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Development and evaluation of microscale apparatus for the generation of kinetic data at high temperatures, applied to the degradation of thiamine

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A microminiaturized tubular heating system, with continuous heating and cooling, for kinetic studies has been developed. The apparatus was used in the temperature range from 110 to 160°C. Heating to holding temperature and cooling to room temperature was accomplished in one second. The sample was injected into the apparatus with an HPLC loop injector. After thermal treatment, a well-defined fraction was withdrawn by a fraction collector. The sample dilution during thermal processing was corrected against an inert standard.

A kinetic study of thiamine degradation in phosphate buffer at pH 7 was performed to evaluate the apparatus. The activation energy (E_a) of the thermal degradation of thiamine was determined to be 119 kJ/mol ($r = -0.9981$).

NOTATION

a_w	Water activity
C'	Constant
d	Diameter (m)
D_f	Dilution factor
E_a	Activation energy (kJ/mol)
Gr	Grashof number
l	Length (m)
n	Constant
Pr	Prandtl number
R	Universal gas constant (8·314 kJ/kmol K)
Re	Reynolds number
R_t	Retained thiamine
t	Time (min)
T	Absolute temperature (K)
u	Velocity (m/s)
α_i, α_o	Heat transfer coefficient (W/m^{2}K)
ΔP	Pressure difference (N/m^2)
λ	Thermal conductivity (W/m^{2}K)
μ	Viscosity of fluid (Ns/m^2)
ρ	Density of fluid (kg/m^3)
Φ	Friction factor

INTRODUCTION

Ultra-high-temperature (UHT) processing and aseptic packaging of milk uses temperatures in the range 135–150°C (Burton, 1988) for a few seconds in order to produce a product which does not require refrigeration for a long shelf life (Ramsey & Swartzel, 1984). In 1989, approximately 15 billion litres of liquid and semi-liquid food products were aseptically packaged in Western Europe and the market is growing at a rate of 3% annually (Beckman, 1991).

However, to optimize thermal food processes and especially to predict the effect of the UHT process upon heat-sensitive food components, reliable kinetic data are required. In order to gather such data, several types of equipment have been developed and evaluated (Davis *et al.*, 1977; Perkin *et al.*, 1977; Burton *et al.*, 1977; David & Shoemaker, 1985; David & Merson, 1990; Swartzel & Jones, 1985; Sadeghi & Swartzel, 1990). The use of capillary tubes was studied by Davis *et al.* (1977) and Perkin *et al.* (1977). Burton *et al.* (1977) compared the results of capillary tube experiments with those obtained with a batch infusion sterilizer and a continuous steam injector sterilizer. They observed that the sterilizers used showed a greater increase in the rate of thermal death with temperature than would be expected from the capillary tube experiments. The differences were greater in the lower UHT region than in the higher. They speculated as to whether their observations could be explained by fundamental differences between the two heating methods, of thermal inactivation of spores in a capillary tube and of thermal inactivation of spores in the bulk of a processing plant, brought about by the close proximity of the surface in the capillaries.

A computer-controlled reactor based on a piston which moves a filter paper saturated with the sample into a steam chamber and then cools the sample by putting it in a chilled buffer solution was used by David and Shoemaker in 1985. This equipment was used in the temperature range between 105·5 and 117·0°C for the study of the inactivation of peroxidase. The equipment had the advantage of extremely short heating times (0·1 s) and rapid cooling, but the authors pointed out that the desorption of enzyme from the filter paper could affect the result. Later this equipment was used (David & Merson, 1990) at higher temperatures (130–145°C) to inactivate *Bacillus stearo-thermophilus*.

A small laboratory sterilizer has been evaluated for kinetic studies (Swartzel & Jones, 1985). The design allowed the examination of the constituents at any time–temperature range appropriate in food processing. Later this equipment was used to examine the kinetics of the inversion of sucrose and the degradation of Blue No. 2 (FD&C Blue No. 2, Indogotine) (Sadeghi & Swartzel, 1990). The results gained with this equipment were compared with those from traditional capillary heating and it was found that capillary heating gave higher values for the activation energy (E_a).

The purpose of the present work was to examine the possibilities and limitations of a miniaturized flow system, optimized with respect to flow properties and heat transfer (indirect heating), and designed to minimize the residence time distribution within the samples. Such a miniaturized system could be used to study reactions in a continuous flow where the access to samples is limited or too costly to use in conventional equipment.

Degradation of thiamine was chosen for the evaluation of the apparatus. The reaction has previously been examined (Feliciotti & Esselen, 1957; Fox *et al.* 1982) and it is well established that the degradation of thiamine by heat is a first-order reaction. pH 7 was chosen in our study since the degradation of thiamine is enhanced with increasing pH. Feliciotti and Esselen (1957) determined the activation energy to be 121 kJ/mol in the temperature range of 109–149°C. Fox *et al.* (1982) altered the water activity (a_w) from a_w = 0·90 to a_w = 1·0 and investigated the degradation of thiamine at 84 and 94°C. They determined the activation energy to be 114 kJ/mol at a_w = 1·0. An agreement with previously published results would be strong evidence that our assumptions concerning the construction of this apparatus are correct.

MATERIALS AND METHODS

The apparatus is shown schematically in Fig. 1. A tubular system was immersed in an arrangement of three vessels: a heating bath (D), a holding bath (E) and a cooling bath (G). Through this tubular system a mobile phase was pumped continuously. The temperatures of the heating bath and of the holding bath were controlled (± 0·1°C) by separate thermostats. The thermostats were calibrated against a Pt 100 sensor. The oil (Shell Thermia Oil B) in the two baths (D,E) was chosen to optimize the heat transfer. The temperature in the heating bath (D) was chosen such that the flow leaving the heating bath had the selected temperature of the holding bath (E). In practice, this meant that the temperature in the heating bath (D) was slightly higher than the temperature in the holding bath (E). The cooling bath (G) contained melting ice and water ≈0°C. The mobile phase was kept in a water bath (A) at 25°C.

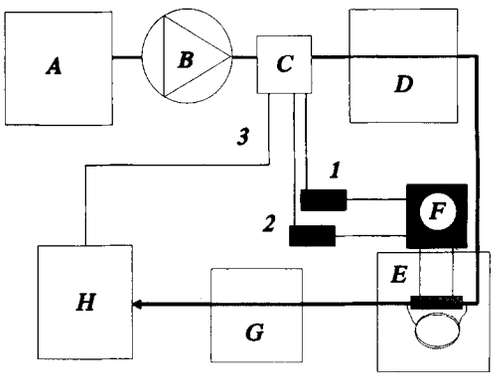

Fig. 1. Schematic diagram of the principle of the apparatus: (A) preheated mobile phase; (B) rebuilt HPLC pump; (C) loop injector; (D) heating bath; (E) holding bath; (F) submerged auto-injector with loop; (G) cooling bath; (H) fraction collector; (1, 2) timers 1 and 2 regulating the operation of the submerged auto-injector; (3) signal from loop injector to start the program of the fraction collector.

The tubular system was made of stainless steel tubes with an inner diameter of 0·35 mm and an outer diameter of 0·60 mm. The size of the tubes facilitated the use of standard HPLC fittings. To resist the high temperatures, the ferrules used were made of vespel/graphite material (SGE, Milton Keynes, UK). The pump (B) used was a rebuilt HPLC pump (Waters M6000 A) with a capacity from 0·225 to 22·275 ml per min and was able to work at pressures up to 6000 psi. To save buffer solution for processing at longer holding times, the pump was stopped 15 s after the sample was injected and restarted 15 s before the end of the holding time.

The sample (96 μl) was injected into the system with a HPLC loop injector (C) (Reodyne).

In the holding bath (E) a auto-injector (F) (Valco WE-10-C6W-special) was submerged. The heated sample entering the holding bath (E) was caught in the loop of the auto-injector and kept there for the desired time. The operation of the auto-injector was controlled by a specially built device (ENPECE, Lund, Sweden) consisting of two timers, the first (1) regulating the time from which the sample was injected up until the auto-injector switched from 'inject mode' to 'load mode' to catch the sample in the loop. With the second timer (2) the actual holding time was selected. The two timers were started by a signal from the loop injector (C) at the moment of injection. The submerged auto-injector was enclosed in a brass cover to protect it from the hot oil. When the auto-injector in the holding bath (E) returned to inject

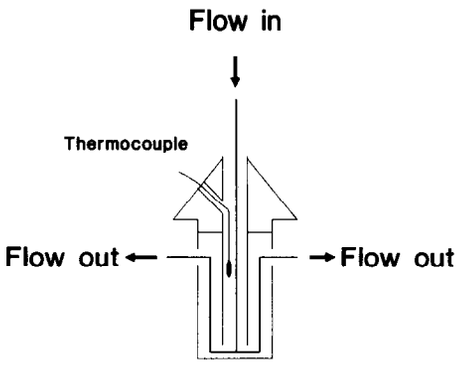

Fig. 2. Prior to the experiments this measuring cell was attached to the tube at the end of the heating bath to measure the temperature of the flow leaving the heating bath. To decrease the influence of the ambient temperature, the flow from the tube, when it had passed the thermocouple, turned at the end of the cell, and was used as insulation between the entering flow and the ambient temperature.

mode, after the holding time, the sample continued with the flow of the mobile phase to the cooling bath (G). After thermal processing a fraction was withdrawn from the flow with a fraction collector (Gilson Model 202) (H). The program of the fraction collector was also started by a signal (3) from the loop injector (C) at the moment of injection. The back pressure to prevent boiling was maintained by the capillary in the cooling bath, 6·18 bar at 160°C (Handbook of Chem. & Phys., 1980).

Since the fraction withdrawn contained both sample and mobile phase in different proportions for each time–temperature combination, the concept of a dilution factor (D_f) was introduced to correct for the non-sample volume in the fraction withdrawn (see below). The dilution factor was determined for every sample.

The measurement of the temperature of the flow inside the tube was made prior to the experiments by attaching a measuring cell at the end of the tube in the heating bath (D) (Fig. 2). The cell contained one small, calibrated thermocouple. The flow from the tube, having passed the thermocouple, was used as insulation between the tube flow and the surroundings.

Technical constraint by the upper temperature limit for the apparatus of this design is 210°C, which is the flame temperature of the oil. The highest temperature tested was 180°C .

Solutions

Sörensen's phosphate buffer at pH 7 (Feliciotti & Esselen, 1957) was used as the mobile phase. A fresh stock solution of thiamine 400 mg/litre (Sigma, USA) dissolved in the mobile phase was made daily. From this stock solution the sample solution was made by diluting it 100 times with mobile phase.

Dilution factor

The dilution factor (D_f) for the samples was determined by processing an inert standard. Sodium benzoate was chosen as standard, since it is stable and easy to measure by absorption in the UV region. The samples of sodium benzoate were processed in exactly the same way as the thiamine samples and the content of sodium benzoate in the fraction withdrawn was measured by absorption at 223 nm in a spectrophotometer (Varian DMG 100). The dilution factor was determined as the average of three replicas of each sample.

D_f was calculated as the quotient between the absorption (ABS) of equal volumes of unprocessed sodium benzoate solution and of the sodium benzoate in the fraction withdrawn after the process (eqn (1)).

$$D_f = \frac{\text{ABS [Sodium benzoate}_{\text{before process}}\text{]}}{\text{ABS [Sodium benzoate}_{\text{after process}}\text{]}} \quad (1)$$

Analysis of thiamine

Thiamine was analyzed by HPLC as described in full by Vidal-Valverde and Reche (1990). The pump used was either a Varian 2510 or a Varian 9010. The detection was made at 244 nm with a Varian 2550 UV–VIS detector. The samples were injected by an autosampler (Varian 9100). The column used was a Waters μBondapack 3·9 mm × 30 cm with 10 μm packing. The composition of the mobile phase was: 69% water, 31%, methanol, 1 ml acetic acid, 3·75 mmol/litre hexasulfonic acid and 1·25 mmol/litre heptasulfonic acid. The flow was 1·5 ml/min and the thiamine peak was eluted in 12 min.

Three samples were analyzed in duplicate for each time–temperature combination. The injected volume was 10–60 μl. A standard curve was made by injecting different volumes of thiamine from the same vial. With this procedure, errors associated with the making of standard solutions of different concentrations were avoided. Retained thiamine (R_t) was calculated as the ratio between the thiamine content in the fraction withdrawn after the process multiplied by D_f and the thiamine content of the unprocessed thiamine solution (eqn (2)).

$$R_t = \frac{D_f \cdot \text{Thiamine}_{\text{after process}}}{\text{Thiamine}_{\text{before process}}} \quad (2)$$

RESULTS AND DISCUSSION

Thiamine degradation

The experiments with the equipment were performed at 110, 120, 130, 140, 150 and 160°C. Linear regression of ln R_t values versus time showed that a first-order kinetic model adequately described the degradation of thiamine. The correlation coefficients for ln R_t values versus time ranged from $r = -0{\cdot}9593$ to $r = -0{\cdot}9969$. This result confirms earlier studies which had also shown that the degradation of thiamine can be described as a first-order reaction.

The activation energy (E_a) (eqn (3)) for the reaction

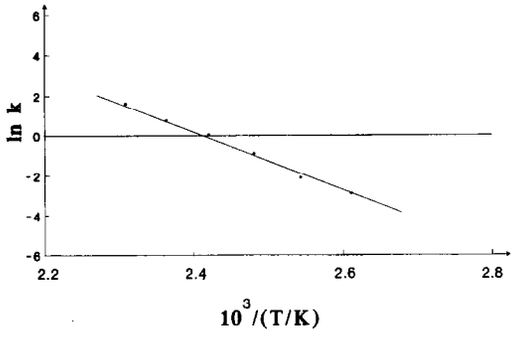

Fig. 3. Arrhenius plot for thiamine degradation in buffer solution at pH 7.

in the temperature region examined was found to be 119 kJ/mol ($r = -0{\cdot}9981$) (Fig. 3).

$$k = A\exp(-E_a/RT) \tag{3}$$

Our results are in good agreement with those of Feliciotti and Esselen (1957) who found E_a to be 121 kJ/mol, and of Fox *et al.* (1982) who determined E_a to be 114 kJ/mol.

Development and evaluation of the apparatus

Pressure drop and heat transfer in the steel tube

The Reynold's number (*Re*) (eqn (4)) is the parameter commonly used to describe the flow properties in a tube. *Re* above 3000 indicates turbulent flow (Coulson & Richardson, 1985). Turbulent flow is preferable because it facilitates heat transfer and gives a more appropriate flow profile.

$$Re = \frac{ud}{\mu} \tag{4}$$

The pressure drop in a single tube is inversely proportional to the cube of the diameter of the tube. Under constant conditions equations for the pressure drop in a smooth tube can be deduced from:

$$\Delta P = (8\Phi)\left(\frac{l}{d}\right)\left(\frac{\rho u^2}{\mu}\right) \tag{5}$$

to,

$$\Delta P = \text{const}\ \frac{1}{d^5} \tag{6}$$

The ideal tube for optimizing heat transfer has no heat resistance in the tube wall. This makes the heat transfer dependent on the heat transfer coefficient of the inner and outer surfaces. The inner heat transfer coefficient (α_i) depends on *Re*, which will be limited by the pressure. The outer heat transfer coefficient (α_o) for a single tube can be deduced (on the same assumptions as above) from:

$$\alpha_o = \frac{C'(G_f Pr_f)^n\ \lambda}{d} \tag{7}$$

to,

$$\alpha_o = \text{const}\ \sqrt{d} \tag{8}$$

Since the heat transfer increases when the diameter approaches zero, a tube with a very small diameter should be selected. However, if the diameter is too small the pressure drop will be too large and it will be impossible to achieve the necessary velocity of the fluid, to get turbulent flow, even with an HPLC pump which is constructed for high pressures. Heating to the desired temperature and cooling to ambient temperature with the tube selected here was achieved in one second.

Residence time distribution

In both laminar and turbulent flow the velocity of the fluid is greater in the middle of a tube than near the wall. This will give rise to a distribution in residence time since the central parts move faster than the more peripheral parts of the flowing liquid. Turbulent flow decreases the residence time distribution, and will make the thermal treatment more uniform.

In a sample that is rapidly injected into a flow and later withdrawn, all parts within the withdrawn fraction have been in the system for the same time. The residence time distribution within such a sample is not greater than the time taken to withdraw the volume. In this case the volumes withdrawn, from each injection, were 30 ~ 100 μl (1 to 3 drops), which gives a maximum residence time distribution of $\frac{1}{3}$ s. Generally a smaller volume was withdrawn when using shorter holding times than when using longer holding times. However, in some temperature–time combinations the fraction withdrawn was increased in order to include the concentration peak of the sample. It is not wise to withdraw a bigger fraction than necessary since this will increase the sample dilution.

Assessment of the dilution factor

The fraction withdrawn contained both sample and mobile phase. For different combinations of time and temperature the proportions of sample to mobile phase changed. This was due to the fact that the smallest change that could be made in the programming of the fraction collector was 0·01 min. This time interval was too long to coincide in a repeatable way with the arrival of the concentration peak at the end of the cooling bath. Accordingly, the same dilution factor could not be attained for the different time–temperature combinations.

However, it is very convenient to investigate a first or pseudo-first order reaction, because the degradation is independent of concentration and only dependent on time (van Boekel & Walstra, 1989), as can be seen from the following rearrangement of eqns (9)–(13) for a first order reaction.

$$\frac{d[A]}{dt} = -k_A[A] \tag{9}$$

$$\ln\frac{[A_t]}{[A_0]} = -k_A t \tag{10}$$

$$[A_0]R_t = [A_t] \tag{11}$$

$$\ln\frac{[A_0]R_t}{[A_0]} = -k_A t \tag{12}$$

$$\ln R_t = -k_A t \quad (13)$$

A second order reaction can be reduced to a pseudo-first order reaction by having the other reactants in such excess that their concentration is not affected by the degradation of the reactant being studied.

Evaluation of the apparatus

To evaluate the apparatus from a functional point of view some basic properties should be defined. We denote the amount of actual test substance, by 'sample', the liquid in which the sample is dissolved by 'solvent', and the mixture of sample and solvent by 'solution'.

Normally, the solvent volume is the same as the solution volume, but by working with a sample which is injected into a continuous flow, the amount of solution required is far less than the total requirement of solvent, and in this way the consumption of sample will be reduced. Our apparatus requires 1·2 litre of solvent per hour when operating continually. The other two apparatuses described using continuous flow (Swartzel & Jones, 1985; Burton *et al.*, 1977) required a flow of 600–1700 litre/h. The non-continuous methods (Perkin *et al.*, 1977; David & Shoemaker, 1985) require less solution than those working with a continuous flow. However, the amount of sample needed in our apparatus is approximately the same as is needed by non-continuous flow equipment. In conclusion, we can say that our equipment combines low consumption of solvent and sample solution with continuous flow. This is accomplished by the introduction of the concept of a dilution factor, which could reduce the use of expensive chemicals.

By the use of a small capillary tube, rapid and controlled heating and cooling times were achieved. Heating into the UHT range and then cooling to below room temperature was accomplished in one second. Our apparatus heats more rapidly than the other methods (Perkin *et al.*, 1977; Swartzel & Jones, 1985) which use indirect heating. Compared to the other two methods that use direct heating (Burton *et al.*, 1977; David & Shoemaker, 1985) our apparatus heated more slowly but cooled faster. Considering the total heating and cooling time we can conclude that our apparatus is better than or at least comparable to any other method.

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