Carbohydrate Polymers 5 (1985) 25-36

Microcalorimetric Studies on the Lyophilic Properties of Pectic Substances

G. Ignatov, N. Markova and Chr. Kratchanov

Higher Institute of Food and Flavour Industries, 4000 Plovdiv, Bulgaria

(Received: 29 March 1983)

SUMMARY

The exothermic effects observed on wetting pectins with water and aliphatic alcohols were studied using a microcalorimeter.

The heat released on wetting 1 g pectin with water was found to be $171 \pm 7{\cdot}5\ J\,g^{-1}$. *It was experimentally established that 1 g of dry pectin exothermically bonded up to 0·57 g of water.*

By using the Gibbs-Helmholtz-Young equation which relates the heat released by wetting to the area of the wetted surface, it was estimated that the surface accessible to water in 1 g of pectin was $1{\cdot}46 \times 10^3\ m^2\,g^{-1}$. *The heat of hydration was independent of the degree of esterification of the pectin. The experimental results revealed that there were about six molecules of energetically bonded water per monomer unit of pectin.*

A specific interaction between methanol and the methoxyl groups of pectin was observed on wetting pectins with methanol and dependence was established between the released heat and the degree of esterification. No similar dependence was reported for the remaining aliphatic alcohols.

INTRODUCTION

The present paper is concerned to extend our understanding of lyophilic characteristics in terms of the capacity of surfaces and groups to bond liquids by molecular forces.

There are a number of methods available for the determination of lyophilic nature. The thermodynamic approach which is used in this

Carbohydrate Polymers 0144-8617/85/$03.30 –

paper is essentially macroscopic and may be applied to the determination of the forces of interaction between polymers and wetting liquids. Such thermodynamic methods may also be used indirectly to obtain information about the location of the adsorption sites on the polymer molecule, and the nature of any bonds.

The thermal phenomena observed in the course of wetting and adsorption are directly dependent on the amount of the liquid bonded and the strength of its retention. Therefore, reduction of the surface energy during the formation of the new interface may serve as a measure of solvent–solute interactions. This energy is condensed as heat of adsorption or hydration.

MATERIAL AND METHODS

A commercial apple pectin was used in this study. The samples were preliminarily washed with 70% alcohol/HCl and then rinsed repeatedly with 75% alcohol until no reaction for chloride ions was obtained. The pectin was first dehydrated with 95% alcohol and then transferred to a vacuum drier at 70°C. Samples of various moisture were obtained by rehydrating for different times over a free water surface in a vacuum desiccator. The wet pectin was sealed in glass phials and left for at least 48 h for the moisture to equilibrate.

The pectins were analysed by the method of Kertesz (1951) to determine the degree of esterification, equivalent weight, purity, ash, density and the pH of a 1% solution.

Absolute alcohols were obtained by successive dehydration of laboratory grade materials by CaO and Mg (Vulfson, 1964). The purity of the dehydrated alcohols and the concentration of the aqueous–alcoholic mixtures were refractometrically measured.

The solvent binding capacity of the pectin in contact with various liquids was followed by determining the heat of hydration in a microcalorimeter having a diathermic wall of the Thianne–Calvet type (Calvet & Prat, 1956). The heat flow due to the exothermic process was measured using 630 thermocouples recording the temperature differences between the internal and external surfaces of the diathermic wall. This wall formed the microcalorimetric chamber. Such a large number of densely arranged thermocouples was obtained by a copper plating on a constantan wire (Wilson & Epps, 1921; Terishtenko, 1971). The microcalorimeter measured heat flows of the order of 10^{-4} W emitted from each cm^3 of sample. The comparatively short response time (about 50 s) allowed the use of the device as a thermooscillograph. In order to avoid external troubles, two differentially connected microcalorimeters were used.

In the case of complete hydration of 1 g of substance, the released heat could be expressed by the Gibbs-Helmholtz-Young relation:

$$Q = S\sigma_{1,2} - TS\frac{\mathrm{d}\sigma_{1,2}}{\mathrm{d}T} \tag{1}$$

where S is the relative surface of the disperse system ($m^2\ g^{-1}$) and $\sigma_{1,2}$ is the surface tension between the wetting liquid and air ($J\ m^{-2}$).

Based on the above relation, the relative surface and thickness of the adsorption layer could be determined.

The heat of hydration is known to be independent of temperature. This assumption may be easily proven by introducing the known linear dependence of surface tension on temperature in eqn (1), i.e.:

$$\sigma_{1,2} = \sigma_0[1 - \Gamma(T - T_0)] \tag{2}$$

where Γ = the surface excess. Therefore,

$$Q = S\sigma_0[1 - \Gamma(T - T_0)] - TS\frac{\mathrm{d}[\sigma_0(1 - \Gamma T + \Gamma T_0)]}{\mathrm{d}T} \tag{3}$$

or

$$Q = S\sigma_0 + S\sigma_0\Gamma T_0 = \text{const.} \tag{4}$$

Hence, if the experimentally observed thermal process is independent of temperature, the phenomenon studied is hydration. For the case where the exothermic hydration processes are functions of temperature, the phenomenon also involves the heat of solution.

The above assumption served as the criterion for adopting the temperatures at which the thermal effects of dissolution and of swelling could be neglected. For that purpose the heat of hydration was measured as a function of the temperature (Fig. 1). The heat released on wetting the pectin was found to be independent of temperature for temperatures lower than 10°C ($Q = f(T) = \text{const.}$), i.e. at these temperatures the thermal effects of dissolution of pectin and swelling become unnoticeably small. Hence, only the exotherm of wetting, which was independent of the temperature, was observed.

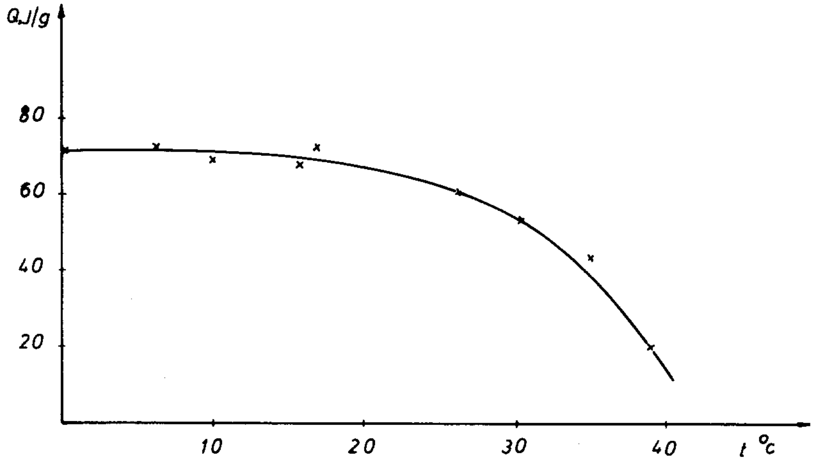

Fig. 1. Dependence of heat of hydration of apple pectin with a moisture of 6·5% on temperature.

Subsequent measurements of the heat of hydration were made at a temperature of 0°C.

HEAT OF HYDRATION OF PECTIC SUBSTANCES

The magnitude of the exotherm observed on wetting the colloid depended on the amount of water bound. The more adsorption sites bound in the preliminary wetting during preparation of the materials, the lower was the amount of bound water and energy released.

In order to estimate the state of the hydrophilic centres in pectins of various degrees of esterification, two pectins of completely different degrees of esterification, were studied. Figure 2 shows the dependence $Q = f(W\%)$ for apple pectins with degrees of esterification of 75·5 and 35%, respectively, within a moisture range of 0–27%.

It is seen that the heat of hydration released as a function of the initial moisture was independent of the degree of esterification. The integral heat of hydration of 1 g dry pectin, Q, was determined by extrapolating the curve $Q = f(W\%)$ to $W = 0$ (Fig. 2). It is evident that $Q_i = (Q)_{W \to 0} = 171 \pm 7{\cdot}5$ J g^{-1}. In order to determine the maximum

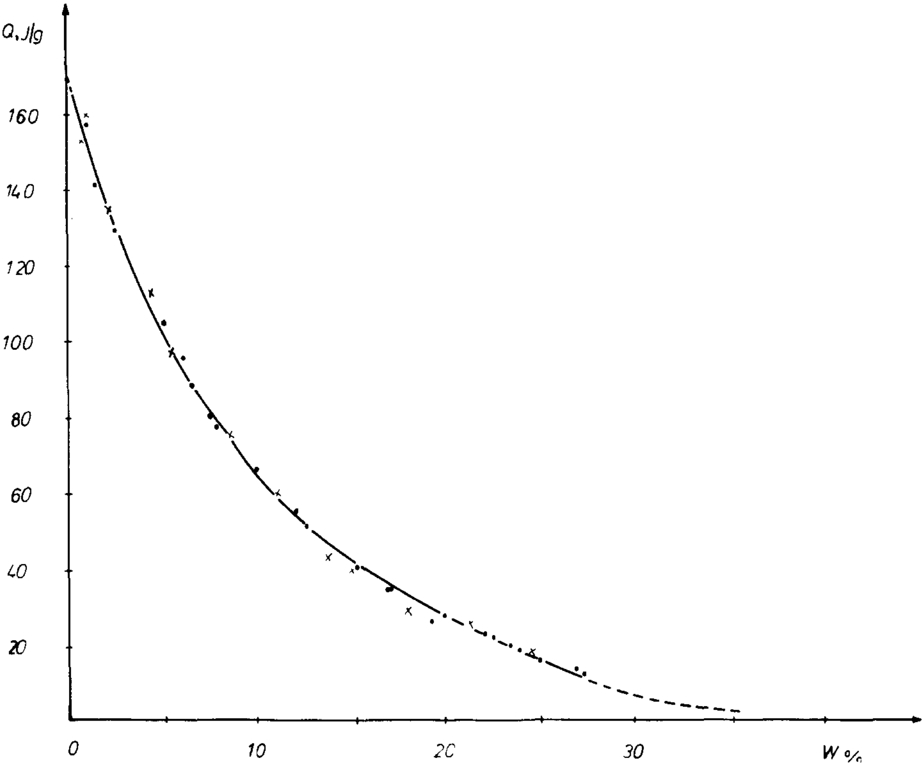

Fig. 2. Dependence of heat of hydration of apple pectin, with various degrees of esterification, on moisture content. (•) Degree of esterification 75·5%; (x) degree of esterification 35·0%.

amount of moisture which could be bound exothermically, the same curve was extrapolated to those moisture values where $Q = 0$. To obtain a more precise extrapolation, the linear dependence $Q = f(\log W)$, given in Fig. 3, was used. This dependence was valid for moisture contents exceeding 20%.

In this instance the maximum moisture was found to be

$$W_i = (W)_{Q \to 0} = 36{\cdot}4 \pm 0{\cdot}5\%$$

or 1 g dry pectin bound to 0·57 g water with emission of heat. This value of water of hydration was designated X_c (g g^{-1}).

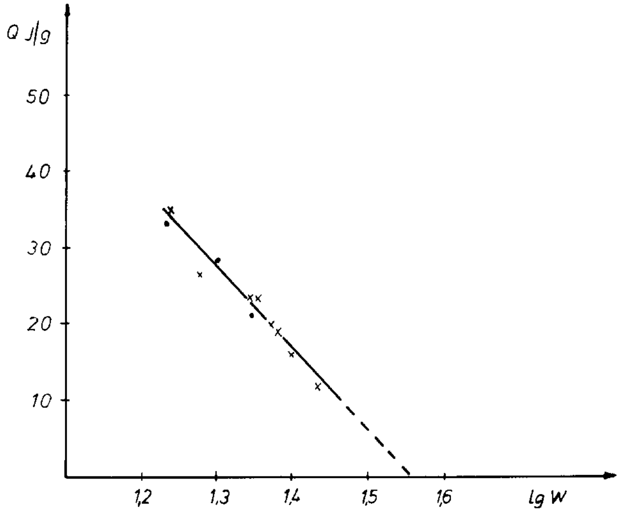

Fig. 3. Extrapolation of the dependence for $Q = 0$. (•) Degree of esterification 75·5%; (x) degree of esterification 35·0%.

From eqn (1), the heat of hydration of 1 m^2 of solid substance (q) was derived:

$$q = \sigma_{1,2} - T\frac{d\sigma_{1,2}}{dT} \tag{5}$$

In water, hydration at $T = 273$ K with $\sigma_{1,2} = 75{\cdot}49 \times 10^{-3}$ J m^{-2} and $d\sigma_{1,2}/dT = -0{\cdot}151 \times 10^{-3}$ J m^{-2} K^{-1}, yielded a value of $q = 116{\cdot}7$ J m^{-2}. Upon dividing the heat of hydration (Q_i) by the heat per unit area (q), a value for the relative surface $S_0 = Q_i/q = 1{\cdot}46 \times 10^3$ m^2 g^{-1}, representing the surface in 1 g pectin accessible to water, was obtained. As the total amount of bound water X_c was known, the thickness of the water layer, d, which caused the exothermic process was determined:

$$d = X_c/\rho \,.\, S_0 = 3{\cdot}9 \times 10^{-10}\ \text{m} \tag{6}$$

In these calculations, the density, ρ, of the water bound to the colloid was assumed to be close to 1×10^3 kg m^{-3} (Volarovich *et al.*, 1950). In the case of such density the monomolecular layer should be $3{\cdot}44 \times 10^{-10}$ m thick. Therefore, the exothermic process was caused by a layer slightly thicker than the monomolecular one.

THERMAL EFFECTS OF WETTING OF PECTINS WITH ETHANOL AND AQUEOUS-ETHANOL SOLUTIONS

Studies of this kind of solvent–solute interaction are of theoretical value for determining the interaction between the aqueous–ethanol medium and the disperse phase but the problem is also of industrial importance as solid pectins are usually obtained by precipitating pectin from aqueous solution using ethanol.

Figure 4 shows the dependence between the heat released on wetting pectins at a moisture of $W = 2{\cdot}5\%$, $6{\cdot}6\%$ and $15{\cdot}3\%$, with aqueous-ethanol solutions having concentrations of ethanol in the range 0–100%. The results showed that the thermal effect began to quickly decrease

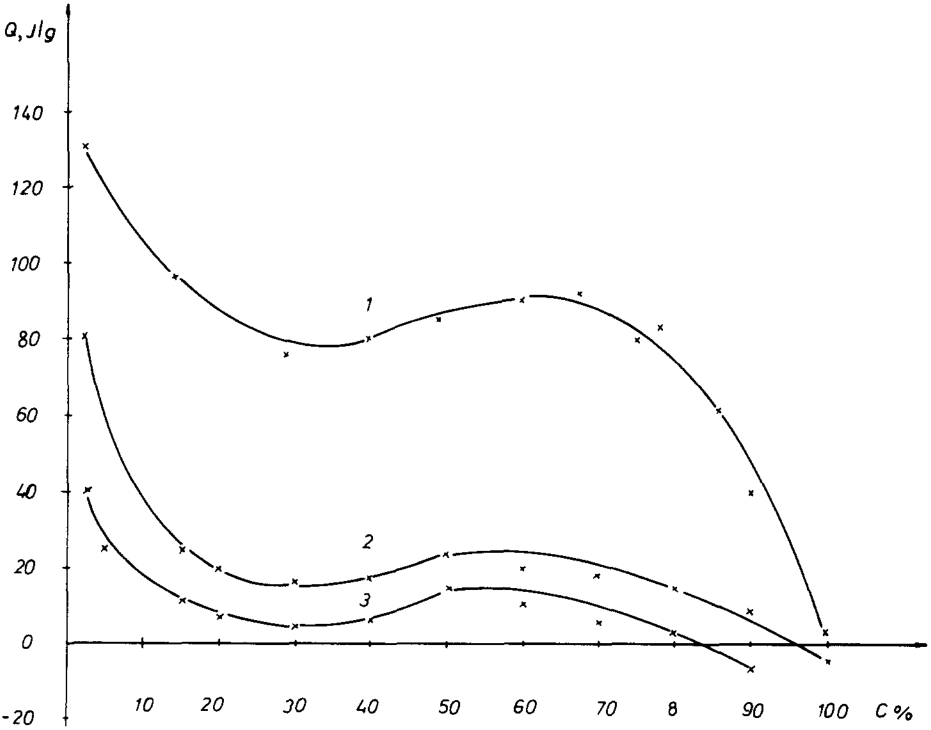

Fig. 4. Heat of hydration of apple pectin with aqueous-ethanol solutions. 1, Sample moisture of 2·5%; 2, sample moisture of 6·6%; 3, sample moisture of 15·3%.

on increasing the concentration of ethanol in water, and a local minimum in the heat released was observed with concentrations of 17–45%. Upon further increasing the ethanol concentration, a local maximum occurred within the range 45–75%. At higher concentrations the thermal effect quickly fell and, depending on the water/ethanol content of the sample studied, in some instances reached negative values. The observed endothermic effect was, presumably, due to the desorption of water from the wet pectin at the higher ethanol concentrations.

The S-form of the isotherm $Q = f(C\%)$ at $W = \text{const.}$ can be explained by the occurrence of characteristic hydrates in the aqueous–ethanol solutions of various concentrations.

It is of interest that isotherms of similar S-shape were obtained in our unpublished studies of other colloids carried out under the same experimental conditions. It shows that the type of isotherm was determined by the properties of the aqueous–ethanol solution and not by the colloid being wetted.

In order to determine the lyophilic characteristics of pectin in relation to ethanol, the heat of hydration of dry pectin with absolute ethanol was also measured.

In the case of a pectin with a degree of esterification of 46·8%, a value of $Q_i = 7{\cdot}5\ \mathrm{J\ g^{-1}}$ dry pectin was obtained. If we use a value for the surface tension of the ethanol $\sigma_{1,2} = 23{\cdot}9 \times 10^{-3}\ \mathrm{J\ m^{-2}}$ and a value for the temperature coefficient of surface tension $\mathrm{d}\sigma_{1,2}/\mathrm{d}T = -0{\cdot}08 \times 10^{-3}\ \mathrm{J\ m^{-2}\ K^{-1}}$, then the heat released in the complete wetting of 1 $\mathrm{m^2}$ solid surface with ethanol, $q = 46{\cdot}2 \times 10^{-3}\ \mathrm{J\ m^{-2}}$, may be determined in accordance with eqn (5).

In this instance, the wetting surface of 1 g dry pectin was estimated as follows: $S_0 = Q_i/q = 1{\cdot}62 \times 10^2\ \mathrm{m^2\ g^{-1}}$.

WETTING HEAT OF PECTINS WITH SATURATED ALIPHATIC ALCOHOLS

The results given in Fig. 2 show that the energy released and hence the bound water were independent of the degree of esterification of the pectin. The water molecules were obviously bound with the hydrophilic centres not located on the carboxyl groups.

TABLE 1
Heat of Hydration for Pectins of Various Degrees of Esterification (DE) with Saturated Aliphatic Alcohols

Alcohol	Q_i $(J\,g^{-1}$ *of apple pectin*)		
	DE = 37·4%	*DE = 46·8%*	*DE = 71·6%*
Methyl alcohol	51·60	58·73	78·66
Ethyl alcohol	7·69	7·50	7·31
n-Propyl alcohol	6·70	7·19	6·82
iso-Propyl alcohol	7·15	–	6·64
n-Butyl alcohol	7·46	–	8·32
iso-Butyl alcohol	5·82	5·50	5·46
sec-Butyl alcohol	5·62	–	5·41
1-Pentanol	6·61	–	7·20
iso-Amyl alcohol	8·53	–	8·57

A liquid which wetted only the methoxylated hydrophobic groups of the pectin was required. In this instance, the released wetting heat would be proportional to the number of methoxylated groups.

For that purpose, the heat of hydration of apple pectins with various degrees of esterification and saturated aliphatic alcohols was measured. The results obtained are given in Table 1.

It is seen that the heat of hydration of pectin with methanol exceeded by one order of magnitude that of the remaining alcohols. Furthermore, the wetting heat was dependent on the degree of esterification: the greater the number of methoxyl groups, the higher the released heat and hence the larger the wetted surface. In the case of the remaining alcohols from the same order, the released heat was independent of the degree of esterification.

CONCLUSIONS

The binding of water on natural colloids is of great importance for the food industry. Studies on the mechanism of this phenomenon have elucidated a number of essential properties relevant to the stability of disperse systems, swelling, drying, gelling power, syneresis, flotation, etc.

The studies showed that the heat of hydration of the pectin (171 J g^{-1}) was comparable to that of other natural colloids. According to Dumansky (1975), the heat of hydration for starch and agar-agar was 117 J g^{-1} and 176 J g^{-1}, respectively. At the same time, the pectin bound more water (0·57 g g^{-1}) as compared to other hydrophilic colloids. For example, agar-agar, gelatine and starch bound 0·55, 0·44 and 0·34 g g^{-1} water, respectively, as reported by Dumansky (1975).

The heat Q_d released during the sorption of 1 g water from an infinite amount of colloid could be determined using the dependence $Q_i/X_c = Q_d$. This heat represented the differential wetting heat. The following value was obtained for the pectin: $Q_d = 300 \pm 14$ J g^{-1}. Within the interval of error, this value coincided with the constant introduced by Dumansky for the differential heat of hydration of colloids $Q_d = 335 \pm 25$ J g^{-1}.

The calorimetric method, even if macroscopic, may give an idea of the distribution of water molecules on the hydrophilic centres. As there were 0·57 g of adsorbed bound water in 1 g pectin with a degree of esterification of 75%, 0·57/18 mol water would be distributed in 1/186 mol pectin. Therefore, in this instance, approximately six molecules of water were distributed in one D-galacturonic residue of pectin. A similar result was obtained when other experimental data were used. Based on the water hydrated surface of 1 g pectin ($1{\cdot}46 \times 10^3$ m^2 g^{-1}), the hydrated surface of 1 mol ($2{\cdot}73 \times 10^5$ m^2 M^{-1}) was determined and the hydrated surface of one elementary unit of pectin amounted to $4{\cdot}55 \times 10^{-19}$ m^2. When the effective area of 1 molecule of water was assumed to be $8{\cdot}4 \times 10^{-20}$ m^2, approximately 5·5 molecules of water were again interacting with one unit of pectin.

The experimental results showed that pectins of various degrees of esterification, thus having a varied number of carboxyl groups, had the same heat of hydration. This suggests that the carboxyl groups were engaged in intramolecular or intermolecular links and they were not hydrated. In this instance, each hydroxyl group in the elementary unit of pectin was quite likely to be hydrated with three molecules of water.

This conclusion agrees with the results of Passinsky who has applied an ultrasonic technique to show that one OH-group binds three molecules of water in a number of organic compounds.

The results obtained in hydrating the surface of pectins with aliphatic alcohols revealed that with the exception of methanol all the remaining alcohols yielded almost the same heat of adsorption. As this heat was independent of the degree of esterification, it may be concluded that these alcohols, on the analogy of water, were bound with the adsorption sites not located on the free or methoxylated carboxyl groups. It is most likely that they interacted with the hydroxyl centres of the pectin molecule as they alone did not change in number upon varying the degree of esterification.

The comparatively small heat of the process (5–8 J g^{-1}) points to significant steric obstacles in wetting due to the large cross dimensions of the molecule of alcohol. The molecules of water formed hydrogen bonds with the same centres but with their small sizes they entered the micropores and established contact with a great number of hydroxyl centres. This is why, it is believed, the heat of hydration by water is more than 20 times that with aliphatic alcohol.

In the case of wetting by methanol, the heat released increased on increasing the number of esterified groups. Our studies showed that there was a strict linear dependence between the released heat and the number of methoxylated groups. With such proportionality, hydrophobic adsorption of methanol was quite likely to occur on the methoxylated centres.

It is known (Walkinshaw & Struther-Arnott, 1981) that interactions were reported between the methyl groups of various pectin molecules. Similar forces are also quite likely to occur between methanol and methoxylated groups of the pectin. The comparatively small methanol molecules penetrated easily and the released heat was one order higher than that on wetting with the remaining alcohols. As water and ethanol were adsorbed on the hydrophilic centres of pectin while the methanol was intensively adsorbed by the hydrophobic methoxylated centres, in a water–ethanol–methanol mixture, no competition would occur between methanol and the other two components for common adsorption centres. This assumption and the more intensive binding of methanol as compared to ethanol led to our suggesting a method of adsorption of methanol from water–ethanol–methanol mixtures using pectin adsorbents (Ignatov *et al.*, 1978). The active adsorption of methanol was also observed by Lokota *et al.* (1978) by means of spectroscopic methods.

REFERENCES

Calvet, E. & Prat, H. (1956). *Microcalorimétrie*, Masson et Cie, Paris.

Dumansky, A. V. (1975). *Koloidnyi Zhurnal* **37** (2), 345-8.

Ignatov, G. I., Kratchanov, Chr., Markova, N. *et al.* (1978). *Patent No. 26603 C12 H1/02, Bulgaria.*

Kertesz, Z. J. (1951). *The Pectic Substances*, Interscience, New York.

Lokota, Ya. G., Klimovich, V. M. & Kurilenko, O. D. (1978). *Koloidnyi Zhurnal* **40**, 5.

Terishtenko, O. A. (1971). *Osnovy teplometrii*, Izd. Naukovaya duma, Kiev.

Volarovich, M. P., Gramayunova, N., Vassileva, L. & Vassileva, Yu. (1950). *Koloidnyi Zhurnal* **12** (5), 319.

Vulfson, N. S. (1964). *Preparativnaya org. chimiya*, Chimaya, Moskva-Leningrad, pp. 156-8.

Walkinshaw, M. D. & Struther-Arnott, G. (1981). *J. Mol. Biol.* **153**, 1075-85.

Wilson, W. H. & Epps, T. D. (1921). The construction of thermocouples by electrodeposition, *Phys. Soc. Proc.* **32**, 5.