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Variability in the phenol–sulphuric acid assay for sodium carboxymethylcellulose

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Sodium carboxymethylcellulose (CMC) is used commonly in foods, detergent powders, and pharmaceuticals. It can be analysed rapidly by the phenol–sulphuric acid colourimetric method (Mallory and Porter, 1972; Milwidsky, (1973). This method has been used recently in experimental pharmaceutical systems (Law and Kayes, 1983). Examination of the data in the original papers indicates a coefficient of variation (CV) for the method of 3–5%. In our hands the CV of this method was typically 7% but on occasions unacceptably variable results were obtained (CV 10–20%). We report here that this variability is due to variation in the rate of addition of the sulphuric acid, and that by controlling this rate a CV of about 1% can be achieved and aberrant results do not occur. In addition, the assay is more sensitive.

The method consists of adding a fixed volume (0.1 ml) of liquified phenol (80% w/w) to the CMC solution (e.g. 2.5 ml of a ca. 0.005%) followed by 5 ml of concentrated sulphuric acid (98%). A brownish-yellow colour develops almost immediately and its absorbance is measured at 490 nm against an appropriate blank. The concentration of CMC is determined from a standard curve for the same CMC. In the original method the acid was added by allowing it to flow from a pipette down the side of the beaker.

The mechanism of the reaction is not known and the method is not specific for CMC. All carbohydrates (e.g. monosaccharides, starches, celluloses) appear to react. In fact the method was developed from one for the determination of lactose in milk and cheese (Barnett and Tawab, 1957). Furthermore, it is reported that the colour depends on the type of CMC analysed (e.g. degree of substitution). In the experiment described below we show that it depends also on the rate of addition of the acid, and that this is a major source of variation in the assay.

A 0.008% CMC (Sigma low viscosity grade) solution was analysed by the standard method except the acid was added by three different methods: (i) all at once and rapidly from a positive displacement pipettor (Brand 5 ml macro-transferpettor); (ii) in four equal portions at 5-s intervals then mixed in; or (iii) all at once and rapidly but left to stand for 30 s before mixing. Assays were performed in triplicate and analysed in a Cary 219 spectrophotometer 10 min after mixing. All reagents were of analytical grade.

The following mean absorbances and coeffi-

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TABLE 1

REGRESSION ANALYSIS OF CALIBRATION DATA FOR THE ANALYSIS OF CMC BY THE PHENOL-SULPHURIC ACID METHOD

CMC (%)	Absorbance (490 nm)
0.001	0.153, 0.138, 0.147
0.002	0.288, 0.297, 0.293
0.003	0.426, 0.419, 0.435
0.004	0.563, 0.576, 0.572
0.005	0.718, 0.704, 0.706

Effect	Degrees of freedom	Sum of squares	Mean square	*F*-value
Total	15	3.35283		
Mean	1	2.76061		
Slope	1	0.59165	0.59165	13500
Residual	13	0.00057	0.000044	
Nonlinear	3	0.00008	0.000028	0.57
Error	10	0.00049	0.000049	

Slope ± S.D. = 140.4 ± 1.2
Intercept ± S.D. = 0.0077 ± 0.004

cients of variation were obtained: (i) 1.180, 0.4%; (ii) 0.776, 6.3%; and (iii) 1.183, 0.3%. These results demonstrate that the absorbance depends on the rate of acid addition (1.180 cf. 0.776). Faster addition leads to a higher absorbance and hence a more sensitive assay. Furthermore, the variability was far greater when the acid was added by the slower method. These results supported our previous attempts to use this assay where little control was exercised over the addition of acid and large CV values were obtained. The third result shows that the time between acid addition and mixing is not critical. Apparently, sufficient mixing occurs on addition of the acid.

The reason why rate of acid addition is so important is not clear. Milwidsky (1973) has reported that the heat of dilution of the acid plays a part in the reaction. However, we have used 50%, 80%, and 98% sulphuric acid and heated the resultant solutions for 10 min at 60°C. For 50% acid no colour developed whereas the colour for 80% acid was only 2% of that for 98% acid. Thus it would seem that heat is not the only critical factor.

The time between addition of acid and reading the absorbance is important but not critical. For example, the mean absorbance readings for solution (iii) above taken at 10 min, 1 h, and 2 h after mixing were 1.183, 1.181, and 1.172, respectively. There was no change in the CV values. Thus little accuracy is sacrificed provided samples are analysed within 1 h after mixing.

A calibration was carried out over the range 0.001 to 0.005% CMC (Table 1). Regression analysis (Draper and Smith, 1968) showed that the intercept was not significantly different from zero, the slope highly significant and that there was no significant curvature. The pooled standard deviation was 0.007 absorbance units corresponding to an average CV of 1.6%.

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