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DEVELOPMENT AND EVALUATION OF NEFOPAM HYDROCHLORIDE MICROCAPSULES

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في هده الدراسة تم إجراء محاولة لحوصلة عقار النيفوبام هيدروكلوريد كعقار يتميز بشدة ذوبانه في الماء وذلك باستخدام طريقة معدلة لتبخير المذيب من المستحلب ، أسلوب الاستخلاص باستخدام البوليمر سليلوز اسيتات البيوتيرات للتغليف. كما تم دراسة تأثير نسبة العقار للغلاف (١:٢، ١:١، ٢:١) علي كمية المنتج من الحويصلات الدقيقة وكمية العقار المتحوصل والتوزيع الحجمى وكذلك خصائص الانطلاق المعملي للعقار بالإضافة إلي دراسة صفات السطح للحويصلات المحضرة عن طريق المسح الالكتروني. ولقد وجد أن الحويصلات الدقيقة المنتجة لها نسبة تحوصل عالية (أكثر من ١٠٠%) ، كما أن معدل انطلاق العقار كان بطيئا سواء في الوسط المشابه للوسط الحامضي (ذو أس هيدروجيني ١,٢) أو الوسط المشابه للوسط القلوي (ذو أس هيدروجيني ٧,٤). ومن ناحية أخري فإن نسبة التحوصل قد زادت (من ١٠٤,٦٦ إلي ١٤١,٢٦)، عند استخدام نسبة الغلاف إلي العقار (١:١) ومعدل انطلاق العقار قد قل مع زيادة حجم الجزيئات من ٢٥٦ إلي ٥١٢,٥ ومع تقليل نسبة العقار المتحوصل نظريا. ولقد تم دراسة التقييم الكينيتي لمعدل انطلاق العقار من الحويصلات باستخدام طرق حسابية مختلفة. وقد وجد أن طريقة الانطلاق كانت بواسطة الانتشار.

An attempt was performed to encapsulate Nefopam hydrochloride, a highly water soluble drug, by a modified emulsion solvent evaporation / extraction technique, using cellulose acetate butyrate (CAB) as a coating polymer. The influence of core/coat ratio (1:2, 1:1 and 2:1 ratio) on the yield, drug loading, size distribution as well as the release characteristics and surface topography of the prepared microcapsules was investigated. The obtained microcapsules exhibited higher encapsulation efficiency and a decreased release rate in simulated gastric fluid (S.G.F. pH 1.2) and simulated intestinal fluid (S.I.F. pH 7.4). On the other hand, the entrapment efficiencies increased (from 104.66 to 141.26, core coat ratio 1:1) and the release rate decreased with increasing microcapsule size (from 250 to 512.5 µm) and/or theoretical drug loading of microcapsules. Kinetic assessment of the release rate of

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microcapsules using different mathematical models has shown that the release rate followed Ritger-Peppas diffusion release kinetics.

INTRODUCTION

Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions[1]. Today microcapsules have been widely used in many fields including food, agriculture, medicine and pharmacy[2-4].

A multitude of compounds have been incorporated in microcapsules and microspheres by several different techniques, in order to stabilize them, convert them into powders, to mask undesirable taste, or to provide modified release properties[5-9]. Multiparticulate systems have gained great interest in oral drug delivery, as they showed several advantages over single-unit dosage forms, such as a lower variability in the G.I. transit time, better drug dispersion, and the possibility of mixing particles with different release properties[10&11]. In addition, they distribute more uniformly in the G.I. tract, thus resulting in more uniform drug absorption, thereby reducing patient to patient variability. Multiparticulate systems minimize the risk of local irritation and possible intestinal retention of non-digestible polymeric materials upon repeated dosing. They have greater flexibility in dose titration and are cost effective. Moreover, formulations have greater flexibility in achieving desired drug release patterns, for example, simply by mixing particles of varying drug-to-polymer ratios or coating thickness, and hence, achieving different release profiles[12&13].

Microencapsulation of highly water-soluble drugs is a challenging task, both regarding the preparation technique, and in attaining particles with the desired release properties[14].

In fact, systems with such compounds are less common than with lower-water-solubility ones, as a great number of the pharmaceuticals belongs to this latter group[5].

To avoid premature leaching in storage solutions, and to enable slower release in the G.I.tract, highly water soluble drugs are often bound to ion-exchange resin particles, prior to coating with a polymer film or they are incorporated into osmotic devices, among other systems[15].

Regarding the microencapsulation technique, methods based on phase-separation are quite attractive, due to their simplicity and modest requirements in equipment. Variations of these methods have been used to encapsulate a highly water-soluble drug with different water insoluble polymers[14,16&17].

These studies concentrate mainly on the development of emulsion formulations that improve drug

incorporation efficiency. The solvent evaporation method is a commonly employed procedure for the preparation of polymeric microspheres. This method involves the emulsification of a solution containing polymer and drug into another medium in which the drug and polymer cannot be dissolved by using a suitable dispersing agent[18&19].

There are several formulation and process parameters affecting microsphere properties and performance during the preparation of microspheres by the solvent evaporation method. Some of these parameters are the aqueous solubility of the drug, the type and concentration of the dispersing agent, the aqueous and organic phases' volume, the ratio of polymer-drug and solvent and the stirring rate of the emulsion system[20-23]. Water soluble drugs such as the theophylline, caffeine, salicylic acid and verapamil HCl could not be entrapped within microspheres when using an o/w emulsion system. In order to improve the drug loading efficiency of relatively water-soluble compounds, a w/o emulsion system has been successfully employed[24].

Nefopam HCl is a non-opioid analgesic considered to act centrally, although its mechanism of action is unclear. It has also antimuscarinic and sympathomimetic action. Nefopam HCl is used for the relief of moderate acute and chronic pain[20]. Its pharmacokinetics after oral administration is characteristicsed by a relatively short elimination half-life about 4 hours[21]. In addition, Nefopam hydrochloride like most of the analgesic drugs has an ulcerative effect on the stomach[20]. In the light of these problems, the properties of this drug warrant the development of a controlled release formulation.

The aim of this study was to investigate, for the first time, the use of a modification of an emulsion-solvent evaporation / extraction technique as a potential means to sustain the release of this freely water soluble drug. A specific object was to achieve a useful loading with minimal drug loss, which occurs during washing procedures. Another objective was to evaluate the release profiles from Nefopam HCl-loaded microcapsules, which, as a continuation of this work could be embedded throughout the matrix of a secondary delivery system such as tablets for further additional sustained-release action.

EXPERIMENTAL

Materials

The following reagents were purchased from suppliers, as indicated, Nefopam HCl (Sigma Chemical Co., St. Louis, USA), cellulose acetate butyrate (CAB) (171-155, 29.5% w/w acetyl, 17% w/w butyryl and 1.5% w/w hydroxyl content, Mw = 65000) (Eastman Chemical Co., Kingsport, TN, USA), polyethylene glycol 4000 (Fluka Act, CH-9470 Buchs, Switzerland), sorbitan trioleate (Span 85) (ICI Surfactants, Cleveland, UK) and magnesium stearate (Fisher Scientific Atlanta, GA). All other chemicals

were of reagent grades and were used as received.

Methods

Preparation of microcapsules

Nefopam HCl-loaded cellulose acetate butyrate (CAB) microcapsules were prepared by a modification of an emulsion-solvent microcapsules extraction (ESE/E) method, according to the following basic procedure[25]: a sufficient amount of CAB (10% w/v) was dissolved in acetone. Polyethylene glycol 4000 at 5% w/w concentration was used as a plasticizer. The complex particles were dispersed in 10 ml of the polymer solution (internal phase) at different core/coat ratios of 1:2, 1:1 and 2:1 followed by emulsification of this phase in 100 ml of liquid paraffin containing 1.0% w/v sorbitan trioleate and 0.5% w/v magnesium stearate. The resulting emulsion was maintained at 25°C and agitated at 1000 r.p.m with a propeller stirrer. After emulsification for 2 hr., 25 ml of n-hexane (non-solvent) was added drop wise at a constant rate of 1 ml/min to extract acetone and precipitate the coat around the particles. Agitation was continued for three hours until the complete evaporation of acetone was accomplished. The microcapsules were collected by filtration, washed with n-hexane and allowed to dry at 37°C in an incubator for 24 hr. The microcapsules were sized through standard sieves (JPX) and they ranged from 120-710 µm. The fraction of microcapsules remaining on each sieve was collected for further study.

Determination of drug loading

Twenty five milligrams of the loaded microcapsules of 100 mg Nefopam HCl were accurately weighed and comminuted in a clean mortar, then pulverized by the aid of a small amount of either 0.1 N HCl (pH 1.2) or phosphate buffer of pH 7.4. The pulverized microcapsules were transferred into a 100 ml volumetric flask and completed with 0.1 N HCl or phosphate buffer to the appropriate volume. An aliquot was withdrawn, filtered and suitably diluted and assayed spectrophotometrically at 265 nm using the same medium as a blank. The drug content for each fraction size of the prepared micro-capsules was also determined.

Scanning electron micrography (SEM)

The shape and surface characteristics of microcapsules were observed by a scanning electron microscope (Jeol-JSM-5200). Micro-capsules were tested onto-double-sided tape, which was placed onto a sample carrier in the shape of a cylinder (5 mm height, 10 mm diameter) and were coated with gold under vacuum with a SPI sputter coater (SPI supplies, Division of Structure Probe, Inc., West Chested, PA, USA) to a thickness of 50 mm. The samples were imaged using a 15 Kv electron beam.

In-vitro release studies

The USP rotating paddle dissolution apparatus (Model DT-06, Erweka, F.R.G.) was used at 50 r.p.m. Fifty milligrams of the microcapsules

were accurately weighed and sprinkled over 250 ml of the release medium (0.1 N HCl or $Na_2HPO_4.2H_2O$ / $NaH_2PO_4.12H_2O$, buffer (pH 7.4 at 0.154 M Na^+ ions) containing 0.02% w/v tween 80) maintained at 37°±0.2°C. Five milliliter samples were withdrawn at specific time intervals and were replaced by equivalent volumes of release medium kept at 37°±0.2°C. The drug released from the microcapsules was determined spectrophotometrically at 265 nm. All release studies were run at least in triplicate for each experiment and the average was used for calculating the amount of the drug released.

X-ray diffraction analysis

The X-ray diffraction patterns of drug, polymer and microcapsules were obtained using a Phillips X-ray diffractometer (Phillips Generator PW-1710, Netherlands) with a Ni-filtered Luk_α-radiation at a scanning speed of 5°C/min.

RESULTS AND DISCUSSION

Physicochemical characteristics of microcapsules containing Nefopam hydrochloride

Several systems have been reported for preparation of microspheres by the solvent evaporation method[26&27].

Table 1 depicts the effects of core/coat ratio and average size on drug content and entrapment efficiency of microcapsules. It is evident that increasing core/coat ratio (microcapsule size: 256 µm) from 1:2 to 2:1 resulted in a corresponding increase in drug content (from 37.37% to 71.68%). At a core/coat ratio of 1:1, increasing average size of microcapsules from 256 to 512.5 µm resulted in an increase in drug content (from 52.34% to 70.632%) and entrapment efficiency (from 104.68 to 141.26).

Figure 1 shows the typical particle size distribution of Nefopam HCl-loaded CAB microcapsules. Obviously, microencapsulation using a core/coat ratio of 1:2 yielded the highest percent of smaller microcapsules (256 µm, 35%) whereas core / coat ratios of 1:1 and 2:1 produced the highest percent of larger microcapsules (362.5 µm ≈ 30%). The utilization of a core/coat ratio of 1:2 resulted in lowering the mean size of microcapsules to a higher degree than other core/coat ratios. Thus, the lower concentration of drug was noted to produce a narrower particle size distribution with more than 30% of microcapsules in the size of 181 µm. The results obtained can be explained on the basis that the fluidity of the polymer solution can be increased by the use of a lower concentration of drug and thus provides a practical mean to control the average microcapsule size.

Drug release from microcapsules containing Nefopam hydrochloride

Absorption of a drug contained in a pharmaceutical oral dosage form is preceded by its release and dissolution in the GI fluids.

Table 1: Micrometric properties of Nefopam-HCl loaded CAB microcapsules and kinetic assessment of their in-vitro release data (pH 7.4).

Core / coat ratio	Average size (µm)	Drug content (%)	Entrapment efficiency (%)	First order		Ritger-Peppas Log T vs. Log Q		
				r	K (hr^{-1})	r	n	intercept
1:2	256.0	37.37	112.2	0.9956	0.1833	0.9903	0.144	1.666
	362.5	27.20	81.6	0.834	0.8375	0.996	0.176	1.73
	512.5	38.66	116.8	0.994	0.1198	0.993	0.116	1.65
1:1	256.0	52.34	104.68	0.5697	37.91	0.9566	0.157	1.802
	362.5	56.59	113.18	0.8518	27.34	0.9744	0.255	1.624
	512.5	70.632	141.26	0.9501	13.62	0.9941	0.218	1.502
2:1	256.0	71.680	107.52	0.9559	-	0.9588	0.1032	1.8302
	362.5	53.361	80.05	-	-	-	-	-
	512.5	55.77	83.66	0.8021	-	0.8851	0.104	1.805

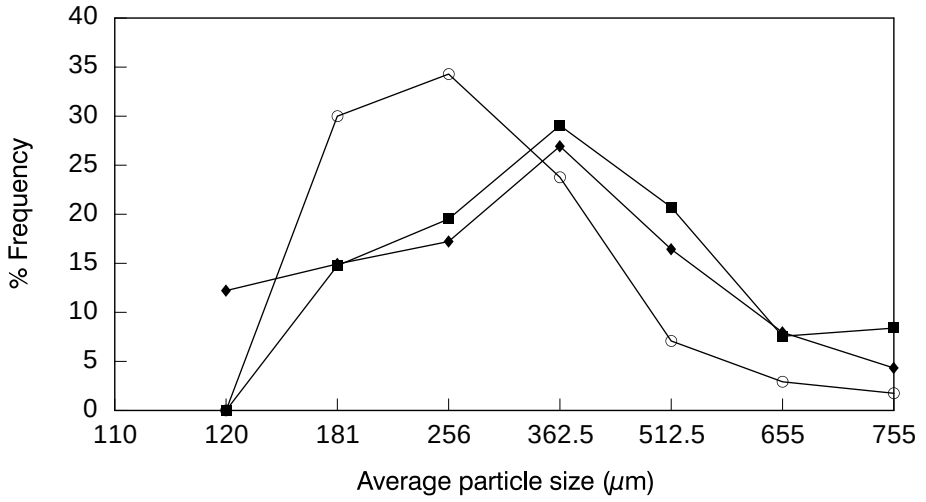

Fig. 1: Particle size distribution of Nefopam HCl-loaded CAB microcapsules prepared at core/coat ratios of (o) 1:2, (■) 1:1 and (♦) 2:1.

Table 1 and Figures. 2-4 show the release properties of Nefopam HCl-loaded CAB microcapsules (425-600 µm average diameters). The divergence in release profiles illustrates performance difference between the effect of core/coat ratios (Figure 2) on drug release, whereby on increase in core/coat ratio resulted in increase in the drug release rate (for example 0.1198 and 13.62 hr^{-1} for 1:2 and 1:1 core/coat ratio respectively). This may be attributed to the increase in drug loading with

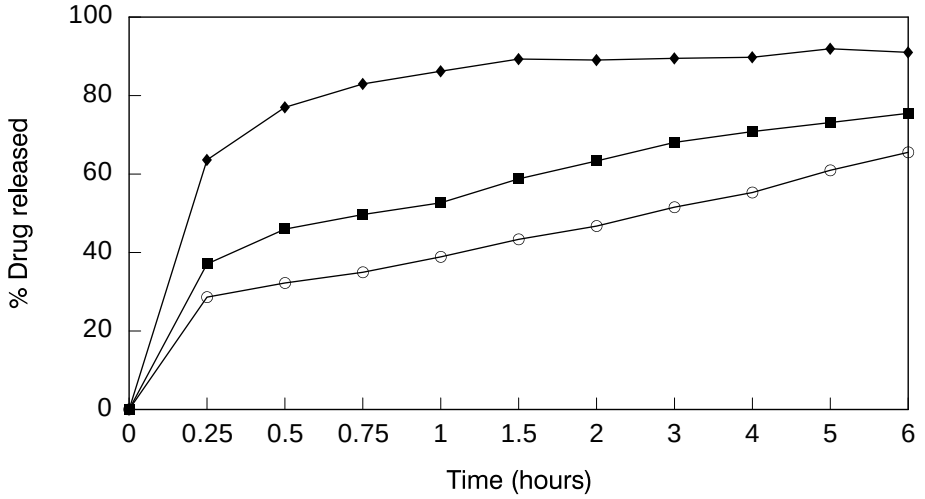

Fig. 2: *In-vitro* release of Nefopam-HCl from its microcapsules (425-600 µm) prepared at core/coat ratios of (o) 1:2, (■) 1:1, (♦) 2:1 (release medium at pH 7.4).

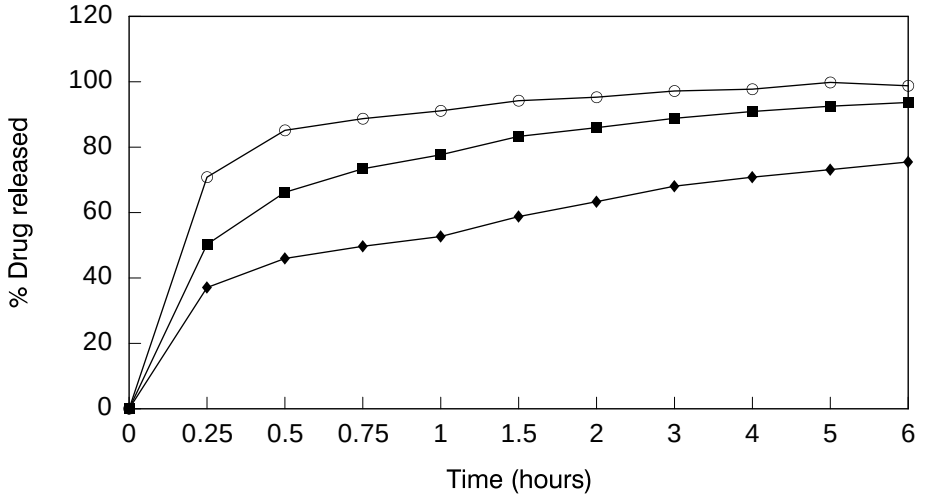

Fig. 3: Effect of particle size on release of Nefopam-HCl from its CAB microcapsules (core/coat ratio 1:1). Particle size: (o) 212-300 µm, (■) 300-425 µm and (♦) 425-600 µm (release medium at pH 7.4).

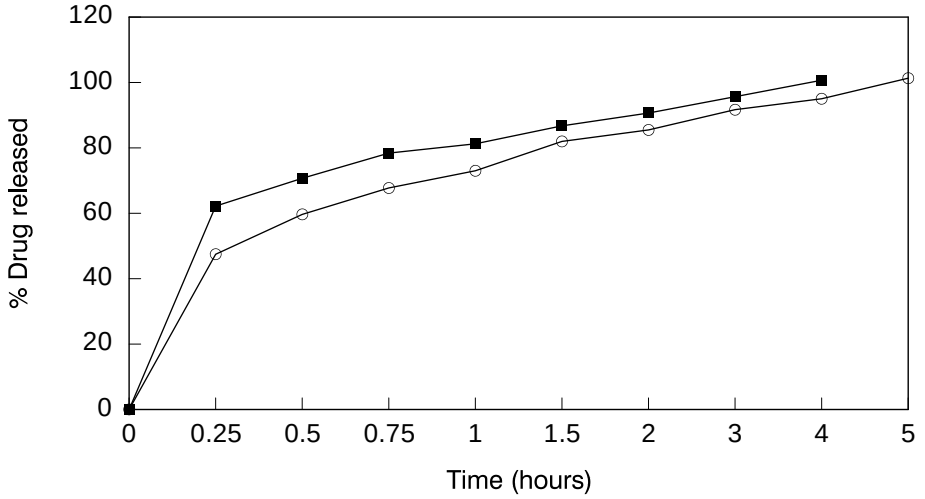

Fig. 4: Effect of pH of the release medium on the release of Nefopam-HCl from its CAB microcapsules (core : coat ratio 1:2, average size: 362.5 µm) (release medium at pH 1:2 (o) and pH 7.4 (■).

the increase in core/coat ratio. The results obtained indicated similarity with those obtained by Bhalerao *et al.*[28] who studied the effect of drug / polymer ratio on the release of diltiazem HCl from microcapsules prepared by the water-in-oil emulsion solvent evaporation technique.

At lower drug / polymer ratio, the mean particle size of the microcapsules was less than that at higher drug / polymer ratios. This was not in consistent with the general rules, i.e. small size of microcapsules provides large surface area for faster drug release[28].

Figure 3 shows the effect of particle size (average size: 212-300 µm, 300-425 µm, 425-600 µm) of microcapsules (core/coat ratio 1:1) on the release of drug from such microcapsules. Evidently, a decrease in microcapsule size was accompanied by an increase in release rate due to an increase in surface area of microcapsules. Similar results were obtained by El-Gibaly *et al.*[29], who studied the effect of particle size on the release of ketoprofen from CAB / polystyrene composite microcapsules prepared by an emulsion-solvent evaporation technique.

The effect of the release media pH on the release of Nefopam HCl from its CAB microcapsules (core/coat ratio 1:2, average size: 362.5 µm) was also shown in Figure 4. Results revealed that a slight rapid release was observed at pH 7.4 as compared with pH 1.2 during the first hour of release testing.

The variation in the release pattern of the microcapsules can be verified by the scanning electron micrographs

given in Figure 5. Representative CAB microcapsules (core/coat ratio of 1:2) are relatively spherical in shape and have smooth surfaces. Higher magnification (X3, 500) has shown the spongy-like and less porous morphology of the outer wall and the absence of surface drug crystals on the microcapsule surface. This is correlated with the decreased release rates of CAB microcapsules (Table 1 and Figure 2). On the other hand, CAB microcapsules (core/coat ratio 1:1) appeared to have different surface morphologies (Figure 5B). Discrete micropores with impregnated structure and numerous drug crystals are clearly seen within microcapsules surfaces. Higher resolution (X1000, 3,500) showed a more porous structure for such microcapsules and the existence of numerous pores within the microcapsule wall. This explains the relatively fast release rates of the CAB microcapsules (core/coat ratio 1:1). The porous structure is probably introduced by rapid vaporization of the solvent and the higher theoretical drug loading resulting in subsequent formation of bubbles during the fabrication process and puncturing the microcapsule membrane[30].

Kinetic interpretation of the release data

Analysis of the release data of Nefopam HCl-loaded CAB microcapsules was carried out according to first order kinetics (Table 1). A simple empirical exponential relation (equation 1) was also proposed by Peppas[31&32] to describe the general solute release behavior of controlled release polymer devices according to a diffusion controlled model for planar matrix.

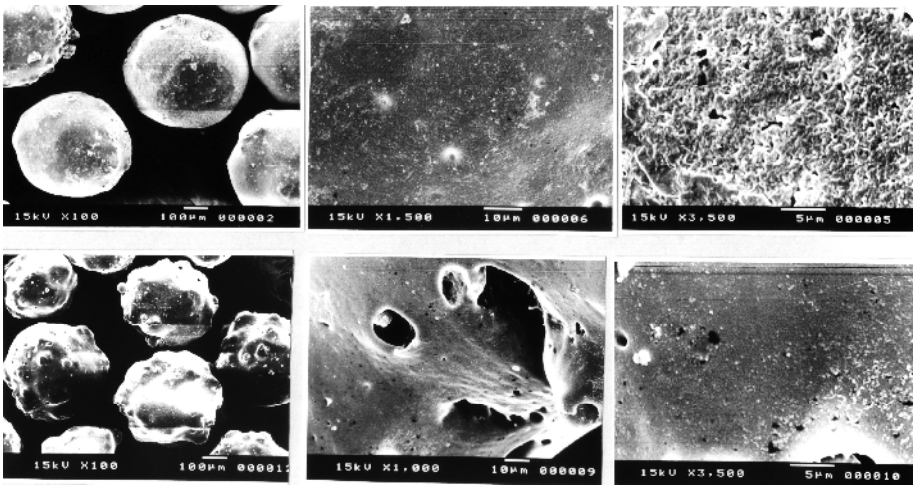

Fig. 5: Representative scanning electron micrographs of Nefopam HCl-loaded CAB microcapsules prepared at core : coat ratios of (A) 1:2 and (B) 1:1.

$$\frac{M_t}{M_\infty} = Kt^n \quad (1)$$

where M_t/M_∞ is the fractional release of the drug, T is the release time, K is a constant incorporating structure and geometric characteristics of the controlled-release device and n is the release exponent, indicative of the mechanism of drug release. Reportedly, the value of n for a spherical sample is 0.43±0.007 for Fickian diffusion, 0.85±0.02 for case II transport (zero-order kinetics) and <0.85 and >0.43 for anomalous (non-Fickian) transport[32]. The values of n, release rate constants and the corresponding determination coefficients (r^2) for the release data of microcapsules are listed in Table 1. However, it is appeared that the release pattern of Nefopam HCl from microcapsules was found to be best explained by a Fickian-diffusion kinetics with n values ranging from 0.104 to 0.25. The lower value of n computed from the Ritger-Peppas relation supported the expected diffusional release kinetics and also negates the erosion or solubilization of the wall-matrix driven phenomena[33&34].

In the case of polydisperse spherical systems, the values of n will be lower than expected. Further reduction of n values has also been reported by Soppimath *et al.*[34] for Nifidipine-loaded cellulose-based matrix microspheres. This indicates that the square root of time relationship for a matrix diffusion-controlled mechanism (Higushi-model) was operative. This equation may be used only for a granular inert matrix system which maintains a constant planar surface area, where the drug diffusion coefficient is clearly concentration-independent and the effect of solubility is implicit. The suggested model has been applied successfully to drug release from CAB microcapsules, containing ketoprofen[29]. Also the results obtained are in consistent with those obtained by Moldenhauer and Nairn[35] who found that micro-encapsulated theophylline fitted a $T^{½}$ plot when M_t/M_∞ is 20.3 thereby, suggesting particle diffusion control.

To understand the physical state of the drug inside the CAB microcapsules (which has an influence on release kinetics), X-ray analysis were performed on intact drug, drug-polymer mixture, and the representative CAB microcapsules (core/coat ratio 1:1), the X-ray diffractogram of the drug-loaded microcapsules showed the presence of crystalline peaks of Nefopam HCl and the data of polymer, indicating that the drug retains its crystalline state within the microcapsules and is present as a dispersion within the microcapsules. This finding supports the suggested release mechanism where a granular inert matrix system (a diffusion-controlled mechanism) was operative (Fig. 6).

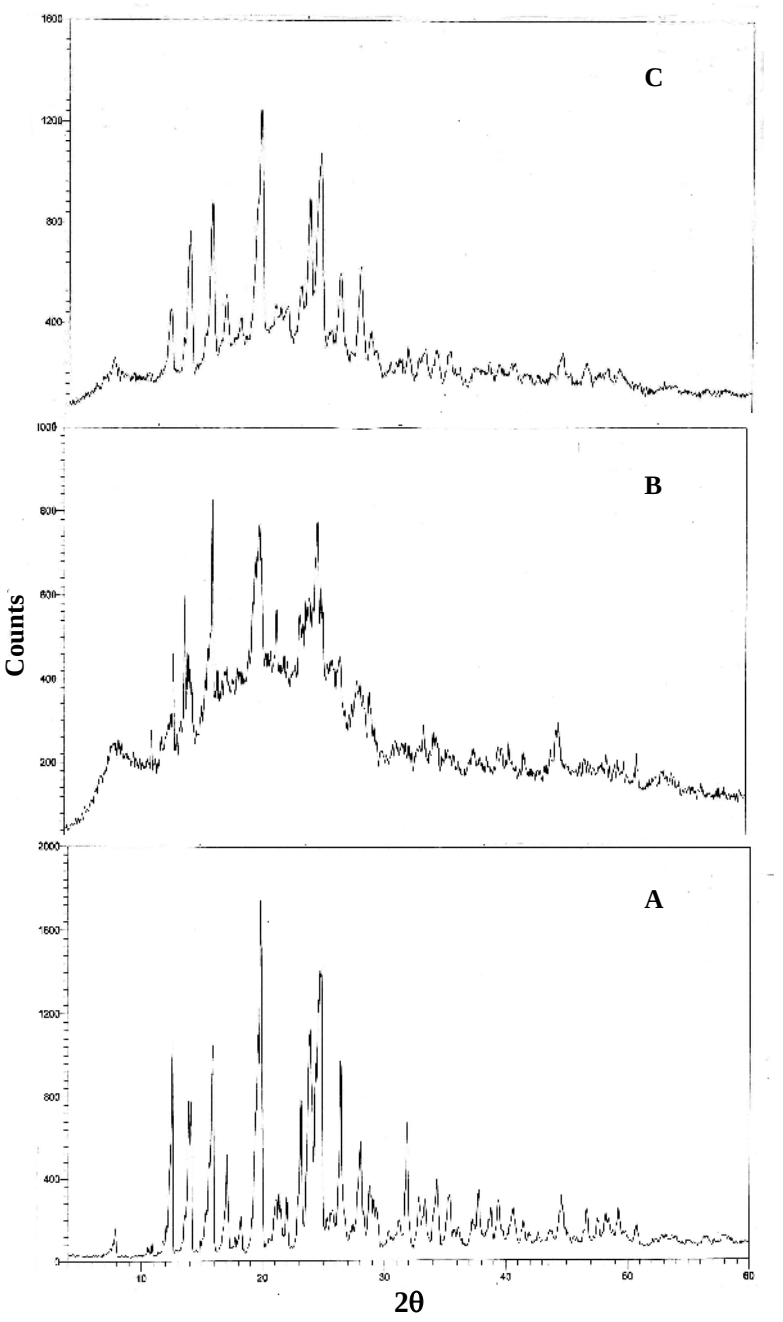

Fig. 6: X-ray diffractometric analysis of (a) crystalline nefopam, (b) drug-polymer mixture (1:1 ratio) and (c) drug-loaded CAB microcapsules (core/coat ratio 1:1).

Conclusion

For the first time, Nefopam HCl was successfully micro-encapsulated by a modified emulsion-solvent evaporation-extraction technique using CAB polymer. The prepared microcapsules offered a prolongation of the drug release rate and showed potential as a sustained release drug delivery system for Nefopam HCl. The variation in drug release rates with core/coat ratio, particle size and pH of release media indicates a potential for *in-vivo* variability. Drug release data of such microcapsules fitted better to diffusion-controlled release mech-anism for a planar matrix system.

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