J. Pharm. Pharmacol. 1995, 47: 188–192
Received April 18, 1994
Accepted October 26, 1994

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Calorimetric Evaluation of Hydration of Cholestyramine

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Abstract

We have investigated the mechanism of hydration of cholestyramine, a water-insoluble resin used pharmaceutically.

Two types of water of hydration (freezing and non-freezing) and the amounts of heat evolved or absorbed during the hydration of cholestyramine were determined. From differential scanning calorimetry, 0·57 g water was observed to be tightly bound per gram of resin (non-freezing water). The hydration of dry cholestyramine was found to be exothermic. The heats of hydration of cholestyramine with chloride or nitrate counter-anions were found to be −6·05 and −3·46 cal g^{-1}, respectively. Some of the partially hydrated cholestyramine samples showed absorption of heat during hydration.

The data generated in the study were utilized to better understand the mechanism of hydration and swelling of cholestyramine.

Interaction of polymers and drug molecules with water is of special interest because of the consequences of hydration on the physicochemical properties (drug stability, transport of drug molecules through polymers) of these compounds (Frank 1972). Slade & Levine (1991) reviewed the effects of water on the development of modern food science technology: food quality, safety, stability and technological performance of food. Joshi & Wilson (1993) recently studied the mechanism of dissolution of hydroxypropylmethylcellulose (HPMC E5) in water. There are possibly three types of water of hydration. Type I water is free water which freezes and its frozen form melts at 0°C. Type II water is the water weakly bound to hydrophilic groups on the substrate, which freezes and its frozen form melts at about −15°C to −35°C. Type III water is tightly bound to hydrophilic and ionic groups on the substrate and does not freeze (Jhon & Andrade 1973). Joshi & Wilson (1993) found that the heat of solution of HPMC E5 was associated mainly with the addition of tightly bound water to the polymer. Endothermic and exothermic components were believed to be present with the dissolution of HPMC E5 and a new model for the dissolution of hydrophilic polymers in water was proposed.

Cholestyramine, an anion exchange resin, is widely used as an orally-active cholesterol-lowering agent. Binding of bile acids and other anions has been studied in detail (Lindenbaum & Higuchi 1975; Kos et al 1991; Benson et al 1993). Cholestyramine consists of a styrene-divinyl benzene copolymer containing quaternary ammonium groups. Cholestyramine is water-insoluble, but absorbs large quantities of water and swells considerably. Because of its wide use, cholestyramine was selected as a model to study the thermodynamics of the hydration process of polymeric resins. Hydration of water-insoluble resins such as cholestyramine was expected to involve the following processes: absorption of water at the most easily accessible reactive sites; breaking of polymer-polymer bonds; creation of polymer-water bonds; and dispersion/swelling of the hydrated polymer chains.

In this study, different types of water in hydrated cholestyramine were determined by differential scanning calorimetry (DSC). Heats of hydration of the hydrated cholestyramine were determined by means of an isoperibol calorimeter. Volume changes in cholestyramine resulting from the absorption of water were also monitored.

Materials and Methods

Drying of cholestyramine

Cholestyramine obtained had chloride as a counter-anion. Cholestyramine with nitrate as the counter-anion was prepared in the laboratory. The resin samples were dried at 70°C under 125 mmHg for three days. Cholestyramine resin is very hygroscopic and, therefore, during weighing of the samples, special precautions were taken to minimize the absorption of moisture (e.g. weighing as quickly as possible and not working on a humid day).

Preparation of cholestyramine nitrate from cholestyramine chloride

Cholestyramine (chloride counter-anion) was hydrated with distilled water and filtered through Whatman filter paper No. 1. Sodium nitrate solution in water (18%, w/v) was passed over the hydrated cholestyramine bed in the funnel to prepare the nitrate salt.

Characterization of types of water in hydrated cholestyramine

The different types of water of hydrated cholestyramine resins were characterized by DSC (Perkin Elmer, DSC-4, Norwalk, CT). The DSC was calibrated using an indium standard. Standard aluminium pans (Perkin Elmer) for volatile liquids were used in the experiments. Samples were weighed with an automatic electrobalance (model # 23,

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Cahn Industries, Inc., Cerritos, CA). Partially hydrated cholestyramine samples were prepared by weighing the dry resin into the aluminium sample pans to which various amounts of water were added. The partially hydrated cholestyramine samples, thus prepared, were sealed hermetically and stored at room temperature (21°C) overnight to attain an equilibrium between water and the resin. The scans were obtained from −50 to 20°C at a scanning rate of 2·5°C min^{-1}. The enthalpies of fusion were calculated by thermal analysis software from MC^2 Thermal Systems (Troy, NY) for an IBM/PC interfaced with the DSC using the peak area of the melting endotherm. Whenever the pre-melting point and post-melting point baselines were different, a nonlinear baseline was constructed during integration of the endotherm to calculate the heats of fusion. Such correction was unnecessary for samples of pure water and nearly fully hydrated polymer samples. The onset temperature (melting point) of frozen water was determined by extrapolating the leading edge of the melting endotherm of water to the baseline. When the onset temperature was less than 0°C, the enthalpy of fusion of the ice was corrected using the following equation (Zhang et al 1989):

$$\Delta H_f(T) = \Delta H_f(273) - \int C_p DT \qquad (1)$$

where Cp is the heat capacity of ice at T°K and ΔH_f is the heat of fusion value at temperatures T°K or 273°K. Estimates of the amounts of different types of water in each sample were calculated from the heat of fusion values. A detailed procedure has been reported earlier (Joshi & Topp 1992).

Heats of hydration: solution calorimetry

The heats of hydration of cholestyramine were determined using a Tronac isoperibol (titration) calorimeter (model 450, Orem, UT). The instrument was checked for calibration with potassium chloride samples. Samples weighed into the sample holders were submerged in 50 mL distilled water in the Dewar flask. The temperature of water in the Dewar flask was monitored with the thermistor after the resin was exposed to water. The analogue voltage was converted to a digital signal (A-D convertor, Laboratory Micro Systems, Inc., 860 Series Interface, Model 860, Troy, NY), imported into LOTUS 123 running on an IBM PC, and processed. Cholestyramine samples with different percentages of water were prepared by weighing dry resin into the sample holders followed by addition of selected amounts of water to the resin. The samples were capped, sealed and stored at room temperature for a minimum of 1 h so that the heat produced by the addition of water was dissipated in the atmosphere.

Volume changes after hydration

To known amounts of dry cholestyramine (1–6 g) in a beaker, water was added. The powders were stirred vigorously long enough (approx. 3–5 min) to provide uniform distribution of water. The beakers were weighed to determine the amounts of water added and the R values (weight ratios of water/resin) were calculated. The partially hydrated cholestyramine samples were transferred to graduated cylinders. The graduated cylinders were tapped several times and the volumes of powders were read. The volumes of partially hydrated cholestyramine were calculated with respect to dry weight of cholestyramine.

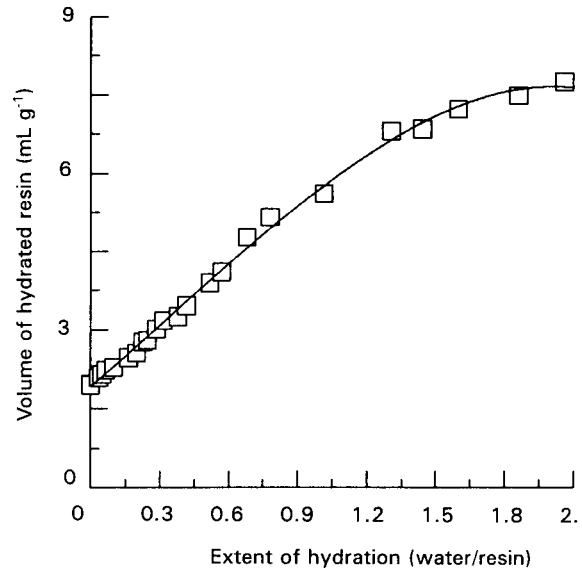

FIG. 1. Volume of cholestyramine in resin (mL g^{-1}) after partial hydration. The extent of hydration is described as R, i.e. weight ratios of the amount of water added to the dry resin. The line drawn through the points represents the fitted equation $y = 1{\cdot}920 + 3{\cdot}748 \times 0{\cdot}522^2 - 0{\cdot}481^3$, $r^2 = 0{\cdot}997$ between 0 and 2·1.

Results and Discussion

Volume changes in cholestyramine hydration

Cholestyramine is known to swell after absorption of a large quantity of water. Attempts were made to study the absorption of water and swelling of cholestyramine under a microscope. The resin absorbed water almost instantaneously and it was very difficult to produce partially hydrated resin and determine volume changes microscopically. The method adopted here was crude, but reliable enough to draw gross inferences for the purpose of this study. Fig. 1 shows the changes in the volume of cholestyramine (chloride counter-anion) caused by the absorption of

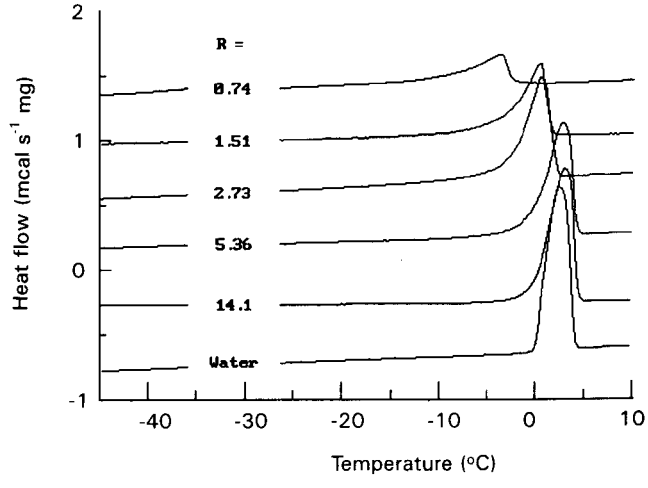

FIG. 2. DSC thermograms of partially hydrated cholestyramine (chloride counter-anion). R = water added to the sample of cholestyramine (g g^{-1}).

water. The volume changes in the resin were apparently linear with increase in R values of up to 1·2. The slope of the initial linear portion was found to be 3·8 mL g^{-1}. The distribution of water molecules on the hydrophilic sites of the resin may explain the increase in the volume.

Different types of water in hydrated cholestyramine

The apparent average melting point of distilled water used in the experiments was 0·17 ± 0·2°C (n = 7). The average value of the heats of fusion for water was 79·33 ± 0·28 cal g^{-1}. Fig. 2 shows representative DSC scans for cholestyramine samples (chloride counter-anion) with various values of R. A melting endotherm of pure water has been included in the figure for comparison. For high R values (e.g. R = 14·1), only one melting endotherm was obtained with a melting point close to that of pure water. This endotherm can be ascribed to Type I water (free, freezing water). With a decrease in R value (e.g. R = 0·74), a fronting (asymmetry of the endotherms at lower temperatures) of the melting endotherms appeared.

The endothermic heat changes (cal g^{-1}) due to the melting of water were determined by the division of the amount of heat involved in the melting transition of water with the weight of water in the sample. Fig. 3 shows the experimental heat-flow values of various partially hydrated cholestyramine (chloride salt) samples. For R = 0·74, the heat-change value was as low as 25·8 cal g^{-1}. As the R value increased, the endothermic heat-change value approached the heat of fusion of pure water.

Fig. 4 summarizes the onset melting points of water in the partially hydrated cholestyramine samples. For the sample with R = 0·74, the onset melting temperature was found to be −9·4°C. The onset melting points of water in the samples increased with an increase in R values and eventually, the melting points approached that of pure water.

Data in Figs 3 and 4 suggested the presence of a fraction of unfrozen water in the experiment. Joshi (1991) maintained sodium hyaluronate samples (R = 0·85 and 8·63) at −50°C for periods of time ranging from 0 to 29 h. The onset temperature and the measured heat flow remained unchanged. Joshi (1991) proposed the presence of different types of water to be a thermodynamic phenomenon, and not a kinetic event. For samples with lower R values, there were larger fractions of water bound to the hydrophilic sites as judged from the melting points of water significantly lower than 0°C. The phase diagram for water under high pressure suggests seven polymorphic forms, some melting below 0°C (Castellan 1983). There is some degree of probability of the presence of polymorphs of water in the hydrated resin samples; however, the samples were never kept under pressure and the experimental conditions did not distinguish between polymorphic forms of water. The lowering of the melting point of water must be due to binding of water to the hydrophilic sites on the resin. From the data, a mean value for non-freezing, tightly-bound water (Type III) was found to be 0·57 ± 0·11 g g^{-1} over the range of R from 0·7 to 14 (Table 1). Overall, the data indicated the existence of freezing and non-freezing water in the hydrated cholestyramine. The exact identity of the Type II water, if present, could not be characterized further by DSC.

Calorimetry

Isoperibol calorimetry provided a direct method to monitor the changes in heat flow due to the addition of water to the dry or partially hydrated resin samples. The mean heats of hydration values of dry cholestyramine with chloride and nitrate counter-anions were found to be −6·05 and −3·46 cal g^{-1}, respectively. Thus, the hydration of dry cholestyramine (chloride or nitrate counter-anions) can be

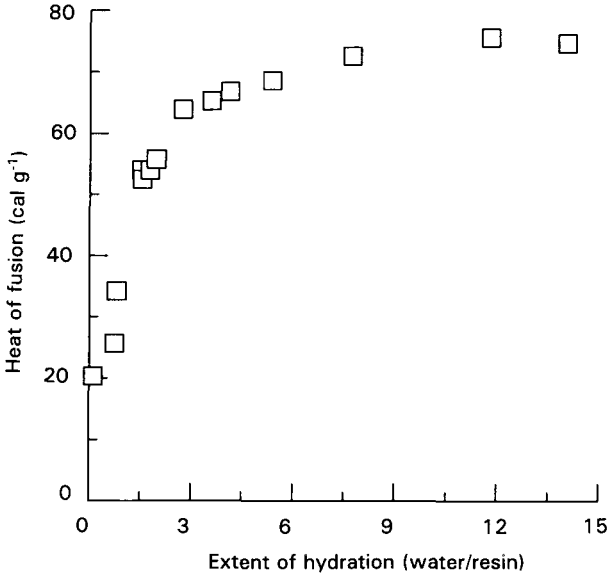

FIG. 3. Heat of fusion values (cal g^{-1}) of the partially hydrated cholestyramine (chloride counter-anion) samples. The values were determined from the peak areas of the melting endotherms of water in the samples using DSC. The heat of fusion of distilled water was found to be 79·33 ± 0·28 cal g^{-1} (n = 7).

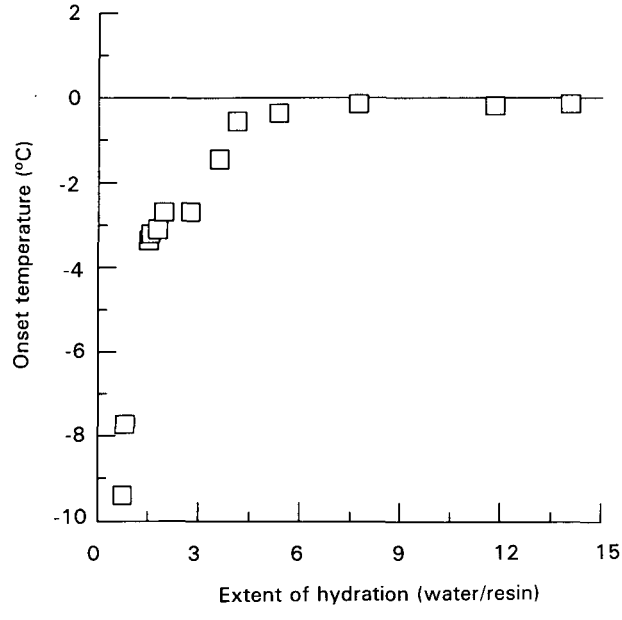

FIG. 4. Onset melting temperatures (°C) of water in the partially hydrated cholestyramine samples. The temperature range in the DSC experiments was −50–20°C with a scanning rate of 2·5°C min^{-1}. The onset temperature of distilled water was found to be 0·17 ± 0·20°C (n = 7).

Table 1. Representative DSC data used in the calculations of determination of the amount of water bound to cholestyramine. The table also lists the weights of water and polymer in the samples, ratios of water to polymer (R), and onset melting temperatures of water in the samples.

Water (mg)	Resin (mg)	R	Onset temperature (°C)	Water frozen (%)	Water non-frozen (%)	Water bound to resin ($g\,g^{-1}$)
2·966	4·029	0·74	−9·4	34·6	65·4	0·48
3·17	2·097	1·51	−3·36	69·5	30·5	0·46
5·031	1·84	2·73	−2·70	82·0	18·0	0·49
4·688	1·309	3·58	−1·44	83·0	17·0	0·61
11·622	0·983	11·8	−0·18	95·3	4·7	0·56

termed an exothermic process. Fig. 5 shows the heats of hydration of these resin samples as a function of R values. Although, the hydration of dry cholestyramine is an exothermic process, hydration of some of the partially hydrated resin samples was found to be endothermic (Fig. 5, R = 0·1–2).

By knowing the total heat evolved or absorbed during the hydration of dry cholestyramine, and the heat evolved or absorbed from samples with different R values, it should be possible to calculate the amount of heat involved with the addition of different amounts of water to the resin sample. For cholestyramine with chloride counter-anion, the heats of hydration were −6·05 and −2·05 $cal\,g^{-1}$ for R values of 0 and 0·054, respectively. This means that 4 $cal\,g^{-1}$ less heat was evolved from the sample which had previously adsorbed 0·054 $g\,g^{-1}$ water, showing that a large heat change was due to the process of adding a small amount of water to the very dry resin. This amount of heat was the net result of the exothermic binding of water and endothermic swelling of the resin. For R equal to 0·092, the net heat of hydration was 0·71 $cal\,g^{-1}$ for the resin (chloride counter-anion). Thus, for samples with R values of 0·054 and 0·092, the hydration process was exothermic and endothermic, respectively. The net heats of hydration values were maximum at R equal to

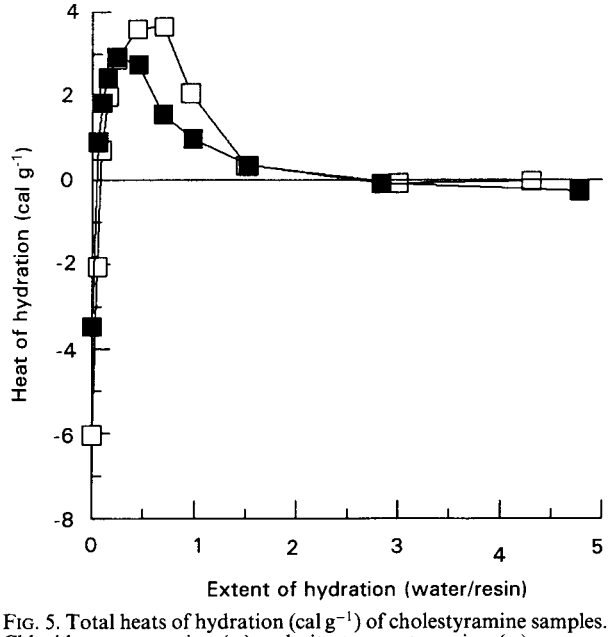

FIG. 5. Total heats of hydration ($cal\,g^{-1}$) of cholestyramine samples. Chloride counter-anion (□) and nitrate counter-anion (■).

0·7 and then decreased with the addition of more water to the sample. The heat of hydration of cholestyramine with large R values was expected to be zero and the present results (for R > 3–4) were consistent with this expectation within experimental variation. Cholestyramine with nitrate as the counter-anion showed a similar heat of hydration vs R profile (Fig. 5).

Joshi & Wilson (1993) proposed that the net heat involved during the hydration of HPMC was the sum of the heats involved in different sub-processes (addition of water to different types of sites). In the present study, the ionic nature of the resin with chloride or nitrate counter-anions, would require an additional term for the heat involvement due to interaction of water with ions. For electrolytes, if the heat of hydration is sufficient to overcome crystal lattice energy, the salt dissolves (Martin 1993). The heats of solution of sodium chloride and sodium nitrate have been reported to be 16·0 and 57·6 $cal\,g^{-1}$, respectively. The heats of solution of potassium chloride and potassium nitrate have been reported to be 55·2 and 82·6 $cal\,g^{-1}$, respectively (CRC Handbook 1992–93). Similar to these pairs of salts, a change in the counter-anion on the resin was expected to alter the heat of hydration of the resin and cholestyramine, with the nitrate counter-anion showing a more positive heat of hydration. The hydration of dry cholestyramine with the nitrate counter-anion evolved less heat compared with dry cholestyramine with the chloride counter-anion, which is equivalent to a more positive ΔH value.

The net heat of dissolution of HPMC E5 was previously observed to be $-24{\cdot}1 \pm 1{\cdot}1\,cal\,g^{-1}$ (Joshi & Wilson 1993) and this large exothermic heat of solution may have helped HPMC E5 to dissolve in water. In the current study, the low heat of hydration (−6·05 $cal\,g^{-1}$ for cholestyramine, chloride counter-anion) may not be sufficient for the resin to make room for itself in the liquid water lattice. The large molecular weight of cholestyramine and its preponderant hydrophobicity may also explain its insolubility in water. However, the exothermic hydration of counter-anions and the hydrophilic groups on the resin generated sufficient energy to provide for the endothermic swelling process.

Changes in free energy can be written as:

$$\Delta G = \Delta H - T\Delta S \qquad (2)$$

where G is Gibbs free energy, H is enthalpy and S is entropy. For a spontaneous reaction to occur, ΔG should be negative. The addition of water to dry resin causing swelling of the resin suggests a negative free energy value. The heat of hydration was negative for the dry resin, but became

positive for the partially hydrated samples (Fig. 5). In the case of dry resin, if the entropic term is positive, it will support the spontaneous hydration of the resin. However, if the entropic term is negative, the product $T\Delta S$ has to be smaller than $\Delta H_{hydration}$ to allow a spontaneous hydration. Swelling of resin molecules may suggest a positive entropy. However, it can also be argued that tightly bound or loosely bound water is more ordered compared with liquid water, suggesting a negative entropy. Instead of looking at entropic changes of water and resin individually, it is important to determine the entropic change in the entire system (water, resin and water-resin). In the case of partially hydrated resin, the heat of hydration was observed to be positive. Thus, only a positive entropic term would ensure a spontaneous hydration.

If it is assumed that the initial quantity of water added is tightly bound to the resin and hydrates the counter-anions, Figs 1 and 5 indicate a linear increase in volume by the addition of tightly-bound water. After the addition of tightly-bound water, the water is added only to the sites for the loosely-bound water and free water. The interaction strength decreases in the progression: ion-ion > water-ion > water-polar = water-water > water-hydrophobic (Rowland & Kuntz 1980). The amount of heat evolved is proportional to the interaction of water with the hydrophilic groups and the ions. This means that the addition of loosely bound or free water would have smaller exothermic components compared with the tightly-bound water. In Fig. 1, the volume change due to the addition of water slowed for $R > 2{\cdot}1$. The heat of hydration data (Fig. 5) support these results. The heat of hydration approached a value of zero at $R = 2{\cdot}1$, indicating fulfilment of all the binding sites with water.

In the previous study with a similar experimental design, dissolution of dry HPMC E5 in water was observed to be exothermic (Joshi & Wilson 1993). The heats of dissolution of the partially hydrated HPMC E5 samples were less negative compared with the heat of solution of dry HPMC E5. However, the net heat of solution of dry or partially hydrated HPMC E5 was never a positive number. Contrary to the results with HPMC E5, the data on cholestyramine demonstrated the presence of positive heats of hydration for partially-hydrated samples. Overall, the data clearly indicated the involvement of various exothermic and endothermic processes during the hydration of cholestyramine. However, the current DSC and solution calorimetry data were insufficient to quantify these different types of heat processes.

Acknowledgement

Steve Head provided expert technical assistance for these studies.

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