FULL REPORT

Testing for Formaldehyde in Carrageenan and PES (Processed Eucheuma Seaweed)

Background

All seaweeds used to manufacture carrageenan and PES are either well-dried (15-30% moisture depending on natural salt content) for long-term stability (up to 2 years) for shipment/storage, or semi-dried (35-50% moisture depending on local environment) for shorter-term stability (up to 3 weeks) for shipment storage. As drying is essentially all done by solar means, the cost to realize stable seaweed is attractively low and easy to achieve. All these seaweeds are re-hydrated during the first process step (washing) for carrageenan and PES.

The use of fresh wet seaweeds is completely avoided. The drying process tightens all the molecular structures comprising the wet seaweed, which in turn renders the non-carrageenan components insoluble, providing the means for a purer carrageenan extract. Extraction of these non-carrageenan components by using fresh seaweed aggravates centrifugation and filtration of carrageenan extracts. Also, drying results in the crystallization of sea salt, which in turn ruptures the plant structures to provide the means for a more rapid and complete extraction of the carrageenan.

Consequently, as formaldehyde is only applied for the preservation of fresh seaweeds, and such seaweeds are not desired or used by the carrageenan or PES industries, formaldehyde is not used in the processing of seaweeds for carrageenan or PES production.

Formaldehyde Testing

Based on the above industry position, formaldehyde data on carrageenan and PES products should indicate zero levels. However, low formaldehyde levels have been detected, and the causes for these data have been investigated.

Formaldehyde has been shown to be a natural component of most marine algae including red seaweeds (Yang, et al, 1998). The levels detected in fresh red seaweeds used for carrageenan extraction were 44 mg/Kg for Chondrus crispus and 289 mg/Kg for Gigartina stellata / Mastocarpus stellatus.

Traditional formaldehyde testing (e.g. AOAC: 931.08, or MBTH/Hach) involves direct acid hydrolysis of the carrageenan or PES samples. Carrageenans and PESs contain up to about 0.3% nitrogen depending on the seaweed source and process used. This nitrogen is present as natural amines, amino acids, peptides, and proteins (about 0.1 – 1.6%). The carrageenan and nitrogen / protein molecules form simple ionic cross links and are co-precipitated. A chemical pathway has been developed to show that the acid hydrolysis step in the traditional formaldehyde test can produce formaldehyde from only these basic carrageenan and protein molecules (Stapelfeldt, 2007).

Consequently, it is not surprising that traditional formaldehyde testing produces background data indicating the presence of formaldehyde, even though formaldehyde has not been used to treat / preserve the seaweed.

It was deemed essential to develop and validate a new method specific to carrageenan and PES for accurate free formaldehyde measurement.

History of the Analytical Test Program

Initial Testing

Initial testing in 2008 concluded that:

(a) the traditional MBTH/Hach method for measuring formaldehyde in carrageenan and PES gave invalid results due to the formation of formaldehyde during the acid hydrolysis step in the test method, and also because the MBTH/Hach method is non-specific with respect to formaldehyde, measuring all aldehydes present,

(b) a new method had been developed and validated by FMC/ISP (Farrell, 2007) for measuring formaldehyde in alginates, this method eliminating the hydrolysis step by applying a pure IPA extraction as the first step in the test procedure,

(c) this new method had measured formaldehyde levels below 2.0mg/Kg on all commercial alginates (n=5) and carrageenans (n=5) tested, and

(d) this new method had measured formaldehyde levels in commercial PES samples (n=7) in the range 2.0-5.5mg/Kg.

In addition, comparisons between the new method (FMC/ISP) and the standard MBTH/Hach method on the same commercial alginate samples lowered measured formaldehyde levels from 10.0mg/Kg to 0.5mg/Kg on average, and on the same commercial carrageenan samples from 3.0mg/Kg to 0.8mg/Kg on average, but on the same commercial PES samples raised from 2.0mg/Kg to 4.0mg/Kg on average.

Method Development Suspended

The technical program on formaldehyde method development was put “on hold” between March and November, 2008 as DG Sanco was deciding on the levels of formaldehyde to allow in alginates and carrageenans/PESs.

On February 13, 2009, the EC published new monographs for alginate and carrageenan/PES (EC Directive 2009/10/EC) with maximum levels of 50mg/Kg for alginate, and maximum levels of 5mg/Kg for carrageenan/PES.

As these new specifications would become effective on February 13, 2010, Marinalg's objective was to have an acceptable test procedure in place for carrageenan and PES no later than that date.

Method Development Resumed

The new method developed and validated for alginates by FMC/ISP (Farrell, 2007) was accepted as the primary focus to achieve the above objective, because the method is straightforward, relatively simple to set-up in a QC/QA, and inexpensive to run. In addition, this method avoids the potential interferences of generating formaldehyde during the hydrolysis step of the traditional methods. It measures "free" formaldehyde in the commercial samples. The application of the method to date has been to simply use 5g of carrageenan or PES in place of 5g of alginate at step 1 of the method.

The key issue with the new method was the high background data points being measured for PES (2.0-5.5mg/Kg), this against a proposed specification of maximum 5mg/Kg. Background levels for carrageenan were similar to those for alginate (0.5-1.5mg/Kg).

We concluded that we first needed to confirm that the new method was really measuring formaldehyde, and eventually found a laboratory facility (NFL, California) that could and were willing to apply GC/ECD to our specific problem. For this initial probe using GC/ECD, we used a standard validated EPA method for the measurement of aldehydes in drinking water (EPA method 556.1), and applied this to the water-diluted IPA extract from the initial step of the FMC/ISP method.

Two samples were selected to screen, one gel-press (GP) carrageenan and one PES (Cottonii) with the intent to (a) confirm that formaldehyde was being measured, (b) identify the differences between carrageenan and PES, and (c) identify other compounds in the IPA extract.

The report by NFL (FMC, 2009) concluded that no formaldehyde was present in either the carrageenan or PES samples based on a LOD/LOQ of 50ppb (0.05mg/Kg). Several other aldehydes were detected, but all at very low levels. Comparisons of the FMC/ISP and GC/ECD data were as follows.

Sample	FMC/ISP Method	GC/ECD
Carrageenan	0.5mg/Kg formaldehyde	0.8mg/Kg total aldehydes*
PES	5.5mg/Kg formaldehyde	0.3mg/Kg total aldehydes*

* sum of all the aldehyde levels reported by NFL.

The data on the carrageenan sample were very close by both methods at 0.5mg/Kg and 0.8mg/Kg. However, the data for PES were quite different (5.5mg/Kg versus 0.3mg/Kg).

The EPA method for drinking water had to be adapted for our R&D work. IPA controls were necessary to eliminate any impurities from that source; all the reported results were incremental above the IPA control. All LOD/MDL levels were quantified using spiking concentrations in the IPA control.

The levels of formaldehyde in the carrageenan and PES samples by the GC/ECD method were essentially zero (less than 50ppb or 0.05mg/Kg), and therefore the data using the FMC/ISP Method, assuming it was measuring the total level of all aldehydes present, should be less than 1mg/Kg for both carrageenan and PES. This was true for the carrageenan sample, but not with PES, and this needed to be resolved.

Attempted Validation of the GC/ECD Method

Our attempts to validate the GC/ECD method failed using either carrageenan or PES. Accurate recovery of standard formaldehyde additions to the samples could not be made. Recoveries from adding the standard formaldehyde to the IPA extracts was acceptable, which indicated that either (a) the formaldehyde was physically lost by evaporation, or (b) the formaldehyde reacted with one of the components in the commercial carrageenan/PES and was removed with the solids on filtration. After several attempts, we concluded that this method could not be validated and it was dropped from our program.

Current Status

The traditional methods (AOAC: 931.08 and MTBH/Hach) were dropped from consideration due to interference by formaldehyde produced in test.

The GC/ECD method based on EPA Method 556.1 has been excluded following standard formaldehyde recovery failures during attempted validation.

The FMC/ISP method (Farrell, 2007) based on pre-extraction of formaldehyde with pure IPA, validated for alginates, is being reviewed for validation for carrageenan, and the expectations are that this will be successful, as data to date indicate similar background readings as per alginates, of the order of 0.5-1.5 mg/Kg versus the specification of “no more than 5 mg/Kg”. This gap of about 4 mg/Kg is certainly adequate with respect to the detection of formaldehyde levels in commercial carrageenans below the specification. However, the application of the FMC/ISP method to PES remains doubtful, as the background readings of up to 5.5 mg/Kg overlap the specification limit of 5 mg/Kg.

A method based on a different process to separate the formaldehyde has been discovered and will be evaluated (NIOSH 2016). In this case the free formaldehyde is extracted out of the sample by a through-flow of air which then passes through a cartridge containing silica gel coated with 2,4-dinitrophenylhydrazine which absorbs the formaldehyde. The formaldehyde is calculated by measuring the amount of reagent used up via UV spectrophotometer. This method is under review for development and validation.

William R. Blakemore, February 2010.

References

1. AOAC Method 931.08, “Formaldehyde in Food”.
2. Hach Method 8110, “Formaldehyde”.
3. EPA Method 556.1, “Determination of carbonyl compounds in drinking water by fast gas chromatography”, Revision 1, 1999.
4. NIOSH 2016, “Formaldehyde”.

5. Yang, et al, 1998, “Formaldehyde from Marine Algae”, Biochemical Systematics and Ecology, 26, 117-123.

6. Farrell, 2007, “Development of a method for determination of residual formaldehyde in finished alginate products”.

7. Stapelfeldt, 2007, “Detection of Formaldehyde in Non-treated Carrageenan, Part 2”.

8. FMC, 2009, “NFL Report CL1549-0”, Carrageenan 3 = PES (cottonii), Carrageenan 4 = Gel Press Kappa-carrageenan.