CHAPTER 15

CREATINE – ITS CHEMICAL SYNTHESIS, CHEMISTRY, AND LEGAL STATUS

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Abstract: Creatine, a small molecule present in muscular tissue of many vertebrates, evolves to one of the most widely used and successful dietary supplements of recent decades (Graham and Hatton, 1999). Importantly, in the industrial manufacturing process, a high quality standard must be maintained. Validated analytical methods capable of providing reliable and consistent analysis of the main substance, side products and potentially harmful impurities must be employed. The principles of those determinations and the nature of possible by-products will be elucidated in this chapter. In addition, the pure creatine produced may be unstable under certain conditions, e.g. within special formulations or galenical forms. Some hints how to deal with this fact and how to avoid instability will also be discussed. Thus, this chapter will serve as a survey of the paths of chemical synthesis of creatine, its chemistry, properties, stability, analytical determination methods and legal status

1. THE HISTORY OF CREATINE

In 1832, the French scientist Chevreul discovered a new ingredient of beef tea and muscular tissue, which he named creatine, according to the source from which it was extracted (κρέας, Greek for flesh) (Chevreul, 1834). The German scientist von Liebig confirmed that creatine is a regular constituent of flesh. Creatine levels in wild animals were ten times higher compared to captive animals suggesting that physical activity might have an influence on the amount of creatine present in flesh (Liebig, 1847). Animals were exclusively used as a source for creatine for about the next century. Thus, meat extracts (Liebigs Fleischextrakt) and urine – via isolation by means of creatinine-zinc chloride double salt, followed by alkaline conversion of creatinine to creatine (see below) – were employed as sources for creatine (Mulder and Mouthaan, 1869; Folin, 1914; Benedict, 1914). If particularly pure material was needed, e.g. for organic preparations and analytical standards, creatine was prepared from meat extract. Twenty-five kilograms of fat-free flesh contains 1 kilogram of this

G.S. Salomons and M. Wyss (eds.), Creatine and Creatine Kinase in Health and Disease, 291–307.

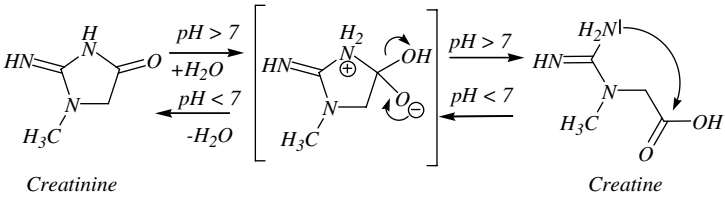


Figure 1. pH-dependent conversion between creatine and creatinine.

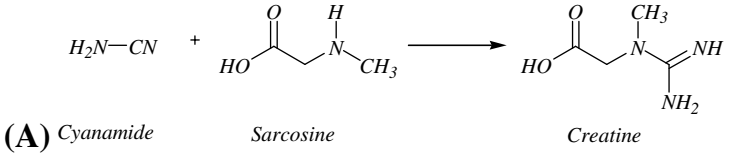
extract which, after extraction for 3 times with 2 litres of absolute ethanol each and re-crystallization from water, yields 25-30 grams of pure creatine (Steudel, 1921).

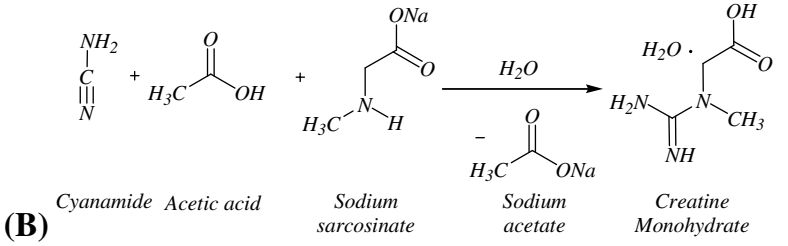
Dessaignes (1857) and Liebig (1858) found that creatine can be obtained from creatinine in basic solutions, e.g. with lime milk (Figure 1). The chemists Strecker and Volhard both described in 1868 the synthesis of the guanidino compound creatine by reacting sarcosine with cyanamide (Figure 2) over several hours or days, in slightly ammoniacal alkaline aqueous (Strecker, 1868) or in heated alcoholic solutions (Volhard, 1868). Those non-optimized reactions led to numerous by-products, so for the next decades, the more efficacious preparation was still the isolation from meat extract. In the first half of the 20th century, with an increase in knowledge about production of cyanamide and other guanylation reagents, technical manufacturing of creatine could be realized.

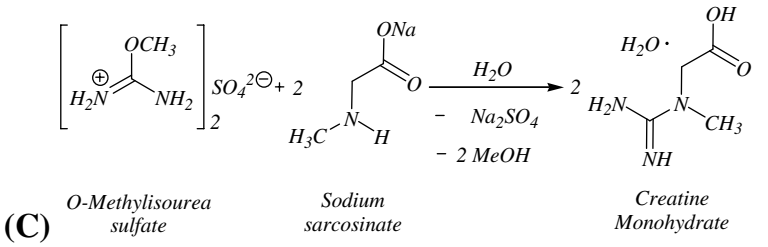
2. CHEMICAL SYNTHESES AND INDUSTRIAL PRODUCTION OF CREATINE

Nowadays, due to the growing interest in natural or synthetic guanidine compounds including creatine as potential pharmaceutical and bioactive leads, a large number of guanylation reagents are described in literature (Gastner, 2002; Yet, 1999). Medical chemistry and pharmaceutical research are the main driving forces in the development of gentle synthetic pathways for novel chemical structures including artificial peptide molecules and peptidomimetics possessing guanidino functionalities and having biological or even medicinal activity (Berlinck, 2002; Baker *et al.*, 2000). Most of the synthetic routes are of academic value and are used for lab scale preparation of new molecules.

Among the various synthetic paths described today, only a few could be implemented within an industrial setting for the production of large quantities of creatine monohydrate. All of the herein elucidated technical processes are based on guanylation, precisely called amidination, of the amino acid sarcosine or its salts (sarcosinates). The guanylation agents (Grambow *et al.*, 2003) are cyanamide (Volhard, 1868), O-methylisourea (Kapfhammer and Müller, 1934) or S-methylisothiourea salts (Schütte, 1943). Interestingly, mainly cyanamide is used in Western countries, e.g. Europe and the United States, whereas in the Eastern world, e.g. Japan and China, O-methylisourea or S-methylisothiourea salts are preferably used for commercial manufacturing of creatine.







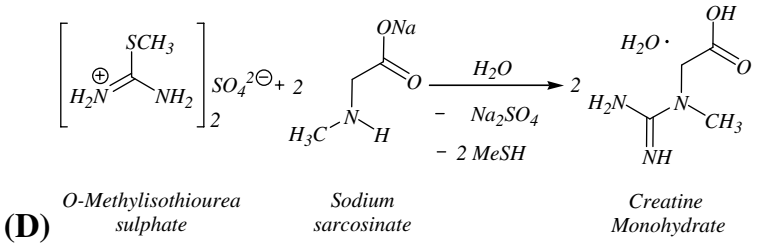


Figure 2. Chemical methods of creatine synthesis. (A) Early chemical synthesis of creatine described by Strecker (1868) and Volhard (1868). (B) Creatine synthesis from sarcosinate and cyanamide. (C) Creatine synthesis from sodium sarcosinate and O-methylisourea sulphate. (D) Creatine synthesis from sodium sarcosinate and O-methylisothiourea sulphate.

2.1. Creatine Synthesis from Sarcosinates and Cyanamide

Based on the early discovery by Strecker and Volhard, creatine can be obtained by reaction of cyanamide and sarcosinates using water as an inexpensive solvent. The raw materials of this process are readily available as basic chemical components. Sarcosinate sodium or potassium salts are produced on an industrial scale by reaction

of methylamine, formaldehyde and sodium or potassium cyanide. On the other hand, cyanamide is derived from calcium cyanamide, which became industrially available by nitrogenation of calcium carbide, known as the Frank-Caro process established around 1900 (Hartmann and Zieke, 1954).

A state-of-the-art manufacturing process for creatine can be summarized as follows (Weiss and Krommer, 1995) (Figure 3): Technical aqueous sodium sarcosinate solution is adjusted with acetic acid under intensive cooling and vigorous stirring to a pH value of about 10. The reaction takes place in a stirring vessel at a temperature around 80 °C by careful addition of aqueous cyanamide solution under agitation followed by a time period of stirring to complete the reaction. After cooling, the crystalline creatine monohydrate is filtered or centrifuged off and washed several times in warm water to remove the reaction solution. Subsequently, the product is dried under vacuum at slightly elevated temperature. The yield of this process is about 75% of HPLC-pure creatine monohydrate.

A variety of manufacturing processes for creatine based on cyanamide are disclosed in the patent literature. Vassel and Garst (1953) describe generally the guanylation of amino acids, including sarcosine, using calcium cyanamide or free cyanamide. Kessel *et al.* (2004) disclose the use of carbon dioxide as acid to adjust the pH value of

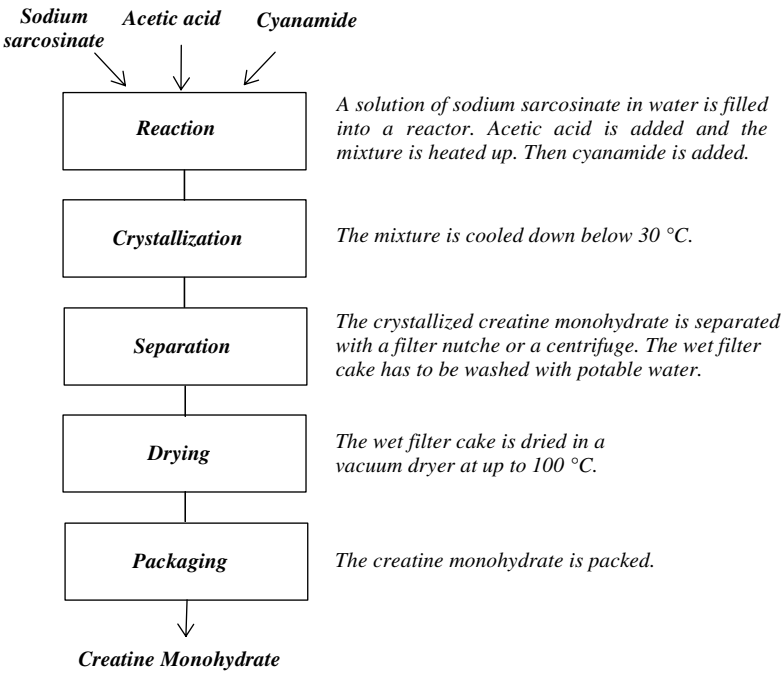


Figure 3. Industrial process flow chart for the synthesis of creatine monohydrate (Weiss and Krommer, 1995).

the reaction solution, whereas Kessel *et al.* (2003) describe a continuous process for creatine production.

2.2. Creatine Synthesis from Sarcosinates and O-alkylisourea

The use of O-alkylisourea as a guanylation agent for amino compounds was described for the first time by Kapfhammer and Müller (1934). Both a Japanese (Iwai and Tsunoda, 1980) and a German patent (Kessel and Kluge, 1998) describe creatine synthesis by continuous introduction of O-alkylisourea, especially O-methylisourea sulphate, into an aqueous sarcosine solution at a constantly maintained pH from 10 to 12 and at 5 to 25 °C. Creatine monohydrate crystals are obtained and purified via a sequence of washing steps using water and alcohols. A German patent discloses a similar process for preparing creatine by O-methylation of urea and reaction of the resultant O-methylisourea salt with sodium sarcosinate. The resultant creatine monohydrate crystals are washed with water (Greindl *et al.*, 1999).

2.3. Creatine Synthesis from Sarcosinates and S-alkylisothiourea

The first synthesis of guanidino acids from amino acids employing S-methylisothiourea was described by Wheeler and Merriam (1903). Although the guanylation agent is readily available and cost-efficient, the reaction has the disadvantage of the liberation of methyl mercaptan, a toxic and environmentally hazardous gas that needs to be annihilated before discharging the reaction vessel after the reaction is completed.

A Chinese patent discloses a process for the chemical production of creatine monohydrate (An *et al.*, 1999), which can be summarized as follows: To a diluted unprocessed aqueous solution of sodium N-methylglycinate, concentrated hydrochloric acid is added under stirring and cooling below 15 °C to adjust the pH to 9.5. At a temperature of less than 35 °C, S-methylisothiourea sulphate is slowly added into the solution under stirring. At about 30 °C, the reaction is completed in several hours. Then hot vapor is fed into the reaction solution. The crystalline mass is separated by centrifugation, followed by several washing steps with water, acids, and alcohol. After drying under vacuum at 40 °C, creatine monohydrate is obtained at a yield of 72.0% and a purity (by HPLC) of 99.3%.

All manufacturing processes described so far, especially those disclosed in the patent literature, can be performed under a rather wide range of process conditions, but generally lead to creatine monohydrate in yields of around 75%. Two additional patents on creatine production shall be mentioned here as well. Kessel *et al.* (2002) describe a purification procedure for creatine, whereas Greindl and Scherr (1999) disclose a process for creatine synthesis based on haloformamidium salts as guanylation agents.

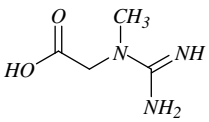
3. PROPERTIES OF CREATINE

Creatine crystallizes from water as monoclinic prisms holding one molecule of water of crystallisation per molecule of creatine. The crystals easily loose this water of

crystallisation at around 100 °C. The solubility of creatine in water increases with temperature and the correlation between solubility and temperature is almost linear. One litre of water dissolves 6 g of creatine at 4 °C, 14 g at 20 °C, 34 g at 50 °C, and 45 g at 60 °C. Creatine, as a small ampholytic substituted amino acid, is only very slightly soluble in pure ethanol (1 part in 9410 parts) and insoluble in ethyl ether. Table 1 shows the major features and key data of creatine.

One of the most important chemical reactions of creatine is related to its stability in aqueous solutions. Creatine is not stable in aqueous solution due to intra-molecular cyclisation to creatinine (see Figure 1). The velocity of creatine degradation is not dependent on its concentration, but on pH (the lower the pH, the faster) and temperature (the higher the temperature, the faster). This conversion is known as the creatine-creatinine-equilibrium and was previously thoroughly investigated by Edgar and Shiver (1925) and Cannan and Shore (1928). A more practical approach to this topic was undertaken by Howard and Harris (1999): their patent discloses formulations of creatine in acidic compositions. Figures 4 and 5 confirm, in correspondence with older experimental data, a decrease in creatine concentration in water under various conditions; for instance, they reveal degradation of creatine at 25 °C over a period of up to three days at different pH values (3.5–7.5) and at 4 °C over a period of up to thirty days at pH values between 3.5 and 7.0. The pH of the samples was adjusted to the desired value using acetic acid or potassium hydroxide, and the pH of the samples was also tested to ensure that pH did not change during the experiment. It was shown that creatine in aqueous solution is reasonably stable for up to 8 h at 25 °C and at a pH of 6.5 or 7.5. The breakdown observed after 3 days at pH 5.5, 4.5 and 3.5 was

Table 1. Characteristics of creatine. CAS = Chemical Abstract Service index name; http://www.cas.org .

Chemical structure	
Chemical name (CAS, 2006)	N-(aminoiminomethyl)-N-methyl-glycine
Trival names	Engl. creatine; Ger. Kreatin; Fren. créatine
Synonyms	(α-methylguanido)acetic acid; methylguanidino-acetic acid; N-amidinosarcosine; α−methyl-guanidino acetic acid; N-methyl-N-guanylglycine; methylglycocyamine; N-amidino-sarcosine; N-(aminoiminomethyl)-N-methyl-glycine
Chemical formula	$C_4H_9N_3O_2$
Molecular mass	131.13 g/mol
CAS number	[57-00-1]
EINECS number	200-306-6
Melting point	decay at 303 °C

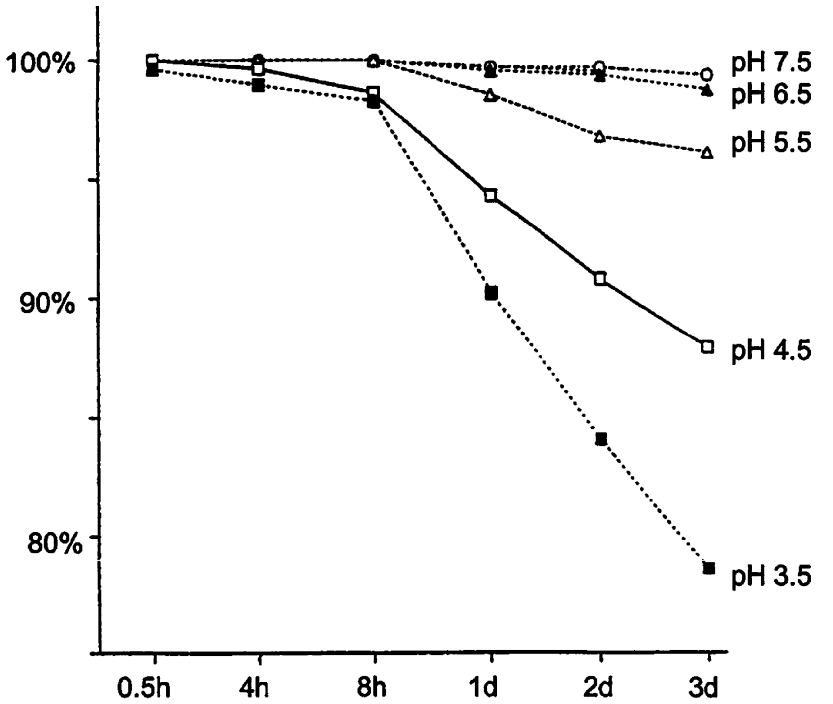


Figure 4. Degradation of creatine over time (0–3 days) at 25 °C and at different pH values (3.5–7.5) (Howard and Harris, 1999).

4%, 12% and 21%, respectively. At 4 °C, the reaction slows down so that even at pH 3.5 and after 30 days, the concentration of creatine in the solution is still above 90%.

Metal salt solutions and acids favour conversion of creatine to creatinine (Beilstein Crossfire Research, 2006, http://www.beilstein.com). Finally, the treatment of creatine with strong oxidative agents and bases cleaves the molecule to small nitrogenous compounds or oxidized molecules like carbonic acids.

Therefore, in respect to aqueous creatine formulations, their use should be as immediate as possible. Otherwise, after preparation, such formulations should be stored at a low temperature to slow down the conversion to creatinine. The solubility of creatine monohydrate at the chosen temperature should be considered, and is 14 g per litre at 25 °C and 8.5 g per litre at 4 °C.

Recently, an intensive investigation into the interesting characteristic of creatine as an anti-oxidant contributed to the understanding of its cell protective activity. Lawler *et al.* (2002) showed that creatine is able to scavenge a number of reactive oxygen species (ROS) having physiological significance in living matter.

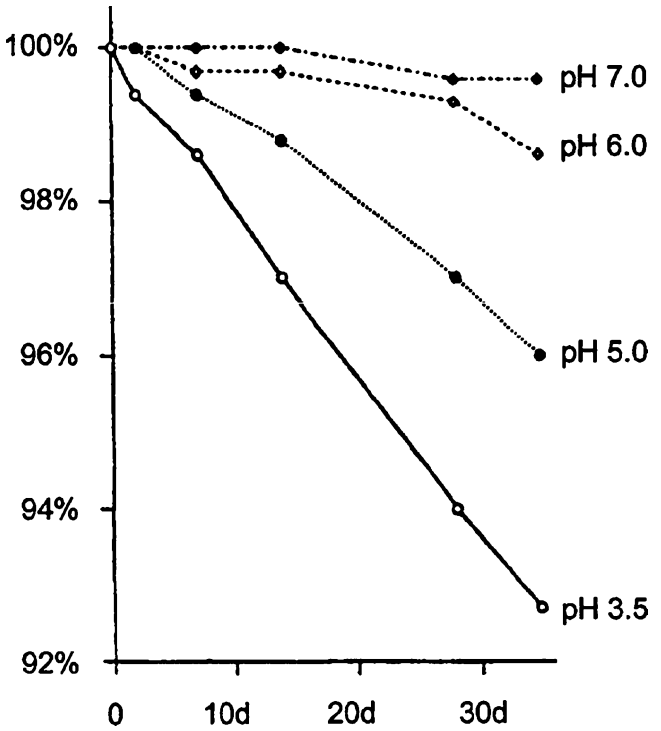


Figure 5. Degradation of creatine over time (0–30 days) at 4 °C and at different pH values (3.5–7.0) (Howard and Harris, 1999).

4. CHEMICAL ANALYSIS OF CREATINE

Various chemical analytical methods for creatine have been developed already. Soon after the discovery of creatine, gravimetric analysis was introduced which was based on formation of insoluble precipitates from creatine and a particular reagent, followed by separation of this precipitate and evaluation of its weight which correlated with the creatine content in the analyzed solution. Those methods became obsolete after the introduction of spectrometric procedures, like photometry, infrared, and magnetic resonance spectroscopy. These methods are still used on their own or in combination with highly efficacious separation methodologies, especially liquid chromatography. Some of the numerous methods are described here with respect to creatine analysis, especially those that have proven their utility in an industrial setting at Degussa.

4.1. Photometric Determination Methods for Creatine

Photometry is a method in which the concentration of an analyte, usually in solution, is rendered measurable by the addition of a reagent that forms a coloured product with the analyte under investigation. The intensity of the colour is dependent on the analyte's

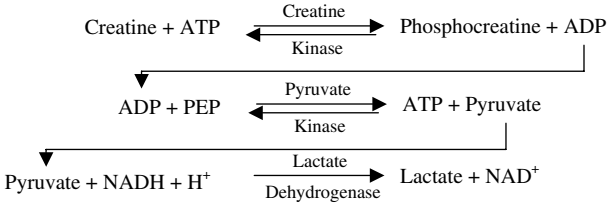


Figure 6. Cascade of enzymatic reactions for creatine determination. ATP: adenosine triphosphate; ADP: adenosine diphosphate; PEP: phosphoenolpyruvate; NADH: nicotine adenine dinucleotide (reduced form); NAD^+: nicotine adenine dinucleotide (oxidized form); H^+: proton.

concentration, and hence can be used to measure it. Photometric determination of creatine can be achieved according to the Folin method (Folin, 1904) which is based on the previously discovered Jaffé colour reaction of creatinine and picric acid in alkaline solution (Jaffé, 1886) which forms an orange-red complex that can be measured at about 500 nm (Merck, 1974, 1987). Creatine needs to be converted to creatinine by acid treatment in solution before measurement. This method is still commonly used to analyze creatine as well as creatinine in biological samples like blood serum, tissue and urine (Davidsohn and Henry, 1969). Due to the fact that this reaction is not highly specific and can be disturbed by other organic compounds found in body fluids, more specific methods for creatine determination were developed.

4.2. Enzymatic Analyses of Creatine

In general, enzymatic determination methods are more specific and accurate than photometric ones based on colour reactions. Enzymes are used to catalyse a specific biochemical reaction. The change in concentration of an involved constituent of the reaction or reaction cascade is measured commonly by a photometer.

Bernt *et al.* (1970) described a method based on Tanzer and Gilvarg (1959) where creatine is converted with ATP and creatine kinase into creatine phosphate, followed by a reaction cascade shown in Figure 6. The associated NADH decrease is photometrically measured at a wavelength of 340 nm, and is proportional to the creatine concentration. An enzymatic procedure developed by Kodak Ektachem uses the enzyme creatinine iminohydrolase, which catalyzes the hydrolysis of creatinine to N-methylhydantoin and ammonia. The ammonia reacts with bromophenol blue to form a blue dye that is measured by reflectance photometry (Blick and Liles, 1985). Several other enzymatic determination methods for creatine are described in literature, e.g. based on creatine kinase (Beyer, 1993).

4.3. Chromatographic Methods for Creatine Determination

Chromatography involves passing a sample – i.e. a mixture which contains the compound in question dissolved in the mobile phase, mostly a solvent – through the stationary phase that retards the passage of the components of the sample by adsorption

phenomena. The components of the sample become separated in time because their passage through the system happens at different rates, so that they finally can be detected successively.

In earlier times, methods for detection of creatine by means of paper and thin layer chromatography were developed but they do no longer play a role nowadays. More advanced determination methods were developed and are described hereafter.

4.3.1. High performance liquid chromatography (HPLC)

Recently, Dash and Sawhney (2002) developed a simple liquid chromatographic method with UV detection for the analysis of creatine and creatinine and applied it to various commercially available creatine supplement formulations. In this method, the mobile phase consists of 0.045 M ammonium sulphate in water. A C-18 column is employed for the chromatographic separation at ambient temperature. The eluent is monitored at 205 nm. As an internal standard, 4-(2-aminoethyl) benzene sulfonamide is used. This method requires less than 7 min of chromatographic time. The method can be used to determine ~1–100 mg/ml of creatine or creatinine. The precision and accuracy for creatine were found to be sufficient for measurements of creatine in various marketed products as well as for analyses of the solubility of various creatine salts and of the stability of creatine in aqueous solution.

Reliable and accurate determination of sample components can be accomplished with high-performance liquid chromatography. HPLC techniques employ fine particles of highly porous materials as an absorbing stationary phase packed typically in steel columns. A solvent phase is forced at high pressure through the packed column with concurrent separation of the transported constituents of the sample. HPLC-determination of creatine can be achieved, e.g., by a method developed by Degussa AG (Manfred Wildenauer, personal communication) which is also suitable for the simultaneous determination of the potential impurities dicyandiamide, creatinine and dihydrotriazine. The mobile phase consists of 0.2 M ammonium dihydrogen phosphate in water which is adjusted to pH 4 with phosphoric acid. Chromatographic separation is achieved at ambient temperature on a nucleosil column 5 SA (ET 250/4, Macherey-Nagel). The flow rate is maintained at 1.0 ml/min and the effluent is monitored at 225 nm. The concentration of creatine is calculated through comparison with external standards. The retention time of creatine is approx. 3.5 min (dicyandiamide approx. 2.8 min, dihydrotriazine approx. 5.4 min, and creatinine approx. 8.6 min).

4.4. NMR and IR Spectroscopy of Creatine

4.4.1. ^{1}H-NMR-spectroscopy of creatine

Nuclear magnetic resonance, or NMR, is a physical phenomenon based upon the magnetic property of an atom's nucleus. It allows the determination of the number, type and relative positions of certain atoms in a molecule. NMR spectroscopy is a powerful instrumental technique used to obtain physical, chemical, electronic and

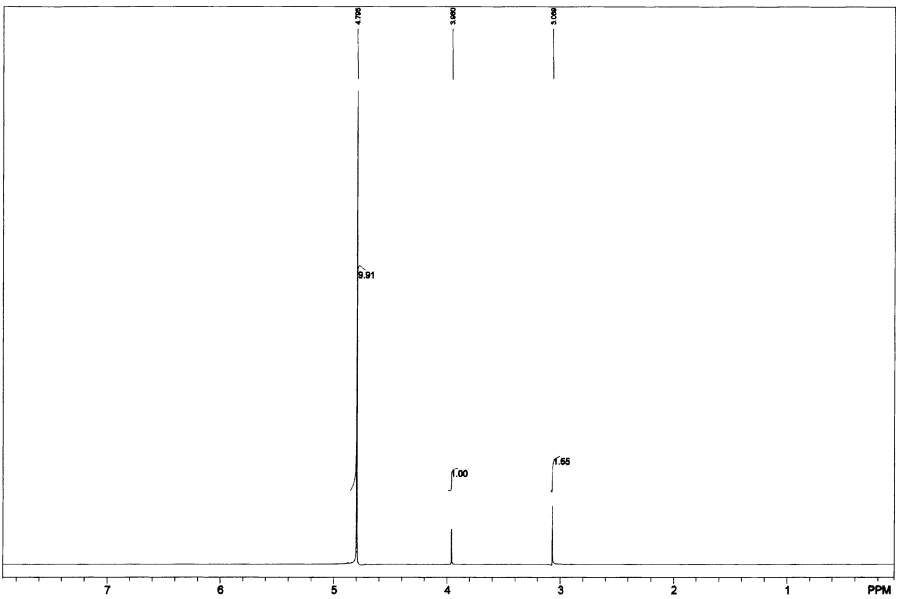


Figure 7. ^{1}H-NMR spectrum of creatine monohydrate in D_2O.

structural information about a molecule. NMR spectroscopy can be applied to atoms having nuclear spin; the most important for NMR analysis of living matter are the hydrogen (^{1}H), carbon (^{13}C) and phosphorus (^{31}P) atoms.

Figure 7 shows a ^{1}H-NMR spectrum of creatine monohydrate. The singlets at 3.069 ppm and 3.960 ppm are caused by the protons of the N-CH_3 group and the methylene protons of the acetate group, respectively. As expected, the integrals indicate a ratio of 3:2. The signal at 4.795 ppm represents the sum of the NH-protons, the protons of water of crystallization, and the protons of HDO contained in D_2O.

4.4.2. ^{13}C-NMR-spectroscopy of creatine

The ^{13}C-NMR spectrum (Figure 8) consists of 4 signals. The signals at 36.935 ppm and 53.875 ppm are caused by the carbon atoms of the methyl group and the methylene group, respectively. The chemical shifts of the guanidine carbon at 157.127 ppm and the carboxylate carbon at 174.604 ppm are in agreement with expectations.

4.4.3. IR-spectroscopy of creatine

Infrared (IR) spectroscopy measures the vibrations of molecules. Each functional group, or structural characteristic, of a molecule has a unique vibrational frequency that can be used for its identification in a sample. When the effects of all the different functional groups are taken together, the result is a unique molecular "fingerprint" that can be used to confirm the identity of a sample.

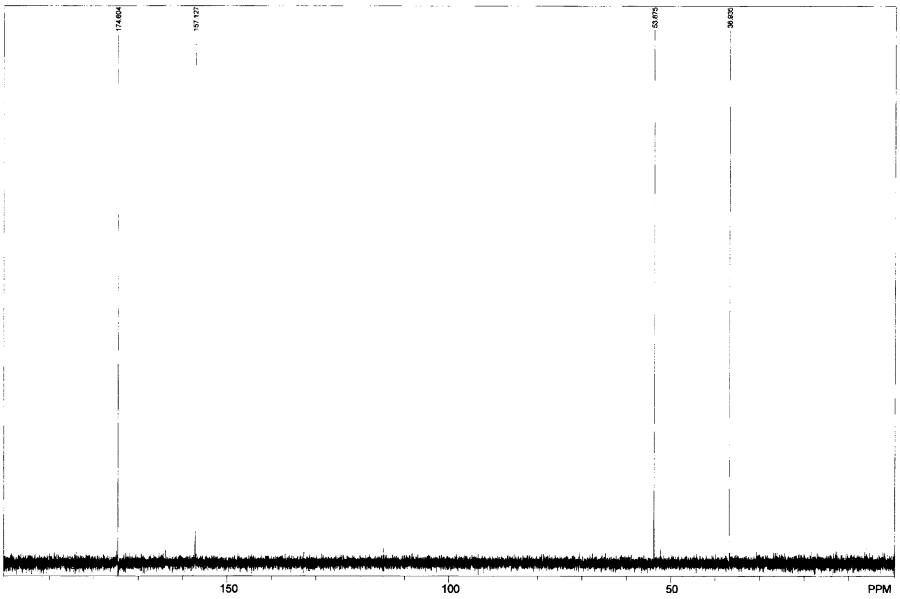


Figure 8. ^{13}C-NMR spectrum of creatine monohydrate in D_2O.

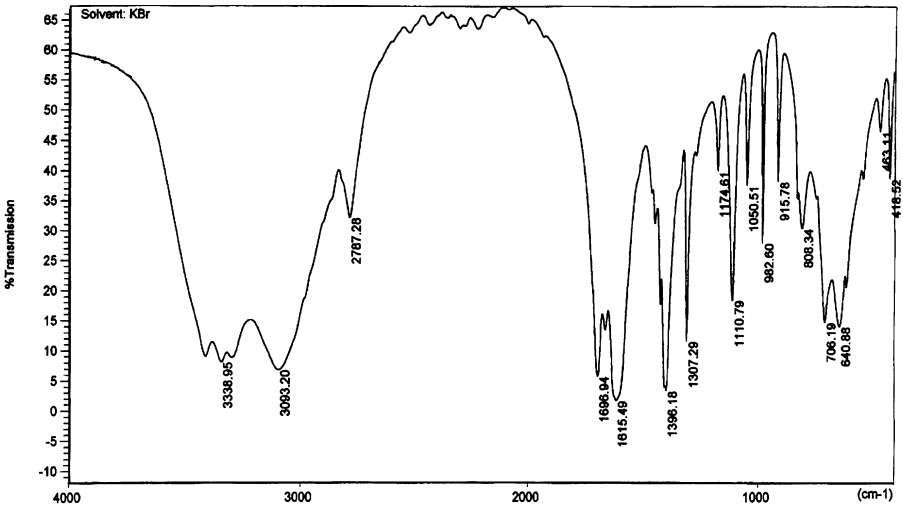


Figure 9. IR-spectrum of creatine monohydrate.

The infrared spectrum of creatine monohydrate in KBr is shown in Figure 9. The broad and intense absorption band between 3500 and 2800 cm^{-1} is due to the valence vibrations of the O-H and N-H bonds of crystal water and the guanidine group, respectively. The absorption band at 2787 cm^{-1} is caused by the valence vibrations of the C-H bonds of the methyl group. The intense bands between 1700 and 1600 cm^{-1} are caused by the valence vibrations of the C–O bonds of the carboxyl group and the C–N bonds of the guanidine group.

5. POTENTIAL IMPURITIES

The analytical methodologies described above are tools for the investigation of synthesised products like creatine monohydrate and its derivatives. This includes both evaluation in respect of the product's quality, more specifically its purity, and the detection of possible impurities. Due to the rather high amount of creatine monohydrate that is consumed for its sports application or when used as supplementation in certain disease conditions, it is obvious that even small proportions of foreign matter, by-products or impurities may be harmful.

As described in section 3 of this chapter, several processes are currently in use to manufacture creatine monohydrate on an industrial scale. Therefore, creatine monohydrate may be contaminated with specific impurities depending on the used process. Analytical investigation of commercially available creatine formulations was undertaken using HPLC to elucidate the differences in quality among marketed products (Figure 10). Distressingly, among those marketed products, some were found to contain the following levels of impurities (for the chemical structures, see Figure 11): up to 54,000 ppm (5.4%) dicyandiamide, 860 ppm (0.09%) dihydrotriazine, and 13,000 ppm (1.3%) creatinine. In addition, elevated levels of heavy metals like mercury and

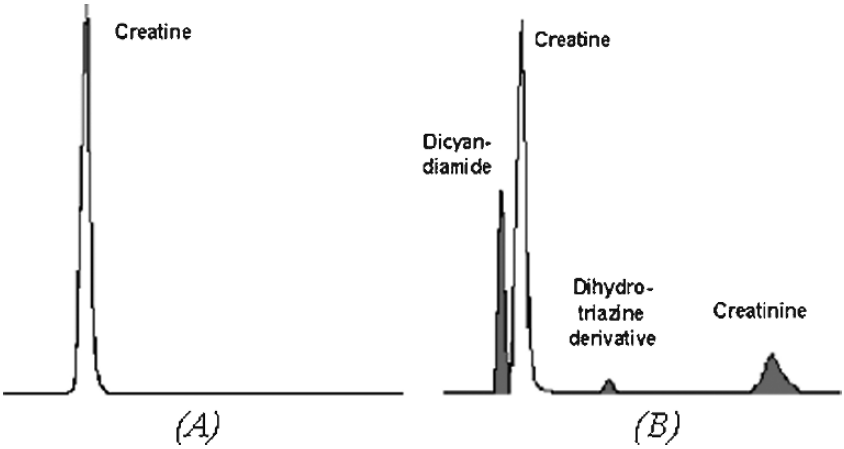


Figure 10. HPLC spectrum of two commercial creatine monohydrate products: (A) pure; (B) contaminated.

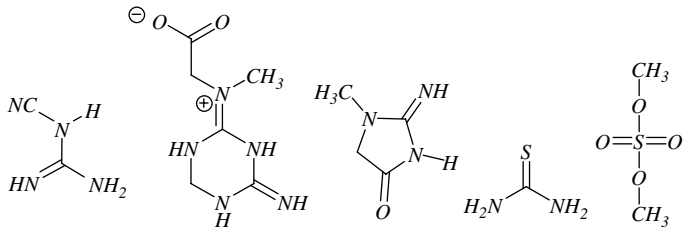


Figure 11. Potential impurities found in commercial creatine monohydrate preparations due to poorly controlled manufacturing processes.

lead have been detected (http://www.creapure.com, August 2006). The contaminations can be explained by poorly controlled synthesis or unsuccessful purification during production. The chemical mechanisms for the formation of by-products during manufacturing have been elucidated and experimentally confirmed at Degussa (Thomas Gastner, unpublished data).

If sodium sarcosinate and cyanamide or O-methylisourea are used as raw materials, the potential by-products are dihydrotriazine and creatinine. Creatinine is a conversion product of creatine, especially when kept in acidic conditions. Dihydrotriazine is formed from contaminations within the sarcosinate solution and has unknown pharmaceutical and toxicological properties. Dihydrotriazine might be one of the most dangerous impurities in creatine monohydrate, since structurally related compounds are known to be carcinogenic. Furthermore, if cyanamide is used as a raw material, the dimerization product dicyandiamide can be formed, especially when cyanamide is used in excess or introduced into the reaction medium at a too high pH value.

If sodium sarcosinate and O-methylisothiourea are used as raw materials, the potential by-products are dihydrotriazine, creatinine, dimethyl sulphate and thiourea. O-Methylisothiourea is made by reaction of thiourea with dimethyl sulphate which explains potential contamination with these two compounds. Thiourea and dimethyl sulphate are harmful if ingested and are known carcinogens. For that reason, it is an obvious and unavoidable postulate not only to control and guard the manufacturing process, but also to implement an appropriate quality assurance policy to maintain high quality standards.

6. RECENT DEVELOPMENTS

In the last decade, a number of innovations and developments were described to overcome technical limitations that creatine bears. One major obstacle of creatine as an ampholytic amino acid is its rather low solubility in water. A solution to this was the formulation of more soluble creatine salts and complexes based on mono-, di- or tricarboxylic acids to form for instance the citrate, maleate, fumarate, tartrate (Negrisoli and Del Corona, 1997), pyruvate (Pischel and Weiss, 1996), ascorbate

(Pischel *et al.*, 1999) or orotate salts (Abraham and Jiang, 2005) of creatine. A few of those novel chemical compounds have – aside from a higher solubility – other advantages, like additive or synergistic effects, e.g. a combination of the ergogenic effect of creatine with anti-oxidative properties of ascorbic acid. The creation of carnitine creatinate (Fang, 1999) was likely undertaken to combine the benefits of both constituents, creatine and carnitine. Similar advantages are claimed for creatine hydroxycitrate that may be used as a dietary supplement for the purposes of reducing adiposity and of appetite suppression, as well as for improvement of muscle and exercise performance (Gardiner *et al.*, 2006). Carnazzo (1999) describes in his patent the use of edible creatine citrate and its formulation for effervescent tablets. Solid and stable creatine/citric acid compositions with carbohydrate were developed by Purpura *et al.* (2005).

Recently, creatine products with an alkaline pH value came onto the market, which claim a higher stability and better bioavailability. However, no clinical studies have been performed and there is no scientific evidence supporting these claims. Due to their weak buffering capacity, these products are also adjusted to an acidic pH in the stomach and will therefore be degraded in the same way as pure creatine monohydrate. For creatine monohydrate, it has been shown that less than 0.1 g of a 5 g dose is lost within 1 hour at pH 3.5. The stability of creatine at pH 1 is even better. Therefore, conversion of creatine to creatinine in the gastrointestinal tract is minimal regardless of transit time (Persky *et al.*, 2003).

7. REGULATORY STATUS OF CREATINE MONOHYDRATE AND ITS SALTS

In the European Union, creatine and its salts fall under the definition of "food" according to Art. 2 of regulation 178/2002 (http://eur-lex.europa.eu/LexUriServ/site/de/oj/2002/l_031/l_03120020201de00010024.pdf) of the European parliament and of the council as they are a "substance or product intended to be, or reasonably expected to be ingested by humans." Taking Art. 1 of Council Directive 89/107/EEC (amendment 1994; http://www.fsai.ie/legislation/food/eu_docs/Food_additives/Dir89.107.pdf) concerning additives approved for use in foodstuffs intended for human consumption into consideration, it is clear that creatine and its salts are not food additives. Their most precise definition is found in the food supplements directive 2002/46/EC (http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_183/l_18320020712en00510057.pdf) as they meet the criteria of Art. 2 (a): "foodstuffs… which are concentrated sources of nutrients or other substances with a nutritional or physiological effect …". In the United States, creatine monohydrate and its salts are classified as "dietary supplements" under the 1994 "Dietary Supplements Health and Education Act" (http://www.fda.gov/opacom/laws/dshea.html).

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