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Report

An Unexpected pH Effect on the Stability of Moexipril Lyophilized Powder

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Because of the limited stability of moexipril (RS-10085; **1**) in aqueous solution, lyophilized parenteral formulations were evaluated as a function of pH in this study. In general, the lyophilized powder of **1** showed about two orders of magnitude less reactivity at 50°C than in aqueous solution at pH values below 3 or above 6. At pH 5.1, however, the lyophilized powder had maximum reactivity, with the rate actually comparable to that observed in aqueous solution. When the distribution of the two major products, diketopiperazine (DKP) **2** and ester hydrolysis analogue **3**, was compared to the observed kinetics as a function of pH, it was clear that removal of water via lyophilization suppressed the spontaneous k_1 cyclization process, the spontaneous k_3 hydrolysis process, and the specific base-catalyzed k_4 hydrolysis process. The overall spontaneous k_2 cyclization process, however, was not affected by lyophilization. The latter result is accounted for by the increased equilibrium constant for the formation of the tetrahedral intermediate, To, as a result of lyophilization. This study demonstrates that stability data in solution can not be used for predicting the stability of moexipril in lyophilized powder form.

KEY WORDS: lyophilization; stability; pH effect; moexipril.

INTRODUCTION

The development of a parenteral formulation of a drug that is unstable in solution often requires the use of lyophilized powder in order to achieve acceptable shelf life (1). The choice of the drug solution(s) to be lyophilized is usually determined by drug stability and solubility requirements and limitations. For example, sodium prasterone sulfate is lyophilized from aqueous solutions with pH values above 3.3 to avoid acid-catalyzed hydrolysis (2). Heroin is lyophilized from an aqueous solution of pH 5, where maximum aqueous stability is observed (3). Lixazinone (RS-82856) (4) and Rhinoxin (NSC-332598) (5) are lyophilized from t-butanol/water solutions for drug solubility enhancement. The acetate salt of morphine is chosen for lyophilization because of its better aqueous solubility than the sulfate salt (6).

Moexipril (RS-10085; **1**) is an *N*-carboxyalkyl dipeptide angiotensin converting enzyme (ACE) inhibitor being developed for treatment of antihypertension and congestive heart failure (7). In order to compare oral versus intravenous bioavailability of moexipril, a 5.0 mg/ml parenteral formulation is required for use in clinical trials. The aqueous stability of **1** has been reported in a previous account (7). It was found that the rate of degradation of **1** as a function of pH followed the rate law:

$$\text{Rate} = k_{obs}(1)$$
$$k_{obs} = k_1 f_{1c} + k_2 f_{1z} + (k_3 + k_4 a_{OH}) f_{1a} \quad (1)$$

where f denotes the fraction of each species and a_{OH} is the activity of the hydroxide ion. The product analysis in aqueous solution showed that both k_1 and k_2 processes led primarily to the diketopiperazine (DKP) **2**, and both k_3 and k_4 processes led primarily to the ester hydrolysis product, **3** (Scheme I). All four kinetic processes were found to be fairly rapid, resulting in an estimated 25°C t_{90} (time to reach 90% remaining) of only ~2 months at the pH of maximum stability, pH 4.5. The undesirable aqueous stability therefore promoted the development of a lyophilized formulation.

EXPERIMENTAL

Materials

Moexipril hydrochloride (**1**), moexipril diketopiperazine (**2**), and hydrolysis product (**3**) were obtained from the Institute of Organic Chemistry, Syntex Research. High-performance liquid chromatographic (HPLC)-grade acetonitrile and tetrahydrofuran were obtained from American Burdick and Jackson and nanopure water was used to prepare the mobile phase. Mannitol was obtained from Emulsion Engineering and all other chemicals were analytical grade and used as received.

Solubility Measurement

The solubility of **1** in water as a function of pH was determined by adding an excess amount of drug to ~6 ml of

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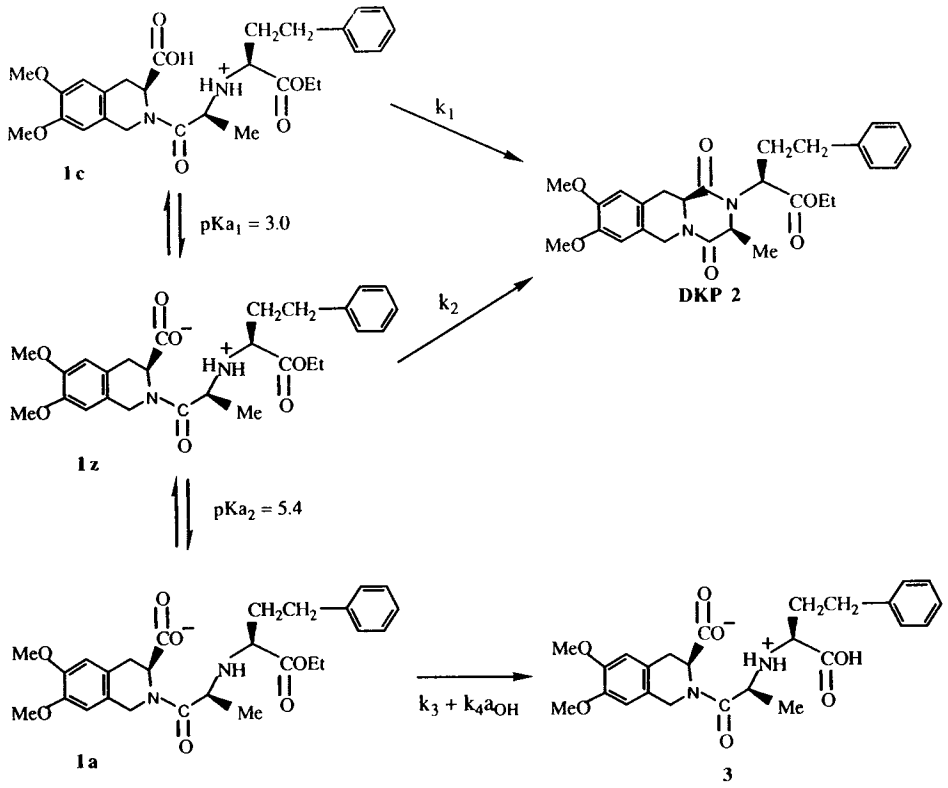


Scheme I

deionized water at 25°C and stirring vigorously for at least 30 min. Aqueous HCl or KOH solutions were added as needed to give the desired pH. After each addition, equilibration was attained after 30 min of stirring. A 0.5-ml aliquot was withdrawn and filtered through a 0.45-μm Millipore filter and the pH of the filtrate was measured using a Radiometer PHM64 Research pH Meter. The filtrate was then diluted with water and assayed by HPLC.

Lyophilization Studies of 1

Six batches of 5.0 mg/ml moexipril solutions containing 5% mannitol at pH 2 to 11 were prepared by weighing and transferring the required amount of moexipril, mannitol, and buffer (0.01 *M* sodium acetate, potassium phosphate, or sodium borate) to the vessel containing 80% of the required water for lyophilization. After all the compounds were completely dissolved, the pH of the solutions was adjusted with NaOH or HCl solution to the desired value. The solutions were then brought to final volume with water and a final pH adjustment was made, if necessary. The solutions were then filtered through 0.22-μm filters and 4 ml of the filtrates was filled into 10-ml type I glass vials. The vials were partially covered with lyo-stoppers and placed on the same tray for lyophilization (lyophilizer Edwards SO_4, Edwards High Vacuum Institute, NY). The lyophilization was performed according to the following procedure: After a 30-min equilibration period at 5°C, the shelf temperature was reduced to −40°C over 1 hr. The freezing temperature of −40°C was maintained for a minimum of 2 hr before vacuum (100 μm) was initiated, whereupon the shelf temperature was raised to a terminal drying temperature of 25°C (9.3°C/hr). Total cycle time was 48 hr. After completion of the cycle, the vials were sealed with aluminum caps and stored at different temperatures for physical and chemical stability evaluations.

Water Content of Lyophilized Powder

To evaluate the water content of the lyophilized powder, two vials from each pH were analyzed by an Aquastart C2000 titrator (EM Scientific, Cherry Hill, NJ). The vials were weighed before and after reconstitution with 10 ml methanol. Approximately 1 ml of the sample was then withdrawn from each individual vial, weighed, and injected into the titrator. After recording the reading of the titrator, the percentage water content of the vial was calculated.

Analytical Method

Sample preparation consisted of reconstituting the lyophilized powder with 4 ml of water or 1:1 water:methanol. The pH was measured and a 250-μl aliquot of the solution was diluted to 25 ml with water to give a ~50 μg/ml drug solution that was analyzed by HPLC. The HPLC method employed an Altex Ultrasphere-ODS 5-μm column and a mobile phase of (55/35/10) 0.05 *M* ammonium phosphate buffer, pH 2.0/acetonitrile/tetrahydrofuran with a flow rate of 1.0 ml/min. Detection was selected at 220 nm with a sensitivity of 0.1 AUFS. The HPLC equipment consisted of an Altex Model 110A pump, a Micromeritics Model 728 autosampler, a Kratos Model 757 variable-wavelength detector, and a Spectra-Physics 4100 computing integrator. The relative molar HPLC response factors for 1, 2, and 3 using

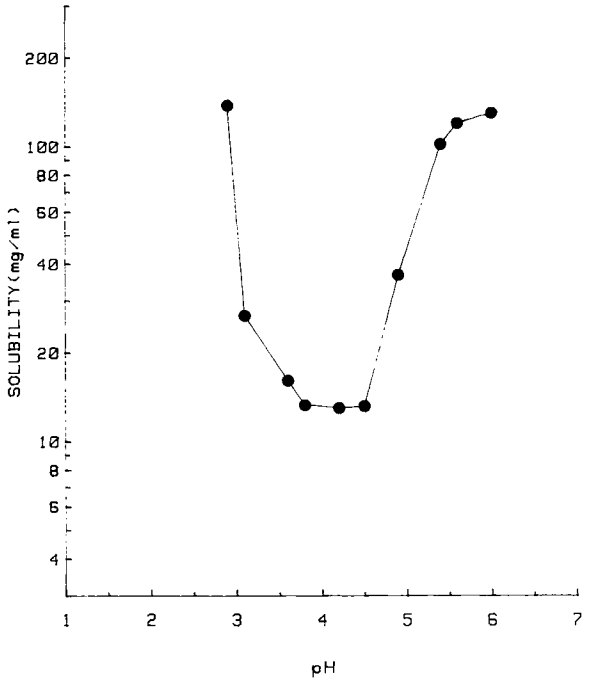


Fig. 1. Aqueous solubility of 1 as a function of pH at 25°C.

authentic samples were determined to be 1:0.82:0.43, respectively.

RESULTS AND DISCUSSION

Aqueous Solubility

The aqueous solubility of 1 was determined at 25°C as a function of pH. Inspection of the results summarized in Fig. 1 reveals that the drug has a U-shaped pH–solubility profile with a minimum solubility of 13 mg/ml at pH 4.2. The pK_a values of the carboxylic acid and amine functional groups of 1 have been determined to be 3.0 and 5.4, respectively (7) (Scheme I). Thus, in the pH region of 3.0–5.4, the drug is mainly in its zwitterionic state (**1z**) with net zero charge and therefore is the least soluble. At pH's below the pK_{a_1} value of 3.0 the drug is positively charged (**1c**), and at pH's above the pK_{a_2} value of 5.4 the drug is negatively charged (**1a**). Both charged species have solubilities greater than 100 mg/ml (Fig. 1). These data indicate that solubility should not pose any problem for lyophilizing a targeted 5.0 mg/ml solution of 1.

Lyophilization Studies

The formulation components of various lyophilization solutions of 1 are summarized in Table I. For isotonicity and integrity of the final product, 5% mannitol was included in each formulation. The pH values of the bulk solutions prior to lyophilization were controlled by the buffer agents or adjusted with HCl or NaOH solutions (Table I). After lyophilization, all formulations yielded good cakes and could be readily reconstituted with sterile water. The moisture content of the lyophilized powder was determined by Karl Fisher method and was found to be less than 1% for each formulation (Table I). The pH value of the reconstituted solution changed slightly from that of the bulk solution in each case and the reason is not obvious as the HPLC analysis of the former solution revealed no apparent degradation (not shown).

The stability of the lyophilized powder of 1 was evaluated at 50°C by a stability-specific HPLC method. Semilog plots of percentage drug remaining versus time at various pH values are shown in Fig. 2. The initial rate method was used to analyze the observed kinetics because (i) drug degradation in most pH regions was less than 20% in the 2-month duration of the study and (ii) when the degradation was severe, such as at pH 5.1, the kinetics were not first-order. A plot of the log of the pseudo-first-order rate constants of the lyophilized powder using the initial rate method as a function of pH is shown in Fig. 3. The log (rate)–pH profile of 1 in aqueous solution extrapolated from the previous study (7) at 50°C is also plotted in Fig. 3 for comparison. To our surprise, the lyophilized powder yielded the minimum stability at pH 5.1, close to where the maximum stability in aqueous solution was observed (pH 4.5) (17). *Furthermore, the degradation of the lyophilized powder at pH 5.1 actually had a rate comparable to that in aqueous solution.*

In an effort to understand the unexpected kinetics described above, the material balance of the degradation was studied using the HPLC response factor method (see Experimental). In all cases studied, DKP (2) and hydrolysis product 3 were the two major degradation products in the solid

Table I. Formulation of Moexipril Solutions for Lyophilization

Components	Formulation					
	A	B	C	D	E	F
Moexipril, 1	5 mg	5 mg	5 mg	5 mg	5 mg	5 mg
Mannitol	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
Sodium acetate	—	0.01 *M*	0.01 *M*	—	—	—
Potassium phosphate	—	—	—	0.01 *M*	—	—
Sodium borate	—	—	—	—	0.01 *M*	—
pH adjusted with HCl or NaOH	2.0	4.5	6.0	7.5	9.0	11.0
Water for injection q.s. to	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
pH of solution before lyophilization	2.0	4.5	6.0	7.5	9.2	11.2
pH of reconstituted solution	2.6	5.1	6.7	7.4	8.0	10.2
% water content of lyophilized powders	0.19	0.66	0.35	0.41	0.88	0.63

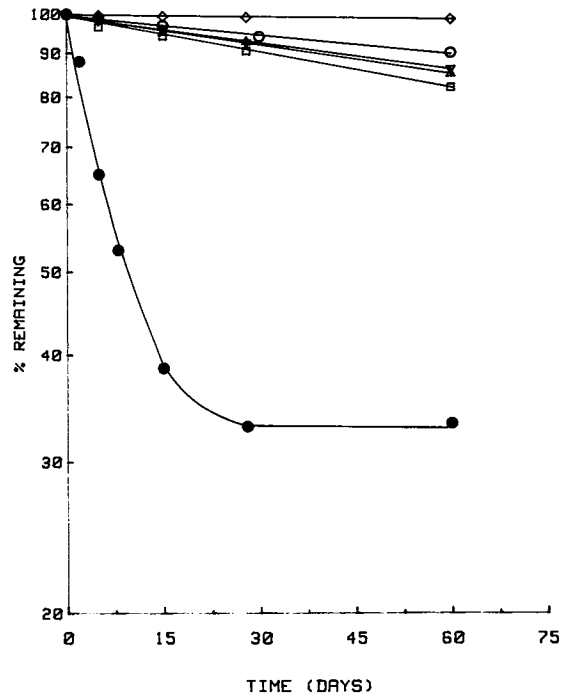


Fig. 2. Semilog plots of percentage drug remaining versus time for the degradation of lyophilized powder of 1 at 50°C and pH 2.6 (□), pH 5.1 (●), pH 6.7 (▽) pH 7.4 (○), pH 8.0 (◇), or pH 10.2 (△).

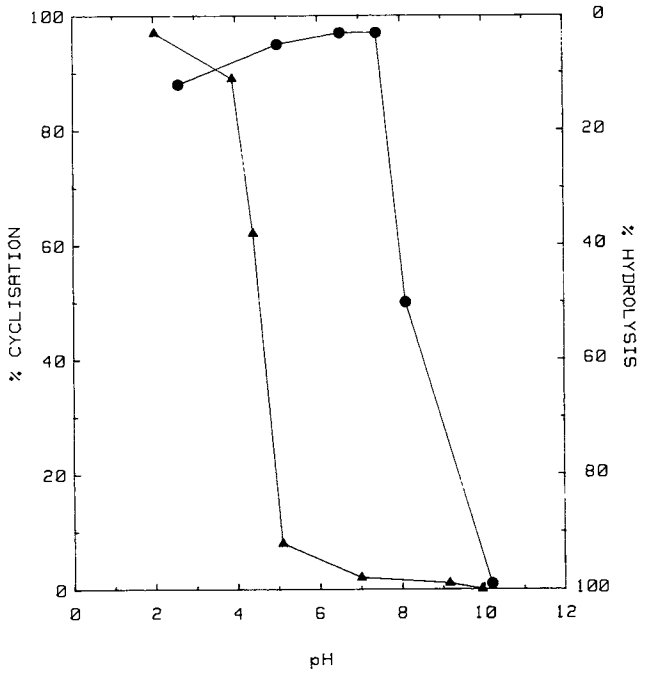


Fig. 4. The distribution of cyclization versus hydrolysis of the degradation of 1 at 50°C in lyophilized powder form (●) and in aqueous solution (▲).

state. The distribution of these two products as a function of pH is compared to that obtained in aqueous solution in Fig. 4. Thus, similar to the reaction in solution, DKP (2) is the major product for the lyophilized powder at pH values below 3. The rate of DKP (2) formation in the solid state at pH values below 3, however, is about two orders of magnitude slower than that in solution (Fig. 3), indicating the suppression of the spontaneous k_1 cyclization process by lyophilization.

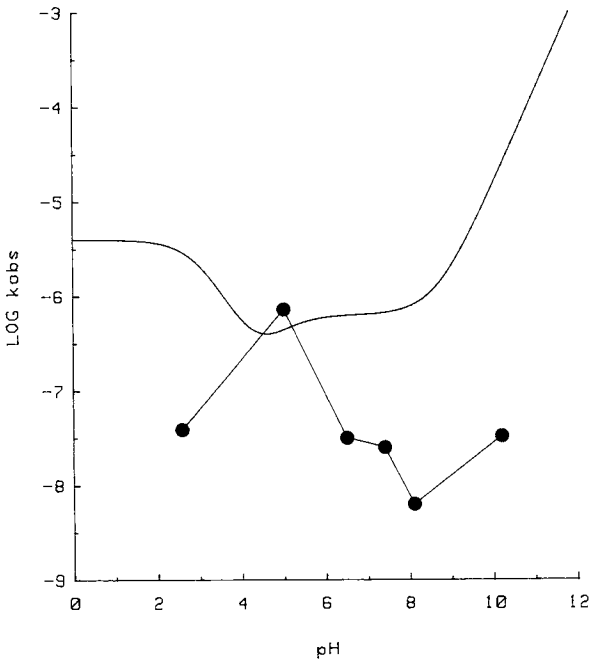


Fig. 3. Log(rate)–pH profiles for the degradation of 1 at 50°C in lyophilized powder form (line segment with data points) and in aqueous solution (solid curve). Standard deviations of the rate constants are ~15%.

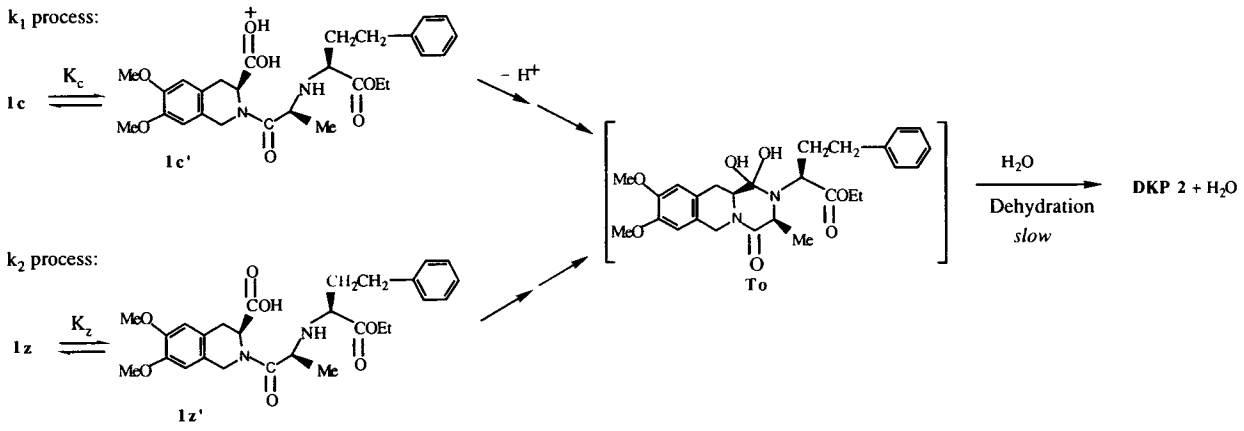
In aqueous solution from pH 4 to pH 8, product distribution indicates that both the spontaneous cyclization to DKP (2) (k_2) and hydrolysis processes to 3 (k_3) are contributing to the degradation of 1, with the former process favored at low pH's and the latter process at high pH's (Fig. 4). In the solid state, however, DKP (2) continues to be the chief product even at pH 8.2, clearly demonstrating that hydrolysis to 3 (k_3), but not the cyclization pathway (k_2), is suppressed by lyophilization.

As that in aqueous solution, the hydrolysis product 3 becomes the predominant product in the solid state at pH values above 8, indicating the emergence of the specific base-catalyzed hydrolysis process (k_4). Comparison of the rate in the solid state to that in aqueous solution at pH values above 8 (Fig. 3) shows a reduction in the k_4 process by two orders of magnitude after lyophilization.

Mechanisms

Solvolysis reactions such as hydrolysis in the solid state are believed to occur only in the adsorbed moisture layer (1). Since the drug is always saturated in this layer, the available water controls the rate of the reaction. This is why both the spontaneous (k_3) and the specific base-catalyzed hydrolysis (k_4) processes [Eq. (2)] of 1 were found to be suppressed by lyophilization.

$$\text{RCOOEt} + \text{H}_2\text{O}\ k_3,\ k_4\ a_{\text{OH}}\ \text{RCOOH} + \text{EtOH} \quad (2)$$



Scheme II

The mechanism of cyclization in solution involves initially the tautomers Ic′ and Iz′ for the k_1 and k_2 processes, respectively (7), because the ammonium groups in cation Ic and zwitterion Iz are not nucleophilic. Both tautomers, Ic′ and Iz′, can then undergo a series of reactions leading to a common tetrahedral intermediate To, which dehydrates in a rate-determining step to yield the final product, DKP (2) (8).

$$\text{rate} = k_{obs}\,[\text{To}][H_2O] \qquad (3)$$

The lyophilized powder of 1 was found to have reactivity comparable to that in solution at pH ~5 (Fig. 3) with DKP (2) as the sole product. This indicates that the k_2 cyclization process (Scheme I) occurred not in the adsorbed moisture layer but rather in the solid state. One possible reason could be that lyophilization significantly destabilized zwitterion Iz by reducing the solvation energy provided by water to the positive and negative charges in Iz. The equilibrium process (K_Z) is thus shifted to favor the uncharged tautomer Iz′, resulting in the overall higher equilibrium concentration of the tetrahedral intermediate, To, in the lyophilized powder (Scheme II). The rate-determining dehydration step of To, on the other hand, requires water as a catalyst [Eq. (3)] even though cyclization generates one equivalent of water. The increase in [To] and the decrease in [H_2O] must effectively compensate each other, as no decrease in reactivity was observed in lyophilized powder at pH ~5.

At pH values above 5, both Iz and Iz′ deprotonate to anion Ia, which will not undergo cyclization because of the carboxylate anion does not have a good leaving group. This and the fact that the k_3 hydrolysis process is suppressed by lyophilization provide reasons for the observed rate decrease from pH 5 to pH 8 in the solid state even though DKP (2) is the major product (Fig. 4).

Interestingly, the cyclization process at pH 5.1 appeared to be completely suppressed at the advance stage of the degradation (33% drug remaining) (Fig. 2), suggesting that some moisture was still required at the site of reaction. This is again consistent with the mechanism proposed in Scheme II. Thus, when surface water is not available for the reaction in the inner layers, the dehydration of the tetrahedral intermediate, To, becomes impossible.

Finally, at pH ~2, lyophilization would destabilize both Ic and Ic′ by reducing the solvation energy they enjoy in aqueous solution. Since both Ic and Ic′ are singly charged, the destabilization of these two species may be similar. This indicates that the equilibrium constant K_c may not be significantly affected by lyophilization. Thus, whether the k_1 cyclization process occurred in the adsorbed moisture layer or in the solid state, it would be suppressed by lyophilization.

CONCLUSIONS

Removal of water by lyophilization was found to have a dichotomous effect on the k_2 cyclization process, which resulted in unexpected reactivity of 1 in the solid state. Nonetheless, due to the suppression of the hydrolysis process by lyophilization, neutral or slightly basic lyophilized powder (pH > 6) of 1 could yield a stable parenteral product. Depending on the mechanism of the degradation, the pH of maximum stability in solution may not be the optimum pH for lyophilization, as demonstrated in the case of moexipril (1).

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