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Interactions in iodinated contrast media solutions

Part 1. A thermodynamic study

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Abstract Contrast media are synthetic molecules often characterized by the presence of heavy atoms, such as iodine, widely used in diagnostic studies. In the framework of a study on the physico-chemical of non-ionic contrast media (NICM), this study reports the calorimetric data for a characterization of the thermodynamic behavior of the aqueous solutions of three NICMs, namely, iopamidol, iomeprol, and iopromide, characterized by the presence of three iodine atoms in the benzene ring. Hydrophilicity is provided by three side arms with polar groups. Here, the results of a calorimetric investigation on the heat of dilution of iopamidol, iomeprol, and iopromide and on iomeprol–iopamidol mixture are illustrated. Despite the very similar chemical structures, the dilution process of iopamidol and iopromide was found exothermic, while an endothermic dilution is shown by iomeprol. The results are discussed in terms of the few other literature data and on the basis of structural and conformational properties.

Keywords Iodinated contrast media · Iopamidol · Iomeprol · Heat of dilution · Solution thermodynamics

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Introduction

Non-ionic contrast media (NICM) are nowadays considered mature investigative drugs of great relevance in delicate radiological analysis like angiography, urography, and myelography [1]. The generic structure of NICM is characterized by a 2, 4, 6 tri-iodo benzene substituted in 1, 3, 5 with non-ionic highly hydrophilic arms containing both amido linkages and hydroxyl substituents [1]. The success of these molecules mainly resides in some peculiar physico-chemical properties that associate relatively low-osmolality with relatively low-viscosity up to high concentration in water [2–4], as well as in the stability of the aromatic iodine substitution and the high concentration of iodine per molecule (up about 50% in weight). The side chains are significantly important not only for increasing the aqueous solubility, but also because they decrease the toxicity and raise the elimination of substances from the patient body [3]. Several investigations have been carried out in the recent times to introduce variations in the structure of the side arms and to improve the solubility without decreasing the iodine weight content [1, 5].

Iopamidol, iomeprol, and iopromide are injectable iodinated contrast agents, and they belong to the class of non-ionic contrast media [6]. The molecular structure of these compounds is given in Fig. 1.

Despite the large use in the clinical practice and the great interest from the molecular viewpoint of these molecules, literature studies on the physico-chemical properties are scarce and almost limited to some properties related to the debate about the association equilibria [7–10] and to phenomenon of atropisomerism characteristic of the crowded substitution on the aromatic ring [1, 11]. As a consequence, the understanding of the correlations between

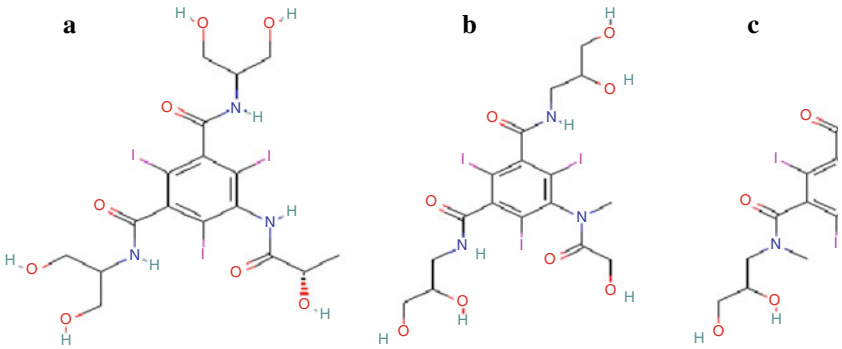

Fig. 1 Chemical structures of iopamidol (**a**), iomeprol (**b**), and iopromide (**c**). Note the structural differences in the three hydrophylic side arms

molecular structure and peculiar physico-chemical properties does not appear satisfactory.

A study aimed at characterizing the properties of some NICMs in solution and in the solid state by means of thermodynamic and spectroscopic methods has been carried out. The solution thermodynamics investigation concerns the correlation of the osmolality (free-energy) with calorimetric (enthalpy) and density (volume) properties of the solutions as a function of concentration. Since many molecular descriptors of the solution properties of these molecules are conformation-dependent and, therefore, complicated by the existence of metastable conformers population, a detailed ^{13}C-NMR study on iopamidol, iomeprol, and iopromide and on common simplified primers has also been carried out in parallel in order to assigning the most relevant conformers (L. Fontanive, unpublished results). This preliminary valuable information is useful for the discussion of the macroscopic effects of the concentrated solution of NICMs.

Although calorimetry has been used even recently for studying subtle interactions occurring in aqueous solutions of drugs [12] and other pharmaceutical excipients, including the elucidation of polymorphism [13], to the best of our knowledge, contrast media have not been investigated yet. Here, a report is presented about the heats of dilution of the solutions of the three NICMs, iopamidol, iomeprol, and iopromide, in the concentration range up to approximately 1.6 m at 25 and 37 °C. These data help to understand some subtle differences in the solution behavior of the three different compounds, which is not fully evident at first glace from other experimental results.

Experimental

Materials

Iopamidol, iomeprol, and iopromide solutions are commercially available as aqueous solutions at molal concentration (m) of 1.48, 1.67, and 1.52 m, for iopamidol, iomeprol, and iopromide, respectively, and were used for the calorimetric measurements without any further purification. In addition to commercial solutions, an aqueous solution of iopamidol was also prepared from solid powder at the same concentration of vials. The solution was prepared by dissolving the iopamidol powder by stirring the weighed solid in a weighed amount of water at 65 °C for 30 min and then heating up to 85 °C for 10 min. These measurements were used as a control for assessing any possible influence on the thermal properties due to the trace presence of excipients in the commercial solutions.

Methods

Heats of dilution measurements on NICM solutions were carried with an LKB 10700 batch-type microcalorimeter equipped with gold cells and were performed at two temperatures, 25 and 37 °C. The electric circuit of the control unit of the calorimeter was modified to allow it to operate up to 45 °C. Electrical calibration of the heat effects was performed and averaged over many independent data points, to give a calibration constant $K = 1.77 \times 10^{-5}$ J/W. The temperature, once set, was checked directly inside the thermostatted air bath. The output signal of the thermopiles, amplified by a Keithley 150B microammeter, was connected to a PC through a Picolog^{r} A/D data acquisition interface. The capability of the digital acquisition was limited to 1 datapoint/s as an average over 10 points. Mathematical analysis of the calorimeter data (baseline correction, integral) was carried out using the Microcal ORIGIN® graphic package.

In each measurement a weighed amount of solution (2 mL) at initial molal concentration m_i was mixed in the sample cell with a weighed amount of water (2 or 1 mL) to give a solution of known final concentration m_f Dilution series were carried out starting from the commercially available aqueous solutions and were continued, at each step diluting the previously obtained sample, until the limit of experimental accuracy was reached, in order to obtain the most accurate evaluation at infinite dilution limit. Given the viscosity of the samples, in particular of the most concentrated ones, a single mixing step of rotating drum

containing the cells was not sufficient to obtain a complete dilution of the sample, as revealed by a significant heat effect still observed by performing a second rotation. Therefore, subsequent rotational movements (up to 15, for the most concentrated samples) of the calorimetric unit (at time intervals of about 2 min) were performed until no residual heat effect was observed. Figure 2 shows the calorimetric curves of an endothermic heat dilution series, overlapped for comparison purpose. In the figure, bumps corresponding to rotations of calorimetric cell are well visible especially for the curves of systems at high concentration, with changes in the shape and the area of the curves upon dilution.

Results and discussion

Isothermal calorimetric dilution measurements on NICM solutions were collected as described in the Experimental part and for each experiment the heat of dilution per mole of solute was calculated for the dilution step from m_i to m_f. Table 1 reports all the relevant experimental data for the systems and the conditions investigated. Iopamidol solution from commercial vials at initial molal concentration of 1.48 was subjected to subsequent dilutions performed at 25 and 37 °C, providing a thermal response from +1.73 to about 0.02 J. Similarly, heat of dilution data of iomeprol solution (initial concentration $m = 1.67$) and iopromide solution (initial concentration $m = 1.52$) as a function of concentration have been collected at 25 and 37 °C in the same experimental conditions of iopamidol. Table 1 reports also the data of the dilution experiments for solutions freshly prepared by dissolving the anhydrous iopamidol powder. It is clear that all the experimental heats of dilution data are constantly exothermic for iopamidol and iopromide solutions and endothermic for iomeprol solutions.

Given the method of subsequent dilution, treatment of the raw calorimetric data has been simply carried out by

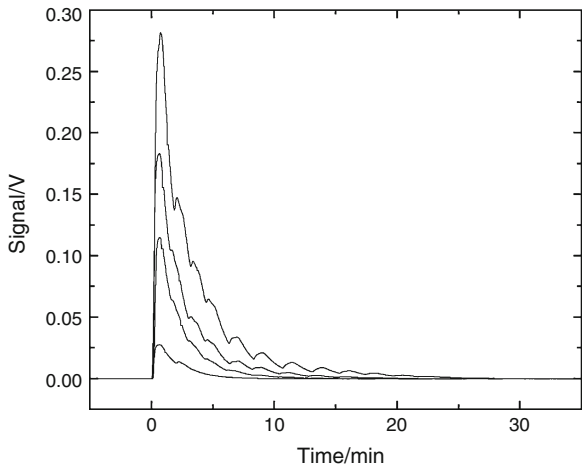

Fig. 2 Calorimetric curves of heat of dilution series at 37 °C (iomeprol); the original curves are overimposed starting at the same initial time of the mixing process. Initial concentration of iomeprol solution decreases from top to bottom curve

Table 1 Heat of dilution and enthalpy data for iopamidol, iomeprol, and iopromide solutions at 25 °C and 37 °C

Sample	m_i	m_f	Q_{dil}/J	ΔH_{dil}/J mol^{-1}
Iopamidol at 25 °C	1.48	0.839	+1.73	−883
	0.839	0.507	+0.740	−570
	0.507	0.317	+0.331	−383
	0.317	0.152	+0.331	−383
	0.151	0.073	$+5.90 \times 10^{-2}$	−205
Iopamidol at 37 °C	1.48	0.838	+1.42	−725
	0.838	0.506	+0.593	−460
	0.506	0.318	+0.258	−299
	0.470	0.217	+0.338	−417
	0.217	0.104	$+8.32 \times 10^{-2}$	−206
	0.104	0.051	$+2.18 \times 10^{-2}$	−108
Iopamidol[a] at 37 °C	1.48	0.836	+1.41	−719
	0.836	0.506	+0.582	−447
	0.506	0.318	+0.252	−292
Iomeprol at 25 °C	1.67	0.925	−0.889	+423
	0.925	0.547	−0.459	+330
	0.547	0.340	−0.240	+263
	0.340	0.160	−0.198	+326
	0.160	0.077	−0.058	+193
Iomeprol at 25 °C	1.67	1.01	−0.952	+362
	1.01	0.603	−0.515	+346
	0.603	0.372	−0.290	+294
	0.372	0.173	−0.234	+358
	0.173	0.084	−0.070	+215
Iomeprol at 37 °C	1.67	0.927	−1.18	+589
	0.927	0.560	−0.543	+388
	0.560	0.351	−0.325	+345
	0.351	0.165	−0.268	+429
	0.165	0.080	−0.082	+261
Iopromide at 25 °C	1.52	0.841	+1.44	−745
	0.841	0.504	+0.585	−460
	0.504	0.313	+0.263	−310
	0.313	0.148	$+5.60 \times 10^{-2}$	−311
	0.148	0.072	$+4.54 \times 10^{-2}$	−161
Iopromide at 37 °C	1.52	0.849	+1.05	−540
	0.849	0.514	+0.377	−292
	0.514	0.321	+0.321	−175
	0.321	0.151	$+8.99 \times 10^{-2}$	−157
	0.151	0.073	$+1.54 \times 10^{-2}$	−53.7

[a] This series of experiments refers to the solution of iopamidol freshly prepared from the anhydrous semicrystalline powder

summing up the heat of dilution for each dilution step to plot the heat of dilution as a function of the final concentration (m_f). Extrapolation of the heat of dilution of the solution to concentration $m_f = 0$ has been done according to standard methods of fitting [14].

Before the analysis of the data, let us comment on the reproducibility of experimental results and of the comparison between the data at 25 °C with the only literature report on these systems. Figure 3 reports two set of data at 37 °C; one experiment is carried out by using the solution of the commercial vials at initial concentration 1.48 m, the other carried out on a solution at same concentration freshly prepared from iopamidol semicrystalline powder by warm dissolution as described in the experimental section. The experiments performed on iopamidol solution directly prepared from powder do not show detectable differences with commercial vials. These results also imply that sample history effects due to atropisomeric non-equilibrium phenomena are excluded or are thermally insignificant.

From the polynomial fitting of the curves of the heat of dilution of the three systems, the apparent molar relative enthalpy, $L\phi$, was calculated as a function of m:

$$L_\Phi = \frac{L - L^0}{n_2} = \frac{L}{n_2} \quad (1)$$

$$\Delta H_{dil} = H_{final} - H_{initial} = L_\Phi(m) - L_\Phi(m_i) \quad (2)$$

According to the formalism introduced by Friedman [15] and thereafter extended by Kauzmann [16] to non-electrolyte solution, the molar apparent relative enthalpy is evaluated from a series of consecutive dilutions to approximately infinite dilution (or extrapolated to this limit) where $m_f = 0$ in the Eq. 2 and then [17]:

$$-\Delta H_{dil} = L\phi(m) = h_{ii}m + 2h_{iii}m^2 + \ldots \quad (3)$$

The values of the coefficients for the three systems are:

$$L\phi\ (\text{Iopamidol}): 2771m - 1054m^2 + 255m^3\ (\text{at } 25\,^\circ\text{C})$$
$$1986m - 570m^2 - 114m^3\ (\text{at } 37\,^\circ\text{C})$$

$$L\phi\ (\text{Iomeprol}): -2947m + 2898m^2 - 1743m^3 + 413m^4\ (\text{at } 25\,^\circ\text{C})$$
$$-3798m + 3523m^2 - 1898m^3 + 401m^4\ (\text{at } 37\,^\circ\text{C})$$

$$L\phi\ (\text{Iopromide}): 2287m - 948m^2 + 242m^3\ (\text{at } 25\,^\circ\text{C})$$
$$876m - 47m^2\ (\text{at } 37\,^\circ\text{C})$$

Figure 4 shows the fitted curves of the apparent molar relative enthalpy of the solution, together with the experimental data points associated to each curve (the experimental values have been subtracted by the values extrapolated at $m = 0$).

The most important result lies in the evidence that dilution of iopamidol and iopromide is an exothermic process, whereas for iomeprol, dilution is endothermic. Such a neat difference must certainly be explained in terms of substantially different balance of the solutions energetics, where both solute–solute and solute–solvent interactions are involved. The rank of the heat of dilution agrees with the order found in the reorientation dynamics of iopromide and iopamidol as measured by NMR as well as in the water/butanol partition coefficient [18]. From the calorimetric data it also appears that the behavior of iopromide dilution at 25 °C is similar to the behavior of iopamidol at 37 °C. As far as the absolute values of heat of dilution are concerned, these are relatively small; in fact,

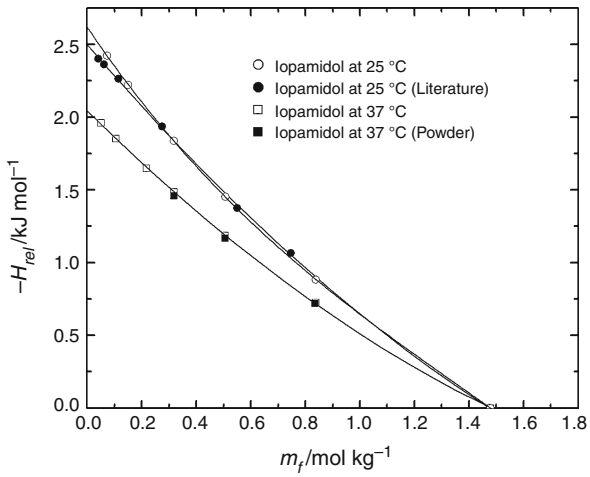

Fig. 3 Results of calorimetric measurements for the dilution of Iopamidol solution at 25 °C and 37 °C compared with the literature data at 25 °C [19] and data of dilution at 37 °C of Iopamidol solution freshly prepared from powder

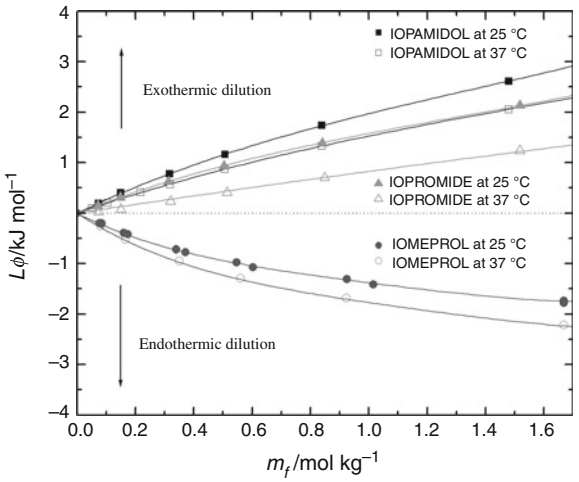

Fig. 4 Change in enthalpy of the solution relative to the enthalpy of pure solvent for iopamidol, iomeprol, and iopromide at 25 °C and 37 °C

the integral heat of dilution does not exceed about 2–3 kJ mol^{-1} for the complete dilution process, starting from concentrated solutions and down to infinite dilution limit. The absolute value of molar apparent relative enthalpy for iomeprol is lower than for iopamidol and iopromide in all range of concentrations. Data for iopamidol at 25 °C were also compared with the literature data at 25 °C [19], showing only a small difference in the integral heats of dilution at low concentration. These literature data where not further processed as they were used to correct the heat of mixing with proteins (fibrinogen or lysozyme). It has been pointed out that a small endothermic effect was measured and ascribed to changes in the solvation properties of the proteins and not to direct interactions, as confirmed by the absence of spectroscopic Raman evidence [19].

The values of the heat of dilution decrease with temperature for all three NICMs, as it can be seen from two sets of experiments at 25 and 37 °C. From these data an evaluation of the heat capacity terms can be approximately carried out. From the value of Fig. 4 the heat capacity of iopromide solution results almost twice of that of iomeprol and iopamidol. This difference is almost surprising in view of the similar chemical structure of the NICMs and should be ascribed to the greater increase of degree of freedom of the solvated species of iopromide respect to iopamidol and to iopromide. Whether the C_p changes are also related to the variation in the potential associative equilibria in solution can only be ascertained by independent measurements. Furthermore, direct C_p experiments need to be carried out.

Before commenting the solution properties of NICMs on the basis of the other literature data, let us report about the calorimetric heat of dilution experiments carried out at 25 °C on mixtures of iomeprol with iopamidol, with the purpose of detecting the change in the heterotactic interactions (if any) of these two similar systems, but with opposite sign of the enthalpic term upon dilution. Measurements were made on mixtures at three solute concentration ratios, in order to evaluate the compatibility of the solutes in aqueous solutions. The results of these experiments are summarized in Table 2.

Figure 5 shows heat of dilution data plotted against the concentration of iomeprol and reports data of iopamidol and iomeprol at 25 °C already calculated. The linearity of plot of Fig. 5 suggests that the *excess* heat of mixing (h_{ij} in the notation of Eq. 3) of iopamidol and iomeprol is almost zero and that their mixed interactions are enthalpically equivalent. The heat of dilution of a 60% iomeprol mixture would be zero, and therefore, such a mixed system is athermic with contributions to the mixing of entropic nature only.

That both intermolecular association and hydration phenomena govern the solution properties of NICMs is shown by many literature evidences. The decrease of the osmotic coefficient for iopamidol and iomeprol [10, 20] is typical of a system undergoing a progressive association, although its analysis is hampered by the clear evidence of strong hydration, which alter the most straightforward analysis commonly used for these processes [21–23]. The extensive hydration of NICMs can be easily deduced from a qualitative analysis of the hydrodynamic volumes evaluated by viscosity measurements. Obviously, both solute–solute interactions and solvent–solute interactions must contribute to the observed thermal effects; the data here reported clearly show that a prevalence of hydrophilic interactions occurs in the solution of iomeprol with respect to the prevalence of less polar interactions occurring in iopamidol and iopromide solution.

In addition, possible concentration-dependent and temperature-dependent conformational effects should also be taken into account. Indeed, the conformational properties of NICMs in solution are dominated by a large number of potential conformers, some of which fall in the category of atropisomerism [1]. Thus, many molecular descriptors of the solution properties of these molecules are conformation-dependent and, therefore, complicated by the existence of metastable conformers population. A detailed ^{13}C-NMR study on iopamidol, iomeprol, and iopromide and on common simplified primers is in progress for the assignment of most relevant conformers in NICMs

Table 2 Heat of dilution of iomeprol/iopamidol mixtures at 25 °C

Iomeprol/iopamidol weight ratio	Q_{dil}/J	ΔH_{dil}/J mol^{-1}
75:25	+0.425	+205
50:50	−0.568	−281
25:75	−1.514	−761

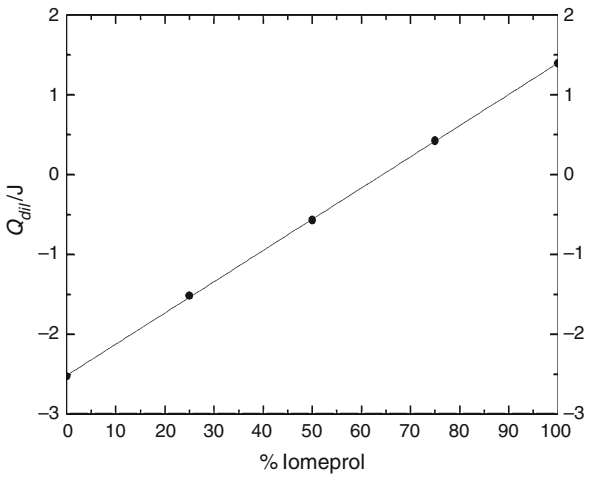

Fig. 5 Heat of dilutions of iomeprol/iopamidol mixture at 25 °C

(L. Fontanive, unpublished results). Without going into the detail of the conformational variability and of the ^{13}C-NMR ^{1}H-NMR investigations carried out for the elucidation of the most probable conformers, the important result is that only six conformers seem to be predominant for iomeprol, while only two for iopamidol. Although these results derive from the parallel study to be published (L. Fontanive, unpublished results), let us capitalize this information in view of the fact that no changes in the population were observed as long as the temperature was changed from 25 to 37 °C. Some preliminary information about the interactions is also available and can be useful for the discussion of the macroscopic effects of the concentrated solution of NICMs. Among the several potential isomers, only intermolecular interactions between polar hydrophilic groups have been detected. Therefore, other non-polar interactions must be detected as inferred by the calorimetric results here presented that allow rationalizing the profile of energetics of the interaction in NICM solutions.

In conclusion, the analysis of the thermodynamic properties of NICM can be fruitfully accomplished in terms of balance between polar and non-polar homotactic interactions and of different contributions from the hydration water. Spectroscopic data are necessary to attribute to specific functional groups the role on interacting moieties responsible for the weak aggregation and, therefore, to both low-viscosity and low-osmolality behavior in solution.

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