DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(15), 2127-2134 (1991)

THE COMPLEXATION OF AMITRIPTYLINE AND IMIPRAMINE BY SODIUM POLYPHOSPHATE.

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ABSTRACT

The complexation of organic cations with sodium polyphosphate (SPP) has received some attention in the past; those workers have focused on research which demonstrated the range of molecules which form insoluble complexes, the in-vitro dissolution of the complexes or in-vivo studies on the absorption of the complexes. This in-vitro study, with amitriptyline and imipramine, presents evidence that structurally related drugs may have substantially different affinities for the SPP. Imipramine has the higher affinity for SPP and the complexation process is unaffected by the presence of up to 0.1M sodium chloride. The maximum stoichiometry obtained with imipramine was 1.13. It is suggested that imipramine is able to interact in a 1:1 ratio with the chain phosphates and in a 2:1 ratio with the terminal phosphates. This mechanism predicts that the net stoichiometry for SPP, with an average chain lenghth of 14, is 1.143.

INTRODUCTION

The complexation of cationic drugs with sodium polyphosphate (SPP) has received some attention in the past [1-4], but those studies have not focused specifically on the effect of the experimental conditions on the process of complexation. The in-vitro study of Patel [1] was the first to show that a wide range of organic cations formed complexes with SPP and also show that the both solid and liquid complexes could form. However,

he did not investigate the effect of the initial drug concentration upon the complexation process. Other workers have employed the poorly soluble complexes formed with quinidine [2] and papaverine [3] in bioavailability studies in dogs; they have shown that the AUC is increased by 10-30% compared to the hydrochloride salts of those drugs. A recent in-vitro report [4], which primarily studied the dissolution of SPP complexes with verapamil, indicated that the complex stoichiometry may depend on the experimental conditions.

The purpose of this communication is to show that, (a) the physicochemical nature of the complex depends markedly on the structure of the cation, and (2) the stoichiometry may be dependent on the experimental conditions. The drugs chosen for this study were amitriptyline and imipramine. These anti-depressent drugs have similar chemical structure and have been shown to adosrb on to Maddrell's Phosphate (Type II), an insoluble sodium polyphosphate; the affinity of amitriptyline was found to be slightly greater than imipramine [5].

MATERIALS AND METHODS

Chemicals

The drugs, amitriptyline HCl and imipramine HCl, were obtained from the Sigma Chemical Company (St Louis, USA). Their identities were confirmed by IR spectroscopy (as a 1% dispersion in KBr) and melting point determination. They were employed as received. Linear Beer's Law plots were obtained in distilled water, for amitriptyline at 250nm and for imipramine at 238nm. The Sodium Polyphosphate was also obtained from the Sigma Chemical Company . The chain length and the total phosphate concentration, measured by inductively coupled plasma spectroscopy (ICPS), have been determined previously [4]. All other chemicals were of analytical reagent grade.

Complexation experiments

Mixtures with constant initial drug concentrations of 0.025, 0.05, 0.075 or 0.1M, various concentrations of SPP (0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04 and 0.05M) were prepared to a constant total volume of 10mL in distilled water. The vials were sealed with Parafilm (American Can Company,) and were equilibrated at 25^{o} C by rotation (20 rpm) in a constant temperature water bath for 4 hours in a completely dark room. After the equilibration period the vials were isothermally centrifuged at 3500 rpm for 15 minutes before samples were removed and diluted with distilled water for UV analysis at the wavelengths specified previously. Preliminary tests showed that the presence of SPP did not interfere with the UV assay.

The tests to determine the effect of the concentration of the sodium ion on the complex stoichiometry were performed in a similar manner except that the initial concentrations of both drug and ligand were constant at 0.05M , and the sodium ion concentration was varied from 0 to 0.1M. The total volume in each vial was 10mL. All other experimental conditions were the same.

RESULTS AND DISCUSSION

The average chain length determined by titration was 14 and the total phosphate content of the SPP is 9.297mmoles/g [4]. The chain length is in good agreement with that claimed by the manufacturer i.e. 15 with a range of 13 to18. However, it must be realized that not only is that estimate d chain lenghth subject to experimental error if the polyphosphate hydrolysis is incomplete, but that it also represents the average chain length of the sample. Determination of the total phosphate content by ICPS provides more useful and more reliable information for these studies. Because of the very high temperature of the plasma, the technique yields a more reliable estimate of the total phosphate content, since it is able to detect the total concentration of phosphate present irrespective of the chain length.

It was visibly apparent that complexation occurred quickly since a dense white precipitate formed immediately on mixing the drug and the SPP. Although both complexes were solid, they differ in their appearance and behaviour. The complex of imipramine (I-SSP) was very fine and even after being allowed to stand undisturbed for about 60 hours had sedimented minimally; centrifugation was essential to obtain sufficient clear supernatant for assay purposes. The complex of amitriptyline (A-SSP) was very coarse and while the largest clumps sedimented quickly a large proportion of the precipitate adhered to the glass vial. The particles of A-SSP were slightly more translucent than the I-SSP which was a solid white material.

Figure 1 shows the complexation diagrams for amitriptyline and imipramine, obtained with initial drug concentrations of 25, 50, 75 and 100mMolar and in which the concentration of SPP ranged from 0 to 50mMolar. The experiments were performed in duplicate at 25^{o} C and showed very good reproducibility. The range of concentrations of SPP over which linearity was displayed increased as the initial drug concentration was increased. This trend which has been observed previously [4].

Figure 2 shows the positive linear relationship between the stoichiometry (obtained from the slopes of the linear portion of the graphs in Figure 1) and the initial concentration of drug over the range of 25 to 75mMolar. It appears that increasing the

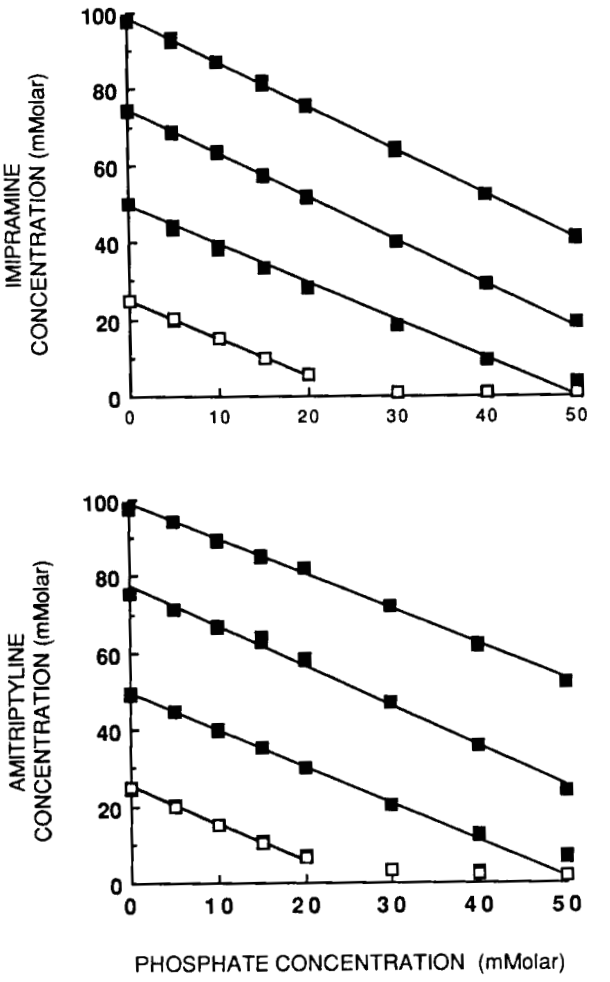

FIGURE 1

The complexation diagrams for amitriptyline and imipramine by sodium polyphosphate at 25°C. The data with an initial concentration of 25mMolar, are drawn different ly to allow them to easily distinguished from the other data.

initial drug concentration to100mMolar causes no further enhancement of the stoichiometry. In fact the stoichiometry of A-SSP is considerably reduced. The precipitate of the complex of amitriptyline at the highest initial concentration was visibly different to that which was obtained at lower concentrations. The precipitate was more translucent and appeared more oily. The complex formed at the high initial concentration may have quite different properties to that which is obtained at the lower initial

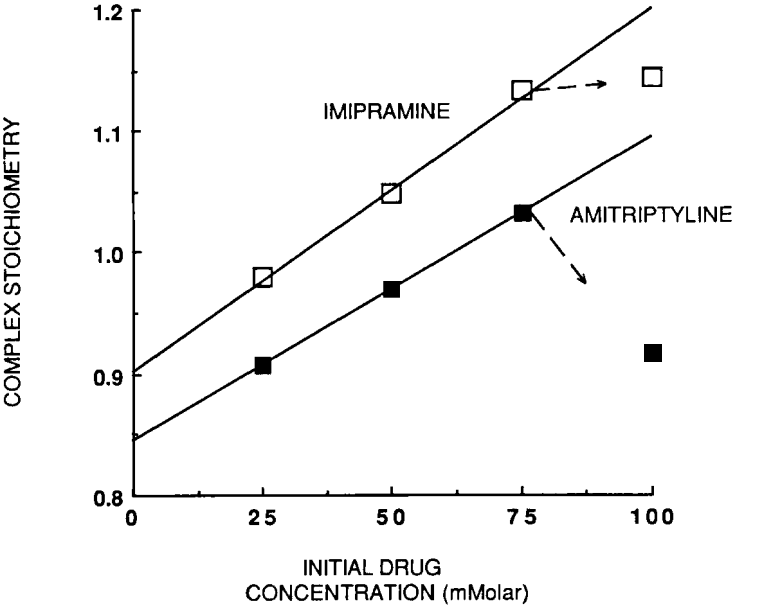

FIGURE 2

The relationship between the stoichiometry of the complexes formed between amitriptyline and imipramine and sodium polyphosphate and the initial concentration of drug.

concentrations. The stoichiometry of the I-SPP appears to have a maximum value of about 1.13. Since the drugs are both monovalent, differences in the stoichiometry can be clearly interpreted as differences in drug/SPP binding ratios or affinities.

The author is not aware of any studies which have established any relationship between the initial cation concentation and the stoichiometry with SPP. Furthermore, previous workers [1-3] have reported stoichiometries of 1 for the drugs studied. The stoichiometry of an amitriptyline complex has been reported [4] to be 0.988 at 25° C. This was obtained with an initial concentration of 35mMolar and is consistent with the trend shown in Figure 2.

It has always been proposed that each anionic phosphate group in SPP is able to complex with one cationic molecule [1,2,4]. However, the terminal groups of the SPP have two potential anionic binding groups , albeit with two substantially different pKa values. Therefore, SPP with an average chain length of 14 may conceivably complex 16 moles of drug. Such a binding relationship would result in a stoichiometry of 1.143. The

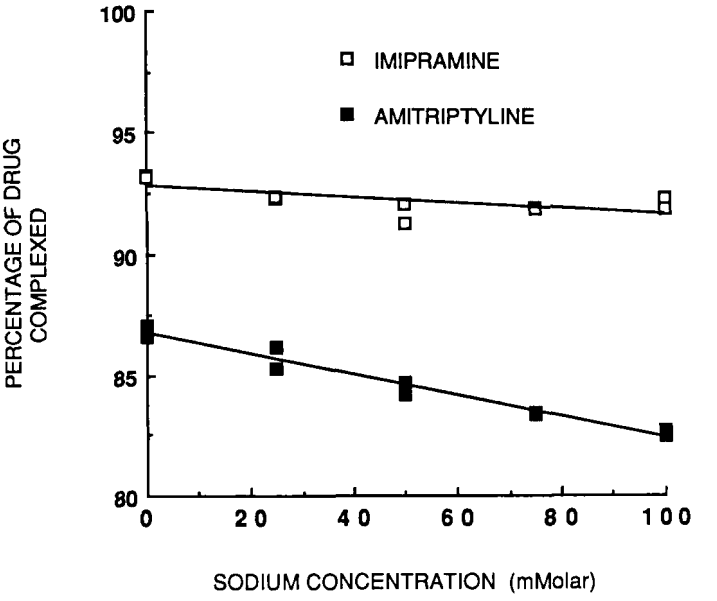

FIGURE 3
The effect of various concentrations of sodium chloride on the complexation of amitriptyline and imipramine with sodium polyphosphate at 25°C.

maximum stoichiometry of I-SPP is 1.13, and it appears that imipramine may be able to undergo this unusual binding process.

It is apparent from Figure 2 that the affinity of imipramine for SPP exceeds that for amitriptyline at all initial concentrations. This is the reverse of the results of Hele [7,8], who used nephelometry to assess the interaction of various phenothiazines and imipramine with a polyphosphate. Unfortunately, neither the source nor the composition of the polyphosphate (which was extracted from the the soluble fraction of RNA from brewer's yeast) were specified, other than to note that the polyphosphate chain-length may have been about 1600.

Logically, the binding affinity of any drug to SPP is influenced by its molecular shape and the different behaviour of amitriptyline and imipramine may be related to structural differences. Both of these drugs have considerably greater angles of flexure and annellation than the planar tricyclic phenothiazine derivatives [6]. The ring systems in these molecules are not the same but they are both quite distorted compared to the phenothiazines. The primary difference between them is the reduced flexibility imposed

on the side chain of amitriptyline by the double bond linkage to the B ring. This may compromise the complexation of amitriptyline with SPP.

Hele [7] also reported that inclusion of relatively high concentrations (1M) of sodium chloride markedly suppressed the formation of a complex between imipramine and the polyphosphate extracted from yeast. Close inspection of the graphical data in her report [7] shows that at lower concentrations of sodium (0-0.1M) there is very little effect on the complexation between the polyphosphate and imipramine. She also noted that with an inorganic polyphosphate (chain length of 12), "lower salt" concentrations stimulated complexation with chlorpromazine but depressed complexation with imipramine. Unfortunately there is no definition made of "lower" and the degree of depression was not specified.

Figure 3 shows the results from the present study. The percentage of drug (from an initial drug concentration of 50mM) complexed by the addition of 50mM of SPP in the presence of concentrations of sodium chloride (which ranged from 0 to 100mM) are shown. The complex of SPP and amitriptyline was marginally suppressed while the I-SSP was unaffected. It is important to note that the concentrations chosen in this study are more modest than those employed by Hele [7]. This data also suggests that the affinity of the imipramine is greater than amitriptyline for the SPP.

CONCLUSIONS

This report presents substantial evidence that the stoichiometry of complexes between organic cations and sodium polyphosphate (with an average chain length of 14) is positively related to the initial cation concentration. It also shows that the affinity of SPP for imipramine is greater than for amitriptyline and that this may be the reason for the observation that the complexation of imipramine is unaffected by modest concentrations of sodium chloride whereas the complexation of amitriptyline is slightly suppressed. To account for the high stoichiometry which may be obtained with imipramine it is proposed that the terminal phosphate groups are able to complex two molecules imipramine.

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