



**TECHNICAL REPORT**

**Title:** DEVELOPMENT OF METHOD FOR DETERMINATION OF RESIDUAL FORMALDEHYDE IN FINISHED ALGINATE PRODUCTS

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**Summary**

Formaldehyde (HCHO) is very soluble in alcohol solutions of various strengths, whereas alginates can be regarded as partially or wholly insoluble, depending on alcohol concentration. This provides a convenient basis for the separation of residual HCHO from an alginate matrix. Solutions of 45% v/v 2-propanol (IPA) and 100% IPA were used to extract residual HCHO from alginate. An x25 aqueous dilution of the filtrate was determined for HCHO content using a standard MBTH colorimetric method (ISP Alginates SOM 24). No significant matrix interference was noted for either extraction method. The 45% v/v IPA extraction produced notable background interference, probably due to dissolution of low molecular weight alginate, whereas the 100% v/v IPA extraction was virtually free of background interference. Further investigation of the 100% IPA extraction method showed that a measurement standard error of +/- 0.5ppm in product could be obtained. The accuracy of the method was validated by determination of recovery solutions containing a certified amount of HCHO. The residual levels of HCHO in the alginate products tested was observed to be very low, with typical levels for Manucol DMF, Manugel DMB, Alginic Acid H/FD and Kelcoloid LVF of 3ppm, 3ppm, 3ppm and 2ppm in product, respectively.

**Robert Farrell**

## 1. EXPERIMENTAL

### 1.1 Hach MBTH method development

#### 1.1.1 Aqueous alcohol extraction

Calibration standards were prepared by adding 0 $\mu$ l, 20 $\mu$ l, 40 $\mu$ l, 60 $\mu$ l and 80 $\mu$ l aliquots of nominal 0.38% w/w HCHO solution to separate 100ml portions of purified water. Each solution was shaken for 1 hour, filtered through Whatman 541 paper, followed by filtration through a 0.2 $\mu$ m syringe filter and diluted x25 with purified water to produce the test solutions.

Standard additions solutions were prepared by adding 0 $\mu$ l, 20 $\mu$ l, 40 $\mu$ l, 60 $\mu$ l and 80 $\mu$ l aliquots of nominal 0.38% w/w HCHO solution to separate 5.00g of sodium alginate (Manucol DMF lot 831613) and adding 100ml 45% v/v IPA. Each solution was shaken for 1 hour, filtered through Whatman 541 paper, followed by filtration through a 0.2 $\mu$ m syringe filter and diluted x25 with purified water to produce the test solutions.

The HCHO in each calibration and standard addition test solution was determined by the standard Hach MBTH method for aqueous effluent (SOM 24).

#### 1.1.2 Pure alcohol extraction

Calibration standards were prepared by adding 0 $\mu$ l, 20 $\mu$ l, 40 $\mu$ l, 60 $\mu$ l and 80 $\mu$ l aliquots of nominal 0.38% w/w HCHO solution to separate 100ml portions of purified water. Each solution was shaken for 1 hour, filtered through Whatman 541 paper, followed by filtration through a 0.2 $\mu$ m syringe filter and diluted x25 with purified water to produce the test solutions.

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The HCHO in each calibration and standard addition test solution was determined by the standard Hach MBTH method for aqueous effluent (SOM 24).

#### 1.1.3 Background interference

Samples of purified alginate product (Manugel GMB) were subjected to the test method using both the aqueous and pure alcohol extraction methods to estimate levels of interference from the alginate. The alginate had previously been purified by washing 6 times with excess 45% v/v and 100% MeOH and allowed to dry at ambient temperature, so that all significant traces of HCHO (and other impurities) were removed. Sample blanks were also prepared and analysed.

#### 1.1.4 Variability

The sources of variability in the procedure were estimated for the 100% IPA extraction method by performing 4 repeat measurements for 4 separate sub-samples of Manucol DMF taken from a bulk sample (c 200g) of lot 831613.

### 1.1.5 Accuracy/Recovery

Two samples (Manugel DMB lot 831582) and two sample blanks were prepared and analysed using the 100% IPA extraction method. 10µl of certified 4000ppm HCHO were added to one of the samples and sample blanks prior to extraction to produce recovery solutions. The results obtained were compared with the theoretical recoveries.

### 1.2 Residual HCHO in alginate products

Products tested were Manucol DMF (lot 831613), Manugel DMB (lot 831582), H/FD (lot 840021) and Kelcoloid LVF (lot 7C12052). 5.00g of each product was extracted with 100ml 100% IPA by shaking for 1 hour, filtered through Whatman 541 paper, followed by filtration through a 0.2µm syringe filter and diluted x25 with purified water to produce the test solutions.

## 2. RESULTS

### 2.1 Hach MBTH method development

#### 2.1.1 Aqueous alcohol extraction

Table 1 gives the concentration of HCHO in the aqueous calibration solutions as determined by the Hach MBTH method.

<b>Aliquot 0.38% HCHO (µl)</b>	<b>[HCHO] in test solution (ppb)</b>
0	0
20	37
40	65
60	95
80	130

Table 1 HCHO concentration in 45% v/v IPA calibration solutions by Hach MBTH method

Table 2 gives the concentration of HCHO in the standard additions solutions by the Hach MBTH method using 45% v/v IPA.

Added 0.38% HCHO ( $\mu\text{l}$ )	[HCHO] in test solution (ppb)
Blank	0
0	102
20	130
40	165
60	192
80	228

Table 2 HCHO concentration in standard additions solutions by Hach MBTH method using 45% v/v IPA

Figure 1 compares the aqueous calibration and standard additions graphs for the MBTH method using 45% v/v IPA extraction. The linear regression results obtained for both straight lines are summarised in Table 3.

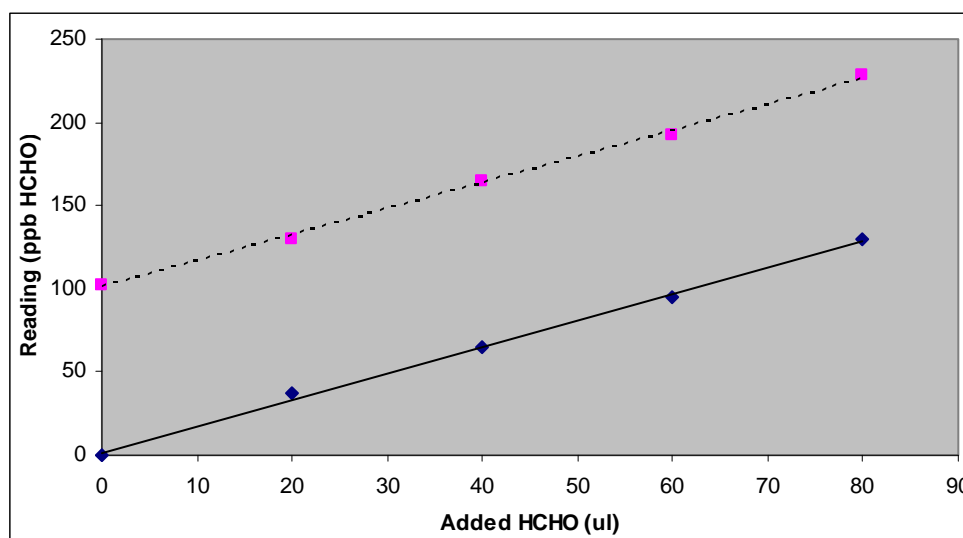


Figure 1 Comparison of graphs for aqueous calibration and standard additions for Hach MBTH method using 45% v/v IPA

Parameter	Aqueous calibration	Standard additions (45% v/v IPA extraction)
$r^2$	0.998	0.998
Slope (ppb/ $\mu\text{l}$ )	1.59	1.57
Intercept (ppb HCHO)	1.8	100.6

Table 3 Linear regression statistics for aqueous calibration and standard additions with 45% v/v IPA extraction

Table 4 shows the HCHO levels in Manugel DMF lot 831613 after extraction in 45% v/v IPA calculated from the direct Hach reading, interpolation of the experimental calibration curve and extrapolation of the standard additions curve.

Source of result	[HCHO] (ppm in product)
Direct ex Hach	51
Ex aqueous calibration	47
Ex Standard additions	49

Table 4 Residual HCHO levels in Manugel DMF lot 831613 by Hach MBTH method using 45% v/v IPA

### 2.1.2 Pure alcohol extraction

Table 5 gives the concentration of HCHO in the standard additions solutions by the Hach MBTH method using 100% extraction IPA.

Added 0.38% HCHO (µl)	[HCHO] in test solution (ppb)
Blank	0
0	6
20	40
40	67
60	95
80	126

Table 5 HCHO concentration in standard additions solutions by Hach MBTH method using 100% IPA

Figure 2 compares the aqueous calibration and standard additions graphs for the MBTH method using 100% IPA. The linear regression results obtained for both straight lines are summarised in Table 6.

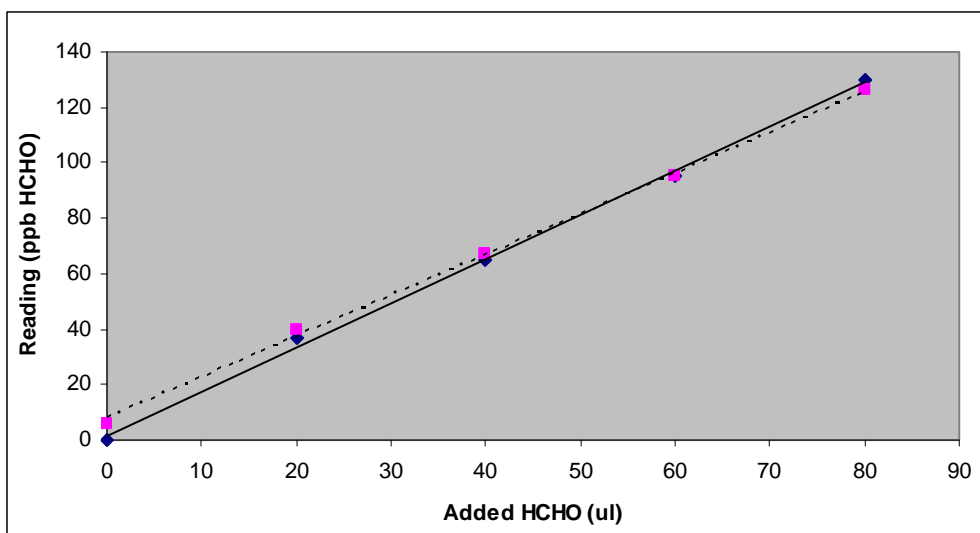


Figure 2 Comparison of graphs for calibration and standard additions for Hach MBTH method using 100% IPA

Parameter	Aqueous calibration	Standard additions (100% v/v IPA extraction)
$r^2$	0.998	0.999
Slope (ppb/ $\mu$ l)	1.59	1.48
Intercept (ppb HCHO)	1.8	7.8

Table 6 Linear regression statistics for aqueous calibration and standard additions with 100% IPA extraction

Table 7 summarises the HCHO levels in Manugel DMF lot 831613 after extraction in 100% IPA calculated from the direct Hach reading, interpolation of the experimental calibration curve and extrapolation of the standard additions curve.

Source of result	[HCHO] (ppm in product)
Direct ex Hach	3.0
Ex aqueous calibration	1.9
Ex Standard additions	2.6

Table 7 Residual HCHO levels in Manugel DMF lot 831613 by Hach MBTH method using 100% IPA

### 2.1.3 Background interference

Table 8 gives the residual HCHO level for purified Manugel GMB by direct reading using the Hach MBTH method after extraction in 45% v/v and 100% IPA.

Extraction method	[HCHO] (ppm in product)
45% v/v IPA	9
100% IPA	0

Table 8 Residual HCHO level in purified Manugel GMB by Hach MBTH method after 45% v/v and 100% IPA extractions

### 2.1.4 Variability

Table 9 lists the replicate analysis results for the test solutions for 4 sub-samples taken at random from the 200g bulk sample of Manucol DMF lot 831613, using 100% IPA extraction.

Sub-sample	Reading (ppb in test solution)			
1	6	7	7	6
2	5	5	5	5
3	7	8	8	8
4	6	5	6	6

Table 9 Replicate analysis results for sub-samples taken from bulk sample of Manucol DMF lot 831613 using 100% IPA extraction

Table 10 summarises the ANOVA analysis for the replicate analysis results.

Source of variation	Variance	F	F (critical)
Measurement	0.21	N/A	N/A
Sample	5.50	26.4	3.49

Table 10 ANOVA analysis statistics

### 2.1.4 Accuracy

Table 11 compares the recovery results obtained by the 100% IPA extraction method with the theoretical recoveries for the test solutions. The added spike should produce an added concentration of 16ppb HCHO in the recovery solutions.

Test solution	Reading (ppb)	Theoretical (ppb)	Recovery (%)
Sample blank	4		
Sample blank recovery	19	20	95
Sample	6		
Sample recovery	21	22	95

Table 11 Accuracy/recovery results for test solutions

## 2.2 Residual HCHO in alginate products

Table 12 gives typical levels of HCHO determined in various alginate products, using the 100% IPA extraction method.

Product	Lot	[HCHO]	
		Solution (ppb)	Product (ppm)
Sample Blank	N/A	2	N/A
Manucol DMF	831613	7	3
Manugel DMB	831582	7	3
AlginicAcid H/FD	840021	8	3
Kelcoloid LVF	7C12052	4	2

Table 12 HCHO in typical alginate products

## **3. DISCUSSION**

### 3.1 Hach MBTH method development

#### 3.1.1 Aqueous alcohol extraction

The 45% v/v calibration and standard additions curves produced slopes of 1.59 ppb/ $\mu$ l and 1.57ppb/ $\mu$ l added HCHO, respectively. The closeness of agreement showed that there was no significant matrix interference in the measurement of HCHO by the Hach method.

#### 3.1.2 Aqueous alcohol extraction

The 100% calibration and standard additions curves produced slopes of 1.59 ppb/ $\mu$ l and 1.48ppb/ $\mu$ l added HCHO, respectively. Although the agreement in the value of the slopes was not as good as for 45% v/v IPA extraction, there was sufficient agreement to show that no significant matrix interference were present in the measurement of HCHO by the Hach method with 100% IPA extraction.

### 3.1.3 Background interference

The unexpectedly high level for Manucol DMF lot 831613 of 102ppb HCHO in the test solution prepared by 45% v/v IPA extraction suggested that there was significant background interference from some substance co-extracted with HCHO. This could conceivably be soluble low molecular weight alginate as each alginate molecule has a reducing aldehyde/hemiacetal end-group which can potentially react with the MBTH reagent. This was tested by the analysis of the sample of the highly purified Manugel GMB. The result of 9ppm HCHO in the purified product support the suggestion that background interference was present and that false positive results may occur if the Hach MBTH method with the 45% v/v IPA extraction is used. The result of 0ppm HCHO in the same alginate sample after extraction with 100% IPA show that the background interference had been reduced to an insignificant level, probably due to the decrease in solubility of low molecular weight alginate in pure IPA. The 100% IPA extraction method was therefore deemed most suitable for routine HCHO determination in alginate products.

### 3.1.4 Variability

Results from the ANOVA statistical analysis show that the estimated within-sample variance, due to measurement, after 100% IPA extraction, was low at 0.21ppb in solution. This corresponds to a standard deviation of 0.46ppb. A single analysis should have a standard error of +/- 1ppb in solution or 0.5ppm in product at a probability level of 95%.

The estimated limit of detection for the method is 1.4ppb in solution, equivalent to 0.7ppm in product. The estimated limit of quantification for the method is 4.6ppb in solution, equivalent to 2.3ppm in solution.

The calculated F value of 26.4 is significantly higher than the critical value of 3.49, indicating that the most significant variability in the method is due to sampling.

### 3.1.5 Accuracy

The recovery of HCHO from the both the sample and sample blank solutions was excellent at 95% indicating that the 100% IPA extraction method is accurate.

## 3.2 Residual HCHO in alginate products

The level of residual formaldehyde found in all 4 samples was low. With the exception of Manugel DMB, the determined levels were all less than the limit of quantification for the method. This indicates that the chemical and heat treatments involved in the manufacturing process are efficient at removing HCHO from the product.

## Principle

Formaldehyde (HCHO) is extracted from alginate product with 100% IPA. The extract is filtered and diluted appropriately to produce a test sample. Excess 3-methyl-2-benzothiazoline hydrazone (MBTH) is added to a test sample and reacts with any formaldehyde present to form an azine. The excess MBTH is oxidised by the addition of a developing solution and reacts with the previously formed azine to produce a blue coloured species. The intensity of the blue colour is measured by absorbance at 630nm and is proportional to the original concentration of formaldehyde present in the effluent sample. The method is based on Hach DR/2000 method number 8110 for formaldehyde in water.

## Apparatus

- 2-decimal place balance
- Hach DR/2000 direct reading spectrophotometer Camlab, P/N 44800-00
- Pair of matched 1 inch 25ml glass cuvettes for above Camlab, P/N 20950-00
- Laboratory shaker
- Whatman type 541 filter papers, 125mm diameter Cat. No. 1541 125
- 10ml disposable syringes
- 0.2µm syringe filters
- 150ml Sterilin container
- Pair of 50ml graduated measuring cylinders with stoppers
- 100µl Eppendorf pipette with tips

## Reagents

- Isopropyl alcohol
- MBTH powder pillows Camlab, P/N 22571-69
- Developing solution for low range formaldehyde Camlab, P/N 22572-49
- RO/DI quality water

## Procedure

1. Weigh 5.00g of alginate product into a 150ml Sterilin container.
2. Add 100ml IPA and tightly close the lid.
3. Place on a laboratory shaker and shake at maximum setting for 1 hour.
4. Filter sufficient solution through a 541 filter paper.
5. Filter sufficient filtrate collected in step 4 above through a 0.2µm syringe filter.
6. Pipette 1.0ml of the filtrate obtained in Step 5 above into a 25ml graduated measuring cylinder.
7. Make up to 25 ml with RO/DI water. Stopper and mix by inverting. This is the test solution.
8. Follow exactly Hach procedure 8110, MBTH method, for Formaldehyde in water, using stored programme number 200 as directed in the Hach DR/2000 spectrophotometer handbook.

Calculation

The Hach DR/2000 reads out formaldehyde concentration directly in µg/litre (ppb).

Concn. HCHO in product (ppm) = Hach DR/2000 reading x dilution factor/1000

For above procedure as written

Concn. HCHO in product (ppm) = Hach DR/2000 reading x 0.5

Limitations

The working range for the method is from 0 to 87mg/l HCHO in effluent.

Precautions

All glassware should be allowed to soak in concentrated HNO<sub>3</sub> before thorough rinsing in RO/DI quality water prior to analysis.