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TECHNICAL NOTE

Compartmental analysis of results of radiotracer experiments in non-living systems in steady state*

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Abstract Compartmental analysis has for many decades been used for the evaluation of results of mainly *in vivo* radiotracer experiments. However, its potential for working out similar experiments, but in non-living systems, has as yet been rather overlooked. This paper aims at reversing this situation. To this end some basic principles of compartmental analysis are given followed by examples of its use in chemistry related laboratory radiotracer studies.

Key words compartmental analysis • radiotracer • non-living systems • steady state

Introduction

In 1962, a book entitled "Basic principles of the radiotracer method. Introduction to mathematical tracer kinetics" appeared. C. W. Sheppard, professor of physiology at the University of Tennessee Medical Units wrote the book [9]. According to the editor: it "is a research monograph which thoroughly reviews the basic principles" of the tracer method. However, "Unlike most books on tracers, it does not tell the reader how to perform a tracer experiment, but it *does tell* – in detail – what may or may not be done with the results in order to obtain meaningful information".

Similar, mainly biology and medicine, i.e. living organisms oriented books dealing with the tracer method soon followed the pioneering Sheppard's book. The titles of a number of such books printed between 1971 and 1992 are given in [7, 10–14]. Each of these books has parts devoted to the compartmental analysis, but there are also books entirely dedicated to it. For examples of such books see [2, 5, 8]. One of them contains (also) some information about the application of compartmental analysis for systems other than *in vivo* ones. An evaluation of the cited books (partly) related to the compartmental analysis approache to radiotracer experiments, points out to a considerable popularity of that approach in biomedical sciences, but almost completely neglects them in other branches of science. Evoking the interest for this approach of scientists performing radiotracer experiments in chiefly non-living systems is the main scope of this paper. To this end the basics of the compartmental analysis will shortly be delineated followed by description of three typical compartmental analysis aided radiotracer studies.

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Theoretical basis

Relevant books: [8–10]. A compartmental system consists of a finite number of compartments interchanging a substance of interest. Within each of the compartments there is an adequate level of homogeneity with regard to that substance. The flow or the rate of transfer, F (in mol or kg per second) to a compartment from another one, i.e. donor compartment is assumed to be directly proportional to the quantity, Q, (in mol or kg) of the substance of interest in the latter compartment, namely: $F = k \cdot Q$, where k (in l/s) is the rate constant. Compartmental systems can be closed, which are isolated from their surroundings, as distinct from the open ones. A closed two-compartment system is the simplest closed system (Fig. 1).

In such a compartmental system, the flows of a substance of interest to compartment a from compartment b and to compartment b from compartment a are: $F_{ab} = k_{ab}Q_b$ and $F_{ba} = k_{ba}Q_a$, respectively. A specific category of compartmental systems are those in dynamic equilibrium or steady state, characterised by stationary state for the quantities of the substance of interest, rate constants and indeed the flows. In the case of a closed, two-compartment system this means that $F_{ba} = F_{ab}$. Moreover, one should realize that the term "compartment" is not necessarily implying a specific volume (e.g. vessel or a part of it). It is merely a kinetically homogeneous, distinct entity.

In order to asses one or more parameters of a two-compartment system, a small quantity, q (in becquerel, Bq or count rate) of the radiotracer for the substance of interest has to be introduced in one of the two compartments at zero time, i.e. $t = 0$. Next the radiotracer quantity, q, is measured as a function of time in one or both compartment.

An optimal, i.e. ideal radiotracer – for example, a molecule labelled with a suitable radionuclide – should mimic the substance under observation in all its relevant chemical and physical properties. Radiotracers are often accompanied by unlabelled material having the same chemical composition as the tracer. The total amount of both substances to be added to a compartment must be negligibly small if compared with the quantity of substance of interest present in that compartment. One should namely avoid any perturbation of the compartmental system under observation.

The rate of change of the quantity of radiotracer in a compartment is established by the composite influence of rates of acquisition and rates of loss of radiotracer. Differential equations delineating these rates of change of q i.e. $q(t)$ in compartments a and b of a two-compartment system are:

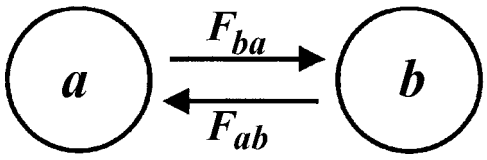

Fig. 1. A two-compartment system comprising of compartments a and b. Two arrows indicate the flows of a substance of interest.

$$(1) \qquad dq_a/dt = k_{ab}q_b - k_{ba}q_a$$

$$(2) \qquad dq_b/dt = k_{ba}q_a - k_{ab}q_b.$$

Note: The rate constant of an ideal radiotracer, $^*k$, for a substance of interest is commonly assumed to be identical with the rate constant, k, pertaining to that substance. Consequently, the symbol k is used for both rate constants.

Integration of differential Eqs. (1) and (2) should be carried out in a way that will enable calculation of k's and F's from the observed time curve for q present in a sampled compartment, i.e. compartment to which the radiotracer was added at $t = 0$. Hence,

$$(3) \qquad q_a(t)/q_a(0) = H_1 \exp(-g_1 t) + H_2$$

$$(4) \qquad q_b(t)/q_a(0) = K_1 \exp(-g_1 t) + K_2$$

where for zero time $q_a(t)/q_a(0) = 1$, $q_b(t)/q_a(0) = 0$ and for $t \geq 0$ $H_1 + H_2 = 1$, $K_1 + K_2 = 0$, $H_2 = Q_a/(Q_a + Q_b)$ and $K_2 = Q_b/(Q_a + Q_b)$. The coefficients H and K emerge from integration as equivalents of complex expressions involving rate constants, k, and the exponential slopes, g [10].

The complexity of equations, similar to the latter ones, increases with the number of compartments comprising a compartmental system. In fact, the equation for compartment a of a closed n-compartment system contains $(n–1)$ exponential terms (each including a coefficient) and a constant. In practice, however, the number of compartments, their sizes (quantities of a substance of interest) and interconnections (substance transport routes) as well as the rates of transfer (flow) of the substance are not known *a priori*. Therefore, a compartmental analysis aided radiotracer experiment should lead to a compartmental model of the system and provide all the Q's and k's of that model. The procedure to be followed includes at least 8 steps, namely:

1. Addition of a radiotracer to one of the compartments (e.g. compartment a) at $t = 0$.
2. Measurement of radiotracer quantity as a function of time, t, in compartment a.
3. Calculation of $q_a(t)/q_a(0)$ for all results and plotting the obtained data as a function of time.
4. Fitting equations of type (3) for $n = 2$, 3, etc. through experimental points and finding n corresponding to the best fit.
5. Calculate all H's and g's.
6. Design of a tentative compartmental model of the system (using already established n) and calculate the k's belonging to that model from g's and H's using explicit equations relating these parameters (see, for example, pages 215–222 in [10]).
7. Calculation of new g's and H's from the obtained k values and composing a new type (3) equation and comparing it with experimental data, i.e. $q_a(t)/q_a(0)$ data.
8. If necessary repetition of steps 6 and 7 until an optimal agreement is being achieved between the calculated curve and the measured data.

Examples of compartmental analysis aided studies

Interfacial exchange of cadmium in CdS suspensions. Closed system

Relevant publications: [3, 4]. In the eighties, the aqueous CdS suspensions were of interest as systems for photocatalytic conversion of solar energy into chemical energy. Applications of such systems require long-term stability of CdS suspensions. This implies the absence of net mass transfer to the aqueous phase from the solid CdS phase. Only an exchange – a balanced bi-directional transport – of cadmium and sulphide ions between CdS and its equilibrium solution may occur. Compartmental analysis aided radiotracer method has been applied for obtaining information on the kinetics of the bi-directional transport of cadmium across the solid/liquid interface in equilibrated acidic aqueous suspensions. To this end a minute amount of ^{109}Cd-labelled $CdCl_2$ as radiotracer was added to the CdS suspension previously equilibrated with dilute H_2SO_4 or $CdSO_4$ solution (all in the dark) and the radiotracer quantity measured at different points in time in the samples of suspension filtrate. Subsequently, the usual time $q_a(t)/q_a(0)$ *vs*. plots were made (Fig. 2).

The evaluation of the experimental results points at an $n = 3$, i.e. a three-compartment system with compartment a being cadmium in solution. As the Q_a is known, one can calculate total solid related exchangeable cadmium, $Q_b + Q_c = Q_s$ using the equation $H_3 = Q_a/(Q_a + Q_s)$. This resulted in a quantity of exchangeable cadmium related to solid equal to 30 ± 4 μmol/m^2; a value of 2.5 ± 0.3 times higher than the theoretically calculated surface cadmium concentration. Further analysis led to a compartmental model with compartment b attached to compartment a, and compartment c attached to compartment b. The corresponding mean residence times or transition times, τ, for cadmium in the latter two compartments were calculated from Q's and F's or k's using the equations: $\tau_b = Q_b/(F_{ab} + F_{cb}) = 1/(k_{ab} + k_{cb})$ and $\tau_c = Q_c/F_{bc} = 1/k_{bc}$. Depending on the initial cadmium concentrations in solutions, the obtained values range from 1.2–4.3 min and 4.9–17.0 days, respectively. Short τ, i.e. τ_b points to cadmium in adsorption layer and long τ, i.e. τ_c indicates cadmium on the surface layer of CdS and probably also in the layer below it.

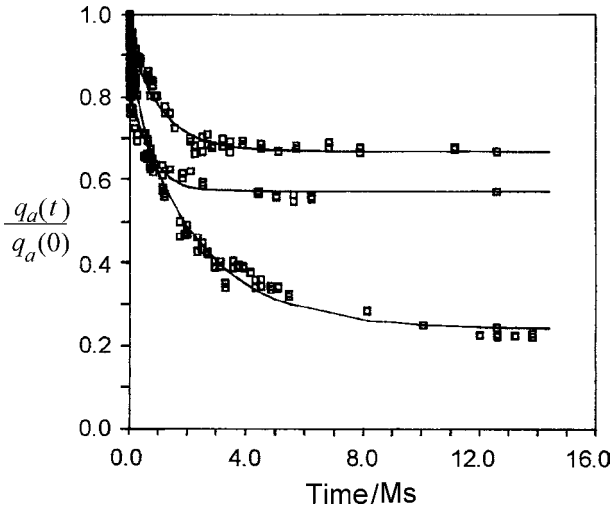

Fig. 2. Relative radiotracer quantity *vs*. contacting time for three suspensions differing in CdS and cadmium concentration. The composition of the suspensions (upper, middle and lower lines) in g CdS per 0.24 L and solution concentrations of cadmium in μmol·l^{-1} are: 0.30, 132; 0.26, 36; and 2.90, 132; respectively. The lines in the figure are obtained by fitting type (3) equations through the experimental points. The optimal fit was obtained for $n = 3$.

Interfacial exchange of phosphate in bovine milk. Closed system

Relevant publication: [6]. Much controversy exists about the state in which the casein bound inorganic phosphate is present. Milk can be considered as a two-phase system of casein micelles in state of dynamic equilibrium, i.e. steady state system with a permanent, bi-directional flow of phosphate ions of "micellar calcium phosphates" with such ions in whey ("milk serum"). The compartmental analysis aided radiotracer experiments may provide information about the number and size of compartments in the system as well as the number of phosphate transport routes in such a system and the corresponding flows, i.e. rate constants.

Phosphate exchange experiments were carried out with bovine milk to which a small amount of ^{32}P-labelled Na_2HPO_4 – an ideal radiotracer for milk phosphates – was added. Milk samples were taken at selected points in time and the serum was separated from casein by ultracentrifugation of samples and the quantity (activity) of radiotracer in the supernatant was measured. A plot of relative radiotracer quantities *vs*. time is shown in Fig. 3. It is evident that the system is the 4-compartment one.

The model showed in Fig. 4 has a central compartment, a, (soluble inorganic phosphate in whey) and three peripheral compartments (belonging to solid phase), b, c and d. The latter 3 compartments are

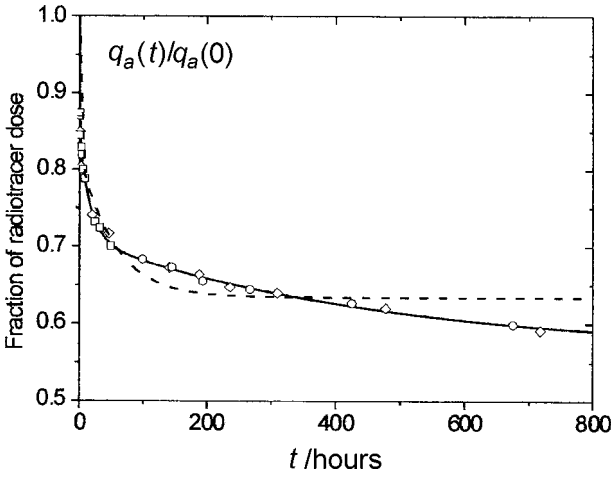

Fig. 3. Time course of the serum fraction, $q_a(t)$, of the initial radiotracer dose, $q_a(0)$. The full line represents the best fit according to equation of type (3), but for $n = 4$; the dashed line is for $n = 3$.

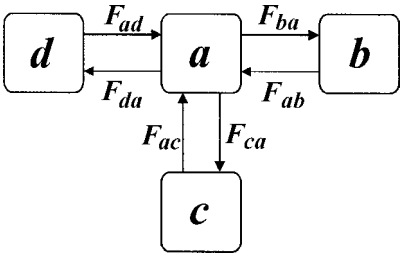

Fig. 4. A four-compartment model of a system pertaining to inorganic phosphate in milk.

assumed not to interact with each other, but with compartment *a* only. No transport of phosphate within the solid phase is expected to occur. The phosphate flows to and from each of peripheral compartments are equal. For example: $F_{ba} = F_{ab}$.

In the present study, another, not yet mentioned algorithm for calculation of rate constants, k, and the sizes Q of the compartments was applied. The rates of change of the relative quantity of radiotracer in four compartments were obtained by dividing by $q_a(0)$ each term of equations analogues to Eqs. (1) and (2), but for $n = 4$ and then simultaneously solving these equations for six rate constants using data points shown in Fig. 3. Starting with a known Q_a and the rate constants resulting from the aforementioned fitting procedure, the missing Q's were also calculated. The most valuable result of this study is the finding of three kinetically different inorganic phosphate entities (compartments) in bovine casein micelles. The corresponding mean residence times ($=1/k$) calculated from k_b, k_c and k_d are 818, 0.24 and 23 h, respectively.

Oxygen transport in an air-water contacting vessel. Open system

Relevant publication: [1]. Gas-liquid contacting in a vessel is one of the major unit operations in the (bio)chemical industry, e.g. aerobic fermentation and oxidation reactions. Hereby, the crucial parameters are the rate of oxygen transfer to liquid phase from gas, the hold-up and the residence time distribution of oxygen in the vessel. Compartmental analysis aided radiotracer experiment may provide this information. Figure 5 shows an experimental air aerated contactor used in the present experiments.

As the diffusion coefficient in water of argon and its "Henry's Law Constants" are very similar to the oxygen ones, ^{41}Ar is selected as the radiotracer for oxygen. At $t = 0$ a small amount of it was injected into the system at the inlet and the radiotracer concentration monitored at the outlet as a function of time. Figure 6 shows a typical experimental $q_f(t)/q_b(0)$ *vs.* time data points and the fitted line corresponding to the next equation which is characteristic for a three-component open system:

$$(5) \qquad q_f(t)/q_b = L_1 \exp(g_1 t) + L_2 \exp(-g_2 t) + L_3 \exp(-g_3 t)$$

where subscripts f and b pertain to freeboard and bubbles, respectively (see Fig. 5).

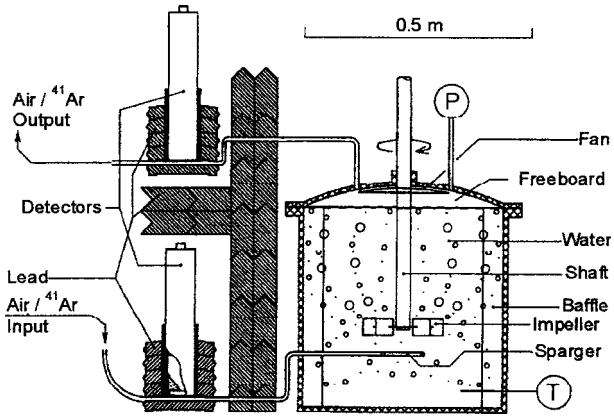

Fig. 5. Experimental set-up consisting of a gas-liquid contractor and two lead-shielded radiation detectors.

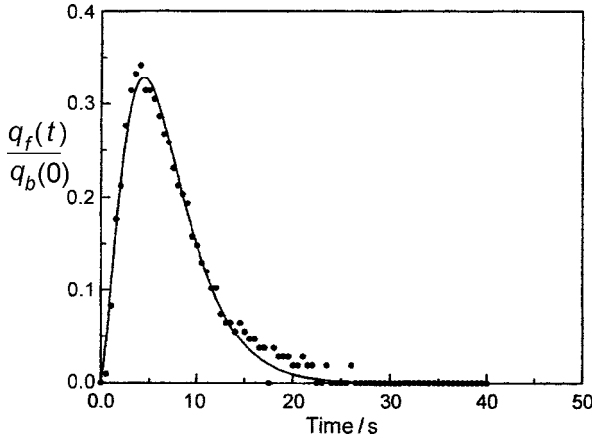

Fig. 6. Typical experimental $q_f(t)/q_b(0)$ values fitted with a three-exponential term function.

Further compartmental analysis combined with some other data eventually led to a (tentative) compartmental model (Fig. 7) for oxygen transport in the aforementioned contactor. Moreover, the values of all

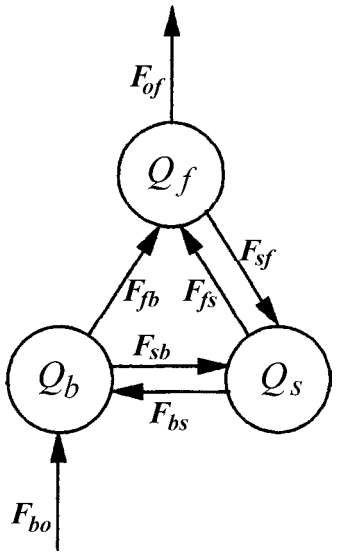

Fig. 7. Compartmental model for oxygen transport in a gas-water contactor. Subscripts *o* and *s* denote the outside and solution, respectively.

model parameters i.e. F's, k's and Q's have also been calculated.

Conclusion

The author of this paper recommends the use of compartmental analysis for extracting a maximum of meaningful and often even unique information from the results of radiotracer experiments in steady state.

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References

1. Abellon RD, Kolar ZI (1993) Utility of ^{41}Ar as radiotracer for oxygen transport studies in air-water contacting systems. Appl Radiat Isot 44:1183–1191
2. Godfrey K (1983) Compartmental models and their application. Academic Press, London
3. Hövell tot Westerflier SWFM van, Kolar Z, Binsma JJM, Stein HN (1987) A radiotracer study of cadmium transport across the CdS/aqueous solution interface. Croat Chem Acta 60:541–548
4. Hövell van Wezeveld en Westerflier SWFM van (1990) Interfacial cadmium exchange in equilibrated aqueous suspensions of cadmium sulphide: a compartmental analysis assisted radiotracer study. Doctoral dissertation. Delft University of Technology, Delft, The Netherlands
5. Jacquez JA (1972) Compartmental analysis in biology and medicine. Kinetics of distribution of tracer-labeled materials. Elsevier Publ Co., Amsterdam
6. Kolar ZI, Verburg TG, Dijk HJM van (2002) Three kinetically different inorganic phosphate entities in bovine casein micelles revealed by isotopic exchange method and compartmental analysis. J Inorg Biochem 90:61–66
7. Norwich K (1977) Molecular dynamics in biosystems. The kinetics of tracers in intact organisms. Pergamon Press, Oxford
8. Robertson JS (1983) Compartmental distribution of radiotracers. CRC Press, Boca Raton, FL
9. Sheppard CW (1962) Basic principle of the tracer method. Introduction to mathematical tracer kinetics. John Wiley & Sons, New York and London
10. Shipley RA, Clark RC (1972) Tracer methods for *in vivo* kinetics. Theory and applications. Academic Press, New York and London
11. Steele R (1971) Tracer probes in steady state systems. Charles C Thomas Publisher, Springfield, IL
12. Welch TJC, Potchen EJP, Welch MJ (1972) Fundamentals of the tracer method. WB Saunders Co., Philadelphia
13. Whicker FW, Schultz V (1982) Radioecology: nuclear energy and the environment. Vol. II. CRC Press, Boca Raton, FL
14. Wolfe RR (1992) Radioactive and stable isotope tracers in biomedicine. Principle and practice of kinetics analysis. Wiley–Liss, New York