# A General Method for the Determination of the Kinetic Stability of Macrocyclic Alkali-metal Complexes with Rates of Decomplexation below $10^{-3}$ $s^{-1}$

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A general method has been developed for the determination of kinetic stabilities of macrocyclic alkali-metal complexes with rates of decomplexation ($k_d$) below $10^{-3}$ $s^{-1}$, by use of radioactive isotopes. This method offers the possibility to study the influence of the solvent polarity and of the salt concentration in solution on the rate of decomplexation of macrocyclic metal complexes. Further advantages are the small amounts of ligand required for these determinations and the simplicity of the method. Furthermore, it is possible by this method to study the 'degenerate' exchange of sodium for sodium and of rubidium for rubidium. By this method the kinetic stabilities of the sodium and rubidium complexes of calixspherands **1–4** were determined. Calixspherand **3** forms kinetically very stable complexes with sodium and rubidium cations in acetone and $Me_2SO$ in the presence of high concentrations of sodium cations in solution; half-life times of exchange are 855 ($Na^+$) and 528 ($Rb^+$) h in acetone and 352 ($Na^+$) and 845 ($Rb^+$) h in $Me_2SO$, respectively. The results of this method were verified by an independent $^1H$ NMR spectroscopic method.

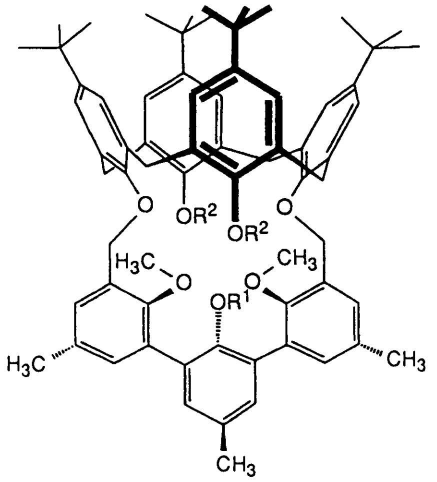
For our programme on the design and synthesis of *kinetically* stable alkali-metal cation complexes for medical applications[1] such as organ imaging (radioimmunoscintigraphy) and therapy (radioimmunotherapy),[2] we need general and sensitive methods to evaluate the kinetic stabilities of these complexes.

Only a limited number of methods is known for the determination of the decomplexation rate constant ($k_d$), which determines the kinetic stability. These are stopped-flow methods,[3] methods based on $^1H$ NMR spectroscopy[4] and NMR spectroscopy of alkali-metal nuclei such as $^7Li$, $^{23}Na$, $^{39}K$, $^{85}Rb$ and $^{133}Cs$,[3] and the competition method used by Cram for the spherands.[5] The stopped-flow methods are limited to slow processes and processes that can be detected by spectrophotometry or conductometry. The NMR methods on the other hand, suffer from quadrupole broadening which decreases the sensitivity. Another disadvantage is that this method often depends on line-broadening and coalescence in a specific temperature window. Kinetically very stable complexes will not show coalescence at temperatures suitable for such NMR experiments, *i.e.*, $T < 150$ °C.[3] The competition method used by Cram for the spherands (we used for the calixspherands)[1,6] comprising the exchange of alkali-metal ions from a non-deuterium-labelled complex to a partially deuterium-labelled complex, is limited to ligands that have in their $^1H$ NMR spectra well separated signals of groups that can be simply introduced as the deuteriated form. Moreover, these groups should exhibit shift differences between the complex and the free ligand.[5] So far this method has only been used in $CDCl_3$ saturated with $D_2O$ and not in more polar solvents. The solvent polarity, however, may have a pronounced influence on the rate of decomplexation of metal complexes, as has previously been shown for the cryptands[7] and cryptahemispherands.[1] More importantly, it is not possible by this method to study the influence of interfering cations in solution. Finally, all the methods lack the possibility of studying the 'degenerate' exchange of sodium for sodium and rubidium for rubidium.

In this paper a general method for the determination of the kinetic stability of alkali-metal complexes is described, which comprises the exchange of radioactive metal ions complexed in a macrocyclic ligand with excess non-radioactive metal ions present in solution. The method allows the study of the influence of solvent polarity and of the salt concentration in solution on the rate of decomplexation of the complexes. Furthermore, the 'degenerate' exchange of sodium for sodium and rubidium for rubidium, can be studied by this method. In order to verify the validity of this method the rates were compared with those obtained with $^1H$ NMR spectroscopy in the same solvent.

## Results

The rates of exchange for the sodium and rubidium complexes of four calixspherands[6] **1–4** (Fig. 1) were determined from an analysis of the exchange of radioactive metal ions complexed in a macrocyclic ligand with non-radioactive metal ions present in solution. Furthermore, the effect of different solvents and of the different alkali-metal cations on the stability of the complexes was studied. To predict the behaviour of the complexes *in vivo*, it would be advantageous to determine the stability in aqueous solution. However, since the calixspherands are insoluble in water, the exchange studies were carried out in acetone or $Me_2SO$. These solvents were selected because of their difference in polarity, and the fact that the calixspherands and the alkali-metal picrates are both sufficiently soluble in these solvents. For the exchange studies, the calixspherands were first complexed with *radioactive* sodium or rubidium cations by the addition of an aqueous solution of *radioactive* salt to an acetone solution of the calixspherand. The free salt was separated from the calixspherand complexes by the addition of chloroform to the solution and fourfold extraction with water. After evaporation of the solvent the *radioactive* complexes (*ca.* $25 \times 10^{-9}$ mol) were dissolved in a 10 mmol $dm^{-3}$ *non-radioactive* salt solution. In this way solutions with a large excess of salt, compared with the amount of the calixspherand (CSP) complex could be applied. At certain time intervals the complex was separated from the free salt and the ratio between complexed and free radioactive salt was determined. These ratios were plotted against time and the kinetic analysis was performed by non-



| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | $CH_3$ | $CH_3$ |
| **2** | $CH_2CH_3$ | $CH_3$ |
| **3** | $CH(CH_3)_2$ | $CH_3$ |
| **4** | $CH_3$ | $CH_2CH_3$ |

**Fig. 1** Structures of the calixspherands **1–4**

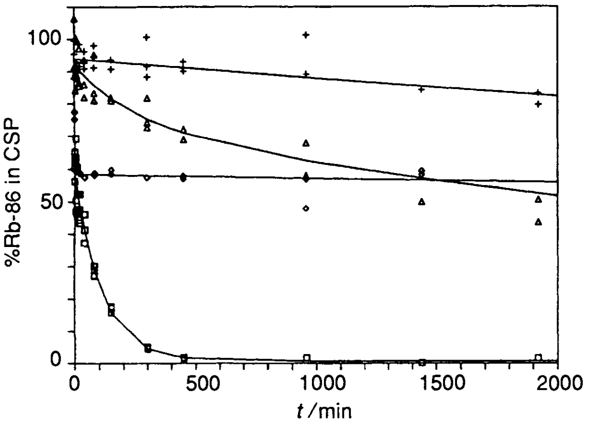


**Fig. 2** Exchange of $^{86}Rb^+$ for $Na^+$ in acetone: □, **1**; +, **2**; ◇, **3**; △, **4**

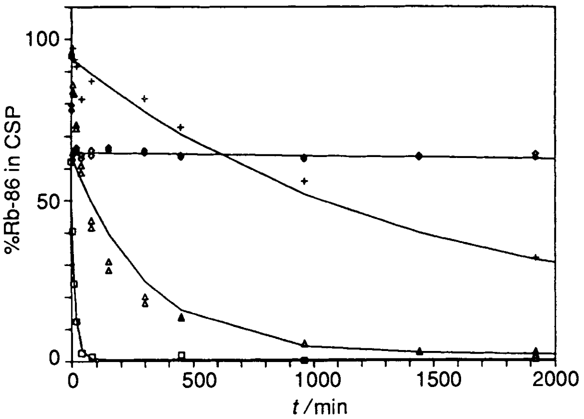


**Fig. 3** Exchange of $^{86}Rb^+$ for $Na^+$ in $Me_2SO$: □, **1**; +, **2**; ◇, **3**; △, **4**

linear curve-fitting[8] to give half-life times for the exchange process. In all cases first-order kinetics was observed.

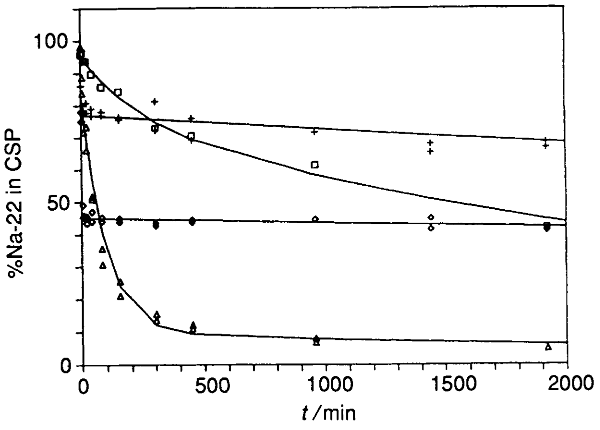


**Fig. 4** Exchange of $^{22}Na^+$ for $Na^+$ in $Me_2SO$: □, **1**; +, **2**; ◇, **3**; △, **4**

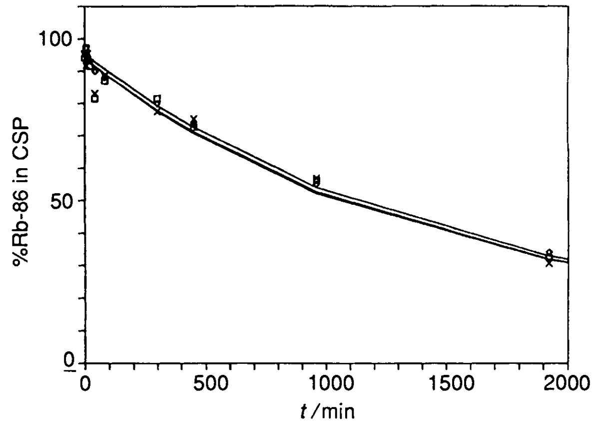


**Fig. 5** Exchange of $[\mathbf{2}\cdot Rb]^+$ with different cations in $Me_2SO$: □, $Na^+$; ◇, $K^+$; ×, $Rb^+$

**Table 1** Half-life times in hours for the exchange of the different calixspherand complexes with *sodium picrate* in $Me_2SO$ and acetone

| | Calixspherand | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | | **2** | | **3** | | **4** | |
| Solvent | $Na^+$ | $Rb^+$ | $Na^+$ | $Rb^+$ | $Na^+$ | $Rb^+$ | $Na^+$ | $Rb^+$ |
| Acetone | 655 | 1.3 | 400 | 175 | 855 | 528 | 125 | 65 |
| $Me_2SO$ | 42 | 0.2 | 192 | 15 | 352 | 845 | 1.1 | 6.3 |

Results of the experiments in which the exchange of complexed $Rb^+$ for excess *non-radioactive* $Na^+$ present in the acetone or $Me_2SO$ solutions was studied, are shown in Figs. 2 and 3, respectively. The low, but stable, level of $[\mathbf{3}\cdot Rb]^+$ is, most likely, due to additional unspecific binding of $Rb^+$ to **3**. This unspecifically bound $Rb^+$ is quickly liberated, whereas the $Rb^+$ complexed in **3** is strongly bound. The same experiments were performed with the sodium complexes of the different calixspherands. For all four calixspherands a slow exchange of $Na^+$ for $Na^+$ in acetone was found. The results obtained in $Me_2SO$ are shown in Fig. 4. Just as for $Rb^+$, unspecific binding of $Na^+$ to **3** was also observed.

The calculated half-life times for exchange in acetone and $Me_2SO$ are depicted in Table 1. From this Table it is clear that the order of stability of the different complexes is the same in both solvents although there are differences in the rates of exchange (*vide infra*).

Besides the influence of the solvent on the stability of the complexes, the influence of the type of cation in solution was studied. For that purpose, potassium and rubidium picrate

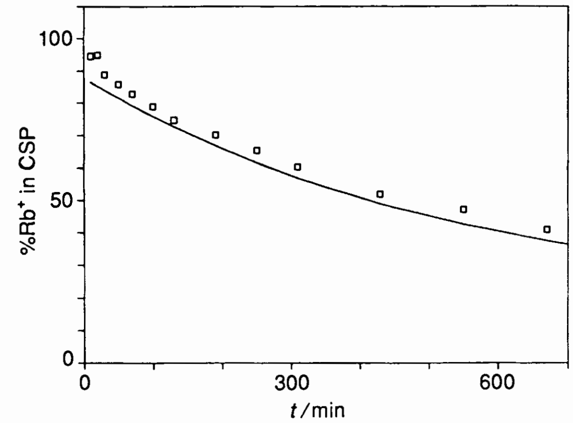


**Fig. 6** Exchange of [**4**·Rb]$^+$ for Na$^+$ in Me$_2$SO: □, points determined by $^1$H NMR spectroscopy; ——, calc. from radioactivity

solutions were used instead of solutions of sodium picrate. From the results for [**2**·Rb]$^+$, as shown in Fig. 5, it can be seen that there is no difference in the rate of exchange, to within experimental error, when different cations are in solution. Similar behaviour was found for the other rubidium complexes and for all the sodium complexes. Finally, experiments were carried out in which no salt was added to the solution (*vide infra*).

To validate the results obtained with the radioactive isotopes, control experiments were conducted using $^1$H NMR spectroscopy. In these experiments a 4 mmol dm$^{-3}$ solution of calixspherand–rubidium complex in Me$_2$SO (0.3 cm$^3$) was mixed with a 20 mmol dm$^{-3}$ solution of sodium picrate in Me$_2$SO (0.3 cm$^3$). The exchange of Rb$^+$ for Na$^+$ for the calixspherands **1** and **4** was monitored by the differences in their $^1$H NMR spectra. Exchange of [**1**·Rb]$^+$ with Na$^+$ in Me$_2$SO was too fast to be followed by $^1$H NMR spectroscopy. A good agreement was found for the exchange of [**4**·Rb]$^+$ with Na$^+$ in Me$_2$SO (Fig. 6). This proves that the exchange method used to calculate the amount of complexed cation is valid.

## Discussion

*Influence of Salts in Solution.*—The observation that, to within experimental error, the type of cation in solution does not influence the rate of exchange is an indication that the rate-determining step in the exchange process is the decomplexation of the complexed cation (Fig. 5). This means that the decomplexation is a simple dissociation [eqn. (1)] of the cation-complex rather than occurring *via* a bimolecular process [eqn. (2)].

$$M_1^+\cdot CSP + M_2^+ \rightleftharpoons M_1^+ + CSP + M_2^+ \rightleftharpoons M_1^+ + CSP\cdot M_2^+ \quad (1)$$

$$M_1^+\cdot CSP + M_2^+ \rightleftharpoons M_1^+\cdot CSP\cdot M_2^+ \rightleftharpoons M_1^+ + CSP\cdot M_2^+ \quad (2)$$

Further evidence for this mechanism was obtained from an experiment in which no salt was added to the solution. Surprisingly disappearance of the complexed rubidium cation, from [**1**·Rb]$^+$ or [**2**·Rb]$^+$, was observed at a rate similar to those in the experiments in which salt was added to the solution. This experiment was repeated with a non-radioactive rubidium complex ([**1**·Rb]$^+$). Examination of the organic layer by mass spectrometry showed that besides the rubidium complex, the sodium and potassium complexes were present; the sodium and potassium ions are most probably liberated from the glassware. This indicates that no influence is exerted by the salt concentration in solution.

It may be concluded that the rate-limiting step in the exchange process is the dissociation, and therefore the observed exchange rates are equal to the rates of decomplexation.

Furthermore, the experiments in which $^{22}$Na$^+$ is exchanged for Na$^+$, and $^{86}$Rb$^+$ for Rb$^+$, indicate that, by this method, it is possible to study the exchange of two different isotopes of one element.

*Influence of Solvent.*—When the half-life times for exchange obtained in acetone are compared with the half-life times obtained in Me$_2$SO, it is obvious that the stabilities of the complexes decrease with increasing polarity of the solvent.

The observed influence may be explained by the fact that the rubidium ion is more weakly solvated than the sodium ion. Therefore, the difference between acetone and the better solvating Me$_2$SO, should be the largest for sodium. The observed effects are comparable to the results obtained by Cox *et al.* for the cryptands,[7] although the stability of the cryptand complexes is influenced much more by the polarity of the solvent. Furthermore, the results indicate that there is no change in the mechanism of exchange on going from the less polar solvent acetone to the more polar Me$_2$SO. Such a change in mechanism was postulated for the cryptahemispherand complexes on going from chloroform to water.[1]

*Influence of the Structure of the Calixspherands.*—Table 1 shows that, for the rubidium complexes, the replacement of one methoxy group on the *m*-terphenyl moiety in **1** by an ethoxy or isopropoxy group or two methoxy groups on the calix[4]arene moiety ethoxy groups leads to a large increase in stability. The half-life time for exchange in acetone increases from 1.3 h for **1** to 65 h for **4**, 175 h for **2** and 528 h for **3**. The effect for **4** thus appears to be less pronounced than for **2** and **3**. Therefore, it can be concluded that the replacement of a methoxy group on the *m*-terphenyl moiety by an ethoxy or isopropoxy group has the largest effect on the stability of the rubidium complex, whereas the replacement of methoxy groups on the calix[4]arene moiety by ethoxy groups has a less pronounced effect on the stability. For the sodium complexes only the introduction of one ethoxy or isopropoxy group onto the *m*-terphenyl unit as in **2** and **3**, respectively, gives an increase in stability compared with **1**, whereas the introduction of two ethoxy groups onto the calix[4]arene unit as in **4** gives a decrease in stability compared with **1**.[6] The change of the order of the stabilities may be caused by the fact that in [**4**·Na]$^+$ the oxygen atom of the rotated phenyl ring is not able to act as a ligating site, because the ethoxy group is too large to allow rotation far enough into the cavity of the calixarene to make contact with the sodium ion. This results in reduced stability whereas, with the larger rubidium ion, there is a good contact with all ligating oxygen atoms.

## Conclusions

Our data show that it is possible to determine kinetic stabilities by using radioactive isotopes. Advantages of this method are the very small amounts of ligand that are required. Furthermore, different solvents can be used and the 'degenerate' exchange of a complexed cation can be studied.

The results also show that it is possible to synthesize rubidium complexes that are stable in solutions with a high salt concentration. The order of stability in such solutions is comparable to the order of stability as determined in chloroform.[6]

Finally, it was shown that the rate-limiting step in the exchange process is a simple dissociation of the complex.

## Experimental

*Materials.*—The synthesis of the calixspherands **1–4** has been described elsewhere.[1,6] The radioactive salts $^{22}$NaCl (specific activity 644 Ci g$^{-1}$) and $^{86}$RbCl (specific activity 1.38 Ci g$^{-1}$) were obtained from Amersham. Solvents were used without purification or drying. The glassware used was standard laboratory equipment and therefore not ion-free.

*Exchange Experiments.*—The free ligands of the calixspherands were loaded with $^{22}$Na$^+$ or $^{86}$Rb$^+$ in the following way. The calixspherand (1 mg) was dissolved in acetone (1.6 cm$^3$) and an aqueous solution of $^{22}$NaCl or $^{86}$RbCl (40 × 10$^{-3}$ cm$^3$; 1 mCi cm$^{-3}$) was added. After half an hour a solution of sodium or rubidium picrate in acetone (10 × 10$^{-3}$ mol dm$^{-3}$; 0.16 cm$^3$) was added. After a further 2 h chloroform (1.6 cm$^3$) was added and the organic solution was extracted with water (4 × 1.6 cm$^3$) and stored before use.

The ion-exchange experiments were performed as follows: For each experiment 10–14 tubes were filled with 20–30 × 10$^{-3}$ cm$^3$ of the solution containing the radioactive calixspherand complex. In this way, each tube contained 6–10 nmol of calixspherand complex and about 5000 counts per min (cpm). The solvent was evaporated off by exposure to air overnight. To every tube were added 100 × 10$^{-3}$ cm$^3$ of a 10 mmol dm$^{-3}$ solution of alkali-metal picrate in acetone or Me$_2$SO, and the mixture was kept at room temperature. At certain time intervals, chloroform (0.7 cm$^3$) and water (0.7 cm$^3$) were added and the tube was vortexed for 10 s, followed by centrifugation for 5 min at 2000 rpm. Part of the organic layer (0.5 cm$^3$) was then transferred to a second tube, the volume in this tube adjusted with water (0.5 cm$^3$), and both tubes counted for 4 min. The exchange was followed for a minimum of 24 h and a maximum of 72 h. The exchange-process was analysed after 10 s, 5, 10, 20, 40, 80, 150, 300 and 450 min, 16 and 24 h, and for the more stable complexes also after 32, 48 and 72 h.

For the calculation of the ratio of complexed and free salt the following assumption was made. All the counts in the organic layer are from the calixspherand cation complex, and all the counts in the water layer are from uncomplexed cations. This number of counts is corrected because only 0.5 cm$^3$ of the organic layer is transferred to a second tube and counted. When acetone was used as the solvent, the total volume of acetone used is taken up by the chloroform layer, but when Me$_2$SO was used as the solvent the total volume of Me$_2$SO used is taken up by the water layer. Therefore, the total volume of the organic layer is 0.8 cm$^3$ for acetone, and 0.7 cm$^3$ for Me$_2$SO, and consequently, the number of counts in the organic layer is multiplied by 8/5, and 7/5, respectively. The percentage of complex radioactive cations (*M$^+$) was calculated in the following way.

For acetone: % complexed *M$^+$ =
$$\{1.6N_{\text{org}}/(N_{\text{org}} + N_{\text{remaining}})\} \times 100\%$$

For Me$_2$SO: % complexed *M$^+$ =
$$\{1.4N_{\text{org}}/(N_{\text{org}} + N_{\text{remaining}})\} \times 100\%$$

$N_{\text{org}}$ = background-corrected number of counts in 0.5 cm$^3$ of the organic layer.

$N_{\text{remaining}}$ = background-corrected number of counts in the remaining layer.

The percentages were plotted against time, and the kinetic analysis was performed using a computer program for non-linear curve-fitting, MULTIFIT,[8] using the Marquardt algorithm[9] or the simplex algorithm[10] for the minimization of the residual weighted sum of squares.

$^1$H *NMR Experiments.*—Solutions of calixspherand rubidium complex (4 mmol dm$^{-3}$) and of sodium picrate (20 mmol dm$^{-3}$) in Me$_2$SO were prepared. Aliquots of each solution (300 × 10$^{-3}$ cm$^3$) were mixed in a NMR tube and equilibrated. $^1$H NMR spectra were taken 10, 20, 30, 50, 70, 100, 130, 190, 250, 310, 430, 550 and 670 min after mixing of the solutions. The ratio between the rubidium and the sodium complex was determined by integration of the signals from the methyl group of one of the ethoxy groups and plotted against time.

*Experiments with Mass Spectrometry.*—[**1**·Rb]$^+$ (*ca.* 70 × 10$^{-6}$ g) was dissolved in acetone (1 cm$^3$) and kept at room temperature for 24 h. Subsequently, 100 × 10$^{-3}$ cm$^3$ of the solution was removed and partitioned between CHCl$_3$ (0.7 cm$^3$) and H$_2$O (0.7 cm$^3$). The CHCl$_3$ layer was diluted 100-fold and analysed by mass spectrometry. The experiment was repeated, using a solution of rubidium picrate in acetone (10 × 10$^{-3}$ mol dm$^{-3}$) instead of acetone.

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