) was prepared in distilled water. DFS (400 mg) was dispersed in this solution and mixed well. The solution was added to the liquid paraffin to form water in oil (w/o) emulsion. The dispersion was stirred at 700 rpm for 5 min. About 20 ml of 0.5% w/v CH solution prepared in 5% v/v acetic acid was slowly added drop wise into a beaker containing the gum-drug-oil emulsion with continuous stirring speed of 700 rpm. At the end, 4 ml of 37% formaldehyde was added and the reaction was allowed to take place for 4 h. The product was filtered and washed with petroleum ether for several times and finally with water and dried at 50°. Microcapsules equivalent to 100 mg of DFS filled in hard gelatin capsule (0 size) were used for further studies. All batches were prepared in triplicate.

In order to study the presence of GK on the formation of microcapsules, a batch of microcapsules were prepared as per the above procedure with out addition of GK and represented as EL. For determining the size distribution of microcapsules, microcapsules in a batch were separated by sieving using a set of standard sieves to sizes. The resulting fractions remaining on the sieves were weighed. The data obtained was subjected to graphical treatments in order to find the mode of size distribution. The mean microcapsule diameter was calculated after sieving[6]. The scanning electron microscopic (SEM) photographs of the microcapsule and its surface were obtained by using Scanning Electron Microscope (Jeol, JSM - 840 A, Japan) with 20 kV accelerating voltage, which are used to evaluate the shape and surface characteristics of the microcapsules. Thermograms of pure DFS and microcapsules were obtained by a Differential Scanning Calorimeter (DSC 220C, SEIKO, JAPAN) at a heating rate of 10°/min from 30 to 300° in nitrogen atmosphere.

Dissolution study:

The dissolution studies were performed using the USP dissolution apparatus II with 100 rpm paddle rotational speed. The dissolution test was carried out at 37±0.5° in 750 ml of 0.1 N HCl (pH 1.2) for 2 h and continued for another 10 h at pH 6.8. The change in pH was achieved by the addition of 250 ml of 0.2 M tribasic sodium phosphate. The samples were periodically removed and analyzed spectrophotometrically at 276 nm. The results given are the means of three determinations. The dissolution data obtained was fitted to zero order[7], first order[8], Higuchi[9], erosion[10] and Korsmeyer-Peppas[11-13] equations to understand the rate and mechanism of DFS release from the microcapsules.

Human bioavailability studies:

The bioavailability studies were carried out on the EF and CF (Difen SR). These studies were carried out on healthy human volunteers. Five healthy male subjects with a mean age of 25.3±1.8 y (ranging from 23 to 27 y), a mean body weight of 72.3±8.4 kg (ranging from 63 to 80 kg) and a mean height of 172.1±5.8 cm (ranging from 166 to 177 cm) were included in the above investigation for EF versus CF. The volunteers were judged healthy on the basis of medical history, physical examination, electrocardiogram and routine laboratory tests. All subjects were free from drugs 14 d before and during the study. All subjects were presented with full details of the investigation, both verbally and in written form, prior to providing written informed consent. An independent Ethics Committee of Andhra University, Visakhapatnam, approved the study, protocols.

The study was of a non-blinded, open-label and double-way crossover design. Subjects were fasted for at least 10 h prior to timing of dose. Each volunteer received 2 different formulations on two different occasions to avoid variations due to time and subject. A week washout period was maintained between the two treatments.

The assigned tablet was swallowed with 200 ml water.

Fluid intake was restricted for atleast 2 h after administration and no food was allowed up to 4 h after administration. A standard lunch was given to all volunteers at 4 h after administration. 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, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 20.0 and 24.0 h following the drug administration. Blood samples, 5-10 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 assay.

DFS plasma levels were determined by a modified HPLC method as previously described by El-Sayed *et al.*[14] In summary, the assay procedure involves protein precipitation of the plasma samples with acetonitrile. Mefenamic acid was used as an internal standard. The chromatographic system consisted of a Model 2800 Bio Rad Solvent Delivery System, a reverse 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:0.01 M potassium dihydrogen phosphate (35:65 v/v, pH 6.3) was used at a flow rate of 1 ml/min. A guard column (Bio Rad Model-1250131) was used. The drug was quantified at 280 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 slope of 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 ($t_{½}$) was calculated using the formula $t_{½}$=0.693/K_{el}. The area under the curve from 0 to 24 h ($AUC_{0\text{-}24\ h}$) is calculated using the trapezoidal rule.

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

Our previous study[5] demonstrated that the GK-CH system is a valuable alternative for microencapsulation of the DFS, which is capable of sustaining the drug release, and the drug release was comparable with that of commercial formulation. It was found that the 1:15 ratio of CH:GK with 25% w/w drug loading and 1.6% w/v of the total polymer concentration, at 700 rpm of stirring speed for 4 h produced uniform, discrete and spherical microcapsules. Hence, these conditions were considered to be optimum and the microcapsules developed under these conditions selected for further studies.

SEM photographs of single microcapsule and its surface of EF as shown in fig. 1 indicated that the microcapsule was spherical, discrete and covered continuously and completely with coacervate of GK-CH as coat material. Micromeritic analysis revealed that the mode of size distribution was log normal in all batches with the average particle diameter of 515.36 µm. The differential scanning thermograms of pure drug as well as microcapsules showed peaks at around 157° corresponding to the melt-

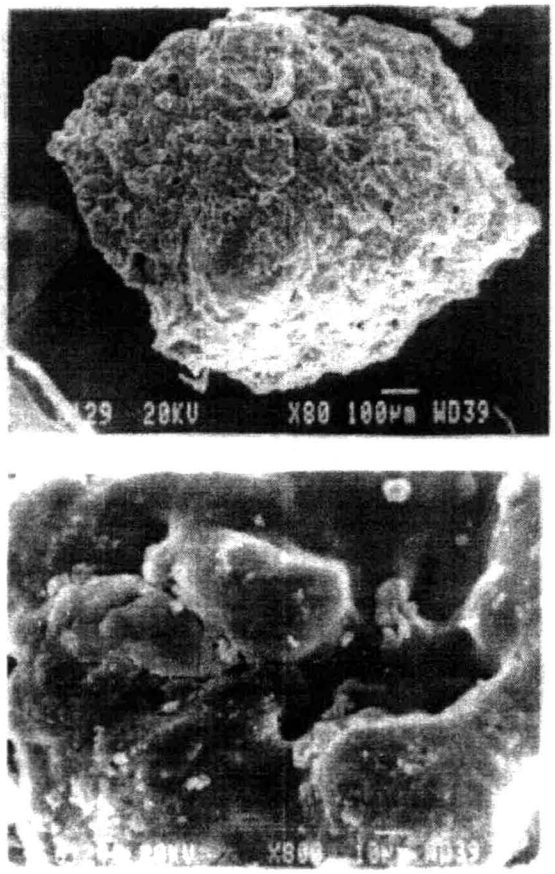

Fig. 1: Scanning electron microscopic photographs of microcapsule (EF)

SEM photographs of whole microcapsule (A) and its surface (B)

ing point of DFS indicated the absence of well defined interaction between DFS and formaldehyde.

In order to study the effect of GK on the properties of microcapsules, batches of microcapsules were prepared with and without addition of GK. Microcapsules prepared without addition of GK were found to be fragile and brittle in comparison with that of microcapsules prepared by the addition of GK. The *in vitro* drug release profiles of DFS from the EL, EF and CF are shown in fig. 2. DFS release from EL was very rapid and fast in comparison with those of EF and CF. From these results, it can be concluded that the addition of GK in the preparation of microcapsules improves the surface

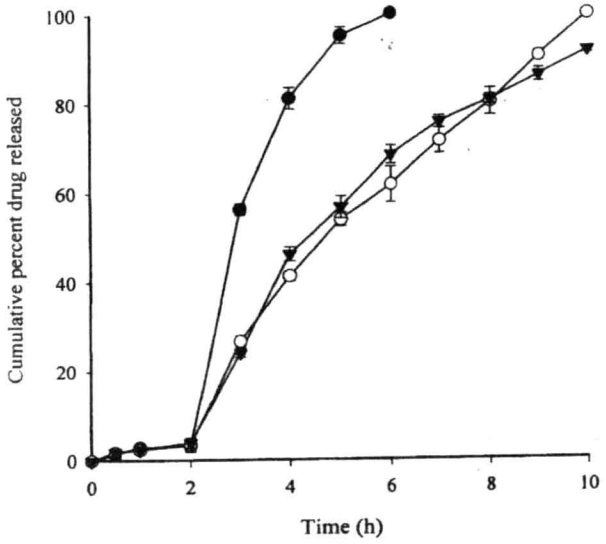

Fig. 2: Dissolution profiles of diclofenac sodium from EL, EF and CF.

Dissolution profiles of diclofenac sodium from the microcapsules prepared by without addition of GK (-●-), experimental formulation (-○-) and commercial formulation (Difen SR) (-▼-) were studied using USP dissolution apparatus II. Diclofenac sodium was estimated spectrophotometrically.

characteristics of the microcapsules and retards the drug release from the microcapsules.

The release profiles of DFS from EF and CF are comparable, indicating that the release rate of DFS from these dosage forms is similar. However, DFS from CF followed first order kinetics with diffusion controlled release mechanism, while from the EF followed zero order kinetics with diffusion as well as erosion mediated release mecha-

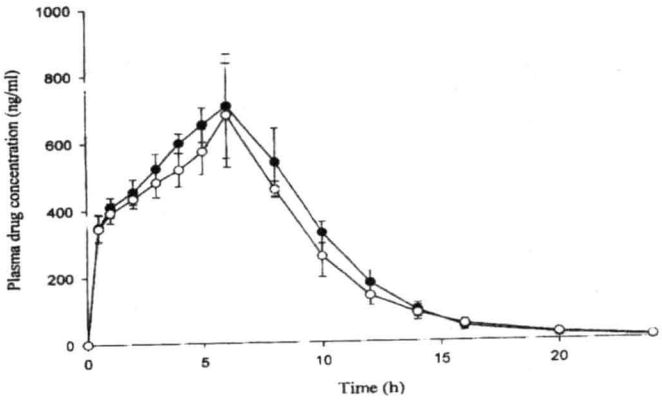

Fig. 3: Mean plasma concentration-time curves of diclofenac sodium

Mean plasma concentrations of diclofenac sodium from experimental formulation (-●-) and commercial formulation (-○-) following the oral administration to healthy human volunteers (n=5)

nism as indicated by the r and n values (Table 1). The release rate constants were found to be 39.059, 31.898% $h^{-1/2}$ for EF and CF respectively indicating that the faster dissolution rate of EF compared to that of CF.

The mean plasma concentrations versus time profiles of DFS following administration of EF and CF 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 periods of time. All the pharmacokinetic parameters ($AUC_{0\text{-}24\ h}$, C_{max}, T_{max}, K_a, $t_{½}$ and MRT) are listed in Table 2. The difference between EF and CF was insignificant for $AUC_{0\text{-}24\ h}$, C_{max}, T_{max}, $t_{½}$ and MRT. However, a statistically significant difference was observed in K_a values. It was found that the K_a of CF was lower than that of EF. This may be due to faster dissolution rate of DFS from EF compared to that of CF as indicated by *in vitro* dissolution studies. Although the K_a values of two formulations were found to be significantly different, the $AUC_{0\text{-}24\ h}$ and C_{max} values of EF were comparable with that of CF.

In conclusion, this study shows that the common pharmacokinetic parameters of DFS from GK-CH microcapsules prepared by using the principle of complex coacervation are not statistically different as compared to that of a commercial SR formulation and hence these microcapsules can be used to prepare sustained release dosage form.

TABLE 1: RELEASE KINETICS OF EF AND CF

Product	Zero order[a]	First order[a]	Higuchi[a]	Erosion[a]	Peppas[a]	n	K (% $h^{-1/2}$)
EF	0.9818	-0.8469	0.9968	0.9549	0.9991	0.621	39.059
CF	0.9542	-0.9963	0.9959	0.9950	0.9963	0.493	31.898

a represents correlation coefficient values (r values), n indicates diffusional exponent derived from Peppas equation, K is release rate constant derived from Higuchi equation, EF represents experimental formulation and CF represents commercial formulation (Difen SR).

TABLE 2: PHARMACOKINETIC PARAMETERS OF DICLOFENAC SODIUM

Parameter	EF	CF
C_{max} (ng ml^{-1})	706.93±155.771	706.16±133.656
T_{max} (h)	5.60±0.548	5.60±0.548
K_a (h^{-1})	0.25±0.016	0.23±0.009
AUC_{0-24} (ng h ml^{-1})	5621.22±411.379	6251.40±702.224
$t_{½}$ (h)	2.46±0.238	2.81±0.270
MRT (h)	6.47±0.234	6.49±0.243

C_{max} is maximum plasma concentration and T_{max} is time of its occurrence, K_{el} is absorption rate constant, $t_{½}$ is elimination half-life, $AUC_{0-24\ h}$ is area under curve from 0 to 24 h and MRT is mean residence time following oral administration of experimental formulation (EF) and commercial formulation (CF) to human volunteers (n=5).

ACKNOWLEDGEMENTS

One of the authors (G.V.M.M.B.) thanks M/S Andhra Sugars Ltd., Thanuku for providing financial assistance. The authors are grateful to M/S Girijan Co-operative Corporation, Visakhapatnam for the facilities and encouragement. The authors express their gratitude to NATCO Pharma Ltd, Hyderabad for providing gift sample of diclofenac sodium.

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