Effect of Carbonate Salts on the Kinetics of Acid-Catalyzed Dimerization of Adefovir Dipivoxil

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Purpose. The chemical stability and product(s) distribution of adefovir dipivoxil (ADV) was examined in the presence of soluble and insoluble carbonate salts.

Methods. Chemical stability of ADV in the solid state at 60°C/30% RH was examined. Stability was also examined in the presence of excess formaldehyde vapor at 23°C/53% RH. ADV and its degradation product(s) were determined by reverse phase HPLC.

Results. Addition of aqueous soluble carbonate salts, such as sodium carbonate, compromised the stability of ADV in solid state. However, aqueous insoluble carbonates, such as calcium carbonate and magnesium carbonate, enhanced the stability of ADV as compared to the control formulation. Pivalic acid, a degradation product of ADV, was shown to accelerate the degradation rate of ADV in solid state. The de-stabilizing effect of this acid on ADV stability was diminished in the presence of magnesium carbonate. Pivalic acid also increased the rate at which ADV dimers were formed in the presence of formaldehyde vapor. Addition of insoluble carbonates reduced the rate of formaldehyde-catalyzed dimerization of ADV.

Conclusions. Addition of insoluble carbonate salts decreased the rate of degradation of ADV by minimizing the extent of formaldehyde-catalyzed dimerization in solid state.

KEY WORDS: adefovir dipivoxil; carbonate salts; dimerization; formaldehyde; pH-modifying agent; chemical stability

INTRODUCTION

The acyloxymethyl spacer group has been applied as a prodrug moiety to various functional groups, such as carboxylates and phosphonates (1). Hydrolysis of the acyloxymethyl ester group leads to the formation of formaldehyde, the corresponding acid, and the parent drug (1–2). Formaldehyde is reactive and is a well-known cross-linking agent for nucleic acids (2–5).

Adefovir dipivoxil (ADV; 9-(2-bis(pivaloyloxymethyl) phosphonomethoxyethyl)adenine) is a bis(pivaloyloxymethyl) prodrug of the antiviral agent, adefovir (PMEA) (6). Lee and co-workers have previously described the degradation kinetics and product distribution of ADV in the solid state (7). The degradation kinetics of ADV is governed by two distinct, but interrelated degradation pathways: hydrolysis of the pivaloyloxymethyl moiety and formaldehyde-catalyzed dimerization of the adenine ring. Hydrolysis of ADV produces one mole each of mono-POM PMEA, pivalic acid, and formaldehyde (Scheme I). The mechanistic scheme describing the dimerization of ADV is depicted in Scheme II. Formaldehyde can further react with the N^6-amine moiety of adenine to form the corresponding carbinolamine intermediate (Scheme II). Dehydration of the carbinolamine intermediate leads to the formation of the reactive imine cation (Schiff base) of ADV, which can react with an additional ADV or mono-POM PMEA molecule to form the methylene-linked dimer (7). Both degradation pathways are known to be pH-dependent in solution.

Addition of pH-modifying agents, such as basic carbonate salts, can alter the micro-environment pH of the formulation and affect the chemical stability of ADV in solid state. The use of carbonate salts to enhance the chemical stability of pharmaceuticals has been previously reported (8,9). Gu and co-workers reduced the rate of intramolecular aminolysis of moexipril hydrochloride by the addition of carbonate salts in a 1:1 ratio at the wet granulation step (8). Soluble carbonate salts, such as Na_2CO_3, demonstrated a better stabilizing effect on moexipril than insoluble carbonate salts, such as $CaCO_3$ (8). Stability of ketorolac was also improved in the presence of carbonate salts, however, a detailed mechanistic explanation was not provided (9).

This study aims to investigate the effect of soluble and insoluble carbonate salts on the solid state degradation kinetics of formaldehyde-catalyzed dimerization of ADV. The degradation kinetics of ADV in the presence of pivalic acid and formaldehyde was also studied to elucidate the role of hydrolytic by-products on the degradation mechanism for ADV. Furthermore, a mechanistic description is provided to explain the effect of soluble and insoluble carbonate salts on the degradation kinetics and product distribution of ADV.

MATERIALS AND METHODS

Materials

ADV was synthesized at Raylo Chemicals (Edmonton, Canada) for Gilead Sciences, Inc. Magnesium carbonate, light powder, USP/NF; calcium carbonate, light powder, USP/NF; sodium carbonate, anhydrous, USP/NF; and sodium bicarbonate powder, USP/NF were sourced from Spectrum Quality Products, Inc. (Gardena, CA). Pivalic acid, magnesium bromide hexahydrate, and magnesium nitrate hexahydrate were obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). Formalin (37% formaldehyde solution) was available from Sigma (St. Louis, MO). All other excipients used to prepare ADV granules were USP/NF grade. Deionized water was used to prepare buffer and HPLC mobile phase solvent systems. All salts and solvents were either reagent or HPLC grade and used as received.

Instrumentation

HPLC assays were performed using a fully automated, computer-controlled liquid chromatograph (Series II 1090, Hewlett-Packard Co., Palo Alto, CA) equipped with a data acquisition system (HPLC^{3D} ChemStation, Hewlett-Packard Co.). A water bath (RM6 Lauda, Brinkman Instruments Inc., Westbury, NJ) was used to control the temperature of the autosampler.

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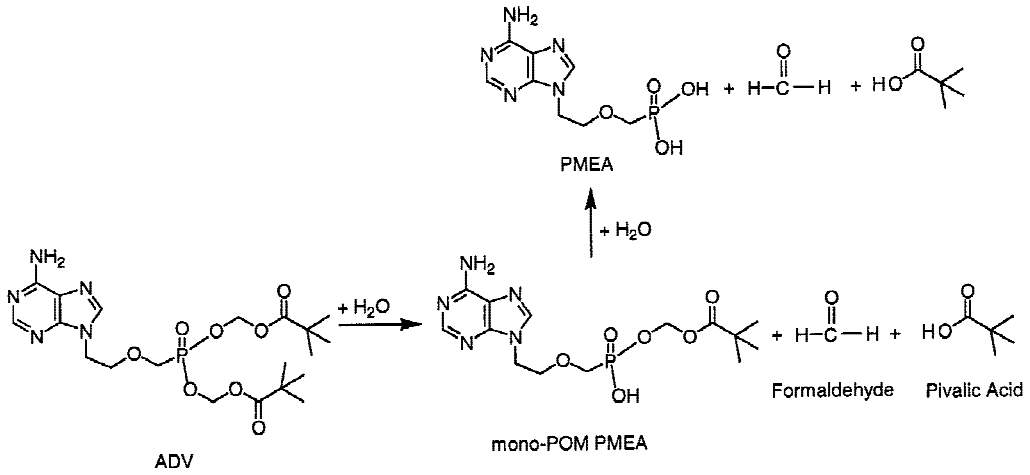


Scheme 1. Hydrolysis of adefovir dipivoxil.

Preparation of ADV Granules

Each batch of ADV granules was prepared using an aqueous wet granulation process, followed by drying, and milling. The formulation components consisted of 15% w/w ADV, 0–2% w/w carbonate/phosphate salts, and 0–0.5% w/w pivalic acid with other commonly used pharmaceutical excipients including a filler, a binder, and a disintegrant. Since the concentrations of ADV, binder, and disintegrant were constant, the concentration of filler was adjusted according to the concentration of carbonate salts and pivalic acid added in the formulations. The control formulation of 15% w/w ADV contained neither carbonate salts nor acid additives.

Preparation of ADV Powder Blends

Three additional batches of powder blends were prepared to examine the effect of adding carbonates extragranularly. A batch of ADV granules containing 15.8% w/w ADV was prepared and mixed with lactose monohydrate, calcium carbonate, and magnesium carbonate in a w/w ratio of 95:5. Each powder blend was mixed for 5 minutes and then passed through a #40 mesh screen twice. The final concentration of ADV was 15% w/w, and the concentration of calcium carbonate and magnesium carbonate was 5% w/w. The batch containing lactose monohydrate served as the control for the other two batches.

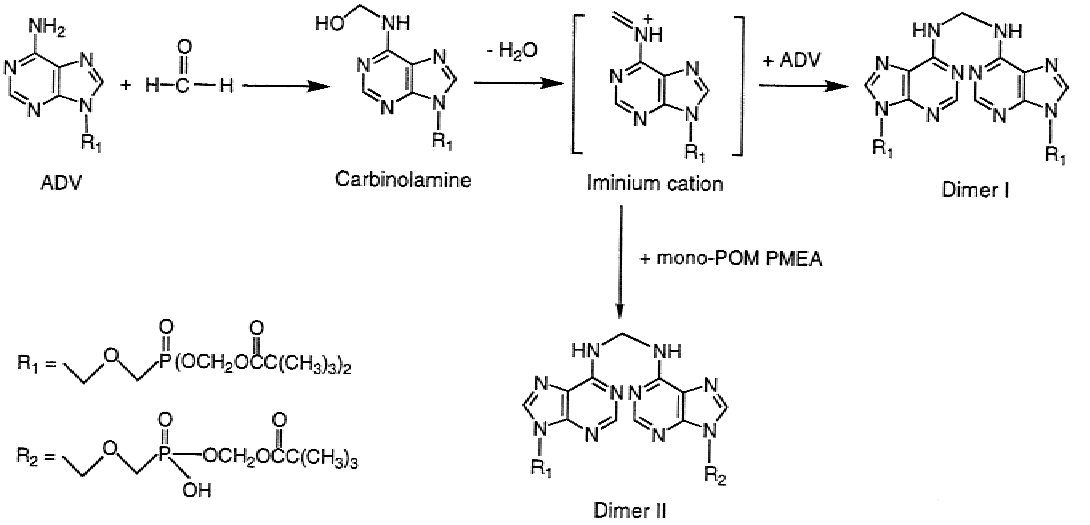
Stability Studies for ADV Granules/Powder Blends

60°C/30% RH

Approximately 2 g of ADV granules/powder blends were placed in a scintillation glass vial (Wheaton Scientific Products, Millville, NJ). These samples were uncapped and stored in a 60°C/30% relative humidity (RH) environment chamber. The 60°C/30% RH condition was controlled by placing saturated magnesium bromide hexahydrate solutions (10) in a closed container, which was placed in a 60°C oven (Model DK-63, Baxter, McGaw Park, IL). Representative samples were removed at each time point and stored in a refrigerator until they were assayed.

Exposure to Excess Amount of Formaldehyde at 23°C/53% RH

In a desiccator, formalin solution was added to an aqueous solution saturated with magnesium nitrate ($Mg(NO_3)_2$),



Scheme II. Dimerization of adefovir dipivoxil.

and the concentration of formaldehyde in the resultant solution was equivalent to 4.8 M. The vapor pressure of formaldehyde was estimated from its concentration in solution with an assumption that the interactions of formaldehyde with $Mg(NO_3)_2$ (*aq*) and water with $Mg(NO_3)_2$ (*aq*) are similar (11). Under this condition, an environment of 53% RH at room temperature (~23°C) was created with a vapor pressure of formaldehyde at approximately 2.8×10^{-4} atm (11). Approximately two grams of ADV granules were placed in a 10-mL open glass vial in the desiccator. At each time point, a 200-mg aliquot of the granules was sampled from each vial and analyzed by HPLC.

HPLC Assay for ADV

A stability-indicating HPLC method was used to determine the percent ADV remaining and degradation product profiles for ADV. The detailed assay method was reported previously (7). This method employed an Alltech mixed model anion exchange™ C_8 column, 7 micron, 100 Å pore size, 4.6 × 250 mm, (Alltech Associates, Inc.) equipped with a Direct-Connect™ Refillable guard column dry-packed with Pellicular C_8 particles, 2 × 20 mm (Alltech Associates, Inc.) at ambient temperature with a flow rate at 1.2 mL/min and UV detection at 260 nm. The mobile phase A consisted of 30% acetonitrile and 70% pH 6.0, 200 mM phosphate buffer, and the mobile phase B consisted of 50% acetonitrile and 50% pH 6.0, 200 mM phosphate buffer. The gradient profile was 100% mobile phase A for one minute, followed by a 19-minute linear gradient to 100% mobile phase B, then held at 100% mobile phase B for 10 minutes. A 10-minute equilibration at 100% mobile phase A was employed between injections. A 20 μL sample was injected to the system. The temperature in the autosampler was controlled ~10°C. Baseline resolution was obtained for ADV and the degradation products reported in the manuscript.

Approximately 200 mg of granules were weighed and transferred to a 100-mL volumetric flask. Twenty mL of pH 2.8 (25 mM phosphate) buffer was added and the mixture was sonicated for 15 minutes. Acetonitrile was added to the flask to approximately 2 cm below the volume marker and the mixture was sonicated for another 15 minutes. After equilibrating the mixture to room temperature, acetonitrile was filled to the exact volume, and mixed well. The solution was filtered through a 0.45 μm Nylon® 66 membrane filter unit (Rainin, Woburn, MA). The first 2 mL aliquot was discarded, and ~3 mL was collected in a disposable scintillation vial. The final concentration of ADV was ~0.3 mg/mL.

RESULTS AND DISCUSSION

Effect of Intragranular Carbonates on the Stability of ADV Formulations

The effect of pH modifying excipients on the degradation kinetics and product distribution of ADV was studied by incorporating soluble (2% $NaHCO_3$ or 2% Na_2CO_3) and insoluble (2% $CaCO_3$ or 2% $MgCO_3$) carbonate salts intragranularly in the ADV formulations. Table 1 lists the percent ADV remaining and its major degradation products including mono-POM PMEA, PMEA, and dimers in the presence of soluble and insoluble carbonate salts stored at 60°C/30% RH. Addition of $NaHCO_3$ and Na_2CO_3 accelerated the rate of ADV degradation relative to the control formulation (Table 1). After 13 days of storage at 60°C/30% RH, the percent ADV remaining in formulations containing 2% $NaHCO_3$ and 2% Na_2CO_3 were 74.9% and 56.9%, respectively. In comparison, the percent ADV remaining in the control formulation was 95.9%. Chromatographic analysis of the reaction products revealed that addition of soluble carbonates to the formulations accelerates the rate of hydrolysis of ADV, while reducing the extent of dimerization (Table 1). The mono-POM PMEA to dimer ratio was 0.5 in the control formulation and 8.4 in the formulation containing 2% Na_2CO_3. This ratio can be used to estimate the relative rates of hydrolysis and dimerization. Hydrolysis of the pivaloyloxymethyl ester moi-

Table 1. Percent Adefovir Dipivoxil (ADV) Remaining and Its Degradation Products[a] in Formulations Containing Soluble and Insoluble Carbonate Salts and Pivalic Acid (PVA) Stored at 60°C/30% RH

Additives	Time (days)	ADV (%)	Mono-POM PMEA (%)	PMEA (%)	Carbinolamine (%)	Dimer(s) (%)
Control	0	99.6	0.4	0	0	0
	13	95.9	1.4	0	0	2.7
	17	91.7	2.8	0	0.2	5.5
	21	84.3	5.0	0	0.4	9.5
2% $NaHCO_3$	13	74.9	14.5	2.4	0	1.6
2% Na_2CO_3	13	56.9	23.4	5.0	0	2.8
2% $MgCO_3$	13	96.3	1.7	0	0.2	1.7
	17	94.6	2.1	0	0.4	2.9
	21	90.5	3.2	0	0.2	5.0
2% $CaCO_3$	13	96.2	1.6	0	0.1	2.0
	17	94.3	2.0	0	0	3.7
	21	89.0	3.4	0	0.3	6.4
0.5% PVA	17	84.8	4.5	0	0.5	9.1
	21	72.4	8.1	0	0.7	17.0
0.5% PVA/2% $MgCO_3$	17	93.9	2.6	0	0	2.4
	21	90.8	3.2	0	0	4.7

[a] The lack of mass balance was attributed to the presence of another known degradation product, N^6-pivaloyl ADV, an additive of pivalic acid to the N^6-amine on the adenine ring of ADV. For example, 2.5% and 4.8% of N^6-pivaloyl ADV was presented in the formulations containing 2% $NaHCO_3$ and 2% Na_2CO_3, respectively, after 17 days of storage.

ety is subject to both acid and base catalyses (1). Addition of soluble carbonate salts, such as $NaHCO_3$ and Na_2CO_3, presumably increases the micro-environment pH in the ADV formulation, leading to an accelerated hydrolysis rate of the pivaloyloxymethyl group.

In contrast, incorporation of insoluble carbonate salts, such as $CaCO_3$ and $MgCO_3$, enhanced the stability of ADV as compared to the control formulation. In particular, dimer formation was reduced considerably (Table 1). After 17 days of storage at 60°C/30% RH, formulations containing $MgCO_3$ showed 2.9% dimer formation compared to 5.5% for the control formulation. Addition of carbonate salts also reduced the formation of mono-POM PMEA from 2.8% in the control formulation to 2.1% in the carbonate formulation (Table 1). Both $CaCO_3$ and $MgCO_3$ are basic salts, but possess low aqueous solubilities. The solubility products (K_{sp}) of $CaCO_3$ and $MgCO_3$ in water at room temperature are 4.96×10^{-9} and 6.82×10^{-6}, respectively (12). The reason for stabilization of ADV by $CaCO_3$ and $MgCO_3$ is the maintenance of pH near the optimal pH range. At a neutral pH condition, $CaCO_3$ and $MgCO_3$ are poorly soluble and do not affect the micro-environment pH of the ADV formulation. As a result, ADV is not subjected to base catalyzed hydrolysis in the presence of insoluble carbonates. This is consistent with lower mono-POM PMEA levels observed in the presence of insoluble carbonates as compared to soluble carbonates. Although $CaCO_3$ and $MgCO_3$ are practically insoluble in water, they are soluble under acidic solution. The pH-dependent solubility profile of $CaCO_3$ and $MgCO_3$ allows these carbonates salts to act as buffering agents and neutralize any acidic degradation products (pivalic acid and mono-POM PMEA) of ADV. The more soluble carbonate salts also neutralize the acidic by-products and they further increase the pH which leading to base-catalyzed hydrolysis of ADV.

Effect of Extragranular Insoluble Carbonates on the Stability of ADV Formulations

The effect of insoluble carbonates as an extragranular excipient was also studied to further understand the mechanism of stabilization. Two powder blends were prepared containing either 5% extragranular $CaCO_3$ or 5% extragranular $MgCO_3$. After 21 days of storage, the percent ADV remaining in formulations containing $CaCO_3$ (86.3%) and $MgCO_3$ (86.8%) was similar to the control formulation (86.4%), suggesting that the extragranular addition of $CaCO_3$ and $MgCO_3$ did not affect the stability of ADV. The degradation product distribution of these two powder blends containing $CaCO_3$ and $MgCO_3$ was also similar to the control formulation. Therefore, if $CaCO_3$ and $MgCO_3$ are used in ADV formulations, they should be incorporated at the intragranular phase during the wet granulation process, presumably to increase the physicochemical interaction between ADV and the carbonates.

Effect of Acidic Degradation Products on the Stability of ADV Formulations

The effect of pivalic acid and magnesium carbonate on the stability of ADV was examined by comparing the stability of ADV in formulations containing 0.5% pivalic acid to those containing a mixture of 0.5% pivalic acid/2% $MgCO_3$. The quantity of pivalic acid added intragranularly represented the extent of pivalic acid formation after ~16% hydrolysis of ADV. Addition of pivalic acid accelerated the degradation rate of ADV as compared to the control formulation (Figure 1). Addition of $MgCO_3$ to the pivalic acid containing formulation significantly improved the stability of ADV as depicted in Figure 1. The formulations containing 0.5% pivalic acid/2% $MgCO_3$ exhibited better stability than the control formulation, presumably because of the added amount of $MgCO_3$ neutralized both the external 0.5% pivalic acid and any intrinsic acid generated from ADV degradation.

Effect of Excess Formaldehyde Vapor on the Stability of ADV Formulations

Decomposition kinetics of ADV are governed by two distinct but interrelated degradation pathways: hydrolysis of the pivaloyloxymethyl moiety and formaldehyde-catalyzed dimerization of the adenine ring. Exposure of ADV formulations to an excess amount of formaldehyde vapor (2.8×10^{-4} atm) allows an evaluation of the effect of carbonate salts on the kinetics of ADV dimerization in the absence of significant hydrolysis. Figure 2 depicts the degradation versus time curves for the control formulation and formulations containing 2% $CaCO_3$ and 2% $MgCO_3$, when exposed to the formaldehyde vapor in a 53% RH environment at 23°C. Under these conditions, hydrolysis of ADV is negligible (<0.3% mono-POM PMEA formed during the 17-day study period), and ADV is rapidly converted to the carbinolamine intermediate. As shown in Figure 2, addition of $CaCO_3$ and $MgCO_3$ reduces the rate of ADV disappearance. Figure 3 shows the rate of carbinolamine and dimer formation in ADV granules, with and without $MgCO_3$, exposed to high vapor concentration of formaldehyde. Addition of $MgCO_3$ reduces the formation rate of both the carbinolamine and dimer conjugates.

The two ADV formulations containing 0.5% pivalic acid and 0.5% pivalic acid/2% $MgCO_3$ were also exposed to formaldehyde vapor (2.8×10^{-4} atm) at 23°C/53% RH. Exposure of ADV formulations containing pivalic acid to high concentration of formaldehyde vapor results in faster rate of dimer-

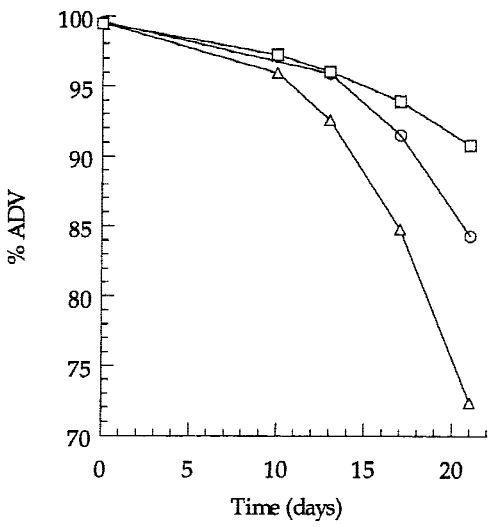


Fig. 1. Degradation-time curves of ADV in granules at 60°C/30% RH: the control (○), 0.5% pivalic acid (△) and 0.5% pivalic acid/2% $MgCO_3$ (□).

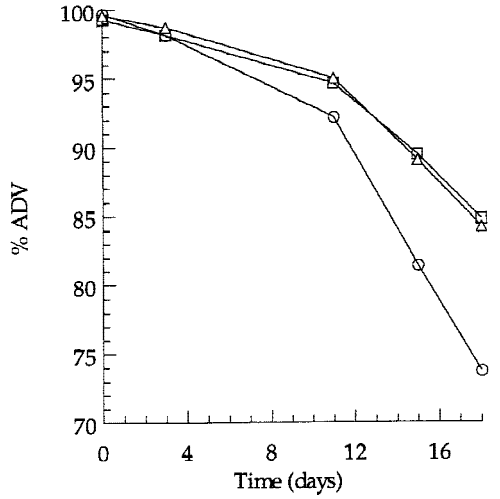


Fig. 2. Degradation-time curves of ADV in granules exposed to 2.8×10^{-4} atm of formaldehyde at 23°C/53% RH: the control (○), 2% $MgCO_3$ (△) and 2% $CaCO_3$ (□).

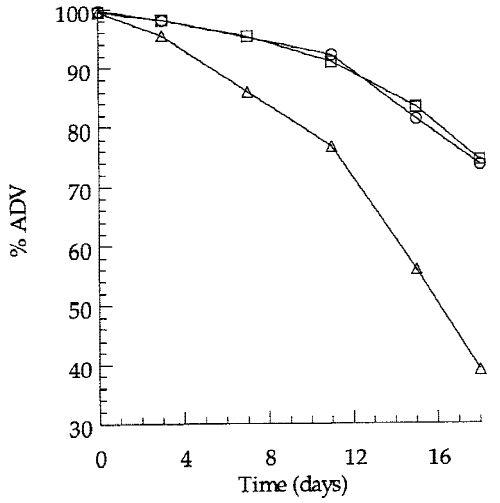


Fig. 4. Degradation-time curves of ADV in granules exposed to 2.8×10^{-4} atm of formaldehyde at 23°C/53% RH: the control (○), 0.5% pivalic acid (△) and 0.5% pivalic acid/2% $MgCO_3$ (□).

ization of ADV compared to control (Figure 4). After 17 days of exposure, the percent ADV remaining in either a formulation containing 0.5% pivalic acid or the control formulation was ~38% and ~74%, respectively. However, incorporating 2% $MgCO_3$ into the formulation containing 0.5% pivalic acid diminished the rate of dimerization of ADV in the presence of pivalic acid. The degradation versus time curves of carbinolamine and dimer formation in ADV granules formulated with 0.5% pivalic acid and 0.5% pivalic acid/2% $MgCO_3$ are illustrated in Figure 5. Again, $MgCO_3$ suppressed the formation of carbinolamine and dimer and enhanced the stability of ADV even in the presence of pivalic acid.

Mechanism of Formation of ADV Carbinolamine and Imine Derivatives

The formation of dimer conjugate(s) is a multi-step reaction involving general acid-catalysis of the addition step to produce a carbinolamine intermediate and the dehydration step to produce a reactive iminium cation as illustrated in Scheme II (13–16). A general mechanism for the formation of the carbinolamine intermediate consistent with kinetic and structure-reactivity data has been described by Sayer and coworkers (15,16). The predominant pathway for carbinolamine formation of a weakly basic amine is via a general acid-catalyzed "concerted" mechanism (15). The N^6-amine group on the adenine ring of ADV is a weak base with a pK_a value of about 3.8 (17). Therefore, mildly acidic conditions can enhance the rate of formation of carbinolamine. The dehydration step of carbinolamine to form the corresponding iminium cation is also acid catalyzed (13). Thus, the rate of the dimerization reaction is enhanced under mildly acidic conditions.

Hydrolysis of ADV leads to the formation of pivalic acid and mono-POM PMEA, which can acidify the surroundings of ADV and catalyze the formation of the carbinolamine in-

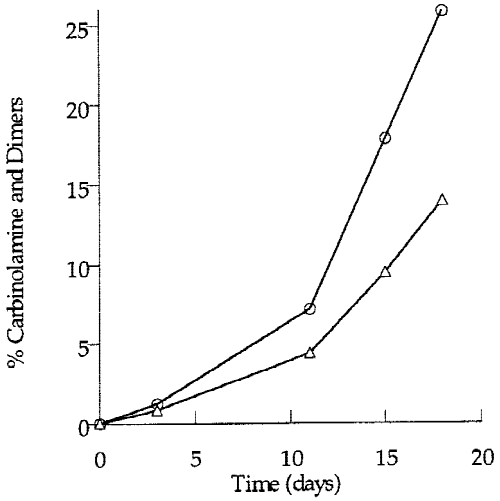


Fig. 3. Formation of carbinolamine and dimer in the control (○) and 2% $MgCO_3$ (△) formulations exposed to 2.8×10^{-4} atm of formaldehyde at 23°C/53% RH. The corresponding degradation-time curves of ADV were shown in Figure 2.

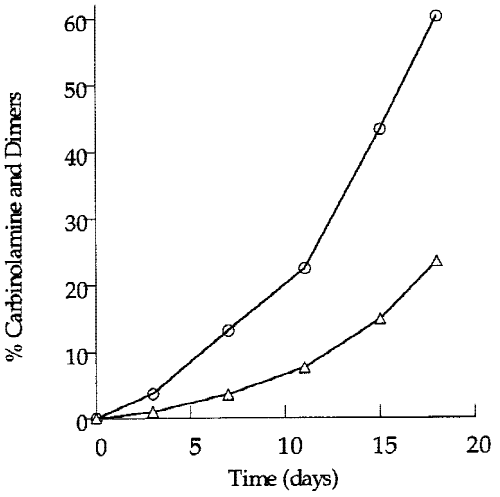


Fig. 5. Formation of carbinolamine and dimer in the formulations containing 0.5% pivalic acid (○) and 0.5% pivalic acid/2% $MgCO_3$ (△) exposed to 2.8×10^{-4} atm of formaldehyde at 23°C/53% RH. The corresponding degradation-time curves of ADV were shown in Figure 4.

termediate and the ensuing reactive iminium cation. Addition of $CaCO_3$ and $MgCO_3$ neutralizes the acidic by-products and reduces the rate of dimerization of ADV. Both $CaCO_3$ and $MgCO_3$ act as a buffering reservoir to maintain the neutral surroundings of ADV. Therefore, the acidic environment generated from hydrolysis of ADV can be continuously maintained to neutral pH conditions through dissolution of the required amount of insoluble carbonates.

CONCLUSIONS

The effect of soluble and insoluble carbonate salts on the kinetics of ADV degradation was studied. Soluble carbonate salts, such as $NaHCO_3$ and Na_2CO_3 , were shown to enhance the rate of ADV hydrolysis by alkalinizing the microenvironment of the formulation. In contrast, incorporation of insoluble carbonates, such as $CaCO_3$ and $MgCO_3$, effectively decreased the degradation rate of ADV. Addition of $CaCO_3$ and $MgCO_3$ neutralizes the acidic by-products of ADV generated during hydrolysis and thereby reduces the extent of acid-catalyzed dimerization.

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