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DEVELOPMENT AND EVALUATION OF A PROLONGED-RELEASE MATRIX TABLETS OF DICLOFENAC SODIUM RESINATE

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لقد تم تحضير أقراص طويلة المفعول تحتوى على معقدات عقار الديكلوفيناك صوديوم مع الراتنج ($Dowex\ 1\text{-}X8.Cl^-$) بإتباع طريقة الكبس المباشر لمعقدات العقار / الراتنج مع عديد من الإضافات المنظمة لإنطلاق العقار (أفيسيل ب.هـ ١٠١ وسكر اللبن وكربوكسى ميثيل السليلوز الصوديومى والكمبريتول). ولقد كان لصياغات الأقراص المنتجة صفات ميكانيكية جيدة. وقد استخدم جهاز قياس معدل الذوبان ذو التحريك الدورانى لتعيين معدل إنطلاق العقار من معقدات العقار مع الراتنج المحملة بتركيزات مختلفة من العقار وكذلك أقراص معقدات العقار مع الراتنج المحضرة من ذوات معدلات إنطلاق تتراوح من ٠,٩٢ إلى ٥,٥٢ مجم.دقيقة$^{-٠,٥}$. وقد أوضحت النتائج إن إنطلاق العقار من هذه الصياغات كان أبطأ من إنطلاقه من أقراص عقار الديكلوفيناك صوديوم بمفرده والتى لها معدلات إنطلاق تتراوح من ٦,٥٥ إلى ١٥,١ مجم.دقيقة$^{-٠,٥}$ أو أقراص عقار الديكلوفيناك صوديوم التجارية الممتدة المفعول والتى لها معدل إنطلاق يقدر بـ ٨,٢٣ مجم.دقيقة$^{-٠,٥}$. ولقد كان لأقراص معقدات العقار مع الراتنج المحتوية على كربوكسى ميثيل السليلوز الصوديومى أو الكمبريتول أبطأ معدلات إنطلاق وهم ٠,٩٢ و ١,٢٧ مجم.دقيقة$^{-٠,٥}$ على التوالى. وقد تم تقدير النظام الحركى (الكينيتى) لإنطلاق العقار من معقدات العقار مع الراتنج على أساس قانون الكتلة. وفى معظم الحالات كانت المنحنيات الخاصة بتأثير نسبة العقار إلى الراتنج وتركيز أيونات الصوديوم على إنطلاق العقار من معقدات العقار مع الراتنج لها معدلين للإنطلاق: معدل إنطلاق سريع خلال الفترة الأولى يتبعه معدل إنطلاق بطئ مما يرجح أن عملية الانطلاق كانت بالإنتشار من خلال جزيئات الراتنج.

وتم فحص نتائج الإنطلاق من الأقراص بإستخدام النظام الحركى (الكينيتى) ذو الرتبة صفر وكذلك أنظمة الإنتشار المضبوط. وأوضحت قيم العوامل الأسية المحسوبة (قيم –ن) أن نظام إنطلاق العقار كان بالإنتشار الحركى التابع لقانون فيكس (علاقة هيوجوشى الخطية للجذر التربيعى للزمن) مؤكدا فاعلية آلية الإنتشار المضبوط .

ولكى نقيم نظام أقراص معقدات العقار مع الراتنج فقد تم إختبار تأثير كل من أفضل صياغات للأقراص والمنتج التجارى الممتد المفعول لعقار الديكلوفيناك صوديوم والعقار بمفرده على تكوين القرح فى معدة الأرانب وقد أثبتت النتائج أن معقدات العقار مع الراتنج وأقراص معقدات العقار مع الراتنج المحتوية على مادة الأفيسيل (ب.هـ ١٠١) كانت أفضل من العقار بمفرده والمنتج التجارى وكذلك أقراص معقدات العقار مع الراتنج المحتوية على مادتى الكمبريتول أو كربوكسى ميثيل السليلوز الصوديومى. وفوق كل شئ فقد أوضحت الدراسة أهمية إستخدام أنظمة المعقدات الأيونية فى عمل الأقراص بغرض حمايتها من الوسط الحامضى للمعدة وكذلك أهمية خصائص المواد المكونة للأقراص وكذا تاثيرها على فاعلية العقار وأيضا الفاعلية الحيوية للأقراص.

The preparation of a potential prolonged-release matrix tablets (IER-tablets) containing diclofenac sodium - anion exchange resin ($Dowex\ 1\text{-}X_8.Cl^-$) complexes has been performed by direct compression of the drug-resin complex (drug resinate) with various release-regulating excipients (Avicel PH 101, lactose, sodium carboxymethylcellulose, and compritol). All the formulations produced tablets with good mechanical properties. The in-vitro release rate of diclofenac sodium from the drug-resin complex samples with different loaded concentrations

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and from the prepared IER-tablets (K_h= 0.92-5.52 mg/min$^{-0.5}$) was determined using a rotating paddle dissolution apparatus. The results obtained showed that drug release from these formulations was slower than from the tabletted diclofenac sodium alone (K_h= 6.55-15.10 mg.min$^{-0.5}$) or a commercial sustained-release tablet formulation of diclofenac sodium (K_h= 8.23 mg.min$^{-0.5}$), and that IER-tablets containing sodium carboxymethylcellulose or compritol exhibited the lowest release rates (K_h= 0.92 and 1.27 mg/min$^{-0.5}$, respectively). The kinetics of the drug release from resinate was evaluated on the basis of the mass low. In most of cases, the profiles for both the effect of drug/resin ratio and sodium ion concentration upon drug release from the resinates exhibited two release rate processes: rapid release during the initial period, followed by a decreasing release rate, suggesting a particle diffusion release process. The release rate data of the tablets were investigated by using zero-order and the matrix-diffusion-controlled kinetics. The calculated exponential release exponents (n values) revealed that release behaviour of all IER-tablets was a Fickian-diffusion kinetics (i.e., The Higuchi-linear square root of time relationship), confirming that a matrix diffusion-controlled mechanism was operative. To evaluate the feasibility of the drug resinate tablet system, the optimum IER-tablet formulations, drug resinates, sustained-release commercial tablet product and plain drug were examined for their ulcerogenic activity in rabbits. The results proved the superiority of the drug resinate and IER-tablets containing Avicel PH 101 over the plain drug, commercial product or IER-tablets containing compritol or sodium carboxymethylcellulose. Overall, this study demonstrated the significance of using ionic complex systems in offering a simple gastro-protected tablets and showed the characteristics of the matrix materials and their influence on the drug activity as well as tablet performance in-vivo.

INTRODUCTION

Diclofenac sodium is a non-steroidal anti-inflammatory drug widely employed in the long-term treatment of a variety of rheumatic disorders, such as osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and other forms of articular degenerative conditions.[1] The drug is almost completely absorbed from the gastro-intestinal tract following oral administration. The main adverse effects are gastro-intestinal disturbances which include irritation with ulceration and bleeding, nausea, vomiting and diarrhoea. The short plasma half-life of the drug (1-2 h) and its side-effects associated with frequent administration of conventional dosage forms point to the need for an efficient sustained-release product of an effective blood level for a reasonably long time.[1,2]

Many formulation studies for controlling diclofenac release have been reported so far. Thus, the drug has been formulated as prolonged-release microcapsules using different microencapsulation techniques and various polymeric materials.[3-5] Arica *et al.*[2] have reported enteric coated diclofenac sodium-carboxymethylcellulose microspheres having sustained-release properties. A biodegradable diclofenac sodium microsphere system using chitosan has been prepared and evaluated in vitro and in vivo.[6]

Hirotani *et al.*[7] used a phospholipid to produce controlled-release granules of diclofenac sodium. Moreover, wax matrix granules containing diclofenac sodium and a rate-controlling agent such as hydroxypropylcellulose or methacrylic acid copolymer L (Eudragit L-100) were prepared.[8]

Prolonged-release hydroxypropylmethyl-cellulose (HPMC)-based matrix tablet formulations for diclofenac sodium have been reviewed by Sheu *et al.*[9] who found that the release of the drug from the hydrophilic HPMC matrices follows a non-Fickian transport in all dissolution media. However, Acartürk[10] employed chitosan as an agent to prepare the prolonged-release matrix tablets by both direct-compression and wet granulation methods. Recently, monoliths containing diclofenac in the chemical form of acid or the sodium salt or the N-(2-hydroxyethyl)pyrrolidine salt were

prepared using ethylcellulose, polytetrafluoroethylene and polymethylmethacrylate for obtaining a gastro-protected form of diclofenac.[11] More recently, an oral sustained release multiple-unit dosage form for diclofenac sodium in the form of mini-matrix tablet formulations enclosed in a hard gelatin capsules was prepared by the use of different natural hydrophilic gums and various excipients as mini-matrix materials.[12]

Ion-exchange resins contain positively or negatively charged sites and are thus classified as either cationic or anionic exchangers which function for some drugs as reliable controlled drug delivery systems.[13] However, improvements of their release properties can be affected by coating the resin beads using wax[14] or various microencapsulation techniques with a variety of polymeric materials.[15-17] The loading and release properties of drugs from strong anionic exchange resins (Dowex type) were also reported.[16,18] Torres *et al.*[19] studied the formulation and in vitro evaluation of a system based on the microencapsulation of diclofenac-resin complexes with hydroxypropylmethylcellulose phthalate. However, wax-coating or microencapsulation for suppressing release may lead to drug loss from the resin beads by the used solvents.

Compritol (glyceryl behenate), a new commercially available agent, could be used as a rate controlling excipient[20]. El-Sayed[21] prepared theophylline tablets containing 50% theophylline, 30% compritol and 20% lactose that remained intact during the dissolution study and released less than 50% of the drug in 6 h.

Therefore, the objective of the present study was to prepare tablets of the drug-resin complexes using different release-regulating excipients including compritol, sodium carboxymethylcellulose, Avicel PH 101 and lactose, and to study the in vitro dissolution behaviour and release kinetics of the prepared resinates and tablets. The in-vivo performance of the drug resinates and tabletted resinates was also examined in comparison with that of a commercial slow release formulation by testing the ulcerogenic activity in rabbits.

MATERIALS AND METHODS

Materials

The following materials were obtained from commercial sources: Dowex 1-X_8 (Dow Chemical Co., Midland, MI, USA), diclofenac sodium (Sigma Chemical Co., St. Louis, USA), Avicel PH 101 (Fluka AG, CH-9470 Buchs, Switzerland), sodium carboxymethylcellulose (NaCMC), lactose (El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt), compritol (Gattefossé S.A., France) and magnesium stearate (Malinckrodt, St. Louis, MO, USA). All other materials were of reagent grade and used as received.

Methods

Equilibrium studies

A series of drug solutions (12.5 mg/ml) were prepared, put in a 125 ml bottles together with 125 mg of the purified and regenerated resin in the chloride form and mixed using a magnetic stirrer (J.P. Selecta, s.a., Spain). At the specified time intervals, the contents of each bottle were filtered, diluted and the drug concentration in the filtrate was determined spectrophotometrically (Shimadzu double-beam spectrophotometer 150-02, Japan) at 276 nm.[2] The time required for a constant amount of the drug to react with the resin was taken as the equilibrium time.

Preparation of the drug-resin complex

Different drug/resin ratios, for example, 0.5:1, 1:1, 2:1 and 3:1 were prepared by a batch process as follows: an accurately weighed amount of the resin (125 mg) was added into the solution of diclofenac sodium in deionized water, and the system was stirred magnetically for 24 h. The drug-resin complex was collected by filtration and washed with deionized water to remove any unreacted drug. The resin particles were dried at 50°C for 24 h. The amount of free drug in the filtrate was assayed spectrophotometrically at 276 nm.[2] and the loaded drug in the complex (R-D) was calculated as:

$$R\text{-}D = D_i - D_f$$

where D_i is the initial amount of drug added and D_f is the amount of free drug.

The adsorption isotherm for diclofenac sodium on to Dowex 1-X_8 was also determined by the equilibration study (Fig. 1).

Infrared spectroscopy

Studies of the infrared spectra of diclofenac sodium, Dowex 1-X_8.Cl^- and the prepared drug-resin complexes were conducted with an infrared spectrophotometer (IR-470, Shimadzu, Japan) using th KBr disc method.

Differential scanning calorimetry (DSC)

DSC analysis for diclofenac sodium, resin, and the prepared drug-resin complexes was carried out using Shimadzu DSC-50 (Kyoto) connected with TA-501. Nitrogen was used as burge gas (40-50 ml/min). A scanning speed of 10°C/min was employed. The sample size in the aluminium sample pan was in the range of 3-5 mg.

Powder X-ray diffraction

The powder X-ray diffraction patterns of the drug, resin and the prepared-drug-resin complexes were measured using Philips 1710 X-ray diffractometry (Netherland-Endhoven) with the following conditions: target Cu, filter Ni, voltage: 40 Kv, current: 30 mA, scanning speed: 2°/min, and a chart speed of 40 mm/min.

Tablet preparations

An amount of diclofenac sodium resinate equivalent to 50 mg of the drug was mixed with 70% w/w excipient for 10 minutes to produce a homogenous preparation. Avicel PH 101, lactose, sodium carboxymethylcellulose and compritol were used as fillers. Magnesium stearate (1.5% w/w) was added as a lubricant and mixed for 5 minutes with other ingredients. Matrix tablets were prepared by the direct compression method using a single punch tablet machine (Korsch-Beflin CK/O, Frankfort, Germany) equipped with flat-faced punches having a diameter of 12 mm.

To prepare the control tablets, the drug alone, excipients (70% w/w) and magnesium stearate (1.5% w/w) were blended, then compressed into tablets by using a single punch tablet machine with 8-mm flat-faced punches. Each tablet contained 50 mg of diclofenac sodium. Three batches were prepared for each formulation and only those tablets that were within $\pm$10 mg of the target weight were used in this study.

Physical tests

The prepared tablets were evaluated for the weight uniformity (U.S.P.), thickness using a starrett portable dial hand micrometer, crushing strength (hardness) by Erweka hardness tester (Erweka TBH28, F.R.G.) and friability by Roche friabilator and the mean values of five determinations as well as their percent relative standard deviations (% R.S.D) were investigated. Disintegration testing (USPXX) was carried out on four tablets in distilled water at 37$\pm$0.5°C using Erweka disintegration apparatus and the results were reported as a mean disintegration time.

Dissolution studies

The dissolution studies were performed using a standard USP rotating paddle dissolution apparatus (Erweka, Model DT-D6, F.R.G.) at 80 rpm. 500 ml of both the simulated gastric fluid (SGF, pH 1.2) and the simulated intestinal fluid (SIF, pH 7.4) at 37°C were used separately as the dissolution medium into which an amount of the drug resinate (equivalent to 20 mg drug) or the prepared IER-tablet which contains an amount of the drug resinate equivalent to 50 mg drug was placed. A 5 ml sample was withdrawn and replaced with fresh medium at fixed time intervals. The concentration of diclofenac sodium was determined from the absorbance at 276 nm.[2]

Dissolution studies of the control tablets containing 50 mg drug or the commercial preparations (conventional fast-release tablets (50 mg drug)) or sustained-release tablets (Tablets (SR), 75 mg drug)) were performed in a similar manner.

In evaluating the effect of concentration of Na^+ ions on drug release from the prepared resinates, the samples were equilibrated with solutions of sodium chloride over a

concentration range of 0 to 0.15 M for 6 h.

Ulcerogenic activity

The rabbits (1.5-2 kg) were separately dosed with 50 mg of diclofenac sodium powder, or an equivalent dose of the drug in the form of diclofenac sodium resinate, IER-tablets and a commercial sustained-release product of the drug (tablets (SR), 75 mg drug)). Each dose was filled into a hard gelatin capsule and one capsule daily was given to each rabbit for 5 days.

The animals were kept free for feeding. At the end of the experiment, the rabbits were sacrificied and the excised stomachs were opened longitudinally along the lesser curvature and their contents were washed out with normal saline. Each stomach was streteched out and examined with a magnifying lens for the presence of any pathological changes such as petechial haemorrhages, mucosal sloughing or ulceration and then photographed.

RESULTS AND DISUCSSION

Characterization of the prepared diclofenac sodium-resin complexes

Adsorption isotherm

The adsorption isotherm of diclofenac sodium on to Dowex 1-X_8.Cl^- at 25°C linearized according to the Langmuir isotherm expressed in Equation (1) is shown in Fig. 1.

$$\frac{Ce}{(X/m)} = \frac{Ce}{Q_{max}} + \frac{1}{(KQ_{max})} \qquad (1)$$

where Ce is the equilibrium concentration of diclofenac sodiun, X/m is the adsorption density expressed as amount of diclofenac adsorbed (mg)/gram of resin, K is the adsorption affinity parameter with units of reciprocal concentration and Q_{max} is the estimated maximum adsorptive capacity expressed as mg of drug adsorbed per gram of the resin. Figure 1 shows that the data conform to the Langmuir model. The linear plot (r= 0.9994) indicates that Q_{max} has a value of about 649.60 mg of the drug/gram of resin. The linearity of Langmuir plot indicates monomolecular adsorption of drug onto the tested resin and a chemisorption adsorption mechanism. Thus, the positive charge carried by the resin particles in aqueous suspension plays a role in binding the negative charge of drug anionic moiety.

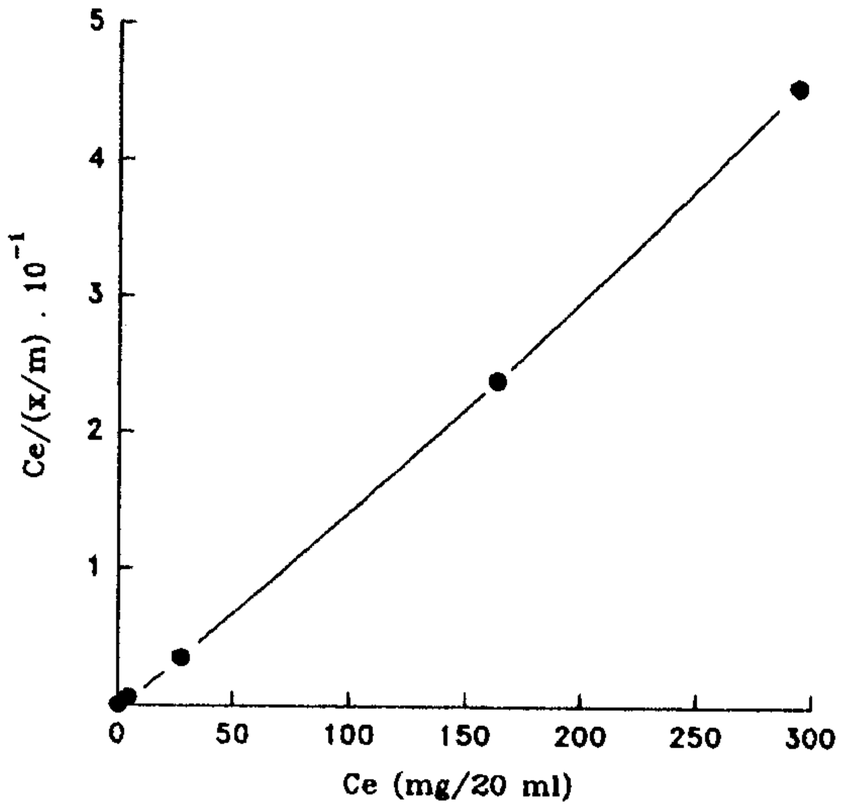

Fig. 1: Plot of Langmuir adsorption isotherm of diclofenac sodium by Dowex 1-X_8.Cl^-. (r^2 = 0.9988).

IR, DSC and powder X-ray diffraction analysis

Infrared absorption bands of the pure drug, ion-exchange resin and diclofenac sodium resinate at drug/resin ratios of 0.5:1 and 1:1 are shown in Fig. 2a-d. The results indicated interactions between the drug and the ion-exchange resin (i.e., the drug in the anionic form displaced the chloride ion of the resin) as indicated by disappearance of the characteristic absorption bands at 1564 cm^{-1} corresponding to the carbonyl group of the drug in the Na^+ form[22] as well as absorption bands at 737 and 758 cm^{-1} corresponding to tri and ortho-substituted benzene,[22] respectively (Fig. 2c,d).

The differential theraml analysis revealed that a sharp endotherm was seen at 286°C, corresponding exactly to the melting point of diclofenac sodium[23] (Fig. 3a). However, in the case of the melting phase transition of the drug resinate, the maxima of the peak of drug resinate were broader and shifted to a lower temperature (195°C) than that of the drug melting point (Fig. 3c,d). This should be attributed to the presence of the drug in the complex form as a solid solution state.

X-ray diffraction analysis for the drug resinate showed no steep peaks as the parent

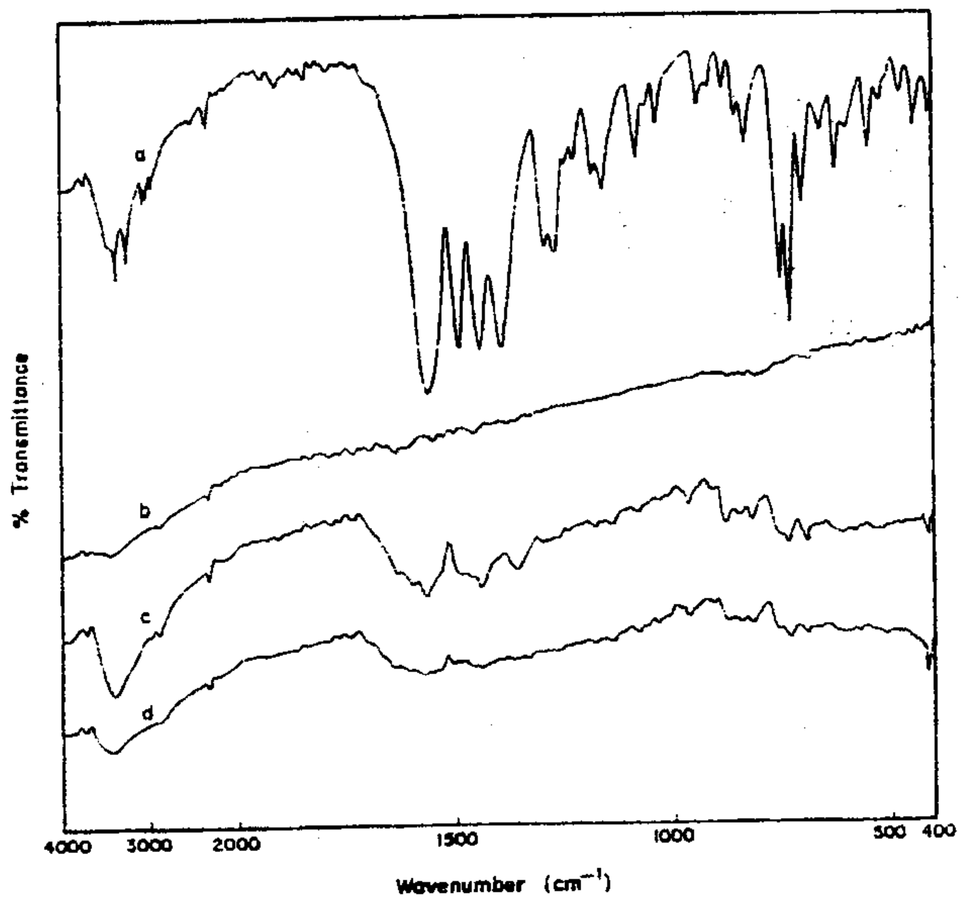

Fig. 2: IR spectra of (a) diclofenac sodium alone; (b) Dowex 1-X_8.Cl^- alone; (c) diclofenac sodium resinate (drug/resin ratio of 0.5:1) and (d) diclofenac sodium resinate (drug/resin ratio of 1:1).

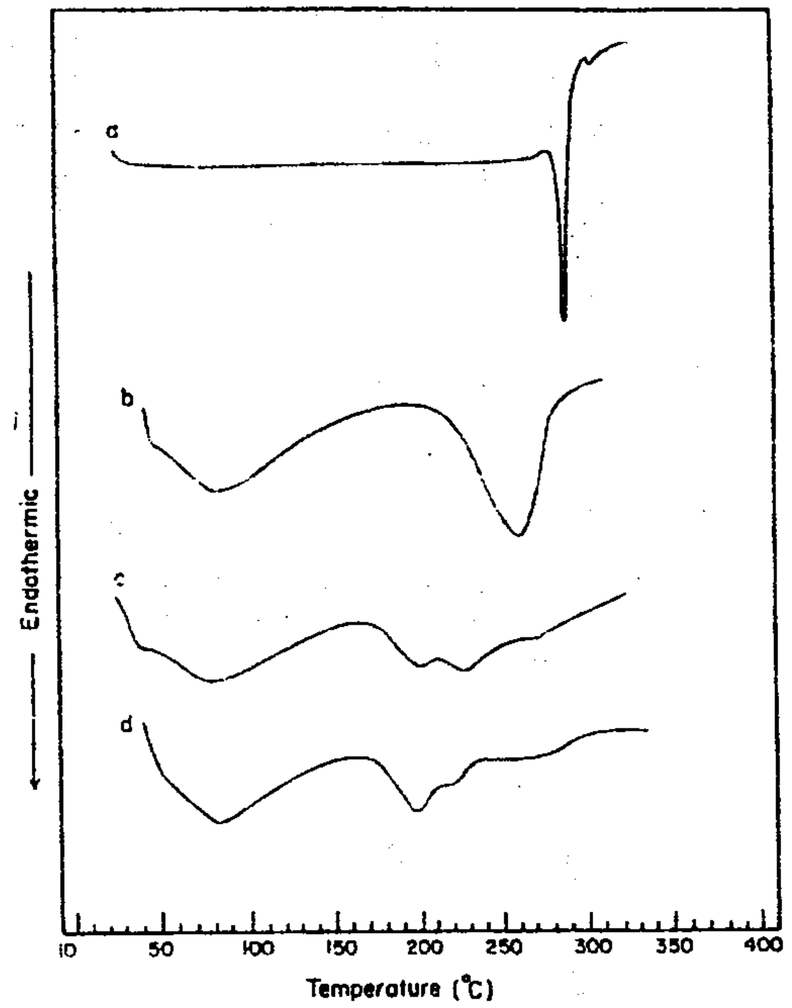

Fig. 3: DSC patterns of (a) diclofenac sodium alone; (b) Dowex 1-X_8.Cl^- alone; (c) diclofenac sodium resinate (drug/resin ratio of 0.5:1) and (d) diclofenac sodium resinate (drug/resin ratio of 1:1).

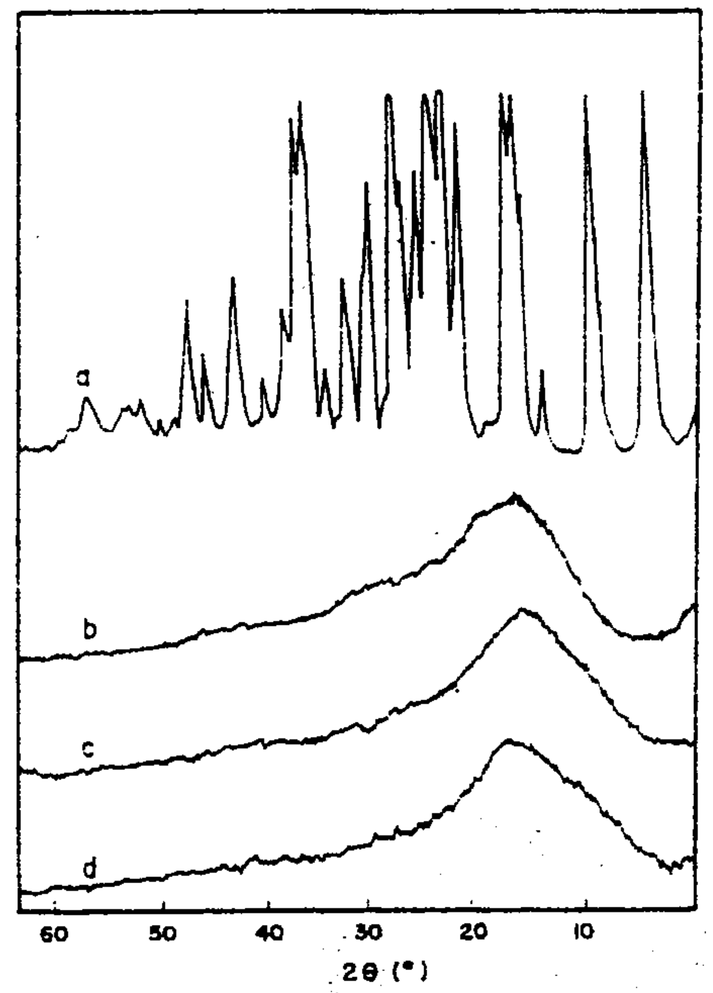

Fig. 4: X-ray diffraction patterns of (a) diclofenac sodium alone; (b) Dowex 1-X_8.Cl^- alone; (c) diclofenac sodium resinate (drug/resin ratio of 0.5:1) and (d) diclofenac sodium resinate (drug/resin ratio of 1:1).

drug (Fig. 4c,d). This confirmed the above mentioned interactions illustrated by IR absorption spectroscopy and DSC analysis.

Loading characteristics of the drug on Dowex 1-X_8.Cl^-

The time required for equilibrium to be reached for the uptake of the drug on the used resin was found to be 24 h. The relation between the drug uptake and the reaction time is shown in Table 1.

Table 2 shows the relation between the initial drug concentration and the amount of drug that reacted with the resin. Evidently, the amount of drug reacted increases as the initial drug concentration increases up to a drug/resin ratio of 1:1. With higher drug/resin ratios (2:1 and 3:1), a slight reduction in the amount of drug loaded and drug content was observed (Table 2).

Release characteristics of the prepared drug-resin complexes

Since diclofenac sodium has a pKa value of 4, the solubility in acidic medium such as gastric juice is extremely low.[9] Accordingly, the dissolution studies of the drug-resin complexes prepared with different loaded drug concentrations were conducted in simulated intestinal fluid (S.I.F., pH 7.4) as shown in Fig. 5. The obtained results revealed that drug/resin ratio of 2:1 exhibited the lowest release rate and that increasing drug/resin ratio from 0.5:1 to 1:1 had little influence on drug release rate (Table 3). Apparently, the drug-resin complexes had the ability to achieve a prolonged release pattern of diclofenac sodium with only 28-42% of diclofenac sodium was released in the first 8 h (Fig. 5), and a further 30-34% was released between 8 and 20 h (data not shown).

Table 1: Effect of reaction time on the uptake of diclofenac sodium on Dowex 1-X_8.Cl^-.

Time (hours)	Amount loaded mg/0.125 gm	Loading capacity mg/gm	Loading[a)] %	Drug content %
1	40.920	327.36	32.74	24.22
4	68.740	549.92	54.99	34.94
8	81.767	654.38	65.44	39.55
16	97.630	781.04	78.10	43.85
24	99.350	794.79	79.48	44.28

- Drug/resin ratio: (1:1)

[a)] Loading (%) = W/W_o x 100, where W: amount of drug loaded, W_o: initial drug amount.

Table 2: Loading characteristics of diclofenac sodium on Dowex 1-X_8.Cl^- as a function of drug concentration.

Initial drug conc. mg/ml	Drug/resin ratio	Amount loaded mg/0.125 gm	Loading capacity mg/g	Loading[a)] (%)	Drug content (%)
3.125	0.5:1	62.390	499.120	99.85	33.40
4.688	0.75:1	89.460	715.680	95.46	41.71
6.250	1:1	97.472	779.776	77.98	43.81
12.500	2:1	85.980	687.870	34.40	40.75
18.750	3:1	80.885	647.680	21.57	39.29

- Equilibrium time: 24 hr.

[a)] Loading (%) = W/W_o x 100, where W: amount of drug loaded, W_o: initial drug amount.

Table 3: Kinetic constants of the release rate of diclofenac sodium calculated according to the mass low.

Variable	Initial release period[a]		Terminal release period	
	r^2	Slope x 10^{-2}	r^2	Slope x 10^{-2}
Drug/resin ratio[b]				
0.5:1	0.97850	0.2007	0.9784	0.07280
0.75:1	0.98500	0.1725	0.9988	0.07266
1:1	0.92060	0.1896	0.9938	0.08905
2:1	0.91979	0.1827	0.9967	0.04930
3:1	0.90970	0.2160	0.8779	0.06399
NaCl concentration (M)[c]				
0	-	-	0.9355	0.01504
0.01	0.91090	0.0621	0.9329	0.05160
0.05	0.90701	0.4220	0.9441	0.12300
0.075	0.9805	0.6431	0.9706	0.19020
0.15	0.9611	0.7373	0.9696	0.31960

[a]: Initial release period: 0-60 minutes.
[b]: Release medium: simulated intestinal fluid (S.I.F., pH 7.4).
[c]: Drug/resin ratio of 1:1.

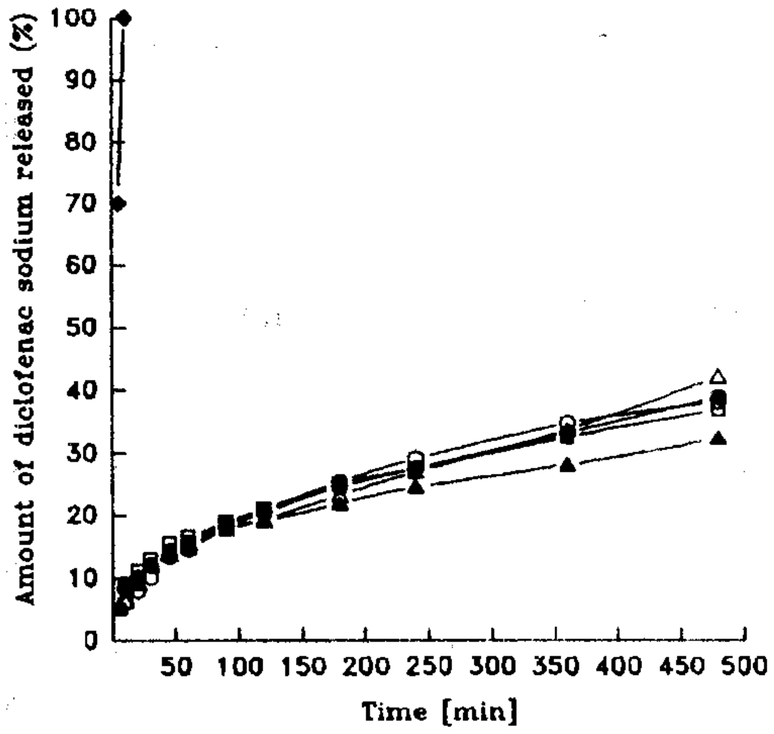

Fig. 5: In-vitro release of diclofenac sodium from its resinates prepared at different drug/resin ratios (S.I.F., pH 7.4).
Key: Intact drug (◆). Drug/resin ratio: (○) 0.5:1, (●) 0.75:1, (△) 1:1, (▲) 2:1 and (□) 3:1.

Kinetics of the drug release from resinates can be described as a chemical phenomenon.[24] For the case of two monovalent ions, the mass low applies to the exchange process when written as:

$$A^- + BR \rightleftharpoons B^- + AR \qquad (2)$$

Where A^- and B^- are the exchanging monovalnet anions and where R refers to the insoluble resinates. When the concentrations of A^- and B^- in solution are maintained constant, the drug release kinetics can be described by Equation 3:[24]

$$Ln(1-F) = -K.t \qquad (3)$$

If several mass action rate processes occur independently, the individual rate constants can be obtained by analyzing a Ln(1-F)-time plot in the same manner as for the decay of a mixture of radioactive species.[24]

When plotting the results according to the mass low (Equn. 3). The graphs of the influence of the drug/resin ratio and sodium chloride

concentration on drug release rate can be described by two release rate processes (Table 3 and Figs. 6&7): rapid release during the initial period ($Kx10^{-2}$= 0.0621-0.73731 min^{-1}), followed by a decreasing release rate ($Kx10^{-2}$= 0.01504-0.31960 min^{-1}) suggesting a particle diffusion release process for diclofenac sodium. However, the deviation from linearity is most pronounced with higher concentrations (0.075 and 0.15 M) of sodium chloride (Fig. 7).

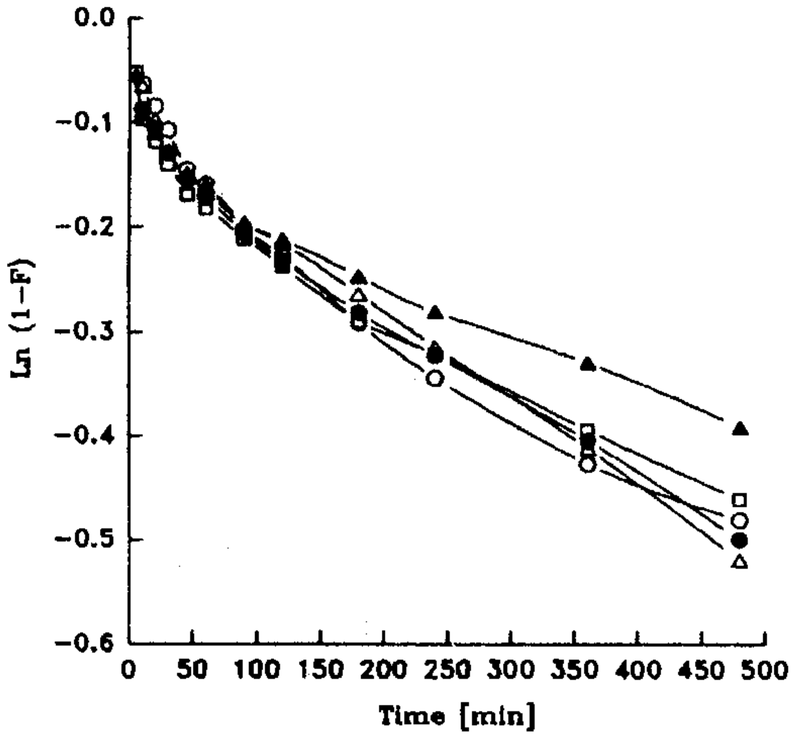

Fig. 6: Effect of drug/resin ratio on release rate of diclofenac sodium from its resinates according to the mass low (S.I.F., pH 7.4).
Key: Drug/resin ratio: (○) 0.5:1, (●) 0.75:1 (△) 1:1, (▲) 2:1 and (□) 3:1.

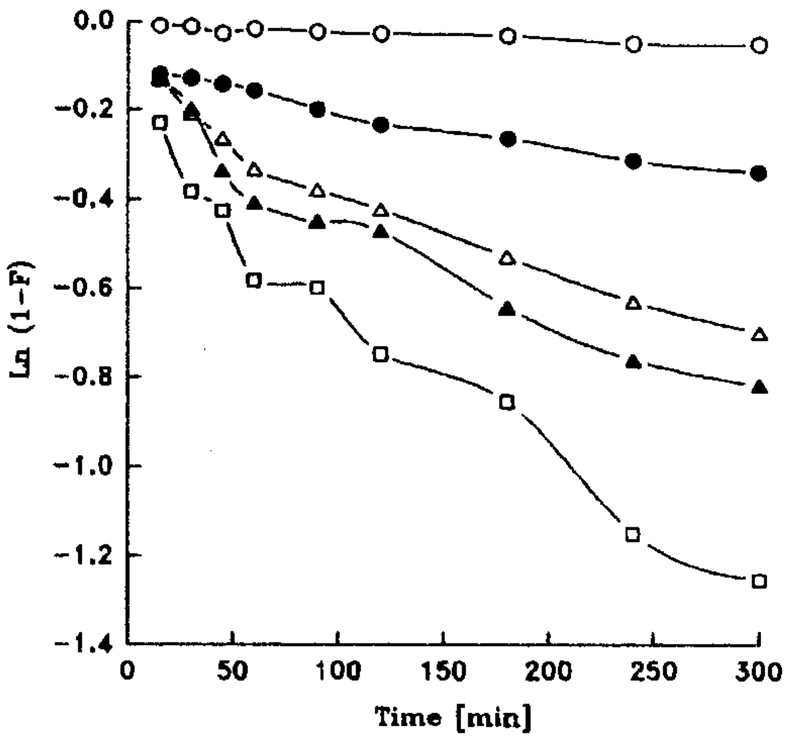

Fig. 7: Effect of sodium ion concentration on release rate of diclofenac sodium from its resinates according to the mass low.
Key: Na^+ ions concentration: (○) none, (●) 0.01 M, (△) 0.05 M, (▲) 0.075 M and (□) 0.15 M. (Drug/resin ratio: 1:1).

Bimodal relationships of this type were noted previously during studies on the release of several drugs from ion-exchange resins.[25,26] The calculated values of the individual rate constants presented in Table 3 revealed that the rate of drug release increased with increasing the concentrations of Na^+ ions in solution. This indicates that the exchange process is chemically rate controlled.[24]

Characterization of the matrix tablet formulations containing diclofenac sodium or its resinates

Physical properties of the tablets

The compression properties of the prepared tablets in comparison with the commercial tablets of diclofenac sodium are presented in Table 4. All the tablets prepared were found to satisfy the USPXXII requirements for weight uniformity and thickness (%RSD ≤ 0.75) as well as the friability test (%F < 1%). The friability of IER-tablets (tablets containing diclofenac sodium resinates) was lower than those of the control tablets (tablets of diclofenac sodium alone), indicating that tabletting of the drug-resin complexes is an acceptable technique for producing prolonged-release tablets.

The tested excipients produced tablets successfully with hardness values of 4.25-15 kg. However, IER-tablets containing Avicel PH 101 (70% w/w) exhibited the highest hardness value (15 kg) and the lowest friability (% friability= 0.15). These results are in agreement with those reported by Yu *et al.*[27] who found that as the content of microcrystalline cellulose in paracetamol tablets was reduced, the tensile strength decreased and the friability percentage increased.

Tablets containing lactose possessed the lowest values of hardness (4.25-4.5 kg) and the highest values of friability percentage (0.86-0.96). However, the use of compritol as an excipient even at a higher percentage (70% w/w) provided good tablets without any signs of capping or sticking.

Disintegration study

Tablets containing Avicel PH 101 or lactose showed rapid disintegration within <20 minutes, while tablets containing sodium

Table 4: Physical properties of the tablets containing diclofenac sodium or its resinates (mean ± RSD).

Formulation type[a]	Excipient type	Weight (g)	Thickness (mm)	Diameter (mm)	Hardness (kg)	Friability (%)	HFR[b]	Disintegration time (h)
I- Diclofenac sodium tablets	Avicel PH 101	0.1810 (0.60)	2.45 (0.55)	8	8.25 (3.2)	0.80	10.31	0.25
	Lactose	0.1800 (0.74)	2.45 (0.60)	8	4.25 (4.2)	0.96	4.43	0.12
	Sodium carboxy-methyl cellulose	0.1820 (0.27)	2.48 (0.31)	8	12.50 (2.7)	0.90	13.89	>4
	Compritol	0.1849 (0.36)	2.50 (0.45)	8	5.50 (2.8)	0.74	7.43	>4
II- Diclofenac sodium resinates tablets	Avicel PH 101	0.6185 (0.51)	4.00 (0.36)	12	15.00 (3.6)	0.15	100	0.30
	Lactose	0.6201 (0.60)	4.05 (0.37)	12	4.50 (2.9)	0.86	5.23	0.15
	Sodium carboxy-methyl cellulose	0.6159 (0.24)	4.00 (0.27)	12	15.00 (3.4)	0.64	23.44	>4
	Compritol	0.6205 (0.35)	4.10 (0.41)	12	6.25 (2.4)	0.60	10.42	>4
III- Commercial tablets[c]								
Product I	-	0.2261 (0.25)	4.00 (0.32)	-	7.50 (2.7)	0.18	41.67	2.05
Product II	-	0.2166 (0.34)	3.80 (0.46)	8.4	11.33 (3.6)	0.10	113.3	0.25

[a]: The prepared tablets contained 50 mg of diclofenac sodium or an amount of diclofenac sodium resinates equivalent to 50 mg of the drug.
[b]: HFR refers to hardness / friability ratio.
[c]: Product I is sustained release tablets containing 75 mg of diclofenac sodium.
Product II is conventional tablets containing 50 mg of diclofenac sodium.

carboxymethylcellulose or compritol remained intact with no signs of disintegration after >4 h. The commercial fast-release and sustained-release tablets disintegrated after 0.25 and 2.05 h, respectively (Table 4).

Release behaviour of the tablets

Figures 8 and 9 indicate the effect of various release-regulating excipients (Avicel PH 101, lactose, sodium carboxymethylcellulose and compritol) on the release rate of diclofenac sodium from the control tablets and IER-tablets in simulated gastric fluid (S.G.F., pH 1.2) and simulated intestinal fluid (S.I.F., pH 7.4). Evidently, the drug release rates were found to be generally higher in S.I.F. (pH 7.4) than in S.G.F. (pH 1.2). Specifically, excipient type showed minimal effect on drug release at the acidic pH. Thus, the prepared tablets released less than 10% of the drug in S.G.F. (pH 1.2) within 4 h (Figs. 8A, and 9A). This may be most likely due to the limited solubility of diclofenac acid form (3.55-3.78 μg/ml) in a particular hydrogen ion environment (pH 1.08-2.5).[9]

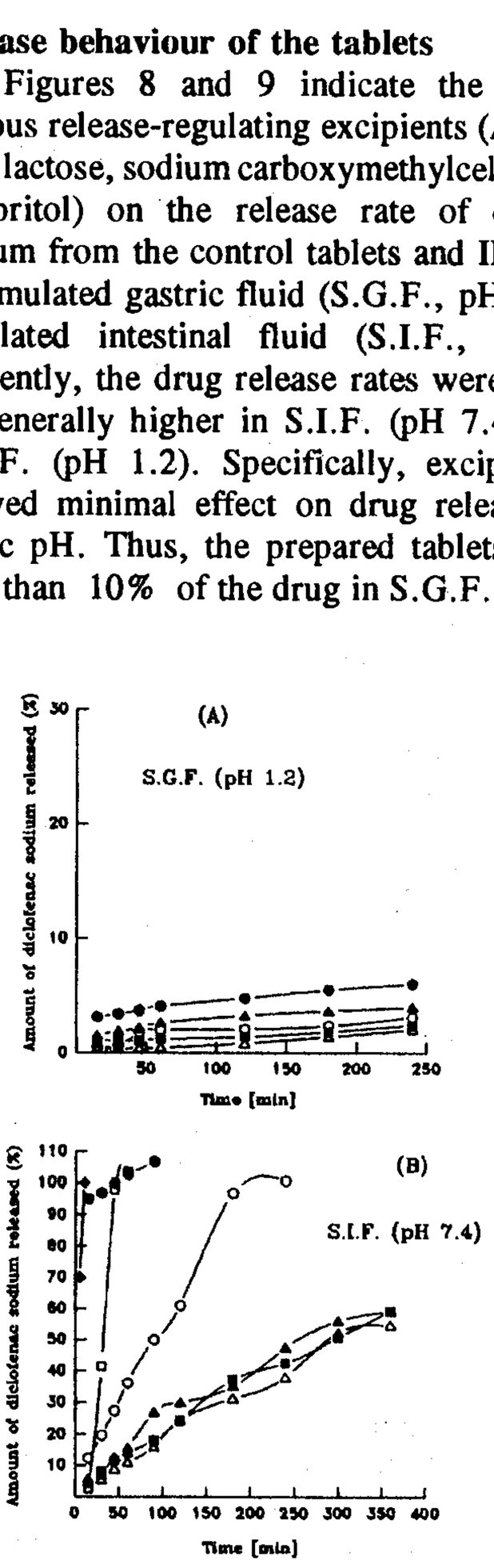

Fig. 8: In-vitro release profiles of diclofenac sodium from its matrix-tablet formulations prepared using various release-regulating excipients.
Key: (◆) Intact drug. Release-regulating excipients: (○) Avicel pH 101, (●) lactose (△) sodium carboxymethylcellulose and (▲) compritol. Commercial diclofenac sodium tablet formulations: (□) conventional tablets (50 mg drugs), (■) sustained-release tablets (75 mg drug).

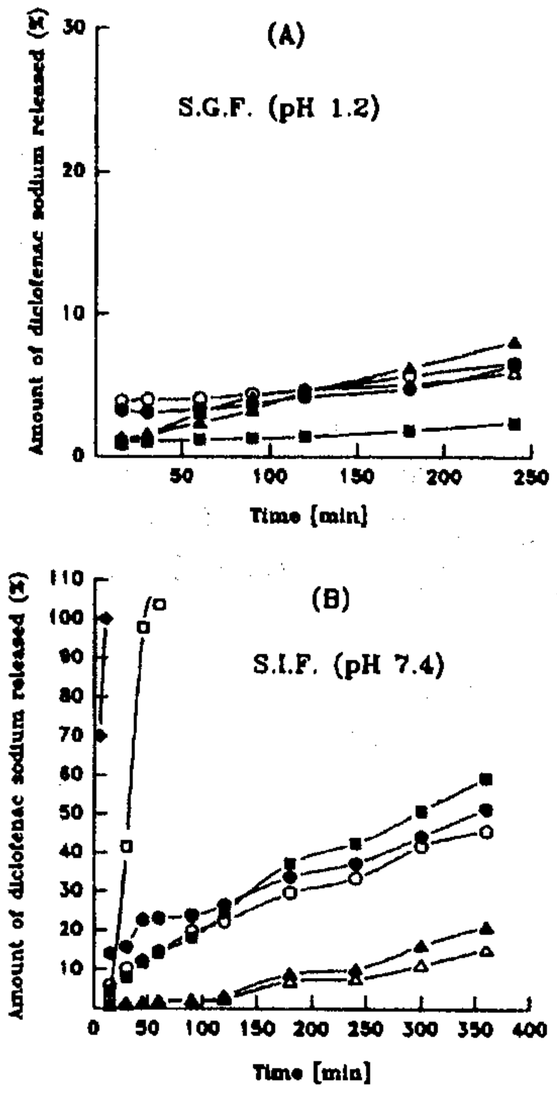

Fig. 9: In-vitro release profiles of diclofenac sodium from its matrix-tablet formulations prepared using the drug resinates with various release-regulating excipients.
Key: (◆) Intact drug. Release-regulating excipients: (○) Avicel pH 101, (●) lactose (△) sodium carboxymethylcellulose and (▲) compritol. Commercial diclofenac sodium tablet formulations: (□) conventional tablets (50 mg drug), (■) sustained-release tablets (75 mg drug).

The drug release profiles of the prepared and commercial tablets in S.I.F. (pH 7.4) are shown in Figs. 8B and 9B. It is apparent that there was a different dissolution behaviour for tablets prepared with lactose compared with other tablet formulations. The control tablets containing lactose as well as the conventional tablet formulation released almost all the drug within 45 minutes, while those containing Avicel PH 101 released only 27.37% of the drug during this period (Fig. 8B). When sodium carboxymethylcellulose or compritol were used as excipients, the produced tablets achieved a prolonged release pattern of diclofenac sodium

and released less than 60% of the drug in 6 h (Fig. 8B).

Comparison among Figs. 8B and 9B revealed that all IER-tablets containing the drug resinates exhibited a pronounced prolonged-release behaviour in comparison with the control tablets or the commercial tablet formulations containing 50 mg of the drug (conventional tablets) or 75 mg of the drug (sustained-release tablets).

IER-tablets prepared using Avicel PH 101 or the water soluble excipient, lactose achieved 45.61 and 51.31% total release of diclofenac sodium in 6 h, respectively. During the course of dissolution, IER-tablets containing lactose disintegrated rapidly within <8 min., leading to an increase in the exposed fresh surface area of the tabletted resinates for dissolution. In comparison, the percent released of diclofenac sodium from tablets containing sodium carboxymethylcellulose and compritol (waxy matrix) decreased to 15.08 and 21.07% in 6 h, respectively. This might be attributed to the physical properties of these tablets which did not disintegrate during both dissolution tests. The appearance of tablets containing compritol at the end of the dissolution test showed no signs for swelling or gel formulation due to its waxy nature. This indicates that compritol contained in the tablet appeared to create an inert matrix through which the drug might diffuse.[20] Basically, sodium carboxymethylcellulose tablets swelled and behaved as a gel-like system, thereby creating a gelatinous barrier through which the drug must diffuse during the dissolution process. The formation of such a gel layer or matrix around the resin particles might account for the significantly decreased release rate of tablets containing hydrophilic polymeric substances.[28]

Kinetics of the drug release

Analysis of the release data of the prepared tablets and commercial tablets was carried out according to zero-order kinetics[29] and Higuchi matrix diffusion-controlled mechanism[30] (Table 5). A simple empirical exponential relation (Equn. 4) was also proposed by Ritger and Peppas[31,32] to describe the relative availability of drugs from swellable and non-swellable matrix systems:

$$\frac{M_t}{M_\infty} = Kt^n \qquad (4)$$

where M_t/M_∞ is the fraction of drug released at time t, K is the release-rate constant incorporating structural and geometric characteristics of the tablets, and n is the diffusional exponent indicative of the release mechanism. The value of n for a cylinder is 0.45 for Fickian diffusion, 0.89 for case II transport (zero-order kinetics) and >0.89 for super case II transport.[31,32] The values of n, correlation coefficient (r) and the time for 50% of drug released ($T_{50\%}$) for all formulations are given in Table 5. The diffusion models were sufficiently linear and the differences between them were noted to be minimal. It was found that (n) values ranged from 0.455 to 0.519 for all the IER-tablets containing the drug resinates or the control tablets containing compritol, confirming a Fickian-diffusion kinetics (square root of time relation) and that release of diclofenac sodium appears to be generally controlled by its low diffusivity in the gel layer formed as the tablet swells. All the above results are consistent with those reported before.[9,20,33,34] Their results revealed that the release of a drug from an inert, heterogeneous matrix was linearly related to the square root of time relationship indicating that a matrix diffusion-controlled mechanism was operative. However, n takes on values of 0.791 and 0.837 for diclofenac sodium tablets (control tablets) containing Avicel PH 101 or sodium carboxymethylcellulose, respectively (Table 5). Phenomenologically, therefore, these tablets behave as near zero-order release systems ($n\rightarrow1$ for case II transport) which exhibited higher correlation coefficients than the diffusion model during the first 60% of the drug release regardless of the specific molecular mechanisms of drug transport. This may be due to the larger surface area to volume ratio of the smaller control tablets (weight: 0.18 gm, thickness: 2.46 mm and diameter: 8 mm (Table 4) which provided an optimum balance between

Table 5: Kinetic assessment of the release data of the tablets containing diclofenac sodium or its resinates (S.I.F., pH 7.4).

Formulation type	Excipient type	Zero order		Higuchi-diffusion model		Ritger-peppas model		$T_{50\%}$ (h)
		K_o ($mg.min^{-1}$)	r^2	K_h ($mg.min^{-½}$)	r^2	Diffusional release exponent (n)	r^2	
I- Diclofenac sodium tablets	Avicel PH 101	1.04	0.999	12.05	0.990	0.791	0.997	1.50
	Lactose	1.64	0.996	15.10	0.980	0.053	0.958	<0.25
	Sodium carboxy-methyl cellulose	0.167	0.995	6.55	0.960	0.837	0.987	4.87
	Compritol	0.188	0.972	8.02	0.995	0.512	0.990	4.35
II- Diclofenac sodium resinates tablets	Avicel PH 101	0.121	0.909	4.62	0.998	0.515	0.992	>6
	Lactose	0.146	0.872	5.52	0.932	0.455	0.990	5.75
	Sodium carboxy-methyl cellulose	0.083	0.984	0.92	0.991	0.519	0.997	>6
	Compritol	0.110	0.979	1.27	0.983	0.476	0.989	>6
III- Commercial tablets[a]								
Product I	-	0.172	0.995	8.23	0.994	0.818	0.998	5.00
Product II	-	2.780	0.963	16.74	0.916	1.880	0.960	0.54

[a] Product I is sustained release tablets containing 75 mg of diclofenac sodium.
Product II is conventional tablets containing 50 mg of diclofenac sodium.

the diffusion and dissolution mechanisms of the swellable matrix to achieve case II transport.[12] Furthermore, the presence of greater drug concentration would enhance the dissolution mechanism and compensate for the decrease in release due to matrix swelling. Similar results were obtained with natural gum mini-matrix tablets formulations containing diclofenac sodium.[12] When this equation was applied to the release of diclofenac sodium from the commercial sustained-release tablets (product I, Table 5), a similar drug release behaviour was noted. The results obtained are in agreement with those obtained by Sheu *et al.*[9] who found that diclofenac release from voltaren SR is essentially a zero-order process. However, the conventional tablets produced a faster release rate with n values >0.89, thereby exhibiting a super case II transport.

Ulcerogenic activity

Table 6 shows the ulcer data of rabbits administered free diclofenac sodium, the drug resinate, IER-tablets (tablets containing the drug resinates) and a commercial sustained-release product of diclofenac sodium (tablets SR, 75 mg drug). Representative photomicrographs of the rabbit stomachs are shown in Figure 10. The control animals developed no signs of gastric ulcerations (Fig. 10A). The macroscopic gross appearance of pyloric and pre-pyloric gastric mucosa revealed that distinict ulcers occurred in the stomachs of all test rabbits. However, the numbers and severities of ulcers were found to be different in most of animals administered different treatments (Fig. 10B-E).

Administratin of diclofenac sodium alone was accompanied by an increase in the number and size of ulcers and resulted in the appearance of massive haemorrhaging, deep ulceration in the region of lesser curvature, mucosal sloughing and numerous pin-point ulcers in the greater curvature area (Fig. 10B). This may be attributable to the very limited solubility of diclofenac sodium, as a propionic acid derivative (pKa 4.7), at acidic pH- values resulting in a longer duration of contact between drug particles and gastric mucosa and hence, a higher gastric activity. Meshali *et al.*[35] reported that the rapidly absorbed form of the anti-inflammatory drugs

Table 6: Ulcer development in rabbits administered different formulations of diclofenac sodium.

Treatment	Number of ulcers in the ulceration region				Total number of ulcer
	Fundus	Lesser curvature	Greater curvature	Pylor-ous	
- Control	-	-	-	-	-
- Free diclofenac sodium	1 (6)	10 (1.5-6)	a	-	>11
- Diclofenac sodium resinates	-	2 (2-3)	-	2 (1-2)	4
- Diclofenac sodium resinate tablets containing:					
- Avicel PH 101	-	2 (2-4)	-	2 (1-2)	4
- Sodium carboxymethylcellulose	-	-	-	1 (11)	1
- Compritol		3	a	1 (10)	>4
- Commercial tablets:					
- Product I[b]	2 (1-3)	9 (1-3)	a	-	>11

- Data between parantheses represent the mean size of ulcer (mm).
a: Means numerous pin-point ulcers.
b: Product I is sustained release tablets containing 75 mg of diclofenac sodium.

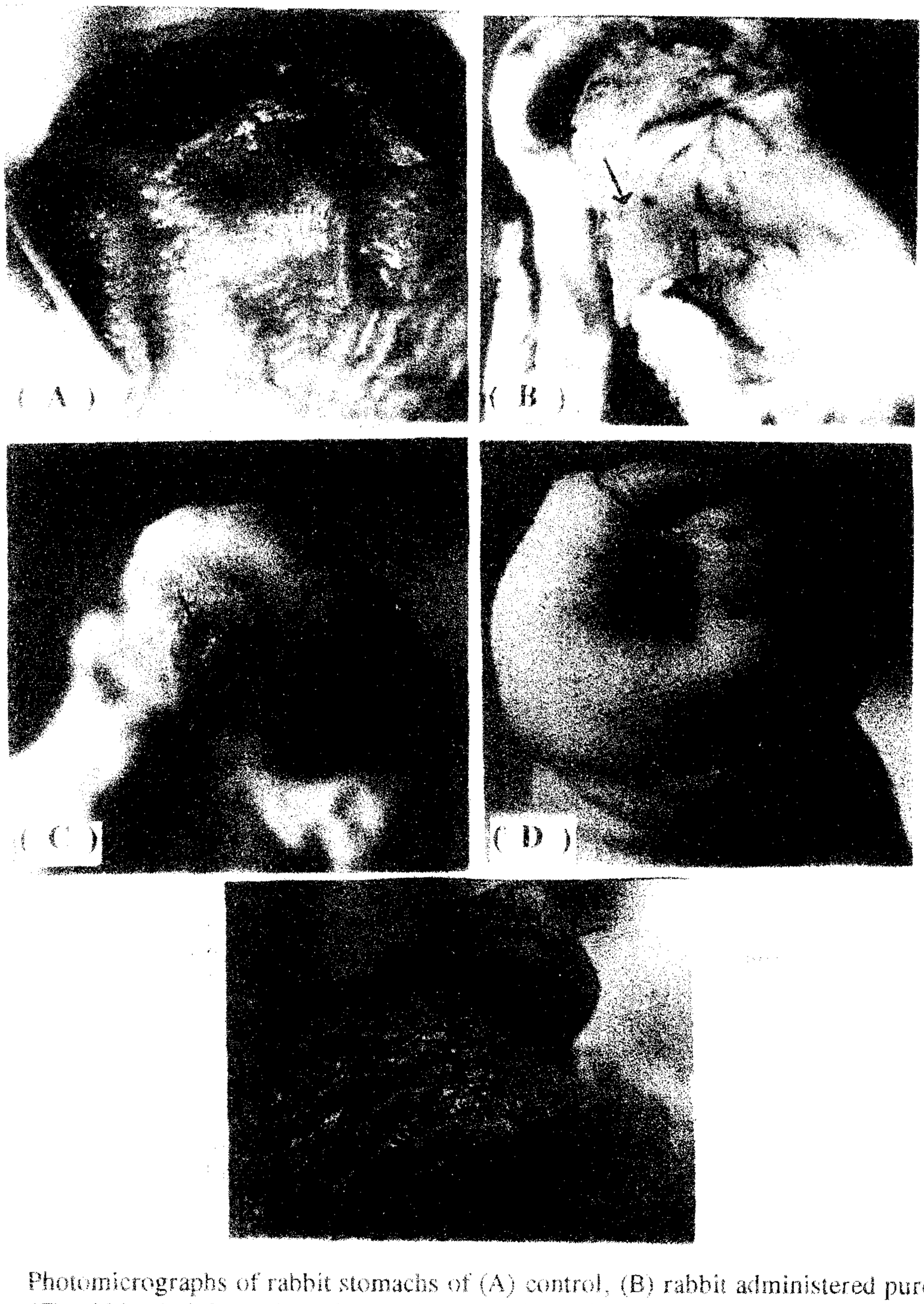

Fig. 10: Photomicrographs of rabbit stomachs of (A) control, (B) rabbit administered pure drug, (C) rabbit administered the drug resinates, (D) rabbit administered IER-tablets containing the drug resinates and sodium carboxymethylcellulose and (E) rabbit administered the commercial sustained-release product.

had a shorter contact time with the gastric mucosa and hence had lower ulcerogenic activity.

Diclofenac sodium resinate and IER-tablets prepared using Avicel PH 101 as an excipient demonstrated lower ulcerogenic activity as compared with the drug alone or the commercial sustained release product (Table 6 and Fig. 10B-E). The number of ulcers decreased from more than 11, mostly in the lesser and greater curvature areas of rabbits administered free drug or the commercial products to 4 for rabbits given diclofenac sodium resinate or IER-tablets containing Avicel PH 101 (Table 6). However, the size of ulcers (1-4 mm) of the drug resinate, IER-tablets containing Avicel PH 101 and the commercial product was found to be smaller than that of the free drug (1.5-6 mm). The differences in the ulcerogenic activities between the free drug, drug resinate. IER-tablets containing Avicel PH 101 and the commercial product can possibly be explained by the physical and sustained-release properties of the prepared drug-resin complexes and their Avicel PH 101 tablet form. The resinate form of diclofenac sodium subdivided the drug in the stomach into very fine particles of the drug-resin complex having a larger surface area and decreased direct contact between gastric mucosa of the stomach and the drug in the sustained-release resinate form whose release properties are dependent on ion-exchange with the counter ions of the stomach. Meanwhile, Avicel PH 101 tablets containing the drug resinate demonstrated similar ulcerogenic activities to those of the untabletted drug resinate (Table 6). A result that may be attributed to the expected rapid disintegration of Avicel PH 101 tablets (estimated disintegration time = 0.3 h) into smaller fragments of resinate particles embeded in the tablet matrix.

Although the ulcers number (4 ulcers, 1-4 mm) was larger in the case of the drug resinate or IER-tablets containing Avicel PH 101, it appeared far less ulcerogenic than IER-tablet containing sodium carboxymethylcellulose (one deep ulcer of an average size of 11 mm in the pyloric region) due to the scattering and less severity of their ulcers (Table 6 and Fig. 10C,D). However, the ulcerogenic activities of IER-sodium carboxymethylcellulose tablets might be attributed to their longer disintegration time (> 4 h) and mucoadhesion properties[36] that might result in the residence of tablets on the stomach wall for a longer period of time. This could be explained on the basis that anionic polymers which have ionizable groups (carboxylic acid function) in the molecule such as sodium carboxymethylcellulose have been reported to be an excellent mucoadhesive materials on hydration due to hydrogen bond formation between the mucoadhesive material and mucin.[36,37] Thus, the matrix tablet might form a gel-like disk on hydration, thereby, neceissiating a longer time for disintegration.

While it might be the first time to assess compritol as an excipient for studying the ulcerogenic activities of diclofenac sodium, the ulcer development data revealed the appearance of an ulcer of larger size (10 mm) in the pyloric region, in addition to smaller ulcers and numerous pin-point ulcers in the lesser and greater curvature regions, respectively. This may be due to the residence of the tablet (disintegration time: >4 h) on the gastric mucosa for a longer duration of time, thereby, resulting in a localized drug release at the points of contact.

Conclusion

From the results obtained, it can be concluded that the prepared ionic complex was of high drug loading and exhibited a sustained-release properties. We have shown that diclofenac sodium release from the prepared drug resinate is a function of the Na^+ ions concentrations indicating a chemically rate-controlled exchange process that resulted in two release rate profiles, thereby, suggesting a particle diffusion release process.

The compression of the drug resinates into tablets using various release-regulating excipients (Avicel PH 101, lactose, sodium carboxymethylcellulose and compritol) resulted in further prolongation of the drug release rate indicating that tabletting of the drug resinate is

a potentially acceptable technique for preparing prolonged-release tablets.

The dramatic increase in drug release rate from the control diclofenac sodium tablets containing lactose could be reduced greatly by replacement of the drug by its ionic complexes confirming that the release-limiting step is that of the ionic exchange process. It was also found that a Fickian diffusion-type kinetics (the Higuchi-linear square root of time relationship) was the best model to describe the drug release kinetics from the IER-tablets containing the drug-resin complex.

Administration of the drug resinates or its tablets containing Avicel PH 101 into rabbits resulted in reduction of the gastric ulcerogenic activity of diclofenac sodium compared to sodium carboxymethylcellulose tablets, compritol tablets or the commercial sustained-release tablets. In this way, it was possible to modify the release characteristics of ionic complexes and avoid the harmful effects of diclofenac on the gastric mucosa.

REFERENCES

1- Martindale: The Extra Pharmacopoeia, 31th Ed., J. E. F. Reynolds (ed.) Royal Pharmaceutical Society, London, pp. 36-37 (1996).
2- B. Arica, M. Y. Arica, H. S. Kas, A. A. Hincal and V. Hasirci, J. Microencapsulation, 13, 689 (1996).
3- S. Bhatnagar, S. Nakhare and S. P. Vyas, J. Microencapsulation, 12, 13 (1995).
4- M. E. Palomo, M. P. Ballesteros and P. Frutos, Drug. Deve. Ind. Pharm., 23, 273 (1997).
5- H. Ichikawa, Y. Fukumori and C. M. Adeyeye, Int. J. Pharm., 156, 39 (1997).
6- M. AçikgÖz, H. S. Kas, Z. Hascelik, Ü. Milli and A. A. Hincal, Pharmazie, 50, 275 (1995).
7- Y. Hirotani, Y. Arakawa, Y. Maeda, A. Yamaji, A. Kamada and T. Nishihata, Chem. Pharm. Bull., 35, 3049 (1987).
8- Y. Miyagawa, T. Okabe, Y. Yamaguchi, M. Miyajima, H. Sato and H. Sunada, Int. J. Pharm., 138, 215 (1996).
9- M. T. Sheu, H. L. Chou, C. C. Kao, C. H. Liu and T. D. Sokoloski, Int. J. Pharm., 85, 57 (1992).
10- F. Acartürk, Pharmazie, 44, 547 (1989).
11- A. Fini, G. Fazio, I. Orienti, V. Bertasi, V. Zecchi and I. Rapaport, Eur. J. Pharm. Biopharm., 38, 66 (1992).
12- J. Sujja-Areevath, D. L. Munday, P. J. Cox and K. A. Khan, Int. J. Pharm., 139, 53 (1996).
13- S. Borodkin, Ion-Exchange Resin Delivery Systems. In: Polymers for Controlled Drug Delivery (P.J. Tarcha, ed.), CRC Press, Inc., Boca Raton, Fl, pp 215-230 (1991).
14- A. S. Geneidi and H. Hamacher, Pharm. Ind., 42, 198 (1980).
15- D. Torres, B. Seijo, G. Garcia-Encina, M. J. Alonso and J. L. Vila-Jato, Int. J. Pharm., 59, 9 (1990).
16- S. MOtycka, C. J. L. Newth and J. G. Nairn, J. Pharm. Sci., 74, 643 (1985).
17- Y. Raghunathan, L. Amsel, O. Hinsvark and W. Bryant, J. Pharm. Sci., 70, 379 (1981).
18- T. Kondo, E. Hafez, H. Abdel-Monem, N. Muramatsu, S. El-Harras and I. El-Gibaly, Powder Technology, 88, 101 (1996).
19- D. Torres, G. Garcia-Encina, B. Seijo, J. L. Vila-Jato, Int. J. Pharm, 121, 239 (1995).
20- M. M. Meshali, G. M. El-Sayed, M. M. Abd El-Aleem and Y. El-Said, S.T.P. Pharma Sci., 5, 429 (1995).
21- G. M. El-Sayed, Pharmaceutical Study on Formulation and Evaluation of Sustained Release Tablets Containing Certain Drugs, Ph.D. Thesis, Mansoura University (1993).
22- K. Florey (ed.), Analytical Profiles of Drug Substances, Academic Press, Inc., Vol. 19, pp. 127-129 (1990).
23- A. C. Moffat (ed.), Clarke's Isolation and Identification of Drugs, 2nd ed., The Pharmaceutical Press, London, p. 533 (1986).
24- G. E. Boyd, A. W. Adamson and L. S. Myers, J. Am. Chem. Soc., 69, 2836 (1947).
25- J. A. P. Vercammen, Int. J. Pharm., 87, 31 (1992).
26- V. Khouw, H. G. Giles and E. M. Sellers, J. Pharm. Sci., 67, 1329 (1978).

27- M. C. M. Yu, M. H. Rubinstein and I. M. Jackson, J. Pharm. Pharmacol., 40, 669 (1988).
28- C.H. Llu, Y. H. Kao, S. C. Chen, T. D. Sokoloski and M. T. Sheu, J. Pharm. Pharmacol., 47, 360 (1995).
29- A. Martin, J. Swarbrick, H. Camarata (eds.), Physical Pharmacy, Lea and Febiger, Philadelphia, p. 352 (1983).
30- W. I. Higuchi, J. Pharm. Sci., 57, 274 (1968).
31- P. L. Ritger and N. A. Peppas, J. Control. Rel., 5, 23 (1987).
32- P. L. Ritger and N. A. Peppas, J. Control. Rel., 5, 37 (1987).
33- J. C. Bain, S. B. Tan, D. Ganderton and M. C. Solomon, Drug. Deve. Ind. Pharm., 17, 2 (1991).
34- T. P. Foster and E. L. Parrott, J. Pharm. Sci., 79, 10 (1990).
35- M. Meshali, H. El-Sabbagh and A. Foda, Acta Pharm. Technolog., 29, 217 (1983).
36- M. J. Tobyn, J. R. Johnson and P. W. Dettmar, Eur. J. Pharm. Biopharm., 42, 331 (1996).
37- J. D. Smart, I. D. Kellaway and H. E. C. Worthington, J. Pharm. Pharmacol., 36, 295 (1984).