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.

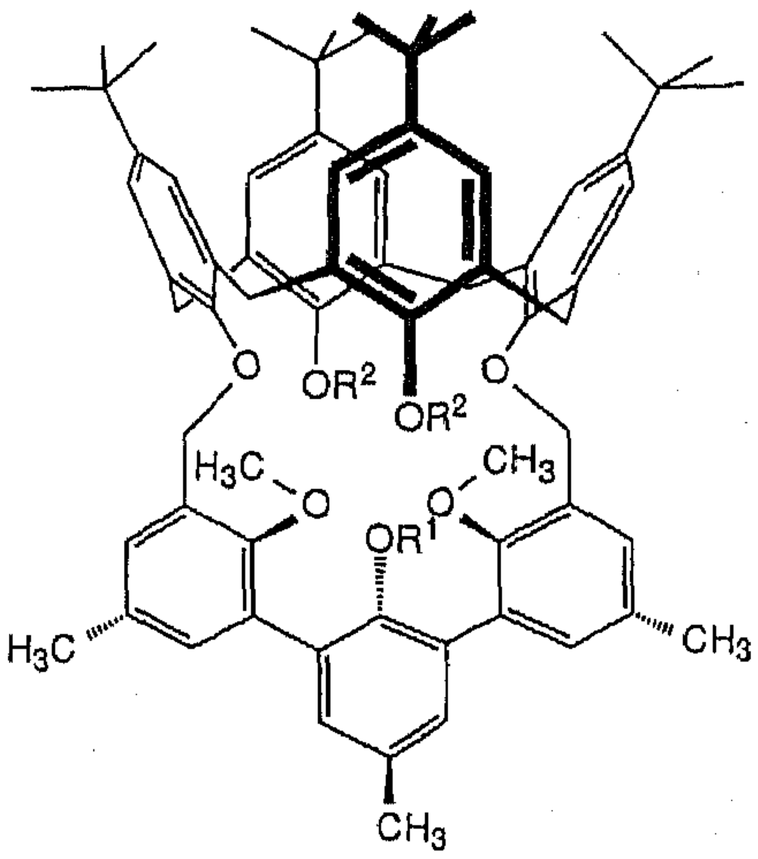
For our programme on the design and synthesis of *kinetically* stable alkali-metal cation complexes for medical applications[1] such as organ imaging (radioimmunoscintigraphy) and therapy (radioimmunotherapy),[2] we need general and sensitive methods to evaluate the kinetic stabilities of these complexes.

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.* 25×10^{-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**

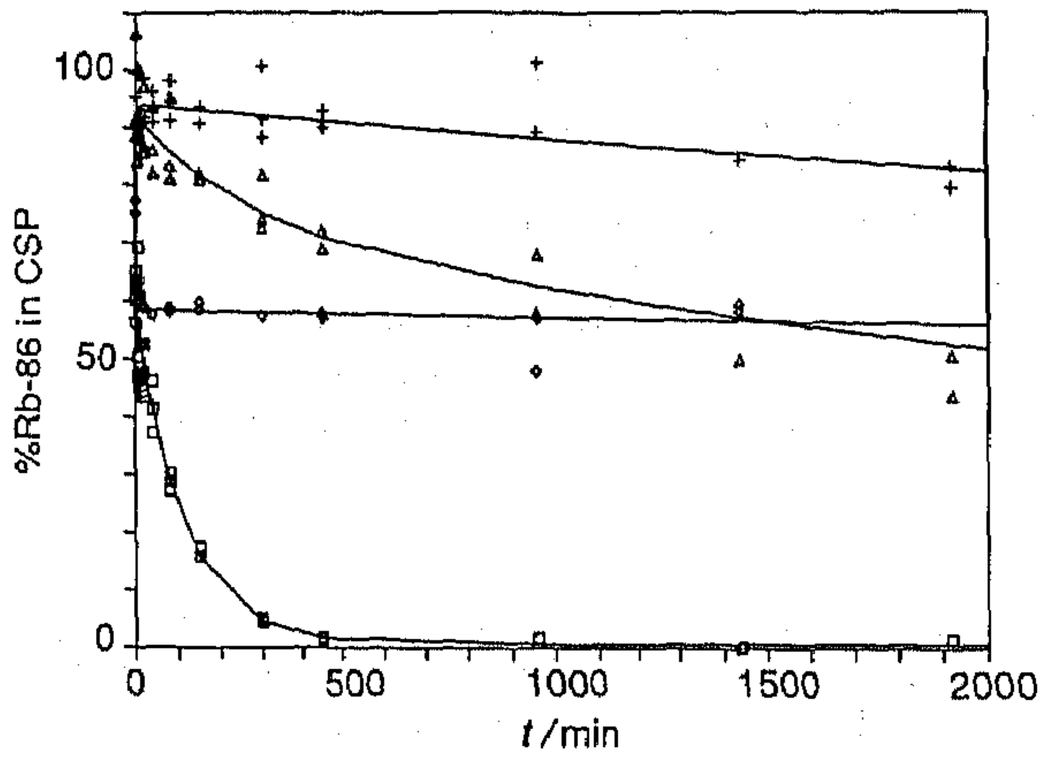


Fig. 2 Exchange of $^{86}Rb^+$ for Na^+ in acetone: □, **1**; +, **2**; ◇, **3**; △, **4**

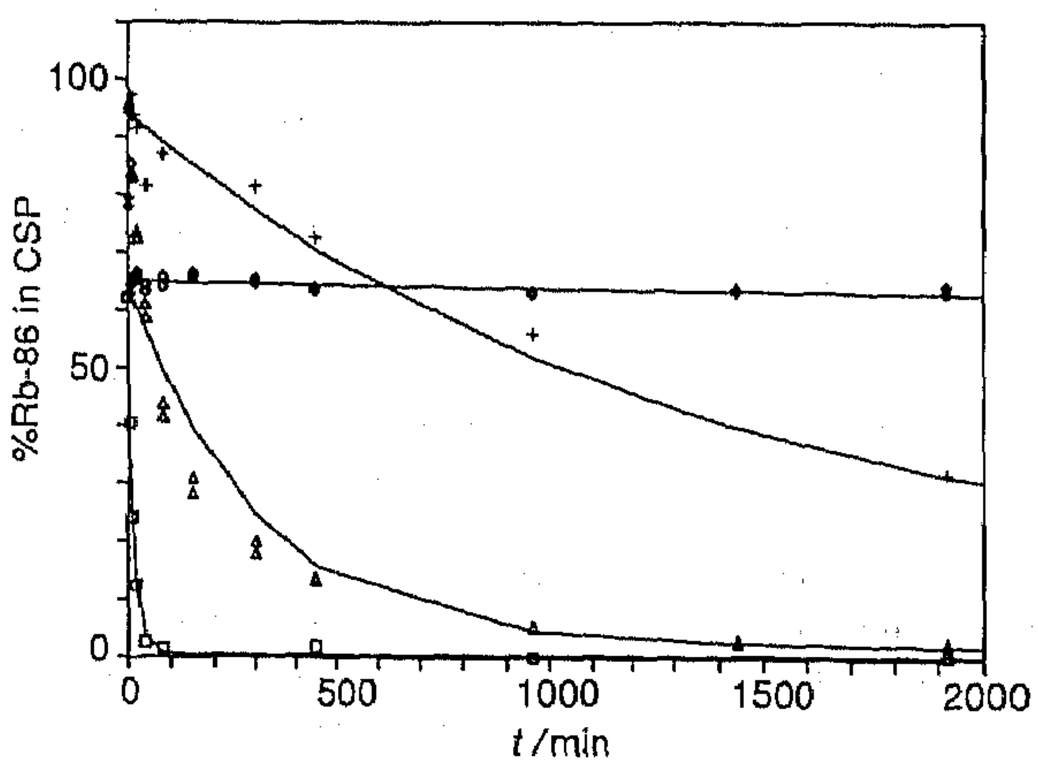


Fig. 3 Exchange of $^{86}Rb^+$ for Na^+ in Me_2SO: □, **1**; +, **2**; ◇, **3**; △, **4**

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

Results of the experiments in which the exchange of

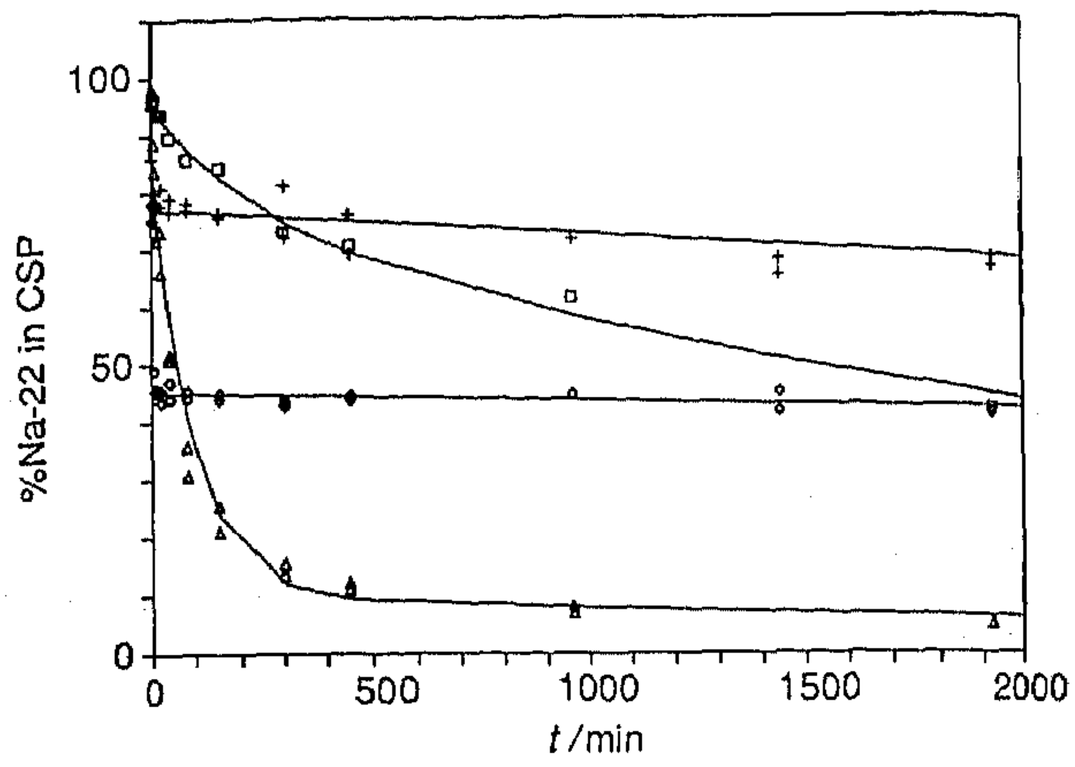


Fig. 4 Exchange of $^{22}Na^+$ for Na^+ in Me_2SO: □, **1**; +, **2**; ◇, **3**; △, **4**

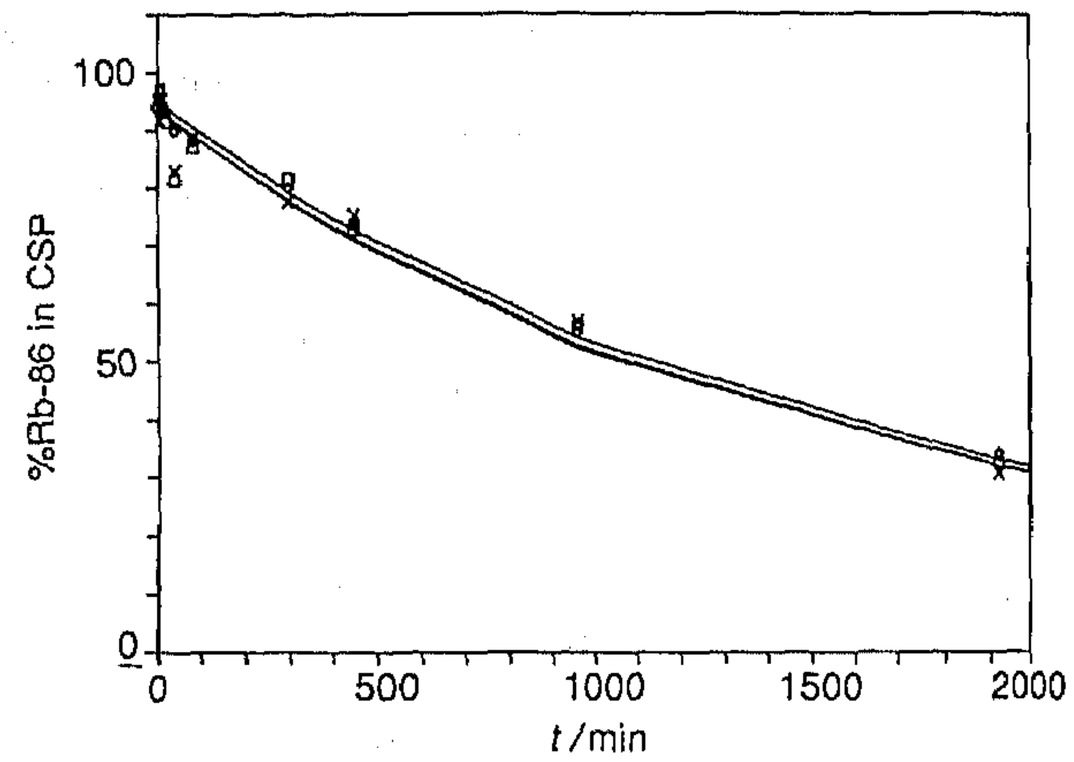


Fig. 5 Exchange of $[2{\cdot}Rb]^+$ with different cations in Me_2SO: □, Na^+; ◇, K^+; ×, Rb^+

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

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 $[3{\cdot}Rb]^+$ 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

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_2SO, 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_2SO was used as the solvent the total volume of Me_2SO 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_2SO, 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 $^*M^+$ =

$$\{1.6N_{org}/(N_{org} + N_{remaining})\} \times 100\%$$

For Me_2SO: % complexed $^*M^+$ =

$$\{1.4N_{org}/(N_{org} + N_{remaining})\} \times 100\%$$

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

$N_{remaining}$ = 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_2SO 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_2O (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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