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Preparation and evaluation of microcrystallized cellulose xanthate-metal-oxytetracycline complexes as antibacterial agents with prolonged antibacterial activity

Hyosik Kong[1], Youna Lee[1], Yonghyun Lee, Young Mi Kim and Yunjin Jung[†]

Laboratory of Biomedicinal/Medicinal Chemistry, College of Pharmacy, Pusan National University, Pusan 609-735, Republic of Korea

[1]*These authors contributed equally to this work*
[†]*To whom correspondence should be addressed*

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ABSTRACT – Microcrystallized cellulose xanthate-metal-oxytetracycline complexes (MCX-metal-OTC) were prepared and evaluated as a controlled release system for the antibiotics. Microcrystallized cellulose (MC) was chemically modified to xanthated microcrystallized cellulose (MCX). One-bath method, where MCX was reacted with OTC-metal complexes, afforded greater amount of OTC bound to the polymeric matrix than did two-bath method, where MCX-metal complexes were treated with OTC. The OTC release from MCX-metal-OTC was greatly sustained compared with that from a mixture of MC/metal/OTC. Furthermore, MCX-metal-OTC manifested antibacterial activity, which lasted for 11-18 days. These results suggest that MCX-metal-OTC is a polymeric antibiotics with prolonged antibacterial activity.

Key words – Controlled release, Coordinate bond, Polymeric drug, Cellulose, Prolonged antibacterial activity and Oxytetracycline

Introduction

There has been a growing interest in pharmacologically active macromolecular compounds during recent years. The development of polymeric drugs is based on their postulated advantages over simple drugs, such as the prolongation of action, the variation in reactivity and toxicity and a possible change in the normal distribution of the drugs.[1-3] A major approach to control the release of small molecular bioactive agents leading to prolonging the duration of their actions has involved their chemical attachment to synthetic or naturally occurring macromolecules[4]. Thus, various agents have been bound via degradable covalent linkages to many different polymeric systems. However, there is no absolute requirement that this highest energy type of bond be the linkage used. Conceptually at least, it should be possible to develop controlled release system based on other types of chemical bonds with lower energy. These could comprise the van der Waals forces (ΔH 1 Kcal/mol), ionic bonds (ΔH 10~15 Kcal/mol) and coordinate or chelate bonds (ΔH 50 Kcal/mol). Unlike covalent bonds, coordinate covalent bonds of chelate type vary in binding stability over orders of magnitude depending on the atomic characteristics of metals and ligands that are involved in the coordinate bond. Therefore, utilizing vast body of background chemical information on the stability constants of chelates, a polymeric controlled release system attaching the bioactive agent to a polymer via a coordinate bond is likely to have various release profiles of a bioactive agent[5]. In previous reports, we showed that chemical modification, xanthation, of cellulosic polymers markedly increases the metal binding capacity of the polymer matrix leading to attachment of a large amount of bioactive agents via chelation between the metal-bound polymer and the bioactive agents. Further, the duration of action of the bioactive agents is prolonged by controlled release of them from the polymer matrix[6,7]. In this study, oxytetracycline (OTC), which is the most widely used antibiotics for the control of bacterial diseases in aquaculture of fish, was attached to chemically modified microcrystallized cellulose (MC) via metal chelation and the polymeric antibiotics was evaluated as a controlled release system for the antibiotics. Our data demonstrate that MCX-metal-OTC complexes maintained the physical integrity of the matrix and xanthation of MC increased the metal binding capacity leading to effective coupling of the active agent to the matrix via metal chelation. The release of OTC from MCX-metal-OTC was remarkably sustained compared with MC/metal/OTC where the metal does not act as a chelating linker between cellulose and OTC. In parallel with

[†]본 논문에 관한 문의는 이 저자에게로
Tel : 051-510-2527, E-mail : jungy@pusan.ac.kr

the release result, the antibacterial activity of MCX-metal-OTC lasted longer than that of free OTC.

Materials and methods

Microcrystallized cellulose (Sigmacell, particle size 50 μm), oxytetracycline hydrochloride and oxytetracycline dihydrate were purchased from Sigma Chemical Co. (U.S.A). Aluminum chloride, zinc chloride and cupric chloride were obtained from Junsei Chemical Co.(Japan) and used as received. The ingredients of the medium used for antibacterial test came from DIFCO Laboratories (U.S.A). The bacteria source was *Staphylococcus aureus* (ATCC 6538). UV spectra were taken on a Shimadzu UV 2101-PC spectrophotometer (Japan).

Preparation of microcrystallized cellulose xanthate

For preparation of microcrystallized cellulose xanthate (MCX), MC (7.5 g) was suspended in a reaction flask containing 2M NaOH (100 mL) followed by addition of carbon 5 ml of disulfide and the mixture was stirred at room temperature. As the reaction proceeded, the reaction mixture turned into orange color. After reaction for 24 h, the pale-yellow product was centrifuged, washed thoroughly with distilled water, acetone in turn, and dried in air. The resultant dry material, MCX was stored in freezing room until used. The products, MCX, maintained the original physical appearance except it was pale-yellow in color. The degree of substitution (DS) of carbon disulfide was estimated by Volhard method. DS for MC was 1.3. DS represents the number of xanthate group per 10 glucose units.

Coupling of OTC to microcrystallized cellulose xanthate by one-bath method

MCX (0.68 g) was placed in 50 mL of metal-OTC complex solutions at various [OTC mM]/[metal mM] concentration ratios 2:1, 1:1 and 1:2 and stirred at 10°C. The concentration of OTC remaining in the solution was measured at the predetermined time intervals. When the amount of OTC bound to MCX reached a maximum (at 1 h), the material was removed from the solution, washed three times with distilled water and 20 mL of acetone, and dried in a stream of cool air. The resulting products, MCX-metal-OTC, were a yellow color for MCX-Al-OTC and MCX-Zn-OTC and a green color for MCX-Cu-OTC and maintained the physical integrity of the matrix.

Coupling of OTC to microcrystallized cellulose xanthate by two-bath method

MCX (0.68 g) was placed in 25 mL of 0.1 M metal solutions and stirred at 10°C. As the reaction proceeded, the color of the MCX turned from pale-yellow to yellow for MCX-Al and MCX-Zn or green for MCX-Cu. After stirring for 1 h, the material was removed, washed three times with 100 mL of distilled water and 20 mL of acetone and dried. The products, MCX-metal, were immersed in 50 mL of OTC solution at 5 mM and shaken on a shaker at 10°C. The concentration of OTC in the solution was measured at the predetermined time intervals. When the amount of OTC binding reached a maximum (at 1 h), the material was removed from the reaction medium, washed three times with distilled water and 20 mL of acetone, and dried by blowing a stream of cool air. The resulting products, MCX-metal-OTC, were a yellow color for MCX-Al-OTC and MCX-Zn-OTC and a green color for MCX-Cu-OTC and maintained the physical integrity of the matrix.

Determination of OTC amount bound to MCX-metal-OTC

The concentration of OTC in the solution was determined by UV spectrophotometry by measuring the absorbance at 364 nm where the presence of metal ions does not interfere with the analysis. A standard calibration curve was constructed from the different concentrations of OTC solution. The amount of OTC bound to the cellulosic polymer was estimated by subtracting the amount of OTC in the bulk solution from that of OTC initially used for the binding experiment.

Release profile of OTC from MCX-metal-OTC

The release experiment was carried out as described previously[7]. Briefly, a 0.5 g amount of MCX-metal-OTC, which was obtained by one-bath method at [OTC]/[metal] concentration ratio 2:1, was placed in 50 mL of distilled water and stirred for 5 min at 20°C. After centrifugation, OTC in the supernatant was analysed. This process was repeated with the residue. The corresponding MC (0.5 g)/metal (0.08 mmol)/OTC (40 mg) mixtures, which were subjected to the washing process once, were used for the same experiment. For the antibacterial test of the supernatants, a 50 mL portion of the supernatant was mixed with the same volume of molten agar medium. The mixture was placed in the holes formed as described under "Antibacterial activity test", which was incubated for 24 h.

Antibacterial activity test

Antibacterial activity was measured indirectly by the agar diffusion method[6]. To a culture plate (diameter: 90 mm, height: 15 mm), was added 30 mL of nutrient agar inoculated with cultures of S. aureus and was placed a ring (o.d.: 10 mm,

height: 10 mm). After solidifying, the ring and the contents were removed to form two cylindrical holes. The sample (equivalent to 2.5 mg OTC) was planted in each hole and added a few drops of molten agar medium upon it. The plates were incubated at 37°C for 24 h and the diameter of inhibition zone was measured. In order to investigate the duration of the antibacterial activity of MCX-metal OTC (containing 2.5 mg of OTC), the contents of the cylindrical holes, after 24 h of incubation, were removed and transferred to holes of a freshly prepared plate as described above, incubated again for 24 h and measured the diameter of the inhibition and this procedure was continuously repeated until the inhibition of growth was no longer noticed. The same procedures were followed using free OTC (2.5 mg) dissolved in agar to compare the duration of antibacterial activity of the free and matrix-bound drug.

Results

One bath method affords a greater binding of OTC to microcrystallized cellulose xanthate.

In previous reports, xanthation of cellulosic polymers (microcrystallized cellulose and cotton cellulose) affords chelating sites to the polymers, thus increasing metal binding capacity and the metals bound to cellulose xanthate-metal chelates act as a linker for binding of antibacterial agents such as neomycin, tetracycline to the polymer matrix[7]. Furthermore, the polymeric antibacterial agents release the antibiotics in a controlled manner and exhibit prolonged antibacterial activity. Since development of controlled release system of OTC, which is most widely used antibiotics for aquaculture of fish[8], is desirable in that it can reduce the frequency of administration and the fluctuation of chemical concentration, thus improving environmental side effects and bioactivity[9], it was investigated whether OTC could be attached to cellulosic polymers using the previous method. Microcrystallized cellulose xanthate was prepared by treatment of microcrystallized cellulose with carbon disulfide. The MCX was added to metal solutions followed by reaction with OTC. The same experiment was done with MC. As shown in figure 1, the order of binding amount of OTC for MCX-metal-OTC complexes was Zn(II)>Al(III)≈Cu(II). OTC binding to MC that was not xanthated was negligible, indicating that the previous method is applicable to preparing polymeric OTC. A previous report demonstrate that one-bath method, where a drug-metal complex is reacted with cellulose xanthate, affords greater binding of the drug to the polymer matrix than does two-bath method, where the metal is reacted with cellulose xanthate followed by treatment with the drug[6]. To examine whether one bath method could increase binding capacity of OTC, MCX was treated with various ratios of metals and OTC measuring binding amount of OTC. The same experiment was done with MC. As shown in figure 2, the maximum binding of OTC for MCX was observed at the [OTC]/[metal] concentration ratio of 2:1 and, consistent with the previous report, the maximal binding amount of OTC by one-bath method was greater than that by two-bath method. The order of binding capacity of OTC was Cu(II)>Al(III)>Zn(II) for MCX-metal-OTC. The binding of OTC to MC was negligible, which is consistent with the result from two-bath method.

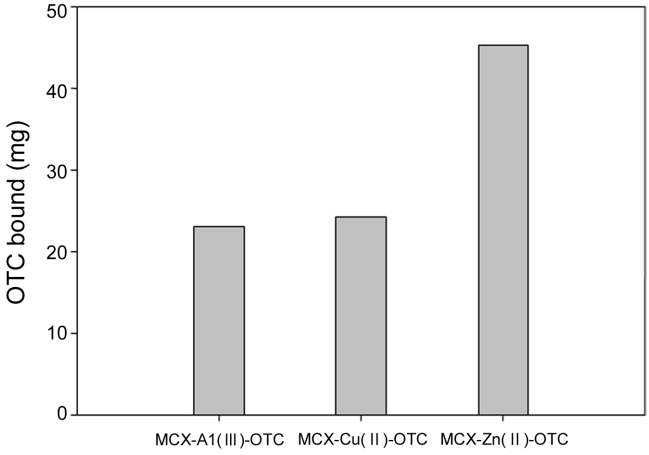


Figure 1−Amount of OTC bound to MCX-metal chelates in two-bath method
MCX (0.68 g) was placed in 25 mL of 0.1 M metal solutions and stirred at 10°C. After stirring for 1 h, the material was removed, washed three times with 100 mL of distilled water and 20 mL of acetone and dried. The products, MCX-metal chelates, were immersed in 50 mL of OTC solution at 5 mM and shaken on a shaker at 10°C for 1 h. The amount of OTC bound to MCX-metal chelates was deduced by subtracting the amount in the final solution from the one initially used for the experiment. Data represent the mean of three separate experiments.

Release of oxytetracycline from MCX-metal-OTC is sustained

We examined whether the polymeric antibiotics released OTC in a controlled manner. MCX-metal-OTC, which were obtained in the maximal binding condition, were placed in 50 mL of distilled water, stirred for 5 min and centrifuged. The concentration of OTC in the supernatants was measured. To the residue, distilled water was added again and the same process was repeated. The same experiment was done with the corresponding MC/metal/OTC mixtures. As shown in the upper panels of figure 3∼figure 5, OTC was completely washed off from MC/metal/OTC mixtures by 2∼3 cycles of the washing process. On the contrary, OTC release from MCX-metal-OTC was sustained up to at least ten-cycles of

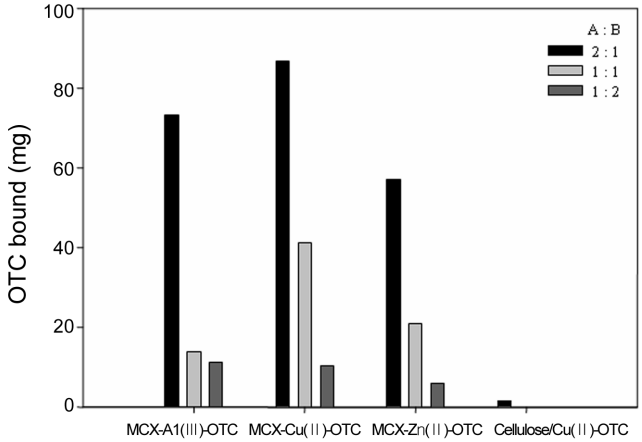
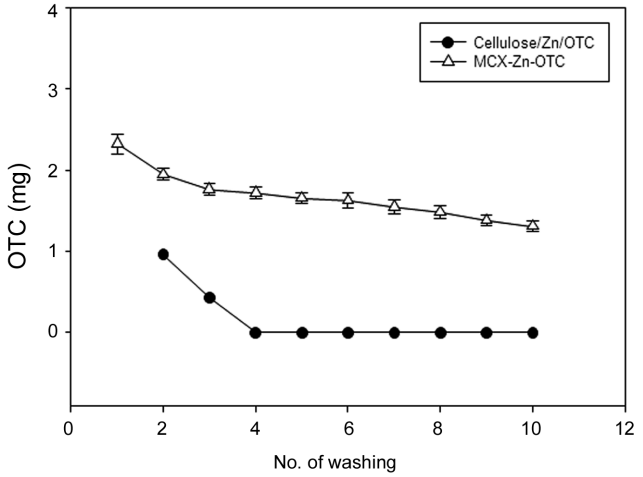
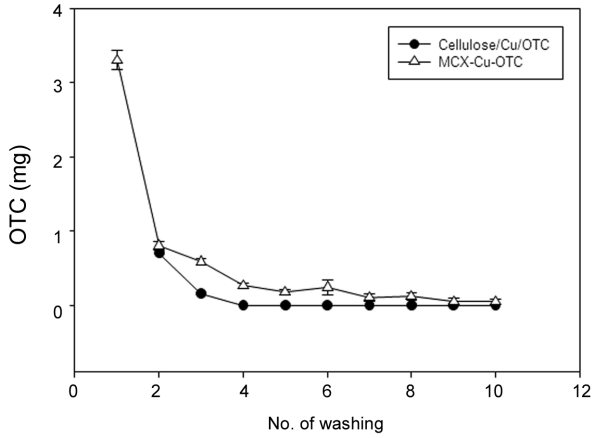


Figure 2−Amount of OTC bound to MCX in one-bath method. MCX (0.68 g) was placed in 50 mL of metal-OTC complex solutions at various [OTC mM (A)]/[metal mM (B)] concentration ratios 2:1, 1:1 and 1:2 and stirred at 10°C for 1 h. The amount of OTC bound to MCX was deduced by subtracting the amount in the final solution from the one initially used for the experiment. Data represent the mean of three separate experiments.



	Diameter of inhibition zone (mm)									
Cellulose/Zn(II)/OTC	28	26								
MCX-Zn(II)-OTC	37	34	34	34	34	30	30	30	30	29

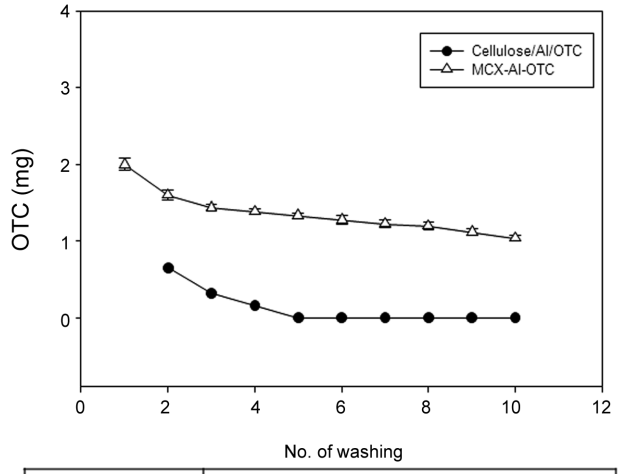
Figure 3−The release profiles of OTC from MCX-Zn(II)-OTC and antibacterial activity of the washing supernatants.
MCX-Zn(II)-OTC (0.5 g) was placed in 50 mL of distilled water and stirred at 20°C for 5 min. After centrifugation, OTC in the supernatant was analysed. This process was repeated with the residue. The same experiment was done with a mixture of MC (0.5 g)/Zn(II) (0.08 mmol)/ OTC (40 mg), which had once been subjected to the washing process. Data represent the mean +/- S.E. (n=3). ∇: MCX-Zn(II)-OTC; ● : Cellulose/Zn(II)/OTC mixture. For the antibacterial test of the supernatants, the supernatant (50 mL) was mixed with molten agar medium (50 mL). The antibacterial activity of the mixture was tested as described under "Antibacterial activity test". Data represent the mean of three separate experiments.



	Diameter of inhibition zone (mm)									
Cellulose/Cu(II)/OTC	27	21								
MCX-Cu(II)-OTC	32	29	29	28	28	28	28	25	24	23

Figure 4−The release profiles of OTC from MCX-Cu(II)-OTC and antibacterial activity of the washing supernatants.
MCX-Cu(II)-OTC (0.5 g) was placed in 50 mL of distilled water and stirred at 20°C for 5 min. After centrifugation, OTC in the supernatant was analysed. This process was repeated with the residue. The same experiment was done with a mixture of MC (0.5 g)/Cu(II) (0.08 mmol)/ OTC (40 mg), which had once been subjected to the washing process. Data represent the mean +/- S.E. (n=3). ∇: MCX-Cu(II)-OTC; ● : Cellulose/Cu(II)/OTC mixture. For the antibacterial test of the supernatants, the supernatant (50 mL) was mixed with molten agar medium (50 mL). The antibacterial activity of the mixture was tested as described under "Antibacterial activity test". Data represent the mean of three separate experiments.

washing. To examine whether OTC released from MCX-metal-OTC exhibited antibacterial activity, the supernatants obtained after centrifugation of suspension of MCX-metal-OTC were subjected to an antibacterial activity test. As shown in the lower panels of figure 3 ~ figure 5, consistent with the release results, while it was not observed from the third supernatant of the mixtures, antibacterial activity was manifested until the end of the release experiment (ten cycles of washing) for MCX-metal-OTC. To rule out the possibility that sustained release of OTC from the polymeric antibacterial agents was elicited by dissolution control of OTC base with low solubility, the release experiment was done with mixture of cellulose/Zn(II)/OTC dihydrate. As shown in figure 6, although OTC dihydrate was released from the mixture until 7 washing cycles, which was prolonged compared with cellulose/Zn(II)/OTC hydrochloride, the release occurred much faster than that of cellulose-Zn(II)-OTC. The same experiment was done using cellulose/Cu(II)/OTC dihydrate and cellulose/Al(III)/OTC dihydrate. Similar results were obtained (data not shown). These results suggest that the release property of the polymeric antibacterial agents depends



	Diameter of inhibition zone (mm)									
Cellulose/Al(Ⅲ)/OTC	26	22	20							
MCX-Al(Ⅲ)-OTC	34	32	32	30	30	30	29	29	29	28

Figure 5–The release profiles of OTC from MCX-Al(III)-OTC and antibacterial activity of the washing supernatants
MCX-Al(III)-OTC (0.5 g) was placed in 50 mL of distilled water and stirred at 20°C for 5 min. After centrifugation, OTC in the supernatant was analysed. This process was repeated with the residue. The same experiment was done with a mixture of MC (0.5 g)/ Al(III) (0.08 mmol)/OTC (40 mg), which had once been subjected to the washing process. Data represent the mean +/- S.E. (n=3). ▽: MCX-Al(III)-Tc; ● : Cellulose/Al(III)/OTC mixture. For the antibacterial test of the supernatants, the supernatant (50 mL) was mixed with molten agar medium (50 mL). The antibacterial activity of the mixture was tested as described under "Antibacterial activity test". Data represent the mean of three separate experiments.

on the bond between metal and OTC rather than dissolution of OTC.

MCX-metal-OTC present prolonged antibacterial activity.

Although the above results suggested that MCX-metal-OTC would show prolonged antibacterial activity by liberating OTC in a controlled manner, it remained unclear whether it would indeed exhibit such property. To examine this, the durability of antibacterial activity of MCX-metal-OTC was evaluated by the agar diffusion method where an inhibition zone produced by the diffusion of the antibacterial agent released from the matrix into the agar was measured with transferring the samples to a freshly prepared plate once a day. The results are summarized in Table I. Whereas the inhibition zones exhibited by free OTC decreased rapidly on each successive test period and disappeared in 4 days, those by MCX-metal-OTC decreased gradually, which lasted for 11~18 days. In a control experiment, MCX and MCX-metal chelates did not show an antibacterial activity (data not shown).

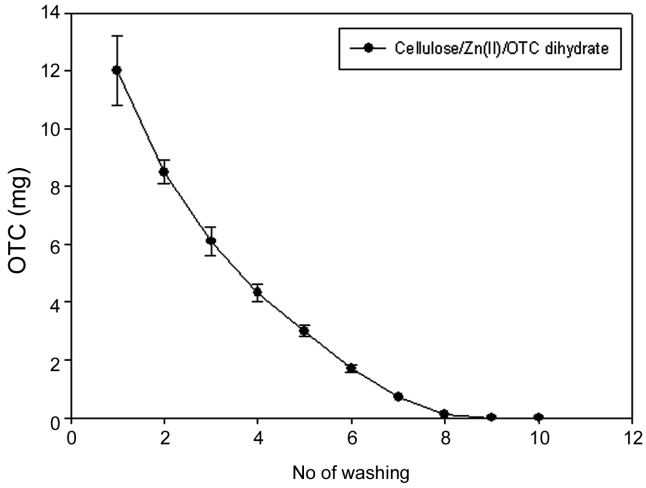


Figure 6–The release profile of OTC from the mixture of MC/ Zn(II)/OTC dihydrate
MC/Zn(II)/OTC dihydrate (0.5 g/0.08 mmol/40 mg) was placed in 50 mL of distilled water and stirred at 20°C for 5 min. After centrifugation, OTC in the supernatant was analysed. This process was repeated with the residue. Data represent the mean +/- S.E. (n=3). ● : Cellulose/Zn(II)/OTC mixture.

Table 1–*Antibacterial Activity of MCX-metal-OTC*

Strains	Sample[a]	Inhibition zone[b] : diameter[c] (mm)
S. aureus	OTC	57 56 39 31
	A	52 48 45 40 37 38 38 39 38 35 36 34 32 27 28 25 22 22
	B	51 54 40 41 37 39 37 38 29 25 23
	C	53 50 46 45 40 38 40 42 40 39 37 37 36 30 24 21

[a]: 1.25 mg of free oxytetracycline and MCX-metal-OTC containing 1.25 mg OTC were used
[b]: The inhibition zone was measured once a day
[c]: Data represent the mean of three separate experiments.
OTC: Free oxytetracycline, A: MCX-Zn(II)-OTC, B: MCX-Cu(II)-OTC and C: MCX-Al(III)-OTC

Discussion

In this study, it was investigated that OTC bound to microcrystallized cellulose xanthate (MCX) via metals was released in a controlled manner. Our data demonstrated that one bath method, where metal-OTC complexes were treated with MCX, afforded greater binding of OTC than did two-bath method, where metals were reacted with MCX followed by treatment with OTC. The release of OTC from MCX-metal-OTC was sustained, the extent of which was dependent on metals. In parallel with the release results, the antibacterial activity of MCX-metal OTC in the agar diffusion method was prolonged, which lasted for 11~18 days.

Xanthation of microcrystallized cellulose and subsequent reactions for the binding of OTC took place readily with little change in physical appearance except for the color. Our data demonstrate that the amount of OTC bound to the matrix differed greatly depending on the coupling method. One bath method afforded a greater amount of OTC bound to the matrix that did two bath method. The extent of formation and type of complex in the presence of different kinds of ligands will be dependent on the avidity of the metal ion for the ligands. If the affinity of the metal ion for the ligands is substantially different, a complex with ligands with greater affinity should be formed. If the affinity of the metal ion for the ligands is about the same magnitude, formation of a mixed complex will be statistically favored by a factor of 2 over a simple complex. The ligand groups capable of forming chelate bond with metal ion are hydroxyl, amine, ketone and carboxamide group in OTC and xanthate or hydroxyl groups in MCX. In the one-bath method, metal ions were first treated with the solution of OTC. In the solution of metals and OTC, a portion of metal ions may be complexed with the ligands in OTC, water molecules or anionic species such as chloride. Upon addition of MCX to metal-OTC solution, formation of insoluble MCX-metal-OTC complex would take place by the exchange of such ligands groups with the ligands in MCX, such as a xanthate or hydroxyl group. In a two-bath method, metal ions are first treated with MCX, and a portion of metal ions may be complexed with the ligands in MCX, water molecules or anionic species such as chloride. Upon addition of MCX-metal to OTC solution, formation of MCX-metal-OTC would take place by the exchange of such ligand groups with the ligands in OTC. As mentioned above, formation of MCX-metal-OTC would be statistically favored if the affinity of the metal ions for the ligands in MCX and OTC is about the same magnitude. If the affinity of the metal ions for the ligands in MCX is substantially greater than those in OTC, formation of MCX-metal-MCX would be favored instead of MCX-metal-OTC. This tendency will prevail predominantly in the two-bath method where MCX is treated with excess metal ions in the first step. As noticed in the present study, bindings of OTC to the matrix in the two-bath method was low compared with the result from one-bath method, which suggested the preferred formation of MCX-metal-MCX instead of MCX-metal-OTC. However, the formation of MCX-metal-MCX would not take place in one bath method as much as in two bath method since OTC-metal complexes bind to the xanthate group on the matrix and the metals complexed with OTC should be less available for formation of MCX-metal-MCX. Thus, our results demonstrating that binding amount of OTC was greater in one-bath method is likely to be due to this effect of one-bath method. In line with this explanation, it was observed that, in one bath method, the OTC binding amount got greater as the ratio of [OTC]/[metal ions] increased in which the one bath effect may get strengthened. Even if the affinity of metal ions for the ligands in OTC is greater than those in MCX, formation of OTC-metal-OTC would be favored, and yet formation of MCX-metal-OTC would take place since the insolubility of MCX-metal-OTC acts as a driving force, which displaces the equilibrium in favor of its formation in the present experiment. In general, a one-bath method seems more favorable than a two-bath method for the binding of drug molecules to the insoluble matrix such as the cellulosic polymers as far as the metal ion and the drug molecule form soluble complex solution. If the metal ion and the drug molecule do not form soluble complex, a two-bath method may be applicable as far as the affinity of the metal ion for the ligand in the drug molecule is greater than that in the matrix.

Release of OTC from MCX-metal-OTC prepared by the one-bath method was investigated by washing samples repeatedly. Our data show that the OTC release from MCX-metal-OTC was observed up to ten washings while that from MC/metal/OTC mixtures was not detected after three cycles of washing. This observation strongly suggests that MCX-metal-OTC could release OTC in a controlled manner. Although the OTC release from MCX-Cu(II)-OTC lasted up to ten washings, the OTC release pattern of MCX-Cu(II)-OTC was shown less sustained than those of the other MCX-metal-OTC whose release levels of OTC were maintained greater. In line with this observation, when the washing supernatants were subjected to an antibacterial activity test, the diameters of inhibition zone for MCX-Zn(II)-OTC and MCX-Al(III)-OTC were relatively greater than that for MCX-Cu(II)-OTC. We do not have an exact explanation on the reason why MCX-Cu(II)-OTC to which the most OTC of the three MCX-metal-OTC was attached exhibited poor controlled release property compared with the other complexes. Since the release of OTC from MCX-Cu(II)-OTC requires exchange of OTC or Cu(II) with a ligand in the washing solvent, it is likely that the binding between Cu(II) and OTC or Cu(II) and xanthate is not easily displaced with the ligand, which may lead to very limited release of OTC. However, this explanation does not address the rapid initial release of OTC from MCX-Cu(II)-OTC, more elaborate experiments are required for interpretation of the phenomenon.

Our data showing that OTC release during the washing process formed inhibition zones indicate that OTC released from MCX-metal-OTC is available to inhibit bacterial growth.

Furthermore, the results of the antibacterial activity test in Table I visualizes that the sustained release of OTC confers the prolonged antibacterial activity on MCX-metal-OTC. In the experiment, we observed that MCX-metal-OTC as well as free OTC showed antibacterial activity represented by inhibition zone and MCX-metal-OTC presented prolonged antibacterial activity compared with free OTC and, moreover, the antibacterial activity of MCX-Zn(II)-OTC and MCX-Al(III)-OTC was a little more durable than that of MCX-Cu(II)-OTC, which is in parallel with the result of the release experiment. If the binding between the drug and the polymer matrix is so weak that most of the active agent is released in the first experimental period, prolonged activity may not be observable. On the other hand, if the binding is too strong to release sufficient amount of the active agent on each experimental day, prolonged activity may not be observable either. If the drug is released in a controlled manner on each successive experimental period from the matrix, prolonged activity may be observable as long as the concentration exceeds the minimum inhibitory concentration. Therefore, our data suggest that OTC tenaciously bound to MCX via the metals released the active agent in a controlled manner to exhibit prolonged antibacterial activity.

Considering that the use of OTC for aquaculture of fish brings environmental side effects including ecotoxicity and development of resistant bacterial populations[10], the polymeric antibacterial material controlling release of the antibiotics, MCX-metal-OTC, may be applicable to aquaculture of fish with improving the environmental problems by reducing frequency of administration and fluctuation of the chemical concentration.

Abbreviations

MC : Microcrystallized cellulose, MCX : Microcrystallized cellulose xanthate, MCX-metal-OTC : Microcrystallized cellulose xanthate-metal oxytetracycline complexes, OTC : Oxytetracycline

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