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Molecular Apparatus for Automatic Titrations

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Apparatus is a collection of instruments, machines and tools used for a particular purpose.[1] A molecular apparatus is a collection of chemical devices working at a molecular level. Herein, we propose the first example of a molecular apparatus formed by a molecular titrator[2] and a molecular pH meter working together in the same environment as a substitute for all the physical devices necessary for the spectrophotometric automatic determination of the pK_a of a drug.

The determination of kinetic or thermodynamic parameters (activation parameters, elementary rate constants, equilibrium constants, etc.) very often require a series of experiments carried out in various environmental conditions (different values of temperature, pH, ionic strength, etc.). Automatic variable-parameter determinations enable one to obtain the same results by carrying out single experiments in which the value of the parameter (T, pH, etc.) is changed in a controlled way. In kinetics,[3–13] the mathematical model describing the process is given by Equation (1):

$$-\frac{d\lambda}{dt} = k_{obs}[Par_i(t)](\lambda - \lambda_\infty) \quad (1)$$

in which λ is an instrumental parameter linked to the concentration of the studied reacting species and $k_{obs}[Par_i(t)]$ is the specific reaction rate varying with the parameter, varying with time ($k_{obs}(Par_i)$ = dependence function; $Par_i(t)$ = modulating function). The fit of the experimental data to Equation (1) gives the terms regulating the dependence ($\Delta S^{\neq}$, $\Delta H^{\neq}$, k_i, etc.). In thermodynamic determinations, a general mathematical model can be formulated in the form of Equation (2):[2]

$$\lambda = D_K[Par(t)] \quad (2)$$

In this case, λ varies only as a consequence of the environmental parameter changing with a dependence function D_K containing the thermodynamic parameter to be determined (for example, K_e). The fit of the experimental data to Equation (2) gives the thermodynamic parameter in a single automatic scan (for example, as it is usually done in automatic determinations of acidic constants).

Recently, we proposed a new kind of molecular device that makes it possible to carry out variable-parameter kinetic (VPaK) experiments[14,15] without using physical devices (mechanical autoburettes, electronic temperature programmers, etc.) to change an environmental parameter (T, pH, I, etc.) in a controlled way inside a reaction vessel. [1.1.1]Cryptand[16–18] was used as a variable-pH device to carry out variable-pH kinetic (VpHK) experiments and obtain, in a single run, the pH–rate profile of a drug.[14] Acetic anhydride was used as a variable-temperature device to carry out a variable-temperature kinetic (VTK) experiment and obtain, in a single run, the activation parameters of a reaction of a metal–organic complex.[15] Moreover, [1.1.1]cryptand was used as a molecular titrator to carry out an automatic spectrophotometric pK_a determination of a molecule.[2] The need to classify these novel devices led to the term "kinetic molecular devices" (KMDs), that is, molecular systems able to perform a specific function in a molecular environment by means of the kinetics of the process in which they are involved. We distinguished them, for reasons that will be discussed later, as "simple" and "composite", depending on whether they use one or more molecular systems When using simple KMDs as substitutes for physical devices, two chemical systems work together in the same reaction vessel: one to modify the parameter and the other reacting conditioned by the change and giving an analytical signal containing information about the dependence on the parameter. When a molecular apparatus is used more chem-

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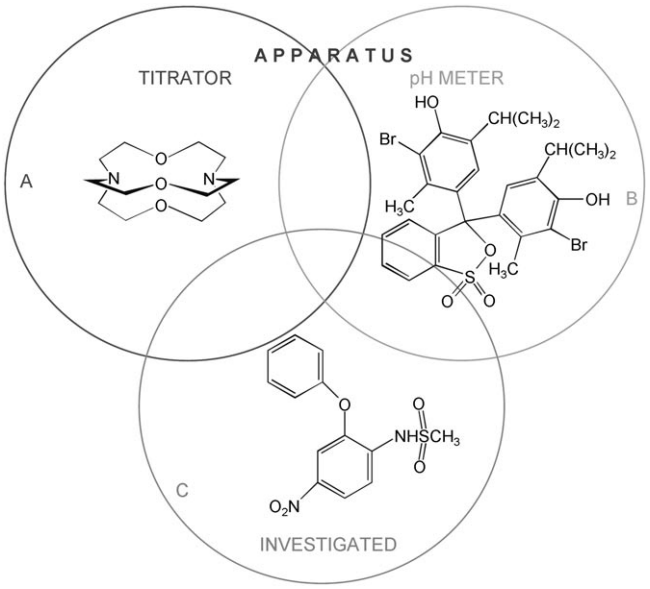

Scheme 1. Schematic of the methodology used for a molecular apparatus for automatic titrations.

ical systems work together. Scheme 1 describes the three chemical systems present in the reaction vessel in which our apparatus was used. A) The VpH-KMD [1.1.1]cryptand acts as a molecular titrator hydrolysing rapidly and reversibly and then reacting slowly and irreversibly to give a proton-entrapped species,[16–18] generating an almost linear increase of pH for about 2–3 pH units in the range 4–11.[2,14,19] B) Bromothymol blue (BTB) acts as a pH meter that, changing the equilibrium between its acid and basic forms by detecting the pH modulated by the cryptand, gives an absorbance signal directly linked to the pH. C) The investigated drug, nimesulide,[20] is sensitive to the change of pH generated by A and measured by B to give an absorbance linked to the pH by a mathematical model useful for data processing.

An experiment was carried out under a nitrogen atmosphere in a quartz cuvette inserted into the thermostatted compartment ($T=298.2$ K) of a Perkin–Elmer λ45 spectrophotometer, containing distilled water (3 mL), [1.1.1]cryptand (ca. 0.01 M), BTB (ca. 1×10^{-4} M), nimesulide (ca. 1×10^{-4} M), HBF_4 in a suitable amount to have a pH_i value of approximately 5.6 and KCl (0.01 M). The absorbance values at $\lambda_1=615.8$ nm (λ_{max} of BTB deprotonated species) and at $\lambda_2=322.7$ nm (one of the isosbestic points of BTB) were automatically acquired and stored on a computer.

To verify the performance of the molecular pH meter, an automatic titration of BTB was carried out by using the procedure described in reference [2], however, the absorbance variation in the range 200–700 nm was recorded (Figure 1). The pH data obtained from the Henderson–Hasselbalch equation [Eq. (3)] with $A_{615.8}$ (at $\lambda=615.8$ nm $A_a=0$) correlates well with those measured by a glass microelectrode inserted inside the cuvette and connected to a metrohm 691 pH-meter ($pH_{mis}=1.03pH_{calcd}-0.23$; $R=0.99994$) with deviations contained within the instrument resolution (0.01). The pK_a obtained by fitting the $A_{615.8}$-t data to Equation (4) (containing the dependence function $pH(t)=5.94+2.20\times10^{-4}t-7.97\times10^{-9}t^2+9.05\times10^{-14}t^3$) using pH data measured by the glass microelectrode was 7.11 ± 0.01 versus 7.10[21] confirming the good function of the method. The spectral change shows that in the range 565–700 nm BTB is free of interferences and can give a good signal for the pH measurement. The cryptand absorbs in the range 200–300 nm. The spectral window 300–565 nm can be used to follow the titration if the spectral change of the titrated species is in correspondence of one of the BTB isosbestic points.[22] Nimesulide, like several other molecules, has a good spectral change in that zone[20] so that its titration can be followed at 332.7 nm.

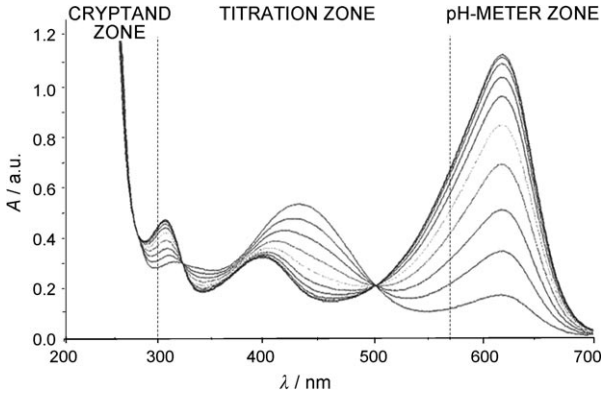

Figure 1. Spectral change during the automatic titration of bromothymol blue (BTB) at $T=298.2$ K in which the pH was varied with time by the molecular titrator [1.1.1]cryptand (selected spectra at scan time = 30 min; $pH(t)=5.94+2.20\times10^{-4}t-7.97\times10^{-9}t^2+9.05\times10^{-14}t^3$ (pH units s^{-1})).

$$pH = pK_a + \log\frac{A}{A_b - A} \quad (3)$$

$$A = \frac{K_a A_b + 10^{-pH} A_a}{10^{-pH} + K_a} \quad (4)$$

Figure 2 shows the change in absorbance at $\lambda_1=615.8$ nm and $\lambda_2=322.7$ nm during the automatic titration of nimesulide using the described molecular apparatus (molecular titrator: [1.1.1]cryptand, pH meter: BTB). The two curves have typical sigmoidal shapes. The fit of the $A_{nimesulide}$-t data to the Equation (4) was done by using the MicroMath SCIENTIST program[23] with Marquadt algorithm[24] with K_a, A_a and A_b as the parameters to be optimised. A, A_a and A_b, in this case, contain the absorbance of BTB at its isosbestic point, but a correction is not necessary because this does not influence the K_a value. The modulating function was $pH(t)=5.63+1.07\times10^{-4}t-1.75\times10^{-9}t^2-1.63\times10^{-14}t^3$ (pH units s^{-1}). The obtained value was $pK_a=6.52\pm0.01$, very similar to that reported in the literature for spectrophotometric measurements carried out in slightly different conditions (6.55 ± 0.01).[20] In fact, by using the molecular apparatus, the acquisition of the two absorbance data (and then

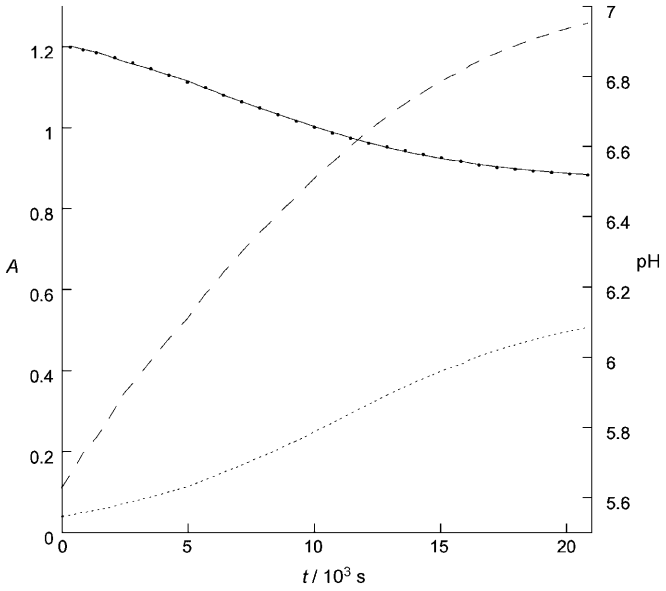

Figure 2. Change in absorbance (dotted line: BTB, $\lambda_1 = 615.8$ nm; plain circles: nimesulide, $\lambda_2 = 322.7$ nm, selected points) during the automatic titration of nimesulide in water, at $T = 298.2$ K, using a molecular apparatus ([1.1.1]cryptand as the titrator and BTB as the pH meter). The dashed line shows the increase of pH as calculated by the BTB absorbance using the Henderson–Hasselbalch equation. The solid line refers to the theoretical curve obtained by the fit to the mathematical model [Eq. (4)].

the pH) are synchronised so that a direct fit of the $A_{\text{nimesulide}}$ versus pH data can be done. The results are identical within experimental error.

This example shows that the molecular apparatus used for the automatic titration works well. The advantages are various: 1) The entire physical apparatus currently used for automatic titrations is eliminated, confining the molecular one inside the spectrophotometric cuvette so that automation becomes very simple. 2) Thermal and chemical homogeneity and constant ionic strength are assured. 3) It is possible to use the molecular apparatus for multiple automatic determinations of slightly different pK_a values, for example, of molecules belonging to homologous series or of the same molecule in different environmental conditions (ionic strength, etc.).

The use of an acid–base indicator for spectrophotometric determination of pH is certainly not novel, but it finds an optimal application in a composite KMD because the chemical homogeneity and the constant volume during the experiment enable continuity of measurements and the simplification of data processing (with suitable software it can be done in real time). Many other molecules can be used as pH meters, chosen for their role as indicators or synthesised for this purpose, so that the measuring zone can be shifted further and the titration zone can be free from interference.

Of course, this apparatus can also be used for automatic variable-pH kinetic experiments for the determination of the dependence of k_{obs} on pH. However, in this case, care must be used in the titration zone (or kinetic monitoring zone) of the spectrum because the change in absorbance of the reacting substrate can be accompanied by a change due to the different partition between acid and basic species during the experiment. Hopefully, with optimisations and further analytical refinements, molecular apparatus of this kind can become routine instruments of investigation in many fields, in which physicochemical phenomena dependent on pH and characterised by a spectral change have to be studied (e.g., determination of stability and pK_a in high-throughput physicochemical profiling in pharmaceutical analysis).[25]

In the light of this new application, a useful classification of KMDs can be done (Figure 3). In Figure 3 simple KMDs include variable-parameter devices, that is, variable-temperature (VT), variable-pH (VpH), variable ionic strength (VI), variable nucleophilic concentration (V[Nu]) devices, for example. VpH-KMDs include VpHK devices[14] and molecular titrators[2] (for example, [1.1.1]cryptand) and VT-KMDs include temperature programmers[15] (for example, acetic anhydride). Composite KMDs (Figure 3) are a new type of KMDs formed by a simple KMD joined with other molecular devices (KMD or not). They are categorised as 1) cooperative, that is, different molecular devices each performing a specific work in the same environment to obtain a common result (for example, a Titration Apparatus[26]); 2) synergic, that is, different devices enhancing each other's specific performances; and 3) integrated, that is, different devices working together, interacting in a way to achieve a different performance from each one.

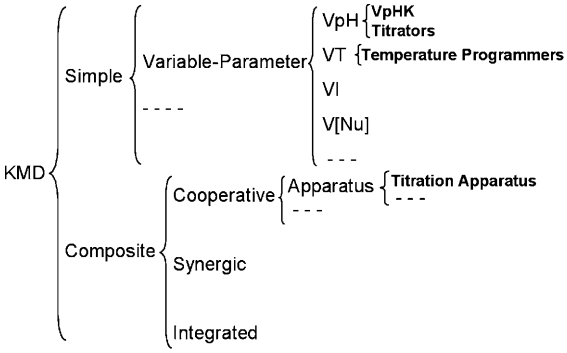

Figure 3. Classification of kinetic molecular devices.

Composite KMDs open new perspectives to this kind of research. Mixing simple KMDs with each other or with different devices can lead to interesting results that are worth exploring. Moreover, like simple KMDs, composite KMDs can be inserted in more complex molecular devices[27] where they can perform a time-dependent role.

Keywords: automatic titrations · cryptands · kinetics · molecular devices

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