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FORMULATION AND EVALUATION OF KETOPROFEN CELLULOSE ACETATE MICROCAPSULES

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يعتبر الكيتوبروفين أحد مضادات الالتهاب غير الإستيرودية الفعالة والتى تستخدم فى علاج مختلف الأمراض الروماتيزمية والحالات المصحوبة بألم . يتميز الكيتوبروفين بسرعة امتصاصه بغض النظر عن طريقة تناوله ويتميز بقصر فترة نصف العمر (من ساعة إلي ساعتين) فى حالة تناوله عن طريق الفم. لذلك كان الهدف من هذا البحث هو صياغة وتقييم العديد من الكريات والكبسولات الدقيقة والمحتوية على الكيتوبروفين وذلك للتحكم في انطلاق العقار وتم اختيار خلات السيليولوز لهذا الغرض. تم تحضير كبسولات دقيقة للكيتوبروفين باستخدام خلات السيليولوز باستخدام طريقة تبخر المذيب فى وجود مادة استحلاب وهى عديد فينيل الكحول. وقد تم تقييم الكبسولات المحضرة من حيث دراسة معدل انطلاق العقار ودراسة خصائص السطح لها كما تمت دراسة فاعلية الكيتوبروفين المضادة للالتهاب. وقد دلت النتائج علي أن انطلاق الكيتوبروفين من كبسولات خلات السيليولوز الدقيقة يعتمد على قيمة الرقم الايدروجيني. كما أن زيادة كمية البوليمر و زيادة حجم الطور العضوي قد أدت إلي نقص معدل انطلاق الكيتوبروفين. كما أوضحت النتائج أن زيادة حجم الطور العضوي قد أدت إلي نقص متوسط قطر الكبسولات الدقيقة المحضرة بينما أدت زيادة كمية البوليمر إلي زيادة متوسط قطر الكبسولات الدقيقة. ويستخلص من هذه الدراسة أن صياغة الكيتوبروفين في صورة كبسولات دقيقة باستخدام خلات السيليولوز يمكن أن تكون طريقة مناسبة للتحكم في انطلاق العقار.

Ketoprofen is a potent non-steroidal anti-inflammatory, analgesic, and antipyretic drug used for treatment of various rheumatic diseases and painful conditions. It is rapidly absorbed regardless the route of administration. Its half life is only 1-2 hrs after oral administration. The aim of this work was to formulate some controlled release oral dosage forms containing ketoprofen. Cellulose acetate was chosen for this purpose. Ketoprofen-cellulose acetate microcapsules were prepared by solvent evaporation technique. Polyvinyl alcohol was used as an emulsifier. The prepared microcapsules were evaluated in terms of their release and surface characteristics. Also, the anti-inflammatory activity of ketoprofen was evaluated using the carrageenan-induced rat's paw

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edema method. The release of ketoprofen from the microcapsules was pH dependant and decreased by increasing the polymer content and by increasing the organic phase volume. The particle size of the produced microcapsules was decreased by increasing the volume of organic phase, whereas it was increased by increasing the polymer content. In conclusion, these results suggested that microencapsulation of ketoprofen using cellulose acetate could be a useful approach for controlled release of the drug.

INTRODUCTION

The emulsion solvent evaporation technique has been widely used in the formulation of different drugs into microcapsules using various polymeric materials[1]. This technique is simple and factors affecting microcapsule size distribution and drug release are easily modified[2] including, type and concentration of emulsifier, volume of organic solvent and polymer composition.

Polyvinyl alcohol (PVA) is by far the most commonly used emulsifier in the O/W emulsion method.

Acetone was the volatile organic solvent of choice for this O/W process.

Cellulose acetate is a cellulose derivative that is soluble in acetone, methylene chloride, chloroform, and poorly soluble in dimethyl formamide and insoluble in heptane and water[3].

EXPERIMENTAL

Materials

Ketoprofen: kindly supplied by Alexandria Co. for pharmaceuticals (Alexandria, Egypt); Cellulose acetate: Sigma chemical Co. U.S.A.; Polyvinyl alcohol M.W. app. 14.000 LR (PVA): Aldrich Co., U.K.; Acetone: El-Nasr Chemical Co. Egypt; Carboxy methyl cellulose: Aldrich Co. U.K.; Carrageenan: Sigma Chem. Co. U.S.A., and Urethane: Sigma Chem.Co. All the other reagents are of analytical grade and used without further purification.

Methods

Preparation of microcapsules

The microcapsules were prepared by solvent evaporation method[4] in 250 ml beaker, using a mechanical stirrer at 400 rpm. The calculated amount of the polymer cellulose acetate (CA) was dissolved in the specified volume of acetone followed by dissolving the calculated amount of Ketoprofen to form the internal phase. The external phase was prepared by dissolving the specific amount of the emulsifier in 120 ml distilled water.

The internal phase was added drop wise to the external phase, using a 20 ml syringe at a rate of 0.5 ml/min. After complete addition of the internal phase, the solvent was

allowed to evaporate. The microcapsules were collected by filtration, washed twice with distilled water, left to dry at ambient conditions for 24 hrs and then stored in a dessicator until used.

Scanning electron microscopy

Images of the microcapsules surface were taken using the electron microscope.

Determination of the particle size of the microcapsules

The dried microcapsules were weighed and sized using USP standard sieve set (400 – 100 μm). The fraction of microcapsules remaining on each sieve was collected and the mean particle size of the microcapsules was assigned as the percentage of microcapsules retained at each sieve multiplied by the average particle size of this sieve.

Determination of the yield of the microcapsules

The yield of the microcapsules was determined by dividing the weight of the prepared microcapsules by the original amount of the polymer and drug used and the results were expressed as a percentage.

Determination of the content of the microcapsules

Ten milligrams of the microcapsules were added to 25 ml phosphate buffer (pH 7.4) in a 25 ml volumetric flask and left overnight.

The withdrawn samples were properly diluted and measured spectrophotometrically at λ_{max} 261 nm against phosphate buffer (pH 7.4) as a blank. The experiment was done in triplicate.

***In-vitro* release studies**

Dissolution testing of the prepared microcapsules equivalent to 100 mg Ketoprofen was performed with the rotating paddle apparatus at paddle speed of 50 rpm and a temperature of 37°C ± 0.5 in dissolution medium of 500 ml. Regarding the dissolution medium, the pH shift method[5] was used as follows:

Solutions of pH 1.2 (each of 500 ml) were used as the release medium for two hours, followed by the addition of (5-7 ml) of 1 M KH_2PO_4 containing 16.75% (w/v) NaOH, in order to change the pH of the medium to pH 5.2. Release was continued in this medium for further three hours. Finally, pH was changed to pH 7.4 by the addition of (3.5–5.0 ml) of the previously added mixture and the experiment was continued for further three hours. Filtered samples, 3 ml each, were removed at specified intervals throughout the whole 8 hours, namely at 5, 15, 30, 45, 60, 90, 120, 135, 150, 180, 240, 300, 330, 360, 420, 450, and 480 minutes. Each sample was diluted with a solution having the same pH value of the release medium from which the sample was withdrawn, and the absorbance was measured at λ_{max} 261 nm against a blank of the same medium.

Evaluation of the anti-inflammatory activity of Ketoprofen-cellulose acetate microcapsules

The anti-inflammatory activity of the selected formulations was evaluated using carrageenan-induced rat's paw edema method[6].

Method

The animals were equally and randomly allocated in four groups as follows:-

- Group one*:* Control group
- Group two*:* Ketoprofen orally, (a dose of 0.5 mg/kg)[7]. (Treatment I).
- Group three*:* Ketoprofen cellulose acetate microcapsules prepared at 1:1 drug to polymer ratio using 0.5% poly vinyl alcohol (PVA) as an emulsifying agent, (a dose of 0.5 mg/kg). (Treatment II).
- Group four: Ketoprofen cellulose acetate microcapsules prepared at 1:1 drug to polymer ratio using 1% PVA, (a dose of 0.5 mg/kg). (Treatment III).

The selected formulations and Ketoprofen powder were suspended in 1% carboxymethylcellulose solution[8]. The rats were anaesthetized by urethane (a dose of 0.5 ml intraperitonial). Each group of animals received the specified drug product by an oesophageal tube[9].

Inflammation was induced by subcutaneous injection of 0.1 ml of 1% W/V carrageenan solution into the subplanator tissue of one hind paw[6]. The thickness of the paw edema was measured by thickness micrometer[10].

The anti-inflammatory effect was expressed as percentage of inhibition of edema thickness compared with control according to the following equation[10&11]:

$$\% \text{ of inhibition of edema} = [(T_0 - T_t) / T_0] \text{ X } 100$$

Where: T_0; is the edema thickness in the control group. T_t; is the edema thickness in the tested group.

RESULTS AND DISCUSSION

Microcapsules morphology

According to Figure (1), the obtained Ketoprofen cellulose acetate microcapsules were discrete, spherical, and freely flowing. Scanning the microcapsules surface at magnification power of 2000x using the electron microscope showed that the microcapsules surface is smooth at both drug to polymer ratios of 1:1 and 1:2.

The particle size of the microcapsules

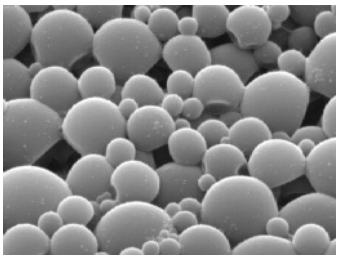
Table (1) shows that increasing the organic phase volume from 30 to 50 ml decreased the mean particle diameter of the produced microcapsules. The values of mean particle diameter decrease at 1:1 D:P ratio were 139.73, 89.21, and 59.4 μm at 0.5, 1, and 2% PVP respectively. In case of 1:2 D:P ratio, the values of mean particle diameter decrease were 8.52, and 4.7 μm at 1 and 2% PVP respectively. The increase in the organic phase volume

at 1:2 D:P ratio increase the mean particle diameter by 20.14 μm using 0.5% PVP. Whereas, the increase in the drug to polymer ratio using 50 ml acetone increased the mean particle diameter (the values of mean particle diameter increase were 116.85, 51.49, and 33.89 μm at 0.5, 1, 2% PVP respectively).

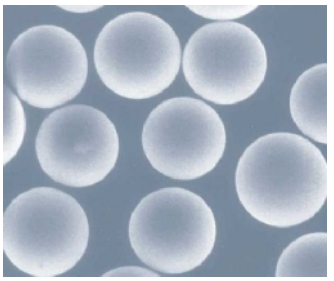
Upon using solvent evaporation technique, highly viscous internal phases produce larger microcapsules due to more difficult dispersion in the external phase[12].

The Yield of the Microcapsules

Table (2) and Figure (2) show that the increase in the amount of the polymer decreased the microcapsules yield (using 50 ml acetone, the values of yield of microcapsules decrease were 8.5, 6, 4.7 % at 0.5, 1, 2 % PVP respectively). The increase in the organic phase viscosity may be the cause. No marked effect was observed for the change in organic phase volume on the percentage yield of the microcapsules.



(a) morphology of microcapsules



(b) smooth surface of microcapsules

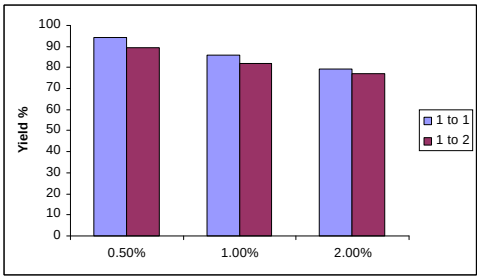
Fig. 1: Scanning electron micrographs of Ketoprofen cellulose acetate microcapsules

Table 1: Particle diameters (μm) of Ketoprofen cellulose acetate microcapsules.

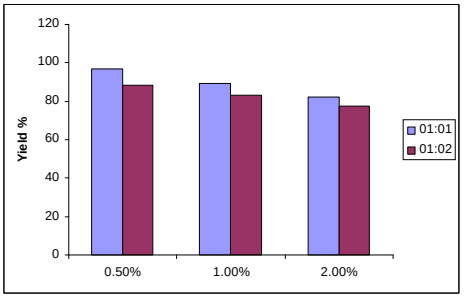
D : P ratio	acetone volume	Microcapsule diameter (μm) at different polyvinyl alcohol concentrations		
		0.5%	1.0%	2.0%
1:1	30 ml	294.33	11.62	171.71
	50 ml	154.6	122.41	112.31
1:2	30 ml	251.31	182.42	150.9
	50 ml	271.45	173.9	146.2

Table 2: Yield of Ketoprofen-cellulose acetate microcapsules.

D : P ratio	acetone volume	Yield % at different Polyvinyl alcohol concentrations		
		0.5%	1.0%	2.0%
1:1	30 ml	94.30	86.10	79.40
	50 ml	96.70	89.10	82.00
1:2	30 ml	89.50	82.00	76.90
	50 ml	88.20	83.10	77.30



(a)
Polyvinyl Alcohol Concentration (%)

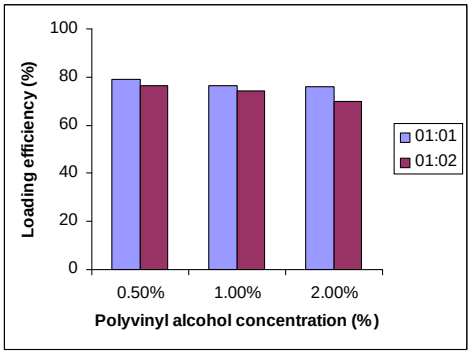


(b)
Polyvinyl Alcohol Concentration (%)

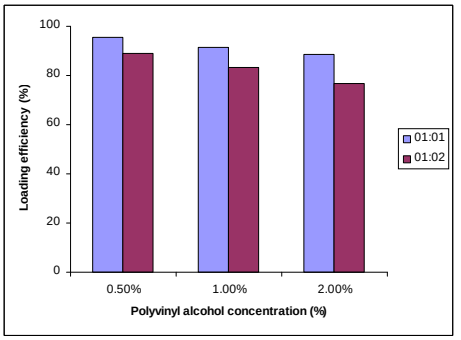
Fig. 2: Effect of drug to polymer ratio and emulsifier concentration on microcapsule yield using (a) 30 ml acetone (b) 50 ml acetone.

The drug content of the microcapsules

The results showed in Tables (3) and (4) and Figure (3) illustrate that the loading efficiency increased with a consequent increase in the acetone volume while the change in drug to polymer ratio has no marked effect on drug loading efficiency.



(a)



(b)

Fig. 3: Effect of drug to polymer ratio and emulsifier concentration on microcapsule drug loading efficiency using (a) 30 ml acetone and (b) 50 ml acetone.

Table 3: Drug loading efficiency of Ketoprofen - cellulose acetate microcapsules for 1:1 drug to polymer ratio.

Acetone volume (ml)	30			50		
PVA concentration (%)	0.5	1	2	0.5	1	2
Theoretical drug content (%)	50	50	50	50	50	50
Actual drug content (%)	38.50	38.30	37.90	47.80	45.70	44.30
Loading efficiency (%)	79.00	76.60	75.80	95.60	91.40	88.60

Loading efficiency % = (Actual drug content / theoretical drug content) ×100

Table 4: Drug loading efficiency of Ketoprofen - cellulose acetate microcapsules for 1:2 drug to polymer ratio.

Acetone volume (ml)	30			50		
PVA concentration (%)	0.5	1	2	0.5	1	2
Theoretical drug content (%)	33.33	33.33	33.33	33.33	33.33	33.33
Actual drug content (%)	25.50	24.80	23.20	29.60	27.80	25.60
Loading efficiency (%)	76.50	74.40	69.66	88.80	83.40	76.80

Loading efficiency % = (Actual drug content / theoretical drug content) ×100

***In-vitro* release of Ketoprofen from the cellulose acetate microcapsules**

The release of Ketoprofen from cellulose acetate microcapsules was pH dependent as shown in Figure (4). The release was faster in alkaline medium compared to acidic medium due to greater solubility of *cellulose acetate* at higher pH values.

The drug release from the microcapsules decreased by increasing the amount of the polymer, as shown in Figures (5) and (6). This delay in drug release could be attributed to the formation of thicker coating membranes or to decrease in number of pores at the microcapsules surface at higher polymer to drug ratios.

Ruiz et al.[13], prepared micro-capsules of terbutaline sulfate with cellulose acetate butyrate and reported that the release was decreased as the amount of polymer increased.

The drug release rate from the microcapsules prepared at 1:2 drug to polymer ratio was slower at higher acetate volume as shown in Figures (6) and (7).

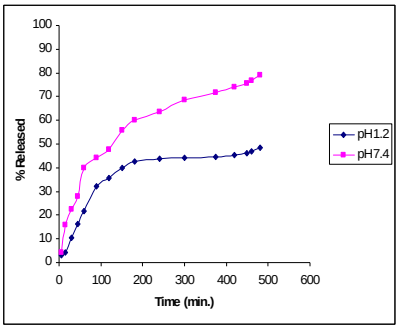


Fig. 4: In-vitro release profile of Ketoprofen cellulose acetate microcapsules prepared at drug to polymer ratio of 1:2 using 0.50% PVA and 50 ml acetone at different pH values.

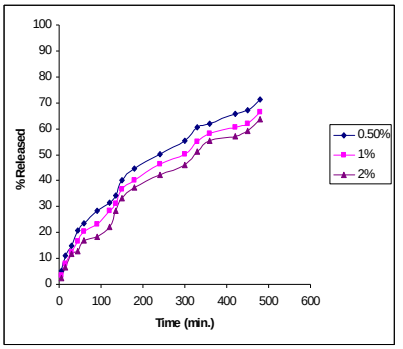


Fig. 6: In-vitro release profile of Ketoprofen cellulose acetate microcapsules prepared at drug to polymer ratio of 1:2 using 30 ml acetone at different PVA concentrations (0.5, 1, 2%).

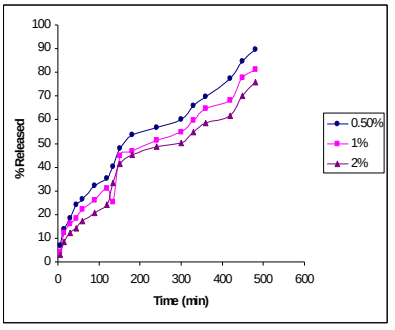


Fig. 5: In-vitro release profile of Ketoprofen cellulose acetate microcapsules prepared at drug to polymer ratio of 1:1 using 30 ml acetone at different PVA concentrations (0.5, 1, 2%).

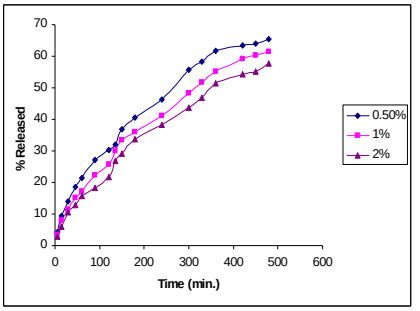


Fig. 7: In-vitro release profile of Ketoprofen cellulose acetate microcapsules prepared at drug to polymer ratio of 1:2 using 50 ml acetone at different PVA concentrations (0.5, 1, 2%).

Kinetic analysis of the release data

It was found that the drug was released from the microcapsules according to first order kinetics at different pH values as shown in Tables (5) and (6).

Table 5: Kinetic analysis of the release data for Ketoprofen cellulose acetate microcapsules prepared at drug to polymer ratio of 1:1 using 30 ml acetone.

PVA concentration (%)		0.5		1.0		2.0	
pH value		1.2	7.4	1.2	7.4	1.2	7.4
Zero-order	K_z	9.955	22.559	11.213	23.424	13.681	29.591
	R_z	0.926	0.928	0.922	0.923	0.947	0.952
First-order	K_f	0.431	0.623	0.402	0.587	0.695	0.551
	R_f	0.996	0.993	0.997	0.994	0.996	0.994
Higuchi equation	K_h	31.321	36.241	45.918	41.872	53.157	44.34
	R_h	0.991	0.980	0.971	0.991	0.919	0.983

K_z ($mg.hr^{-1}$), K_f (hr^{-1}) and K_h ($mg/cm^2.\ hr^{½}$) are the release rate constants of zero–order, first order and Higuchi model kinetics, respectively, as well as R_z, R_f and R_h are their corresponding correlation coefficients.

Table 6: Kinetic analysis of the release data for Ketoprofen cellulose acetate microcapsules prepared at drug to polymer ratio of 1:1 using 50 ml acetone.

PVA concentration (%)		0.5		1.0		2.0	
pH value		1.2	7.4	1.2	7.4	1.2	7.4
Zero-order	K_z	11.421	20.972	11.972	27.452	9.783	21.742
	R_z	0.942	0.926	0.966	0.977	0.972	0.953
First-order	K_f	0.414	0.347	0.533	0.432	0.730	0.591
	R_f	0.998	0.999	0.995	0.993	0.997	0.998
Higuchi quation	K_h	33.180	35.910	40.726	25.72	32.742	23.647
	R_h	0.991	0.978	0.993	0.965	0.940	0.992

K_z ($mg.hr^{-1}$), K_f (hr^{-1}) and K_h ($mg/cm^2.\ hr^{½}$) are the release rate constants of zero–order, first order and Higuchi model kinetics, respectively, as well as R_z, R_f and R_h are their corresponding correlation coefficients.

The anti-inflammatory activity of Ketoprofen-cellulose acetate microcapsules

Table 7 shows that treatment II was the best one as it demonstrated the highest anti-inflammatory activity because the amount of drug released using 0.5% PVA was greater than in case of 1% PVA leading to higher percentage of edema inhibition.

Conclusion

The results of this study indicate that the release pattern of the Ketoprofen from the microcapsules is greatly affected by the amount of the polymer and the volume of organic phase.

Also, the results revealed that microcapsulation of Ketoprofen using cellulose acetate could be a useful approach for controlled release of the drug.

Table 7: Inhibition % of different ketoprofen formulations on the carrageenan-induced edema in the kindpaw of rats.

Treatment No.	Dosage Form	Inhibition % of Edema after:				
		1 hr	2 hr	3 hr	4 hr	5 hr
1	Ketoprofen Oral Suspension	63.8 ±10.3	77.3 ±9.1	61.3 ±8.7	73.5 ±16.1	37.5 ±5.4
2	Ketoprofen cellulose acetate microcapsules prepared using 0.5 % PVA	72.4 ±6.0	81.8 ±9.1	84.8 ±5.3	91.4 ±8.9	71.9 ±9.4
3	Ketoprofen cellulose acetate microcapsules prepared using 1% PVA	63.8 ±13.3	77.3 ±11.8	79.5 ±8.7	64.8 ±12.4	62.5 ±18.7

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