# Intercalation of Anticancer Agent Cytarabine and Its Related Compounds into γ-Titanium Phosphate

**Mayumi Danjo, Yukari Mizuguchi, Yukari Yagita, Kazuyo Kakiguchi, Tomomi Yanagida, and Mitsutomo Tsuhako***

Department of Chemistry, Kobe Pharmaceutical University, Motoyama, Kitamachi, Higashinada-ku, Kobe 658

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The intercalation of nucleic acid-related compounds which have the pyrimidine structure into the layered titanium phosphate, $Ti(H_2PO_4)(PO_4)\cdot 2H_2O$ (γ-TiP), was examined. The intercalation of cytosine derivatives such as anticancer agent cytarabine was successful. In addition, cytarabine release tests from the obtained cytarabine intercalation compound of γ-TiP were performed in various kinds of solutions containing sodium ions. The release of cytarabine became slower by intercalation into the layered phosphate. Cytarabine release was strongly influenced by the pH value and the concentration of sodium ions in the test solution. In the reaction in aqueous solutions with higher concentrations of sodium ion and/or higher pH values, the ion-exchange of $Na^+$ and protonated cytarabine in the intercalation compound and the disintegration of the layered structure of γ-TiP occurred. On the other hand, in the solutions with the lower sodium ion concentration and the lower pH value, the layered structure of γ-TiP was disintegrated and cytarabine in the interlayer region rearranged.

The interaction of inorganic layered crystals, such as layered phosphates of tetravalent metal and mica-type layered silicates, with various organic compounds by intercalation and ion-exchange reaction has been widely investigated.[1,2] Recently, more information on the physicochemical characteristics of the intercalation compounds newly formed has been desired, in addition to the mechanism of the reaction and the arrangement of organic compound in the interlayer region. Particularly, the intercalation compounds of significant guests such as drugs and bactericides[3] have attracted extensive attention. Their intercalation into inorganic layered compounds may lead to new functional materials that are characterized by controlling the release of guest compound, maximum duration of effectiveness, and good thermal stability. For example, Watanabe et al.[4] have reported the interaction between synthetic inorganic mica and phosphatidylcholine in a solid dispersion with indometacin, with the aim of application as a novel drug delivery system. However, most previous papers have been limited to only proving the intercalation of the guest compound.

We have investigated the intercalation of a wider range of heterocyclic compounds into layered phosphates to obtain more detailed information on the intercalation of drugs and bactericides with heterocyclic structure.[5] Through the investigation, it was found that some nucleic acid related compounds were readily intercalated into $Ti(H_2PO_4)(PO_4)\cdot 2H_2O$ (γ-TiP). Such a simple intercalation reaction without cumbersome compound such as pillar will simplify attempts to discuss the applications as drug delivery system. Consequently, 4-amino-1-β-D-arabinofuranosyl-2(1*H*)-pyrimidinone (cytarabine or arabinosylcytosine), which is one of nucleic acid related compounds, was selected as the main guest compound in the present study. Since cytarabine acts as a competitive inhibitor of DNA polymerase and an antineoplastic agent for acute lymphocytic leukemia and acute myelocytic leukemia, its intercalation into inorganic layered phosphate is very significant, if cytarabine intercalation compound can be applied to drug delivery system. In the present study, the intercalations of cytarabine and other nucleic acid-related compounds into γ-TiP were examined and their physicochemical properties were characterized. In addition, cytarabine release tests from the cytarabine intercalation compound were performed in the various kinds of solutions containing sodium ions as the preliminary investigation for the application as a drug delivery system. Sodium, which accounts for about 90% of total cations in blood serum, is one of the most inorganic elements and performs important biochemical actions. Consequently, the effect of sodium ion in the solution on drug release behaviors is very interesting.

It is well known that the layered phosphates can ion-exchange with various metal ions. The metal ion-exchange properties of layered phosphates have been described in detail by Clearfield et al.,[6] and the kinetic studies on the ion-exchange of alkali-metal ions by α-zirconium phosphate, $Zr(HPO_4)\cdot H_2O$, have been reported by Mikami et al.[7] In the present study, if the release of cytarabine from the intercalation compound occurs, it may be ascribable to the ion-exchange of $Na^+$ and the protonated cytarabine in the cytarabine intercalation compound of layered phosphate. Since we expect that the release of cytarabine will be influenced by the pH value of the solution, NaOH and NaCl solutions were adopted in the cytarabine release test.

## Experimental

**Chemicals.** $\gamma$-TiP was prepared according to the procedure described in a previous paper.[8] Cytosine, thymine, uracil, cytidine, and cytarabine were of reagent grade from Yamasa and Kohjin Co., Ltd., respectively. All other chemicals were purchased from Wako Chemical Industries Ltd. The structural formulas of the guest compounds used in the present study are shown in Fig. 1.

**Intercalation Procedures.** $\gamma$-TiP (1.0 g) was suspended in 0.1 $dm^3$ of 0.1 mol $dm^{-3}$ aqueous solution of nucleic acid-related compounds in an Erlenmeyer flask and the suspension was stirred at room temperature or 70 °C for 1—72 h. The resultant products were filtered, washed with distilled water, and dried in air.

**Cytarabine Release Test.** The amount of cytarabine released from 0.1 g of the cytarabine intercalation compound, obtained by the reaction of $\gamma$-TiP (1.0 g) with 0.1 $dm^3$ of 0.1 mol $dm^{-3}$ aqueous cytarabine solution at room temperature for 3 d, into 0.1 $dm^3$ of various solutions ($H_2O$, $1.0\times10^{-1}$ and $1.0\times10^{-3}$ mol $dm^{-3}$ NaOH and NaCl) after 0.25, 0.5, 1, 2, 5, and 9 h, was determined by HPLC.

**Analytical Procedures.** The X-ray diffraction patterns were measured with Rigaku Denki Rint 2000 using Ni-filtered Cu K$\alpha$ radiation to monitor all new phases and measure these interlayer distances. A Rigaku Denki Differential Thermogravimetric Analyzer, TG 8110, was used with a platinum–rhodium pan in an air flow for the DTA and TG measurements. Elemental analyses of C and N were done with a Sumigraph NC-90-A. HPLC analysis was carried out with a JASCO 802-SC system (Tokyo, Japan). The column used was a Fluofix (Neos Co., Ltd., Japan) and the column temperature was maintained at 40 °C. An aliquot (0.01 ml) of the reaction mixture was injected. Flow rate was 1.0 ml $min^{-1}$. The eluent was 0.02 mol $dm^{-3}$ potassium dihydrogenphosphate with 2 mmol tetrabutylammonium bromide added. The absorbance of effluent was continuously monitored at 254 nm.

## Results and Discussion

**Intercalation of Nucleic Acid-Related Compounds.** We have reported that pyrimidine was not intercalated into $\gamma$-TiP, and described that the intercalations of heterocyclic compounds into layered phosphates were influenced by their $pK_a$ values and structures (property and bonding position of functional group).[5,9] Some substitutions of functional groups for pyrimidine enabled to be intercalated. Figure 1 shows the structure of nucleic acid-related compounds, which have the pyrimidine structure, used as guest compounds. Their intercalations into $\gamma$-TiP were proved by the X-ray diffraction patterns. The X-ray diffraction patterns of their intercalation compounds, obtained at 70 °C for 5 h (Fig. 2b, c) and at room temperature for 3 d (Fig. 2d, e), are shown in Fig. 2. Figure 2b—e illustrates that new diffraction peaks corresponding to the intercalation compounds of nucleic acid-related compounds appeared at a lower angle than that ($d = 11.6$ Å) of host $\gamma$-TiP (Fig. 2a). Such a low angle shift in the diffraction peak clearly demonstrates that these guest compounds are intercalated into $\gamma$-TiP with the expansion of the interlayer distances specified in Fig. 2.

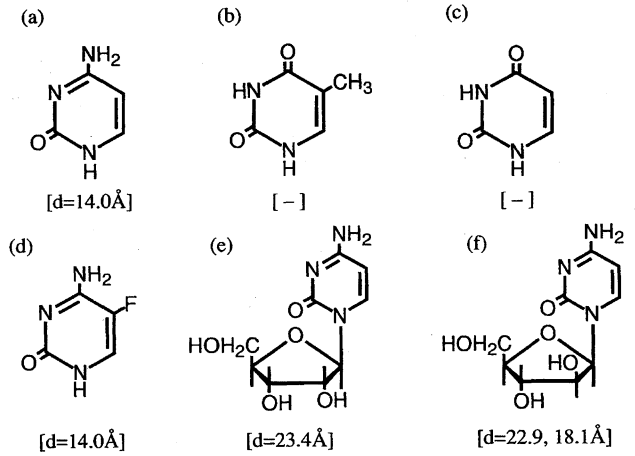


Fig. 1. Structural formulas of the nucleic acid related compounds used as guest. (a), cytosine; (b), thymine; (c), uracil; (d), 5-fluorocytosine; (e), cytidine; (f), cytarabine.

The interlayer distances of the intercalation compounds and the contents of nucleic acid-related compounds into $\gamma$-TiP were listed in Table 1. As can be seen, cytosine and its derivatives (Fig. 1a, d—f) were intercalated into $\gamma$-TiP although thymine and uracil (Fig. 1b, c) were difficult to intercalate. Such results suggest that the substitution of the amino group for pyrimidine is effective for the intercalation in the present system. We have reported that the nitrogen atom in the amino group more preferentially interacts with the hydrogen phosphate group (P–OH) of the layered phosphate than that in pyridine ring, in the case of 2-aminopyridine.[9] Consequently, it is reasonable that the amino group of cytosine derivative is the binding site with the hydrogen phosphate group of the layered phosphate, considering the structure of the guest compounds and the results of previous studies.

Cytosine and 5-fluorocytosine molecules must adopt the same arrangements in the interlayer region of $\gamma$-TiP if one takes account of the interlayer distances being the same. The distance between an amino group and the atom which is most apart from the amino group in cytosine and 5-fluorocytosine is about 5.6 Å, assuming that the bond angle of HNH and the bond length of N–H in their molecules are almost equal to those in 2-aminopyridine.[9] The thickness of the layers of $\gamma$-TiP is 9.2 Å, assuming it is equal to the interlayer distance of the anhydrous $\gamma$-TiP.[10] The increase of the layered distance ($14.0 - 9.2 = 4.8$ Å) is near to the molecular size (5.6 Å) of cytosine and 5-fluorocytosine. Consequently, cytosine and 5-fluorocytosine must form a monolayer in the interlayer

Table 1. Interlayer Distances ($d$) and Contents of Nucleic Acid Related Compounds into $\gamma$-TiP

| Nucleic acid related compound | $d$ / Å | Content / mmol $g^{-1}$ of $\gamma$-TiP |
|---|---|---|
| Cytosine | 14.0 | 1.73 |
| Thymine | 11.6[a] | 0 |
| Uracil | 11.6[a] | 0 |
| 5-Fluorocytosine | 14.0 | 0.91 |
| Cytidine | 23.4 | 1.39 |
| Cytarabine | 22.9 18.1 | 1.62 |

a) The same interlayer distance with $\gamma$-TiP.

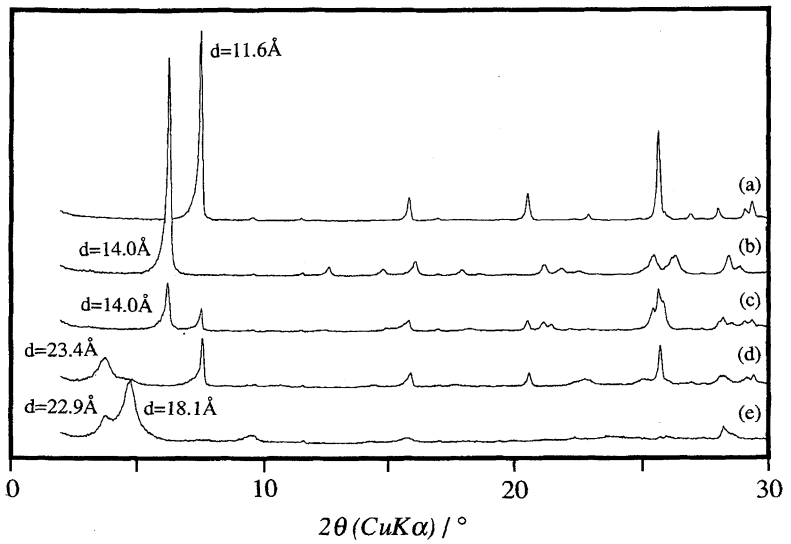


Fig. 2. X-ray diffraction patterns of the intercalation compounds of γ-TiP. (a), γ-TiP; (b), cytosine; (c), 5-fluorocytosine; (d), cytidine; (e), cytarabine.

region of γ-TiP, in which the amino group at the pyrimidine ring interacts with the phosphate group of γ-TiP.

On the other hand, cytarabine formed the phases with the interlayer distances ($d$=22.9 and 18.1 Å) and cytidine formed a phase with $d$=23.4 Å. Cytarabine in the phase ($d$=22.9 Å) must adopt the same arrangement in the interlayer region with cytidine, by taking account of the near interlayer distances. The phase with $d$=18.1 Å was formed in the only cytarabine intercalation compound. The distance between an amino group and the atom which is most apart from the amino group in cytarabine and cytidine is less than 11.2 Å, which is a molecular size (ca. 5.6 Å) of cytosine plus that (ca. 5.6 Å) of arabinofuranose or ribofuranose.[11] The increases of the layered distance are 22.9−9.2=13.7 Å, 23.4−9.2=14.2 Å, and 18.1−9.2=8.9 Å. Cytarabine in the phase with $d$=18.1 Å probably forms a monolayer in the interlayer region, but the arrangement of cytarabine and cytidine in the phase with $d$=ca. 23 Å is difficult to discuss from only the interlayer distance.

We had to controll temperature in the intercalation reaction to get the intercalation compounds of cytosine derivatives. Although cytosine and 5-fluorocytosine were difficult to intercalate into γ-TiP at room temperature, they were readily intercalated at 70 °C and this led to the intercalation compounds having the interlayer distances shown in Table 1. Cytidine and cytarabine, however, were directly intercalated into γ-TiP even at room temperature. Figure 3 shows the time course of the concentration of residual free cytidine or cytarabine on suspending γ-TiP (1.0 g) in 0.1 $dm^3$ of 0.1 mol $dm^{-3}$ aqueous cytidine or cytarabine solution at room temperature and 70 °C. Amounts of cytidine and cytarabine in aqueous solution decreased up to ca. 5 h and were approximately constant after that at room temperature. Final adsorptions of cytidine (after 48 h) and cytarabine (72 h) by γ-TiP were calculated at 2.0 mmol and 2.3 mmol per g of γ-TiP, respectively, by the analysis of HPLC. On the other hand, at 70 °C, although more cytidine was once intercalated

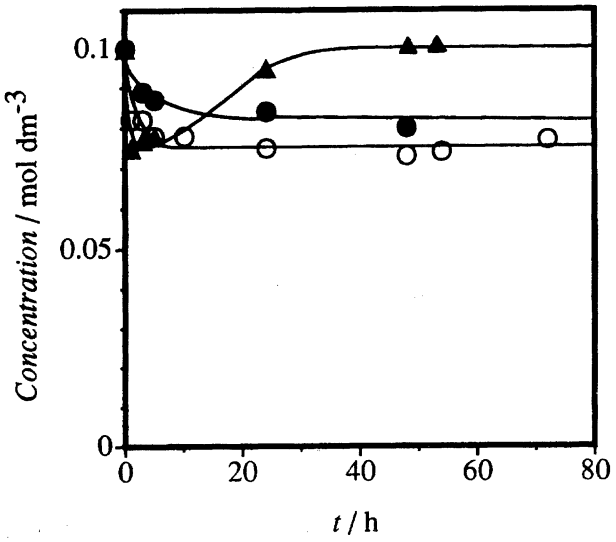


Fig. 3. Time course of concentration of residual free cytidine or cytarabine. ●, cytidine at room temperature; ▲, cytidine at 70 °C; ○, cytarabine at room temperature.

into γ-TiP, it oozed out of γ-TiP into aqueous solution again. This is ascribable to disintegration of the layered structure of γ-TiP, because X-ray diffraction patterns of the product after 24 h indicated an amorphous phase.

**Thermal Stability of Intercalation Compound.** Since organic compounds having thermolability are expected to become more stable by being held in the interlayer region of layered phosphate, the thermostabilities of nucleic acid related compounds intercalated into γ-TiP were investigated. Figure 4 shows DTA and TG curves for the intact γ-TiP, the cytarabine intercalation compound of γ-TiP, and intact cytarabine. In the DTA curve of the intact γ-TiP (Fig. 4a), two endothermic peaks with 17.1% weight loss due to the dehydration of water of crystallization appeared at about 100 °C, and an endothermic peak with 8.4% weight loss attributable to the condensation of hydrogen phosphate group

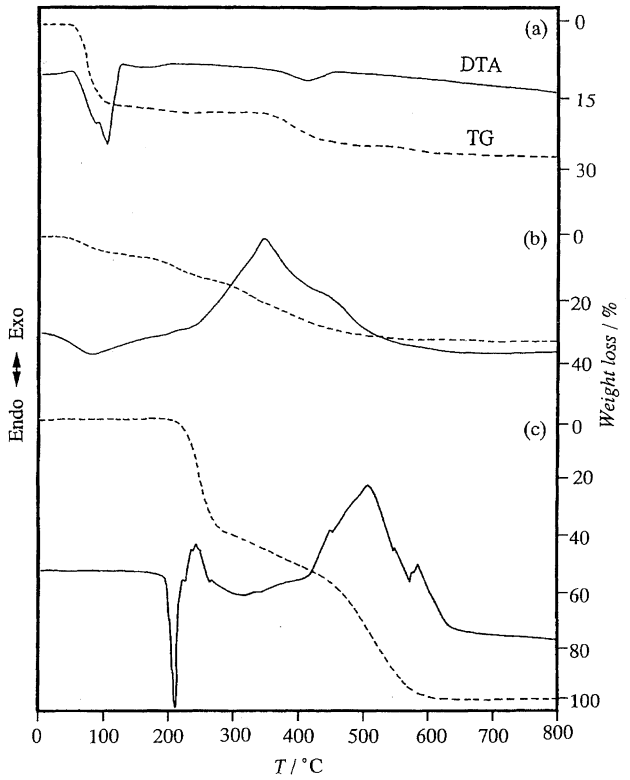


Fig. 4. DTA and TG curves of cytarabine intercalation compound of $\gamma$-TiP. (a), $\gamma$-TiP; (b), cytarabine intercalation compound; (c), cytarabine.

appeared at 390 °C.[12]

On the contrary, in the cytarabine intercalation compound (Fig. 4b), two large exothermic peaks appeared at about 350 and 450 °C with about 35.0% weight loss. They must be ascribable to the thermal degradation of cytarabine intercalated into $\gamma$-TiP. In intact cytarabine (Fig. 4c), endothermic peaks due to decomposition appeared at about 210 °C. Thus, it was proved that the stability of cytarabine up to the high temperature of 350 °C increased by intercalating into layered phosphate.

**Cytarabine Release from Intercalation Compounds.** Cytarabine release behaviors from the cytarabine intercalation compound (referred as $\gamma$-TiP-cytarabine) were investigated in various kinds of solutions. The cytarabine content in $\gamma$-TiP-cytarabine was measured by elemental analyses of C and N, and was 1.16 mmol per g of $\gamma$-TiP-cytarabine. The drug content found by elemental analysis was lower than that by the analysis of HPLC, because cytarabine physically adsorbed on the surface of $\gamma$-TiP was removed by washing $\gamma$-TiP-cytarabine with distilled water. Figure 5 shows the cytarabine release profiles from $\gamma$-TiP-cytarabine into various kinds of solutions ($H_2O$, $1.0\times10^{-1}$ and $1.0\times10^{-3}$ mol dm$^{-3}$ NaOH and NaCl). The dissolution rate of intact cytarabine is faster for every solution in the present experiment. For example, 0.116 mmol of cytarabine dissolved in 0.1 dm$^3$ of 0.1 mol dm$^{-3}$ NaCl in an instant (closed lozenge in Fig. 5). However, cytarabine release from $\gamma$-TiP-cytarabine was much slower than that of intact sample, because cytarabine were held into the interlayer region of layered material. Further, their release rates depended on the composition of the solutions. Cytarabine release rates were affected by the pH value and the sodium ion concentration in the solutions. The cytarabine release rate (quadrate) in NaOH solution was faster than that (circle) in NaCl solution of the same concen-

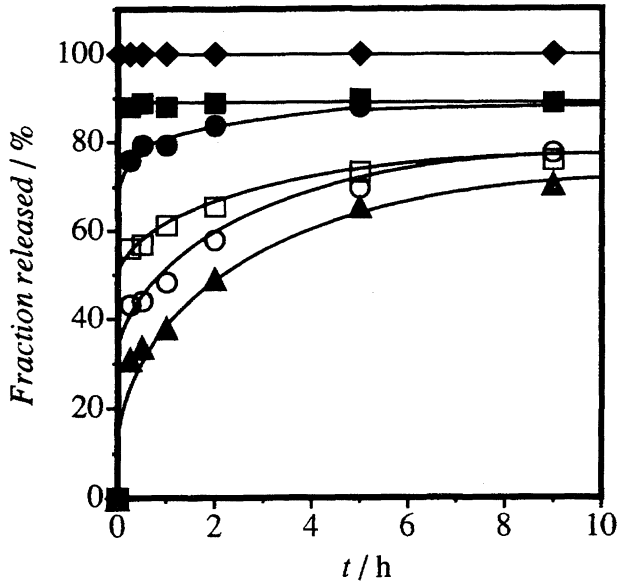


Fig. 5. Cytarabine release profiles from cytarabine intercalation compound of $\gamma$-TiP into various solutions. ◆, cytarabine not intercalated in $\gamma$-TiP; ■, $1.0\times10^{-1}$ mol dm$^{-3}$ NaOH; ●, $1.0\times10^{-1}$ mol dm$^{-3}$ NaCl; □, $1.0\times10^{-3}$ mol dm$^{-3}$ NaOH; ○, $1.0\times10^{-3}$ mol dm$^{-3}$ NaCl; ▲, $H_2O$.

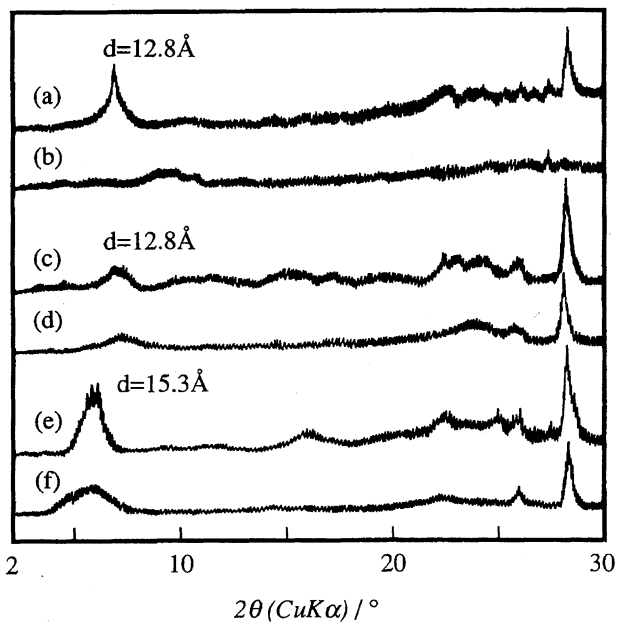


Fig. 6. X-Ray diffraction patterns of solids obtained by the reactions of cytarabine intercalation compounds of $\gamma$-TiP with various solutions. (a), after 0.25 h in $1.0\times10^{-1}$ mol dm$^{-3}$ NaOH; (b), after 48 h; (c), after 0.25 h in $1.0\times10^{-1}$ mol dm$^{-3}$ NaCl; (d), after 48 h; (e), after 0.25 h in $H_2O$; (f), after 48 h.

tration of sodium ions, that is, that in the solution of higher pH was faster. And the cytarabine release rate was faster in the higher $Na^+$ concentration, as can be seen in comparing closed and opened symbols (quadrate and circle) in Fig. 5. It is well known that the drug release was composed of the reaction process of drug on the surface and the diffusion of drug in the solution. In the present study, the drug release from $\gamma$-TiP-cytarabine may be governed by the reaction process of drug on the surface of intercalation compound, which meant the ion-exchange of $Na^+$ and the protonated cytarabine in $\gamma$-TiP-cytarabine, since the solubility of cytarabine and the surface area of $\gamma$-TiP-cytarabine were both sufficient higher values.

On the other hand, Fig. 6 shows the X-ray diffraction patterns of the solids after 0.25 and 48 h drug release tests. The X-ray diffraction pattern of original $\gamma$-TiP-cytarabine is shown in Fig. 2e. The diffraction patterns of the solids obtained by the drug release in $1.0\times10^{-3}$ mol dm$^{-3}$ NaOH and NaCl solutions were very similar to those in $1.0\times10^{-1}$ mol dm$^{-3}$ NaOH solution and $H_2O$, respectively, and are omitted. As can be seen, the crystallinity of all samples decreased after cytarabine release test. Further, two types of diffraction patterns were observed on these X-ray diffraction patterns. One is that the phase with an interlayer distance of 12.8 Å appeared after 0.25 h release test and then came to be amorphous after 48 h in $1.0\times10^{-1}$ mol dm$^{-3}$ NaOH and NaCl solutions (Fig. 6a—d) and $1.0\times10^{-3}$ mol dm$^{-3}$ NaOH solution. The other is that the phase with $d=15.3$ Å appeared but was not completely amorphous after 48 h, but the crystallinity decreased in $H_2O$ (Fig. 6e, f) and $1.0\times10^{-3}$ mol dm$^{-3}$ NaOH solution. The interlayer distance of the phase in the former was identical with that of the sodium form of $\gamma$-TiP represented by the fundamental form, Ti$(Na_2PO_4)(PO_4)\cdot2H_2O$,[13] implying that the ion-exchange of $Na^+$ and the protonated cytarabine in the intercalation compound occurred in this system. On the other hand, in the latter, a part of the cytarabine was released and its arrangement in the interlayer region of $\gamma$-TiP was changed, that is, an other phase with narrower interlayer distance ($d=15.3$ Å) was formed by the disintegration of the layered structure of $\gamma$-TiP.

## Conclusion

Some nucleic acid-related compounds containing anticancer agent cytarabine were readily intercalated into the inorganic layered titanium phosphate ($\gamma$-TiP). The cytarabine release became slower by intercalation into the layered phosphate. The cytarabine release behavior was influenced by the pH value and the concentration of sodium ion in the test solution, indicating that drug release from $\gamma$-TiP depended on the surrounding environmental conditions. In the reactions in aqueous solutions with the higher concentrations of sodium ion and/or the higher pH values, the ion-exchange of $Na^+$ and the protonated cytarabine in $\gamma$-TiP-cytarabine and the disintegration of the layered structure of $\gamma$-TiP occurred. On the other hand, in the solutions with the lower sodium ion concentrations and the lower pH values, the layered structure of $\gamma$-TiP was disintegrated and cytarabine in the interlayer region rearranged. Of course, more detailed information on the physicochemical properties and discussions on pharmaceutics of the drug intercalation compound of the layered phosphate are necessary for the practical applications as a drug delivery system. However, the present study will give some useful information concerning the specific drug release from layered compound.

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