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Enantioselective Release Behavior of Ketoprofen Enantiomers from Alginate-metal Complexes, Monitored by Chiral HPLC

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Alginate-metal complexes were prepared with divalent (Ca, Ba, Zn) and trivalent metals (Fe, Al) via congealing method in form of beads. Alginate mixed metals (Ca and Fe) complexes were also prepared by simultaneous and consecutive congealing. The studied beads were blank beads and racemic ketoprofen (KTP) loaded beads. Metal content was determined by atomic absorption spectroscopy and was 1.8% to 30.4%. The IR spectra showed interactions between ketoprofen carboxylic OH and alginate hydroxyl OH, thus chiral interaction is suggested. Chiral HPLC was used to monitor enantioselective release (ESR) of racemic ketoprofen enantiomers in a phosphate buffer solution (PBS) at pH = 7.4. ESR is expressed as the relative chromatographic area of R-enantiomer to S-enantiomer (R/S ratio). For divalent metal complexes, over the first 50 min of release, R/S was < 1; with starting value of 0.53 in case of calcium, indicating important ESR, but less important in case of barium. However, in case of zinc, R/S was >1 with starting value of 1.1, indicating weak ESR. No significant results of ESR were obtained for trivalent metal complexes, where R/S is almost 1 in case of iron and aluminum. For alginate beads, which simultaneously congealed with (Ca and Fe), R/S was >1. Nevertheless, consecutively congealed alginate gave opposite ESR behavior; R/S was <1 for Ca then Fe congealed alginate, while R/S was >1 for Fe then Ca congealed alginate.

Keywords: ESR, Alginate, Ketoprofen, Enantiomers, HPLC, *In vitro*

INTRODUCTION

Scientists have been fully aware of the effect of chiral drugs on the pharmaceutical practices [1-3]. This awareness also takes into account the role of chiral drugs interactions with chiral excipients in the ESR of enantiomeric drugs from their formulations [4], which would, logically, be extended to a subsequent effect on the pharmacology and bioavailability of chiral drugs. Therefore, investigating possible interaction between chiral drugs and chiral excipients, especially in the field of ESR applications, has become a research focusing area [5-20]. However, ESR applications in pharmaceutical practices have not been accomplished yet, as far as we know.

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Different forms of alginate were widely used in pharmaceutical applications [21-25]. Alginate structure contains two uronic acids; α-D-mannuronic acid (M) and β-L-guluronic acid (G). These acids constitute homopolymeric blocks MM or GG, and blocks with an alternating sequence (MG blocks) as illustrated in Fig. 1. Sodium alginate can form hydrophilic gels by interaction with multivalent metal ions leading to cross-linked structures. An egg-box model was proposed to illustrate how the metal binds to alginate chains as shown in Fig. 2 [26,27]. Thus, gel beads, of different drugs, were prepared by dropping solutions of sodium alginate into solutions of ion metal chloride, then each drug release behavior was studied [20,28-33].

Ketoprofen, 2-aryl propionic acid derivative, is widely used as a non-steroidal anti-inflammatory drug (NSAID) [34]. It is a chiral molecule due to the asymmetric carbon

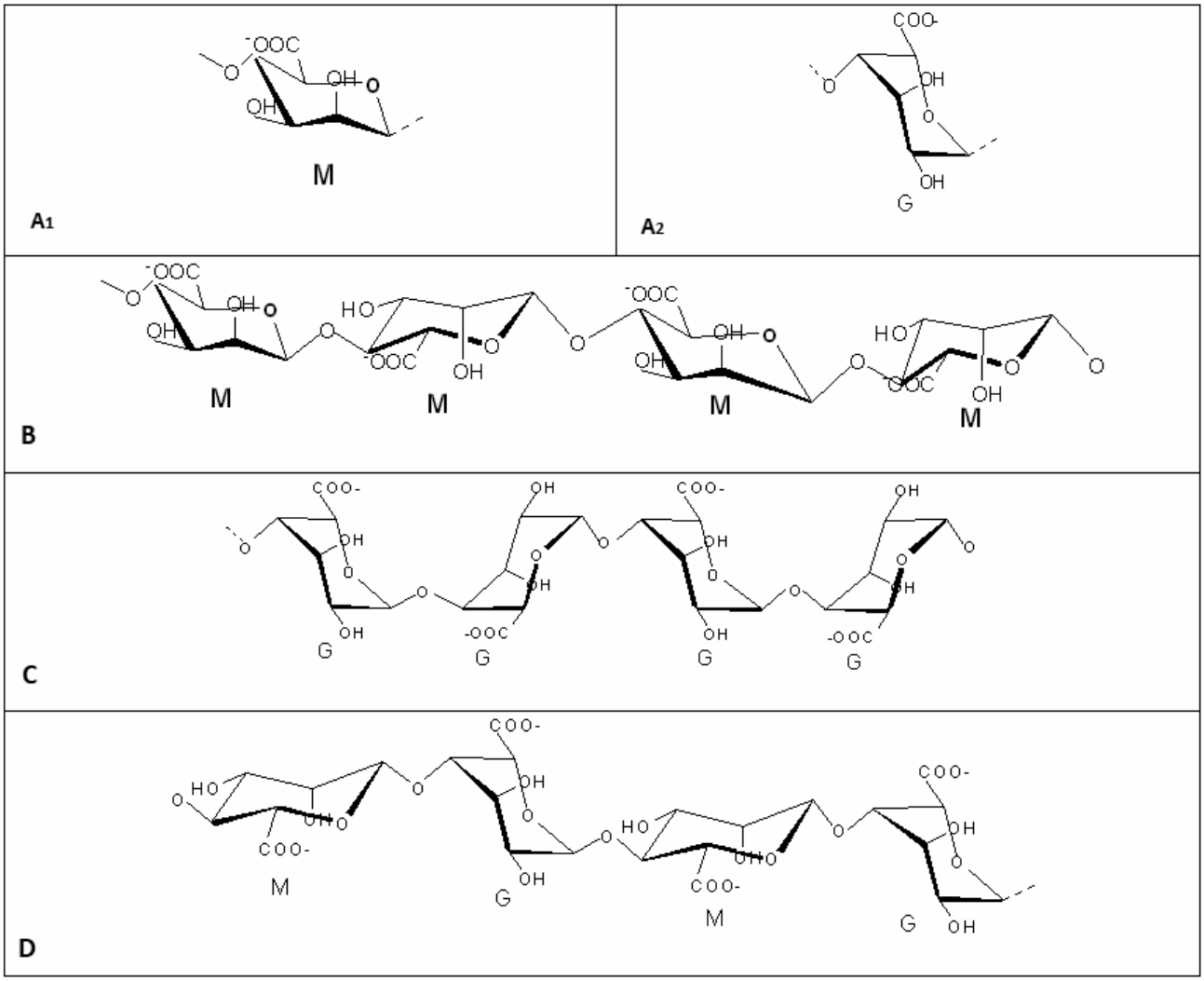


Fig. 1. Structure of alginate sodium salt. A_1: D-mannuronic acid, A_2: L-guluronic acid, B: Repeated β-D-mannuronic acid blocks, C: Repeated α-L-guluronic acid blocks, D: Repeated β-D-mannuronic-α-L-guluronic acid blocks.

C-2 as shown in Fig. 3. It is also known for short half-life time. Previous studies attended to the clinical pharmacokenetics of ketoprofen enantiomers, where therapeutic effect is attributed to the S-enantiomer [35-38].

The primary aim of this paper is to prepare different alginate-metal complexes in beads form and evaluate the role of these complexes in the enantioselective release of ketoprofen enantiomers. The second aim is to draw attention to the fact that ESR would affect the enantiospecific or enantioselective pharmacological and bioavailability studies, especially in case of fast absorption of chiral drugs, where erroneous conclusions could result. Hence, this may help also in designing a stereoselective dosage form.

EXPERIMENTAL

Materials

Alginic acid sodium salt and (S)-Ketoprofen (assay 99%) were purchased from Sigma-Aldrich. Calcium

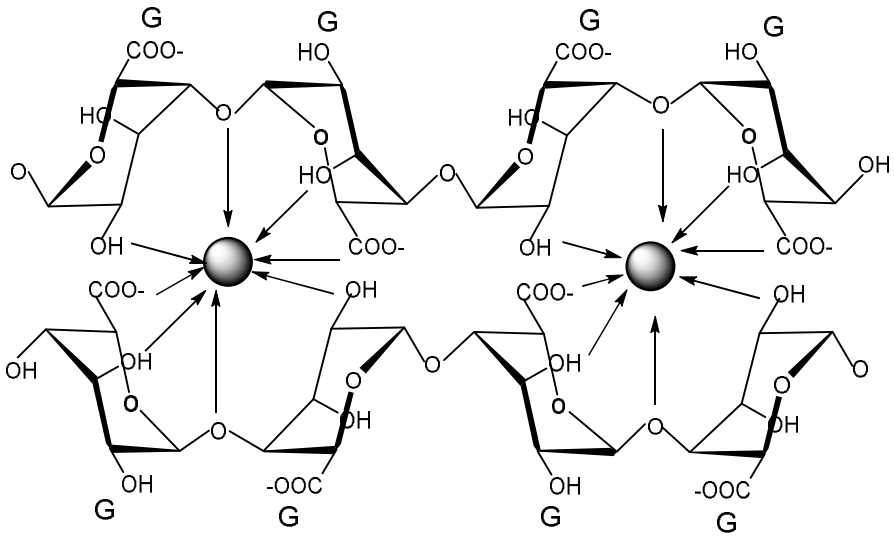


Fig. 3. Proposed egg-box model for alginate-multivalent metal complexes.

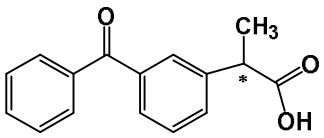


Fig. 3. Chemical structure of KTP, chiral center C-2 labeled with*.

chloride, iron chloride, aluminum chloride, barium chloride dehydrate and zinc chloride were obtained from Merck. Racemic keteprofen was supplied from Infinity laboratories Private Limited (Infinity, India) 99.6%. Hexane, isopropanol and trifluoroacetic acid (TFA) were of analytical grade or HPLC grade and were obtained from Merck.

Instrumentations and Methods

Fourier transform infrared (FTIR) spectroscopy. The FTIR spectra of sodium alginate, blank alginate beads, loaded alginate beads, and pure KTP were recorded in Bruker FTIR spectrometer (Bruker-vector 22) from 4000-400 cm^{-1} using KBr disks. The disks were made by applying a pressure of 10 ton cm^{-2} in a hydraulic press.

Atomic absorption spectrophotometer (AAS). The metal contents in alginate complexes were determined using AAS-Shimadzu-AA6800 atomic absorption spectrophotometer. A quantity (6-10 mg) from each alginate-metal complex, was treated with 25 ml HCl 1 N. The obtained solutions were left at room temperature for 24 h and 48 h, to confirm the transformation of the alginate metal complexes into acidic form (alginic acid). As the acid HCl is much stronger than the uronic acids of alginate (pK_a equals to 3.38 and 3.65 for M and G acids respectively), a cationic exchange between the ion metals in the complexes and the acid proton occurred. Subsequently, the obtained solutions contained the metal ions quantitatively. After filtration, the solutions became ready to measure the concentrations of Na^{+}, Ca^{+2}, Zn^{+2}, Ba^{+2}, Fe^{+3} and Al^{3+}.

HPLC. KTP enantiomers were monitored using HPLC-LC-20AD Shimadzu-Japan, liquid chromatography equipped with photo-diode array detector, manual Rheodyne injector (model 7125, 20 µl loop) and Lab solution software. The used mobile phase contained hexane, isopropanol and TFA with 90:10:0.1 v/v% ratios, respectively. It was degassed prior to use for 30 min. The

isocratic flow rate was 1 ml min^{-1}. The chiral column used was Kromasil®-5-amy coat (250 × 4.6 mm i.d) 5 µm. It was equilibrated for at least 30 min at temperature (30 °C) with the mobile phase flowing through the chromatographic system before starting the analysis. The samples were monitored at 254 nm.

Beads preparation via alginate-metal complexation. Blank beads and KTP loaded beads were prepared using ionotropic congealing method. For blank beads, sodium alginate was dissolved in aqueous PBS, pH = 7.4 to obtain 2% (Weight/Volume) alginate solution. The obtained solution was then extruded as droplets, through a glassy tube (inner diameter of 1.0 cm), into the congealing bath. The bath contained a continuously stirred solution of ion metal chloride (3% w/v) at room temperature. The tube was positioned at 5 cm above the surface of metal chloride solution. The formed beads were stirred continuously in the solution for 24 h, separated and washed with PBS. A further washing was carried out with distilled water for 1 min, then, the beads were dried at 40 °C for 48 h. For KTP loaded beads, racemic KTP was dissolved in sodium alginate aqueous solution PBS, pH 7.4. The obtained solution was then congealed in a (3.0%, w/v) metal chloride solution. The ratio of KTP to sodium alginate was 1 to 3.75 (w/w). Other type of beads were congealed, in a simultaneous way, with two different metal ions. The concentration in the bath was 3% for each ion. In addition, some beads were congealed in a consecutive way, where the congealing process took place with the divalent cation Ca^{2+} for 3h. After this period, the formed beads were removed and dropped immediately in the second congealing bath of the trivalent cation Fe^{3+} for 21 h. This experiment was repeated differently by altering the sequence of congealing (*i.e.*, with Fe^{3+} for 3 h then with Ca^{2+} for 21 h). Figure 4 illustrates the alginate congealing method used in this study [27,28,30].

Bead size. The size of beads was measured using a millimeter scaled ruler. The diameters were measured using at least three beads. Table 1 shows the obtained results.

Drug loading and loading efficiency. To evaluate the quantity of loaded drug (W_l), drug loading efficiency (L%) and drug content (KTP%) for the prepared beads, it was necessary to determine the residual KTP in the congealing solution. The concentration of residual KTP (C_{res}) in the congealing solution was determined (in mg ml^{-1}) using quantitative chiral HPLC. As the total volume of the solution is known (V ml), the residual drug weight W_{res} (mg) was directly calculated (W_{res} = V * C_{res}). Subsequently, provided that the drug weight used for beads preparation is W_t (mg), the weight of loaded drug W_l will be given by (W_l = W_t - W_{res}), the loading efficiency L(%) = (W_l/W_t) * 100, and the drug content KTP% = (W_l/ W_{db}) * 100, where W_{db} is the total weight of drug loaded beads after drying. The obtained results for this section are summarized in Table 2.

***In vitro* drug release study.** A quantity of dried beads W_d (mg), contained W_{KTP} (mg) drug, was placed in volume V_{rl} of *in vitro* release solution (V_{rl} = 5 ml). At the initial time (t = 0 min), the KTP enantiomers were entrapped in the taken dried beads. Hence, the initial concentration of each enantiomer in the solution is practically zero. However, the content of each enantiomer in W_d is half the KTP content, because the loaded drug is racemic; *i.e.*, W_R = W_S = 0.5 W_{KTP}. After 10 min stirring, aliquots (V_{al} = 0.2 ml) were taken from the release solution at different time intervals, and a fresh PBS solution (V_{PBS} = 0.2 ml) was immediately replenished in the release solution to permanently preserve the release volume V_{rl} = 5 ml. Each taken aliquot was extracted with 1 ml of cyclohexane. The obtained extract was injected in the HPLC system using 20 µl loop. When a KTP quantity Q (Q < W_{KTP}) releases from the beads to the medium, the corresponding enantiomers concentrations are Q_R/V_{rl} = C_{alR} and Q_S/V_{rl} = C_{alS}. However, the concentrations in the extract, C_{exR} and C_{exS}, will be diluted five times (supposing 100% extraction recovery) as the ratio of extract volume V_{ex} to the aliquot volume V_{al} is (1/0.2) = 5; *i.e.*, C_{alR} = (1/0.2) C_{exR} gives C_{alR} = 5C_{exR}. Table 3 shows data related to the release experiment.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of different prepared beads of alginate-metal complexes; congealing conditions, metal content as percentage and mole/w, bead size and shape. The shape and size of beads varied depending on the used ion metal; the divalent ion beads are smaller in size (5-7 mm diameter for wet and 2-3 mm for dry beads) than trivalent ion beads (7-10 mm diameter for wet, 2-3.5 mm

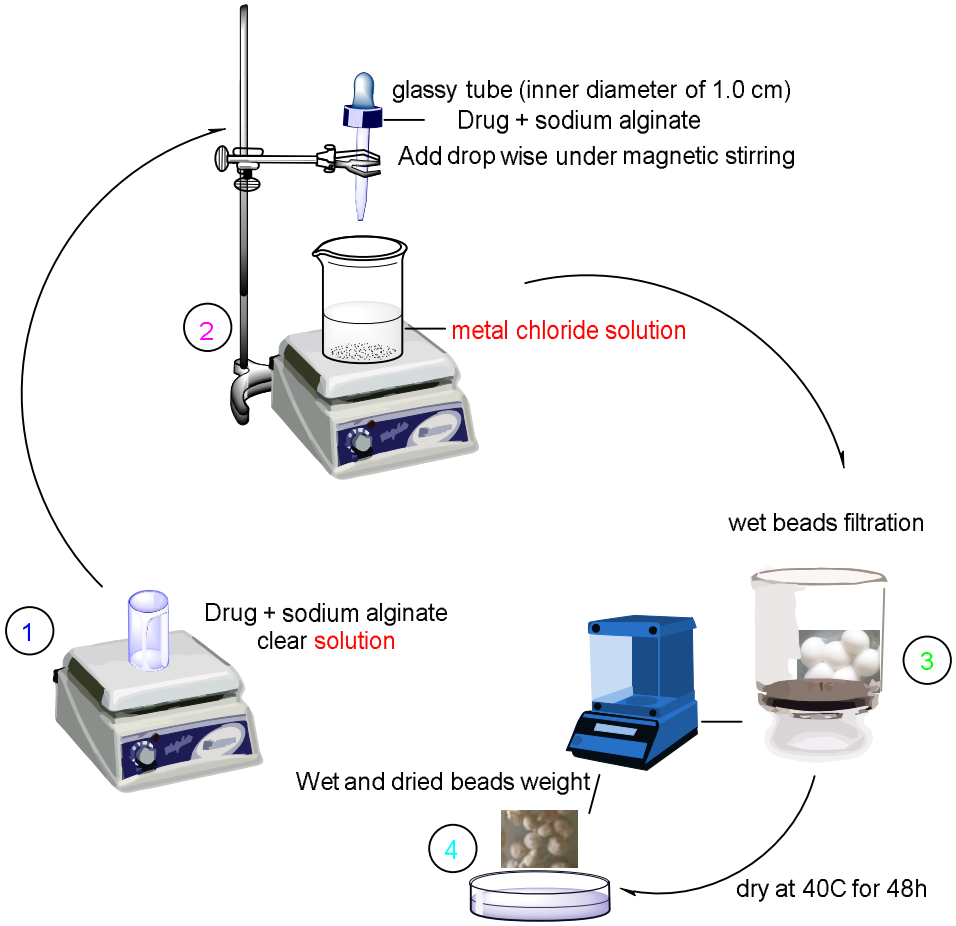


Fig. 4. Congealing method for alginate beads preparation.

for dry beads). The shape was not always spherical; it is tear-like for Ca and Ba beads and collapsed for Fe beads.

Metal content (molar to 100 g) in the prepared beads varied, interestingly, compared to Na content (0.39) of the starting sodium alginate. Thus, if the congealing process occurs *via* full exchanging between Na^+ and other metal ions, then it would be expected to obtain values of (0.19) and (0.13) for divalent and trivalent ions, respectively. However, the actual results show much excess for the examined metal ions. This may be explained in term of egg-box formation ability; i.e., multivalent ions form egg-box do not necessarily obey the same stoichiometric rule of sodium, as sodium does not have egg-box formation ability. In other words, alginate's affinity towards the studied multivalent metals is an important factor influencing the metal content in the formed complexes. It has been shown, for divalent metals, the following order of decreasing alginate's affinity Pb > Cu > Cd > Ba > Sr > Ca > Co, Ni, Zn > Mn [39,40]. The current divalent metal content results showed the order Ca (0.32) > Zn (0.30) > Ba (0.22), which is not in consistence with the above mentioned affinity order. A possible explanation for this is that metal ions might be entrapped inside the formed beads as a result of ions equilibrium between two media: the congealing solution and the solution inside the congealed beads. However, the metal content in blank beads does not significantly differ from that of KTP loaded beads, and it follows the same order, Ca > Zn > Fe > Ba > Al. Nonetheless, the case of alginate-Al beads makes an exception, where Al content is the lowest and differs

Table 1. Congealing Conditions and some Characteristics of the Prepared Beads

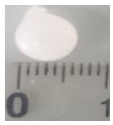
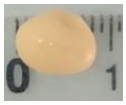
Type	Name	Congealing solution/ Congealing time (h)	Metal content (%)	Metal content Mol/100 g	Bead size (mm) & shape
Ketoprofen loaded alginate-metal beads	ACaK	$CaCl_2$ 3% 24 h	12.8 (Ca)	0.32	
	ABaK	$BaCl_2$ 3% 24 h	30.4 (Ba)	0.22	
	AZnK	$ZnCl_2$ 3% 24 h	20.0 (Zn)	0.30	
	AAlK	$AlCl_3$ 3% 24 h	1.8 (Al)	0.06	
	AFeK	$FeCl_3$ 3% 24 h	15.7 (Fe)	0.28	
Blank alginate-metal beads	ACa	$CaCl_2$ 3% 24 h	13.9 (Ca)	0.34	
	ABa	$BaCl_2$ 3% 24 h	30.1 (Ba)	0.22	
	AZn	$ZnCl_2$ 3% 24 h	20.4 (Zn)	0.31	
	AAl	$AlCl_3$ 3% 24 h	4.4 (Al)	0.16	
	AFe	$FeCl_3$ 3% 24 h	15.1 (Fe)	0.27	
	ANa	–	8.9 (Na)	0.39	

Table 1. Continued

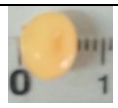
Ketoprofen loaded alginate-mixed -metals beads	A_1K	$CaCl_2$ 3% + $FeCl_3$ 3% 24 h		-	-	
	A_2K	$CaCl_2$ 3% 3 h	$FeCl_3$ 3% 21 h	-	-	
	A_3K	$FeCl_3$ 3% 3 h	$CaCl_2$ 3% 21 h	-	-	

Table 2. Data and Results for Drug Loading and Loading Efficiency of the Prepared Beads

Name	Before congealing		After congealing			After drying		
	W_{Alg} (mg)	KTP W_t (mg)	Total wet beads (g)	KTP W_{res} (mg)	KTP W_l (mg)	KTP L%	Total dried beads W_{db} (mg)	KTP%
ACaK	328	83	9.182	6.7	76.3	91.9	0.446	17.1
ABaK	313	88	8.612	23.1	64.9	73.8	0.518	12.5
AZnK	324	87	8.375	11.5	75.5	86.8	0.454	16.7
AAlK	315	87	10.472	5.1	81.9	94.1	0.577	14.2
AFeK	340	80	12.306	3.9	76.1	94.9	0.585	13.0
A_1K	325	82	11.261	25.8	56.2	68.7	0.785	7.2
A_2K	322	83	8.210	12.5	70.5	84.9	1.015	6.9
A_3K	328	82	11.380	16.4	65.6	80.1	0.604	10.9

W_{Alg}: the quantity of alginate used to form the 2% (w/v) solution in PBS.

between blank and loaded beads. Actually, there are not sufficient studies about determining metal content in alginate metal complexes using atomic absorption spectroscopy. However, metal cations (Ca^{2+}, Na^{1+}, Mg^{2+}, K^{1+}) were determined using this technique in alginate-zeolite composite after cationic exchange under acidic conditions of ammonium chloride [41].

Table 2 shows the KTP loaded as the percentage and the corresponding loading efficiency. The results show higher KTP contents for alginate-metal beads with single metal compared to alginate-mixed-metals beads. Generally speaking, KTP loading is 6.9 to 16.9 percent.

FTIR Analysis

In order to explore the interactions between entrapped KTP and the prepared alginate metal complexes, IR spectra

Table 3. The Summarized Data of the Release Experiment

Name	V_{rl} (ml)	W_d (mg)	KTP (%)	W_{KTP} (mg)	$0.5W_{KTP} = W_R = W_S$ (mg)	V_{al} (ml)	V_{PBS} (ml)	V_{ex} (ml)
ACaK	5	219	17.1	37.4	18.7	0.2	0.2	1
ABaK	5	211	12.5	26.4	13.2	0.2	0.2	1
AZnK	5	210	16.7	35.0	17.5	0.2	0.2	1
AAlK	5	251	14.2	35.5	17.8	0.2	0.2	1
AFeK	5	295	13.0	38.2	19.1	0.2	0.2	1
A_1K	5	290	7.2	20.8	10.4	0.2	0.2	1
A_2K	5	306	6.9	21.0	10.5	0.2	0.2	1
A_3K	5	317	10.9	34.6	17.3	0.2	0.2	1

Table 4. IR Data for Analyzed Substances in View of Expected Interactions

	Type	OH Hydroxyl (cm^{-1})	OH Carboxyl (cm^{-1})	C=O carboxyl KTP (cm^{-1})	C=O Carboxyl Alg (cm^{-1})	C=O Keton KTP (cm^{-1})
KTP			2250-3250	1692		1650
ANa	b	3523 (3000-3800)	*		1622 (1500-1800)	
ANa-K	p	2500-3750		1696	OV (1500-1800)	1652
ACa	b	3434 (3000-3750)	*		1636 (1500-1800)	
ACa-K	p	2500-3750		1697	OV (1500-1800)	1651
ACaK	l	3000-3750		OV (1500-1800)	1633	OV (1500-1800)
ABa	b	3434 (3100-3700)	*		1612 (1500-1800)	

Table 4. Continued

ABa-K	p	2250-3750		1699	OV (1500-1800)	1654
ABaK	l	2500-3750		OV (1500-2000)	1612	OV (1500-2000)
AZn	b	3544 (3100-3750)	*		1626 (1500-1750)	
AZn-K	p	2500-3750		1696	OV (1500-1800)	1653
AZnK	l	3000-3750		1699	OV (1500-1850)	1664
AFe	b	3416 (3000-3750)	*		1622 (1500-1750)	
AFe-K	p	2250-3750		1701	OV (1500-1750)	1648
AFeK	l	3000-3750		1633 (1500-1850)	OV (1500-1850)	1595
AAl	b	3419 (3000-3750)	*		1629 (1500-1800)	
AAl-K	p	2250-3750		1691	OV (1500-1800)	1653
AAlK	l	3000-3750		1699	OV (1500-1800)	1660

b: blank, p: physical mixture, l: loaded, *: carboxylic OH of alginate metal complexes is not expected because the proton OH is replaced with the ion metal.OV: the corresponding signal is not resolved but overlapped with other signal in the determined range below.Peak top and peak base values are given when appropriate.

were recorded for all kinds of prepared beads and for physical mixtures of KTP with blank beads. Expected interactions are mainly hydrogen bonding between the carboxyl group of KTP and the carboxyl and the hydroxyl groups of alginate. It is also expected to have a dipole-dipole interaction between carbonyl groups of all kinds. Table 4 represents IR data of all analyzed substances, while Fig. 5 to Fig. 8 represent the corresponding IR spectra.

KTP spectrum shows a large medium absorption band of carboxylic OH between 2250-3250 cm^{-1} and sharp intense bands for carboxylic carbonyl C=O and ketone C=O at 1692 cm^{-1} and 1650 cm^{-1}, respectively.

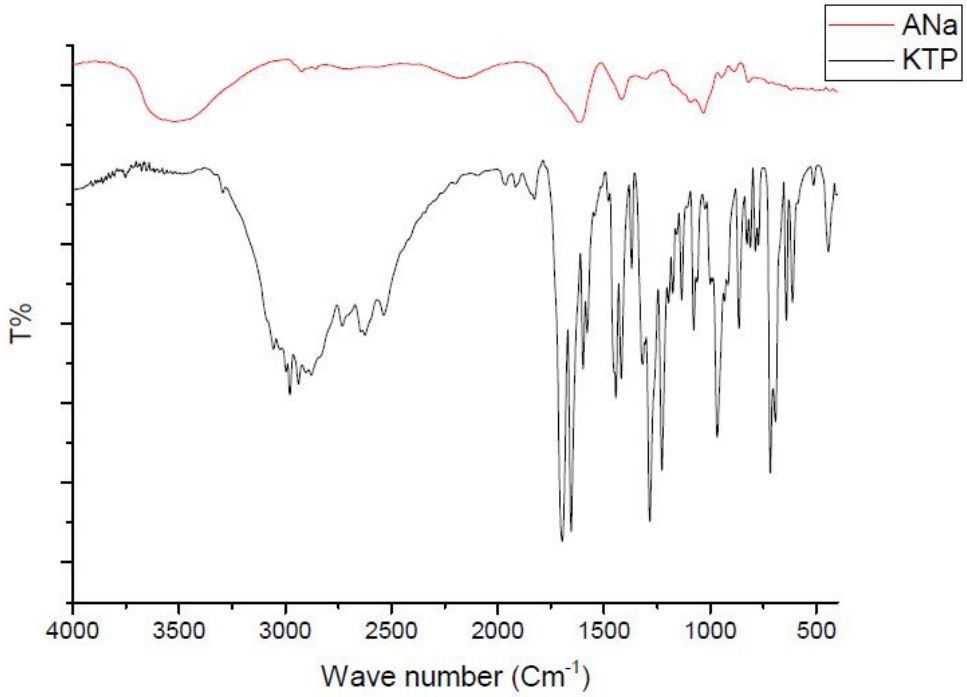


Fig. 5. IR spectra of sodium alginate ANa and ketoprofen KTP.

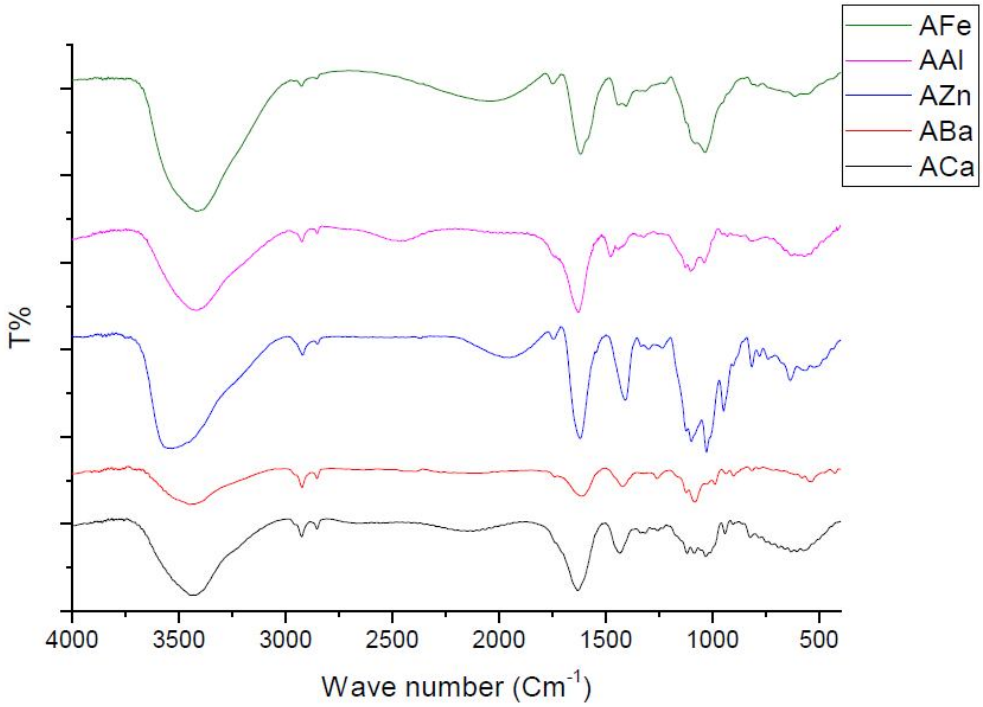


Fig. 6. IR spectra of blank alginate metal complex.

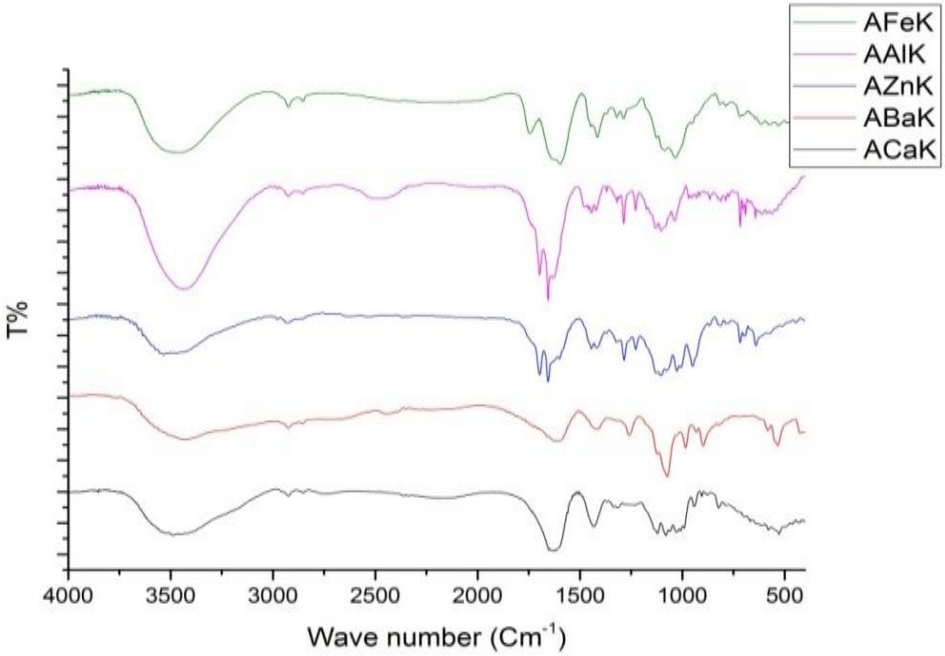


Fig. 7. IR spectra of KTP loaded alginate metal complex.

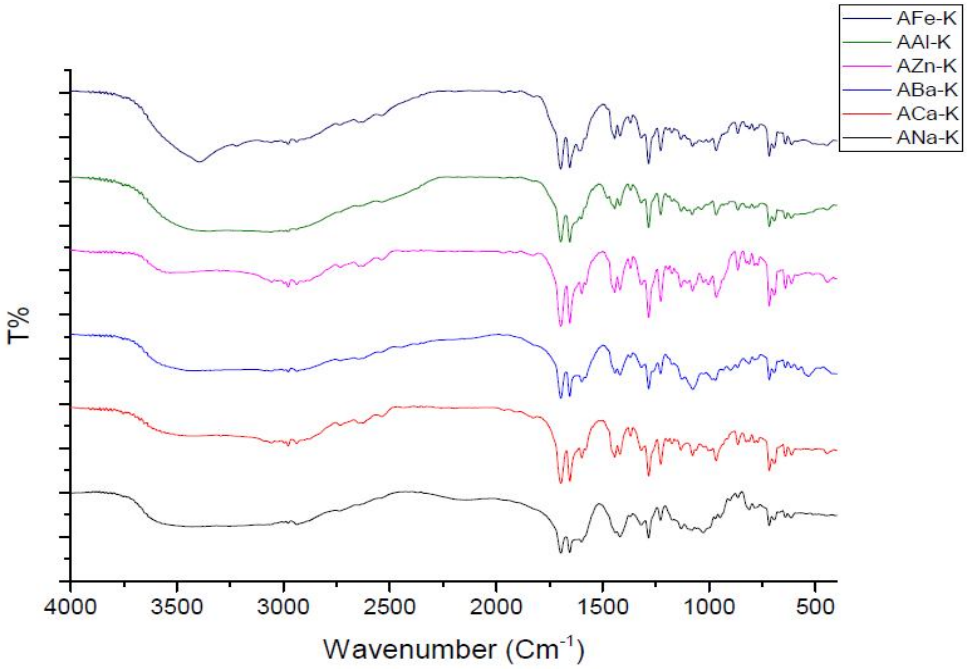


Fig. 8. IR spectra of physical mixture of KTP with blank beads.

Blank alginate metal complexes beads, generally, show a broad and intense absorption band of OH in the region 3000-3750 cm^{-1}. Carboxylic carbonyl C=O of alginate complexes absorbs in the region 1610-1630 cm^{-1} with medium to intense and relatively broad signal (1500-1800 cm^{-1} at the base line). Two types of C=O participate in this signal, the C=O mannuronic and the C=O guluronic. They may be resolved depending on the metal ion of complex, but distinguishing the two types is not a goal of this work. Actually, previous work attempted to elucidate the metal-carboxylate interactions, as well as, the structure of alginate-metal complexes using IR spectroscopy. According to the frequencies of characteristic peaks of carboxylates (around 1600 and 1400 cm^{-1}) for different metal complexes, it was proposed a 'pseudo bridged' unidentate coordination with intermolecular hydrogen bonds for the complexes in the polyguluronic regions while a bidentate bridging coordination was proposed for the polymannuronic regions [42]. The coordination between metal ion (Pb^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}) and the oxygen atoms of carboxyl and hydroxyl groups was also ascertained in alginate- metal complexes [43].

Physical mixtures of KTP with blank beads show different features of IR spectra. Generally, there is a very large medium of very strong band in the region 2500-3750 cm^{-1} corresponding to the KTP hydroxyl (2500-3250 cm^{-1}) beside the OH of alginate complexes (3250-3750 cm^{-1}). The characteristic peaks of carboxylic and keton C=O are present and very clear, while the characteristic C=O of alginate cannot be distinguished because of overlapping with KTP signals.

KTP loaded alginate metal complexes have IR spectra comparable to those of unloaded complexes, with exception of the case of Zn and Al, where characteristic signal of KTP can be shown clearly. In the case of Fe, an important signal observed at 1741 cm^{-1} may correspond to C=O carboxyl of mannuronic or guluronic acid. As a result of spectra comparison, the signal of carboxylic OH of pure KTP disappears and becomes one signal with the hydroxyl OH signal of alginate in the loaded beads, due to hydrogen bonding interaction, suggesting a chiral interaction that can affect KTP release and lead to an enantioselective release during release experiments. However, it is important to note that the overall hydrogen bonding interactions depend on the metal ion nature; *i.e*., size and valency, as the formed egg-box has a metal impact on oxygen atoms participating in hydrogen bonding as discussed above.

In Vitro ESR Study

ESR was monitored using chiral HPLC and expressed as the chromatographic area ratio of R to S enantiomers. Typical chromatograms are shown in Fig. 9 and Fig. 10, where enantiomers are well-resolved. ESR values for divalent and trivalent alginate complexes were calculated for an experiment time of 350 min, and the obtained results are depicted in Fig. 11. Practically, within the first 50 min of the release experiment, divalent complexes showed a noticed ESR compared to trivalent complexes. For divalent metal complexes, ACaK beads showed ESR <1 with starting value ESR = 0.53, meaning that R enantiomer is more retained in the beads than the S-enantiomer because of stronger chiral interactions; *i.e*., hydrogen bonding mainly. After that, ESR became 1 and the release is not stereoselective. Similar trend can be shown in the case of ABaK beads, where ESR < 1 (starting ESR = 0.7) meaning lower selectivity towards S-enantiomer compared to the ACaK case. However, ESR values fluctuate because the released concentrations of ABaK beads were very weak leading to imprecise integration values. Differently, AZnK beads gave ESR > 1 all over the experiment time, indicating selectivity towards R-enantiomer. In fact, this is interesting, because altering the ESR behavior means altering the nature of chiral interactions, which would result only when Zn forms a different egg-box stereochemistry from this of ACaK and ABaK. For trivalent metal complexes, no significant results were obtained for ESR. All over the experiment time, the release was practically racemic for AFeK beads (ESR = 1) and AAlK beads (ESR = 1.01).

For alginate mixed metals (Ca and Fe), Fig. 9 shows varied ESR behavior depending on the preparation method. No significant ESR was observed for simultaneously congealed alginate beads A_1K, where ESR > 1 all over the experiment time. For consecutively congealed alginate beads, ESR had opposite behaviors; ESR < 1 for A_2K beads (Ca then Fe) but ESR > 1 for A_3K beads (Fe then Ca). Thus, A_3K and A_1K had similar ESR behaviors in contradiction to A_2K. These current results suggest different egg-box

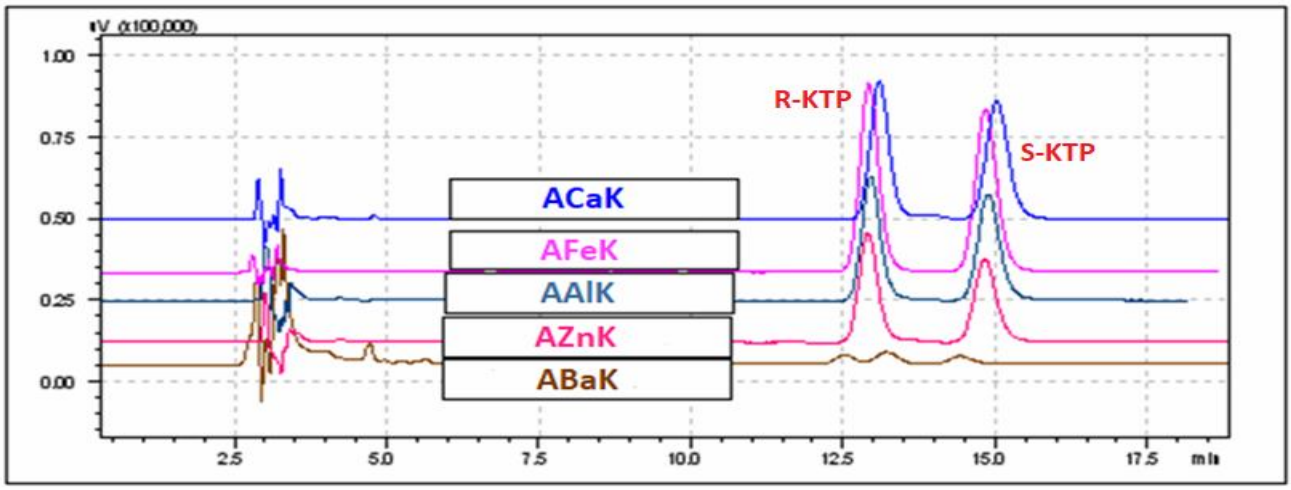


Fig. 9. Typical chiral HPLC chromatograms for alginate beads complexed with single metal. R-KTP and S-KTP enantiomers are well resolved.

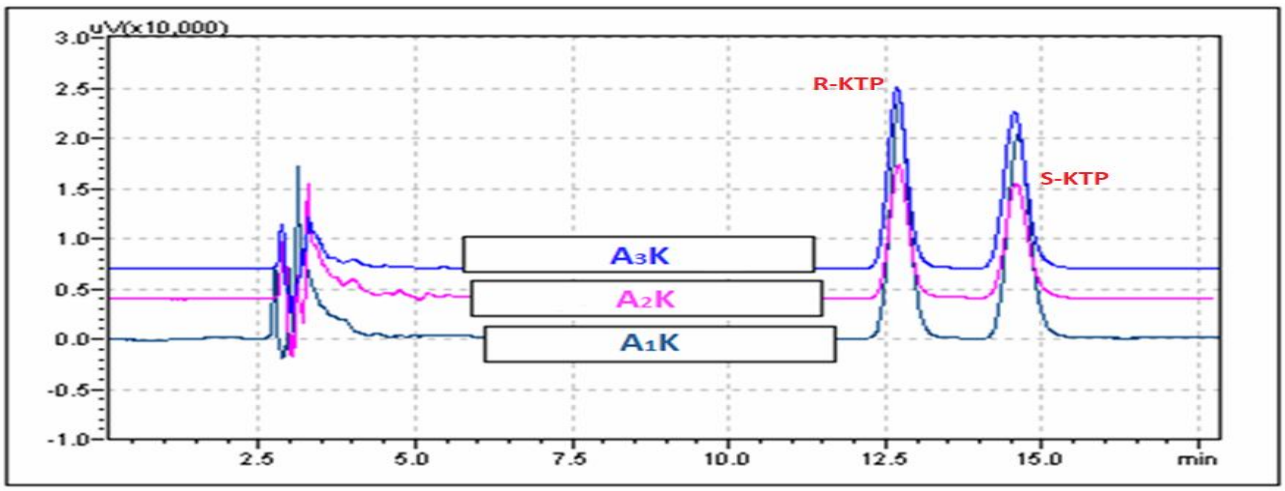


Fig. 10. Typical chiral HPLC chromatograms for alginate beads complexed with two metals. R-KTP and S-KTP drug enantiomers are well resolved.

stereochemistry depending on the order of alginate congealing. In other words, it is possible to alter the stereoselectivity by altering the sequence of congealing with Fe and Ca.

CONCLUSIONS

In this work, different kinds of alginate-metal complexes were prepared in form of beads which were studied for ESR *in vitro*. The obtained results showed a noticeable ESR in case of divalent metal complexes, but not for trivalent metal complexes. ESR values where either >1 or <1 depending on the metal complexes indicating the ability of tuning selectivity towards R or S enantiomer by choosing the metal or by mixing metals. As ketoprofen is known for its short half life time, the ESR results, especially

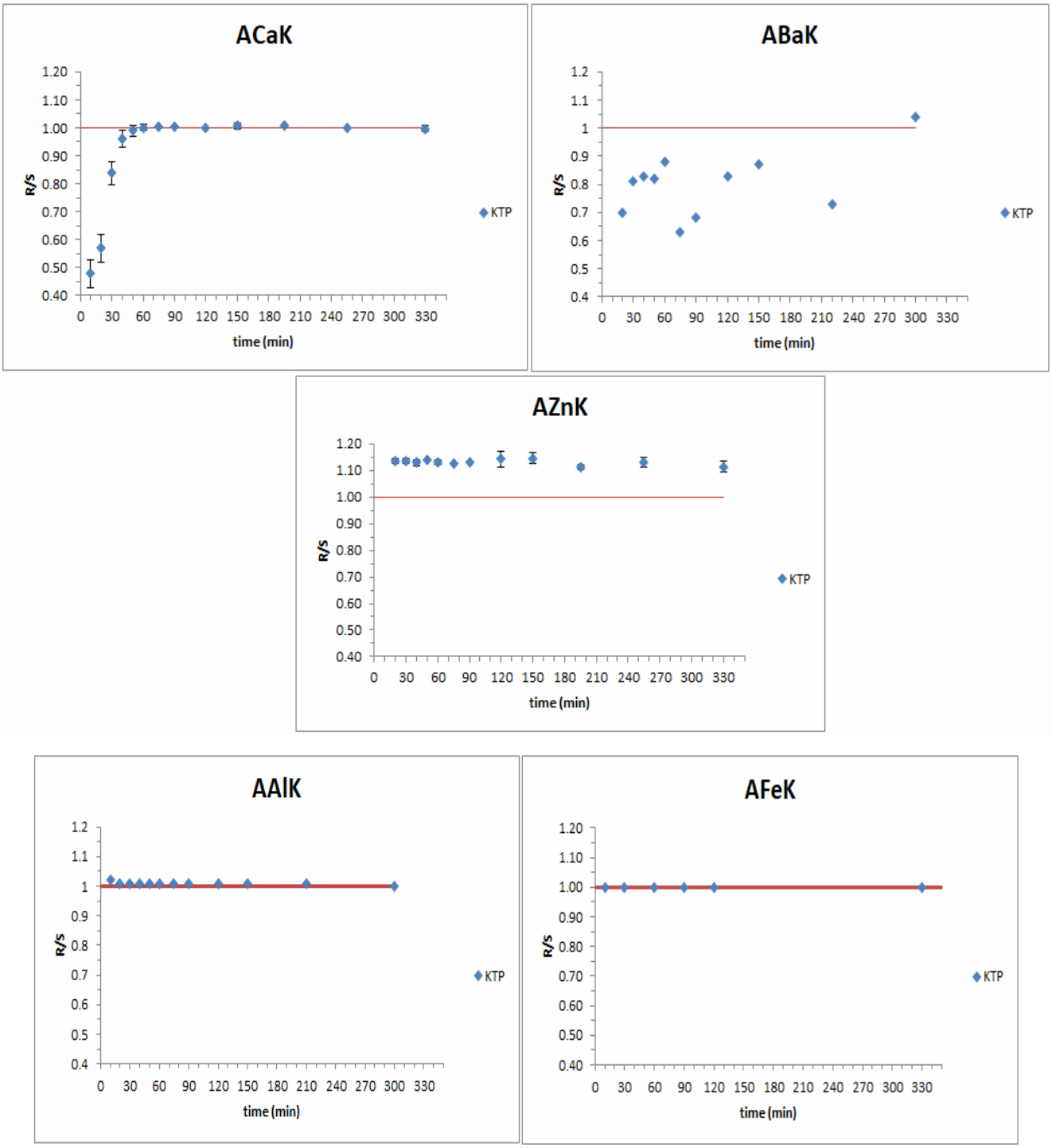


Fig. 11. ESR for divalent and trivalent alginate-metal complexes beads monitored by chiral HPLC as R/S ratio.

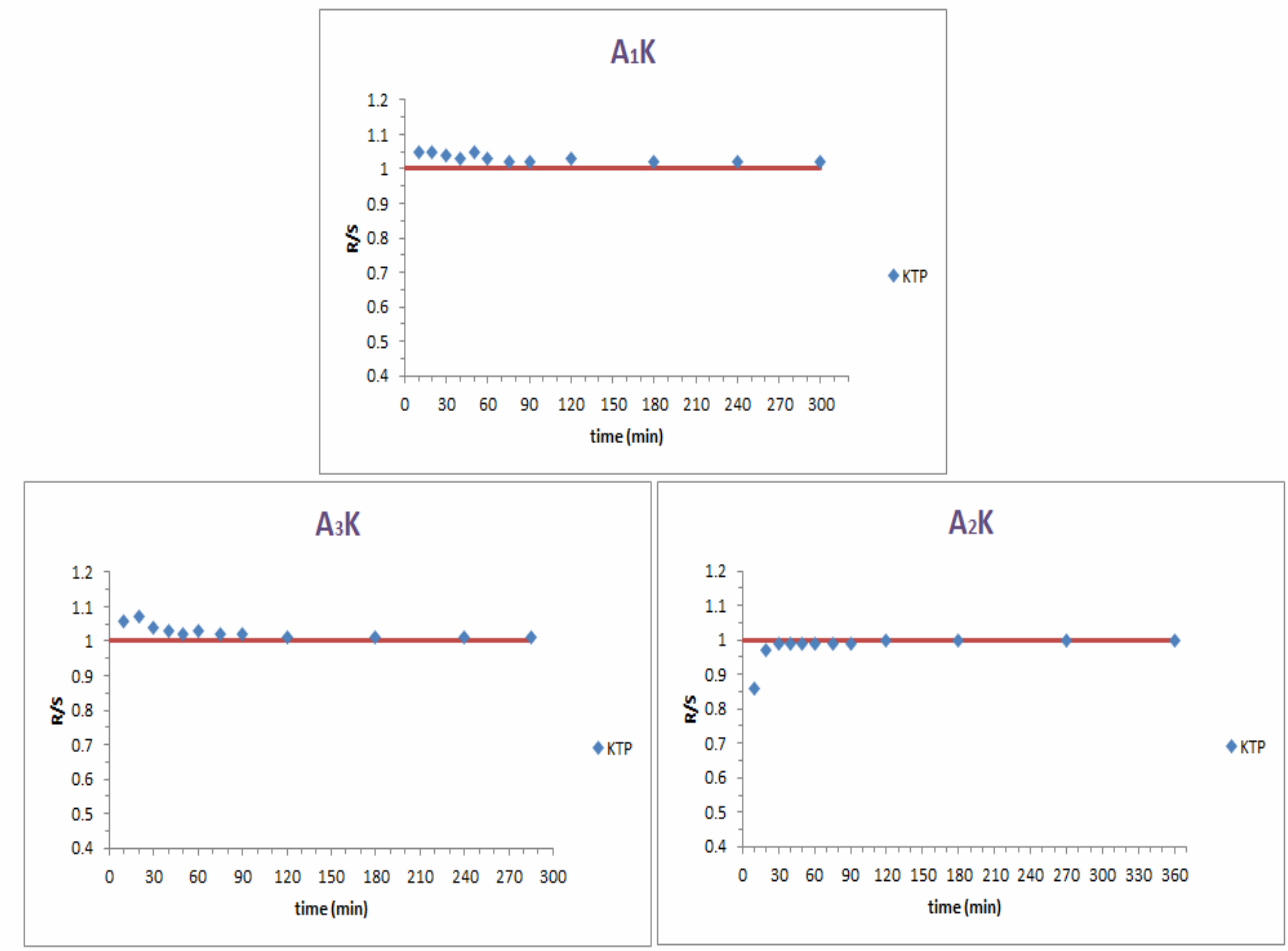


Fig. 12. ESR for alginate complexed with two metals beads monitored by chiral HPLC as R/S ratio.

in case of alginate-calcium complex, draw the attention to the idea that using such chiral excipient should be taken into consideration, as it could affect the drug bioavailability and pharmacokinetic.

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