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Evaluation of the Stability of Creatine in Solution Prepared From Effervescent Creatine Formulations

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ABSTRACT

The objectives of this study were to determine the cause of the crystallization in a large volume creatine supplement solution made from effervescent powders containing di-creatine citrate, and to characterize these crystals using thermal analyses and x-ray diffractometry. Creatine effervescent powders were dissolved in deionized water (pH 6.2) and stored both at room temperature (RT) (25°C) and refrigerated condition (4°C) over a period of 45 days. Creatine concentration was determined using high-performance liquid chromatography (HPLC). Intrinsic dissolution and saturated solubility of creatine, creatine monohydrate, and di-creatine citrate in water were determined and compared. Crystal growth was detected only in the refrigerated samples on the seventh day of storage. Differential Scanning Calorimetry (DSC) and x-ray diffraction (XRD) studies revealed that the crystals formed were of creatine monohydrate. Ninety percent creatine degradation was observed within 45 days for RT samples. However, at refrigerated condition this degradation was 80% within the same time period. The pH of the RT samples also increased from 3.6 to 4.5 during storage. No such increase was observed in the case of refrigerated samples. The intrinsic dissolution rate constants of the compounds decreased in the following order: di-creatine citrate > creatine > creatine monohydrate. In conclusion, di-creatine citrate used in effervescent formulation dissociates to creatine in aqueous solution and eventually crystallizes out as creatine monohydrate. Significant decrease in solubility and effect of pH contribute to this crystallization process.

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KEYWORDS: Di-creatine citrate, creatine, creatine monohydrate, creatinine, stability, effervescent creatine

INTRODUCTION

Creatine is a naturally occurring guanidino compound found in the skeletal muscles and plays an important role in the metabolism of proteins.[1] Use of creatine supplement increases lean body mass, high-intensity power output, and strength in humans.[2] There has been a growing interest among athletes and researchers concerning the therapeutic application of creatine and benefits of using such a supplement.[3] Studies have shown that creatine uptake in muscle is dependent on sodium and not on insulin spike as was believed earlier.[4] Thus, formulations containing high-calorie sugars are not necessary for creatine transport and absorption. Creatine is administered orally either as a solid dosage form or as a solution. Mechanism of oral absorption of creatine is still unclear.[3] Investigations of creatine permeability across Caco-2 monolayer have shown that neither the P-glycoprotein nor multidrug resistance-associated protein is involved in the enhanced basolateral to apical transport of creatine.[5] This lack of movement of creatine may also be due to the absence of amino acid transporters specific for creatine or the absence of paracellular transport in creatine absorption.[3]

Creatine monohydrate has been extensively used as the salt of choice for commercial creatine supplement formulations. Its poor aqueous solubility still poses difficulty during formulation.[6] Therefore, di-creatine citrate with better aqueous solubility has been utilized in effervescent preparations. Two creatine effervescent products investigated in this study used di-creatine citrate salt to enhance its solubility and thereby its palatability.[7] Large volume solutions are often prepared from these effervescent products during big athletic events. The concentration of this solution is usually 15.6 mg/mL (15 grams per 960 mL of deionized water). According to the manufacturer (FSI Nutrition,

Omaha, NE, personal communication, February, 2002), such solution when stored under refrigerated condition has been shown to develop crystals.

Stability of creatine both in solid-state as well as in solution has been reported. Creatine is converted to creatinine in water under acidic conditions.[8,9] This process requires an intramolecular cyclization followed by removal of 1 mol of water. The solid-state properties of creatine and its stability in the solid-state have been described elsewhere.[10] According to this report, creatine monohydrate dehydrates to form anhydrous creatine from 97°C to 125°C with a subsequent phase change. Anhydrous creatine then undergoes intramolecular cyclization with removal of 1 mol of water to form creatinine. The creatinine formed finally melts with decomposition.[10]

The objectives of this study were (1) to determine the cause of the crystallization in a large volume creatine supplement solution prepared from effervescent powders containing di-creatine citrate, and (2) to characterize these crystals using thermal analyses and x-ray diffractometry. This study also attempted a viable solution of this crystallization problem during storage under refrigerated condition.

MATERIALS AND METHODS

Materials

Creatine (Lot: 08912PS), creatinine (Lot: A011608003), and creatine monohydrate (Lot: 08523DS) (Aldrich, Milwaukee, WI); and 4-(2-aminoethyl) benzene sulfonamide (Lot: 02816TP) (Sigma, St Louis, MO) were used as received. Creatine Edge (CE) and Creatine Clear (CC), the 2 effervescent creatine formulations used, were obtained from FSI Nutrition (Omaha, NE). Besides di-creatine citrate, the other components present in these formulations may include fructose, sodium bicarbonate, potassium bicarbonate, citric acid, ascorbic acid, vitamin B12, artificial/natural orange flavor, FD&C yellow #6, FD&C red #40, and sucralose. Di-creatine citrate (FSI Nutrition), water (HPLC grade, Lot: 007403), and ammonium sulfate (Lot: 50K0246) (Fisher Scientific, Springfield, NJ) were used as received. The 0.45-µm syringe filters (Lot: N6SMB345X) used in this study were obtained from Millipore Inc (Bedford, MA).

Determination of Solution Stability

Creatine Edge and Creatine Clear were used for this purpose. Three packets of each, containing 5 g of creatine per packet, were dissolved in 960 mL of water placed in screw-capped plastic bottles. The final creatine concentration in solution was 0.0156 g/mL. Two such solutions were kept at room temperature (25°C) and 2 others at refrigerated condition (4°C). Samples were collected on day zero, day 1, day 2, day 4, day 7, day 10, day 21, and day 45. To obtain a 200 times dilution, 10 µL of the samples were diluted to 2 mL. Creatine concentration in the diluted sample was determined by HPLC.[11] The pH of the solutions as well as any visual color changes was monitored over this time period.

Determination of Saturated Solubility of Creatine, Creatine Monohydrate, and Di-creatine Citrate in Water

The saturated solubility of creatine, creatine monohydrate, and di-creatine citrate was determined at 3 different temperatures (4°, 25°, and 37°C). An excess amount of each sample was placed in borosilicate screw-capped glass bottles containing 5 mL of water (pH = 6.2). These solutions were shaken periodically for 48 hours. The samples were then centrifuged at 1000 rpm for 10 minutes and the supernatant was collected. Diluted solutions of the supernatant samples were analyzed using HPLC.

Intrinsic Dissolution Studies

Intrinsic dissolution was carried out for creatine, creatine monohydrate, and di-creatine citrate using a modified Wood's apparatus.[12] Powders (0.7 g) were weighed out and poured into constant surface area dies. The powder sample was compressed in the die using a Carver press at 900 psi with a dwell time of 15 seconds. The remaining empty space of the die was filled with molten beeswax. The surface area of the compressed discs was 1.54 cm^2. Dies containing these discs were then mounted on USP dissolution apparatus containing 500 mL of deionized water at 37°C as the dissolution media. The discs were rotated at 100 rpm using an SR2 speed control system (Hanson Research Corp, Chatsworth, CA). Samples were collected through 0.45-µm syringe filters at definite time intervals over a period of 6 hours and analyzed using HPLC. All studies were carried out in triplicate.

Table 1. Change in Creatine Concentration in the Solution With Time at Different Storage Conditions and Over a Period of 45 Days*

Days	Room Temperature % Creatine Remaining in Solution (wt/vol)					Refrigerated Conditions % Creatine Remaining in Solution (wt/vol)				
	pH†	CC	CC	CE	CE	pH†	CC	CC	CE	CE
Day 1	3.6 ± 0.0	100	100	100	100	3.6 ± 0.0	100	100	100	100
Day 2	3.6 ± 0.1	99.4	99.3	98.8	98.8	3.6 ± 0.1	98.2	100	74.4	100
Day 4	3.7 ± 0.1	59.9	85.1	96.4	94.1	3.5 ± 0.1	98.2	97.9	72.4	100
Day 7	3.9 ± 0.1	44.3	49.4	94.1	85.3	3.5 ± 0.1	96.3	93.6	66.8	98.1
Day 10	3.9 ± 0.1	32.9	38.3	40.1	36.5	3.5 ± 0.0	38.8	49.2	36.2	47.5
Day 21	4.2 ± 0.1	24.0	29.2	38.3	31.8	3.5 ± 0.0	29.4	37.9	26.6	37.7
Day 45	4.5 ± 0.0	10.2	13.2	12.5	12.9	3.7 ± 0.1	27.5	28.8	18.1	24.7

*CC indicates Creatine Clear; CE, Creatine Edge.
†Mean ± SD; n = 4.

Di-creatine Citrate Degradation Studies

The stability of di-creatine citrate in solution was monitored at an elevated temperature (60°C). A 100-µg/mL solution of di-creatine citrate was prepared in deionized water. Two milliliters of the solution was put into eighteen glass ampules and sealed with a propane torch. The ampules were kept at 60°C in a controlled temperature oven (Blue M Electrical, Blue Island, IL) for a period of 2 weeks. On various predetermined time intervals, the ampules were broken open, and the samples were analyzed using HPLC. All data were collected in triplicate.

Analysis Using HPLC

For the analysis of creatine, creatine monohydrate, and di-creatine citrate, a liquid chromatography method, previously reported by Dash and Sawhney, was used.[11] This method was also used for the determination of citric acid and creatinine, 2 possible degradation products from di-creatine citrate solution. The chromatographic separation was achieved on a C-18, 250 × 4.6-cm column with UV detection at 205 nm. The system was operated using 0.045 M ammonium sulfate as the mobile phase at a flow rate of 0.75 mL/min. As an internal standard, 4-(2-aminoethyl) benzene sulfonamide was used.

RESULTS AND DISCUSSION

Stability of Creatine in Solutions Prepared from Effervescent Formulations

CC and CE are the 2 effervescent formulations used in this study. As per the manufacturer's specification, these formulations contain only di-creatine citrate as the creatine source. Most of the creatine supplements in the market usually contain creatine monohydrate salt as the creatine source. Creatine monohydrate has a poor aqueous solubility and palatability. The use of di-creatine citrate salt on the other hand eliminates these problems and enhances consumer compliance. Solutions made from these effervescent powders, when kept under refrigerated condition at a concentration level of 15.6 mg/mL (corresponding to 3 packets of effervescent powders in 960 mL of water), develop crystals at the bottom of the container on the seventh day of storage. Determination of the stability of creatine in solutions prepared from these effervescent powders was carried out both at room temperature (RT) and under refrigerated condition (4°C). The changes in creatine concentration over time are shown in **Table 1**. Greater degradation occurred in RT samples as compared with the refrigerated ones. Ninety percent degradation was observed within 45 days for RT samples. However, at refrigerated condition this degradation was around 80% over the same time period. Appearance of crystals was observed in refrigerated samples beginning on the seventh day. No such crystals were observed in RT samples over the entire period of study.

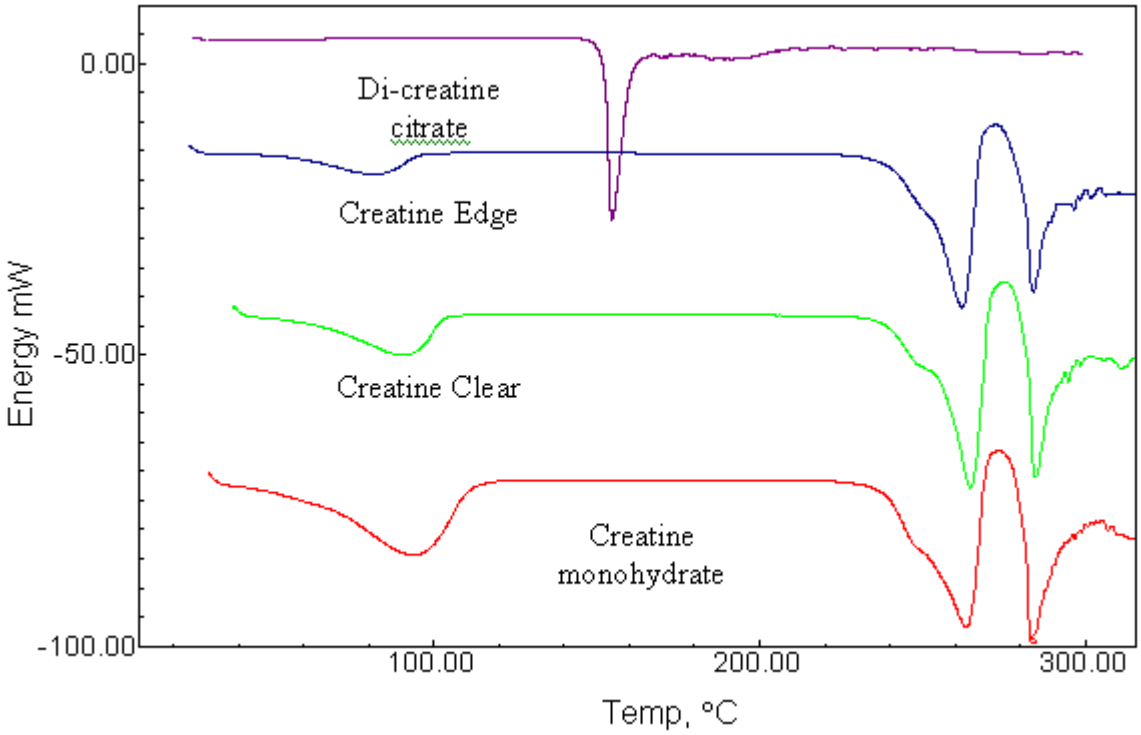

Figure 1. DSC curves of di-creatine citrate, Creatine Edge, Creatine Clear (crystallized samples from large volume solution kept at refrigerated condition [4°C]), and creatine monohydrate.

These crystals were collected after filtration and subjected to DSC and x-ray diffractometry. **Figure 1** depicts the DSC curves of the crystals obtained from refrigerated samples of both CE and CC after the end of the stability studies. The DSC curves for both samples were identical as shown in **Figure 1**. DSC results indicated that crystals formed from CC and CE samples were identical. However, the DSC curves of these samples were different from the curve of the starting material (di-creatine citrate) but identical to creatine monohydrate. Therefore, the underlying hypothesis for the above findings was that di-creatine citrate used in the formulation is converted to creatine in aqueous solutions. Creatine in presence of water forms creatine monohydrate, which eventually crystallizes out from solution because of its poor solubility. Powder x-ray diffraction patterns of these samples were then obtained and compared to test this hypothesis. The XRD patterns of both crystallized samples obtained after filtration are shown in **Figure 2**. Both the XRD patterns of the samples were identical but different from the XRD patterns of di-creatine citrate (patterns not shown). However, these patterns were identical to that of creatine monohydrate as already published elsewhere.[10]

During the stability studies, an increase was observed in the pH of the solutions kept at RT. This increase was at least 1 pH unit over a period of 45 days (**Table 1**). However, no such increase was noted in the case of the refrigerated samples. Our explanation for this pH change in the case of room temperature samples is as follows. During preparation of solutions from effervescent formulation, the production of carbon dioxide is expected due to the chemical reaction between acid and bases present in the formulation with water. According to Henry's law, the solubility of a gas (carbon dioxide in this case) decreases with increase in temperature.[13] Therefore, one should expect the solubility of carbon dioxide in the refrigerated samples to be much higher as compared with the RT samples. The presence of carbon dioxide in water will form carbonic acid, which, in turn, makes the pH of the solution more acidic as seen in the case of the refrigerated samples. However, in the case of the room temperature samples, loss of carbon dioxide over time might have contributed to the pH shift. A change in color of the solution was also noticed in the case of RT samples. This color change was not observed in the case of refrigerated samples. The presence of ascorbic acid in the formulations and its photochemical degradation may contribute to this color change only in the case of the RT samples. Further work is necessary to confirm this speculation.

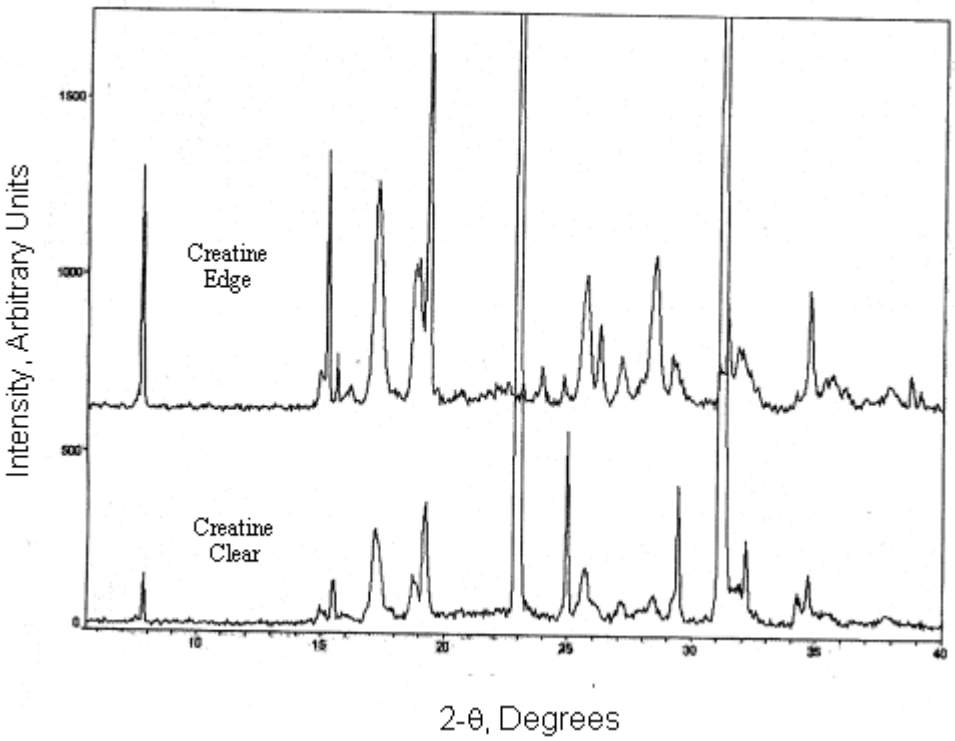

Figure 2. X-ray diffraction patterns of Creatine Edge and Creatine Clear crystallized samples from large volume solution kept at refrigerated condition (4°C).

Table 2. Saturated Solubility of Creatine, Creatine Monohydrate and Di-creatine Citrate in Deionized Water at Different Temperatures

Name of Compound	4°C		25°C		37°C	
	pH	Solubility (mg/mL)	pH	Solubility (mg/mL)	pH	Solubility (mg/mL)
Creatine	5.5 ± 0.2	7.6 ± 1.7	5.4 ± 0.0	19 ± 2.8	5.8 ± 0.2	38 ± 3.5
Creatine monohydrate	5.8 ± 0.0	6.1 ± 2.4	5.6 ± 0.1	17 ± 0.3	5.7 ± 0.0	36 ± 7.5
Di-creatine citrate/creatine	4.9 ± 0.1	65 ± 8.9	4.5 ± 0.3	84 ± 5.8	4.3 ± 0.0	71 ± 1.1

Saturated Solubility Study

The saturated solubilities of creatine, creatine monohydrate, and di-creatine citrate at different temperatures are shown in **Table 2**. The saturated solubility of di-creatine citrate is higher than that of creatine or creatine monohydrate at all 3 temperatures. However, the differences in solubility between creatine and creatine monohydrate were found to be minimal at all 3 temperatures. The results from this study further confirm the poor solubility of creatine monohydrate at all 3 temperatures investigated. The drop in solubility value of di-creatine citrate at 37°C can be explained by the fact that the pH of this solution was more acidic as compared with the other 2 solutions. Conversion of creatine to creatinine is expected to be high at this acidic pH.[8] The conversion of creatine to creatinine will definitely reduce the creatine concentration in the solution. Determination of creatinine levels in these samples was found to be high (15 ± 1.5 mg/mL) as compared with other samples. This study further confirms the conversion of creatine to creatinine in an acidic environment.

Intrinsic Dissolution Study

Intrinsic dissolution studies were performed for creatine, creatine monohydrate, and di-creatine citrate using a "disc" method.[14] Saturated solubility results at

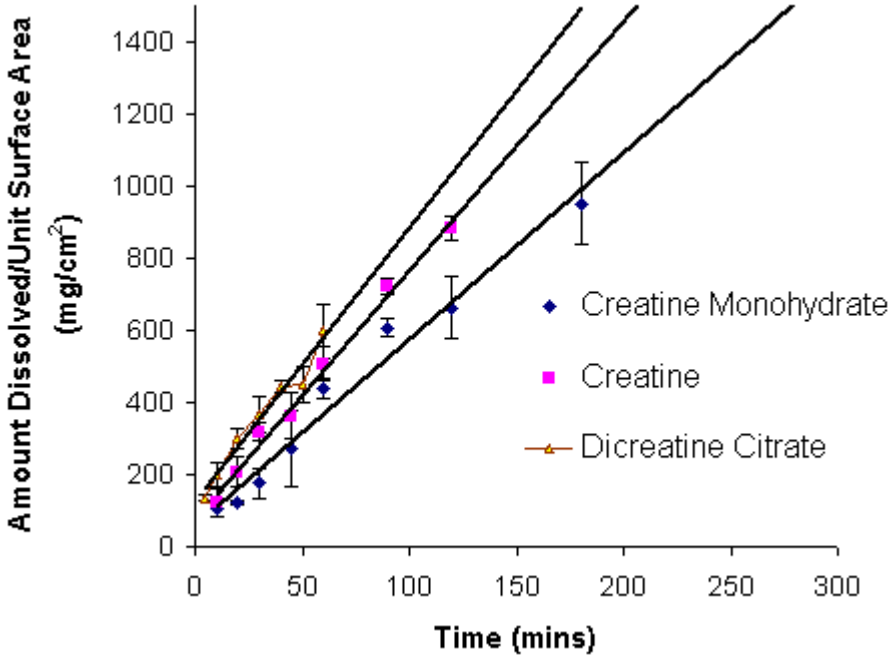

Figure 3. Intrinsic dissolution profiles of di-creatine citrate, creatine monohydrate, and creatine. Vertical bars represent mean ± SD; n = 3.

37°C for each compound were used to determine the amount of compound needed to make the disc to maintain sink condition throughout the dissolution experiment. The plots of the amounts of creatine dissolved per unit surface area versus time were linear with $r^2 > 0.97$ (**Figure 3**). The amount of creatine dissolved was measured as the total amount per area of the dissolution surface of the discs. The intrinsic dissolution rate constant can be calculated from the slope of this plot. The slope was found to be higher for di-creatine citrate as compared with creatine and creatine monohydrate. The intrinsic dissolution rate constants of the compounds may be arranged in descending order: di-creatine citrate > creatine > creatine monohydrate. In the case of di-creatine citrate, the intrinsic dissolution rate was 7.61 $mg.cm^{-2}$ $.min^{-1}$; in the case of creatine, 6.92 $mg.cm^{-2}$ $.min^{-1}$; and in the case of creatine monohydrate, 5.18 $mg.cm^{-2}$ $.min^{-1}$.

Di-creatine Citrate Degradation

Degradation of di-creatine citrate in water was performed at elevated temperature to aid in rapid degradation. HPLC was used to measure creatine and degradation product if any. Di-creatine citrate in aqueous solution dissociates to creatine, and citric acid. Finally creatine is converted to creatinine in solution. The HPLC method developed earlier was found to be suitable to determine all 3 compounds.[11] Citric acid concentrations within the range of 5 to 100 µg/mL were found to be linear. The linearity ranges for creatine and creatinine along with all the validation parameters have been published elsewhere.[11]

The degradation results for di-creatine citrate in solution are shown in **Figure 4**. The data presented reflect only creatine concentration as opposed to di-creatine citrate. When di-creatine citrate was added to water, dissociation of this salt to creatine was observed by HPLC, as expected. There was a steady decrease in creatine concentration in solution. However, there was an increase in both citric acid and creatinine concentration in the solution as shown in **Figure 4**. This increase in concentration may be attributed to the fact that di-creatine citrate dissociates in solution to creatine, leading to the release of citric acid into the solution. Some creatine may convert to creatine monohydrate in presence of water, and some may convert to creatinine, while the rest remains as creatine in solution. Creatine monohydrate, formed during the process, crystallizes out from solution because of its low solubility. However, at RT, the di-creatine citrate though gets converted to creatine monohydrate, the amount of creatine monohydrate formed may be below its solubility limit at this higher temperature (6 times higher than the refrigerated one) and therefore does not crystallize out

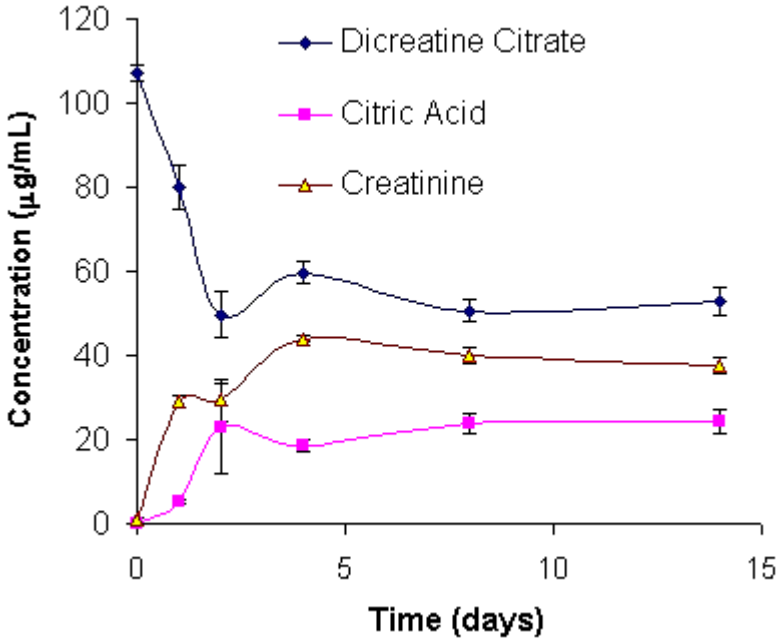

Figure 4. Degradation of di-creatine citrate in solution in sealed ampules at 60°C. Vertical bars represent mean ± SD; n = 3.

from solution. Studies conducted using creatine concentration below its solubility limits (2 packets of effervescent powder equivalent to 10 g of creatine in 960 mL water) showed no sign of crystallization over a 45-day period under refrigerated condition. This result further supports the hypothesis and that crystallization of creatine monohydrate from solution can be avoided by maintaining its concentration below its solubility limit at a particular temperature. The possible conversion of di-creatine citrate to creatine at various humidity conditions was also investigated. In this study, di-creatine citrate powders were exposed to 4 different controlled relative humidity (RH) chambers at RT (31%, 45%, 79.3%, and 100% RH). Thermal analyses as well as x-ray diffraction studies confirmed no conversion of di-creatine citrate to creatine in the solid state at all the humidity conditions tested. This study further confirms that such conversion was only observed in solution.

The degradation data obtained from this investigation were modeled using SAAM II software system (SAAM Institute, Seattle, WA). The 2 proposed models tested are depicted in **Figure 5**. **Figure 5A** represents model #1, in which di-creatine citrate dissociates to produce creatine and citric acid. Creatine in solution is converted to one of its degradation products, creatinine. **Figure 5B** depicts model #2, which represents the simple creatine-creatinine equilibrium. The plots of the predicted values determined by the SAAM II program as well as the experimental values are plotted and shown in **Figure 6**. Comparing both plots (**Figure 6A** and **B**), it was quite clear that model #1 (**Figure 6A**) describes the experimental data much better than model #2.

CONCLUSION

Di-creatine citrate has a higher solubility and dissolution rate constant as compared with both creatine and creatine monohydrate. Effervescent formulation containing di-creatine citrate, when dissolved in a large volume of water and stored over a period of 1 week, undergoes significant degradation. Crystallization of creatine monohydrate from such a solution is observed under refrigerated temperature and when the concentration of creatine in solution exceeds 15.6 mg/mL, a popular concentration used by athletes (15 g creatine/960 mL water). Di-creatine citrate dissociates to creatine in aqueous solutions with the release of citric acid. Some of the creatine formed crystallizes out from solution as creatine monohydrate, some converts to a more water-soluble form creatinine, and rest remains as creatine in solution. Of these 3 possible products, creatine monohydrate has the lowest solubility and therefore it crystallizes out of solution. This study further reveals that the solubility problem of creatine

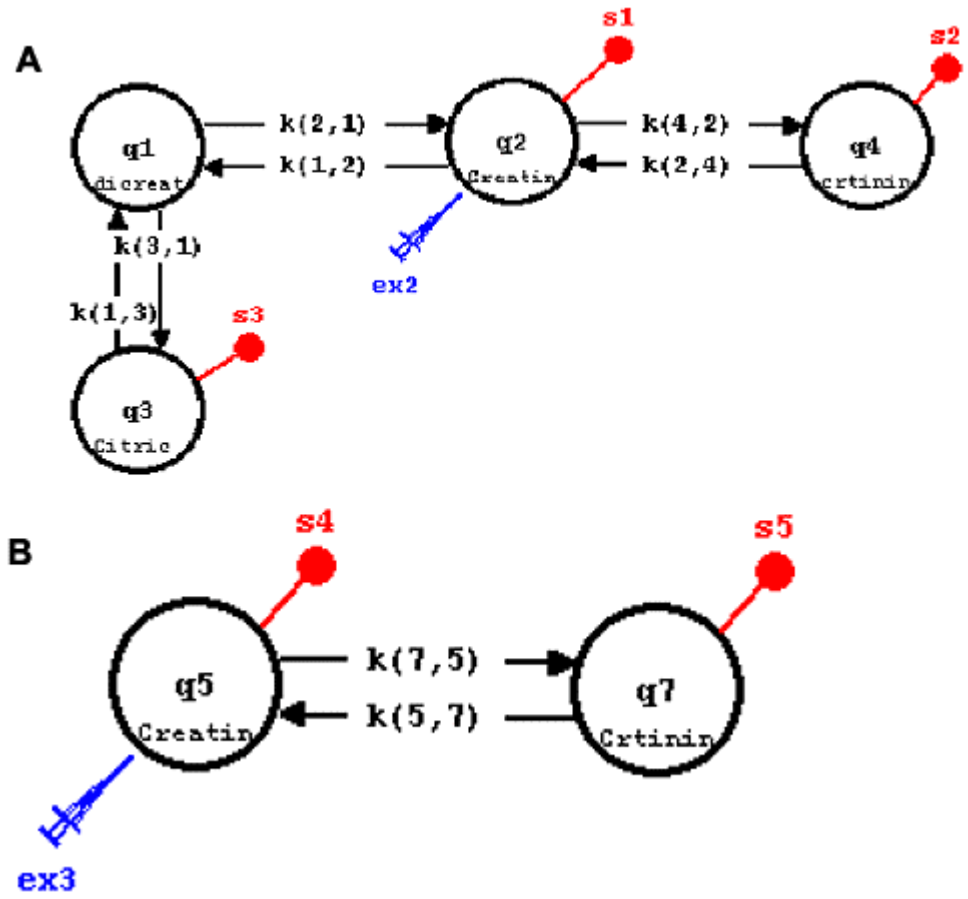

Figure 5. A) Modeling of di-creatine citrate stability data using SAAM II software. Model #1: q1 (dicreat)= di-creatine citrate; q2 (creatin)= creatine; q3 (citric)= citric acid; q4 (crtinin)= creatinine. The dose is ex2; s1, s2, and s3 are the sampling sites, respectively; B) Modeling of di-creatine citrate stability data using SAAM II software. Model #2. q5 (creatin)= creatine; q7 (crtinin)= creatinine. The dose is ex3; s4 and s5 are the sampling sites, respectively.

monohydrate can be avoided by using these formulations within its solubility limit. Instead of using 3 packets of CC or CE per 960 mL of water, this study suggests using only 2 packets per 960 mL of water if the solution is to be refrigerated. However, higher concentration (3 packets/960mL water) can be stored at RT without any crystal formation. For stability reasons, this solution should be used within 6 days of the date of its preparation.

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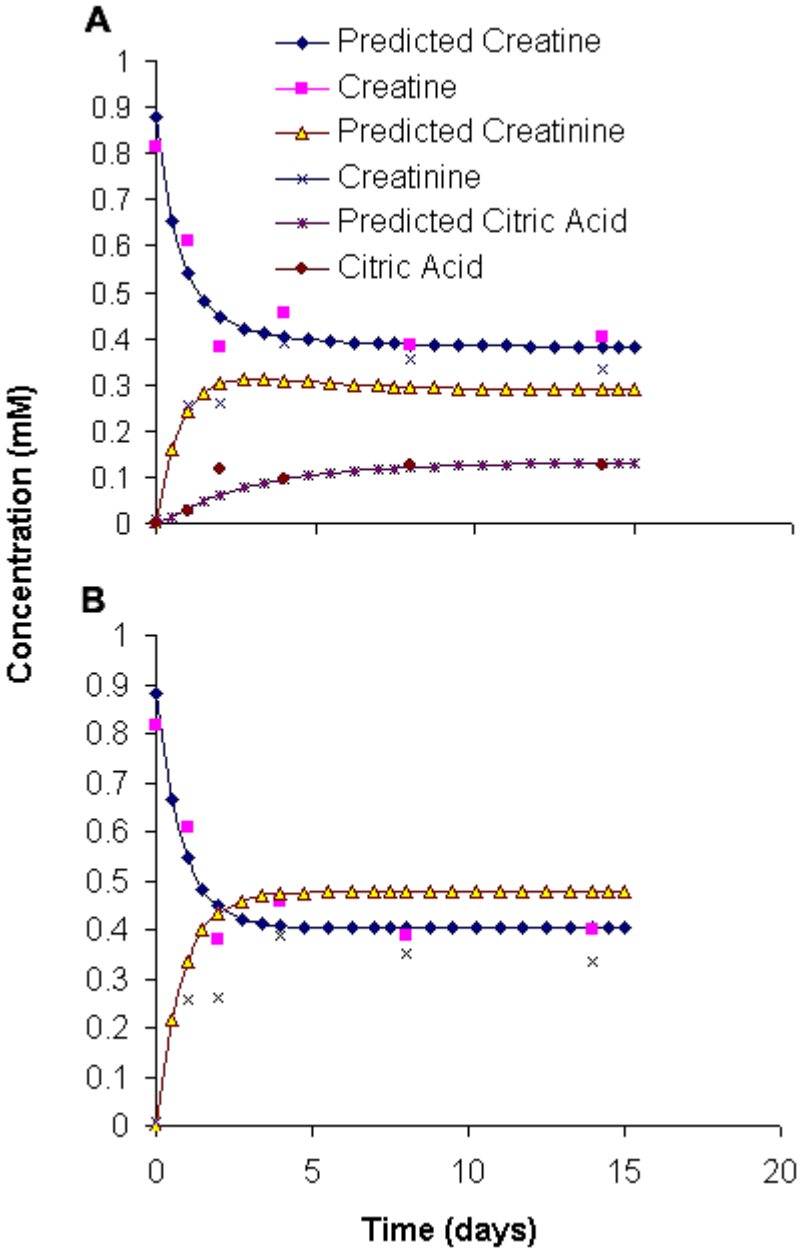

Figure 6. A) Plot of the predicted (using SAAM II software system) versus experimental data according to model #1; B) Plot of the predicted (using SAAM II software system) versus experimental data according to model #2.

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