Thermochimica Acta, 171 (1990) 239–244
Elsevier Science Publishers B.V., Amsterdam

MICROCALORIMETRIC CONTROL OF MICROBIOLOGICAL PROCESSES. I. ANALYTICAL DETECTION OF GLUCOSE AND SOME OTHER CARBOHYDRATES.

M.V. REKHARSKY [1], O.A. RODIONOVA [2], Yu.I. TARASOV [1], A.A. MAVRIN [1], A.M. EGOROV [1] and S.V. BELYAEV [2]

[1] *Department of Chemistry, Lomonosov State University, Moscow 117234 (U.S.S.R.)*
[2] *Shemyakin Institute of Biorganic Chemistry, U.S.S.R. Academy of Sciences, Moscow (U.S.S.R.)*

(Received 29 March 1990)

ABSTRACT

Microcalorimetric methods have been developed for continuous analytical control of glucose, lactose and sucrose concentrations in solutions in biotechnological processes. The methods are based on the Malprade reaction. The carbohydrate concentrations were detected on a flow–mix microcalorimeter. The accuracy of measurements of the carbohydrate concentration (2–4 mM) in solution is 1–2%. Other components common to biotechnological solutions (amino acids, ammonium ions, urea and acetate) have virtually no influence on the results.

INTRODUCTION

The conditions for growing of recombinant microorganisms need to be carefully controlled [1]. In order to cultivate such microorganisms, raw materials containing glucose and, more rarely, sucrose and lactose are used as a carbon source [2].

As is known, a bioactivity monitor (Thermometric AB, Sweden) can be applied to obtain thermograms of the growth of different cultures [3–5]. Our aim was to develop procedures for detection of glucose, sucrose, lactose by using this instrument.

Microcalorimetric detection of carbohydrates in microbiological processes is based on the Malprade reaction, i.e. oxidation of carbohydrates with periodate in phosphate buffer. This reaction is well studied in analytical chemistry [6], and the method can easily be adapted for certain industrial solutions (e.g. to find out how amino acids affect the detection, see below).

The Malprade reaction has been investigated by thermochemical methods [7–11]. Bark et al. [7–9] performed indirect calorimetric titration of aqueous solutions of different carbohydrates: periodate was added in excess and after

0040-6031/90/$03.50

the reaction was completed the excess periodate was titrated with hydrazine sulphate. De Oliveira and Rodella [10], and Volf et al. [11] undertook direct calorimetric titration. They showed that the malaprade reaction includes two thermochemical stages: one rapid (2–5 min) then one slow.

The published methods [7–11] have in our opinion the following disadvantages. The indirect method requires preliminary complete oxidation of the carbohydrate sample which sometimes, e.g. in the case of sorbose, takes 18 h. In the direct method [10–11] batch equipment was used. Since this is a two-stage reaction (see above), the heat effect cannot be referred to the first step, i.e. directly to the analytical reaction.

We think it is reasonable, first, to use the direct method in order to avoid the preliminary preparation of samples, and secondly, to apply the flow microcalorimetric technique, which allows strict limitation of the time the reaction mixture is in the microcalorimetric cell and thus permits measurement of the thermal effect at the first stage.

EXPERIMENTAL

The experiments were performed on a Bioactivity Monitor, with a flow–mix cell. The solutions were delivered to the cell by an LKB-2132 pump at a rate of 9.81 ml h^{-1}. We varied the following parameters: buffer composition and pH, the IO_4^- concentration, and components of the biotechnological processes.

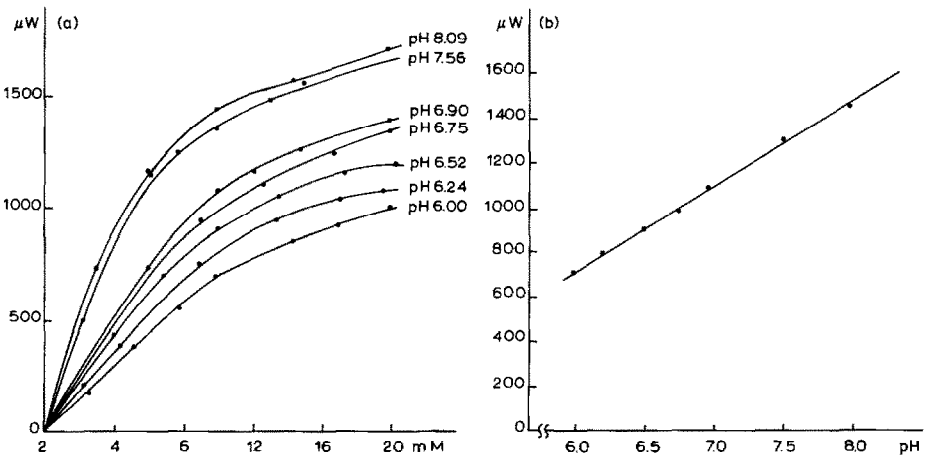

Fig. 1. a, Heat release effect (μW) of interaction of glucose (concentration 0.00123 M) with sodium periodate (concentration 0.001 M to 0.020 M) between pH 6 and 9). b, Heat effect (μW) of interaction of glucose (0.00123 M) with sodium periodate (0.010 M) as function of pH.

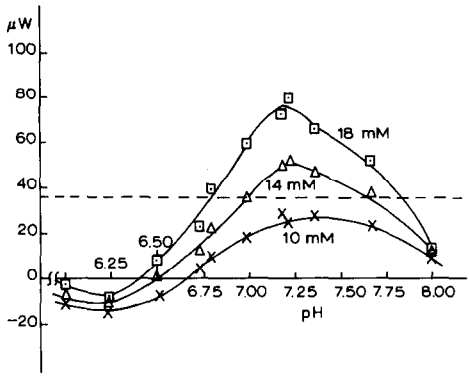

Fig. 2. Heat effects (μW) of dilution of sodium periodate (concentrations 10, 14 and 18 mM) vs. pH. The dotted line corresponds to 1–2% of the total heat release of the analytical reaction (Fig. 3).

Figure 1 shows graphically the magnitude of the heat effects of interaction of periodate (0.001–0.020 M) with glucose (0.001 23 M) at pH 6–8. Because of the poor solubility of potassium periodate the reaction was conducted in sodium phosphate buffer (Fig. 1 and the following figures). At

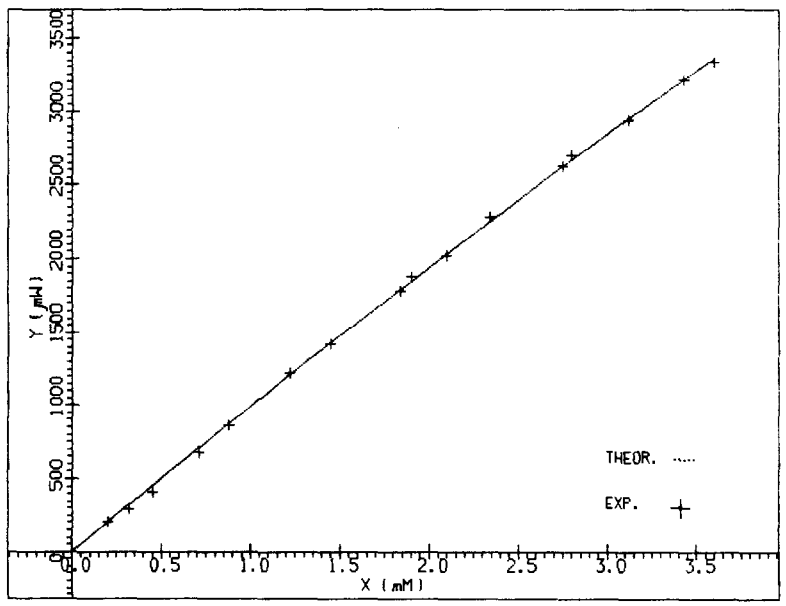

Fig. 3. Calibration curve for detection of glucose concentration by heat effect of the Malprade reaction: +, experimental data (Y_{ex}); (· · ·, theoretical calculated dependence of heat effect (Y_{th}) on glucose concentration (x, mM), Y (μW) $= 1018X - 23.55X^2$. The mean square error of a single measurement calculated from this curve is $\sigma = [\Sigma(Y_{ex} - Y_{th})^2/(n - 1)]^{1/2} = 22$ μW, which corresponds to an accuracy of 1% in the analysis of glucose solution (2–3 mM).

low concentrations of periodate ion (1–10 mM) the heat effect increases sharply with increase in the periodate concentration (Fig. 1a). Further increase in concentration somewhat inhibits the heat effect. Consequently, with the increase in the periodate concentration, the precision of analytical determinations improves, since the same error in preparing a solution of a given concentration affects the results less at higher concentrations (more than 10 mM). In addition, the heat effect of the reaction increases steadily with increasing pH at the same periodate concentration (Fig. 1b).

When developing microcalorimetric procedures one should take into account thermal side-effects as well, particularly those on dilution of the reagents [12]. Figure 2 presents the magnitude of heat effects of periodate dilution under various conditions (periodate concentration 10, 14 or 18 mM, depending on pH).

To obtain reliable analytical data by microcalorimetry the heat effects from reagent dilution should be minimal; optimum values should not exceed 1–2% of that of the analytical reaction. Taking into account these facts and that at $6.3 < pH > 7.5$, the buffer capacity of the solution is low, a pH of 6.8–7.0 is considered to be optimal.

Thus we selected the following conditions (Figs. 1 and 2): sodium

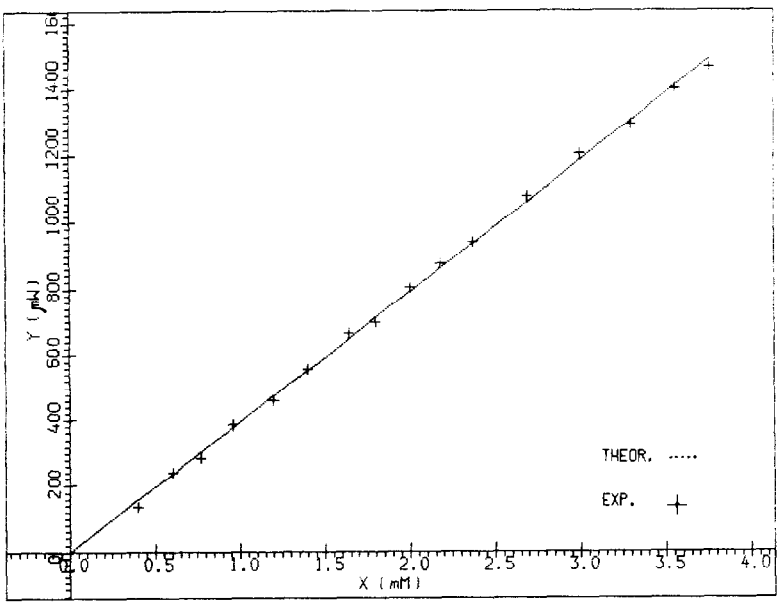

Fig. 4. Calibration curve for detection of lactose concentration by heat effect of the Malprade reaction: +, experimental data (Y_{ex}); ···, theoretically calculated dependence of heat effect (Y_{th}) on lactose concentration (X, mM), Y (μW) = 394.8X. The mean square error of a single measurement calculated (see Fig. 3 caption) from this curve is 15 μW, which corresponds to an accuracy of 1% in analysis of lactose solution (3–4 mM).

phosphate buffer solution (0.025 M; pH 6.88), periodate concentration 0.014 M.

DISCUSSION

The calibration curves for microcalorimetric detection of common carbohydrates under the above conditions in biotechnological processes are presented in Fig. 3 (glucose), Fig. 4 (lactose) and Fig. 5 (sucrose). It is noteworthy that one experiment takes 10–15 min.

The experimental data (Figs. 3–5) are processed on a personal computer (IBM PC-AT). According to the Fischer criterion the experimental data for sucrose and lactose are satisfactorily described by a simple model, $y = aX$; for glucose (Fig. 3) it is reasonable to use another model, $Y = aX + bX^2$.

In all cases we observed an almost proportional dependence of the heat effect of the reactions on the concentration of the substance being determined. The heat effect of the glucose reaction is approximately 2.5 times higher than that of lactose and 16 times as high as that of sucrose.

These techniques are applicable for controlling biotechnological processes in which only one carbohydrate provides the carbon source for the micro-

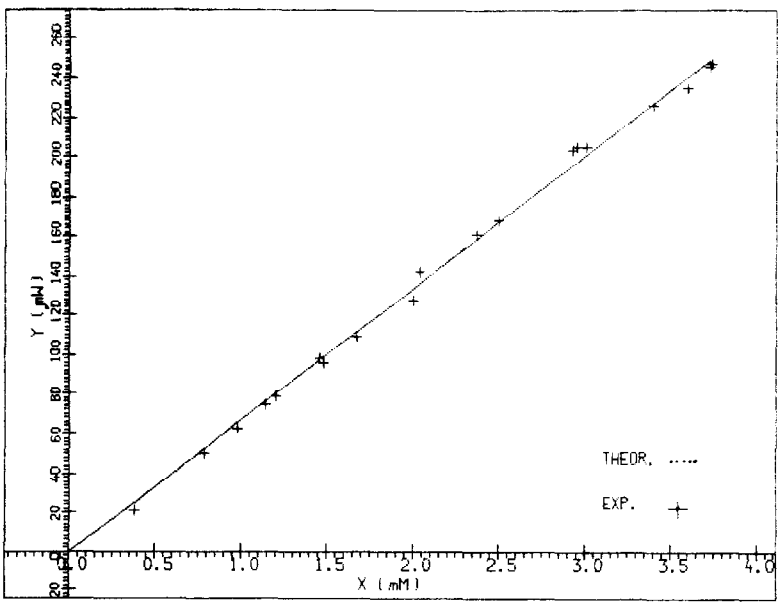

Fig. 5. Calibration curve for detection of sucrose concentration by heat effect of the Malprade reaction. +, experimental data (Y_{ex}); ···, theoretically calculated dependence of heat effect (Y_{th}) on sucrose concentration (X, mM), Y (μW) = 66.8X. The mean square error calculated (see Fig. 3 caption) from this curve is 4 μW, which corresponds to an accuracy of 2% in the analysis of sucrose solution (2.5–3.5 mM).

organisms. For more complex mixtures of several carbohydrates, it is reasonable to use microcalorimetric enzymatic methods [13–14].

We carefully considered the problem of how admixtures influence the results of analytical detection. Thus, for example, of all the amino acids, only serine and tryptophan react with periodate ion at a measurable rate [6]. Very small concentrations of amino acids are usually found in the culture liquid [15], therefore they have practically no influence on the results of microcalorimetric detection of carbohydrates (particularly, the standard nutrition medium Minimum Essential contains tryptophan in a quantity lower by 2 orders of magnitude than that of glucose [15], and the measured heat effect at practical concentrations is at least 3 orders of magnitude less than the total heat effect of the reaction).

It is also noteworthy that the presence in large concentrations (comparable to those of carbohydrates) of amino acids which are not oxidized by periodate ion, such as alanine, leucine, lysine, valine, glycine and glutamine (these amino acids are present in nutrition media in the largest concentrations), as well as analogous amounts of the most typical products of microorganism metabolism (acetate, ammonium ions and urea), do not affect the microcalorimetric detection.

REFERENCES

1 J.L. Ingraham, O. Maale and F.C. Neidhardt, Growth of the Bacterial Cell, Sinawer Associaties, Sunderland, MA, 1983.
2 I. Beily and D. Ellis, Principles of Biochemical Engineering, Vols. and 1 and 2, World, Moscow, 1989, (in Russian).
3 I. Wadsö, Thermochim. Acta, 137 (1988) 1.
4 R. Bar, Trends Biotechnol., 6 (1988) 55.
5 I. Carmel, Thi Cong To and A. Beanbien, Anal. Chim. Acta, 213 (1988) 165.
6 I.M. Koltgof, V.A. Stenger, R. Bleher and J. Matsuyma, Volumetric Analysis, Vol. 3, State Scientific Technology Publishers Chemical Literature, Moscow, 1961 (in Russian).
7 L.S. Bark, D. Edwards and P. Prachuabpaibul, Proc. Soc. Anal. Chem., 11 (1974) 170.
8 L.S. Bark, P. Prachuabpaibul, Anal. Chim. Acta, 72 (1974) 196.
9 L.S. Bark and D. Edwards, Fresensius Z. Anal. Chem., 272 (1976) 202.
10 W.A. de Oliveira and A.A. Rodella, Talanta, 26 (1979) 965.
11 R. Volf, M. Stastny, J. Vulterin and M. Waldman, Chem. Prum., 28 (1978) 513.
12 J. Jordan and J.W. Stahl, in J.K. Grime (Ed.), Chemical Analysis, Vol. 79, Analytical Solution Calorimetry, Wiley–Interscience, Englewood Cliffs, NJ, 1985, pp. 17–57.
13 C.F. Mandemins, L. Bülew, B. Danielesson and K. Mosbach, Appl. Microbiol. Biotechnol., 21 (1985) 135.
14 M.V. Rehkharsky, O.A. Rodionova, E.N. Nemykina, S.I. Kharitonov and S.V. Belyaev, J. Biochem. Biophys. Methods, 19 (1989) 253.
15 R. Ham and W.L. McKeehan, in W.B. Jakoby and I.H. Pastan (Eds.), Cell Culture, Methods Enzymol., 58 (1979) 44.