- Report -

Hydrolysed protein

Determination of Surface Tension

acc. to OECD 115 for Testing of Chemicals (1995)
and Council Regulation (EC) No. 440/2008, Method A.5

Sponsor

SICIT Group SPA
Via Arzignano 80
Chiampo (VI)
Italy

Author

Jens Lange

Test Facility

Noack Laboratorien GmbH
Käthe-Paulus-Str. 1
31157 Sarstedt
Germany

Study ID

acc. to GLP

190311BY / CPT18671

Study completion date

2019-12-11

Responsibilities

TEST FACILITY Noack Laboratorien GmbH
Käthe-Paulus-Str. 1
31157 Sarstedt
Germany

Phone: +49 (0) 5066 706 70
Fax: +49 (0) 5066 706 789
Email: info@noack-lab.de

Management Dr. Christian Maeß (Chemist)

Head of QAU Gudrun Möhrmann-Kalabokidis (Biologist)
Email: gudrun.moehrmann-kalabokidis@noack-lab.de

Study director Jens Lange (Technical Expert)
Address: see above
Email: jens.lange@noack-lab.de

Statement of GLP Compliance

Title	Hydrolysed protein Determination of Surface Tension
Guidelines	• OECD Guideline 115 (1995) • Council Regulation (EC) No. 440/2008, Method A.5
Test Item	Hydrolysed protein (Batch number: 19070125)
Test Facility	Noack Laboratorien GmbH Käthe-Paulus-Str.1, 31157 Sarstedt, Germany Phone: +49 (0) 5066 706 70, Fax: +49 (0) 5066 706 789, E-mail: info@noack-lab.de

I declare that this study was conducted and reported in compliance with present OECD, EC and German principles of Good Laboratory Practice.

2019-12-11
.....
(Date)

.....
(Jens Lange, Study Director)

Statement of the Quality Assurance Unit

Title	Hydrolysed protein Determination of Surface Tension
Guidelines	• OECD Guideline 115 (1995) • Council Regulation (EC) No. 440/2008, Method A.5
Test Item	Hydrolysed protein (Batch number: 19070125)
Study Director	Jens Lange

The study was verified and reported to the study director and test facility management as follows:

Inspected study phase		Inspection date	Date of report
Study plan		2019-08-07 2019-08-27	2019-08-07 2019-08-27
Study plan amendment		2019-09-27	2019-09-27
Experimental phase	Test system	2019-09-27	2019-09-27
Report		2019-10-14 2019-10-28 2019-12-11	2019-10-14 2019-10-28 2019-12-11

It is confirmed that the reported results accurately and completely reflect the raw data of the study. Also methods, procedures, and observations are accurately and completely described in the report. The accordance of the study with its study plan and the principles of Good Laboratory Practice is guaranteed.

2019-12-11
.....
(Date)

.....
(Christine Bruhnke, QAU)

Table of Contents

List of Tables

List of Figures

Abbreviations / Definitions

CAS	Chemical Abstracts Service
CoA	Certificate of analysis
Date notation	YYYY-MM-DD (Year-Month-Day)
EC	European Council
OECD	Organization for Economic Co-operation and Development

1 Summary

The surface tension of a 1 g/L aqueous solution of the test item Hydrolysed protein (batch number: 19070125) was determined according to OECD Guideline No. 115 (1995) and Council Regulation (EC) No. 440/2008 Method A.5 with the OECD harmonized ring method on 2019-09-27 at the test facility.

The determination was carried out with a tensiometer in ten successive measurements with a standard deviation ≤ 0.05 mN/m at a temperature of 20 °C.

The **surface tension** of a 1 g/L aqueous solution of the test item **Hydrolysed protein** was determined to be:
52.33 mN/m (standard deviation ± 0.05 mN/m) at 20 °C.

Considering that distilled water has a surface tension of 72.75 mN/m at 20 °C and the test item showed a surface tension below 60 mN/m under the conditions of this referring method, the test item should be regarded as being a surface-active material.

2 Test Item

2.1 Test Item Properties

Test item	Hydrolysed protein
Batch number	19070125
CAS no.	9015-54-7
Purity	44.1% w/w
Appearance	Brown liquid
Water solubility	>1000 g/L
Stability under test conditions	Not specified
Expiry date	2024-02-18
Recommended storage	Protect the product from direct sunlight. Store the product in the original container at room temperature.

The test item and the information concerning the test item were provided by the Sponsor.

2.2 Test Facility Actions

Receipt	2019-06-12
Identification parameters	Name, batch number, state and color
Retention sample	At least 1 g of the test item has been retained on 2019-06-14 and stored at 6 ± 2 °C.
Storage conditions	18 – 25 °C, dark, in tightly closed original container

3 Method

3.1 General Information

TEST GUIDELINES	• OECD Guideline No. 115 for the Testing of Chemicals (1995), 'Surface Tension of Aqueous Solutions' • Council Regulation (EC) No. 440/2008, Method A.5, 'Surface Tension' The study was performed in compliance with GLP. For the respective guidelines please refer to the chapter 'Literature / References'.
TYPE AND PURPOSE OF THE STUDY	Determination of the surface tension of the test item via tensiometer using the OECD harmonised ring method, which is based upon the standards ISO 304-1985, DIN 53914, ASTM-D-1590 and ASTM-D-1331.

3.2 Test System and Test Procedure

TEST SYSTEM	KRÜSS K11HRX tensiometer: Consisting of a platinum iridium ring (thickness: approx. 0.4 mm, mean circumference: 60 mm, internal no. 1) connected to a precision balance and with a mobile sample table (accuracy of measurements: 0.1 mN/m). KRÜSS GMBH WISSENSCHAFTLICHE LABORGERÄTE Borsteler Chaussee 85-99a 22453 Hamburg, Germany Software: KRÜSS Laboratory Desktop, Version 3.2.2.3068 PC: notebook, Compaq 6710b, HP
Reason for the selection of test system	The selection of the test system is based on the guidelines and is state of the art.
Calibration	When the tensiometer was used, the force measuring system of the apparatus was calibrated daily using a mass calibration procedure.

TEST PROCEDURE

Test item	Hydrolysed protein
Test concentration	1 g/L

Replicates	Single
Control	According to the guidelines, no control item is necessary.
Reference item	A reference study is carried out every year with n-heptane to show the validity of the test design (most recent determination in June 2019, see chapter 5.5).
Test vessel	Glass dish with an inner diameter of ca. 65 mm and a height of ca. 35 mm
Number of measurements	10 consecutive measurements with a standard deviation of ≤ 0.05 mN/m
Preparation of the ring and the test vessel	The ring and the test vessel were rinsed before the start of the test with acetone and water and flamed with the flame of a gas burner. The test vessel was then rinsed with water. This cleaning procedure is in accordance with the operating instructions of the tensiometer and has been shown to be suitable and sufficient based on our experience.
Preparation of the test solution	200 mg of the test item were weighed out (to the nearest 0.1 mg) into a 200 mL measuring flask and filled up to the mark with ultrapure water (test facility device). This solution was stirred in the closed flask for 45 minutes at about 20 °C in an air-conditioned room to prepare 1 g/L test solution (see 'Test concentration'; room temperature was recorded).
Application	The test solution was transferred carefully into the test vessel to avoid foaming. The glass dish was filled up to approx. 5 - 10 mm below the edge and placed on the table of the test apparatus.
Performance of the test (tensiometer program)	After the tensiometer program had been started, the test vessel was automatically raised by the tensiometer until the ring was immersed below the surface of the test solution. The table top was lowered at a rate of 5 mm/min until the maximum force was reached.*[1] This applied force was automatically detected and registered by the tensiometer and subsequent measurements were automatically performed until a constant surface tension value was reached over 10 consecutive measurements.

*[1] without separation of the attached liquid layer from the ring

Temperature (target) during tensiometer measurements	20 ± 0.5 °C (test solution) The temperature in the test solution was kept constant using a refrigerated circulator.
Measuring speed	5 mm/min
Depth of immersion	3 mm
Density via tensiometer for the ring method	The density (20 °C) of the test solution was determined in a preliminary measurement via tensiometer using the buoyancy method. This density was used for the subsequently obtained surface tension values (correction method of HARKINS & JORDAN for the ring method, automatically taken into account by the tensiometer software).
TYPE AND FREQUENCY OF MEASUREMENT	The times at preparation of the test solution, transferring the test solution into the test vessel and the start of the measurements were recorded. After the test vessel was placed on the mobile sample table, the tensiometer program was started. The test solution temperature was recorded automatically with each measurement by the tensiometer.
Equipment	• Analytical balance, KERN • Magnetic stirrer, VARIOMAG • Data logger with temperature sensor, TESTO • Gas burner, CFH • Thermostat (refrigerated circulator), LAUDA • Radio controlled clock, CONRAD ELECTRONIC • Aluminium ruler, STAEDTLER • Milli-Q device, MERCK • Standard laboratory equipment
Reagents	• Ultrapure water (test facility device)

3.3 Calculations

The correction method of HARKINS & JORDAN was used. The results of the measurements were corrected automatically by the tensiometer software.
Further calculations were made in accordance with the test guidelines; the software used was MICROSOFT® EXCEL.

3.4 Validity Criteria

The temperature of the test solution has to be 20 ± 0.5 °C.

4 GLP

Chronological test description	• Preparation of the test solution (experimental starting) • Cleaning of the ring and the test vessel • Transfer of test solution into the test vessel • Determination of the surface tension of the test solution
Deviations from the guidelines	None
Deviations from the study plan	None

DATES

Study initiation	2019-08-27
Study plan amendment	2019-09-20
Experimental starting	2019-09-27
Experimental completion	2019-09-27
Study completion	Please refer to page 1

Archiving

The following will be retained in the archive of the test facility for at least 15 years:

- all raw data
- study plan
- final report
- all records performed by the quality assurance program including master schedules
- sample of the test and reference item

The test facility may use a GLP contract archive.

5 Results

5.1 Determination of the Density via Tensiometer for the Ring Method

The density of the test solution was determined in a preliminary measurement via buoyancy method to be 1.001 g/cm³ (20.0 °C). This density was used for the subsequently obtained surface tension values (correction method of HARKINS & JORDAN for the ring method, automatically taken into account by the tensiometer software).

5.2 Temperature

The temperature of the test solution during the whole series of measurements was 19.8 °C.

5.3 Time Line of the Determination of the Surface Tension

Start of measurement was 39.2 minutes after transfer of the test solution into the test vessel. After that time 21.5 minutes were needed to reach equilibrium at which 10 successive measurements were successfully achieved with a standard deviation of ≤ 0.05 mN/m. In Table 1 the time line of the study is shown. The time dependence of the measured surface tension is shown in Figure 1.

Table 1: **Time Line of the Determination of the Surface Tension**

	Date	Time [hh:mm:ss]	Time from transfer of test solution to start [min]
Preparation[1)] of the test item solution	2019-09-27	10:22	
Transfer into test vessel		11:07:22	
Start of measurement		11:46:36	39.2

1) see section 3.2 for the preparation

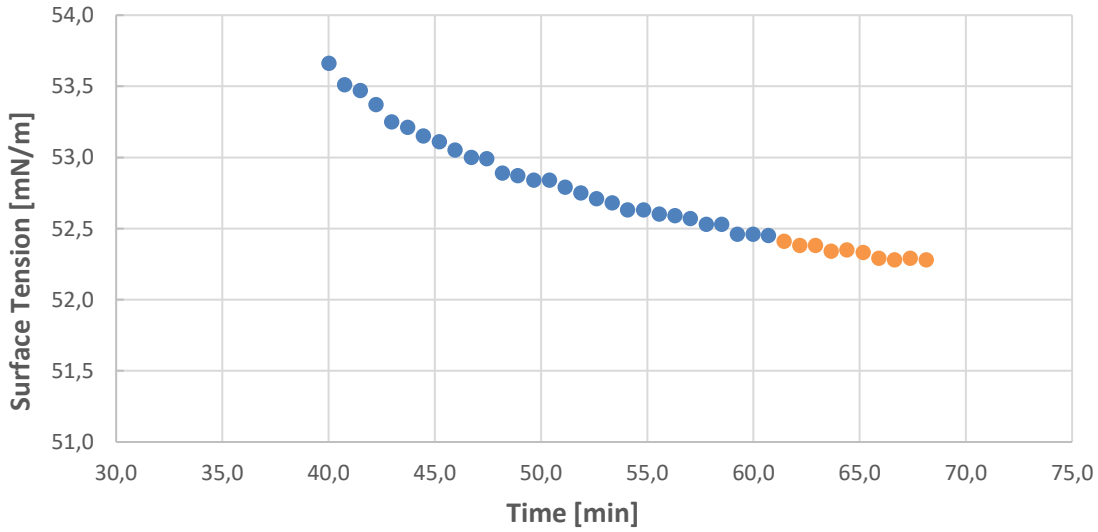

Figure 1: **Time Dependence of the measured Surface Tension of the Test Item**

5.4 Surface Tension of the Test Item

The determination was carried out in ten successive measurements with a standard deviation of ≤ 0.05 mN/m. For further details see Table 2.

Table 2: **Surface Tension, Temperature**

Measurement No.	Time [1] [min]	Time [2] [min]	Surface Tension [mN/m]	Temperature [°C]
1	61.4	22.2	52.41	19.8
2	62.2	23.0	52.38	19.8
3	62.9	23.7	52.38	19.8
4	63.7	24.4	52.34	19.8
5	64.4	25.2	52.35	19.8
6	65.2	25.9	52.33	19.8
7	65.9	26.7	52.29	19.8
8	66.6	27.4	52.28	19.8
9	67.4	28.1	52.29	19.8
10	68.1	28.9	52.28	19.8
Mean	**-**	**-**	**52.33**	**19.8**
SD [±]	**-**	**-**	**0.05**	**0.0**

SD = standard deviation
1) Time from transfer of test solution into test vessel until end of each measurement
2) Time from start of measurement until end of each measurement

5.5 Determination of the Reference Item

The surface tension of the reference item n-heptane was determined on 2019-06-13 as 20.22 ± 0.05 mN/m (20 °C) for the platinum iridium ring RI 21, internal no. 1, and is within the acceptable range of 20.23 ± 0.22 mN/m.

5.6 Validity Criteria

The temperature of the test solution was 20 ± 0.5 °C.

6 Conclusions

The surface tension of a 1 g/L aqueous solution of the test item Hydrolysed protein was determined to be 52.33 mN/m (standard deviation ± 0.05 mN/m) at 20 °C.
Considering that distilled water has a surface tension of 72.75 mN/m at 20 °C and the test item showed a surface tension below 60 mN/m under the conditions of this referring method, the test item should be regarded as being a surface-active material.

7 Literature / References

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/Chem(98)17, Environment Directorate, OECD, Paris, 1999
- Directive 2004/10/EC, The OECD Principles of Good Laboratory Practice (GLP)
- Principles of Good Laboratory Practice – German Chemical Law (ChemG), Annex 1
- OECD Guidelines for the Testing of Chemicals, No. 115 (1995) ‘Surface Tension of Aqueous Solutions’
- Council Regulation (EC) No. 440/2008 Method A.5, ‘Surface Tension’
- ISO 304:1985, ‘Surface active agents - Determination of surface tension by drawing up liquid films’
- Guidance on the Biocidal Products Regulation: Volume I: Identity of the active substance/physico-chemical properties/analytical methodology - Information Requirements, Evaluation and Assessment. Parts A+B+C, Version 2.0, May 2018
- HARKINS, W.D. & JORDAN, H.F (1930). J. Amer. Chem. Soc., 52, 1751.

8 Certificate of Test Item Analysis

SICIT CHEMITECH S.p.A.
Capitale soc. int. vers. € 1.000.000,00
36072 CHIAMPO (Vicenza) - ITALIA - Via Arzignano, 80
Tel. +39 0444 450946 (4 linee r.a.) - Fax +39 0444 453812

Società Unipersonale
Registro delle Imprese di Vicenza, Codice Fiscale
e Partita IVA IT 02821790249 - R.E.A. 278043

CERTIFICATE OF ANALYSIS N. 19005485

Product:	HYDROLYSED PROTEINS
Code:	110590 DO.LA.11
Batch:	19070125
Production date:	18/02/2019
Expiry date:	18/02/2024
Destination:	Noack Laboratorien GmbH
Delivery date	06/06/2019
Packing:	plastic bottle 1 L

PARAMETERS	ANALYTICAL RESULTS	MEASURE UNIT	METHOD	SPECIFICATION
Aspect	Complies			brown coloured liquid
Solubility	>1000	g/l	EPA830-7840	
Dry matter	59,4	% w/w	TGA01	≥ 58,0
Density	1,27	g/ml	DEN01	
Total nitrogen	7,60	% w/w	CNLECO01	
Ammoniacal nitrogen	0,55	% w/w	N03	
Organic nitrogen	7,05	% w/w	calculated	≥ 6,50
Total carbon	24,4	% w/w	CNLECO01	≥ 20,0
Active Ingredient :				
Hydrolysed proteins	44,1	% w/w	calculated	
Free amino acids	9,23	% w/w	HP02	
Ashes	10,6	% w/w	TGA01	
Calcium	0,24	% w/w	HP04	
Sodium	4,07	% w/w	HP04	
Chloride	3,92	% w/w	HP04D	
Sulfate	1,08	% w/w	HP04D	
pH in 10% sol.	6,43		PH01	≥ 6,00 ≤ 7,00

APPROVED Date 10/06/2019 Quality Control Manager
Eliana Franco

9 GLP-Certificate of Noack Laboratorien

Gewerbeaufsicht in Niedersachsen

Staatliches Gewerbeaufsichtsamt Hildesheim

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Abs.1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Noack Laboratorien GmbH Käthe-Paulus-Str. 1 31157 Sarstedt DEUTSCHLAND	**Noack Laboratorien GmbH** Käthe-Paulus-Str. 1 31157 Sarstedt GERMANY

Prüfungen nach Kategorien / Areas of Expertise (gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)

1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	1 - physical-chemical testing
4 - Ökotoxikologische Prüfungen zu Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	4 - environmental toxicity studies on aquatic and terrestrial organisms
5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung	5 - studies on behaviour in water, soil and air; bioaccumulation
6 - Prüfungen zur Bestimmung von Rückständen	6 - residue studies

Ort / Place	Datum der Inspektion / Date of Inspection (Tag.Monat.Jahr / month.day.year)
Sarstedt	**07. – 10. Juni 2016 & 13. Juli 2016 /**
Sarstedt	**Jun 07th – Jun 10th, 2016 & Jul 13th, 2016**

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Hildesheim, 03.01.2017

Staatliches Gewerbeaufsichtsamt Hildesheim
Im Auftrage

Bahn

GEWERBEAUFSICHTSAMT HILDESHEIM