MEASUREMENTS OF THERMOPHYSICAL PROPERTIES OF POLYACRYLAMIDE GEL USED FOR ELECTROPHORESIS

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Density, heat capacity and thermal conductivity were measured for polyacrylamide gel which is used for electrophoresis. Dependence of the density and heat capacity on gel concentration is explained by a model in which the gel is assumed to be a two-component solution of polyacrylamide and water. Dependence of the thermal conductivity on gel concentration is examined by assuming that the gel is composed of multiple thin layers of these components. The specific properties of density, heat capacity and thermal conductivity of polyacrylamide in the gel were obtained from these models and correlated with temperature. These quantities are comparable to those of ordinary plastics. Variations of the thermophysical properties with gel concentration and temperature are well estimated by correlation equations based on the models in this study.

Introduction

Gel electrophoresis is widely used for separating proteins, enzymes and other valuable substances from biochemical materials. When the substances to be separated are thermally weak, the gel must be cooled and kept at a low temperature to protect them from irreversible denaturation or deactivation. In separating proteins by use of isoelectric focusing, it is recommended that water below 5°C should be circulated to cool a gel medium for electrophoresis²⁾. To achieve fast separation successfully, the maximum voltage that can be applied to the cell without causing denaturation or deactivation of biochemical substances should be estimated in advance of electrophoresis on the basis of thermophysical properties of the gel.

A great many studies of electrophoresis have been carried out using gel. In addition, various phenomena such as phase transition are now being investigated for gel with keen interest in many fields⁵⁾. But little is known about the thermophysical properties, particularly the thermal conductivity, of gels. This paper deals with the density, specific heat capacity and thermal conductivity of polyacrylamide gel which is widely used in electrophoresis.

1. Experimental

1.1 Preparation of gel

Polymerization of acrylamide was conducted at 303K in an aqueous solution containing acrylamide, N, N'-methylene-bis (acrylamide) (BIS), ammonium per-

sulfate and N, N, N', N'-tetramethylethylenediamine (TEMED). Polyacrylamide gel (PAG) thus prepared for electrophoresis is conventionally characterized in terms of two quantities, %T and %C³⁾. The former denotes the total concentration of acrylamide

$\%T = 100 \times [\{\text{acrylamide (g)} + \text{BIS (g)}\} / \text{polymer solution (ml)}]$

and the latter denotes the degree of cross-linking by BIS

$\%C = 100 \times [\text{BIS (g)} / \{\text{acrylamide (g)} + \text{BIS (g)}\}]$

In this study PAG of 3%C was prepared by changing %T up to 40. Each polymerization solution contained 4 cm³ of 1.5% ammonium persulfate and 0.05 cm³ of TEMED in a volume of 100 cm³. PAG thus prepared did not exhibit fluidity.

1.2 Measurements

The volume of a PAG sample of known weight was measured from the displacement volume of water when it was immersed into water in a pycnometer and then its density was calculated. The specific heat capacity was measured by differential scanning calorimetry.

The thermal conductivity of PAG was measured, using the vessel shown in Fig. 1. The vessel was a stainless steel pipe of 20 mm inner diameter, 1 mm thickness and 240 mm length. A copper-constantan thermocouple of 1mm sheath diameter with a response time of 0.2 s was inserted into the vessel and fixed at the center and mid-height of the pipe by using a cross-shaped thin plastic plate. Polymerization of PAG for this measurement was carried out in the vessel where the thermocouple had been installed. In this manner PAG without fluidity was filled in the measuring vessel.

The procedure for measuring the thermal conductivity of PAG is as follows. The vessel containing gel is

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Fig. 1 Vessel for measuring thermal conductivity of gel
(1) Stainless steel pipe (2) Thermocouple
(3) Cross-shaped plastic plate for fixing thermocouple
(4) Rubber plug (5) Gel

Fig. 2 Comparison of the variations of temperature with Eq.(2)

kept at a temperature T_1 in a water bath and is then moved quickly into another water bath at a different temperature T_2 . If the heat capacity of the vessel and the resistance to heat transfer through water to the vessel are negligibly small and heat transfer in the gel takes place only in the radial direction, the time variation of temperature T_c at the center of the cylindrical gel is expressed by

$$\frac{T_2 - T_c}{T_2 - T_1} = \sum_{n=1}^{\infty} \frac{2 \exp(-a_n^2 \alpha \theta / R^2)}{a_n J_1(a_n)} \quad (1)$$

where a_n is the n -th root of $J_0(a_n) = 0$ ¹⁾. When θ is large enough to satisfy the condition of $\ln[(T_2 - T_c)/(T_2 - T_1)] < -0.5$, Eq. (1) is well approximated by the following equation.

$$\ln[(T_2 - T_c)/(T_2 - T_1)] = -(5.76 \alpha / R^2) \theta + 0.474 \quad (2)$$

A typical example of measured temperature variations is shown in Fig. 2. The thermal diffusivity α is obtained from the linear variation of $\ln[(T_2 - T_1)/(T_2 - T_c)]$ against

Fig. 3 Dependence of the density of PAG on total concentration of PA and temperature

θ for large θ which satisfies the above-mentioned condition, and the thermal conductivity λ is calculated from the relation $\lambda = \rho C_p \alpha$.

The validity of Eq. (2) was examined by solving the equation of heat conduction numerically. The calculation was carried out for the case where the stainless steel vessel ($\rho = 8 \times 10^3 \text{ kg/m}^3$, $C_p = 0.5 \text{ kJ/(kg}\cdot\text{K)}$, $\lambda = 16 \text{ W/(m}\cdot\text{K)}$ and $\alpha = 4 \times 10^{-6} \text{ m}^2/\text{s}$) containing gel with the same thermophysical properties as those of water ($\rho = 1 \times 10^3 \text{ kg/m}^3$, $C_p = 4.2 \text{ kJ/(kg}\cdot\text{K)}$, $\lambda = 0.60 \text{ W/(m}\cdot\text{K)}$ and $\alpha = 1.43 \times 10^{-7} \text{ m}^2/\text{s}$) was heated by elevating the outer-wall temperature stepwise. The temperature distributions were solved simultaneously in both regions of the vessel wall and gel. By plotting the calculated results of $\ln[(T_2 - T_c)/(T_2 - T_1)]$ against θ , the thermal diffusivity $\alpha = 1.44 \times 10^{-7} \text{ m}^2/\text{s}$ was obtained. This value is very close to the $\alpha = 1.43 \times 10^{-7} \text{ m}^2/\text{s}$ for the sample gel. In addition, it was confirmed that the error in obtaining α does not exceed one percent even if the tip of the thermocouple has been off-centered as much as 0.5 mm in error.

Measurements of thermal conductivity were carried out in the two modes of increasing temperature $T_1 < T_2$ and decreasing temperature $T_1 > T_2$, while keeping $|T_1 - T_2| = 10 \text{ K}$. The thermal conductivity at $T = (T_1 + T_2)/2$ was determined as the mean of those measured in both modes.

In this manner the density, specific heat capacity and thermal conductivity of PAG were obtained at 278.2, 288.2, 298.2, 308.2 and 318.2 K.

2. Results and Discussion

2.1 Density

The density of PAG increases with total concentration as shown in Fig. 3. The density of 40%T PAG is ten to fifteen percent larger than that of water. On the other hand, it decreases slightly with temperature. In other words, a small expansion of PAG takes place with

Fig. 4 Comparison of density variations with Eq. (7)

Table 1. Variations of ρ_A^* , C_{PA}^* and λ_A^* of PA in PAG with temperature

T [K]	$\rho_A^* \times 10^{-3}$ [kg³/m³]	C_{PA}^* [kJ/(kg.K)]	λ_A^* [W/(m.K)]
278.2	1.56	1.34	0.16
288.2	1.54	1.66	0.18
298.2	1.52	1.75	0.21
308.2	1.47	1.90	0.24
318.2	1.45	2.06	0.31

$$\rho_A^* = (1.487 + 0.3063 \times 10^{-2}T - 0.1000 \times 10^{-4}T^2) \times 10^3$$

$$C_{PA}^* = -3.25 + 1.67 \times 10^{-2}T$$

$$\lambda_A^* = 5.37 - 3.84 \times 10^{-2}T + 0.707 \times 10^{-4}T^2$$

increasing temperature.

If PAG is assumed to be a two-component solution of polyacrylamide (PA) and water, the gel volume, v , may be expressed as the sum of the volumes of PA and water,

$$v = v_A + v_W \quad (3)$$

and its mass, m , is expressed by

$$m = m_A + m_W \quad (4)$$

where subscripts "A" and "W" denote polyacrylamide and water respectively. The total concentration %T is converted into the density of PA in the gel, ρ_A [kg/m³], from the relation $\rho_A = 1000x$ (%T/100). The amounts of ammonium persulfate and TEMED were small enough to allow the assumption of a two-component solution. The volume fraction, ϕ , and the mass fraction, ω , of PA are related to the density of PA, $\rho_A^* = m_A/v_A$, and the density of water, $\rho_W^* = m_W/v_W$, in the gel by

$$\phi = v_A/v = \rho_A/\rho_A^* \quad (5)$$

$$\omega = m_A/(m_A + m_W)$$

$$= \rho_A/[\rho_A + \rho_W^*(1 - \rho_A/\rho_A^*)] \quad (6)$$

Using these relations, the gel density ρ is expressed in terms of ρ_A , ρ_A^* and ρ_W^* as

$$1/\rho = (1 - \rho_A/\rho)/\rho_W^* + (\rho_A/\rho)/\rho_A^*$$

$$= 1/\rho_W^* - (1/\rho_W^* - 1/\rho_A^*)(\rho_A/\rho) \quad (7)$$

The data measured at 298K are compared with Eq. (7) in Fig. 4. The density at $\rho_A/\rho = 0$ is that of water, $\rho_W = 0.997 \times 10^3$ kg/m³. The linear variations of the data are well expressed by Eq. (7), which intersects the ordinate

Fig. 5 Dependence of the specific heat capacity of PAG on total concentration of PA and temperature

Fig. 6 Comparison of specific heat capacity variations with Eq. (8)

at $1/\rho = 1/\rho_W$. This means that the density of water in the gel can be taken as equal to that of free water. By applying Eq. (7) with $\rho_W^* = \rho_W$ to the data, $\rho_A^* = 1.54 \times 10^3$ kg/m³ was obtained. The validity of Eq. (7) has also been confirmed for the data at other temperatures. The values of ρ_A^* obtained in this manner are listed in Table 1, in which its correlation with temperature is given.

2.2 Specific heat capacity

Figure 5 shows that the specific heat capacity of PAG decreases with increase in total concentration (%T), but its dependence on temperature increases with %T. The assumption of a two-component solution leads to the following relation for the specific heat capacity of PAG:

$$C_P = \omega C_{P_A}^* + (1 - \omega) C_{P_W}^* \quad (8)$$

The data plotted in Fig. 6 show that Eq. (8) is valid and that the specific heat capacity of water in PAG is taken equal to that of free water. The values of $C_{P_A}^*$ which were obtained by applying Eq. (8) with $C_{P_W}^* = C_{P_W}$ to the data are listed in Table 1. $C_{P_W}^*$ increases with temperature, and its correlation is also given in Table 1.

2.3 Thermal conductivity

Figure 7 shows that the thermal conductivity of

Fig. 7 Dependence of the thermal conductivity of PAG on total concentration of PA and temperature

Fig. 8 Schematic diagrams of gel structure: (a) network structure (b) multiple-layer structure parallel to heat flow direction (c) multiple-layer structure perpendicular to heat flow direction

PAG decreases with total concentration but increases with temperature. To estimate the effects of total concentration and temperature on the thermal conductivity, it is assumed that the structure of PAG is composed of multiple thin layers of PA and water as shown in Fig. 8, although PAG is likely to have a complicated three-dimensional network structure. Two simple alignments of the multiple layers are examined here; one is parallel to the direction of heat transfer (Fig. 8b) and the other is perpendicular to it (Fig. 8c). The thermal conductivity for the former alignment is expressed as

$$\lambda = \phi \lambda_A^* + (1 - \phi) \lambda_W^* \quad (9)$$

and the latter alignment as

$$1/\lambda = \phi/\lambda_A^* + (1 - \phi)/\lambda_W^* \quad (10)$$

where ϕ is the volume fraction of PA in the gel. Equation (9) was used as a basis on deriving an expression for the thermal conductivity of a packed bed by Kunii and Smith⁴). Application of Eq. (9) to the experimental data gave negative values of λ_A^* , hence the former alignment was discarded. On the other hand, Eq. (10) does not yield such a physically meaningless situation, and λ_W^* may be taken equal to λ_W , as shown in Fig. 9. This means that the heat transfer through PAG is mainly governed by the

Fig. 9 Comparison of thermal conductivity variations with Eq. (10)

Table 2. Comparison of thermophysical properties of PA with those of plastics

Material	$\rho \times 10^{-3} [\text{kg/m}^3]$	$C_p [\text{kJ/(kg·K)}]$	$\lambda [\text{W/(m·K)}]$
Polyacrylamide in PAG	1.52	1.75	0.21
Polyethylene	1.14	1.56	0.22
Polyvinylchloride	1.35~1.45	0.84~1.17	0.13~0.29
Copolymer of styrene and acrylonitrile	1.075~1.10	1.34~1.44	0.12
Nylon 6	1.15	1.72	0.25

thermal resistances of water and PA network frame perpendicular to the heat flow direction. The values of λ_A^* , obtained by applying Eq. (10) with $\lambda_W^* = \lambda_W$, are also listed in Table 1 together with its correlation with temperature.

2.4 Prediction of thermophysical properties

The procedure for predicting ρ , C_p and λ of PAG for a given total concentration and temperature is as follows. The properties of free water are substituted for those of water in PAG, i.e., $\rho_W^* = \rho_W$, $C_{p_W}^* = C_{p_W}$ and $\lambda_W^* = \lambda_W$. The correlation equations given in Table 1 are used for estimating ρ_A^* , $C_{p_A}^*$ and λ_A^* . The volume fraction ϕ and mass fraction ω are calculated by Eqs. (5) and (6), in which the value of ρ_A is obtained from the total concentration %T, as mentioned in section 2.1. The density ρ of PAG is estimated by

$$\rho = \rho_A + \rho_W (1 - \phi) \quad (11)$$

and C_p and λ are estimated by Eqs. (8) and (10).

The solid lines in Figs. 3, 5 and 7 represent the calculations by the above-mentioned procedure.

The calculations agree with the measurements with relative errors of 0.3 percent for density, 1.4 percent for specific heat capacity and 5.7 percent for thermal conductivity. The large errors for thermal conductivity may be due to experimental errors that accompanied the difficulties in carrying out homogeneous polymerization in the measuring vessel without mixing at the early stage of reaction and in giving an ideal step change to the wall temperature.

2.5 Comparison with thermophysical properties of plastics

In Table 2, the thermophysical properties ρ_A^* , C_{PA}^* and λ_A^* of PA in PAG at 298K are compared with those of typical plastics, i.e., polyethylene, polyvinyl chloride, nylon and acrylonitrile-styrene-copolymer⁶⁾. It is worth noticing that the thermophysical properties of PA in PAG are comparable to those of the plastics.

Summary

The variations in thermophysical properties of PAG with gel concentration were analyzed by assuming that the gel is a two-component solution of PA and water for the estimation of ρ and C_p and also by assuming that it is composed of multiple thin layers of these components for the estimation of λ . The variations of the experimental data satisfy these assumptions, from which ρ_A^* , C_{PA}^* and λ_A^* of PA in PAG were obtained and correlated with temperature. It was confirmed that Eqs.(8), (10) and (11) with ρ_A^* , C_{PA}^* and λ_A^* are applicable to the estimation of ρ , C_p and λ of PAG.

Nomenclature

C_p	= specific heat capacity	[kJ/(kg·K)]
m	= mass of gel	[kg]

R	= radius of vessel used for measuring thermal conductivity of gel	[m]
T	= temperature	[K]
v	= volume of gel	[m ³]
α	= thermal diffusivity	[m ² /s]
θ	= time	[s]
λ	= thermal conductivity	[W/(m·K)]
ρ	= density	[kg/m ³]
ϕ	= volume fraction of PA in PAG	[-]
ω	= mass fraction of PA in PAG	[-]

<Subscripts>

A	= polyacrylamide
w	= water

<Superscripts>

*	= in PAG
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Literature cited

- 1) Carslaw, H.S. and J.C. Jaeger: "Conduction of Heat in Solids", p. 199, Clarendon Press, Oxford (1959)
- 2) Horio, T. and J. Yamashita ed.: "Tanpakusitsu-Koso no Kiso Jikkenho", p. 260, Nanko-do (1984)
- 3) idem: ibid, p. 271
- 4) Kunii, D. and J.M. Smith: *AIChE J.*, **6**, 71-78 (1960)
- 5) Tanaka, T.: "5 Phase Transition of Polymer Gels", in "Kagaku Sosetsu No. 8", The Chemical Society of Japan ed. p. 38-45, Gakujutsu Shuppan Center, Tokyo (1990)
- 6) The Chemical Society of Japan: "Kagaku-Binran Kisohen I", 3rd. ed., p. 515, Maruzen, Tokyo (1984)