

Determination of nitrogen content

Kjeldahl determination according to USP general chapter <461>

Summary

The Kjeldahl method is used to determine the nitrogen content in organic and inorganic samples. The Kjeldahl analysis consists of three steps: digestion, distillation, and titration. During the catalytic digestion step, organic nitrogen (except nitro- and azo-groups and nitrogen in rings) is converted into ammonium. Sodium hydroxide is added just before the distillation step in order to convert the ammonium into ammonia. Through steam distillation, the latter is transferred into the receiver vessel containing an absorbing agent (e.g., boric acid). Finally, the separated ammonia is titrated with sulfuric acid.

Protein content in samples can also be determined from the nitrogen content obtained by Kjeldahl setup. USP<461> describes the titration method to determine nitrogen content in organic products using Kjeldahl nitrogen setup. This Application Note illustrates nitrogen determination in heparin sodium.

Configuration



2.905.0010 - 905 Titrand

High-end titrator for potentiometric titration with one measuring interface for use with Dosino dosing systems. up to four dosing systems of the 800 Dosino type; dynamic (DET), monotonic (MET), and endpoint titration (SET); Measurement with ion-selective electrodes (MEAS CONC); Dosing functions with monitoring, Liquid Handling; four MSB connectors for additional stirrers or dosing systems; "iTrode" intelligent electrodes; USB connector; For use with OMNIS Software, ti amo software, or Touch Control unit; Compliance with GMP/GLP and FDA regulations such as 21 CFR Part 11, if required;



6.0278.300 - iUnitrode with Pt1000

Intelligent, combined pH electrode with integrated memory chip for storing sensor data and Pt1000 temperature sensor. This electrode is particularly suitable for pH measurements and titrations in difficult, viscous, or alkaline samples; at elevated temperatures; for long-term measurements; The fixed ground-joint diaphragm is insensitive to contamination. Reference electrolyte: $c(\text{KCl}) = 3 \text{ mol/L}$, storage in storage solution. Alternatively: reference electrolyte for measurements at $T > 80^\circ\text{C}$: Idrolyte, storage in Idrolyte. iTrodes can be connected to Titrand, Ti-Touch or 913/914 meters.

Sample and sample preparation

The analysis is demonstrated on heparin sodium. An appropriate amount of heparin sodium is weighed accurately and transferred into a clean 2-neck round bottom flask. Sodium sulfate, copper sulfate, and sulfuric acid are added for the digestion process. The content is gently heated below the boiling point until the frothing ceases. It is then heated again at a higher temperature until the content boils and the solution becomes brown. The contents are cooled and carbon dioxide-free water is added carefully while thoroughly mixing. Sodium hydroxide solution is added through the side-neck of the round bottom flask. Granulated zinc is added

and the flask is connected immediately to the Kjeldahl nitrogen distillation setup. The outlet of the setup is put into a solution of boric acid. The distillation is carried out until approximately 80% of the total volume is transferred into the boric acid solution.

Experimental



Figure 1. Example of a Titrand system consisting of a 905 Titrand and a 900 Touch Control. Alternatively the 905 Titrand can also be connected to a PC and controlled by *tiamo*.

The analysis is performed automatically on a Titrand system consisting of a 905 Titrand. The Unitrode is used for the indication of the titration curve.

The prepared sample is titrated potentiometrically against standardized sulfuric acid until after the first equivalence point.

Results

Sharp titration curves are obtained where the equivalence point is reliably determined by the Touch Control or *tiamo*TM.

The determined nitrogen content of heparin sodium is 1.581% (SD(rel) = 1.48%, n = 5), which is within the nitrogen content specified by USP (1.3% to 2.5%) for heparin sodium.

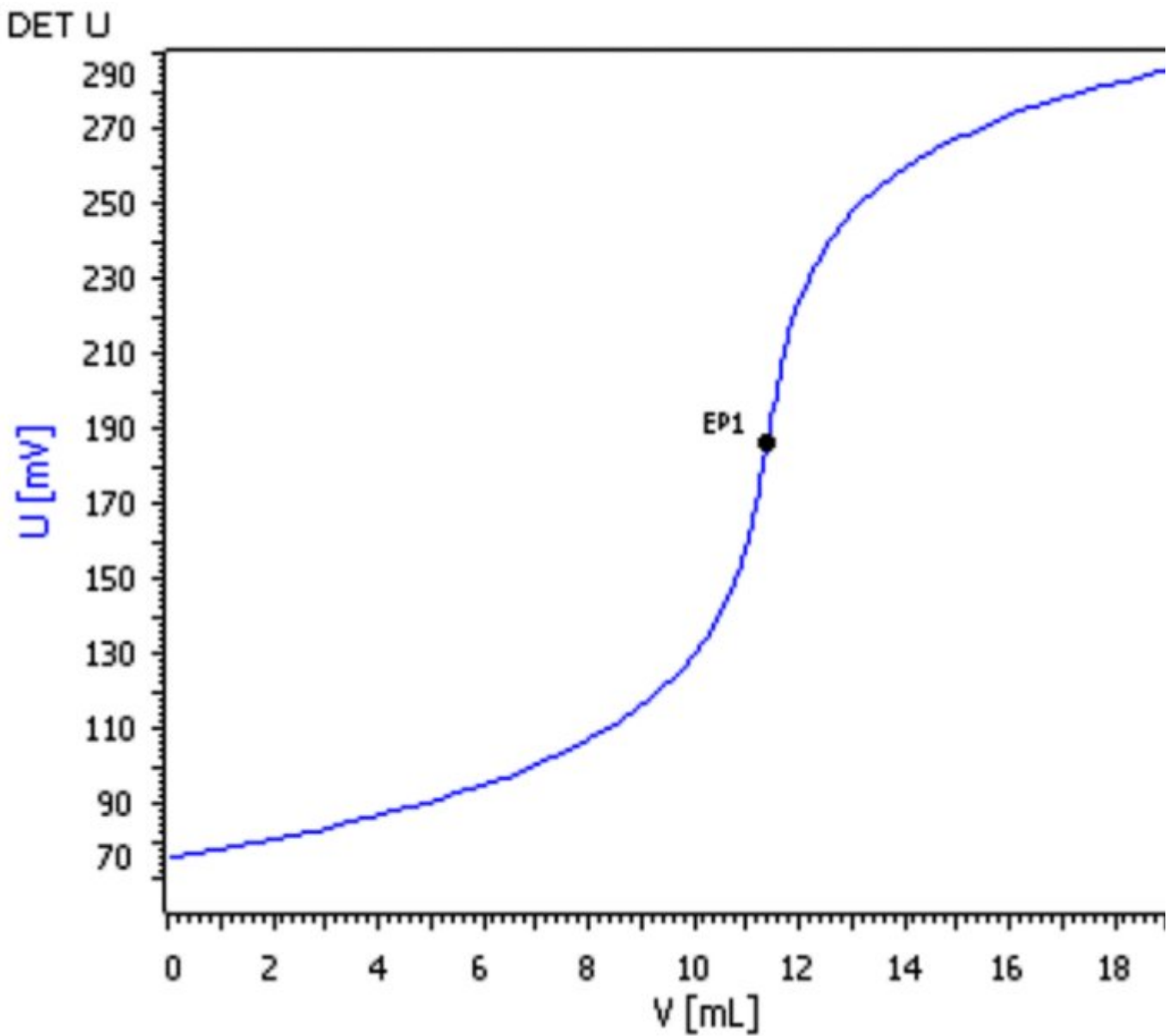


Figure 2. Titration curve of the Kjeldahl determination of heparin sodium according to USP general chapter <461>.

Conclusion

This method shows the possibility to determine the nitrogen content in various kinds of samples automatically, accurately, and reliably by titration according to the **USP general chapter <461>**.

Aside from heparin sodium, the following compounds can also be analyzed with this method:

- Antithrombin III Human
- Beta Glucan

- Cellulose sodium phosphate
- Chlorophyllin copper complex sodium
- Colloidal oatmeal
- Copovidone
- Crospovidone
- Dalteparin sodium
- Dextran 1
- Dextrin
- Dihydroxyaluminum aminoacetate,
- Enoxaparin sodium etc.
- Guar gum
- Mecamylamine Hydrochloride tablets
- Melphalan
- Polyvinyl acetate dispersion
- Povidone iodine
- Povidone
- Psyllium hemicellulose
- Pullulan
- Racemic calcium pantothenate
- Ralbumin human
- Scaffold bovine dermis
- Spirulina tablet
- Taurine
- Thioguanine
- Trehalose
- Wheat bran
- Zein

Metrohm AG

Ionenstrasse

9100 Herisau

<mailto:info@metrohm.com>