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Biological & Biochemical materials

THE USE OF DIFFERENTIAL SCANNING CALORIMETRY IN CHEMICAL EDUCATION
III. (Final) A biochemical experiment

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An overview is given of the potential of DSC in chemical education. A way in which DSC can be introduced into education with a minimum of teacher effort, is explained. A comprehensive description is given of a new biochemical experiment, 'An investigation of the baking process with DSC'. It is shown that here, although signals normally are rather weak, much insight can be obtained into the nature of some important biochemical processes.

Keywords: biochemistry, chemical education, DSC, proteins, starch

Introduction

In preceding papers of this series [1, 2], the potential of DSC in chemical education has been demonstrated. A description was given of the DSC12E, a heat-flux differential scanning calorimeter, especially developed for educational use and introduced by Mettler in 1991 [3]. Also the so-called 'Proficient Pac' [4], an educational tool enabling rapid introduction of thermal analysis (in particular DSC) into the curriculum of educational institutes was comprehensively described.

The main part of this Proficient Pac is a text book, entitled 'The Practice of Thermal Analysis' [5]. In this book, which is divided into two parts, the background principles of the various thermal analysis techniques are first given. In part 2, a comprehensive description is given of 16 illustrative experiments which

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can be performed by the individual student in a limited amount of time. These experiments cover a broad range of chemistry and physics (for an overview [1]). As biochemistry plays an important role within chemical education, it would be attractive to have an experiment also in this field. Fortunately, the specifications of the DSC12E do not present any restrictions to these high-sensitivity measurements and therefore a new biochemical experiment was designed.

Experimental

All experiments were carried out using the Mettler DSC12E differential scanning calorimeter. Egg-white samples were prepared from fresh eggs and homogenised. As a starch sample, flour was taken, and this was dried before use. Measurements were performed in hermetically-sealed aluminium crucibles. Sample weight was approx. 15 mg, heating rate 10 deg·min^{-1}.

Description

In biochemistry, important reactions are the denaturation of proteins and the gelation of starch. To inform the student about these phenomena, an experiment was designed entitled: 'An investigation of the baking process with DSC'. As the title already reflects, this experiment is closely related to daily practice normally a very important issue for students.

The experiment is built up of three parts, which can be carried out separately:

1. Investigation of the denaturation of proteins
2. Investigation of the gelation of starch
3. Study of the baking process

The denaturation of proteins

Proteins form a class of very important molecules. On heating they all undergo a transition at which the spatial conformation of the molecule changes. This transition, which is normally irreversible, is called denaturation. Denaturation is a very subtle process and is associated with breaking of weak hydrogen and sulphur bonds. Therefore, many external parameters influence this reaction. It is the aim of this experiment to gain some insight into the process of denaturation. As the protein sample, use is made of normal chicken egg-white. This egg-white is built-up of various proteins, the most important of which are conalbumine and ovalbumine [6]. In the DSC curve, the denaturation is clearly visible (Fig. 1d).

In order to gain an idea of the influence of the environment on the denaturation process, some measurements are performed where the concentration of external ions is changed. As external ions, H^+, Na^+ and Al^{3+} are taken. During each experiment, the denaturation temperatures and enthalpies are measured. The relationships between these parameters should be explained.

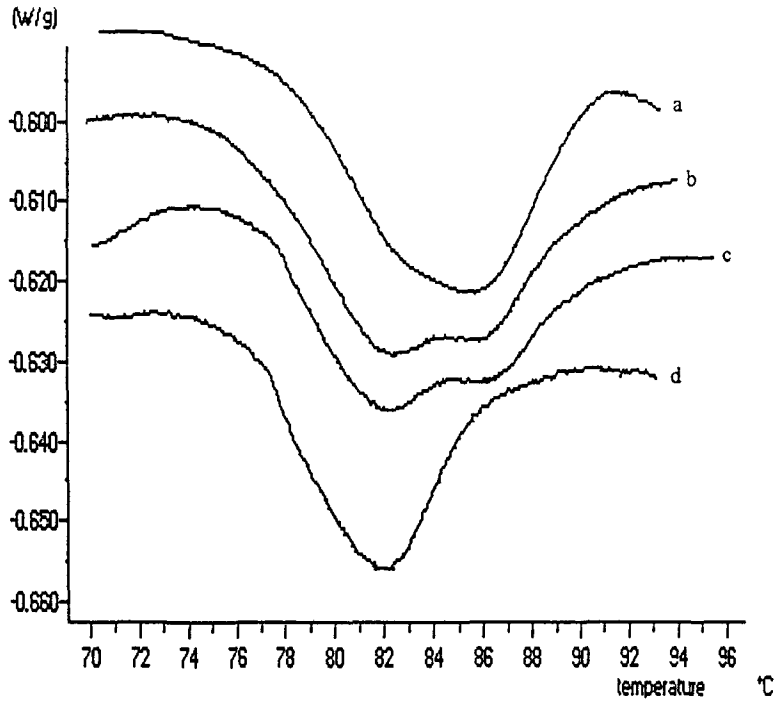

Fig. 1 DSC curves of chicken egg-white of various age: a) 40days, b) 25days, c) 15days, d) 1day

On ageing, the ovalbumine in egg-white shows a transition to a more stable form called S-ovalbumine [6]. With DSC this process can be examined very easily as Fig. 1 shows. Here, as a function of time, the height of the peak at 82°C decreases and that of the peak at 86°C increases. Samples of various ages, between 1 and 50 days, should be prepared and a relationship between the height of the peaks at 82° or 86°C and the age of the sample should be established. In this way, it is possible determine the age of an unknown chicken egg with DSC.

Finally, the potential of DSC for identification of substances can be demonstrated in this field. Samples are prepared of egg-white of various bird eggs. As Fig. 2 shows, the various sources can easily be distinguished.

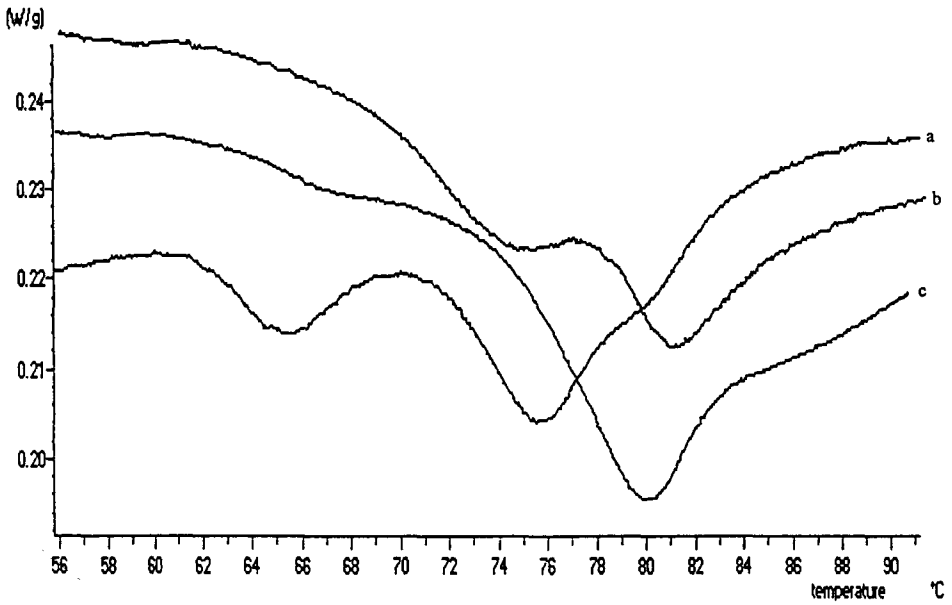

Fig. 2 DSC curves of egg-white of various bird eggs a) quail b) blackbird c) goose

Gelation of starch

Starch normally is a mixture of two types of molecules, amylose (approx. 25%) and amylopectin (approx. 75%). Both molecules can be regarded as semi-crystalline glucose polymers, the main difference between them being the degree of branching. On heating in the presence of water, at a certain temperature the intramolecular hydrogen bonds are broken and intermolecular hydrogen bonds with the water molecules form. As a result, the starch swells and the semi-crystalline structure disappears. This process is called gelation.

Gelation is a complex process, strongly dependent on the type of starch and the concentration of water. This is clearly reflected in the DSC curve, with peaks varying in temperature and area. First, samples are prepared of various types of starch (e.g. corn, oat, potato, rice) and the DSC curve of each sample is measured. Differences between those curves, as given in Fig. 3, are striking.

Secondly, the water content of a sample should be changed and the DSC curve measured as a function of percentage of water in the sample. Some typical results are given in Fig. 4. Changes in the DSC curve should be explained in terms of the gelation mechanism.

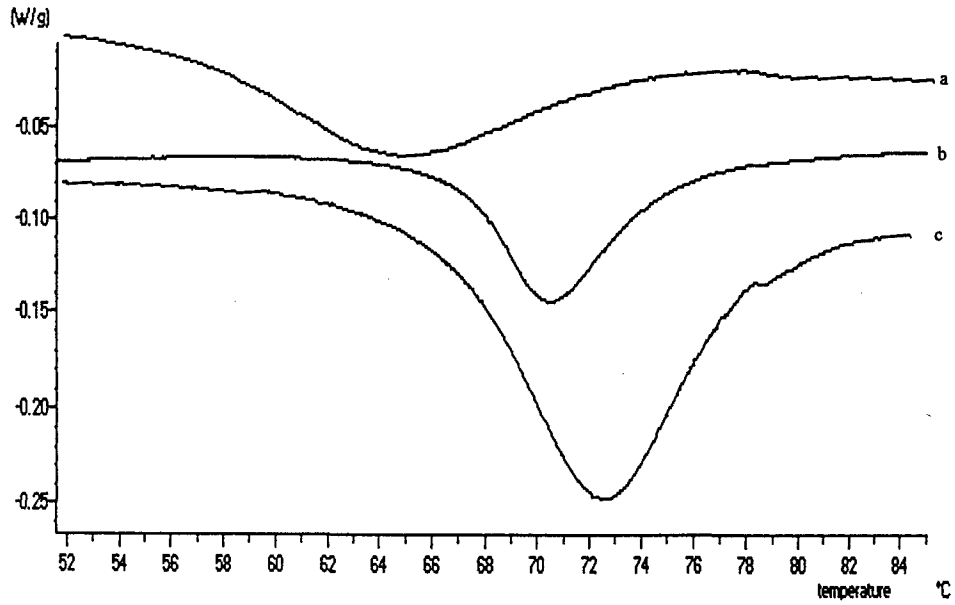

Fig. 3 DSC curves of various starches a) rice b) potato c) corn

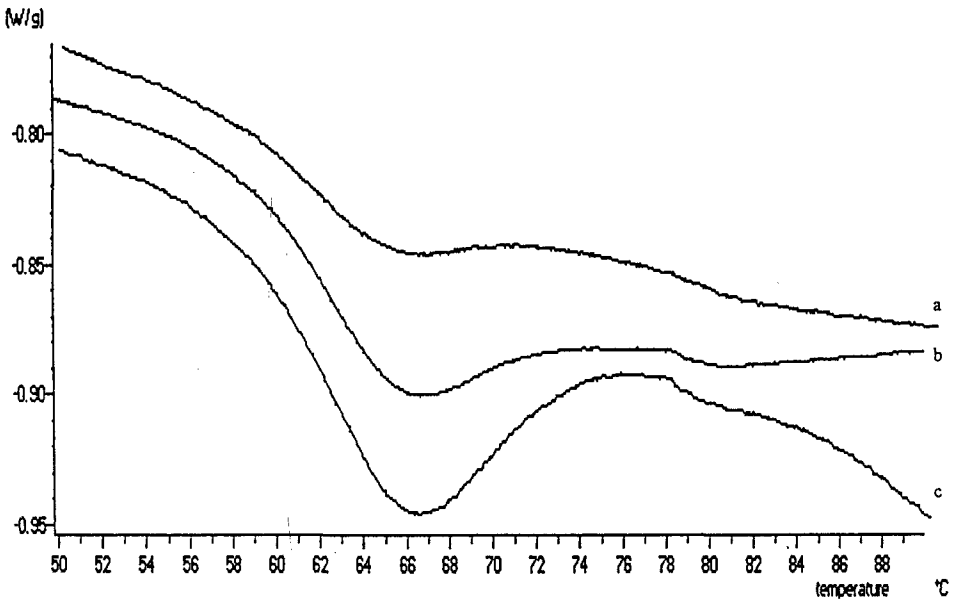

Fig. 4 Dependance of the gelation of corn starch on the water content a) 43% b) 55% c) 68%

The baking process

From a chemical point of view, baking is a combination of the gelation of starch and the denaturation of proteins in an environment containing substances such as water, fat, sugar and salt. Relative amounts of these ingredients determine whether baking yields a good result or not. In this respect, a good result is said to be obtained when on heating a stable framework of low density is formed.

To obtain more insight into the baking process, a recipe is made step by step and after each step the DSC curve is measured. In Fig. 5, the DSC curve of a mixture of egg-white, starch and water is given (ratios 3:1:4). The gelation peak at 67°C and the denaturation peak at 83°C can be seen clearly. When sugar is added, both peaks shift towards higher temperatures and the temperature difference between them decreases with increasing sugar content. As can be seen from Fig.6, at a suger content of 38% the peaks start to overlap (c), and at a sugar content of 47% only one peak is seen (d).

Fig. 5 DSC curve of a mixture of starch, egg-white and water (1:3:4)

The student should come to the conclusion that the relative amounts of egg-white, starch and sugar in an actual recipe are such that in the DSC curve only one peak is visible. Apparently, simultaneous gelation and denaturation is a requirement for a good product.

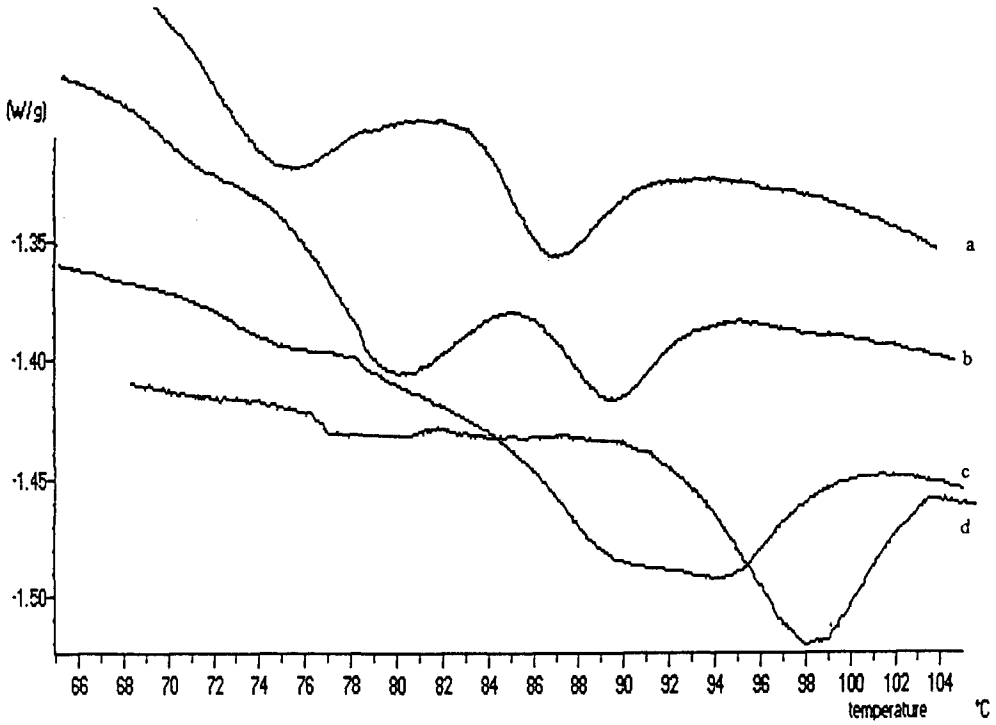

Fig. 6 Influence of sugar on the DSC curve of a starch/egg-white/water mixture a) 17.5% b) 25.0% c) 38.0% d) 47.0%

Conclusions

In daily life, biochemical processes play an important role. DSC is an attractive and powerful technique to gain insight into the nature of some of these reactions. The experiments described are easy to perform and can be carried out in a limited amount of time. The reported experiments serve only as a guideline. Extensions, changes or improvements by the individual teacher are highly welcomed.

References

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Zusammenfassung — Es wird ein Überblick über die Möglichkeiten von DSC im Chemieunterricht gegeben. Es wird ein Weg beschrieben, wie DSC unter minimalem Aufwand im Unterricht eingeführt werden kann. Eine ausführliche Beschreibung eines neuen biochemischen Versuches "Eine Untersuchung des Backprozesses mit DSC" wird beschrieben. Es wird hier gezeigt, daß – obwohl die Signale normalerweise eher schwach sind – ein tieferer Einblick in die Natur einiger wichtiger biochemischer Prozesse erlangt werden kann.