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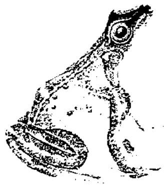

CEN TC 164/WG9 COLLABORATIVE STUDY ON THE DETERMINATION OF ACRYLAMIDE MONOMER IN POLYACRYLAMIDES (DWQ 9007)

Interim report

DoE 3148

JULY 1992

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Interim report

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SUMMARY

WRc has taken part in an interlaboratory study organised by CEN TC 164/WG9/TG8, into the evaluation of the proposed European Standard method for the determination of acrylamide in polyacrylamides. The results of the work are reported in this document along with a discussion section outlining some suggestions for possible further method improvement.

1. OBJECTIVES

CEN TC 164/WG9/TG8 has instigated an interlaboratory study, in which WRc have been asked to participate, to evaluate the proposed European standard method for the determination of acrylamide in polyacrylamides. Each laboratory was provided with three polyelectrolyte samples - cationic, nonionic and anionic - and were asked to extract each sample in triplicate into a mixture of methanol or acetone in water. Five replicate analyses of each extract were then to be carried out by High Performance Liquid Chromatography (HPLC). The methods for the extraction and analysis are detailed in draft European Standards (Draft prEN: Anionic And Non-ionic Polyacrylamides Used For Treatment Of Water Intended For Human Consumption, TG802 - Revised by Permanent Drafting Committee on Feb. 25, 1992 and Draft prEN: Cationic Polyacrylamides Used For Treatment Of Water Intended For Human Consumption, TG801 - Revised by Permanent Drafting Committee on 25 February 1992).

2. METHOD VALIDATION

The draft Standard was followed in detail by WRc, with the exception of the filtration step (Section 5.2.2.5.2.3) which was slightly altered to enable the use of a traditional Buchner flask system and glass fibre filters. Syringe filters were found to be awkward in use and it was not clear whether they were cellulose or glass fibre based. It was decided that the simplest method of filtration would be to use a small Buchner flask fitted with glass fibre filters under vacuum whilst the sample was passing through. By filtering standard solutions through this apparatus it was ascertained that GFC filters neither leach nor retain acrylamide. Cross contamination was minimised by thorough rinsing of the apparatus with the appropriate solvent and using clean GFC filters and pasteur pipettes for each extract.

Prior to analysis of the trial samples, the method was validated using other samples that were available in the laboratory. An old PLRP-S column was originally used but this gave very poor peak separation and so it was necessary to purchase a new column upon which the peak separation was found to be satisfactory. However, separation of acrylamide from other interferences in the polymer extract was not complete (approximately 80%) even when the new column was used. The linearity of the HPLC method was verified and the calibration curve found to be linear over the range of a series of five standards of concentration varying from 1 to 100 mg l^{-1} corresponding to 10 to 1000 mg acrylamide kg^{-1} in a 4 g sample.

3. SAMPLES

Samples were supplied at the meeting of CEN TC 164/WG9/TG8 on 23 April 1992 in plastic containers labelled 'cationic', 'anionic' and 'non-ionic'. These were relabelled 'CEN-anionic', 'CEN-cationic' and 'CEN-nonionic'. They were stored in the dark at ambient temperature, extracted on 15 June 1992 and analysed on 16 and 17 June 1992.

4. APPARATUS AND REAGENTS

The apparatus and reagents used were as detailed in the draft Standard with the exception of the following:

Cobos CM360 Precision Balance
Edmund Buhler KS10 laboratory orbital shaker (500 rev min^{-1})
Buchner filtration apparatus (100 ml)
GFC filters, 4.25 cm diameter
Disposable pasteur pipettes

The HPLC system used consisted of:

Waters model 510 pump
Waters automated gradient controller
Rheodyne sample injection valve with 5 µl loop
Livereel column temperature controller
Cecil CE212 variable wavelength ultraviolet monitor
Hewlett Packard HP3396 electronic integrator
PLRP-S 5u 100A HPLC column (150 x 4.6 mm)

5. PREPARATION OF TEST SAMPLES

It was found that a very large proportion (greater than 90%) of the samples passed a 1000 µm sieve. The remainder was ground using a pestle and mortar and a composite sample used for analysis.

6. EXTRACTION AND ANALYSIS OF TEST SAMPLES

6.1 Extraction of anionic/nonionic polyelectrolytes

Each polymer test sample was weighed (4±0.001 g) into a 250 ml screw topped bottle and 40 ml of 80% methanol/water solvent pipetted in. The bottle tops were screwed on tightly and the bottles agitated overnight on an orbital shaker at 500 rev min^{-1}. After overnight agitation the liquid extracts were filtered under vacuum through a GFC filter. A clean pasteur pipette was then used to transfer 2 ml of the filtrate to a labelled vial. Between samples, the Buchner system was rinsed with 80% methanol/water and a fresh GFC filter inserted. Extractions were carried out in triplicate, and a blank was prepared by adding 40 ml of 80% methanol/water to an empty bottle.

6.2 Extraction of cationic polyelectrolytes

The extraction was carried out as detailed in Section 6.1 above, substituting 80% acetone/water for 80% methanol/water.

6.3 HPLC determination of acrylamide

Samples, standards and blanks were injected via a 5µl injection loop onto the HPLC system previously detailed in Section 4. The area of the peak with a retention time corresponding to that of the acrylamide standard was then measured with the aid of an electronic integrator. Five replicate analysis were carried out on each extract and two blanks were injected with each set of samples. The appropriate calibration standards (20 mg l^{-1} in 80% methanol/water for anionic/nonionic polyelectrolytes and 100 mg l^{-1} in 80% acetone/water for cationic polyelectrolytes) were injected after every five analyses to check performance of the HPLC column.

7. RESULTS

The residual acrylamide monomer content of the original test sample was calculated as described in Section 5.2.2.6.1 of the draft European standard.

The results of replicate analysis are given in Table 7.1.

Table 7.1 Concentrations of acrylamide determined by replicate analysis of polyacrylamides

Sample	Weight (g)	Concentrations of acrylamide (mg kg^{-1}) 1	2	3	4	5
CEN-Cationic:						
Extract-A	4.001	91	83	81	80	79
Extract-B	4.000	76	76	77	74	86
Extract-C	4.000	69	79	72	76	80
CEN-Anionic:						
Extract-A	3.999	250	198	221	210	213
Extract-B	4.000	208	215	216	212	234
Extract-C	4.000	241	212	217	260	253
CEN-Nonionic:						
Extract-A	3.999	244	269	262	294	243
Extract-B	3.999	288	261	250	257	274
Extract-C	4.001	292	298	294	261	268

(Blanks: No measurable peaks)

8. DISCUSSION

1. Sections 5.2.2.4.9.1 and 5.2.2.5.2.1 of the draft standards specify that the accuracy of weighing should be 0.025% or better. This is not consistent with the 0.75% limits specified on the 40 ml of solvent pipetted onto the samples in Section 5.2.2.5.2.1. It was also found that an error was involved in the pipetting of the solvents, it was difficult to transfer the liquid without the formation of an air bubble in the pipette.

2. Section 5.2.2.5.2.3 of the draft standard was considered to be ambiguous in its wording and awkward in practice. After trials with pipette filters it was decided that the most suitable method of filtration of the samples was to use glass fibre filters and filter the samples under vacuum using a Buchner flask system. The vacuum was applied whilst the sample was filtering and removed immediately to restrict evaporation of the solvent. Trials proved that there was no loss or gain of acrylamide from standards filtered by this method.

3. Section 5.2.2.5.3.4 of the draft standard states that a calibration standard should be injected after every four or five samples to check the HPLC column's performance. However there is no advice given on the criteria that should be used to judge it's performance; for example, there should be restrictions on the acceptable peak area and peak width or height variations to ensure that the required accuracy of the analytical results is achieved.

4. In practice the optimum separation of the acrylamide peak from the interfering substances that are also extracted from the polymer was found to be about 80% and this could deteriorate with column use, resulting in less accurate estimation of the acrylamide peak area. It should be possible to modify the HPLC conditions to improve this separation, and in addition, a value for the minimum acceptable column separation efficiency should be included in the specifications.