

## Improved Creatine Stability and pH Profile for Kre-Alkalyn

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**Abstract:** Creatine modification for commercialization has included many variations to increase its stability. The current report assesses the stability of a sodium bicarbonate buffered creatine sold under the brand name Kre-Alkalyn under various conditions. Kre-Alkalyn is shown to have a 1 year stability profile under accelerated testing. It maintains a high pH over time compared to normal creatine and can be augmented with additional stabilizing buffers. This stability and buffering profile may serve useful in providing continued creatine availability.

### 1. INTRODUCTION

Creatine (N-(aminoiminomethyl)-N-methyl glycine) is an important energy producing amino acid based metabolite produced in the liver, kidneys, and pancreas and commonly used by the muscles [1-3]. Creatine in various forms is used as a supplement to increase athletic performance [4-6]. Creatine is pivotally important in delaying ATP depletion during anoxia or ischemia through the creatine-phosphocreatine system, for example [7]. Yet its stored shelf life and half-life in the stomach play a role in its bioavailability after ingestion. Previous reports suggest that the degradation of creatine results from pH changes [2, 8, 9]. At normal physiological pH, 10% of creatine has been suggested to convert to creatinine, its most common byproduct [10].

In this report a commercial creatine monohydrate powder buffered with sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) to a pH of 12 (Kre-Alkalyn® All American Pharmaceuticals) was tested for stability and examined for conserved buffering capacity when compared to traditional creatine. Though understudied in the context of creatine, changes in acid-base regulation have also been shown to effect protein-protein interactions [11], mitochondrial efficiency [12], and cellular signaling and proliferation [13, 14]. Furthermore, Kre-Alkalyn increased cytoprotection against cisplatin-induced cytotoxicity compared to conventional creatine in 293T human kidney cells [8], suggesting another possible utility of in vivo creatine buffering.

Herein, two commercialization properties of Kre-Alkalyn are examined. Specifically, the stability and conversion of Kre-Alkalyn to creatinine and pH profile compared to traditional creatine.

### 2. METHODS

#### 2.1. Stability Testing Procedure:

1.5 grams of Kre-Alkalyn® was mixed in water. Real Time and Accelerated testing was performed. The same lot was used for both test.

#### Step 1:

Kre-Alkalyn® powder was assayed for purity, 1.5 grams of Kre-Alkalyn powder was added to 4 oz of water and stored in lab for real time testing, and 1.5 grams of Kre-Alkalyn powder was added to 4 oz of water and put into incubator for accelerated testing.

#### Step 2:

Both groups of products were tested at 30 day segments.

## 2.2. FTNIR Analysis:

Identification performed by FTNIR, against in-house external library standards obtained from Sigma & produced by HPLC. Quantification by FTNIR against external library standards obtained from Sigma & produced by HPLC (Bran & Luebbe InfraProver II FTNIR).

## 2.3. HPLC Analysis:

Analysis performed by HPLC using Intersil ODS-2 5um (250x4.6mm) and 25 min. gradient elution with 0.1% phosphoric acid buffer in H<sub>2</sub>O and 0.1% phosphoric acid in acetonitrile. External reference standards obtained from Sigma-Aldrich.

## 2.4. Stomacher:

Previously described by Golini [8], but consisting of a glass vessel and liquid acid to mimic the acidic environment of the stomach, creatine or Kre-Alkalyn was first added to water as described above and then added to the acid and pH was assessed over time.

## 2.5. pH Analysis:

Creatine and Kre-alkalyn solutions were tested for changes in pH using a standard calibrated pH meter (ATLAS Bioscience; Tuscon, AZ). Three solutions were added to Kre-Alkalyn, hydrochloric acid (0.1 ml – 1M), sodium hydrogencarbonate/sodium carbonate (11.7 / 13.3 (ml – 1M)), and ethanolamine/ethanolamine hydrochloride (18.6 / 6.3 (ml – 1M)).

## 3. RESULTS

The shelf stability of Kre-Alkalyn is conserved during both real-time and accelerated testing of creatine to creatinine conversion. Only during the accelerated stability test was there a small indication of creatine to creatinine conversion (0.055 after 150 days, the equivalent to 5 years of real time, and 0.1% after 180 days, the equivalent to 6 years of real-time). It has been established that creatine breakdown is a function of pH [2]. Using a systematic mock-up of a stomacher, described above, it was shown that Kre-Alkalyn maintained a significantly higher pH than traditional creatine, in three separate reagents tested. A slow decline from highly basic pH (12) to neutral pH (7) after 45 minutes with creatine in a 0.1 M HCl solution was observed. Even with the addition of hydrogencarbonate/sodium carbonate or ethanolamine/ethanolamine hydrochloride, commonly used biopharmaceutical buffers, which increased the rate of pH reduction, Kre-Alkalyn maintained higher pH levels than traditional creatine.

## 4. DISCUSSION

It has previously been reported that the commercially available sodium bicarbonate buffered creatine Kre-Alkalyn may provide cytoprotective properties, above and beyond traditional creatine when used in the presence of cytotoxic agents such as those used during chemotherapy [8]. In this report, Kre-Alkalyn, was assessed for soluble stability during both a real-time (1 year) and accelerated (6 year) trial. During these trials only a small fraction of Kre-Alkalyn was converted into creatinine at the end of the accelerated 6 year trial. A much higher rate of degradation was observed with traditional creatine in similar trials [15, 16].

Furthermore, the conversion of creatine to creatinine has been directly linked in vivo to changes in pH. As such, stability under acidic conditions, reminiscent to the stomach, were conducted with traditional and buffered Kre-Alkalyn. The results of these tests suggested that the pH buffering capacity of Kre-Alkalyn will maintain a much higher pH in an acidic environment than traditional creatine, even in the presences of additional acid buffering reagents. First water solubilized Kre-Alkalyn was examined in the presence of HCl and only slowly declined to a neutral pH after a period of 45 minutes, ample time for the creatine contained in the supplement to leave the stomach and enter the blood-stream. Second, when tested in the presence of acidifying buffers, Kre-Alkalyn

maintained a significantly higher pH over 45 a minute period then traditional creatine in a normal HCl solution.

The findings of this report suggest that Kre-Alkalyn maintains its stability when mixed with water over a significant time frame and should provide a sustained basic pH in the acidic environment of the stomach necessary for reducing creatine to creatinine conversion. Thus, based on the stability profiles presented, Kre-Alkalyn should provide superior availability in vivo to traditional creatine commonly used in sports supplements.

## 5. REFERENCES

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