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Excipient hydrolysis and ester formation increase pH in a parenteral solution over aging

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Abstract

Recently, the number of drug substances that are poorly water-soluble has increased dramatically. This makes improving solubility one of the most critical tasks in pharmaceutical development today. In this study, the physicochemical stability of an injectable solution of conivaptan hydrochloride salt was investigated. Because its free form is hydrophobic, the drug substance was solubilized in a co-solvent system, 40% of which was composed of different alcohols. Since the free form is also alkaline, lactic acid was added to the co-solvent system to further improve its solubility. Remarkably, the pH of the solution was found to increase gradually over time. Considering the physicochemical nature of the drug substance, uncontrolled increases in pH would pose a potential threat of reducing solubility and forming precipitates. For this reason, a risk evaluation was performed. The evaluation revealed that the pH increase was caused by the hydrolysis of lactic acid oligomers as well as by the ester formation occurring between lactic acid and the alcohols. High concentrations of lactic acid supplied as an excipient usually contain lactic acid oligomers, which are hydrolyzed into lactic acid monomers upon dilution with water. Commercial software was used to determine the pK_a values of the lactic acid oligomers, which were found to be lower than that of lactic acid monomers. This indicates that hydrolysis causes the pH to increase. Ester formation consumes the acid, which also causes the pH to increase. However, both hydrolysis and ester formation equilibrated by the 16-month time point when stored at 25 °C. This information allowed the upper limit of the pH increase to be determined molecularly, thereby ensuring product quality through the prevention of precipitate formation due to reduced solubility. Increased awareness of the importance of risk evaluation in pharmaceutical development is critical as these kinds of chemical reactions between excipients constitute a potential risk factor, but tend to be overlooked.

Keywords: Risk evaluation; Quality management; Lactic acid oligomer; Alcohol; Ester; Hydrolysis

1. Introduction

The number of hydrophobic drug substances has increased dramatically since high throughput screening and combinatorial chemistry were introduced into the pharmaceutical development process. The need to improve drug solubility during formulation design to combat this emerging difficulty has become greater. A variety of methods for improving drug solubility, such as making the drug into a salt and adjusting the pH, has been suggested for parenteral formulations. The use of a co-solvent system is effective for this purpose (Sweetana and Akers, 1996). So far, some 20 excipients are available for use in co-solvent systems, including alcohols such as ethanol, glycerol, propylene glycol, sorbitol, and polyethylene glycol (Akers, 2002). These alcohols enhance the solubility of poorly water-soluble drugs by increasing the hydrophobic quality of the solvents. Careful considerations are, however, necessary for formulation selection because some of these alcohols sometimes exhibit hemolytic activity (Fuet et al., 1987) and promote the degradation of drug substances during storage (Bundgaard, 1990). Alcohol is a labile molecular species that can participate in many kinds of organic chemical reactions, which raises concern about potential chemical reactivity during storage.

Although all drug products must be stable throughout their shelf life, injectable solutions generally have more difficulties with storage stability compared to solid dosage forms. Before parenteral formulations are released, they must pass not only assay and related substances testing, but also the other tests such

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as those for osmotic pressure, insoluble particulates, pH, etc., all of which must be satisfied throughout the storage period. pH is one of the critical parameters that controls the solubility and chemical reactivity of drug substances. Acetic acid, citric acid, tartaric acid, phosphoric acid, and TRIS are often used as pH modulators (Akers, 2002). These molecules sometimes pose stability problems in pharmaceuticals due to their own chemical reactivity. For instance, phosphate anion, particularly dibasic phosphate anion, is a strong nucleophile that is capable of attacking electrophiles such as esters and acid–amide bonds, which results in the production of related substances (Stella, 1986). Phosphoric acid and citric acid can form precipitates by interacting with alkali cations such as calcium and aluminum (Hasegawa et al., 1983). Such reactivity of pH modulators not only gives rise to pH changes in drug products, but also leads to the generation of insoluble foreign matter, which is a risk factor. Poly-lactic acid and its co-polymer polymerized with glycolic acid are often used as an *in vivo*-degrading-polymer. The chemical reactivity of the acids is exploited for useful purposes in this particular case (Fu et al., 2000).

In this study, a poorly water-soluble drug, conivaptan hydrochloride, an intravenously administered inhibitor of vasopressin for the treatment of low blood sodium levels, was formulated as an injectable solution using a co-solvent system containing ethanol and propylene glycol. While it was initially adjusted to around 3.0–3.4, the pH of the solution was found to gradually increase over a long period of time. Since the drug substance free form is alkaline, hydrochloride was used to make it into a salt for improved solubility. High solubility was ensured by adding lactic acid to the solution to maintain a protonated form under acidic conditions. pH increases that occur over time pose a risk of precipitate formation during its shelf life. Therefore, in order to secure product quality, the risk of this occurrence was evaluated by exploring the cause of the pH increase. Since hydrophobic drugs are being chosen for pharmaceutical development more often, and since alkaline drugs constitute a major portion of those synthetic drugs, an increase in pH over time is of great significance in terms of drug solubility. This study demonstrates that chemical reactions involving lactic acid and alcohols give rise to a pH increase over time in an injectable solution, but the increase is limited and the reactions eventually equilibrate. Reaction products produced by excipients are easily overlooked because they are not generally classified as related substances, which are strictly restricted. They are, however, very important in that they can generate solution conditions that might adversely affect drug substances and degenerate product quality. Analytical tools such as LC–MS, IC-MS, IC-ECD, GC-FID, and GC–MS were used to trace the reactions of the excipients occurring in the solution over time.

2. Materials and methods

2.1. Materials

Conivaptan hydrochloride was synthesized in-house. Lactic acid was supplied by Purac (Gorinchem, The Netherlands). Propylene glycol was purchased from Merck (KGaA, Darmstadt, Germany). Ethanol was supplied by Amakasu Sangyo (Tokyo, Japan). *n*-Octane was purchased from Wako (Osaka, Japan). Triethylene glycol was supplied by Nacalai Tesque (Kyoto, Japan). pH standards were purchased from Micro Essential Laboratory (Brooklyn, NY, USA). All the other reagents were purchased from Kanto Chemical (Tokyo, Japan).

2.2. Production of the drug product

Conivaptan hydrochloride was solubilized in a mixture of water, propylene glycol, and ethanol. Bulk solution consisting of about 95% of the final volume of the drug product was prepared. The pH was then adjusted to between 3.0 and 3.4 with lactic acid. After the pH was adjusted, the solution was brought to volume with water for injection (WFI). It was then filtered to remove insoluble foreign matter, and sealed in glass ampoules. The ampoules were then autoclaved at 121 °C for at least 20 min. Any ampoule that underwent the sterilization process was defined as a "fresh drug product", and the pH of this sample was recorded at the initial time point. The final concentration of lactic acid in the drug product ranged between 5 and 25 mM, depending on the amount of lactic acid added. The drug product contained 30% (w/v) propylene glycol and 10% (w/v) ethanol in water.

2.3. pH measurement

Two kinds of buffer salts, (pHydrion buffers: pH 2.00 and 4.00; micro essential laboratory) were dissolved in a mixture of 10% ethanol (w/v) and 30% propylene glycol (w/v) in water. A pH electrode, which had been calibrated according to the standard procedure described in USP ⟨791⟩, was rinsed with water and immersed in each of the alcohol-containing buffer solutions. Then, the pH was adjusted to 2.165 or 4.788 (at 25 ± 0.5 °C) (the original pH meter readings (calibrated using the USP method) were close to the target). The pH of alcohol-containing solutions were always measured after this type of adjustment.

2.4. pK_a determination

The pK_a values of dimer-, trimer-, and tetramer-lactic acid were calculated using commercial software (Advanced Chemistry Development, version 5).

2.5. LC–MS

Sample solution was injected into a polystyrene–divinylbenzene resin based column (Aminex HPX-87H, 7.8 mm ID × 300 mm, BioRad Laboratories, Hercules, CA, USA) fixed in a chromatograph (Alliance 2695, Waters Corporation, Milford, CT, USA) combined with a mass spectrograph (Micromass ZQ, Waters Corporation). The column temperature, flow rate, and mobile phase were 40 °C, 0.3 mL/min, and 0.1% formic acid/acetonitrile (3/2), respectively. The ESI positive mode at the cone voltage of 15 V was used for acquiring mass spectra at $m/z = 149$ of propylene glycol mono-ester. The ESI negative mode at the cone voltage of 20 V was used for acquiring total ion chromatograms (m/z 30–350) of lactic acid. The injection vol-

umes were 5 μL for the analysis of propylene glycol mono-ester and 100 μL for lactic acid, respectively.

2.6. IC-MS

Fifty microliters of 10 mM lactic acid solution was injected into an ion chromatography column (TSKgel IC-anion-SW, 4.6 mm ID × 50 mm, Tosoh, Tokyo, Japan) fixed in a chromatograph (Alliance 2695, Waters Corporation) combined with a mass spectrograph (Micromass ZQ, Waters Corporation). The column temperature and mobile phase were 40 °C, and 1 mM phthalic acid (pH 3.4), respectively. The pH of the mobile phase was adjusted using potassium hydroxide. The flow rate in the column was 1 mL/min, but a splitter was installed at the mouth of the mass spectrograph, which reduced the flow rate to 0.2 mL/min. The analyzed substances were ionized in an ESI negative mode. The cone voltage was 20 V.

2.7. IC-ECD

Fifty microliters of a solution containing lactic acid was injected into an ion chromatography column (TSKgel IC-anion-SW 4.6 mm ID × 50 mm, Tosoh) fixed in an ion chromatograph (IC7000, Yokogawa Electric Corporation, Tokyo, Japan) equipped with an electro-conductivity detector (ECD). The column temperature and flow rate were adjusted to 40 °C and 1 mL/min, respectively. The mobile phase was 1 mM phthalic acid (pH 3.4), adjusted using potassium hydroxide.

2.8. GC-FID

One microliter of a solution was injected into a capillary column (DB-WAX 30 m × 0.53 mm × 1 μm, Agilent Technologies, Palo Alto, CA, USA) fixed in a gas chromatograph (HP6890, Yokogawa Electric Corporation) equipped with a hydrogen flame ionization detector (FID: 280 °C, hydrogen: 50 mL/min, air: 450 mL/min make-up gas: helium). The carrier gas was helium, the flow rate of which was 35 cm/s. The column temperature was initially adjusted to a temperature of 100 °C, then slowly increased to 124 °C at a rate of 3 °C/min, then rapidly raised to 220 °C at a rate of 100 °C/min. The temperature was kept constant at 220 °C for 10 min. The injection splitter ratio and temperature were 5–1 and 200 °C, respectively.

2.9. GC–MS

One microliter of a solution was injected into a capillary column (DB-WAX 30 m × 0.25 mm × 0.5 μm, Agilent Technologies) fixed in a mass spectrograph (Clarus 500, Perkin-Elmer, Wellesley, MA, USA). The carrier gas and its flow rate were helium and 1 mL/min, respectively. The column temperature was initially adjusted to a temperature of 100 °C, then increased to 124 °C at a rate of 3 °C/min, then rapidly raised to 220 °C at a rate of 45 °C/min. The temperature was kept constant at 220 °C for 20 min. The injection splitter ratio and temperature were 5–1 and 200 °C, respectively. The analyzed substances were ionized in electron impact (EI) mode.

Table 1
Formulation of the drug product

Component	Amount per ampoule	Function
Conivaptan hydrochloride	20 mg	Active ingredient
Propylene glycol	1.2 g	Solubilizer (solvent)
Ethanol	0.4 g	Solubilizer (solvent)
Lactic acid	q.s.[a] to pH 3.0	pH adjuster
Water	q.s.[a] to 4 mL	Solvent

[a] Quantity sufficient.

3. Results

3.1. The co-solvent system of conivaptan injectable

The chemical composition of the conivaptan hydrochloride drug product is shown in Table 1. Since the conivaptan free form is poorly water-soluble (solubility, approximately 1 μg/mL at neutral pH), a co-solvent system has been selected for formulation using ethanol and propylene glycol. The drug product was notably stable in all quality control (QC) tests concerning aging at 25 °C, except for pH. Fig. 1 shows the pH increases of three different production lots plotted as a function of storage period. It is obvious that the pH of the solution increased during storage, but it appeared to level off at 15 months in these particular production lots (Fig. 1). Since conivaptan is alkaline and poorly water-soluble, it is theoretically possible that if the pH of the solution happens to increase unchecked, the conivaptan free form may precipitate when deprotonation occurs on a nitrogen atom on the molecule. Thus, pH is the key parameter for long-term stability of the drug product, and elucidation of the cause of the pH increase is an important part of risk evaluation.

3.2. Hypothetical mechanics of pH increases

Functional groups that are capable of acid–base reactions can only contribute to pH change in the solution. Those groups include an alkaline nitrogen atom on the conivaptan free form

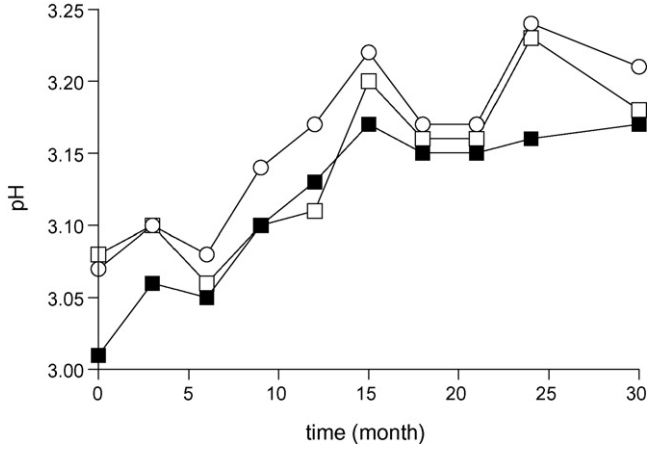

Fig. 1. pH increases observed in three different production lots over time (25 °C).

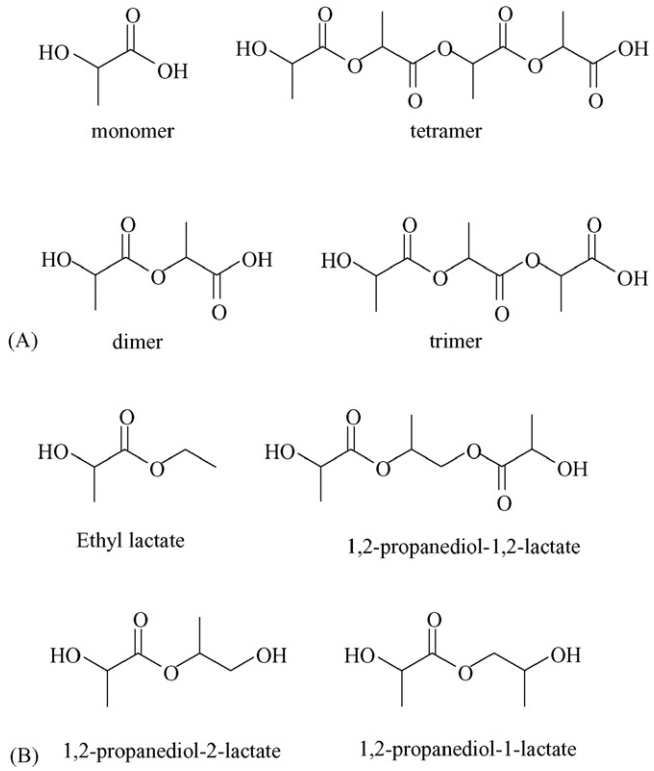

Fig. 2. Chemical structures of the lactic acid monomer and oligomers (A), and the lactates (B).

and a carboxyl group on lactic acid. The question is how these components are involved with the pH increases observed over time. Possible mechanisms involving lactic acid are as follows:

1. It is well known that lactic acid supplied as an excipient contains lactic acid oligomers that are produced by self-condensation (ester formation) (Holten et al., 1971; Fig. 2A). A lactic acid oligomer, as opposed to lactic acid monomer, is defined as a lactic acid polymer consisting of less than 10 lactic acid molecules. If the pK_a of a lactic acid oligomer is comparable to that of a strong acid, and it is smaller than that of lactic acid monomer, the pH can gradually increase over time with the hydrolysis of the lactic acid oligomer. If the pK_a of the lactic acid oligomer is larger than that of the lactic acid monomer, the pH can increase if the lactic acid monomers undergo self-condensation over time. This results in the production of a lactic acid oligomer.
2. If the lactic acid reacts with ethanol and/or propylene glycol to form an ester (Fig. 2B) over time, it means that acid was consumed, which lowers the acid concentration, thereby causing a pH increase.
3. If the lactic acid is unstable and degrades over time, it means that acid was consumed, which lowers the acid concentration, thereby causing a pH increase.

Since conivaptan is a compound that is very stable chemically, no significant changes were observed upon assay and testing for related substances. It is not possible for conivaptan to form an ester bond with lactic acid or alcohol because the drug substance

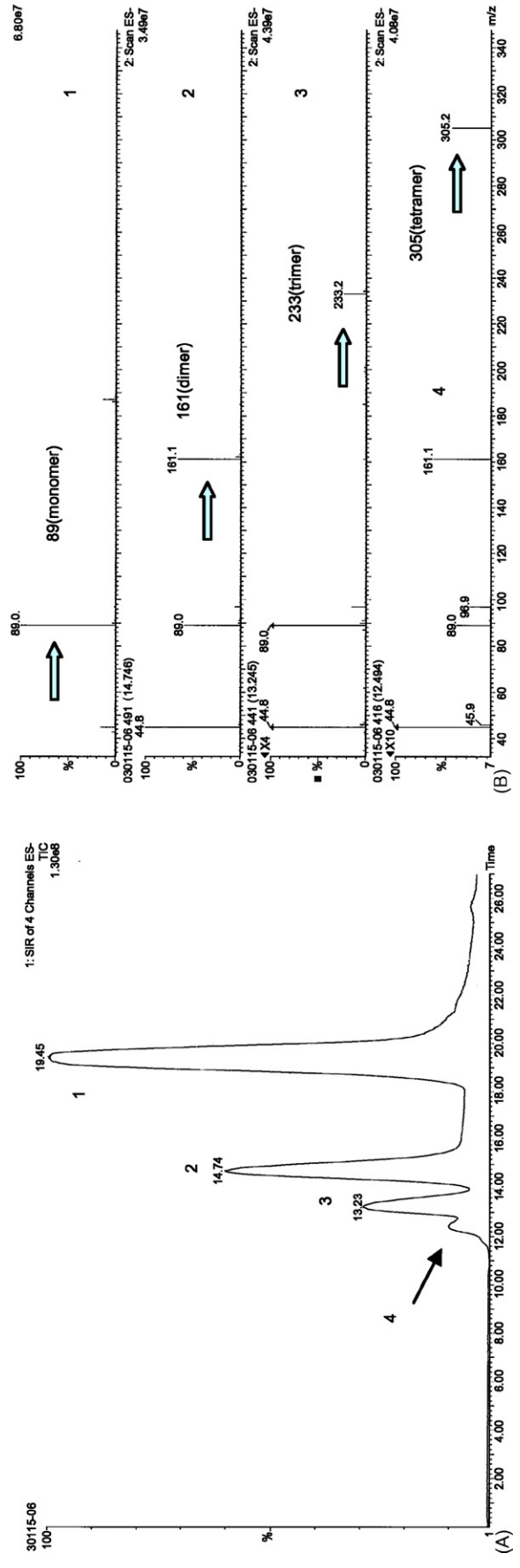

Fig. 3. Analyses of a lactic acid solution using LC–MS are shown: (A) a total ion chromatogram; (B) mass spectra of each peak shown in (A).

Table 2
pK_a of lactic acid and lactic acid oligomers

Lactic acid	pK_a		Acidity
	Calculated value	Merck index	
Monomer	3.91 ± 0.11	3.83	Low
Dimer	2.65 ± 0.10	NM[a]	High
Trimer	2.54 ± 0.10	NM[a]	High
Tetramer	2.54 ± 0.10	NM[a]	High

[a] Not mentioned.

has no hydroxyl or carboxyl groups in it. Naturally, no covalent changes occur with conivaptan, which leaves physicochemical changes as the only possibility.

1. If lactic acid deprotonates to form a transient complex with protonated conivaptan, the pK_a of the lactic acid apparently shifts to that of a strong acid. Then, if the complex dissociates to free the lactic acid, the pH of the solution increases when the weak acid is again protonated.
2. If conivaptan free form is transiently stabilized in the co-solvent system, conivaptan hydrochloride deprotonates to its free form initially. Then, if conivaptan free form gradually protonates over time, the pH of the solution increases.

Elution of alkali from the ampoule walls is another possible mechanism. The mechanics of this theory can hold true more or less independently of the chemical composition of the drug products.

3.3. Hydrolysis of lactic acid oligomer

LC–MS was used to analyze the composition of a lactic acid solution. As shown in Fig. 3, the lactic acid dimer, trimer, and tetramer were detected in addition to lactic acid monomer. The ratio of each component to the total amount (monomer basis) is unclear because the detection sensitivity of each species is unknown. Certainly, there are at least four species in the solution where the monomer predominates, followed by the dimer. Presence of the trimer and tetramer in the solution seemed to be much less.

The pK_a values of the lactic acid monomer and oligomers were then estimated. Since they were not described in the literature, the pK_a values of the lactic acid oligomers were calculated using commercial software. The pK_a values are summarized in Table 2. Obviously, the pK_a values of the lactic acid oligomers were significantly smaller than that of the monomer, which indicates that the oligomers are stronger acids. The pK_a value of the lactic acid monomer is listed in the Merck index, and is comparable to the simulated value. Table 2 demonstrates how the predicted value agrees well with the observed value, which suggests that the predicted pK_a values of the lactic acid oligomers are also reliable. These results indicate that if the lactic acid oligomer slowly hydrolyzes in the drug product, the pH of the solution will increase over time.

Next, a lactic acid solution was analyzed using IC-MS, the results of which are shown in Fig. 4A and B. When a mass chromatogram was obtained with the detection mass number fixed at $m/z = 89$ (lactic acid monomer), distinct peaks were observed at 4.11 min (peak 1) and 11.20 min (peak 2) (Fig. 4A, upper). Another mass chromatogram was then obtained with the detection mass number fixed at $m/z = 161$ (lactic acid dimer), which showed a single peak at 11.17 min (Fig. 4A, lower). These results suggest that under these analytical conditions, the lactic acid monomer is the first peak eluted, and the lactic acid dimer is the second. Higher molecular weight lactic acid oligomers were not detected under these analytical conditions. The peak assignment was checked against their mass spectra to confirm accuracy (Fig. 4B). Since the conditions for separation had been established, the samples were analyzed using an electro-conductivity detector (ECD) for the sake of convenience. Measurements were first made using different concentrations of fresh lactic acid, which indicated excellent linearity for both the monomer and dimer (Fig. 5A). When fresh and aged drug products (25 °C, 16 months) were analyzed under the same separation conditions, the lactic acid dimer in the aged sample had evidently hydrolyzed into the monomer (Fig. 5B and C). The initial ratio of dimer to monomer in the fresh drug product, however, could not be determined because, originally, the electro-conductivities of the molecular species were unknown. The titration method was then introduced to determine the ratio using a fresh lactic acid solution. This was done under the assumption that all the lactic acid oligomer species were dimers, as was the case in a previous study (Lucke and Gopferich, 2003). The proportion of monomer to dimer was found to be 3.4 (monomer basis), indicating that the lactic acid dimer had accounted for 23% of the total lactic acid initially, but had hydrolyzed into the monomer over time.

Now that there was proof that the lactic acid dimer had hydrolyzed after months of storage, the amount of dimer present and the change in pH were soon followed by promoting hydrolysis under stressed conditions (autoclave at 121 °C). The results are summarized in Fig. 6. The drug product undergoes routine terminal sterilization at 121 °C for 25 min (autoclave) when manufactured. Since this process entails stressed conditions that could facilitate hydrolysis, the drug product was autoclaved for a much longer time (360 min) to accelerate aging. The amount of dimer evidently decreased over time as the pH increased (Fig. 6A–C). The initial pH depended on the solvent used for preparation, and the pH was apparently greater in the co-solvent system (Fig. 6D). In Fig. 6D, the pH at 25 min corresponds to the initial pH of a "fresh drug product," as noted earlier in Section 2. At 25 min, the amount of dimer had decreased a little, but the majority remained intact (Fig. 6A–C), with a slightly increased pH (Fig. 6D). The increase in pH from 25 min to 360 min was a little less than 0.2 (Fig. 6D). This value is comparable to the pH increase observed for the drug product over time (Fig. 1). As shown in Fig. 6E, the pH increment was almost independent of the initial concentrations of lactic acid, which is consistent with the fact that the pH increases were independent of the initial pH (Fig. 1). When the autoclave time was extended from 360 to 600 min, no degradation of lactic acid occurred, indicating that lactic acid itself is chemically stable. The fact that the pH of the solution remained unchanged

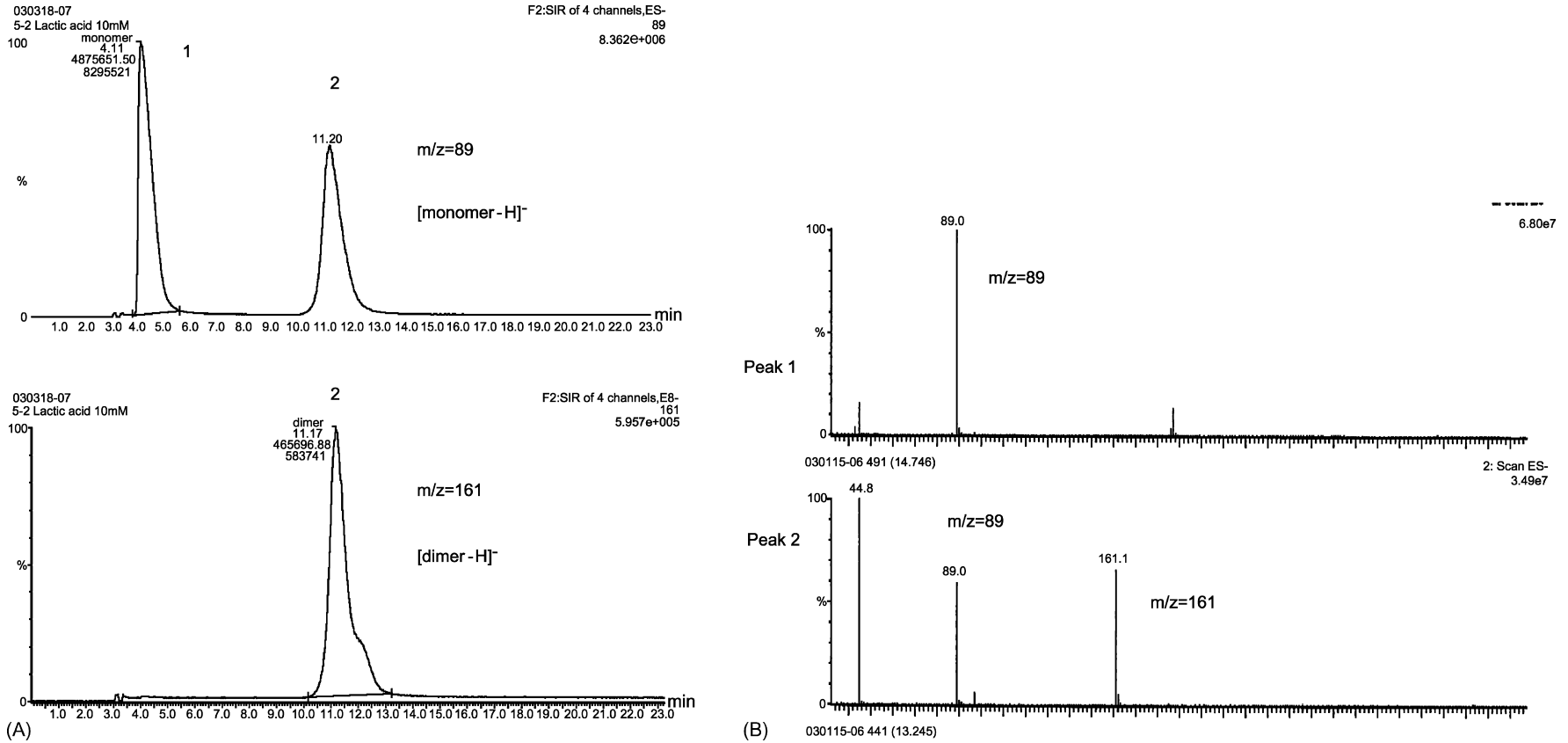

Fig. 4. Analyses of a lactic acid solution using IC-MS: (A) mass chromatograms of a lactic acid solution; (B) mass spectra of the peaks observed in (A).

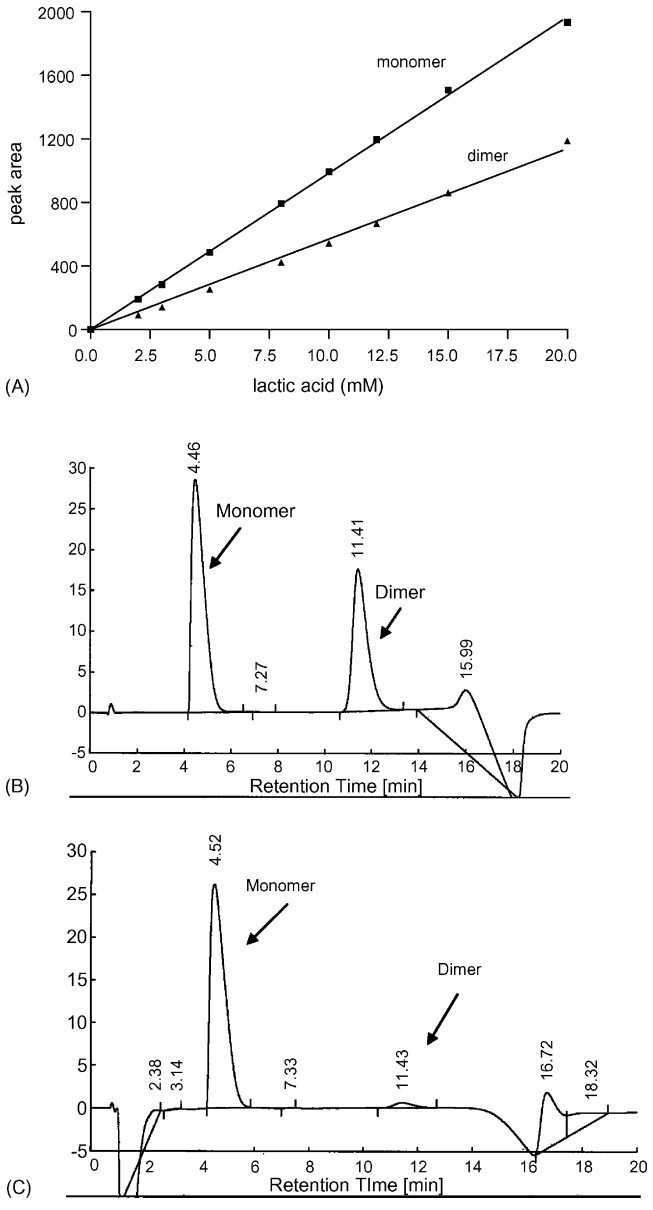

Fig. 5. Analyses using IC-ECD: (A) peak areas of both lactic acid monomer and dimer shown as a function of total lactic acid concentration (monomer basis); (B) an ion chromatogram obtained from a fresh drug product; (C) an ion chromatogram obtained from an aged drug product (25 °C, 16 months).

for 23 days at room temperature (data not shown), together with the autoclaving results, suggest that the hydrolysis of the lactic acid dimer in the drug product advances slowly over a long period of time. This allows for a gradual change in pH, resulting in an increase of a little less than 0.2.

3.4. Ester formation

It is hypothesized that four different esters, including ethyl lactate and three propylene glycol lactates, form in the drug product (Fig. 2B). Ethyl lactate was the first to be analyzed. GC-FID was used for analysis because ethyl lactate is volatile and its pure standard is commercially available. The analytical method was validated for quantification. Fig. 7A illustrates the fine peak obtained from the standard sample at 5.8 min (black arrow), identified as ethyl lactate. When fresh drug product was analyzed, the peak detected was very narrow (data not shown), which indicates that the amount of ethyl lactate present in the sample was very slight. In contrast, when an aged drug product stored at 25 °C for 21 months was analyzed, a distinct peak (black arrow) was detected at 5.8 min (Fig. 7B). The aged drug product was compared with the standard sample and estimated to contain 1.14 mM ethyl lactate. Clearly, ethyl lactate is synthesized in the drug product over time during storage at room temperature. Table 3 summarizes the analytical results obtained at different time points with different production lots. The amounts of ethyl lactate produced depended on the initial lactic acid concentrations, but the reactions at 25 °C equilibrated by the 16-month time point at the latest. At 5 °C, the ester amounts were substantially smaller at the same time points, indicating that ester formation is much slower at the lower temperature.

Propylene glycol lactates were the next to be probed. Since propylene glycol lactates were commercially unavailable, a standard sample was prepared in-house. After propylene glycol was mixed with an equal amount of lactic acid, the mixture was stored at 60 °C for 80 days, after which it was used as a standard sample without purification. As shown in Fig. 7, the standard (Fig. 7C) and an aged drug product (25 °C, 21 months, Fig. 7B) were both analyzed using GC-FID. A comparison of the two chromatograms indicated that unknown peaks (open arrows) were observed at 11.758 and 11.750 min, respectively. Certainly, these peaks represented the same substance. The unknown substance was then analyzed using LC–MS. As presented in Fig. 8A and B, the main peaks on mass chromatograms observed at $m/z = 149$, which corresponds to the molecular weight of a propylene glycol mono-ester, occurred at 18.52 and 18.59 min, respectively. The unknown substance was also analyzed using GC–MS (EI), where the main peaks were observed at around 12.5 min on the total ion chromatograms of both samples (not shown). When the peaks were unfolded to reveal their mass spectra, the same spectral pattern was seen, with the main peak at $m/z = 45$ (Fig. 8C and D). This is expected to occur upon degradation of a propylene glycol mono-ester. The unknown substance was identified as a propylene glycol mono-ester since the standard was prepared using the mixture of lactic acid and propylene glycol, and 148 is the molecular weight of a propylene glycol mono-ester.

It has now been proven that the unknown peak that was detected using GC-FID (Fig. 7B and C, open arrows) represents a propylene glycol mono-ester. The ester found in an aged drug product was then quantified. As stated earlier, the propylene glycol lactate standard was prepared in-house. Since the ester concentration in the standard had not been determined, the effective carbon number (ECN) method was used for quantification (Scanlon and Willis, 1985; Jorgensen et al., 1990) rather than using a calibration curve regressed with the same substance. The ECN method takes advantage of the fact that the detection sensitivity of the FID depends on the kinds of atoms and chemical bonds present in the organic molecules, but not on specific chemical structures. The quantification of the ester was therefore possible by correcting the FID responses obtained using a different organic compound of a known concentration. Table 4

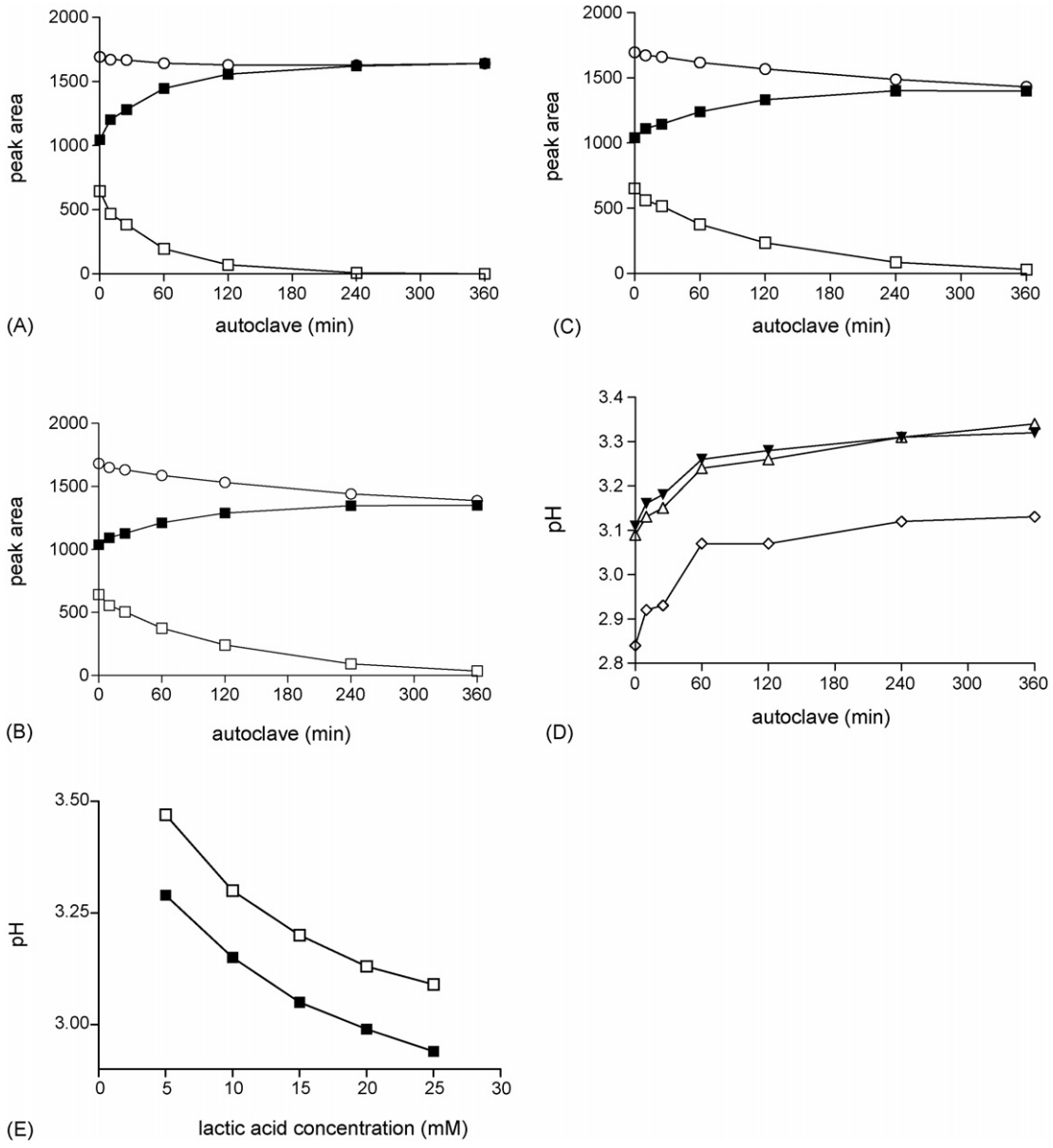

Fig. 6. Hydrolysis of lactic acid dimer and the accompanying pH increases observed under stressed conditions. The lactic acid concentration was 10 mM. The lactic acid was analyzed using IC-ECD: (A) lactic acid aqueous solution (no alcohols); (B) drug product; (C) conivaptan-free solution. The symbols are open square (dimer), closed square (monomer), and open circle (monomer plus dimer), respectively; (D) accelerated pH increases observed in a lactic acid solution (open diamond), a drug product (open triangle), and a conivaptan-free solution (closed inverse triangle); (E) pH values of drug products (40% (w/v) alcohol) containing different concentrations of the acid (monomer basis) observed after 25 min (closed square) and 360 min (open square) autoclave.

summarizes the ECNs of several organic compounds and their peak areas obtained using GC-FID. Fig. 9A plots the peak area as a function of the ECN, which shows good linearity. Fig. 9B demonstrates the ethyl lactate concentration estimated using the ECN method with *n*-octane as a function of that obtained using the calibration method with a pure ethyl lactate standard. There was an excellent correlation between the two methods. *n*-Octane was used for quantification because its ECN was large and the experimental results were highly accurate and precise. Table 3 illustrates the concentrations of the propylene glycol mono-ester found in aged drug products with differing initial lactic acid concentrations. Since it was only barely detectable at the initial time point (data not shown), the ester had to have been synthesized during long-term storage at 25 °C. The amount of ester produced in samples with the same initial concentration was lower when stored at a lower temperature (5 °C). This, again, indicates that the ester was produced during storage, with the result reflecting a slower reaction rate at the lower temperature.

As summarized in Table 3, the proportion of esters that are produced in the drug product compared to the total amount of lactic acid is around 15%, irrespective of the initial lactic acid concentration. This means that 15% of the initial lactic acid was consumed during ester formation, which results in a pH increase of about 0.04, irrespective of the initial pH (Fig. 6E).

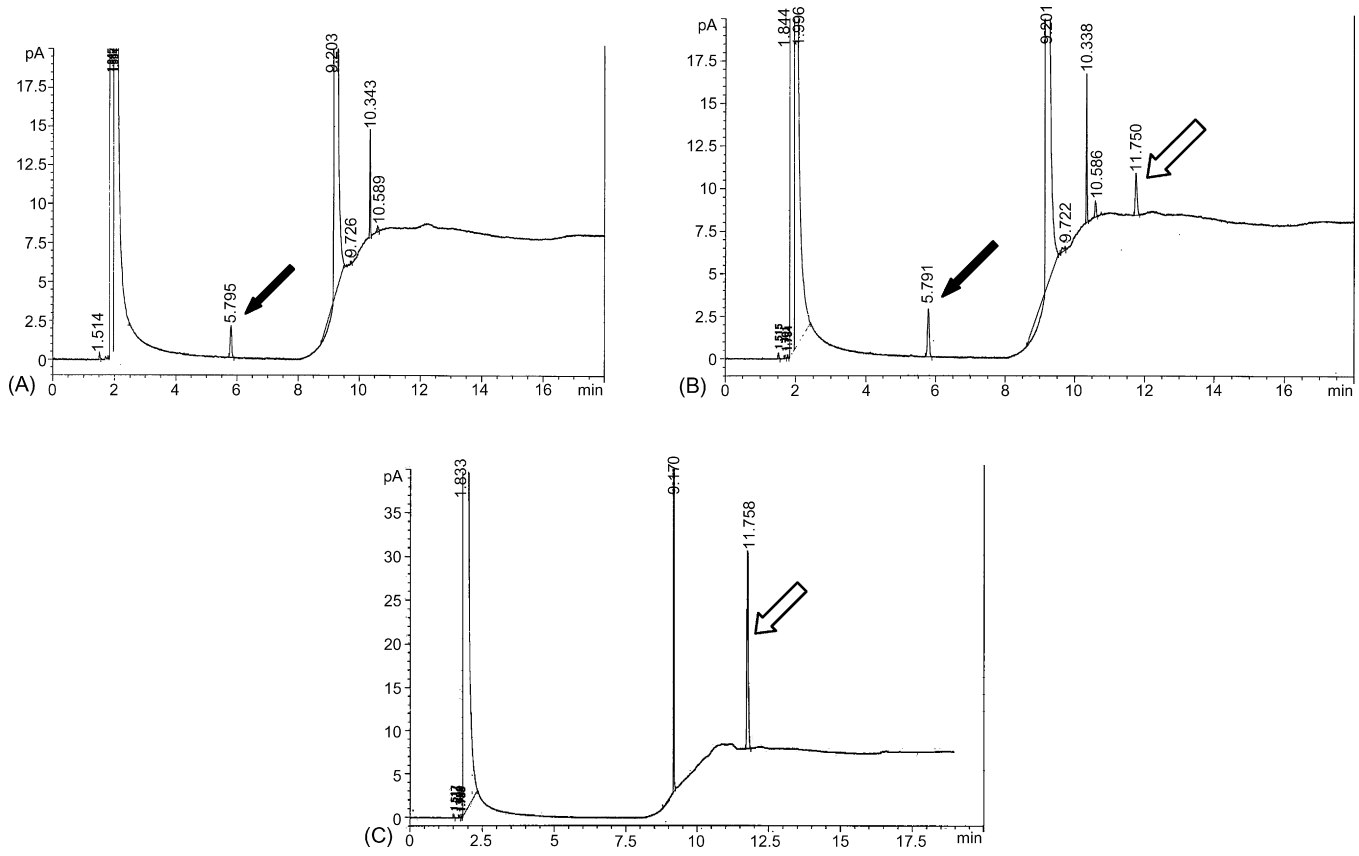

Fig. 7. Analyses of the esters produced in the drug product using GC-FID. The samples were: (A) ethyl lactate pure standard; (B) aged drug product (at 25 °C for 21 months); (C) propylene glycol mono-ester standard prepared in-house, respectively. The black and open arrows indicate ethyl lactate and propylene glycol mono-ester, respectively.

The regression curve obtained with this lactic acid monomer (Fig. 6E, open square) is $Y = 0.7159 \exp(-0.09332X) + 3.021$ ($R^2 = 0.9998$). With initial lactic acid concentrations of 10 and 20 mM, the loss of 15% lactic acid results in pH increases of 0.042 and 0.036, respectively. Thus, the pH increment resulting from ester formation can be estimated as approximately 0.04.

Finally, the production of esters at room temperature over a long period of time suggests that the stressed conditions (autoclaving at 121 °C for 360 min, Fig. 6D) that induced a pH

Table 3
Assay of ethyl acetate and propylene glycol monolactate

Lot	Storage temperature (°C)	Concentration (mM)				Ratio (%) of total lactate to lactic acid (at 21 months)
		Lactic acid	Ethyl lactate		Propylene glycol monolactate	
		Initial	17M[a]	21M[a]	21M[a]	
A	25	5.52	0.40	0.42	0.33	13.6
B	25	12.47	1.15	1.14	0.97	16.9
C	25	7.56	0.74	0.76	0.59	17.9
D	25	7.56	0.73	0.77	0.61	18.3
Lot	Storage temperature (°C)	Concentration (mM)				Ratio (%) of total lactate to lactic acid (at 19 months)
		Lactic acid	Ethyl lactate		Propylene glycol monolactate	
		Initial	16M[a]	19M[a]	19M[a]	
E	25	8.46	0.82	0.80	0.64	17.0
F	25	6.04	0.56	0.56	0.44	16.6
E	5	8.46	0.37	0.42	0.28	8.3
F	5	6.04	0.23	0.28	0.18	7.6

[a] Months.

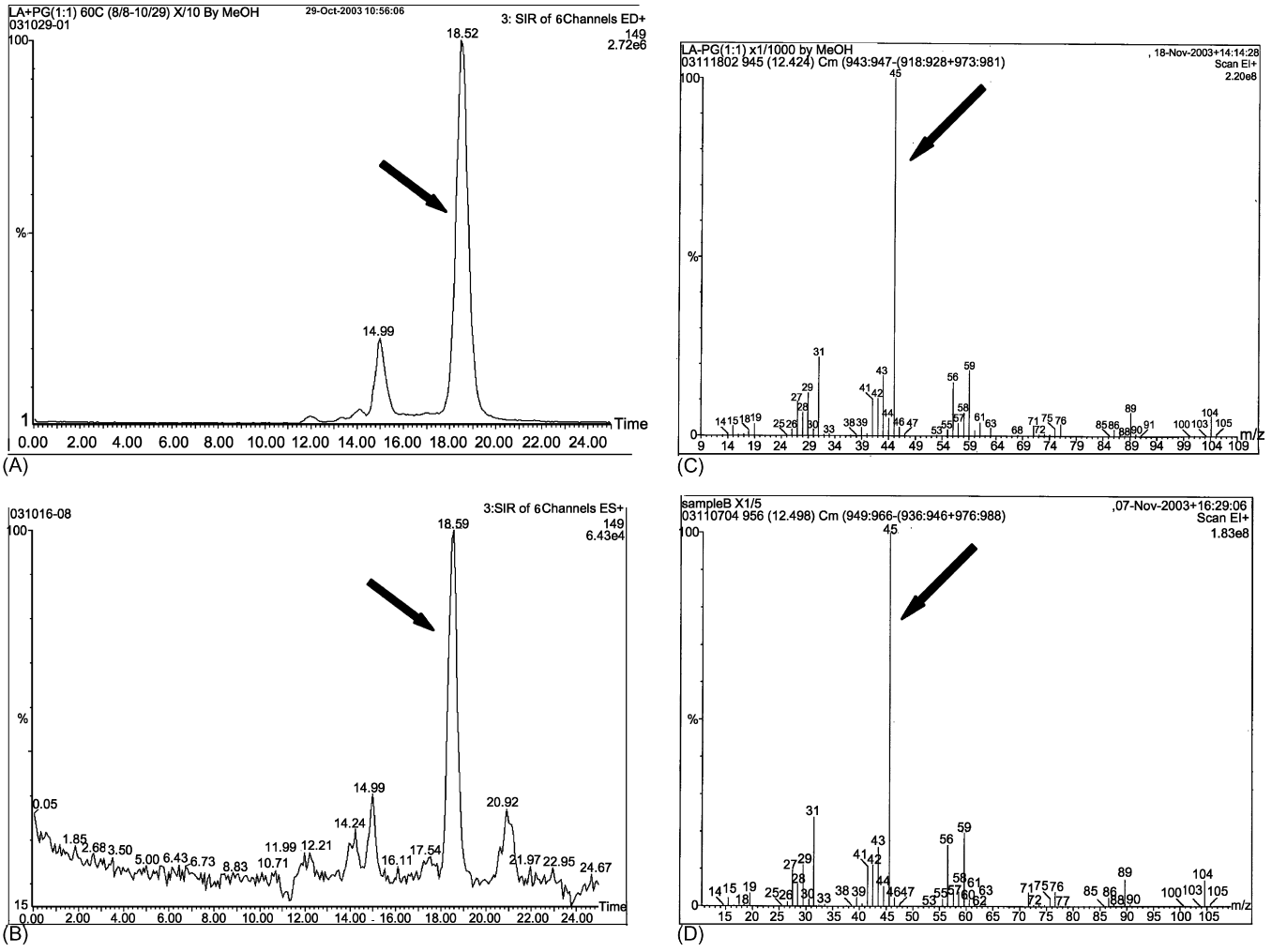

Fig. 8. Analyses of a propylene glycol mono-ester: (A, B) mass chromatograms obtained using LC–MS; (C, D) mass spectra obtained using GC–MS; (A, C) standard prepared in-house; (B, D) aged drug product (25 °C for 21 months).

increase of a little less than 0.2 should also cause ester formation. In actuality, the pH increase caused by ester formation under stressed conditions was estimated to be less than or around 0.04, possibly because equilibrium was reached rapidly (within 360 min). This means that the pH increment (a little less than 0.2) observed under stressed conditions (Fig. 6D) is due in part to ester formation. This suggests that the total pH increase is less than the simple sum of 0.2 (Fig. 6D) and 0.04 (Fig. 6E, open square).

Table 4
Compounds evaluated for ECN-response relationship

Compound	Formula	ECN	Peak area[a]
2-Methoxy ethanol	$C_3H_8O_2$	1.60	306.54327
Propylene glycol	$C_3H_8O_2$	1.65	371.42535
Ethyl formate	$C_3H_6O_2$	1.95	305.48568
Methyl acetate	$C_3H_6O_2$	1.95	328.63403
Triethylene glycol	$C_6H_{14}O_4$	3.20	548.00078
3-Methyl-1-butanol	$C_5H_{12}O$	4.40	761.26890
n-Octane	C_8H_{18}	8.00	1181.90731

[a] Corrected for sample weight.

4. Discussion

4.1. Limited pH increases in the drug product caused by aging

The pH increases observed in the drug product were found to be caused by the hydrolysis of the lactic acid dimer (approximately 0.15), and by the formation of esters between lactic acid and the alcohols (approximately 0.04). The sum of these values agrees well with the maximum pH increases observed in the drug products, although the pH increase profiles varied somewhat, depending on the production lot. The maximum pH increases occurred independent of the initial concentration of lactic acid. The conclusion has thus been reached that the pH increase observed with the drug product is, for the most part, caused by the hydrolysis of the lactic acid dimer along with the ester formation between lactic acid and the alcohols. The changes caused by these two processes created conditions sufficient to produce the pH increase observed. If any other event had contributed to the change in pH, a significantly higher increase would have been observed. The pH value almost levels off after

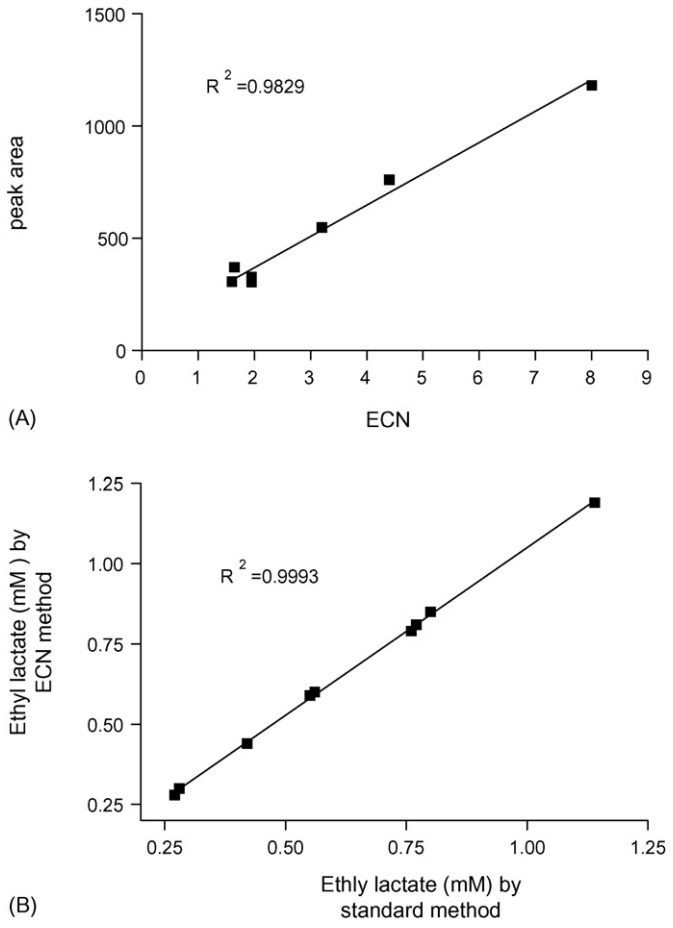

Fig. 9. Validity of the effective carbon number (ECN) method: (A) linearity between the peak area and ECN; (B) linearity between the standard and ECN methods.

16 months at 25 °C, irrespective of the production lot. At this point, the lactic acid dimer has hydrolyzed into the monomer almost completely, and ester formation has equilibrated, with the remaining amount of esters being proportionate to the initial lactic acid concentrations. Accordingly, a limited pH increase is now established on a molecular basis, thus ensuring that the pH will not increase enough to produce precipitates over time. If the pH is adjusted to 3.0 during manufacturing, there is hardly any risk of precipitate production.

4.2. The pK_a of the lactic acid monomer and oligomers

The lower pK_a of the lactic acid dimer compared to that of lactic acid monomer (Table 2) can be explained as follows: when two lactic acid molecules react with each other to form a lactic acid dimer, the α-hydroxyl group of the lactic acid monomer turns into an ester bond. The carbon atom in the carbonyl group in the ester bond is slightly positive (δ^+), which attracts electrons from the oxygen atom of the α-hydroxyl group of the monomer, which in turn renders the oxygen atom all the more electron-attractive. The oxygen atom attracts electrons from the adjacent α-carbon, thus reinforcing its inductive effect (through the α-carbon) on the carboxyl group of the monomer and stabilizes it in a deprotonated form. The fact that the pK_a of propionic acid (4.88) is much larger than that of lactic acid clearly indicates that the electron-attractive property of the α-hydroxyl group has a significant effect on the pK_a of lactic acid. The inductive effect prevails in the lactic acid dimer, but occurs only slightly in the trimer. Therefore, there is no difference in pK_a values of the trimer and tetramer (Table 2).

4.3. Equilibrium and kinetics of the hydrolysis of lactic acid oligomers

Equilibrium and the kinetics of lactic acid oligomer hydrolysis have been investigated previously by Lucke and Gopferich (2003). Lactic acid, a fermentation product, is approximately 90% pure (w/w), and the proportion of dimer to total lactic acid (monomer basis) was determined to be 33.1% using a titration method, assuming that all the lactic acid oligomers were the dimer. In fact, the majority of the lactic acid oligomers was confirmed to be the dimer using HPLC, which is supported by the findings of this study as well (Fig. 3). Since the pK_a values of the trimer and tetramer were smaller than those of the dimer and monomer (Table 2), the ECD sensitivity of the former lactic acids should be higher than that of the dimer or monomer due to the enhanced ionization. This leads to an amplified peak area for each molecule of trimer- and tetramer, rendering the observed peak areas relatively large, in spite of the small amount (Fig. 3A). Lucke and Gopferich (2003) also demonstrated that the hydrolysis equilibrium of lactic acid oligomers is drastically dependent on concentration. Their results stated that the ratio of lactic acid oligomers to total lactic acid (monomer basis) at equilibrium at room temperature was 3.2% at 50% (w/w) lactic acid, 0.4% at 10% (w/w) lactic acid, and 0.1% at 5% (w/w) lactic acid, as compared to 33.1% (w/w) at 90% lactic acid. In a 10 mM lactic acid solution (about 0.09% lactic acid (w/w)), which was the concentration used in this study, the dimer is almost completely hydrolyzed at equilibrium at room temperature (Fig. 6A). Thus, dilution with water results in dimer hydrolysis. However, Lucke and Gopferich (2003) stated that the hydrolysis kinetics were very slow. It took 30 h for equilibrium to occur in a 10% (w/w) lactic acid solution, even at temperatures as high as 90 °C. Hydrolysis was even slower at room temperature; it took 30 days for 50% (w/w) lactic acid to equilibrate, and 50 days for 1% (w/w). Dilution with water slows the hydrolysis at room temperature. In a 10 mM lactic acid solution (0.09% w/w), it is expected that the lactic acid dimer would hydrolyze much more slowly, and equilibrate after months. In actuality, the pH of 10 mM lactic acid solution remained almost unchanged after 23 days (data not shown), which confirms that the hydrolysis of the lactic acid dimer is extremely slow. These lines of evidence agree completely with the pH increase profiles observed with the drug product over months (Fig. 1).

4.4. Equilibrium and kinetics of the ester formation reactions

As shown in Table 3, the ester formation reactions were at equilibrium at the 16-month time point. Fig. 10 demonstrates the ester concentrations as a function of initial lactic acid con-

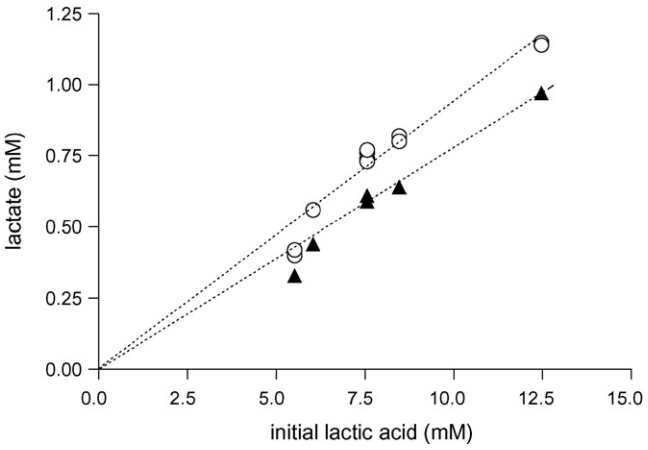

Fig. 10. Ester concentrations obtained after storage at 25 °C as a function of initial lactic acid concentrations. Ethyl lactate (open circle). Propylene glycol mono-ester (closed triangle).

centration. Good linearity was obtained with both ethyl lactate and the propylene glycol mono-ester, which agrees with the conclusion that equilibrium had been reached by 16 months. Using the slopes of the regression lines and the concentrations of the water and the alcohols, the equilibrium constants (K) for ester formation were calculated to be 1.63 for ethyl lactate and 0.81 for the propylene glycol mono-ester. These values are comparable to that of ethyl acetate ($K = 4$) (Roberts and Caserio, 1964), and the differences are less than one order of magnitude. It has been established that ester formation under acidic conditions occurs due to Fisher's nucleophilic substitution and that steric hindrance by the bulky structures of acid and/or alcohol slows the reaction rate (Roberts and Caserio, 1964). It is therefore possible that the somewhat smaller equilibrium constants are related to the facts that propylene glycol is bulkier than ethanol (formation of propylene glycol mono-ester) and that lactic acid is bulkier than acetic acid (formation of both ethyl lactate and propylene glycol mono-ester). The carbon atom of the carbonyl group of the ester bond is more positive (δ^+) than that of ethyl acetate because of the inductive attraction of the electrons of the lactic acid α-hydroxyl group. This leaves the carbon atom more vulnerable to nucleophilic attack by a water molecule, thus promoting the hydrolytic reaction. In any case, the ester formation reactions can reach equilibrium after 16 months at room temperature, and the pH increases caused by the reactions level off at a certain value. Those reactions can occur even at 5 °C. It has been suggested previously that ester formation can advance even in a solid phase at room temperature over a longer period of time (Mizuno et al., 2005). These results indicate that, in an injectable solution, molecular mobility sufficient to cause ester formation is maintained even at room temperature.

4.5. Other species of propylene glycol lactate

In theory, propylene glycol lactate has at least two different mono-esters and one di-ester because of its two different hydroxyl groups. One mono-ester was detected and quantified using GC-FID-ECN, although there was no experimental evidence as to which mono-ester it was. It is highly likely that only one of the possible mono-ester species was produced in the solution because they are both skeletal isomers that can be separated from one another using gas chromatography, but only a single peak was observed. If both mono-esters had been produced, two distinct peaks would have been observed. These peaks would have been in close to each other since the esters have similar physical properties. It is probable that, since esterifying reactions are affected by steric hindrances, the hydroxyl group bound to the 1-carbon reacted with lactic acid. Remarkably, even though the di-ester was not detected, narrow peaks of propylene glycol lactates with three or four lactic acid molecules were detected. These species may contain the lactic acid dimer bound directly to propylene glycol. In any case, with the exception of the mono-ester species quantified using the ECN method, the contribution of propylene glycol lactates toward the consumption of lactic acid was trivial.

4.6. Contribution of other mechanisms toward the increase in pH

Hydrolysis and ester formation with lactic acid constitute sufficient conditions for the pH increase observed over time. This leads to the conclusion that no conivaptan-related or other possible events made any significant contribution toward the pH increase. If conivaptan gradually protonates over time, the pH of the solution will increase. However, this is unlikely to occur because the solution is uniform and composed of a single phase. In such a chemical environment, acid–base reactions are reversible and instantly reach equilibrium. If a complex consisting of lactic acid and conivaptan dissociates over time, the pH of the solution will increase, but this is unlikely to occur. Incidentally, there were no ultraviolet shifts observed between the conivaptan spectra of fresh and aged drug product. It is generally known that alkali elution occurs when ampoules or vials are used during formulation. Alkali elution actually was observed in the conivaptan drug product, but it did not occur to a degree that would significantly affect pH.

5. Conclusion

In this study, we investigated the molecular basis of pH increases observed in an injectable solution over time, and successfully determined an upper pH limit. The pH increases were caused by the hydrolysis of a lactic acid dimer and the ester formation between lactic acid and ethanol and/or propylene glycol. The erroneous assumption that excipients are chemically inert species is often made. Chemical reactions occurring among excipients are prone to be overlooked because the products are not classified as related substances detectable in quality control tests. However, product quality may be at risk if the stability of a drug substance is adversely affected by a change in pH resulting from latent reactions among excipients. It should therefore be emphasized that the most important issues addressed by this study are: (1) the evaluation of risk made by determining the upper limit of the pH increase, and (2) the elucidation of the physicochemical background, but not in the magnitude, of the

pH increase itself. It is important to establish a quality management system in which scientific analyses can assure product quality by determining the risk factors that are not explicitly evaluated by quality control testing. An increasing need will arise for drug development based on both chemical and physicochemical analyses oriented toward risk evaluation.

References

Akers, M.J., 2002. Excipient–drug interactions in parenteral formulations. J. Pharm. Sci. 91, 2283–2300.

Bundgaard, H., 1990. Drug allergy: chemical and pharmaceutical aspects. In: Florence, A.T., Salole, E.G. (Eds.), Formulation Factors in Adverse Reactions. Wright, London, pp. 23–55.

Fu, K., Pack, D.W., Klibanov, A.M., Langer, R., 2000. Visual evidence of acidic environment within degrading poly(lactic-co-glycolic acid) (PLGA) microsphere. Pharm. Res. 17, 100–106.

Fuet, R.C., Lidgate, D.M., Whatley, J.L., MaCullough, T., 1987. The biocompatibility of parenteral vehicles: in vitro/in vivo screening comparison and the effect of excipients on hemolysis. J. Parenter. Sci. Technol. 41, 164–168.

Hasegawa, K., Hashi, K., Okada, R., 1983. Physicochemical stability of pharmaceutical phosphate buffer solutions. V. Precipitation behavior in phosphate buffer solutions. J. Parenter. Sci. Technol. 37, 38–45.

Holten, C., Muller, A., Rehbinder, D., 1971. Lactic Acid: Properties and Chemistry of Lactic Acid and Derivatives. Verlag Chemie, Weinheim.

Jorgensen, A.D., Picel, K.C., Stamoudis, V.C., 1990. Prediction of gas chromatography flame ionization detector response factors from molecular structures. Anal. Chem. 62, 683–689.

Lucke, A., Gopferich, A., 2003. Acylation of peptides by lactic acid solutions. Eur. J. Pharm. Biopharm. 55, 27–33.

Mizuno, M., Hirakura, Y., Yamane, I., Miyanishi, H., Yokota, S., Hattori, M., Kajiyama, A., 2005. Inhibition of a solid phase reaction among excipients that accelerates drug release from a solid dispersion with aging. Int. J. Pharm. 305, 37–51.

Roberts, J.D., Caserio, M.C., 1964. Basic Principles of Organic Chemistry. W.A. Benjamin Inc., New York.

Scanlon, J.T., Willis, D.E., 1985. Calculation of flame ionization detector response factors using the effective carbon number concept. J. Chromatogr. Sci. 23, 333–340.

Stella, V.J., 1986. Chemical and physical basis determining the instability and incompatibility of formulated injectable drugs. J. Parenter. Sci. Technol. 40, 142–163.

Sweetana, S., Akers, M.J., 1996. Solubility principles and practices for parenteral dosage form development. PDA J. Parenter. Sci. Technol. 50, 330–342.