

KINETICS AND MECHANISM OF REACTIONS OF MAIN GROUP METAL IONS WITH BIOLOGICAL CARRIERS

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I. SURVEY ON KINETICS OF METAL COMPLEX FORMATION

Most of the information on rates of ligand substitution in metal complexes has been collected during the last decade following the introduction of modern techniques for the study of rapid reactions, such as e.p.r., n.m.r. and relaxation spectrometry. A condensed survey on characteristic rates of H_2O -substitution in the inner coordination sphere of metal ions is given in *Figure 1.1*,² Looking

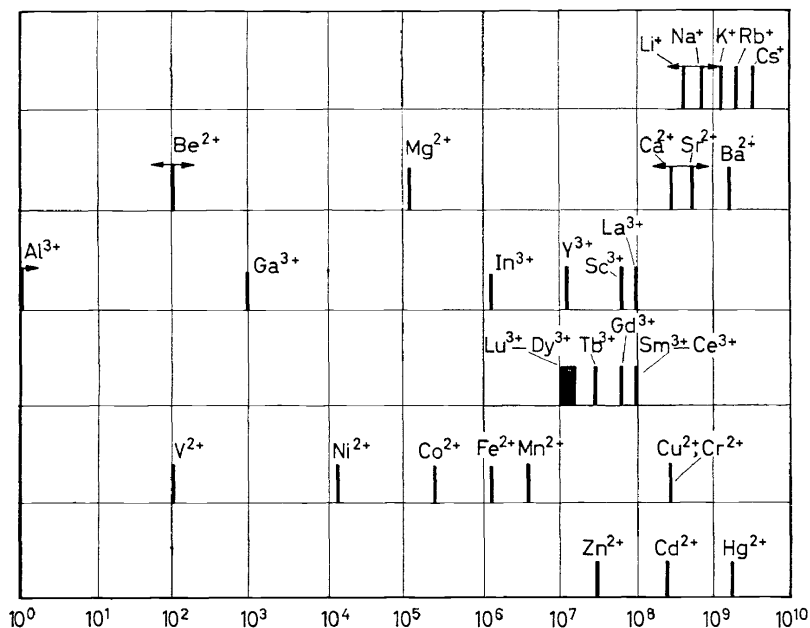


Figure 1. Characteristic rate constants (sec^{-1}) for substitution of inner sphere H_2O of various aquo ions.

at those data it may be justified to ask whether there is anything like a “characteristic rate” associated with a given metal ion, independent of the nature of the substituting ligand. For a large number of metal ions, such “characteristic rates” indeed have been found, at least as far as orders of magnitude (rather than precise numbers) are concerned.

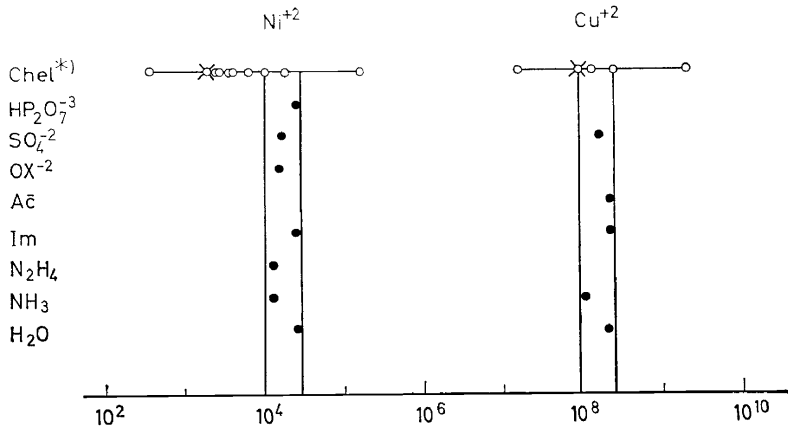
Actually, *Figure 1* includes three groups of metal ions. In the first group, the water molecules in the inner coordination spheres are so labile that substitution will occur at almost every encounter. As a consequence, the overall rate constants for metal complex formation are close to those for diffusion controlled reactions. The step of inner sphere substitution then cannot easily be separated from the overall rate. Thus, those data are only in a trivial sense characteristically associated with the nature of the metal ion.

A second group represents the other extreme: the water molecules in the inner coordination sphere are so tightly bound that hydrolysis, *i.e.* splitting of a water molecule, occurs more readily than substitution. The overall rate constant then is largely influenced by the nature of the ligand, (*e.g.* its basicity) and thus not characteristic of the metal ion only. (Note that a step of internal hydrolysis $\text{Me}(\text{H}_2\text{O})\text{X} \rightleftharpoons \text{MeOH} \cdot \text{HX}$ is not expressed in the pH-dependence of the rate constant). The behaviour of this group of metal ions is not yet very well understood since the overall mechanisms may involve quite a number of non-separable steps. Highly charged and small metal ions such as Be^{2+} , Al^{3+} , Fe^{3+} and most tetravalent ions belong to this group.

The majority of main group and transition metal ions, however, belong to the third category, showing well-defined substitution rates, almost independent of the nature of the substituting ligand. The substitution kinetics of this category of metal ions (including most ions of the first mentioned category) may be characterized by three rules:

1. The rate of substitution of a given solvent molecule in the inner coordination sphere depends mainly on the nature of the metal ion rather than that of the substituting ligand.

In *Figure 2* two examples are given: the substitution rates for nickel and copper complexes.² As far as second order rate constants for complex formation are concerned, they have to be corrected for the ion pair formation



*) Hen^\dagger , Htrien^\dagger , en, phen, 2-Mephen, dipy, tripy, HEDTA^{-3} , PAD, \times Mur

Figure 2. First and second order rate constants for water substitution in the coordination sphere of Ni^{2+} and Cu^{2+} for a series of ligands. The values for simple ligands are corrected to yield the first order rate constants of substitution, whereas overall constants are given for multidentate chelates (values from ref. 2).

in order to yield first order rate constants for the substitution rates. (The stability constant of the ion pair depends, of course, on certain properties of the ligand such as charge and configuration). As is seen from *Figure 2*, the rule holds quite well for simple substitution processes and still might be extended to multiple substitution as far as the first substitution steps are representative for the overall process of chelate formation (*cf.* below). Behaviour according to this rule is equivalent to an S_N1 type of mechanism, in which the main energy barrier results from loosening a metal ion water bond rather than from forming a new bond between the metal ion and the incoming ligand. (This classification is rather formal since a true S_N1 mechanism could represent only an extreme limit for such a "push-pull" substitution process).

2. *If—in multiple substitution—a ligand is more tightly bound than the substituted solvent molecule, it will labilize the rest of the inner sphere solvent molecules, whereas these will be stabilized, if the ligand is less tightly bound.*

Here "overall" charge of the ligand turns out to be less important than "local" charge or binding capacity, as was shown for a number of examples by Margerum,³ Hunt⁴ and Hague.⁵

3. *In general, the rate of substitution is slower the higher the charge and the smaller the ion.*

As seen in *Table 3* the substitution rates of Mg^{2+} -aquo-complexes are by 3 to 4 orders of magnitude smaller than for Ca^{2+} -aquo-complexes. This behaviour again is indicative of a S_N1 -type of mechanism, *i.e.* loosening of the metal ion solvent bond triggers the whole substitution process. Actually, there may be deviations from these rules for non symmetrical arrangement of the ligands. We quote two examples:

(i) The extreme differences in substitution rates for Ni^{2+} and Cu^{2+} or V^{2+} and Cr^{2+} result mainly from the Jahn-Teller effect loosening the axial bonds in d^9 and d^4 metal ions⁶ as opposed to the ligand field stabilisation of the symmetrical complexes of d^8 and d^3 metal ions.^{7, 8}

(ii) The unusually fast substitution in the earth and rare earth series—where Sc^{3+} is found to substitute even faster than a divalent ion of comparable size⁹—is possibly due to an unsymmetrical arrangement of the solvent molecules for coordination numbers larger than 6. Here again labile positions may occur which will substitute more quickly than in a symmetrical arrangement (*cf.* *Figure 3*).

The three rules quoted above must be taken as what they are: rules and not laws. They show clearly certain tendencies, but for a more precise comparison one should rely on more explicit considerations of the different interaction terms involved, such as: metal ion—ligand attraction, including electrostatic (ion—ion or ion—multipole) interaction; polarization and ligand field effects; (electrostatic) ligand—ligand repulsion and van der Waals terms. The energies of all these different interactions are quite large. Since for stability constants and activation parameters they compensate to a few kilocalories, computations of the single terms would require a high accuracy in order to yield reliable results. Actually, most of our knowledge still comes from experimental results like the above rules which might guide further theoretical attempts.

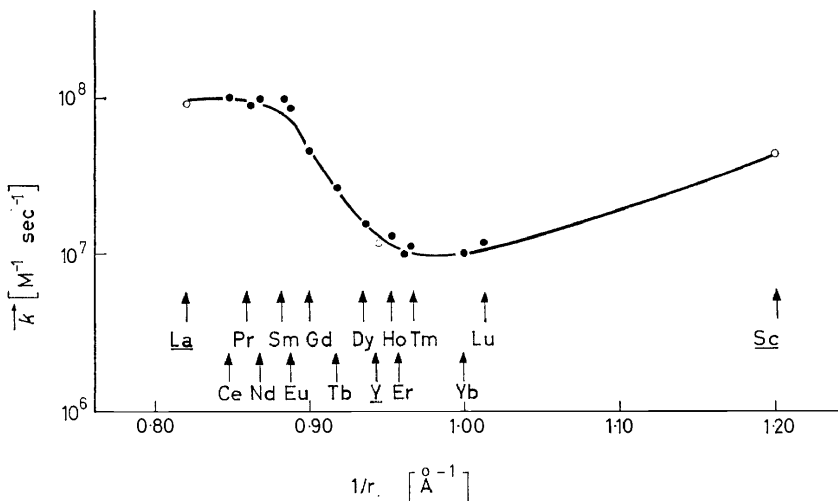


Figure 3. Second order rate constants for the complex formation of the trivalent lanthanide ions and of the earth metal ions with murexide. (from ref. ⁹)

II. COMPLEXES OF MAIN GROUP IONS

A fairly simple picture is to be expected for the ions of main group elements. Due to their "noble gas" like electron configuration they should show a straightforward relationship to simple physical properties such as size and charge. At first glance one might not find it too exciting to look at those complexes since one would not expect any sophisticated "chemistry". However, there are several nontrivial facts which immediately call for a closer investigation.

1. The free energies of solvation as well as the solvation enthalpies lie in the range of hundred to several hundred kilocalories (*cf. Table 1*)¹⁰ and

Table 1. Heat of solvation and free energy of solvation of alkali ions and alkaline earth ions (values from ref. ¹⁰).

	$-\Delta H (\text{H}_2\text{O})$		$-\Delta G (\text{H}_2\text{O})$		$-\Delta G_t (\text{H}_2\text{O} \rightarrow \text{CH}_3\text{OH})$ Strehlow
	Eley and Evans	Latimer	Eley and Evans	Latimer	
Li^+	133	121	123		
Na^+	115	95	108	91	-0.41
K^+	90	76	85	75	-0.03
Rb^+	81	70	75	70	+0.06
Cs^+	73	62		65	+0.15
Mg^{2+}	501		479		
Ca^{2+}	428		412		
Sr^{2+}	381				
Ba^{2+}	347		334		

yet, substitution usually occurs within 10^{-5} seconds (*e.g.* for Mg^{2+}) or even within 10^{-8} to 10^{-10} seconds (as for Ca^{2+} , Sr^{2+} , Ba^{2+} and the alkali ions).

2. The stability constants of the alkaline earth- (and also the alkali) complexes¹¹ usually do not show any simple dependence on the size of the

metal ion. Any simple model would suggest a monotonic decrease for the stability constant with increasing metal ion radius or the inverse of it. In fact, for a number of complexes both types of monotonic dependence are found, but in other cases stability constants show maxima for a certain particular size.

3. Many of the main group metal ions are of primary biological importance. Most striking is their highly specific behaviour in enzyme activation or in bioelectric phenomena. Such a specificity of metal-ion-binding by a "carrier", for instance, is just another example for the above mentioned non-monotonic behaviour.

Experimental data about stability constants and rates of alkaline earth- and especially of alkali-ion-complexes are very scarce in literature. Studies are difficult for three reasons:

(i) Complexes usually are weak and have to be studied at high metal ion concentrations.

(ii) Rate constants are very high, so that half times are in the micro-second range and below—especially at high concentrations, which have to be used for the study of complexes of low stability.

(iii) Reactions cannot usually be followed spectrophotometrically. There are only very few (if any) indicators which can be used.

Actually, a considerably increased sensitivity and time resolution of the relaxation methods was required for the studies reported below. The methods used in these studies were: (a) a T-jump device with a highly increased signal to noise sensitivity (order 10^4 at $1 \mu\text{s}$ risetime), constructed by C. R. Rabl¹² and (b) an electric field-pulse method utilizing travelling waves with a time resolution of 30 nanoseconds, developed by G. Ilgenfritz.¹³

In both methods the relaxation processes are recorded spectrophotometrically. Furthermore some sound absorption studies in the frequency range of 300 kHz to 30 MHz were carried out by F. Eggers¹⁴ using a new resonator method (which requires only 1 ml of sample solution instead of 100 to 1000 ml as required by conventional devices).

Before starting any discussion of experimental data we may ask for possible explanations of size-specificity of the metal ion, *i.e.* the non-monotonic size dependence. Specificity in binding is certainly not a consequence of a peculiar electron configuration as is the case with transition metal ions, such as Ni^{2+} , Cu^{2+} *etc.* We are dealing here with ions of "noble gas" like electron configuration and we must therefore try to base our model on factors such as charge and size of the metal ion and peculiarities of structure of the complexing agent only.

The complex formation involves the substitution of one or several solvent molecules from the inner coordination shell of the metal ion. If the incoming ligands can be arranged as freely as the solvent molecules in the coordination shell, then—at least for a symmetrical, *e.g.* octahedral arrangement—we should expect the two mentioned monotonic size dependencies:

(i) The incoming ligand is *more* tightly bound than the solvent molecule to be substituted: The stability of the complex will decrease with increasing radius of the metal ion. The smaller the metal ion the larger will be the gain in binding energy for the ligand.

(ii) The incoming ligand is *less* tightly bound than the solvent molecule:

Here the size dependence of complex stability will be inverted, since now the smallest metal ion will most strongly prefer the solvent molecules.

It is very important that in these cases with decreasing metal ion size the cavity of the ligand coordination shell can just as freely contract to the metal ion size as the solvent coordination shell. This is very often not possible if the ligands are interconnected as in multidentate chelate complexes. Here the different ligands usually have some freedom of motion only as long as the formed cavity exceeds a certain size. Below this size the ligands will, however, "freeze" into fixed positions since steric hindrances as well as ligand-ligand repulsion will prevent a further contraction of the cavity. We have thus an optimal fit of the cavity for a certain size of metal ion. For all metal ions larger than this critical size the complex stability will increase with decreasing metal ion radius, assuming the ligands are more tightly bound than the solvent molecules. Below the critical size, however, this behaviour will be inverted, since now there will be little or no gain in ligand binding energy with decreasing metal ion radius (since the size of the cavity is fixed), but there will be an increased requirement of energy for desolvation. Note that this optimal fit will produce a maximum in the thermodynamic stability constant but that this does not mean the optimally fitted complex is absolutely the most stable complex. This is clearly demonstrated by *Figure 4*. The lower curve describes the size dependence of the free energy of solvation (r being the metal ion radius). The upper curves represent two different size dependences for the free energy

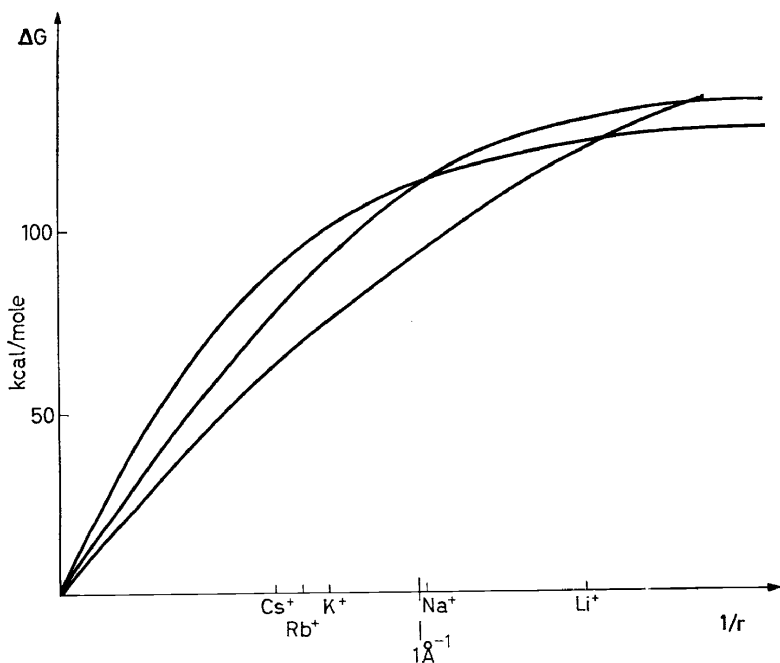


Figure 4. Dependence of free energies for ligand binding and solvation on reciprocal radius of metal ion (schematic).

Table 2. Stability constants of metal complexes.
 (log K_1 in aq. sol., $\mu \rightarrow 0$, $\mu^* = 0.1$; T = 18 — 25°)

	SO ₄ ²⁻	F ⁻	Ac ⁻	IDA ²⁻	NTA ³⁻	EDTA ⁴⁻	DGITA ⁴⁻	Metal Phthal. ⁶⁻	Erio R ³⁻	Tiron ⁴⁻	Oxime ⁻
Mg ²⁺	2.3	1.8	0.8	3.7	7.0	9.1	5.2*	8.9*	7.6*	6.9*	4.7
Ca ²⁺	2.3	<1.0	0.8	3.4	8.2	11.0	11.0*	7.8*	5.4*	5.8*	3.3
Sr ²⁺			0.4		6.7	8.8	8.5*			4.6*	2.6
Ba ²⁺		<0.5	0.4	1.7	..	7.8	8.4*	6.2*		4.1*	2.1
Li ⁺	0.6				3.3	2.8*					
Na ⁺	0.7				2.2	1.7*					
K ⁺	0.9										

of ligand binding (including rest solvation). These curves are monotonic, *i.e.* the smallest ion shows the largest values. However, due to the fact that the free energy of ligand binding bends sooner towards a saturation (brought about by steric fixation of the ligands), the difference of the ligand binding and solvation curve may well show a maximum. It is just this difference which determines the stability constant (K_{stab}) of the metal complex. Note that as long as the increase in free energy of solution exceeds the change in RT. In K_{stab} the free energy of ligand binding will be monotonic, regardless of whether K_{stab} increases or decreases. Note furthermore that the maximum of K_{stab} could occur at any position depending on how soon the ligand binding will reach saturation, and that this position does not necessarily coincide with the $1/r$ -value at which saturation is reached. (The picture of optimal adaptation of a metal ion to the minimal size of the ligand cavity was oversimplified above in order to demonstrate the principle).

We may now return to a discussion of experimental data. *Table 2* shows a compilation of some stability constants, mainly for the alkaline earth ions.² All types of behaviour as discussed above are found. In contrast to the stability constants the rate constants show a much more uniform behaviour as is seen in *Table 3*. Rate constants of complex formation are usually by

Table 3. Rate constants for complex formation reactions of Mg^{2+} and Ca^{2+} .

	Mg^{2+}	k_{form}	Ca^{2+}
SO_4^{2-}	$1 \times 10^5 \dagger$		
$\text{S}_2\text{O}_8^{2-}$	$1 \times 10^5 \dagger$		
CrO_4^{2-}	$1 \times 10^5 \dagger$		$\geq 5 \times 10^7 \dagger$
F^-	3.7×10^4		
HF	$\sim 4 \times 10^4$		
ATP ⁴⁻	1.3×10^7		$\geq 1 \times 10^9$
ATP ³⁻	$\sim 3 \times 10^6$		
ADP ³⁻	3×10^6		$\geq 3 \times 10^8$
ADP ²⁻	1×10^6		
Metal Phthal.	$(\sim 2 \times 10^6)$		$(\sim 7 \times 10^8)$
IDA ²⁻	9×10^5		$2.5 \times 10^8 \dagger$
			$\text{Sr}^{2+}: 3.5 \times 10^8 \dagger$
			$\text{Ba}^{2+}: 7 \times 10^8 \dagger$
Glycine ⁻			$4 \times 10^8 \dagger$
Oxine ⁻	3.8×10^5		$\geq 1 \times 10^8$
Oxine H	$\sim 1 \times 10^4$		

† First order rate constants.

3 to 4 orders of magnitude higher for Ca^{2+} than for Mg^{2+} . This is especially true if we reduce these values to the rate constants of substitution by correcting for the stability of the outer sphere ion pair (which depends on the charge of the complexing agent). Very uniform results are obtained for this step, even for multidentate ligands. One has to assume therefore that the complex formation rate is mainly determined by the rate of substitution of the first solvent molecules from the inner coordination shell, so that the individual differences of stability constants show up in the dissociation rather than the formation rates. This is not necessarily so for all the chelates, especially if chelate formation involves pre-equilibration of initial substitution steps.

REACTIONS OF MAIN GROUP METAL IONS

Data for Sr^{2+} and Ba^{2+} are more scarce. Stability constants usually decrease as compared to Ca^{2+} , so that either a monotonic decrease from Mg^{2+} to Ba^{2+} or a maximum for Ca^{2+} is found. The rate constants always were found to be higher for Sr^{2+} and Ba^{2+} than for Ca^{2+} . The differences, however, are not (and cannot be) very large, since the values are close to the limiting rate constant for a diffusion controlled reaction. The same holds for alkali ions. Some data from earlier experiments¹ are reported in *Table 4*. However, in most cases the assignment of rate and equilibrium constants was not very straight forward since the complexes are very weak, and in some cases overall rate constants, in other cases those of intermediate steps are evaluated from the relaxation data. Those earlier studies were done by the sound absorption method. *Figure 5* shows some sound absorption data

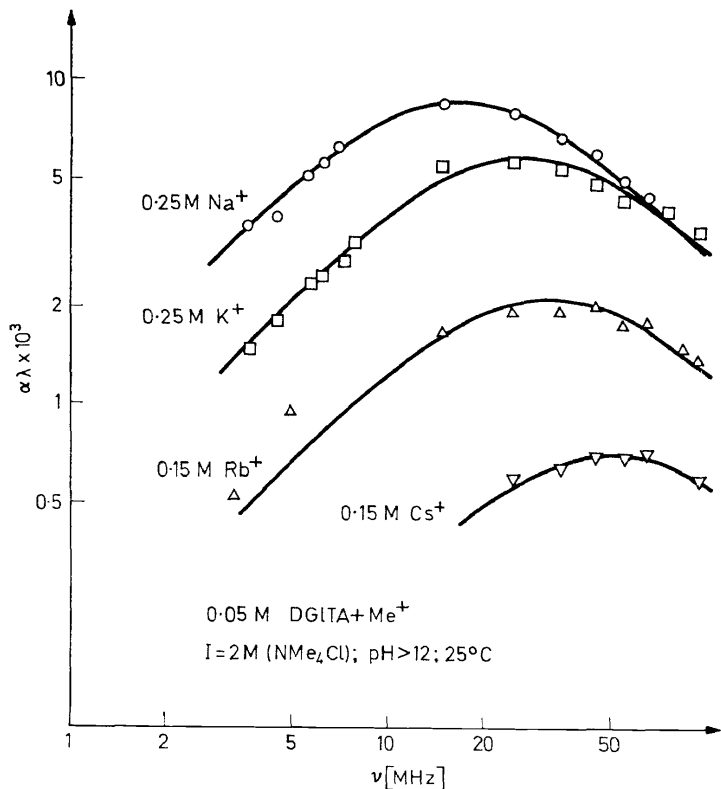


Figure 5. Frequency dependence of the excess sound absorption in aqueous solutions of alkali metal ions with DGITA.

for alkali complexes of DGITA (2,2'-ethylenedioxybis[ethyliminodi(acetic acid)]). The frequency of maximum absorption (per wave length) is represented by the reciprocal relaxation time ($\nu_{\max} = 1/2\pi \cdot \tau$)⁵. The curves show directly the increase of rate with increasing radius of the metal ion.

A much clearer picture of alkali ion complex formation arises if methanol is used as a solvent. Some more recent studies will be reported in the next section.

Table 4. Substitution rate constants for alkali metal ions.
 ($\mu = 2M$ (NMe₄Cl); pH > 12; 25°)

	NTA ³⁻		EDTA ⁴⁻		DGTA ⁴⁻	
	$\vec{k}[M^{-1}sec^{-1}]$	$\overleftarrow{k}[sec^{-1}]$	$\vec{k}[M^{-1}sec^{-1}]$	$\overleftarrow{k}[sec^{-1}]$	$\vec{k}[M^{-1}sec^{-1}]$	$\overleftarrow{k}[sec^{-1}]$
Li ⁺				1.4 × 10 ⁷	1.7 × 10 ⁸	2.8 × 10 ⁷
Na ⁺	1.9 × 10 ⁸	3.1 × 10 ⁷	1.0 × 10 ⁸	2.3 × 10 ⁷	2.0 × 10 ⁸	6.1 × 10 ⁷
K ⁺		1.0 × 10 ⁸	1.7 × 10 ⁸	3.9 × 10 ⁷	2.0 × 10 ⁸	9.0 × 10 ⁷
Rb ⁺		1.5 × 10 ⁸	8.5 × 10 ⁷	1.3 × 10 ⁸		2.3 × 10 ⁸
Cs ⁺		2.1 × 10 ⁸		2.0 × 10 ⁸		3.1 × 10 ⁸

III. ALKALI AND ALKALINE EARTH COMPLEXES IN METHANOL

Methanol as a solvent offers several advantages for the study of alkali ion complexes:

1. Due to the lower dielectric constant of methanol higher complex stabilities are found than in water, especially if charged ligands are involved. Thus a more precise determination of stability and rate constants of otherwise quite weak complexes is possible.

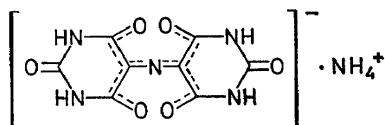
2. On the other hand, the solvation of the cation in water and methanol is quite similar, as was shown by Strehlow¹⁶. There are only small changes of ΔG , ΔH , and ΔS if an alkali ion is transferred from water to methanol. The substitution behaviour (mechanism and rate) might be quite similar for both solvents so that studies in methanol can be considered representative for water also.

3. Biological alkali ion carriers—the main object of our present investigation—due to their lipophilic character are usually quite insoluble in water, but fairly soluble in methanol.

4. Methanol can be used as a solvent for T-jump studies by both the Joule and the microwave heating method. For field effect and sound absorption studies it shows even some advantages. As a consequence of the lower dielectric constant the field effect for charged ligands is larger than in water. For uncharged ligands (*e.g.* the carriers described in section IV) relatively large ΔH -values are to be expected. Thus the equilibrium will be very strongly temperature dependent. Sound waves in methanol involve a pronounced temperature wave, which in water around room temperature is nearly absent (due to the density maximum at 4°).

The first problem was to find a suitable optical indicator for the alkali ions. No such indicator has been reported in literature so far. We remembered, however, that Schwarzenbach^{17, 18} described murexide to be an indicator for Ca^{2+} ions in aqueous solution.

Murexide is the ammonium salt of purpuric acid and has the following chemical structure.



The anion has a strong absorption maximum around 527 m μ ($\epsilon = 11700$ in methanol), which is shifted upon complexation with Ca^{2+} to shorter wave lengths. Complexation occurs most likely between two oxygens next to the N-bridge.

The stability constant for the Ca-complex in aqueous solution, as reported by Schwarzenbach,¹⁸ is not very large ($\text{p}K$ 2.7). Mg^{2+} was found to form no complex to any detectable extent whereas Sr^{2+} and Ba^{2+} showed some tendency for complexation.

Although several other indicators have been reported in literature having much higher stability constants for the alkaline earth ions, murexide seemed to be the best candidate for the indication of alkali ions in methanol due to several features:

1. Murexide has a negative charge (distributed among the four oxygens next to the N-bridge) which is not protonated in the neutral pH-range. Actually, the pK in aqueous solution is around 0 and in methanol around 4. Since alkali ions generally are quite weak in forming complexes it is very important that protons do not compete too strongly for the complexing site.

2. The spectral properties of murexide are quite peculiar. The blue shift of the absorption maximum upon complexation probably is due to some change in the orientation of the two rings relative to each other. Such an effect should be quite sensitive for weakly complexing ions, which otherwise will only be of little influence to the electronic structure of the dye molecule.

Murexide indeed turned out to be an ideal indicator for the alkali ions in methanol, especially with respect to the study of biological carriers.

Figure 6 shows the spectral shift of the absorption maximum upon titration with Na^+ . The well defined isobestic point indicates a simple complexation behaviour. Those titrations were carried out for the alkali and alkaline earth ions.

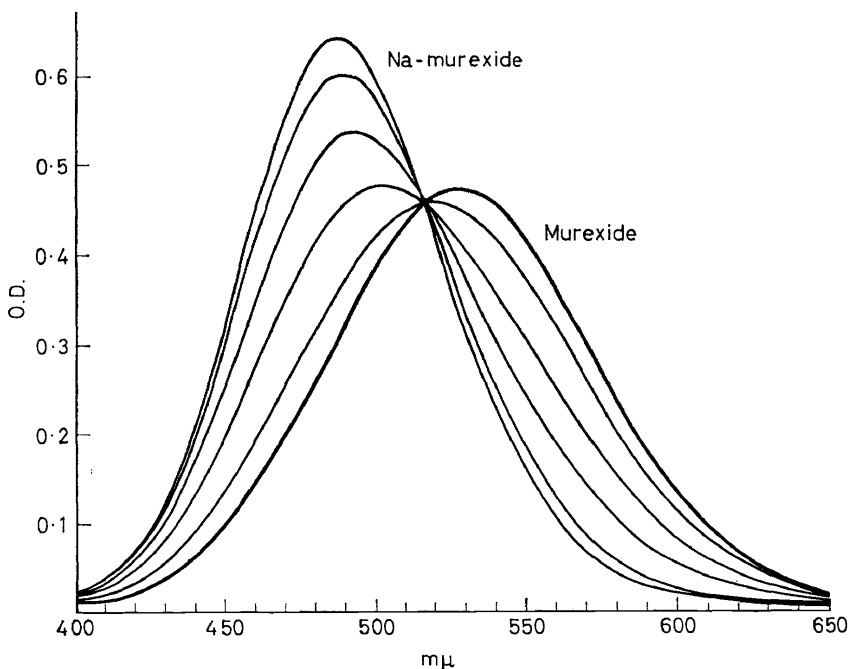


Figure 6. Optical spectra of murexide in methanol upon addition of increasing amounts of Na^+ -ions.

$$(C_{\text{Mur}} = 4 \times 10^{-5}\text{M}, C_{\text{Na}^+} = 1 \times 10^{-4} \text{ to } 8 \times 10^{-2}\text{M})$$

REACTIONS OF MAIN GROUP METAL IONS

In the alkali series the highest pK -values were found for Na^+ , in the alkaline earth series for Ca^{2+} —as was to be expected from Schwarzenbach's results for aqueous solutions. Generally the stability constants in methanol are higher by orders of magnitude than in water (*cf. Table 6*) and show much more clearly the non-monotonic size dependence as described in the preceding section. This maximum for Na^+ and Ca^{2+} results from the balance between ligand binding and solvation energy, whereas the "absolute" stability of the complexes should parallel monotonically the sequence of ionic sizes. This is clearly indicated by the amount of wave length shift and the increase in ϵ . Both $\Delta\lambda_{\text{max}}$ and $\Delta\epsilon$ are largest for the smallest ion and decrease monotonically with increasing size, as seen from *Figure 7*.

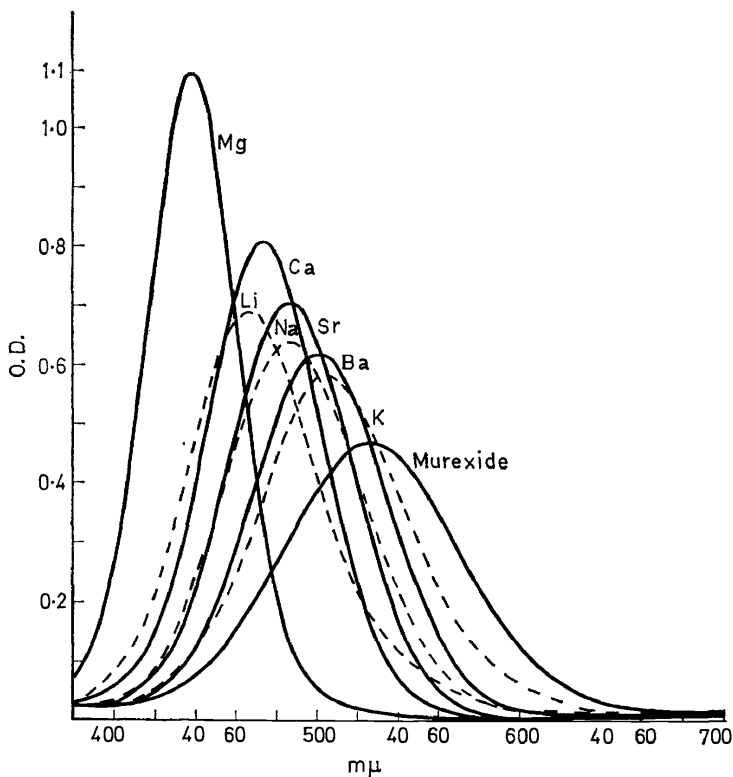


Figure 7. Optical spectra of murexide in methanol and of its complexes with alkali and alkaline earth ions.

$$(c_{\text{MUR}} = 4 \times 10^{-5}\text{M})$$

For the alkali-complexes the stability constants can be easily determined from the titration curves (*cf. Table 5*). Here K_{diss} is always much larger than the indicator concentration and therefore can be immediately obtained from plots of $(E_{\infty} - E)/(E - E_0)$ vs $1/C_{\text{M}}$ where the extinctions E , E_0 and E_{∞} refer to the metal ion concentrations C_{M} , 0 and ∞ (saturation) respectively.

This procedure cannot be used for the alkaline earth complexes. First, the stability constants (except for Mg^{2+}) are so high that they contribute only small correction terms to the titration curve, even at very low indicator concentrations. Second, the titration curves for Ca^{2+} , Sr^{2+} and (partly) Ba^{2+} showed their "halfpoints" (*i.e.* $(E_{\infty} - E_0)/2$) around a metal ion concentration $C_M^{\circ} \approx C_{\text{Ind}}^{\circ}/4$ (*i.e.* close to a fourth of the indicator concentration).

Only Mg^{2+} showed a "halfpoint" close to $C_M^{\circ} \approx C_{\text{Ind}}^{\circ}/2$ as is to be expected for the formation of a 1:1 complex of high stability. However, even here a plot of $(C_M^{\circ})/(E - E_0)$ vs. $1/(E_{\infty} - E)$ ¹⁹ did not yield a straight line except for high ionic strengths, where a $K_{\text{diss}} > C_{\text{Ind}}^{\circ}$ was obtained. Such behaviour is indicative of 1:2 complex formation, which in case of Ca^{2+} is so strong that saturation occurs for any excess of metal ions even at the lowest usable indicator concentrations. Sr^{2+} and Ba^{2+} show also some transition region for the isobestic point which may be interpreted in the same way. Under these circumstances titration curves do not provide much information about the stability constants of the single steps. We therefore used another quantity, namely relaxational amplitudes, to learn more about the complexation of alkaline earth ions with murexide.

A suitable indicator for dynamics studies should have not only pronounced spectral shifts, but also a sufficiently rapid performance. Only then can it be used for the observation of other complexation reactions. Actually, the indicator may still be used if it reacts with a speed comparable to that to be followed. In this case, however, one has to know precisely the rate constants of the indicator for the evaluation of the relaxation spectrum of the coupled reaction system. Therefore it was necessary to carry out rate measurements with the indicator-metal ion system.

For all the alkali ions these reactions turned out to be too fast to be resolved by the temperature jump technique, half times being below the μsec -range. However, a new electric field pulse technique with spectrophotometric observation was just ready in time to be used for these studies. In this technique the perturbation of equilibrium is brought about by a strong electric field utilizing the dissociation field effect. It is applicable to any equilibrium between charged reaction partners. The spectrophotometric observation allows a determination of the equilibrium shift, even if the reactant to be followed is present in low concentration compared to other charged species. (This is the main advantage over the previously used conductometric field technique). The field pulse is produced by cable discharge. The resulting travelling wave has a very steep front.

The present time resolution of this method is 30 nanoseconds. This resolution was just suitable to detect the relaxation effect of the reactions of Li^+ and Na^+ with murexide. In *Figure 8* a typical oscillogram is shown. K^+ (requiring a higher concentration due to its lower stability constant) was already beyond the resolution of this method. However, a lower limit for the rate constant could be derived which almost coincides with the upper limit for a diffusion controlled process. Even the measured value for Na^+ is already very close to this limiting value of about 2 to $3 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$ (which for charged species is somewhat higher in methanol than in water due to the lower dielectric constant of methanol favouring the electrostatic

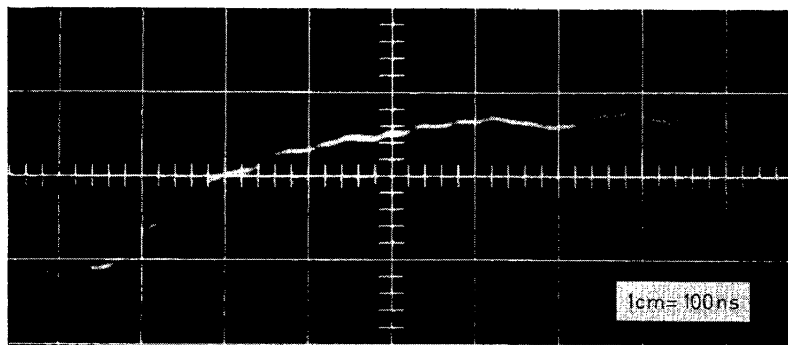


Figure 8. Chemical relaxation in the system Na^+ murexide, brought about by high electric field pulse.

$$(\text{C}_{\text{Mur}} = 4 \times 10^{-5} \text{ M}, \text{I}_{\text{Na}^+} = 1 \times 10^{-4} \text{ M})$$

attraction of the reactants). The present results suggest that the rate constants for K^+ , Rb^+ and Cs^+ are diffusion controlled. Only Li^+ shows a considerably lower value. Table 5 shows a compilation of equilibrium and rate constants for the reactions of alkali ions with murexide in methanol.

Table 5. Murexide complexes in methanol (25°).

	$k_t [\text{M}^{-1} \text{sec}^{-1}]$	$k_D [\text{sec}^{-1}]$	$K_{\text{stab}} [\text{M}^{-1}]$
Li^+	$(4 \pm 1) \times 10^9$	5×10^6	8×10^2
Na^+	1.4×10^{10}	5.6×10^6	2.5×10^3
K^+	$> 1.2 \times 10^{10}$	$> 10^7$	1.2×10^3

We may conclude that from both a static and dynamic point of view murexide appears to be an ideal indicator for alkali ions in methanol. The spectral shifts are characteristic and easily detectable. The stability constants are high enough and in a very convenient range (most convenient for an investigation of biological carriers, *cf.* below). The rates are as high as they could be, *i.e.* diffusion controlled, and thus suitable to couple quickly with any other complex reaction.

Less ideal—but not less interesting—is the behaviour of alkaline earth complexes. Although details about these systems will be reported in a forthcoming paper we may mention some general features which are of interest to coordination chemists.

As shown above the situation is complicated by the very high stability constants and by the tendency for the formation of 1:2 complexes.

The best understood case is that of Mg^{2+} . The substitution rates for Mg^{2+} in water are in the neighbourhood of 10^5 sec^{-1} (*cf.* Table 3). In methanol we should find similar behaviour and thus the relaxation effects should be in

the time range covered by the T-jump method. *Figure 9* contains a typical oscillogram for the relaxation effect of the Mg^{2+} murexide complexes in methanol. Evaluation of the concentration dependence of the measured relaxation times yields a rate constant of $8 \times 10^6 \text{ M}^{-1}\text{sec}^{-1}$ for the recombination of Mg^{2+} with murexide. Considering the fact that in methanol the ion-pair is more stable than in water we estimate a rate constant of substitu-

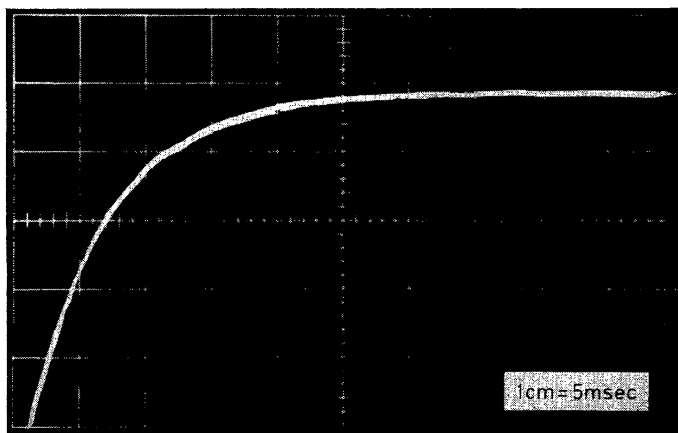


Figure 9. Temperature-jump relaxation effect of the system Mg^{2+} murexide in methanol solution.

$$(C_{\text{Murex}} = 4 \times 10^{-5} \text{ M}, C_{\text{Mg}^{2+}} = 6 \times 10^{-5} \text{ M}, \mu = 0.01 \text{ M})$$

tion of about $5 \times 10^4 \text{ sec}^{-1}$. This is only slightly less than in water and suggests a quite similar substitution behaviour for methanol and water. Correspondingly we should expect rates for Ca^{2+} , Sr^{2+} , and Ba^{2+} higher by about 3 to 4 orders of magnitude. They actually are found in the μ -second region using the electric field pulse technique.

The amplitudes of these relaxation effects pass through a very steep maximum for $C_{\text{M}}^{\circ} \approx C_{\text{Ind}}^{\circ}/2$ as is seen from *Figure 10*. It can be shown that relaxational amplitudes contain all the information about stability constants and reaction enthalpies, much more than titration curves do. In the titration curve the dependence of extinction on metal ion concentration is observed. If the stability constant is large compared to $1/C_{\text{Ind}}^{\circ}$ the titration yields mainly the indicator concentration and only small correction terms containing the stability constant. In the relaxational amplitudes the dependence of the extinction on $\ln K$ is directly measured. Here all terms not containing the stability constant drop out. The use of this method for a determination of stability constants and reaction enthalpies is described in detail in a forthcoming paper (*cf.* also ref. ²⁰).

The measurements yield for methanol the same order of stabilities as found for H_2O by Schwarzenbach,¹⁸ namely $\text{Ca}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+} \gg \text{Mg}^{2+}$, the absolute values being shifted by orders of magnitude.

REACTIONS OF MAIN GROUP METAL IONS

Ca^{2+} -Murexide
 $10\mu\text{s}/\text{cm}$

$[\text{Ca}^{2+}]$

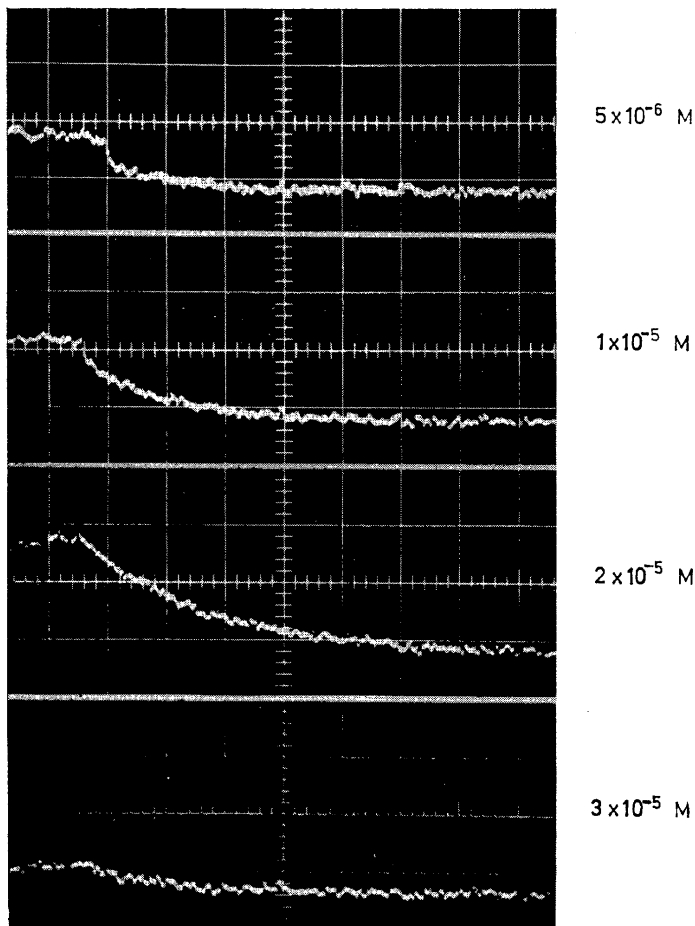


Figure 10. Chemical relaxation in the system Ca^{2+} murexide in methanol, as brought about by high electric field pulses. ($C_{\text{Mur}} = 4 \times 10^{-5} \text{ M}$)

IV. THE STUDY OF BIOLOGICAL CARRIERS

The aim of the studies described in the preceding section was primarily to provide a fast responding optical indicator for the alkali ions. It turned out, however, that these studies reflect already some of the features of a size specific carrier. With this information available we may predict some rules for the design of such a carrier:

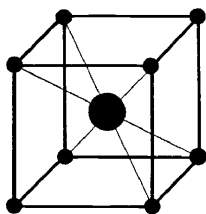
1. As many solvent molecules of the inner coordination sphere as possible should be replaced by the coordinating sites of the carrier molecule. For two ions of different size the reference state may then involve as much as the total difference of free energies of solvation. (At room temperature 1.4 kcal/mole corresponds to one order of magnitude in stability constant).

REACTION OF MAIN GROUP METAL IONS

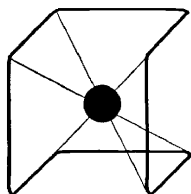
arrangement, whereby the cyclic molecule is wrapped up like a tennisball seam (*cf. Figure 12, 13*). All polar groups are "inside" the cyclic molecule leaving a cavity for the metal ion which is optimally adapted to the size of K^+ as reflected by the stability constants (*cf. Table 6*). The values in *Table 6* were determined by Simon²⁴ using the technique of vapour phase osmometry. As is seen, the Na^+ -complex of monactin is weaker by more than 2 orders of magnitude than the K^+ -complex. Li^+ does not show detectable complex formation at all whereas Rb^+ and Cs^+ complex measurably but weaker than

Table 6. Stability constants of some alkali ion macroretroside complexes in methanol at 30° (values from ref.²⁴)

Ligand	Stability Constant $K[M^{-1}]$		
	Na^+	K^+	K_{K^+}/K_{Na^+}
Nonactin	$(1.3 \pm 0.2) \times 10^2$	$(5.0 \pm 0.7) \times 10^3$	38 ± 5
Monactin	$(1.1 \pm 0.1) \times 10^3$	$(2.5 \pm 1.0) \times 10^5$	230 ± 70



Cubic arrangement



Tennis ball seam

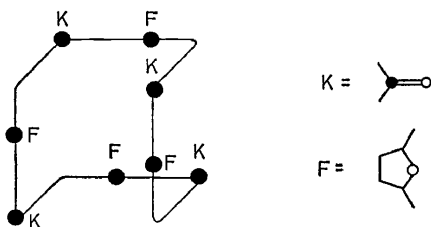


Figure 12. Simplified spatial structure of metal complexes of the macroretroside.



Figure 13. Molecular model of nonactin compared with a hydrated metal ion (K^+). The cyclic nonactin molecule is twisted into a shape similar to the seam of a tennis ball, with the hydrophobic groups at the outside and the coordinating carbonyl and furane oxygens directed to the centre.

K^+ . The complex formation can be followed spectrophotometrically using murexide as indicator. Figure 14 shows the decrease of the Na^+ murexide complex absorption upon addition of monactin. This is the method we have to use in order to follow the reaction behaviour of monactin "instantaneously".

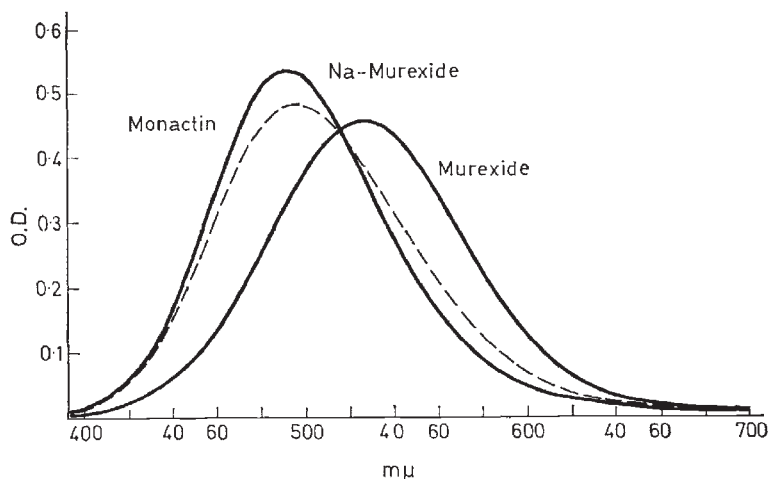


Figure 14. Optical spectra of murexide in methanol in the absence and in the presence of Na^+ ions. The broken line shows the effect of adding monactin to the Na^+ murexide system, indicating a decrease of the free Na^+ ion concentration due to complex formation of Na^+ with monactin.

$$(C_{MUR} = 4 \times 10^{-5} M, C_{Na^+} = 1.4 \times 10^{-3} M, C_{MON} = 3.2 \times 10^{-3} M)$$

How fast does complex formation occur? This is a very interesting question in view of the particular structure of the metal complex. The ligands surround the metal ion completely. How fast can the metal ion strip off all solvent molecules in order to slip into the cavity? Can the carrier molecule "breathe" enough to allow for a stepwise "redressing" process? It was very hard to guess any rate since such a reaction could be quite slow.

From equilibrium studies we knew, that equilibration is complete within seconds. Thus we tried first to measure the rates with the help of the temperature jump technique. Due to the fact that the sample cell represents the resistance of the discharge circuit we could not reach very high time resolution. (Any cation concentration had to be kept small enough not to interfere with the complex formation to be observed). The result of these studies was: any equilibration with the monactin molecule was complete within fractions of milliseconds. That complex formation really occurred during this period followed quite clearly from the amplitude of the concentration shift, which was more than twice as large as with the indicator alone (under corresponding conditions).

The next method we tried was the electric field pulse technique, as used for the Na^+ murexide reaction. However, there is no direct response of the Na^+ monactin equilibration since the process does not involve any change of charge and therefore does not show a measurable dissociation field effect. On the other hand, the application of the high electric field produces some heating within a few microseconds. Here again we found that equilibration with monactin was complete within the heating period (which was $5\mu\text{s}$ with this method).

This result was quite unexpected because a relaxation time this short would impose a lower limit for the rate constant of complex formation of $10^8 \text{ M}^{-1}\text{sec}^{-1}$. The limiting value for a diffusion controlled reaction of *uncharged* species in methanol should be around $3 \times 10^9 \text{ M}^{-1}\text{sec}^{-1}$. If a stepwise stripping would have a 50 per cent probability for each ligand to be exchanged, a maximum value of about $5 \times 10^8 \text{ M}^{-1}\text{sec}^{-1}$ were to be expected. Apparently we are very close to this value and it is now more a matter of sportsmanship to determine any exact number. †

All qualitative conclusions can already be drawn from the results known so far. Apparently the molecule can open up to quite an extent to allow for a stepwise exchange thus yielding optimal carrier performance. Since for Na^+ the reaction is almost diffusion controlled, K^+ can not have a much higher rate. Thus the main differences of specificity in binding will show up in the dissociation rates. Here we see immediately why a high rate of complex formation is required for optimal performance. After transporting the metal ion through a membrane the carrier has to be unloaded fast enough. Assume a forward rate constant of $5 \times 10^8 \text{ M}^{-1}\text{sec}^{-1}$ and a stability constant of $2.5 \times 10^5 \text{ M}^{-1}$ —as was found for K^+ —the resulting rate constant of dissociation then would be $2 \times 10^3 \text{ sec}^{-1}$. Thus the elementary—possibly rate limiting—step of transport through a membrane would not surpass essentially the millisecond range, even for such an optimally behaving system.

† Meanwhile the rate constant could be extrapolated to $3 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$ from sound absorption measurements carried out in collaboration with F. Eggers (to be published).

In conclusion we may say that—although these problems are still far from being solved—some general picture of biological alkali ion transport is emerging. The “Maxwell-Demon” is disappearing. It is replaced by careful structural and dynamic analysis which shows that specificity in alkali ion binding is not due to some obscure yet unknown chemical property of the carrier but rather due to a specific structural design which utilizes the superposition of otherwise well known interactions.

SUMMARY

The factors influencing the complex stability and kinetics of alkali and alkaline earth ions are discussed. A monotonic radius dependence is to be expected for the binding of monodentate ligands. Size specificity, on the other hand, is characteristic for certain chelates in which the ligands freeze into fixed positions forming a cavity of minimal size. Superposition of the free energies for ligand binding and solvation can lead to a pronounced maximum of complex stability for a particular size. The action of biological carriers is based on this principle.

After a survey on available data recent equilibrium and rate studies with the potassium specific carrier monactin using murexide as optical indicator are reported. Murexide is shown to be an ideal indicator for alkali ions in methanol. It forms stable 1:1 complexes with all alkali ions, thereby shifting the absorption peak considerably to shorter wave lengths. Complex formation is nearly diffusion controlled. Alkaline earth ions also form 1:2 complexes.

The macrotetrolide monactin has been shown to bind preferably potassium. The sodium complex is by about two orders of magnitude less stable than the potassium complex, but can conveniently be used for rate studies. Complex formation occurs very rapidly. The fast substitution of the solvent molecules from the inner solvation sphere is most likely effected by a stepwise process, in which the twisted ring molecule opens up to a considerable extent. Such a flexibility of structure establishes another important principle for carrier action. The studies reported in this paper were carried out by relaxation spectrometric methods utilizing time constants and relaxational amplitudes.

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