Effect of Carbon Dioxide on the Thermodynamic State of Water in Collagen

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ABSTRACT

Thermodynamic activities of polar sites of collagen in the presence of CO_2 were observed by inverse gas chromatographic techniques using water as a probe. The interactions between collagen and the water probe were evaluated by determining the specific retention volume (Vg°) and partition coefficient (Kp) at 25°C, 30°C, and 35°C. Thermodynamic parameters were determined from these data. CO_2 exhibited a significant effect on the water binding of collagen as shown by increased Vg° and Kp values as compared to N_2- and He-treated collagen. The thermodynamic parameters of partial molar Gibb's free energy ($\Delta\overline{G}_T^\circ$), partial molar enthalpy ($\Delta\overline{H}_T^\circ$) and partial molar entropy (ΔS_T°) indicated CO_2 significantly increased the average energy of water binding by collagen.

INTRODUCTION

THE EXACT ROLE of water in the structure and function of biopolymers in food systems still remains unclear. The importance of water as a food constituent is recognized, however, the physical chemistry of these systems is poorly understood (Gilbert et al., 1983).

Protein-water interaction is of great importance from a theoretical and practical point of view. At the lower vapor pressure region, specific binding sites are believed to be responsible for this interaction (Coelho et al., 1979). These binding sites are believed to be polar amino acid side chains (Wolfenden et al., 1981). Recently, studies have been undertaken to evaluate changes in thermodynamic parameters such as enthalpy of absorption which indeed is related to binding energy of polar amino acids.

Coelho et al. (1979) reported that the heat of sorption at lower vapor pressure regions was not linearally related to that at a higher vapor pressure region. This indicated that the heterogenic amino acid composition in natural proteins was responsible with the polar amino acids in minor concentrations being most active.

Understanding and determining the effect of CO_2 gas on dry protein-water interaction during the hydration process would provide useful information on bacterial spoilage as well as sensory properties. Therefore, the understanding of the behavior of protein-water interactions in a CO_2 atmosphere could become an indispensable tool for studies of the determination of shelf life and the use of modified atmospheric packaging for low to intermediate moisture products.

Recently, inverse gas chromatography (IGC) has been employed as an effective technique in moisture sorption studies of food systems such as proteins, starches, bakery products and freeze-dried coffee (Helen, 1983; Paik, 1984; Tanaka, 1984; Smith et al., 1982). Due to the high sensitivity of the detector system, more rapid and accurate information regarding specific binding sites at the lower vapor pressure regions is facilitated. The observation and evaluation of a parameter such as specific retention volume (Vg°) enable an in-depth investigation of the thermodynamics of specific binding sites (Coelho et al., 1979).

The objective of this study was to determine the effects of CO_2 and N_2 atmospheres on the thermodynamic state of water in collagen by studying their interactions during inverse gas chromatography. Specific objectives included the calculation of the thermodynamic parameters of CO_2-collagen and H_2O interactions which govern sorption mechanisms and to determine the protein-water interaction at lower moisture where the effect of specific binding sites would be dominant.

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THEORY

Inverse gas chromatography

Inverse gas chromatography (IGC) involves the assay of the changes of a stationary phase by determining the interaction with a mobile phase that is known (Guillet, 1973; Guillet and Galin, 1973; Nakajima and Gocho, 1981; Gilbert, 1984).

Retention values. In gas chromatography, the net retention volume (Vn) is presented by Eq. (1)

$$Vn = Vr - Vo \quad (1)$$

where, Vr = retention volume of probe and Vo = retention volume of an unabsorbed peak (air).

The net retention time can also be presented by Eq. (2)

$$Vn = tr \cdot w \cdot j \frac{Tc}{Ta} \cdot \left(\frac{Po - Pa}{Po}\right) \quad (2)$$

where, tr = empirical column pressure gradient correction factor, w = carrier gas flow rate, Tc = column temperature, Ta = ambient temperature, Po = atmospheric pressure at ambient temperature and Pa = vapor pressure at ambient temperature.

The j factor can be calculated from Eq. (3) (James and Martin, 1952)

$$j = \frac{3}{2}\frac{(Pi - Po)^2 - 1}{(Pi - Po)^3 - 1} \quad (3)$$

where, Pi = column insert pressure.

The specific retention volume (Vg°) which is the net retention volume per unit weight, corrected to O°C, is expressed by Eq. (4)

$$Vg^\circ = \frac{273}{Tc} \cdot Vn \cdot \frac{1}{w_s} \quad (4)$$

where, w_s = weight of polymeric material in a stationary phase.

The retention volume of the probe (Vr) can also be determined by Eq. (5)

$$Vr = Vo + Kp \cdot Vs \quad (5)$$

where, Vs = volume of polymeric material and Kp = partition coefficient, defined as the ratio between the probe concentration in the polymeric material and in the mobile phase. The net retention volume can, therefore, be expressed by combining Eq. (1) and (5).

$$Vn = Kp \cdot Vs \quad (6)$$

Kp can be expressed as

$$K_p = \frac{Vg^\circ \cdot \lambda s \cdot Tc}{273^\circ} \quad (7)$$

where, λ = density of polymeric material.

Thermodynamic parameters from Vg°

Theoretical work on liquid-gas interactions of polymer-solute thermodynamics has been extensively studied using retention values (Varsano, 1971). Khalil (1976) and Orr et al. (1981) expanded this work using a gas-solid mode. Thermodynamic parameters can be calculated for a gas-liquid system by employing Vg°C. The Vg° calculation is based on the assumption that Vg° is related to the partition coefficient (Kp) which is not affected by probe concentration. Vg°, however, generally shows a probe concentration dependence in a gas-solid system, therefore, Vg° must be defined at an infinite dilution to calculate thermodynamic parameters. When the probe concentration is at an infinite dilution, and the vapor pressure of the probe is extremely low, Kp is governed by Henry's Law because probe-probe interaction is negligible.

Orr et al. (1981) based the following discussion on thermodynamic parameters using Vg°. $\Delta\bar{G}_T°$, which constitutes the total standard partial molar Gibb's free energy as given using the following equation:

$$\Delta\bar{G}_T° = -RT \ln Kp \quad (8)$$

where R and T are gas constant and column temperature, respectively. Kp is obtained by Vg° by Eq. (5), as previously cited.

$\Delta H_T°$, which is the total standard partial molar enthalpy is calculated using the following equation:

$$\Delta\bar{H}_T° = R \cdot \frac{d(\ln Vg°)}{d(1/T)} \quad (9)$$

The total standard partial molar entropy, $\Delta S_T°$, is calculated from the Gibb's-Helmholtz equation:

$$\Delta\bar{S}_T° = \frac{\Delta H_T° - \Delta G_T°}{T} \quad (10)$$

MATERIALS & METHODS

Sample preparation

Fresh microcut calf-hide collagen (supplied by Devro, Inc., Somerville, NJ) was used in this study. A collagen dispersion in water was prepared by dropping the pH to 2.5 using dilute acetic acid. A known weight of this dispersion together with a known weight of inert support (Supelcoport 60/70 mesh) and water at 0°C was blended for 5 min. Care was taken to ensure that the temperature of the blend did not exceed 25°C. After freeze-drying for 24 hr, the mixture was further dried by heating in a forced circulation oven at 45°C for 24 hr (Coelho et al., 1979).

The dry stationary phase was passed through a 70 mesh sieve and packed into a 100 × 0.64 cm (o.d.) aluminum column using a vacuum pump and a vibrating device. The amount of stationary phase packed into the column was determined by weighing the column before and after the packing, and also by the difference in the weight of the container holding the stationary phase before and after packing.

To remove most of the moisture (without affecting the structural properties of the collagen) that might have been absorbed during the process of packing, the column was conditioned for 48 hr by passing dry helium (50 cc/min) through it. A separate column for CO_2, N_2, and He was employed to assure the direct affect of each gas on the collagen/H_2O relations.

Inverse gas chromatography (IGC) techniques

A Varian 1700 dual column gas chromatograph with thermal conductivity detector was used for the IGC analysis. Column temperature: 25°C, 50°C, and 35°C; detector temperature: 160°C; injection port temperatures: 150°C; carrier gases: CO_2, N_2, He; carrier gas flow rate: 60 cc/min - pulse, 40 cc/min - frontal; and filament current: 150 mA. High purity grade helium, CO_2 and N_2, manufactured by Union Carbide Company (Linden, NJ) was further purified by moisture and oxygen traps (R&D Separation, N. Highlands, CA) after the respective tanks.

The column temperature was monitored by a thermocouple attached on the surface of the midpoint of the column. The constant temperature was maintained by a circulating water bath around the column in the GC oven. The columns were separated from the heated injection ports and detector oven by spacer columns (5 cm long) filled with siliconized glass wool. Detector signals were recorded by a Hewlett-Packard 7127B stripchart recorder using a chart speed of 1.27 cm/min. The retention time of polar probes and peak area were monitored by an integrator (model C-EIB, Shimazu Company, Kyoto, Japan). Room temperature was monitored by a calibrated mercury thermometer. Carrier gas flow rate was measured at the detector outlet at room temperature by a soap bubble flow meter manufactured by Supelco, Inc. The column inlet pressure was measured by a mercury manometer. An outlet pressure of one atmosphere was used based on prior work.

Calculation of thermodynamic parameters

The specific retention volume Vg° was calculated from Equation 4 and thermodynamic parameters of sorption calculated from Vg° included partition coefficient (Kp), total standard partial molar Gibb's free energy ($\Delta\bar{G}_T°$), total standard partial molar enthalpy ($\Delta\bar{H}_T°$), and total standard partial molar entropy ($\Delta\bar{S}_T°$).

Statistical Analysis

An analysis of variance (ANOVA, confidence level of 95%) was used to determine the correlation between the thermodynamic parameters and the gaseous atmospheres.

RESULTS & DISCUSSION

Effect of gas on the sorption thermodynamics of Supelcoport

Partition coefficient (Kp°), specific retention volume (Vg°) and partial molar Gibb's free energy ($\Delta\bar{G}_T°$) were calculated at an infinite dilution to observe the interaction of the gas with sorption themodynamics of Supelcoport. Vg° values for CO_2, N_2 and He at 25°C, 30°C and 35°C were calculated by the relative retention time (Vn) by using Eq. (2) and (4).

The relationship between probe size, gas and Vg° is presented in Table 1. For all three gases, the relationship between probe size and specific retention volume Vg° indicated independence. The relationship was the same for three gases at a constant temperature. By obtaining Vg° at the infinite dilution, thermodynamic parameters of Kp° and $\Delta\bar{G}_T°$ were calculated. From the above data is was concluded that thermodynamics of the absorption of water on Supelcoport was gas independent.

Retention values

Retention time was calculated as in previous work which utilized the peak front for the observation of retention time for Vg° values (Varsano and Gilbert, 1973; Orr et al., 1981). The relative retention time (Vn) was graphically determined from the chromatograms generated by IGC using air as an unabsorbed peak.

Significant differences in the presence of CO_2, N_2, and He

Table 1—Specific retention volume (Vg°) of Supelcoport at 25°C, 30°C, and 35°C[a]

Amount Injected (μL)H_2O	Vg° 25°C	Vg° 30°C	Vg° 35°C
He			
1.0	2.56	2.98	2.97
2.0	2.57	2.96	2.98
3.0	2.57	2.98	2.98
4.0	2.56	2.98	2.96
5.0	2.55	2.97	2.98
CO_2			
1.0	2.97	2.57	2.55
2.0	2.96	2.57	2.55
3.0	2.97	2.55	2.57
4.0	2.97	2.56	2.57
5.0	2.97	2.57	2.57
6.0	2.96	2.57	2.57
N_2			
1.0	2.95	2.55	2.57
2.0	2.97	2.57	2.56
3.0	2.97	2.57	2.57
4.0	2.97	2.56	2.56
5.0	2.97	2.57	2.56
6.0	2.96	2.57	2.56

[a] Data are means of three determinations.

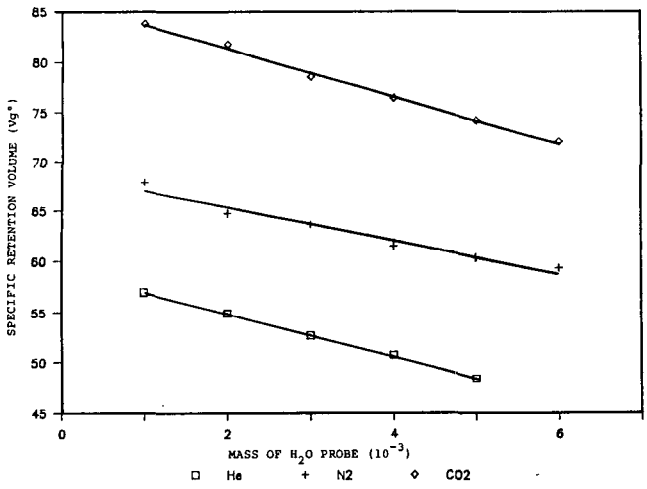

Fig. 1.—Effect of CO_2, N_2, and He on the specific retention volume (Vg°) in collagen at 25°C.

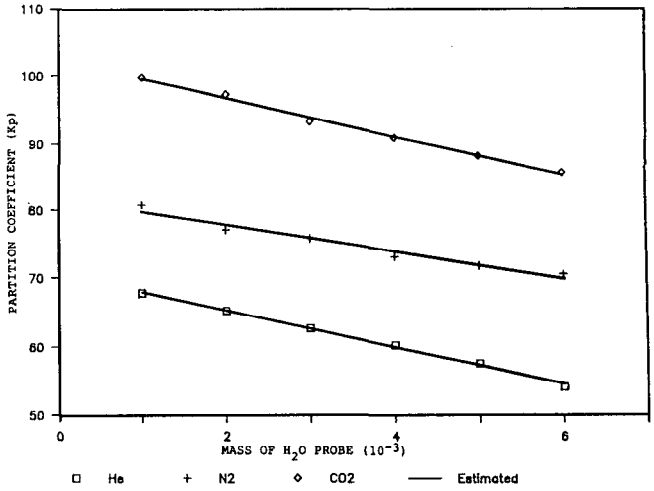

Fig. 2.—Effect of CO_2, N_2, and He on the partition coefficient (Kp) in collagen at 25°C from (Vg°) values.

Table 2—Effect of CO_2, N_2, and He on the specific retention volume (Vg°) of collagen at 25°C, 30°C, and 35°C[a]

Amount injected (μL) H_2O	Vg° 25°C	30°C	35°C
He			
1.0	57.04*	50.54	43.17
2.0	54.88	48.49	37.69
3.0	52.71*	47.36	34.51
4.0	50.67*	45.19	33.37
5.0	48.39*	43.14	32.35
CO_2			
1.0	83.84*	67.80*	50.91*
2.0	81.71*	64.67*	48.89*
3.0	78.46*	63.66*	47.77*
4.0	76.44*	61.53*	45.64*
5.0	74.20*	59.29*	43.51*
6.0	72.07*	58.28*	42.50*
N_2			
1.0	67.86*	52.82	40.89
2.0	64.67*	51.68	38.84
3.0	63.64*	49.63	36.67
4.0	61.37*	46.33	35.65
5.0	60.34*	44.17	33.37
6.0	59.32*	43.03	32.46

[a] Data are means of three determinations.
*Asterisk indicates that means for a comparison between gaseous atmospheres within a specific temperature are significantly different at $p < 0.05$ by ANOVA.

Table 3—Effect of CO_2, N_2, and He on the partition coefficient (Kp) of collagen at 25°C, 30°C, and 35°C[a]

Amount injected (μL) H_2O	(Kp) 25°	30°C	35°
He			
1.0	67.93*	61.20	53.14
2.0	65.36*	58.72	46.40
3.0	62.77*	57.35	42.48
4.0	60.34*	54.72	41.07
5.0	57.63*	52.24	39.82
CO_2			
1.0	99.85*	82.10*	62.66
2.0	97.31*	78.31*	60.18*
3.0	93.44*	77.09*	58.80*
4.0	91.04*	74.51*	56.18*
5.0	88.37*	71.79*	53.56*
6.0	85.83*	70.57*	52.31*
N_2			
1.0	80.82*	63.96	50.33
2.0	77.02*	62.58	47.81
3.0	75.79	60.10	45.14
4.0	73.09*	56.10	43.88
5.0	71.86*	53.49	41.07
6.0	70.64*	52.10	39.95

[a] Data are means of three determinations.
* Asterisk indicates that means for a comparison between gaseous atmospheres within a specific temperature are significantly at $p < 0.05$ by ANOVA.

Table 4—Effect of CO_2, N_2, and He on the total partial molar Gibb's free energy ($-\Delta\bar{G}_T$) of collagen at 25°C, 30°C, and 35°C[a]

Amount injected (μL) H_2O	$-\Delta\bar{G}°_T$ 25°	30°C	35°
He			
1.0	2.50*	2.48	2.44
2.0	2.48*	2.46	2.35
3.0	2.45*	2.44	2.30
4.0	2.43*	2.41	2.28
5.0	2.40*	2.38	2.26
CO_2			
1.0	2.73*	2.66*	2.54*
2.0	2.71*	2.63*	2.51*
3.0	2.69*	2.62*	2.50*
4.0	2.68*	2.60*	2.47*
5.0	2.66*	2.58*	2.44*
6.0	2.64*	2.57*	2.43*
N_2			
1.0	2.60*	2.51	2.40
2.0	2.58*	2.49	2.37
3.0	2.57*	2.47	2.33
4.0	2.55*	2.43	2.32
5.0	2.53*	2.40	2.28
6.0	2.52*	2.38	2.26

[a] Data are means of three determinations.
* Asterisk indicates that means for a comparison between gaseous atmospheres within a specific temperature are significantly different at $p < 0.05$ by ANOVA.

Table 5—Effect of CO_2, N_2, and He on the total partial molar entropy ($-\Delta\bar{S}°_T$) of collagen at 25°C, 30°C, and 35°C[a]

Amount injected (10^{-3})	$-\Delta\bar{S}°_T$ 25°C	30°C	35°C
He			
1.0	7.99	7.92*	7.92*
2.0	7.72*	7.67*	7.89*
3.0	5.81*	5.74*	5.94*
4.0	5.54*	5.51*	5.84*
5.0	5.30*	5.28	5.55*
CO_2			
1.0	10.87*	10.92*	11.13*
2.0	10.60*	10.69*	10.91*
3.0	9.32*	9.41*	9.64*
4.0	9.13*	9.41*	9.60*
5.0	9.09*	9.21*	9.51*
6.0	8.83*	8.91*	9.22*
N_2			
1.0	8.28	8.44*	8.67*
2.0	8.28*	8.44*	8.60*
3.0	8.05*	8.28*	8.59*
4.0	7.79*	8.05*	8.24*
5.0	7.25*	7.55*	7.82*
6.0	6.98*	7.33*	7.59*

[a] Data are means of three determinations.
* Asterisk indicates that means for a comparison between gaseous atmospheres within a specific temperature are significantly different at $p < 0.05$ by ANOVA.

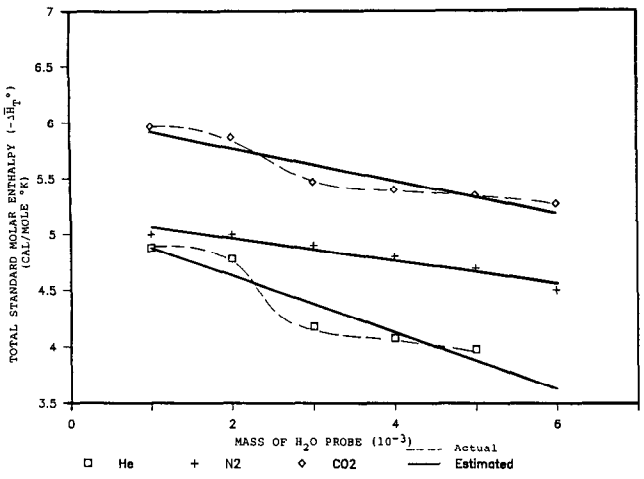

Fig. 3.—Effect of CO_2, N_2 and He on the total standard molar enthalpy (-$\Delta\bar{H}_T$°) in collagen from 25°C (Vg°) values.

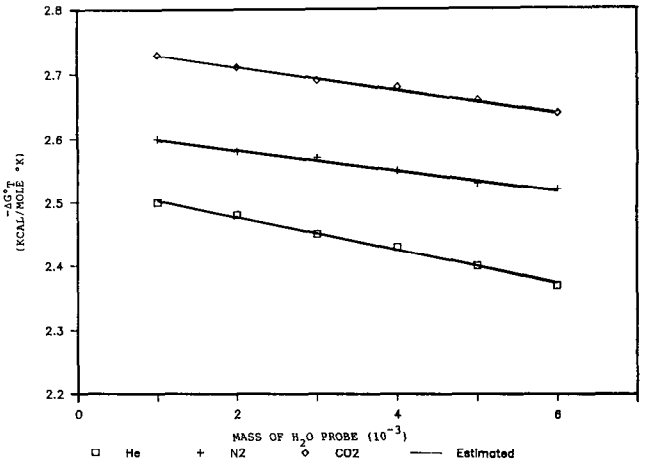

Fig. 4—Effect of CO_2, N_2, and He on the standard partial molar Gibb's free energy ($\Delta\bar{G}_T$°) in collagen at 25°C.

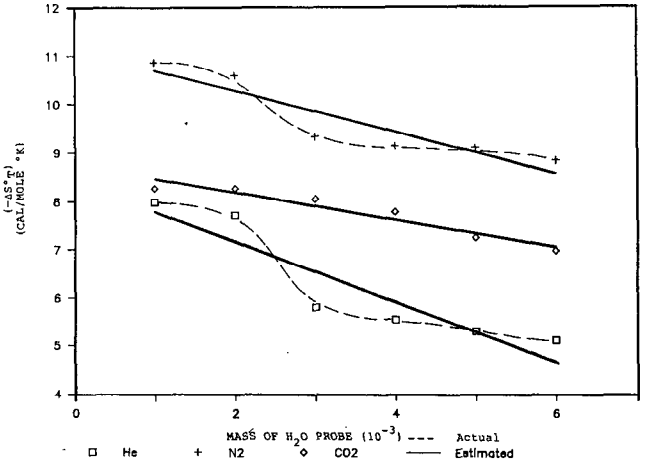

Fig. 5.—Effect of CO_2, N_2, and He on the standard partial molar entropy (Δ_T°) in collagen at 25°C.

were observed in the Vg° values for collagen at 25°C, 30°C and 35°C as shown in Table 2. The CO_2-treated collagen exhibited larger retention volume followed by N_2 and He the lowest (Table 2). This effect appeared to be greatest at 25°C (Fig. 1). The relationship showed that Vg° decreased with a temperature increase. The effect of probe concentration on Vg° among the treatments in shown in Table 2. Vg° volumes increased as the probe concentration decreased. The increased Vg° with CO_2, compared to N_2 and He, indicated an increase in the availability of the polar binding sites in CO_2-treated collagen.

The effect of CO_2, N_2 and He on the partition coefficient (Kp) of collagen at 25°C is presented in Fig. 2. Significant differences were observed between the CO_2-treated collagen and the N_2- and He-treated collagen at 25°C, 30°C, and 35°C (Table 3). Since Kp is defined as the concentration of probe in the stationary phase versus concentration of probe in the mobile phase, it is directly related to Vg°. The Kp of the CO_2-treated collagen was significantly larger over the entire range of sorption than the N_2- and He-treated collagen, indicating a higher binding energy, i.e., transport velocity was low, therefore, more mobile phase was required to achieve elution.

Thermodynamic parameters from Vg°

Calculations of the thermodynamic parameters for observing the effect of CO_2, N_2, and He on the probe protein interactions at 25°C, 30°C, and 35°C were achieved by defining Vg° at the infinite dilution for each atmosphere. The density of collagen, obtained from Devro, Inc., agreed with literature value of 0.091. The assumption was made that there would be no changes in the density due to the gas atmospheres for the calculations of ΔG_T°.

The $\Delta\bar{H}_T$° values were most negative in CO_2-treated collagen at 25°C, 30°C and 35°C followed by N_2 and He (Fig. 3). This indicated that the sorption process of the water probe was most favored in the CO_2 atmosphere directly followed by N_2 and He atmospheres. The $\Delta\bar{S}_T$° values followed a similar trend as $\Delta\bar{G}_T$° (Tables 4 and 5). These data indicated that the probe-CO_2 system was relatively more ordered than the probe-N_2 and probe-He system at 25°C, 30°C, and 35°C. The greatest effect of CO_2 on $\Delta\bar{G}_T$° and $\Delta\bar{S}_T$° was observed at 25°C (Fig. 4 and 5). Probe-He exhibited the least negative $\Delta\bar{G}_T$° and $\Delta\bar{S}_T$° values (Tables 4 and 5). These results indicated that CO_2 significantly increased energy of water binding by collagen at 25°C, 30°C and 35°C. The significant difference in $\Delta\bar{H}_T$° values between CO_2 and N_2 indicated a change of protein-water interaction with CO_2 having a stronger affinity of water and involving binding to the less polar specific sites.

CONCLUSIONS

CO_2 APPEARED to have a significant effect on the H_2O binding of collagen as shown by increased Vg° values as compared to N_2- and He-treated collagen.

The evaluation of the thermodynamic parameters of $\Delta\bar{G}_T$°, and $\Delta\bar{H}_T$°, and $\Delta\bar{S}_T$° from Vg° data at different temperatures indicated that CO_2 significantly increased average energy of water binding by collagen.

Significant differences in moisture sorption in the CO_2-, N_2-, and He-treated collagen were observed at both high and low vapor pressure regions. CO_2-treated collagen appeared to significantly increase water sorption by collagen at 25°C, 30°C, and 35°C. This observation suggested that with the CO_2-treated collagen, more or higher activity sites became available for sorption at the low and intermediate vapor pressure regions.

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