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# Preparation and in vitro evaluation of verapamil HCl and ibuprofen containing carrageenan beads☆

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**Abstract**

The objective of this study was to prepare and evaluate carrageenan beads as a controlled release system for a freely water soluble drug verapamil hydrochloride and a slightly water soluble drug ibuprofen. Beads were prepared by ionotropic gelation method. The influence of formulation factors (drug content, polymer concentration, counterion type and concentration, outer phase volume) on the particle size, encapsulation efficiency and in vitro release characteristics of beads was investigated. The encapsulation efficiency of veraparnil HCl in the beads (34.8–71.1%) was higher than that of ibuprofen (23.6–58%). While about 30% of ibuprofen was released at 6 h, about 70% of verapamil HCl was released in 5 h from the carrageenan beads prepared. 

*Keywords:* Carrageenan; Beads; Verapamil HCl; Ibuprofen; Controlled release

## 1. Introduction

In recent years natural water soluble polymers have been examined for their suitability to prepare multiparticulate controlled release drug delivery systems. Thus the use of organic solvents needed to solubilize the water insoluble polymers can be avoided.

Carrageenan has been used increasingly in pharmaceutical formulation studies. Suzukı and Lım (1994) used ι-carrageenan together with locust bean gum to prepare microcapsules for the sustained release of gentamicin sulphate. The influence of the locust bean gum on the ι-carrageenan microcapsule membrane led to a near zero order release of gentamicin sulphate.

Patıl and Speaker (2000) formulated a model protein, horse radish peroxidase (HRP) in a microcapsule delivery system using ι-carrageenan. This encapsulation system employing the ionic interaction between ι-carrageenan and a series of amines successfully captured the protein, HRP.

Garcıa and Ghaly (1996) used carrageenan in order to control acetaminophen release from spheres prepared by cross linking technique. The results showed that carrageenan could exert control over the rate and amount of drug released from the spheres prepared.

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In this article we have investigated the incorporation of a freely water soluble drug, verapamil hydrochloride and a slightly water soluble drug, ibuprofen in carrageenan beads. The beads were characterized by particle size determination, encapsulation efficiency and release rates of drugs. The influence of preparation technique and of the solubilities of the model drugs on these properties were examined.

## 2. Materials and methods

Verapamil HCl Knoll, ibuprofen Atabay, ι-carrageenan FMC and κ-carrageenan Sigma and Hercules. These substances were all donations of the manufacturers. Other substances used were of pharmaceutical grade.

Table 1
Bead formation studies with different types and concentrations of carrageenan and salts

| Carrageenan type | Carrageenan (%) | Salt type | Salt (%) |
|---|---|---|---|
| Seaspen | 1 | $CaCl_2$ | 4 |
| (ι-carrageenan) | 3 | KCl | 4 |
| Gelcarin GP 379 | 1 | KCl | 4/5/6/7 |
| (ι-carrageenan) | 2 | KCl | 4/5/6/7 |
| | 1.5 | $CaCl_2$ | 2/3/4/5/6 |
| | 3 | $CaCl_2$ | 2/3/4/5/6 |
| | 1.5 | $NH_4Cl$ | 5 |
| | 1.5 | NaCl | 6 |
| Gelcarin GP 359 | 1.5 | KCl | 8/9/10 |
| (ι-carrageenan) | 1.5 | $BaCl_2$ | 10 |
| Gelcarin GP 812 | 3 | $CaCl_2$ | 3/4 |
| (κ-carrageenan) | 4 | $CaCl_2$ | 3/4 |
| | 2.5 | KCl | 4/5 |
| | 2.5 | $Al_2(SO_4)_3$ | 4/5 |
| Sigma C-1263 | 2.5 | KCl | 4 |
| (κ-carrageenan) | 3 | KCl | 4 |
| | 3 | $CH_3COOK$ | 2 |
| | 3 | $CH_3COOZn$ | 3 |
| Gelcarin GP 911 | | | |
| (κ-carrageenan) | 2.5 | KCl | 2/3/4/5/6 |
| | 3 | KCl | 2/3/4/5/6 |
| Genugel | | | |
| (κ-carrageenan) | 2.5 | KCl | 3/4/5 |
| | 3 | KCl | 3/4/5 |
| | 3.5 | KCl | 3/4/5 |

### 2.1. Solubility measurement

Verapamil hydrochloride or ibuprofen in an amount of excess of its solubility was placed in 10 ml of dissolution medium in a bottle maintained at $37 \pm 10.5$ °C and shaken in a constant temperature water bath (Certomat WRB. Braun, Biotech International) for 48 h. At appropriate times aliquots were taken from the dissolution medium and filtered. The drug content of the samples were assayed spectrophotometrically (Shimadzu UV 2100 S, Japan) at 278 and 221 nm respectively. Calibration curves were used for the determination of the amounts dissolved.

### 2.2. Preparation of the carrageenan beads

The compositions of the formulations prepared are shown in Tables 1 and 2. Beads without drug were prepared according to the formulation shown in Table 1 to determine the optimal types and percentages. The formulations of the drug carrying beads are shown in Table 2 where the effects of different factors are also taken into account. Aqueous dispersions of carrageenan were prepared in 15 ml distilled water. The drug was added while stirring. The beads were prepared by dropping this dispersion from a disposable syringe into the aqueous salt solutions stirred magnetically by using a magnetic stirrer (Ikamag RH, Germany). The beads formed were filtered under vacuum, washed with 100 ml distilled water and dried in a hot air oven at 37 °C (Garcıa and Ghaly, 1996).

### 2.3. Determination of the mean bead size

Particle size distribution of the beads was determined by a vibrational sieve analysis system (Retsch, Germany) with sieves having apertures of 0.71, 0.85, 1.18 and 2 mm vibrating at a speed of 50 rpm for 10 min. Mean particle sizes of the formulations were calculated.

Table 2
Codes of verapamil HCl and ibuprofen containing carrageenan beads tested for different parameters

| Code | Drug (%) | Carrageenan (%) | Salt type | Salt (%) | Outer phase (ml) |
|---|---|---|---|---|---|
| Ver 1/Ibu 1 | 0.5 | 3.5 | KCl | 4 | 150 |
| Ver 2/Ibu 2 | 1 | 3.5 | KCl | 4 | 150 |
| Ver 3/Ibu 3 | 1.5 | 3.5 | KCl | 4 | 150 |
| Ver 4/Ibu 4* | 1 | 2.5 | KCl | 4 | 150 |
| Ver 5/Ibu 5* | 1 | 3 | KCl | 4 | 150 |
| Ver 2/Ibu 2 | 1 | 3.5 | KCl | 4 | 150 |
| Ver 6*/Ibu 6* | 1 | 4 | KCl | 4 | 150 |
| Ver 2/Ibu 2 | 1 | 3.5 | KCl | 4 | 150 |
| Ver 9/Ibu 9* | 1 | 3.5 | $CaCl_2$ | 4 | 150 |
| Ver 10/Ibu 10* | 1 | 3.5 | NaCl | 3 | 150 |
| Ver 7/Ibu 7 | 1 | 3.5 | KCl | 4 | 150 |
| Ver 2/Ibu 2 | 1 | 3.5 | KCl | 5 | 150 |
| Ver 8/Ibu 8 | 1 | 3.5 | KCl | 4 | 150 |
| Ver 11/Ibu 11 | 1 | 3.5 | KCl | 4 | 50 |
| Ver 12/Ibu 12 | 1 | 3.5 | KCl | 4 | 100 |
| Ver 2/Ibu 2 | 1 | 3.5 | KCl | 4 | 150 |

* No spherical beads were formed with these fomulations.

### 2.4. Encapsulation efficiency of the beads

Using a mortar and a pestle beads were crushed and a sample of exactly 25 mg was extracted in 25 ml pH 7.4 phosphate buffer solution. After filtration the sample was assayed spectrophotometrically at 278 nm for verapamil hydrochloride and at 221 nm for ibuprofen. Carrageenan did not interfere with the drug absorbances at these wavelengths.

### 2.5. In vitro release studies

The dissolution rates of the pure drugs and the beads prepared were measured according to the USP XXIII paddle method. The dissolution medium was 500 ml phosphate buffer solution (pH 7.4) at $37 \pm 0.5$ °C. Accurately weighed samples of 30 mg for pure verapamil hydrochloride, 10 mg for pure ibuprofen and 100 mg for drug containing beads were used for the test. At preset time intervals aliquots were withdrawn and replaced by the same volume of dissolution medium. The amount of drug dissolved/released was determined spectrophotometrically. The kinetics data obtained from release rates were evaluated.

### 2.6. Statistical analysis

The data obtained from the particle size, encapsulation efficiency and release rate determination studies of verapamil hydrochloride and ibuprofen containing carrageenan beads were analysed statistically with ANOVA and *t*-test by using Sigma Stat.

### 2.7. Scanning electron microscope analysis

Shapes and surface characteristics of the beads were investigated and photographed using Scanning Electron Microscope (SEM; Joel JSM-5200, Japan).

## 3. Results and discussion

The equilibrium solubilities of verapamil HCl and ibuprofen in pH 7.4 phosphate buffer solution were found to be $123.87 \pm 6.07$ and $7.43 \pm 0.51$ mg/ml respectively, indicating the sink condition limits for the dissolution studies.

In order to obtain the optimum bead formulation various salts were investigated with different iota and kappa carrageenan types (Table 1). Con-

trary to κ-carrageenan, no spherical beads were formed with ι-carrageenan formulations using the salt solutions seen in Table 1. This may be the result of the fact that with KCl, κ-carrageenan gives harder gels than ι-carrageenan (The United States Pharmacopeia XXIII, 1995). Experiments with κ-carrageenan types showed that calcium chloride and potassium chloride were suitable for bead formation; best spheres in shape were obtained by dropping 3.5% Genugel dispersion into 4% potassium chloride solution. Using these ratios, drug containing spherical beads having almost a smooth surface were obtained (Fig. 1). To further optimize this formulation with Genugel, effects of drug concentration, polymer concentration, salt type, salt concentration and outer phase volume were investigated. Codes of these verapamil HCl and ibuprofen containing κ-carrageenan beads prepared are seen in Table 2.

By incorporating verapamil HCl in κ-carrageenan beads using potassium chloride, calcium chloride and sodium chloride (Ver 2,9,10) and low polymer concentrations (2.5–3%; Ver 4,5) spherical beads could be obtained. But when using ibuprofen at the same experimental conditions flake like particles were formed, except for Ibu 2. The incorporation of the slightly water soluble drug as a dispersion made the ionic bounding

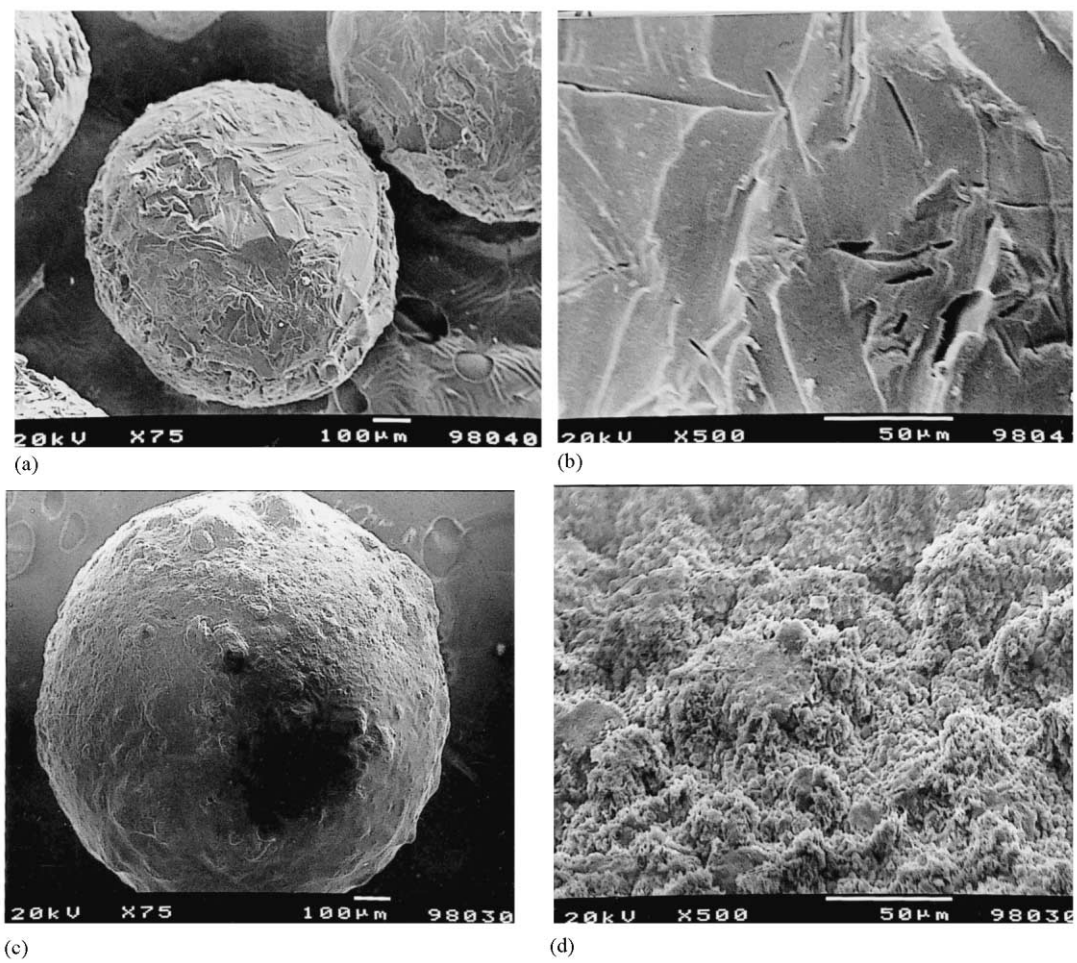


Fig. 1. Scanning electron micrographs of (A) shape and (B) surface of formulation Ver 2 and (C) shape and (D) surface of formulation Ibu 2.

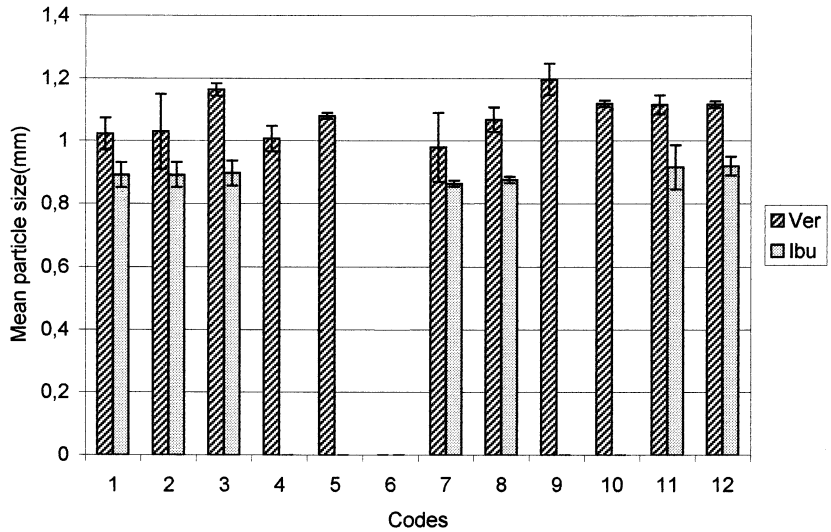


Fig. 2. Mean particle sizes of carrageenan beads ($n = 3$).

with calcium chloride and sodium chloride difficult. The same difficulty was observed with low κ-carrageenan concentrations. The optimum polymer concentration was found to be 3.5% (w/v) for both drugs, a 4% (w/v) polymer dispersion could not be dropped from the injector due to its high viscosity. Controlled release spheres containing acetaminophen are reported to be prepared from a dispersion containing 3% κ-carrageenan; using calcium chloride solution as a cross linking agent gave the slowest drug release (Garcıa and Ghaly, 1996).

Verapamil HCl containing carrageenan beads were in the size range of 0.980–1.197 mm and ibuprofen containing carrageenan beads were in the size range of 0.865–0.921 mm (Fig. 2). None of the variables affected the mean particle sizes of carrageenan beads carrying verapamil HCl and ibuprofen ($P > 0.05$). The mean particle sizes increased as their encapsulation efficiencies increased, although the differences were not significant statistically ($P > 0.05$) also as reported by Bhardwaj et al., 1995.

The encapsulation efficiencies were found to be 34.8–71.1% for verapamil HCl and 23.6–58.0% for ibuprofen containing carrageenan beads (Fig. 3). The encapsulation efficiency of verapamil HCl and ibuprofen increased as their concentrations in the formulation increased ($P < 0.05$; Ver 1,2,3; Ibu 1,2,3). Using different salts potassium chloride, calcium chloride and sodium chloride; (Ver 2,9,10) affected the encapsulation efficiency of verapamil HCl, best results were obtained with KCl ($P < 0.05$). Carrageenan and potassium chloride concentrations had no effect on the encapsulation efficiency of verapamil HCl ($P > 0.05$; Ver 4,5,2,6 and Ver 7,2,8). These encapsulation data are in compliance with those of another water soluble drug, acetaminophen (Garcıa and Ghaly, 1996).

In the preparation of carrageenan beads containing ibuprofen, potassium chloride concentration was effective ($P < 0.05$; Ibu 7,2,8), whereas outer phase volume had no effect on the encapsulation efficiency ($P > 0.05$; Ibu 11,12,2). The encapsulation efficiency of the ibuprofen containing beads prepared with chitosan or sodium alginate using tripolyphosphate and calcium chloride solutions was 98%, using carrageenan the ibuprofen encapsulation efficiency in our study did not exceed 58.8%, although the same method was used. So with chitosan or sodium alginate more efficient encapsulation was achieved (Bodmeıer and Paeratakul, 1989; Bodmeıer and Wang, 1993).

Comparing the mean particle size and encapsulation efficiency data of the corresponding formu-

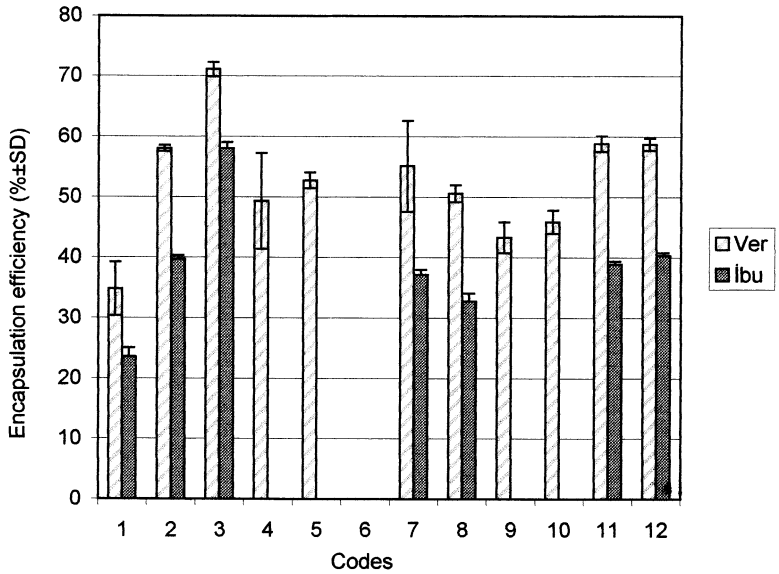


Fig. 3. Encapsulation efficiencies of carrageenan beads ($n = 3$).

lations of verapamil HCl and ibuprofen, it can be seen that the freely water soluble drug was always entrapped in higher ratios, which results in bigger particles.

The dissolution profiles of freely water soluble model drug verapamil HCl and slightly water soluble model drug ibuprofen are shown in Fig. 4.

In vitro release rates of verapamil HCl and ibuprofen decreased as the concentration of the drug increased ($P < 0.05$; Fig. 5). Similar results were reported for the in vitro release of acetaminophen (Garcıa and Ghaly, 1996), but not for ibuprofen (Bodmeıer and Wang, 1993).

According to $t$-test results the carrageenan concentrations of Ver 4 and Ver 5 did not lead to a significant difference in release rate, whereas a statistically significant difference was found between the three groups ($P < 0.05$), showing that the carrageenan concentration is an effective factor (Fig. 6). As mentioned before, ibuprofen containing carrageenan beads could not be obtained with carrageenan concentrations below 3.5%.

In vitro release rates of verapamil HCl and ibuprofen slowed down as the concentration of potassium chloride increased ($P < 0.05$; Fig. 7). Although the profiles of Ibu 2,7 and 8 seem to be very close on the figure, the differences were significant statistically. The increase of the in vitro release rates of verapamil HCl and ibuprofen from carrageenan beads prepared with low concentrations of potassium chloride solutions may be due to the less cross linked structure of the beads. This may result in a more porous matrix and higher drug release (Garcıa and Ghaly, 1996).

Using various types of salts resulted significant differences in the release rate of verapamil HCl ($P < 0.05$). When calcium chloride was used the

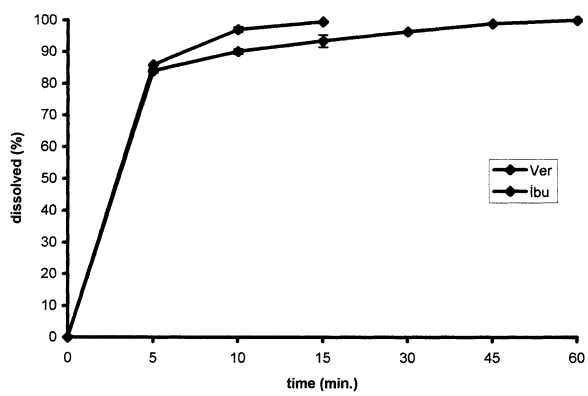


Fig. 4. Dissolution of 30 mg pure verapamil HCl and 10 mg pure ibuprofen at $37 \pm 0.5$ °C in 500 ml pH 7.4 phosphate buffer solution ($n = 3$).

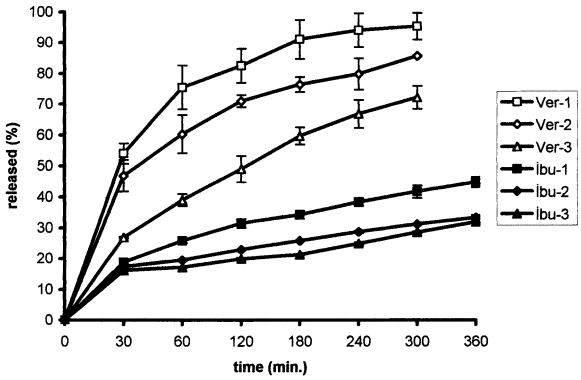


Fig. 5. In vitro release of verapamil HCl and ibuprofen from carrageenan beads with 0.5% (Ver, Ibu 1), 1% (Ver, Ibu 2) and 1.5% (Ver, Ibu 3) drug content ($n = 3$).

release rate decreased, but when sodium chloride was used verapamil HCl was released rapidly (Fig. 8). Ibuprofen containing carrageenan beads could not be prepared with calcium chloride and sodium chloride as mentioned before.

It was observed that outer phase volume had no effect on the release rates of verapamil HCl and ibuprofen from carrageenan beads ($P > 0.05$; Fig. 9).

The release rate of the water soluble drug verapamil HCl from the beads was always higher than that of the slightly water soluble drug ibuprofen.

When the release rate data were evaluated according to the equation $M_t/M_\infty = k.t^n$, it was found out that $n$ is less than 0.5 in all cases and this result shows that the drugs were released from the beads with a mixed mechanism (Peppas, 1987).

Correlation coefficients of different mathematical models for verapamil HCl and ibuprofen that are shown in Table 3 were obtained using the software (DISSOL; Ağabeyoğlu, 1991).

Correlation coefficients of different mathematical models for verapamil HCl leads us to the conclusion that the Modified Hixson Crowell provides the best correlation. Hixson and Crowell model initially proposed as a kinetic model for the dissolution of powders was modified and used for multiparticulate systems (Abdou, 1989).

According to the data in Table 3, it can be seen that correlation coefficients of different mathematical models for formulations of ibuprofen are fitting to Higuchi equation. This equation can be used for homogeneous and heterogeneous matrix systems and was developed to define the release from plastic matrices which do not change their sizes and shapes after the release study (Öner et al., 1984).

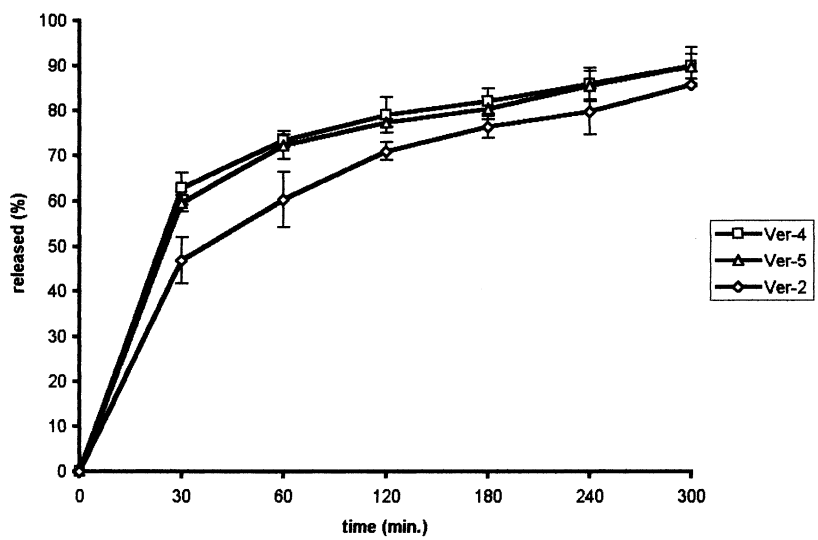


Fig. 6. In vitro release of verapamil HCl from carrageenan beads prepared with 2.5% (Ver 4), 3% (Ver 5) and 3.5% (Ver 2) carrageenan ($n = 3$).

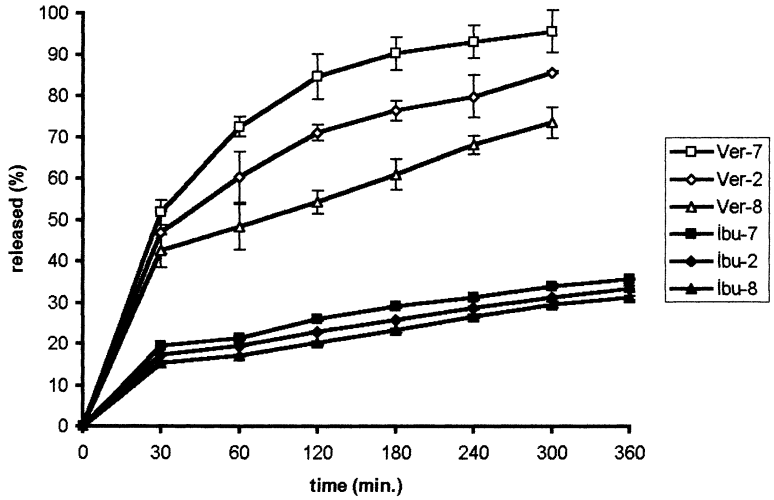


Fig. 7. In vitro release of verapamil HCl and ibuprofen from carrageenan beads prepared with 3% (Ver, Ibu 7), 4% (Ver, Ibu 2) and 5% (Ver, Ibu 8) KCl concentration ($n = 3$).

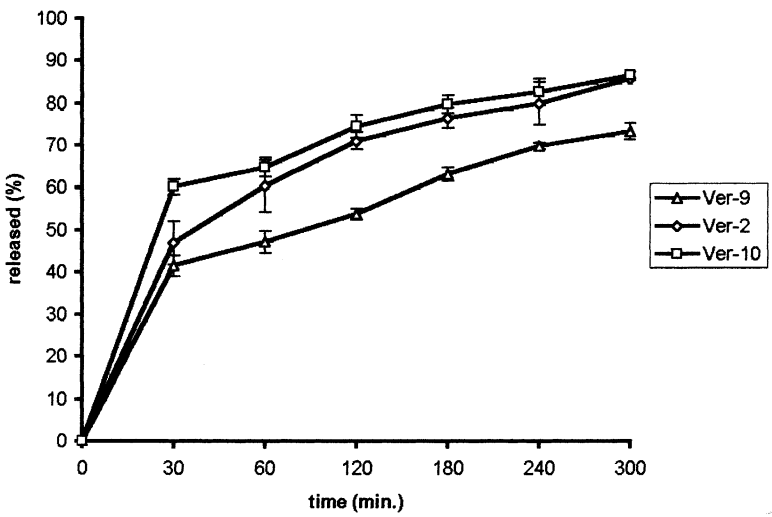


Fig. 8. In vitro release of verapamil HCI from carrageenan beads prepared with KCl (Ver 2), $CaCl_2$ (Ver 9) and NaCl (Ver 10) ($n = 3$).

The explanations above correlate with the observations during and after the in vitro release study of the verapamil HCl and ibuprofen incorporated carrageenan heads, where the sizes and shapes of the beads did not change.

## 4. Conclusion

Drug containing carrageenan beads were prepared by ionotropic gelation method. While about 30% of ibuprofen was released at 6 h, about 70%

of verapamil HCl was released in 5 h from the carrageenan beads prepared. This study has proved the usefulness of the natural polymer carrageenan for the preparation of controlled release systems of both water soluble drug verapamil HCl and slightly water soluble drug ibuprofen.

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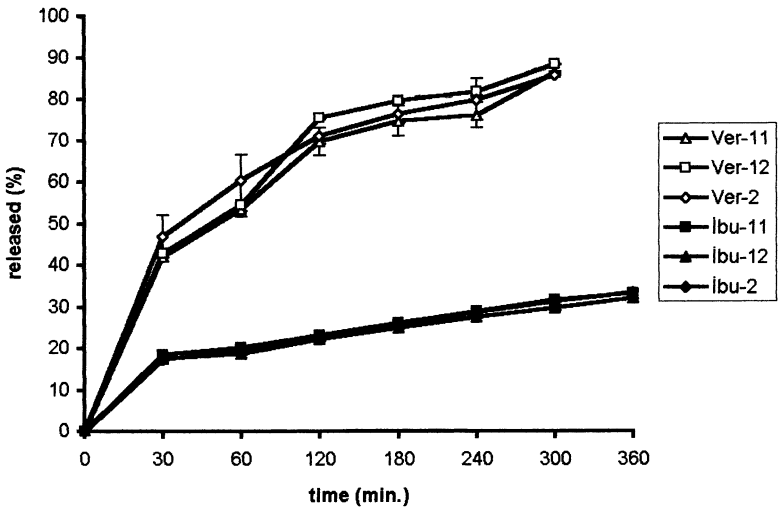


Fig. 9. In vitro release of verapamil HCl and ibuprofen from carrageenan beads prepared with 50 ml (Ver, Ibu 11), 100 ml (Ver, Ibu 12) and 150 ml (Ver, Ibu 2) of outer phase volume ($n = 3$).

Table 3
Correlation coefficients of different mathematical models for verapamil and ibuprofen

| | 0. Kinetics | 1. Kinetics | Hixson Crowell | Modified Hixson Crowell | Higuchi equation | Square root of time $\sqrt{t}$ |
|---|---|---|---|---|---|---|
| Ver 1 | 0.7873 | 0.9646 | 0.9210 | 0.9699 | 0.9355 | 0.8820 |
| Ver 2 | 0.8948 | 0.9730 | 0.9547 | 0.9912 | 0.9754 | 0.9606 |
| Ver 3 | 0.9586 | 0.9936 | 0.9857 | 0.9962 | 0.9980 | 0.9939 |
| Ver 4 | 0.8962 | 0.9719 | 0.9560 | 0.9815 | 0.9667 | 0.9548 |
| Ver 5 | 0.8879 | 0.9657 | 0.9510 | 0.9673 | 0.9632 | 0.9426 |
| Ver 7 | 0.7835 | 0.9718 | 0.9232 | 0.9775 | 0.9402 | 0.8838 |
| Ver 8 | 0.9941 | 0.9945 | 0.9971 | 0.9585 | 0.9908 | 0.9904 |
| Ver 9 | 0.9826 | 0.9937 | 0.9920 | 0.9659 | 0.9925 | 0.9911 |
| Ver l0 | 0.9457 | 0.9891 | 0.9786 | 0.9856 | 0.9860 | 0.9886 |
| Ver 11 | 0.8968 | 0.9470 | 0.9407 | 0.9793 | 0.9508 | 0.9558 |
| Ver 12 | 0.8536 | 0.9456 | 0.9208 | 0.9780 | 0.9433 | 0.9325 |
| Ibu 1 | 0.9491 | 0.9702 | 0.9639 | 0.9913 | 0.9917 | 0.9881 |
| Ibu 2 | 0.9921 | 0.9964 | 0.9952 | 0.9755 | 0.9945 | 0.9953 |
| Ibu 3 | 0.9840 | 0.9775 | 0.9798 | 0.8864 | 0.9810 | 0.9318 |
| Ibu 7 | 0.9760 | 0.9843 | 0.9817 | 0.9848 | 0.9958 | 0.9975 |
| Ibu 8 | 0.9940 | 0.9961 | 0.9956 | 0.9590 | 0.9940 | 0.9858 |
| Ibu 11 | 0.9939 | 0.9968 | 0.9960 | 0.9624 | 0.9975 | 0.9906 |
| Ibu-12 | 0.9944 | 0.9974 | 0.9965 | 0.9613 | 0.9986 | 0.9909 |

of Medicine, Histology Department) for technical assistance in the SEM analysis.

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