AUTOMATED DETERMINATION OF CHLORIDE AND PHOSPHATE IN CANE JUICE, MOLASSES AND SUGAR

W. C. Cheng, H. I. Shiue and H. T. Cheng
Taiwan Sugar Research Institute, Tainan, Taiwan, Republic of China

ABSTRACT

Automated methods for determination of chloride and total $P_2O_5$ in cane juice, sugar and molasses have not been well developed in the sugar industry, although some methods have been applied in other fields, such as chloride in water, plasma, and urine, and phosphorus in meat, feeds, and fertilizer. However, determination of these anions is necessary and important for sugar technology and for research workers, as the conventional manual methods are usually complicated and involve more procedures. Usually the methods are time-consuming methods and may introduce many analytical errors.

In this experiment, rapid and reliable automated methods have been developed based on the development of the coloured ferric-chloro-thiocyanate and ascorbic acid-molybdate-phosphate complex for determination of chloride and phosphate content respectively on the Technicon AutoAnalyzer.

The results show that the standard deviation and the coefficient of variation of the developed automated methods were respectively 2.3 ppm and 0.99% with a measuring range of 0-800 ppm for chloride determination, and 0.305-0.869 ppm and 0.34%-0.63% with a range below 15 ppm for $P_2O_5$ analysis. The determinations are much quicker as both may be run at the rate of 30 samples per hour. The methods are considered acceptable and reproducible and can be applied in the sugar industry.

INTRODUCTION

The major constituents of inorganic anions in cane juice are chloride and phosphate, but, some phosphorus is present in organic form. The determination of these constituents is important in the routine assessment of juice quality for chemical control and research work; total phosphorus analysis is also frequently required in nutritional investigations. Conventional methods for these determinations involve the gravimetric quinoline molybdate method, or colorimetric analysis of phosphate, and back titration by quantitatively precipitating silver salts to determine chloride, etc. These methods are both time-consuming, and high in analytical error.

An automated determination of chloride, inorganic form of $P_2O_5$ and total $P_2O_5$ in cane juice, sugar and molasses has not been well developed. However, it is reported that some auto-determinations using the Technicon AutoAnalyzer have been applied for determining chloride in water, plasma and urine or even for determination of phosphate in meat, feeds, and fertilizer by Mcneal, Law and Hoyl.

The main purpose of this study was to develop a rapid and reliable automated method for determination of chloride and $P_2O_5$ in the sugar industry by...
adapting a suitable colorimetric method to the Technicon Auto-Analyzer. The analysis was performed in a continuously flowing stream to which appropriate diluents and reagents were added automatically. Heating, cooling, and mixing operations and separation by dialysis were carried out as required. The sample solution and reagents were added automatically to the measuring system in predetermined proportion. The performance could be automatically checked by the introduction of a standard sample instead of the unknown at periodic intervals. In this paper the procedures of the new method and the results of the experiment are described.

EXPERIMENTAL PROCEDURE

1. Apparatus and operating conditions
A Technicon Auto-Analyzer, equipped with a set of proportion pumps, sampler, recorder, dialyser, constant voltage transformer, heating bath and colorimeter, was used in this study. The optimum operating conditions have been determined and the flow diagram has been designed as shown in Fig. 1.

2. Reagents
All reagents used were of either guaranteed or analytical grade.
1) Ferric ammonium sulphate solution
Dissolve 12 g ferric ammonium sulphate in 500 ml distilled water, add 77 ml concentrated HNO₃ and dilute to 1 litre with distilled water.
2) Saturated aqueous mercuric thiocyanate solution
Add the mercuric thiocyanate to the distilled water, agitate for several hours, allow to set overnight and filter the solution through a good grade of filter paper to make a saturated aqueous solution (about 2-3 g mercuric thiocyanate is dissolved in 1 litre water).
3) Ammonium molybdate solution
Dissolve 4.7 g ammonium molybdate in 500 ml distilled water, add 35 ml concentrated H₂SO₄, and dilute to 1 litre with distilled water.
4) 1% ascorbic acid
Add 10 g ascorbic acid in distilled water, and dilute to 1 litre then store in the refrigerator.

3. Preparation of sample
1) For determination of chloride
Weigh appropriate quantity of sample (about 1-5 g), dilute to 100 ml with distilled water, and filter.
2) For determination of phosphate and total P₂O₅
It is known that phosphorus is present in raw juice not only in an inorganic form, but also in an organic form, much more being present in the latter form. However, the organic can only be determined by a colorimetric method if the sample is completely decomposed. In this study three sample preparation systems were tried and compared as follows:
   a) Direct dilution
Add 8 ml of 10% trichloracetic acid in appropriate quantity (1-5 g) of sample onto the water bath, and heat to 60 C for 3 minutes. Then dilute to 100 ml with distilled water and filter.
FIGURE 1. Flow diagram of phosphate and chloride analysis.
W. C. Cheng, H. I. Shue and H. T. Cheng

RESULTS AND DISCUSSION

The experimental results are shown as follows:

1. Chloride

This determination is based on the formation of a ferric-chloro-thiocyanate complex. The reactions may be written as follows:

\[ \text{Hg(SCN)}_2 + 2 \text{Cl}^- \rightarrow \text{HgCl}_2 + 2(\text{SCN})^- \]

\[ 3(\text{SCN})^- + \text{Fe}^{+++} \rightarrow \text{Fe}_3(\text{SCN})_6 \]

The sample is mixed with a solution of ferric ammonium sulphate in nitric acid and then with saturated aqueous mercuric thiocyanate, the flow diagram being shown in Fig. 1. The color complex \( \text{Fe}_3(\text{SCN})_6 \) is measured at 480 nm. The results are shown in Table 1, and indicate that the method is acceptable and has a good enough reproducibility, with a measuring range of 0-800 ppm (Fig. 2). It may be run at the rate of 30 samples per hour.

### Table 1. Accuracy of chloride results by automated method.

<table>
<thead>
<tr>
<th>Present in sample ppm</th>
<th>Standard added ppm</th>
<th>Total present ppm</th>
<th>Recovered ppm</th>
<th>%</th>
<th>Standard deviation ppm</th>
<th>Coefficient of variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>500</td>
<td>585</td>
<td>577</td>
<td>98,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>500</td>
<td>580</td>
<td>590</td>
<td>101,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>500</td>
<td>576</td>
<td>576</td>
<td>100,0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>300</td>
<td>372</td>
<td>373</td>
<td>100,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>300</td>
<td>383</td>
<td>378</td>
<td>98,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>300</td>
<td>394</td>
<td>396</td>
<td>100,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av</td>
<td></td>
<td></td>
<td></td>
<td>99,6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Phosphate

The conditions of 3 colorimetric systems for phosphorus determination, i.e., amino-naphthol-molybdate, ammonium molybdate and ascorbic acid molybdate were studied. Results indicated that the ascorbic acid molybdate method could be adopted to trace analysis. The flow diagram is shown in Fig. 1.

As mentioned above, three sample preparation systems, i.e., direct dilution, dry ashing and wet ashing system were compared with AOAC method (i.e., sample prepared by dry ashing, and measured by Pye Unicum Sp 3 000 Automatic U-V spectrophotometer). It is seen in Table 2 that the results from the direct dilution are much lower than others. This is because the amount of phosphate determined by the direct dilution method only included inorganic
phosphate and therefore this method could be used for the determination of inorganic form of $P_2O_5$. Also, the sample preparation systems by dry ashing and wet ashing could be used for determination of total $P_2O_5$.

The value of the $F$-test showed that either the dry ashing or wet ashing procedure may be applied to the Auto-Analyzer without significant difference, the recovery by either method being good. In 26 replicated tests, the standard deviation and the coefficient of variation of dry ashing and wet ashing were 0.305 ppm and 0.869 ppm, and 0.34% and 0.63%, respectively; hence, the reproducibility of these 2 methods is satisfactory. The dry ashing method seems

\[ \text{FIGURE 2. Standard curves for phosphate and chloride.} \]
### TABLE 2. Comparison of methods for phosphate analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery Test</th>
<th>Reproducibility Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Results ppm</td>
<td>Recovery ppm</td>
</tr>
<tr>
<td>AOAC† Method</td>
<td>Dry* Ashing Method</td>
<td>Wet Ashing Method</td>
</tr>
<tr>
<td>A</td>
<td>352</td>
<td>320</td>
</tr>
<tr>
<td>B</td>
<td>1 315</td>
<td>1 350</td>
</tr>
<tr>
<td>C</td>
<td>1 350</td>
<td>1 370</td>
</tr>
<tr>
<td>D</td>
<td>1 700</td>
<td>1 664</td>
</tr>
<tr>
<td>E</td>
<td>1 210</td>
<td>1 170</td>
</tr>
<tr>
<td>F</td>
<td>1 366</td>
<td>1 356</td>
</tr>
<tr>
<td>Average</td>
<td>1 249</td>
<td>1 231</td>
</tr>
</tbody>
</table>

* Dry Ashing Method  
Wet Ashing Method  
Direct Dilution Method measured by Technicon Auto-Analyzer

† AOAC Method: Measured by Pye Unicum Sp 3 000 Automatic spectrophotometer.
to be the easiest and the shortest. The solution thus obtained can also be analysed for metals, either by the conventional titration method or by atomic absorption spectrophotometry. The standard curve is shown in Fig. 2 and its measuring range is below 15 ppm. It may be run at the rate of 30 samples per hour.

The above selected systems could be used for determination of total $\text{P}_4\text{O}_5$ and inorganic $\text{P}_4\text{O}_5$ in sugar processing. The analytical results for the defecation process are listed in Table 3 and the results for the carbonation process are listed in Table 4. These results show an acceptable degree of accuracy for the method, and indicate the advantage in time saved.

**TABLE 3.** Analysis of phosphate and chloride in defecation process.

<table>
<thead>
<tr>
<th></th>
<th>Mixed juice</th>
<th>Clarified juice</th>
<th>Raw syrup</th>
<th>Final molasses</th>
<th>Raw Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>2660</td>
<td>2683</td>
<td>2618</td>
<td>2685</td>
<td>267</td>
</tr>
<tr>
<td>$\text{P}_4\text{O}_5$</td>
<td>1447</td>
<td>385</td>
<td>368</td>
<td>1587</td>
<td>70</td>
</tr>
</tbody>
</table>

**TABLE 4.** Analysis of phosphate and chloride in carbonation process.

<table>
<thead>
<tr>
<th></th>
<th>Mixed juice</th>
<th>1st carbonated juice</th>
<th>2nd carbonated juice</th>
<th>Thin juice</th>
<th>Fine syrup</th>
<th>Final molasses</th>
<th>White sugar (SWC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>1607</td>
<td>1638</td>
<td>1649</td>
<td>1646</td>
<td>1625</td>
<td>6615</td>
<td>58</td>
</tr>
<tr>
<td>$\text{P}_4\text{O}_5$</td>
<td>1806</td>
<td>99</td>
<td>67</td>
<td>64</td>
<td>60</td>
<td>229</td>
<td>1,4</td>
</tr>
</tbody>
</table>

**CONCLUSION**

By adapting suitable sample preparation systems and colorimetric determinations to the Technicon Auto-Analyzer methods have been successfully developed for the determination of chloride and phosphate in juices, sugar and molasses. The results showed that the standard deviation and coefficient of variation of the automated methods were good enough to meet our requirement. The methods are very much shorter and may be run at the rate of 30 samples per hour. Therefore, the developed automated methods are considered useful and reproducible and may be applied in the sugar industry.

**REFERENCES**

W. C. CHENG, H. I. SHIUE AND H. T. CHENG

MEDICION AUTOMATICA DEL CONTENIDO DE CLORUROS Y FOSFATOS EN JUGO DE CAÑA, MIELES Y AZUCAR

W. C. Cheng, H. I. Shiue and H. T. Cheng

RESUMEN

En la industria azucarera, los métodos de medición automática del contenido de cloruros y P₂O₅ total en el jugo de caña, azúcar y mieles, no han sido bien desarrollados, aunque algunos de los métodos han sido aplicados en otros campos como la medición del contenido de cloruros en el agua, el plasma y la orina y el contenido de fósforo en la carne, alimentos para animales y fertilizantes. Sin embargo la medición del contenido de estos aniones es necesaria e importante para los que trabajan en investigación relacionada con la tecnología azucarera. Los métodos convencionales de análisis, efectuados manualmente, son laboriosos y complicados. Algunos de estos métodos requieren de mucho tiempo y están sujetos a posibles errores analíticos.

En este experimento, fué desarrollado un método automático rápido y confiable basado en la coloración del cloro-tiocianato férrico y el complejo de molibdato-fosfato de ácido ascórbico para la determinación de cloruros y fosfatos, respectivamente, utilizando un auto-analizador Technicon.

Los resultados demostraron que la desviación estándar y el coeficiente de variación de los métodos automáticos desarrollados fueron de 2,3 ppm y 0,99% en un rango de 0 a 800 ppm para los cloruros, y de 0,305-0,869 ppm y 0,34%-0,63% en un rango inferior a 15 ppm para el análisis de P₂O₅, respectivamente. Se probó que el tiempo requerido para los análisis es mucho más corto. En los dos casos pueden analizarse hasta 30 muestras por hora. Por lo tanto, los métodos pueden ser considerados factibles y reproducibles y aplicables a la industria azucarera y a labores de investigación azucarera.