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The Interaction of Dopexamine with Various Drugs and Excipients in Parenteral Solutions

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Abstract—The interaction of dopexamine hydrochloride with various excipients and other drugs in parenteral solutions has been investigated by microcalorimetry. The interaction with heparin sodium, in particular, is significant. The interaction is strongest in parenterals containing glucose and is eliminated in normal saline. Divalent cations are more effective than monovalent ones in eliminating the reaction, which is apparently ionic in nature.

Phenolic drugs like dopexamine, a new drug for the treatment of chronic heart failure (Jaski et al 1986), can be expected to oxidize at high pH values giving coloured products. This can be overcome by lower pH, antioxidants and single dose injections. But such drugs are protonated at acidic pH values and the cations readily react with anions of drugs like heparin and frusemide so precipitation may occur. If this does not happen the ion-ion interactions are not readily detectable by spectroscopy or chromatography. It seemed therefore that, as heat is likely to be evolved on interaction, microcalorimetry might offer a useful approach to detecting the ion-ion interactions. It may also be useful for screening possible interactions between a drug and excipients considered in the design of the injection.

Materials and Methods

Dopexamine HCl (powder and injection), mannitol, and sodium metabisulphite were from Fisons, Loughborough, UK. Dobutamine HCl (powder and injection) was from Eli Lilly & Co. (Indianapolis, IN). Verapamil injection 2·5 mg mL^{-1} (Knoll Pharmaceutical Co., Whippany, NJ), furosemide (frusemide) injection USP 10 mg mL^{-1} (LyphoMed, Inc., Melrose, Park, IL), heparin sodium injection derived from bovine lung 10000 USP units mL^{-1} (LyphoMed), heparin calcium injection derived from porcine intestinal mucosa 25000 USP units mL^{-1}, digoxin injection 0·25 mg mL^{-1} (Burroughs Wellcome Co., Research Triangle Park, NC), 0·9% sodium chloride injection USP (Travenol Laboratories, Inc., Deerfield, IL) and 5% dextrose injection USP (Travenol) were all obtained from a local hospital. Sodium and calcium salts of heparin, in powder form, were obtained from CP Pharmaceuticals Limited (Wrexham, UK). Sodium heparin from bovine lung was obtained from Sigma Chemical Co. (St Louis, MO). All other reagents were of analytical grade.

All solutions were used within 4h of preparation. Solutions of frusemide and dopexamine HCl were protected from light by covering the glass vessels containing these solutions with aluminium foil. All solutions prepared from powder sources were filtered using a Gelman 0·45 μm filter (Gelman Sciences, Inc., Ann Arbor, MI).

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Calorimetry

Calorimetric investigations were carried out for the interaction of dopexamine HCl, in dextrose 5% in water (D5W) and 0·9% NaCl in water (NS), with each of the following drugs: digoxin, frusemide, heparin Na, heparin Ca and verapamil HCl. The interaction of dopexamine HCl with heparin was also investigated in the presence of various other inorganic salts. The calorimeters were also used to study the possible interaction between dopexamine HCl and various potential excipients.

Measurements of heat flux were made in either a LKB batch Microcalorimeter Model 2107-111/112 or a LKB flow microcalorimeter 2107-121 (LKB, Bromma, Sweden). Amplification was by a Keithley 150B microvolt ammeter (Keithley, Cleveland, OH). The flow microcalorimeter was modified in that the air bath was replaced by a Tronac model 1055 water bath with a PTC40 temperature controller (Tronac Inc., Orem, UT).

The batch microcalorimeter was a standard LKB 2107 system with gold cells. The heat of mixing of the two drugs was measured in the batch microcalorimeter by loading one compartment of the reaction vessel with 2 mL of a drug solution, the other compartment with 2 mL of another drug solution and each of the compartments of the reference vessel with 2 mL of the solvent used to prepare such solutions. The heat of dilution from each drug was determined by placing 2 mL of the drug solutions in a compartment of the reaction vessel. The other compartment and the reference vessel compartments were filled with 2 mL of the corresponding solvent.

Each run consisted of filling the vessels and allowing the instrument to equilibrate for about 1h. When the baseline was stable, the liquids within each vessel were mixed by rotation of the cylinder. After all the heat had been absorbed (when the baseline was stable again), the cylinder was rotated again to record the difference in friction between the liquids in the two vessels. Finally, each run was calibrated by setting the current and time functions in the control unit.

All the heats of reaction obtained from the batch microcalorimeter were calculated from the areas of the voltage-time curves and the calibration constant obtained by passing a known current for a known time.

The flow microcalorimeter was used to carry out titration procedures. Solutions of heparin sodium and dopexamine HCl were delivered to the mixing cell by two LKB 10200

peristaltic pumps at approximately 15 mL h^{-1}. The dopexamine HCl concentration was kept constant while the concentration of heparin sodium was varied. The calorimeter was calibrated electrically within the expected range and a calibration constant of 0.0575 $\mu V\ \mu W^{-1}$ was used. Flow rates were precisely determined gravimetrically, each day.

The heat was calculated in J mol^{-1} of dopexamine from the voltage output, the calibration constant and the flow rate (Hardee et al 1978). The heat of reaction between the two drugs was corrected for any heat of dilution of the reactants.

In general, the concentrations of the drug solutions were selected to be within the concentrations commonly used when those drugs are administered by intravenous infusions (McEvoy 1986). However, in some instances the drug concentrations were slightly higher than those commonly used in clinical practice so that the heats could be measured more accurately. The concentrations for heparin are generally given in units mL^{-1} since in many cases the injection was used and no conversion to a weight unit was provided by the manufacturer. (It is possible to convert the concentration of heparin Na to mg mL^{-1} by dividing the units mL^{-1} by 169.7 in the case of the heparin from CP Pharmaceuticals Limited and by 149 in the case of the heparin from Sigma.) The preliminary trial of mixing two drugs in a given solvent consisted of observing the resulting solutions for turbidity, discoloration and pH, and did not include any adjustments in pH. Finally, new solutions were prepared with similar pH to the mixture of interest using concentrated HCl or NaOH, and a Model 611 digital pH meter (Orion, Cambridge MA) since the solutions within the calorimeter must preferably be of the same pH.

Ultraviolet spectroscopy

Ultraviolet spectra (200–500 nm) were obtained using a Hewlett Packard 8451 diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA). Solutions of the individual drugs and the drug mixtures, used for calorimetric analysis, were diluted to an appropriate concentration before spectrophotometric analysis. The spectral data were stored on 3.5″ discs using a dual disc drive (HP 7470A). The same 1 cm quartz cell was used throughout.

Fluorescence spectroscopy

Fluorescent intensity measurements were obtained for solutions of various concentrations of heparin Na, dopexamine HCl, and their mixture in D5W at pH 4.9 using a Perkin Elmer LS-5 fluorescence spectrophotometer (Perkin-Elmer, Norwalk, CT) with excitation at 280 nm and fluorescence monitored for the range 280–520 nm.

Ultrafiltration

An Amicon micropartition system MPS-1 (Amicon Corporation, Danvers, MA) was used to study the feasibility of separating the 'free' dopexamine from the dopexamine bound to heparin. Solutions of heparin Na 40 units mL^{-1}, dopexamine HCl 0·4 mg mL^{-1} and a mixture containing 40 units mL^{-1} of heparin Na and 0·4 mg mL^{-1} of dopexamine HCl, using D5W at pH 4·9 as a solvent, were prepared in duplicate. Approximately 1 mL of the solutions was placed in each filtration cell and centrifuged for about 5 min. An exact aliquot of the filtrate was diluted with D5W at pH 4·9, before spectrophotometric analysis.

Results and Discussion

Preliminary work (batch microcalorimetry, UV, fluorescence and ultrafiltration)

Heats of dilution were detected only for the mixing of 2 mL of digoxin 0·2 mg mL^{-1} with 2 mL of either D5W and NS. Further studies indicated that the detected heat was the result of the dilution of cosolvents present in the injection. Calorimetric investigations of the possible interaction between dopexamine hydrochloride and a series of drugs in parenteral fluids suggest interactions in D5W with heparin and frusemide but not with digoxin and verapamil (Table 1). There was apparently interaction between any of the drugs in normal saline. Table 2 shows that heat fluxes were detected for the mixing of heparin Na and dopexamine HCl in all the studied solvents except those solvents with high content of NaCl (0·9%). Addition of EDTA to the mixture of heparin and dopexamine HCl did not change the interaction. No signs of immediate precipitation were observed for any of the

Table 1. Detection of heat for the mixing of 2 mL of selected drug solutions with 2 mL of dopexamine HCl 0·8 mg mL^{-1} measured at 25°C. Final concentration of dopexamine HCl in the mixtures was 0·4 mg mL^{-1}.

Drug	Drug concn before mixing (mg mL^{-1})	Drug concn after mixing (mg mL^{-1})	pH	Heat flux
Dextrose 5% in water				
Digoxin	0·02	0·01	5·0	No
Frusemide	1·0	0·5	6·5	Yes
Heparin[a]	various[a]	various[b]	4·9	Yes
Verapamil HCl	0·20	0·10	4·2	No
Normal saline				
Digoxin	0·02	0·01	5·0	No
Frusemide	1·0	0·5	6·5	No
Heparin	80 umL^{-1}	40 umL^{-1}	4·9 and 5·2	No
Verapamil HCl	0·20	0·10	4·4	No

a Two different sources of heparin sodium and heparin calcium.
b See Table 2.

Table 2. Heat flux of a mixture of 2 mL dopexamine HCl 0·8 mg mL^{-1} ($1·86 \times 10^{-4}$ M) with 2 mL heparin Na in selected solvents at pH 4·9, measured at 25 C. Final concentration of dopexamine HCl in the mixture was 0·4 mg mL^{-1}.

Solvent	Heparin Na Concn (umL^{-1})	No of Trials	Heat (s.d.) ($kJmol^{-1}$ of dopexamine)
Dextrose 5% in water	120	2	4·978 (0·125)
	80	4	4·924 (0·159)
	40	1	2·659
	20	1	1·840
Dextrose 5% and 10^{-4} M EDTA in water	80	2	4·096 (0.168)
Dextrose 5% and 0·9% NaCl in deionized water	80	2	0
Deionized water	80	2	2·243 (0·0191)
0·252% NaCl in deionized water	80	2	1.267 (0·101)
0·9% NaCl in water	80	2	0

concentrations listed for the drug mixtures in Tables 1 and 2.

Table 3 contains the lowest attempted drug concentration for which obvious signs of turbidity were observed. Only three of the studied drug mixtures showed obvious signs of turbidity. At high concentrations of dopexamine HCl (1 mg mL^{-1}) and heparin Na (50 units mL^{-1}), in D5W, some turbidity was seen. If the concentration of heparin Na was kept at 50 units mL^{-1} while the concentration of dopexamine was increased to greater than 1 mg mL^{-1} the turbidity was significantly increased. However, the inverse was not true; when dopexamine was kept constant at 1 mg mL^{-1} and heparin Na was increased to greater than 50 units mL^{-1} no change in turbidity was noted. A similar situation was observed for mixtures in D5W containing high concentrations of dobutamine HCl (1.5 mg mL^{-1}) and heparin Na (50 units mL^{-1}). The observed turbidity in the mixture must be attributed to an ionic interaction between the drugs, as the individual drugs are soluble in D5W at acidic pH. The turbidity of the frusemide—dopexamine mixture is easier to explain since the frusemide is poorly soluble in water at low pH. It is quite possible, however, that the negatively charged frusemide reacts with the positively charged dopexamine, to produce an insoluble salt. All the remaining experiments were designed so that no precipitation occurred.

Absorption spectra (200–500 nm) of the individual drug solutions and the corresponding drug mixtures did not show any spectral changes for any of the studied drug mixtures. Fig. 1 shows the spectra of heparin Na, dopexamine HCl and their mixture in D5W, pH 4·9. The full spectrum of all the drug mixtures is the sum of the spectra of the individual drugs. The emission spectra of heparin Na, dopexamine HCl and their mixture in D5W, pH 4·9, with excitation at 280 nm are shown in Fig. 2. The presence of heparin Na at a concentration of 387 units mL^{-1} with dopexamine HCl $2·3 \times 10^{-4}$ M resulted in quenching of the fluorescence of dopexamine. Increasing the heparin concentration, while keeping the dopexamine concentration constant did not produce any further quenching of the dopexamine fluorescence; these changes were too small for quantitative investigations.

Fig. 3 shows the absorption spectra for the dilutions of the filtrates obtained for the experiments performed using the Amicon micropartition system. The spectra demonstrated that most of the dopexamine was retained by the membrane when dopexamine was present in a mixture containing heparin Na. The results clearly indicate that a significant portion of the heparin was not retained. The unretained portion of the heparin can be attributed to the lower

Table 3. Selected mixtures of two catecholamines with other drugs observed to become turbid in dextrose 5% in water.

Catecholamine	Catecholamine concn (mg mL^{-1})	Drug	Concn
Dobutamine HCl	1·5	Heparin Na	50 umL^{-1}
Dopexamine HCl	1·0	Heparin Na	50 umL^{-1}
Dopexamine HCl	0·75	Frusemide	0·55 mg mL^{-1}

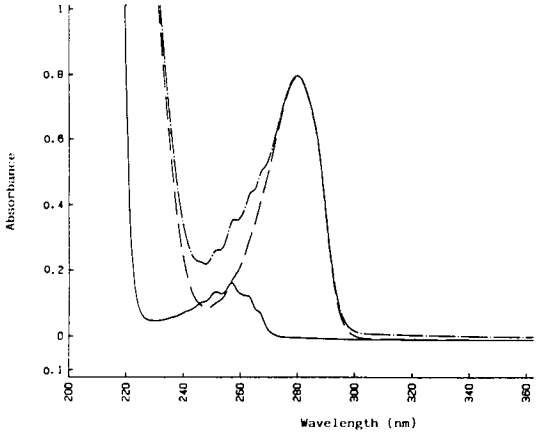

FIG. 1. Ultraviolet spectra of heparin Na 60 units mL^{-1} (——), dopexamine HCl 120 μg mL^{-1} (- - -) and a mixture containing heparin Na 60 units mL^{-1} and dopexamine HCl 120 g mL^{-1} (——·——). Solvent was dextrose 5% in water at pH 4·9.

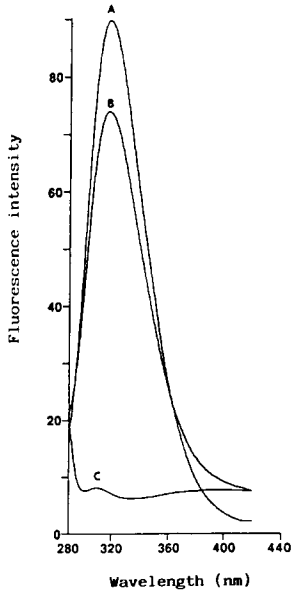

FIG. 2. Emission spectra of (A) dopexamine HCl $2·3 \times 10^{-4}$M, (B) mixture of heparin Na 387 units mL^{-1} and dopexamine HCl $2·3 \times 10^{-4}$M and (C) heparin Na 387 units mL^{-1}. Excitation at 280 nm. Solvent was D5W at pH 4·9.

threshold of the membrane used which is about 30 000 Daltons, since heparin is a mixture containing a molecular weight range of 3000 to 37 500 Daltons (Nachtmann et al 1983). Therefore, this technique clearly shows the binding of dopexamine to heparin but it is not the most suitable for quantitative work.

Flow microcalorimetry

Fig. 4A shows heats of reaction per mole of dopexamine HCl as a function of heparin sodium concentration, expressed in units mL^{-1}. These data clearly show that there are small differences in the heats of reaction depending upon the source of heparin, no matter how the concentration of heparin is expressed. It was therefore considered desirable to conduct the remaining experiments using the same batch of heparin (derived from bovine sources), namely the Sigma source. The plateaux in the graphs are reached when all the dopexamine HCl is bound to heparin. The stoichiometry of the reaction and the affinity constants cannot be estimated from these results because of the many and varied acidic groups present in the mixture of molecules called heparin.

Correlating the data of Fig. 4A with the ultrafiltration evidence of binding, and the fluorescence results, suggests that the heat arises from the binding of dopexamine to heparin.

The effect of various pH values, 3·9, 4·9, 5·9, on the detected heat of reaction are shown in Fig. 4B. Under the experimental conditions, the catecholamine is fully protonated since it has pKa values of 8·65, 9·79 and 10·5 (J. Tillman, personal communication, Fisons Plc., Loughborough, UK, 1986). Similarly, the sulphonic acid and the aminosulphonic acid groupings of the heparin are fully deprotonated, but the extent of ionization of the carboxylic acids, pK_a of 5.7 (Brand & Vert 1985), increases with increased pH. The minor differences of heats as a function of pH may be the result of some interaction of these groupings with the dopexamine. All subsequent investigations were performed at pH 4·9 since the intravenous admixtures containing heparin Na and dopexamine hydrochloride, have

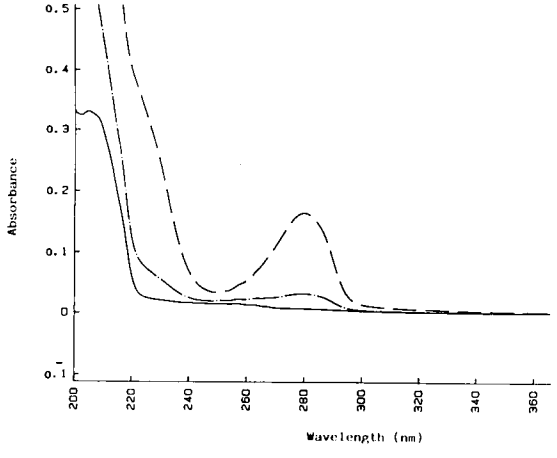

FIG. 3. Ultraviolet spectra obtained with the same dilution factor for the filtrates of heparin Na 40 units mL^{-1} (——), dopexamine HCl 0·4 mg mL^{-1} (- - -) and a mixture containing 40 units mL^{-1} of heparin Na and 0·4 mg mL^{-1} of dopexamine HCL [——·——]. Solvent was D5W at pH 4·9.

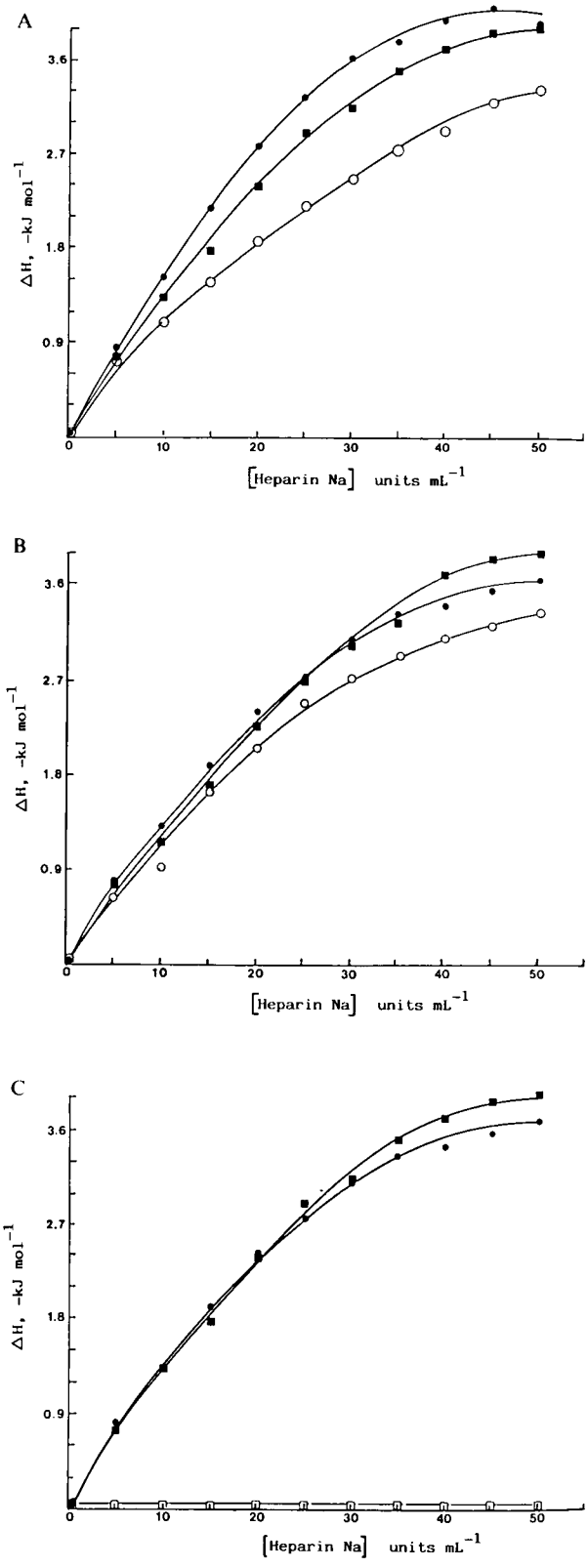

FIG. 4. Heat of reaction ΔH, $-kJmol^{-1}$ dopexamine HCl at 25°C and A pH 4·9, as a function of heparin Na concentration with the dopexamine concentration fixed. Solvent: D5W. Sources of heparin: (●) Lyphomed, (■) Sigma, (○) CP; B as a function of heparin Na concentration with the dopexamine concentration fixed. pH: (■) 5·9, (●) 4·9, (○) 3·9; C with pH 4·9, as a function of heparin Na concentration with the dopexamine concentration fixed. Solvents: (■) D5W, (●) H_2O, (□) NS.

pH values of approximately 4·9 because of the strong acidic nature of dopexamine hydrochloride solutions.

Fig. 4C shows the heats of reaction per mole of dopexamine in D5W, NS and H_2O at pH 4·9. The heats were similar, for the same source of heparin, in D5W and H_20 suggesting no involvement of the dextrose molecules in the reaction. No heats of reaction were detected in the saline solution which can be interpreted as indicating no reaction between the heparin and the dopexamine. The lack of reaction in normal saline could be due to an ionic strength effect or competition between the dopexamine cations and the inorganic sodium ions for the anionic centers in the heparin molecule.

The effect of one:one electrolytes on the heat of the dopexamine-heparin reaction is shown in Fig. 5A. It is obvious that the effect of these electrolytes on the heat of reaction depends upon the nature of the electrolyte. The similarity of the results for KCl and KBr strongly suggests that the anion is of no consequence in the reaction. The reaction seems to be inhibited by cations in the following order of efficacy: $K^+ > Na^+ > Li^+$. Fig. 5B shows that divalent inorganic cations stop the reactions at much lower concentrations than monovalent cations, Ca^{2+} being more effective than Mg^{2+}. The overall effectiveness of stopping the reaction is $Ca^{2+} > Mg^{2+} > K^+ > Na^+ > Li^+$. This is the

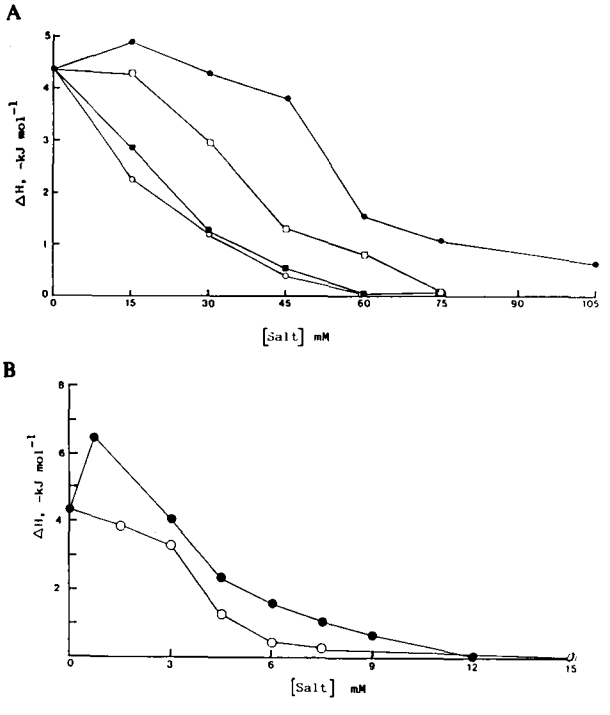

FIG. 5. Heat of reaction ΔH – kJmol^{-1} dopexamine HCl at 25°C and pH 4·9, as a function of salt concentration with both drugs concentrations fixed. Salts A (●) LiCl, (□) NaCl, (■) KCl, (○) KBr. Salts B (●), $MgCl_2$, (○) $CaCl_2$.

Table 4. Heats of interaction for mixtures of dopexamine HCl (10 mg mL^{-1}) and several excipients in H_2O at pH 2·5, measured at 25 C.

Excipient	Cell concn (% wt/vol)	Heat of reaction (kJmol^{-1} dopexamine)
Disodium EDTA $2H_2O$	0·005	+0·008
	0·050	−0·054
Sodium metabisulphite	0·005	0
	0·500	+0·404
Ammonium sulphate	0·010	−0·016
	0·500	+0·431
Sodium citrate	0·010	+0·018
	0·500	+0·162
Mannitol	0·500	+0·098
	2·000	+0·415

accepted order of affinity of these ions for heparin (Dunstone 1962; Brand & Vert 1985) and so the reaction in the presence of these chlorides seems to be a competition between the cationic drug and the inorganic cation for the anionic site on the heparin molecule.

Table 4 shows the interaction of dopexamine with potential excipients of injections. The magnitude of the observed heats is at least an order of magnitude smaller than that observed for the dopexamine-heparin reaction. For the salts, minor changes in interactions between oppositely charged ions can explain the data. Interactions with EDTA could not be investigated at higher concentrations because it precipitated at pH 2·5. In the case of mannitol it is possible that the high affinity of mannitol for water causes changes in the distribution of water with the net results of the absorption of a small amount of heat.

The data reported here are strong evidence that the reaction between dopexamine and heparin is an ion-ion interaction. This reaction can be eliminated in the presence of a sufficient concentration of inorganic cations, including the concentration of sodium ions found in normal saline.

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