DB-ALM Protocol n° 26 : The Zein Test

Skin Irritation and Corrosivity

This method determines the amount of the corn protein, zein, dissolved by a surfactant as mg nitrogen in 100ml of surfactant solution, the value of which is referred to as the "zein number". The method may be applicable to detergent raw materials as a screening test for the evaluation of the relative mildness of surfactants.

Résumé

The "zein test" is based on the solubilisation of the water insoluble corn protein, zein (which resembles the protein present in human skin and hair), by surfactants. Götte (1964) demonstrated that the solubility of zein in surfactant solutions correlated well with skin irritation/skin roughness caused by surfactants. He found that skin irritancy increased with increasing solubility of zein.

Experimental Description

Endpoint and Endpoint Measurement:

SOLUBILITY: solubility of zein; content of solubilised zein determined by nitrogen content

Experimental System(s):

ZEIN (corn protein)

Basic Procedure

A known weight of zein is shaken with an aqueous solution of surfactant containing 10g/l of active matter. The amount of zein solubilised is determined by measuring the nitrogen content of the aqueous solution obtained by filtering the mixture. A sample of sodium dodecyl sulphate (SDS) is also analysed with each batch of test samples as a reference.

Test Compounds and Results Summary

Anionic surfactants
Sodium lauryl sulphate
Sodium alkyl sulphates
Sodium alkyl ether sulphates
Alpha olefin sulphonates (sodium neutralised)
Alkyl sulphosuccinates (sodium neutralised)

Cationic/amphoteric surfactants
Quaternary ammonium compounds
Alkyl betaines
Alkylamidobetaines
Alkyl imidazoline derivatives
Amine oxides
Carboxylates

Discussion

In comparison to conventional animal testing, the zein test is a rapid (4-5 hours), low cost technique, which uses the already existing expertise to be found in a general analytical laboratory.

It has been demonstrated that the zein method yields repeatable results when applied to most classes of surfactants which are in general agreement with literature values. Although, the majority of the work has been carried out with anionic surfactants, the method has been shown to be satisfactory for cationic and amphoteric surfactants.

The method is applicable to detergent raw materials as a screening test for the evaluation of the relative mildness of detergents. The method is based on a procedure which originally came from AZKO but with some minor modifications. Albright and Wilson's method is carried out at ambient temperature rather than at 35^{0}C where the zein has a tendency to coagulate. Also the AZKO method uses both demineralised water and sodium lauryl sulphate for "calibration" purposes.

This method is a considerable improvement on the AZKO method, because the correction for the solubility of zein in the demineralised water is negligible.

It should be noted that when zein is added to a solution of alkyl betaines (containing 10g/l of active matter) it coagulates into one solid mass and will not disperse even with vigorous shaking.

Comparison with other test systems
Kästner and Frosch have recommended the zein test as a screening method for evaluating the skin tolerance of surfactants, because of its good correlation to results obtained on human skin under conditions of the Duhring chamber test - a modified patch testing method.

In general, the zein test has been shown to correlate well with the Duhring chamber test - with the exception of alkyl benzene sulphonate and sodium dodecyl sulphate. High zein numbers were found for surfactants with high scores and low zein numbers for low scores.

Lang and Spengler correlated critical micelle concentrations (CMCs) of surfactants and mixtures of surfactants, with skin irritancy, assessed by Duhring chamber and zein test results. They concluded that a low CMC was indicative of low skin irritancy.

It has been acknowledged that the zein test is probably better associated with skin irritancy evaluation rather than ocular irritation assessment. However, CMC determinations have been shown to be relevant to both skin and eye irritation studies.

Status

In-house development.

OTHER ORGANISATIONS USING THE TEST
The Safety Evaluation section of Boots Pharmaceuticals, Nottingham, UK, are currently evaluating the zein test for efficacy.

Last update: August 1991

PROCEDURE DETAILS, *August 1991*

The Zein Test
DB-ALM Protocol n° 26

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Equipment
Boiling water bath
Laboratory centrifuge, with 50ml polypropylene centrifuge tubes
Mechanical shaker with 8 positions
pH meter, with combined glass electrode assembly for pH measurement
Oven, set at 110 ^{0}C
Glassware - 400ml glass beaker, 2 x 1000ml volumetric flask and stopper, 250ml conical flask and stopper, glass pipettes, B24 ground glass joint, test-tubes, Kjeldahl flask, burettes.
Whatman filter paper, No. 41

Materials
Sodium dodecyl sulphate - BDH
Zein purum - Fluka EGA-Chemie or similar - Use the same batch for each set of determinations
Hydrochloric acid, 0.1N (0.1mol/L) standard volumetric solution
Sodium hydroxide, 0.1N (0.1mol/L) standard volumetric solution

METHOD

(a) *Preparation of sodium dodecyl sulphate solution*
Weigh 10±0.1g of SDS into a 400ml beaker.
Add approximately 300ml of water, warm and stir to dissolve.
Transfer the solution quantitatively to a 1000ml volumetric flask, dilute to the mark, stopper and shake the flask to mix the contents.

(b) *Preparation of sample solution*
Weigh sufficient sample to contain 10±0.1g of active matter into a 400ml beaker.
Add approximately 300ml of water and stir to dissolve the sample, warming if necessary.
Using a pH meter and either 0.1N sodium hydroxide or 0.1N hydrochloric acid, as appropriate, adjust the pH of the solution to 7.0±0.2.
Transfer the solution quantitatively to a 1000ml volumetric flask, dilute to the mark, stopper and shake the flask to mix the contents.

Procedure
Transfer, by pipette, 40ml of the sample solution into a 250ml conical flasks fitted with a B24 ground glass joint. Pipette 40ml of the SDS solution into a second flask. Up to 7 samples may be conveniently analysed simultaneously with the one SDS reference.
Add 2g zein to each flask and stopper the flasks securely.
Shake each flask vigorously by hand for a few seconds then place the flasks on the mechanical shaker and continue the vigorous shaking for 1 hour.
Transfer most of the solution from each flask to centrifuge tubes and centrifuge at about 4000 r.p.m. for 15 minutes.

Filter the centrifuged solutions through No. 41 papers into labelled sample tubes.
Pipette 2ml of each filtered solution into suitably labelled 30ml Kjeldahl flasks and place the flasks on the boiling water bath.
Direct a stream of air into each flask and leave until the solutions have evaporated to dryness.
If a sample contains nitrogen, pipette 2ml of the original sample solution into a labelled Kjeldahl flask and evaporate to dryness as described above.
Place the Kjeldahl flasks in the oven for 15 minutes then allow to cool.

Continue, with each flask, as in steps 2 to 12 in Method G1C1 (Appendix 1), with one reagent blank determination for each set of samples.

Titrate each distillate in turn with 0.1N hydrochloric acid from a 10ml burette, the endpoint being from blue to grey becoming orange when overshot.

RESULTS

Calculations

(a) *For the SDS and sample free from nitrogen*

Zein number = $(T_1 - T_0) \times f \times 70$

Where
T_1 = the titration for the SDS reference or the sample
T_0 = the blank titration
f = the standardisation factor of the hydrochloric acid

The zein number for SDS usually lies within the range 490-530.

(b) *For samples containing nitrogen*

Zein number = $(T_1 - T_2 - T_0) \times f \times 70$

Where
T_1 = the titration for the sample treated with zein
T_2 = the titration for the sample solution without treatment with zein
T_0 = the blank titration
f = the standardisation factor of the hydrochloric acid

The zein number for SDS should be quoted along with the results for the samples.

Classification of results

Table 1: Classification of detergents by irritancy potential as reflected by the zein number

Zein number (mg N/100ml)	*Classification*
<200	non-irritant
200-400	moderate irritant
>400	strong irritant

Experimental Data

PRODUCT/CHEMICAL TYPE	**ZEIN NUMBER**
Anionic surfactants	
Sodium lauryl sulphate (STANDARD)	531, 528, 530, 515, 504, 494, 493, 503, 501

With each set of samples analysed a 10g/L solution of pure sodium lauryl sulphate is analysed as a reference.	Average is 511 with a relative repeatability of 7%.
Sodium neutralised alkyl and alkyl ether sulphates - laboratory prepared	
C12-14 sulphate	507
C12-14 (1EO) sulphate	360
C12-14 (2EO) sulphate	288, 278
C12-14 sulphate - mixture of 1EO + 12EO	279
C12-14 (3EO) sulphate	228, 208
C12-14 (12EO) sulphate	82
C12-18 (6EO) sulphate	113
C12-18 (8EO) sulphate	106
C12-18 (9EO) sulphate	81
Alpha Olefin Sulphonates - Sodium neutralised	
Alkyl chain	
C14/16	388
C14/16	311
C16/18	167
Alkyl sulphosuccinates - Sodium neutralised	
Sulphosuccinate type	
C12-14 (1EO)	314
C12-14 (2EO)	280
C12-14 (3EO)	227
Alkanolamide ethoxylate	137
Cocomonoethanolamide	197
Cationic/amphoteric surfactants	
Quaternary ammonium compounds	
Benzalkonium chloride	217
Alkyldimethylhydroxyethyl ammonium chloride	302
Alkyltrimethyl ammonium bromide	319
C14 trimethylammonium bromide	237
Alkyldimethylethylbenzyl ammonium chloride based on C16-18	137
Alkyldimethylethylbenzyl ammonium chloride based on C12-14	114
Alkyl betaines	
C10 Betaine	0.0
C12 Betaine	0.0
C14 Betaine	0.0
C16 Betaine	0.0
Alkylamidobetaines	

Coco Amidopropyl betaine	76
Coco Amidopropyl betaine (lower free amine)	61
Alkyl imidazoline derivatives	
Mono Carboxyl derivative	25
Dicarboxyl derivative	50
Amine oxides	
C12/C14 amine oxide	91, 74
Cocoamidopropylamine oxide	53
Carboxylates - laboratory prepared	
Laurylmonoethanolamine monocarboxylate	19
Laurylaminoethylethanolamine dicarboxymethylate	51
Laurylaminoethylethanolamine monocarboxylate	0.0
Miscellaneous samples	
Na C12/14 SFAME	295
Na C16/18 SFAME	321
Na cocoyl isethionate	284
N.B. SFAME denotes alpha sulpho fatty acid methyl ester	

SUMMARY

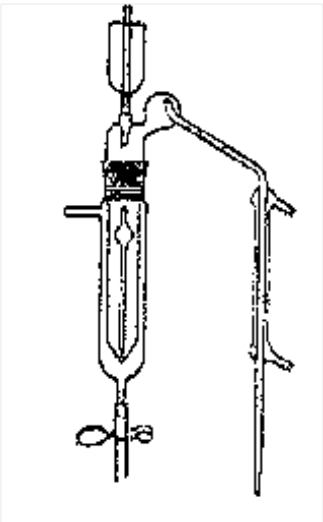
Classification	**Product**
Non-irritant , Zein number <200	
	Sodium salts of ethoxysulphates with greater than 6 moles ethylene oxide
	C16/C18 alpha-olefin sulphonate
	Sulphosuccinates based on alkanoamides
	Alkyl and alkylamido betaines
	Amine oxides
	Carboxylates
	Imidazoline derivatives
Moderate irritant , Zein number 200-400	
	Sodium ethoxy sulphates up to 3 mole
	C14/c16 alpha-olefin sulphonate
	Sulphosuccinates based on 1, 2 and 3 mole ethoxylated alcohols
	Quaternary ammonium compounds
Strong irritant , Zein number >400	
	Sodium C12-14 sulphate

APPENDIX I

ESTIMATION OF NITROGEN CONTENT BY SEMI-MICRO KJELDAHL METHOD

Equipment
Burette, 10ml class A, graduated in 0.02ml
Kjeldahl flask, 30 or 50ml
Conical flask, 100ml
Beaker, 150ml
Long-stemmed funnel
Isomantle and stand for the Kjeldahl flask
Fume cupboard

Hoskin's distillation apparatus (Gallenkamp) with steam generator:



Materials
Kjeldahl catalyst tablets, selenium - BDH
Kjeldahl catalyst tablets, sodium sulphate - BDH
Sulphuric acid, AR grade, concentrated, 98% (m/m) (36N)
Boric acid, 20g/L reagent solution
Sodium hydroxide, 10N (10M) reagent solution
Hydrochloric acid, 0.1N (0.1M) standard volumetric solution
Hydrochloric acid, 0.01N (0.01M) standard volumetric solution
Pipette 25.0ml 0.1N (0.1M) hydrochloric acid into a 250ml volumetric flask, dilute to the mark, stopper and mix. This is an alternative titrant for use with samples of low nitrogen content.
BDH 4.5 indicator solution

Method
1. Accurately weigh the appropriate amount of sample into the Kjeldahl flask, taking sufficient to contain 0.1-0.15g carbon in the case of low nitrogen contents and sufficient to contain 8-13mg N in the case of high nitrogen contents, i.e. N:C greater than 1:12 by weight.

2. Add 2g Na_2SO_4, 0.05g selenium (i.e. one tablet of each kind) and 4ml concentrated sulphuric acid. Add the same reagents to a second flask for a blank and proceed with both.

3. Place the flask on the isomantle situated in a fume cupboard and heat gently at first until frothing ceases. Raise the heat and boil gently until carbonaceous matter is oxidised and for a further 20 minutes. Increase the heat near the end of the digestion so that the refluxing acid washes

the neck of the flask. The overall heating period should be about 45 minutes. During the latter part of the digestion prepare the distillation apparatus as step 5.

4. Allow the Kjeldahl flask to cool. Add 10ml water, washing down the sides of the flask, and swirl until the sodium sulphate has dissolved.

5. Heat the steam generator and pass steam through the Hoskin's apparatus for at least 20 minutes, until ready to continue. Ensure that the stopper is in position in the apparatus, the drain outlet is closed and water is flowing through the condenser. At least 3ml per minute of condensate should be obtained.

6. Remove the source of the heat from the steam generator. The decrease in pressure brought about by condensing steam should cause the inner chamber of the Hoskin's apparatus to be emptied. Drain, and close outlet. Re-commence heating the steam generator.

7. Insert a long-stemmed funnel into the inner chamber of the Hoskin's apparatus and add 20±2ml 10N sodium hydroxide. Take care that no drop of alkali falls into the cup of the apparatus. Replace the stopper.

8. Pass steam into the apparatus for 5-10 minutes at a rate to produce about 3ml of condensate per minute.

9. Measure 10ml 2% boric acid solution into a 100ml conical flask. If the titration is expected to be less than 1ml, use 150ml beakers instead of 100ml conical flasks. Add 2ml indicator, place beneath the condenser and add the minimum amount of distilled water so that the condenser tip is just covered.

10. Pour the contents of the Kjeldahl flask into the cup of the Hoskin's apparatus. Without interrupting the steam supply, carefully raise the stopper and allow to flow into the inner chamber at such a rate that the receiver acid does not rise into the condenser.

11. Rinse the flask and cup with 5ml water and run into the apparatus as before. Repeat the washing.

12. Allow distillation to proceed for 5-7 minutes, then lower the receiver and distil for a further 2 minutes to rinse the inner surface of the condenser. Finally, wash the condenser tip.

13. Titrate the contents of the flask with 0.1N HCl from the 10ml burette. If the titration is expected to be less than 1ml, titrate potentiometrically using 0.01N HCl and adjust the calculation.

14. For the next determination or blank, if done immediately, there is no need to wash out the distillation apparatus; simply proceed from step 6.

Results

Nitrogen =

$$\frac{(t-t_0) \times f \times 0.14}{\text{weight taken}} \% \text{ N}$$

t_0 = ml 0.1N HCl required for blank
t = ml titration for sample
f = factor of 0.1N HCl

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