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Studies on the Inclusion Complex of Diloxanide Furoate-β-Cyclodextrin

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Abstract

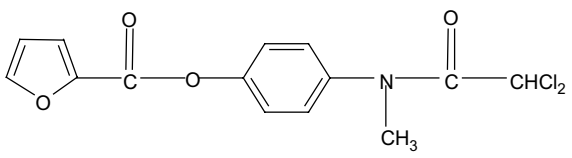
Cyclodextrins are a group of cyclic oligosaccharides which have shown to improve pharmaceutical properties of drugs, such as solubility and stability, by forming inclusion complexes. Diloxanide furoate has direct amoebicide action on the intestinal lumen and little solubility in water. In this work, solubility, NMR, fluorescence techniques, and kinetic methods were used to determine the equilibrium constant for the formation of the β-cyclodextrin–diloxanide furoate complex, $K_{1:1}$. It is shown that the kinetic approach is the best method for such a determination.

Introduction

Cyclodextrins are cyclic oligosaccharides built from glucose units joined together by α-glucosidic bonds α(1,4). They are molecules with a shape of a truncated cone having a relatively apolar inner cavity, while the hydroxy groups, situated on the outer part of the ring, make cyclodextrins both hydrophilic and water soluble (Figure 1) [1]. It is well known that cyclodextrins can form inclusion complexes with a large variety of guest molecules which can change the properties of the encapsulated molecule (solubility, oxidation protection, bioavailability, reduction, racemization, isomerization, polymerization, hydrolysis and/or decomposition of the guest). This ability has been largely exploited in pharmaceutical applications [2–6].

Different methods have been used to characterize the inclusion complexes. In the solid state, the most employed detection methods include thermal analysis, X-ray diffraction, FT-IR, and NMR [3, 7, 8]. In solution, it is necessary to determine the stoichiometry of the complex and the stability or association constant between cyclodextrin and guest molecule. For these determinations spectroscopic methods (such as NMR, ultraviolet or fluorescence) are commonly used but, when the solubility of the guest is low, the solubility method is probably the most common one [3], since the other methods are useless. For a right application of the solubility method it is necessary to guarantee that the equilibrium between the solid and liquid phases has been achieved, requiring long experimental times (usually a week). This also requires that the guest is fully stable in solution. For instance for a guest suffering a slow decomposition (such as hydrolysis) in solution, the kinetic rate constant (accepting a first order reaction rate) must be lower than $10^{-6}\ s^{-1}$ to guarantee a decomposition less than 10% in one week. Such a value is largely exceed for the hydrolysis of esters at neutral pH. Furthermore, it is necessary to take into account that cyclodextrins catalyzed many reactions in aqueous solution, among them the hydrolysis of esters. In such cases, a kinetic method is the best way to obtain information about the stoichiometry an equilibrium formation constant between a guest and cyclodextrin. In the present paper we illustrate that methodology for the diloxanide furoate (furamide) is 4-(*N*-methyldichloroacetamide)phenyl-2-furoate (1)/β-cyclodextrin system. On the other hand, this system presents a pharmacological interest as it has been shown that the inclusion complex of diloxanide furoate in β-CD improves the activity of this drug in infected rats [9, 10]. It is therefore important in the evaluation of the association constant between this drug and β-cyclodextrin.

Furamide exhibits direct amoebicide action on the intestinal lumen and little has solubility in water [11–14].



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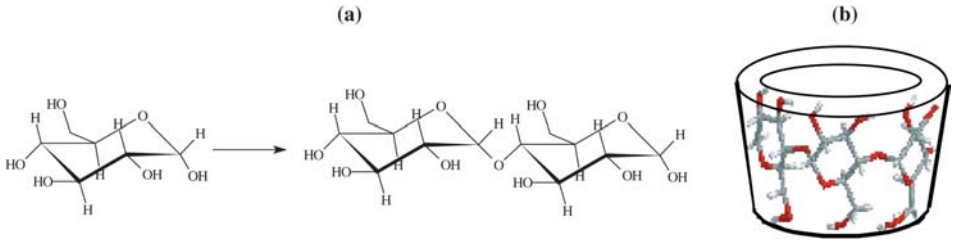


Figure 1. (a) Formation of α-(1,4)-glucosidic bonds in cyclodextrins; (b) the truncated cone structure of α-cyclodextrin (7 *D*-glucose units).

The elevated value of the partition coefficient, as pointed out by Dutta *et al*. [15], along with low solubility are the grounds for the therapeutical application of diloxanide furoate in the acute intestinal amoebiosis and to its limited action in the invasive or chronic form of such disease [11, 13, 14]. Once the drug is dissolved in the gastrointestinal tract, it is susceptible to hydrolysis, thus forming diloxanide, which is rapidly absorbed and eliminated and is less effective when compared to its ester form [12].

Materials and methods

Diloxanide furoate from Lab. Knoll S.A. and β-cyclodextrin donated by Cerestar (USA) were used throughout this work. Diloxanide furoate was used without previous purification based on literature and experimental data, including melting point ($mp_{lit.}$ = 114–116 °C; $mp_{exp.}$ = 110–112 °C) and ultraviolet patterns (UV) with maximum absorption at 258 nm (ethanol) [16, 17]. Although the purity of β-cyclodextrin was over 98%, recrystallization in hot water was carried out.

The solubility diagram was elaborated according to the method proposed by Higuchi and Connors [18]. Using appropriate analytical tubes, excess amounts of diloxanide furoate corresponding to 1.0 mM were added to aqueous solutions of β-CD in concentrations varying from 0.0 to 22.0 mM at pH approximately 7.0. The mixtures were constantly stirred at 37 °C for 7 days and triplicate samples were prepared. The floating component was then filtered using cellulose acetate membranes (Millipore) with pore diameter 0.45 μm. Dilutions of 1/10 were then prepared at constant temperature using the filtrated. The dissolved concentration of the drug was measured from UV tests carried out setting the wavelength to the value used to establish the calibration curve of diloxanide furoate (260 nm; ε 18162 ± 73 M^{-1} cm^{-1}). These values were used to determine the concentrations of the drug dissolved in each tube which contained the concentrations of β-CD represented in the solubility diagram.

A BRÜKER model AC300 spectrophotometer and mixtures of deuterated solvents were used in complexation studies by ^{1}H and ^{13}C NMR, respectively. Since the solubility in water of diloxanide furoate is very low, different ratios of water/methanol and water/acetonitrile solvent mixtures were also tested (20:80, 30:70, 40:60 and 50:50). Methanol:deuterated water (50:50) and equimolar concentrations of diloxanide furoate and β-CD (4 mM) were used in ^{1}H NMR measurements whereas a mixture of acetonitrile and deuterated water (40:60) and equimolar concentrations of diloxanide furoate and β-CD (10 mM) were used in ^{13}C NMR tests.

Fluorescence measurements were carried out in an Edimburgh Model F900 Fluorimeter. Excitation and emission slits of 3.0 and 5.0 mm, respectively, and λ maximum of excitation 338 nm were used. The concentration of diloxanide furoate was 3.0×10^{-5} M (0.2% ethanol) and that of β-CD was 1.2×10^{-2} M.

Kinetic data was gathered using a Varian DMS-80 UV–VIS spectrometer at 260 nm. The hydrolysis reaction of diloxanide furoate (concentration 6.66×10^{-5} M) in aqueous solution of 0.4% ethanol at 37 °C was used to that end. The ionic strength of the solution was 0.5 M (KCl).

Results and discussion

The solubility diagram of diloxanide furoate in β-CD is shown in Figure 2. Measurements were made at pH 7.0 as Nieto Reyes demonstrated that the solubility of diloxanide furoate varies with the pH of the medium [19]. Initially a linear increase of the diloxanide furoate solubility with β-cyclodextrin concentration is observed,

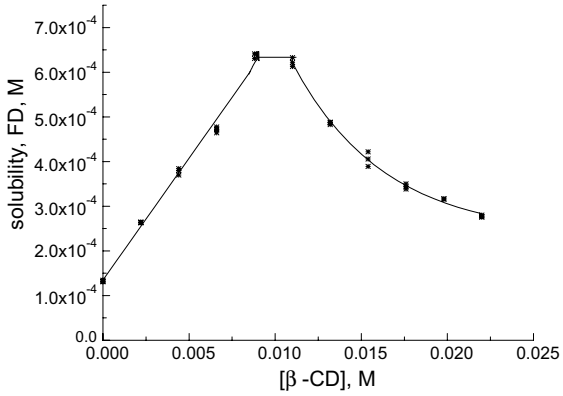


Figure 2. Solubility diagram of diloxanide furoate in β-cyclodextrin (pH 7.0). From the analysis of the first portion of the diagram a value of 427 M^{-1} ($r = 0.9959$) was obtained for the equilibrium constant of the formation of the complex, $K_{1:1}$.

indicating the formation of a complex, followed by a plateau (corresponding to maximum solubility). Finally, the solubility decreases due to the reduction in the concentration of free diloxanide furoate in solution. Therefore this is a classical B_s diagram type (Higuchi and Connors), indicating the formation of the inclusion complex with limited and well-defined solubility [18]. This type of diagrams were recently reviewed by Zughul and Badwan [20] who suggest to analyze all the segments of the diagrams (rising, plateau and descending portions) since it allows to distinguish between the type of complex which precipitates (either stoichiometry 1:1 or 1:2). In the present case, when $S_{eq} \times L_{eq}$ is plotted against ($S_{eq} + L_{eq}$) being S_{eq} being the solubility of the solute and L_{eq} the equilibrium concentration of cyclodextrin, an almost linear plot is obtained suggesting that the complex precipitate has a 1:1 stoichiometry [20]. From the analysis of the linear rising portion of the diagram and Equation (1) [18] the equilibrium constant for the formation of the complex was obtained, resulting a value of 427 M^{-1}, in good agreement with Nieto Reyes [19].

$$K_{1:1} = \frac{b}{S_o(1-b)}, \qquad (1)$$

where b is the slope of rising portion and S_o the solubility of pure diloxanide furoate in the absence of cyclodextrin. However, it must be considered that this technique does not take into account the formation of hydrolysis products (see below) which can compete with the substrate for complexation by β-CD. This fact has been previously documented in literature. A good example is the complexation of the antineoplasic drug melphalan by sulfobutyl ether β-CD and hydroxypropyl-β-CD (HP-β-CD). Two degradation products (hydroxymelphalan and dihydroxymelphalan) were formed after 24 h which competed for the complexation with the cyclodextrins, reducing the apparent solubility of melphalan [21].

The addition of cyclodextrin to an aqueous solution usually results in significant improvement of the corresponding fluorescence pattern. The ensuing change is similar to that observed in compounds dissolved in less polar solvents, such as dioxane and ethanol, suggesting that the compound was transferred from an aqueous environment to the apolar cavity of cyclodextrin [7]. The increase in fluorescence intensity resulting from the complexation process in cyclodextrin has also been qualitatively discussed by MA *et al.* who worked with antineoplasic agents such as melphalan and carmustine [21]. Nevertheless, in the conditions of the present investigation, no significant differences were observed in the excitation or emission patterns of diloxanide furoate regardless of the presence of β-CD. It can be theorized that the slight difference observed corresponds to low solubility in water and little fluorescence of diloxanide furoate.

Nuclear magnetic resonance spectroscopy (NMR) has also been used to characterize inclusion complexes as changes occur in the proton (^{1}H) or carbon (^{13}C) signals of the cyclodextrin or guest molecule, as they are complexed [22–27]. In this work, the sensitivity of NMR was not high enough to detect complexation in the experimental conditions used. Although some experiments were carried out in solvent mixtures (water–methanol and water–acetonitrile), these more hydrophobic environments hindered complexation.

As we have pointed out at the introduction a better value for $K_{1:1}$ can be obtained from kinetic, mainly if the effects of the concentration of cyclodextrin in the degradation rate of a substance are important. In such cases, the stability or formation constant of the complex can be attained from the stabilization or degradability effects exerted by the cyclodextrin [3, 4, 7, 22]. Examples of stabilization of several drugs as indomethacin [28], aspirin [29], melphalan and carmustine [21] by cyclodextrins are well known but the catalysis by cyclodextrins on the cleavage of esters are also well described in the literature [30].

Figure 3 shows that β-CD inhibits the degradation of diloxanide furoate at basic pH. Therefore hydrolysis is hindered at basic pH, confirming a higher drug stability when it is complexed. It suggests that β-CD can shield the compound from the action of esterases present in the gastrointestinal tract [19]. However, the kinetic data also indicates that some hydrolysis of diloxanide furoate at pH 7.0 takes place, the kinetic constant being 1.8×10^{-5} s^{-1}. This means that after seven days, the solubilized diloxanide furoate is almost fully hydrolyzed. Therefore, the equilibrium constant determined by the isothermal method cannot be correct. The hydrolysis reaction and complexation are competing processes which can affect the apparent solubility of the drug.

The first-order velocity constant for the hydrolysis of diloxanide furoate in the presence of β-CD and the

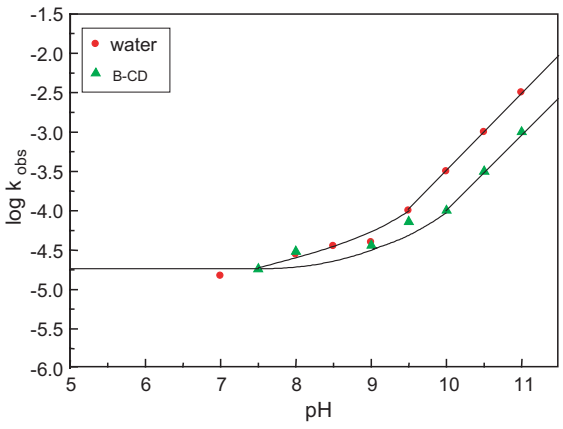
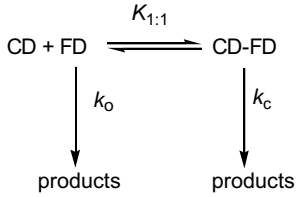


Figure 3. pH dependence of the logarithm of the first-order velocity constant for the hydrolysis reaction of diloxanide furoate in water and β-CD, 37 °C, ionic strength 0.5 M (KCl).

stability, formation or equilibrium constant can be determined from methods which are similar to that proposed by Michaelis–Menten for enzymatic kinetics [31]. Since the kinetic study carried out herein used diluted solutions, the formation of 1:1 complexes can be represented by the following kinetic diagram (Scheme 1);



Scheme 1.

where [CD–FD] refers to the diloxanide furoate–β-cyclodextrin complex, k_o is the rate constant of the non-catalyzed reaction, k_c is the rate constant of the reaction in the presence of β-CD and $K_{1:1}$ is the equilibrium or formation constant of the CD–FD complex.

The kinetic expression for the hydrolysis of the active drug may be described by Equations (2) and (3). The observed first-order rate constant for the total disappearance of the drug (k_{obs}) is then the weighted average of k_o and k_c:

$$\frac{-\mathrm{d}[\mathrm{FD}]_T}{\mathrm{d}t} = k_{obs}[\mathrm{FD}]_T = k_o[\mathrm{FD}] + k_c[\mathrm{CD-FD}]. \quad (2)$$

Considering that the kinetics studies were realized under pseudo-first-order conditions; $[CD]_T >> [FD]_T$ and $[FD]_T = [FD] + [CD–FD]$, $[CD]_T = [CD] + [CD–FD]$, so it was straightforward to deduce the Equation (3)

$$k_{obs} = \frac{k_o + k_c K_{1:1}[\mathrm{CD}]_T}{1 + K_{1:1}[\mathrm{CD}]_T}, \quad (3)$$

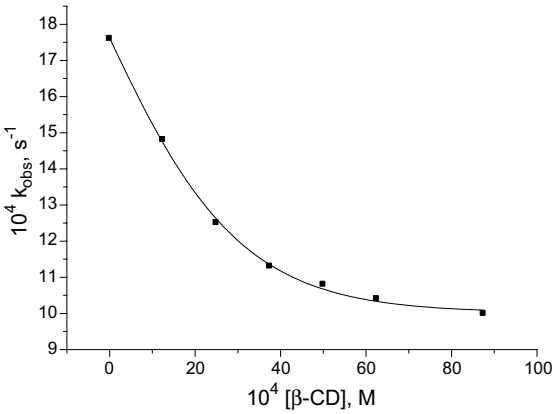


Figure 4. First-order velocity constant as a function of the increasing contents of β-CD for the hydrolysis reaction of diloxanide furoate (6.66×10^{-5} M), pH 10.75, $\mu = 0.5$ M (KCl), $\lambda = 260$ nm and 37 °C; $k_o = 1.75 \times 10^{-3}$ s^{-1}.

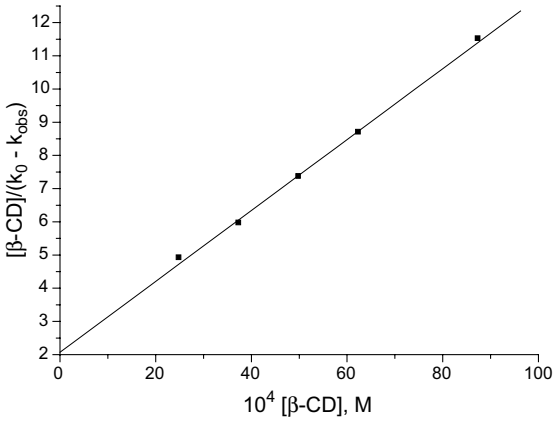


Figure 5. $[CD]_T/(k_o–k_{obs})$ profile as a function of $[CD]_T$ of the hydrolysis reaction of diloxanide furoate (6.66×10^{-5} M), pH = 10.75, $\mu = 0.5$ M (KCl), $\lambda = 260$ nm and 37 °C.

which has been rearranged as follows [7],

$$\frac{[\mathrm{CD}]_T}{k_o - k_{obs}} = \frac{1}{K_{1:1}(k_o - k_c)} + \frac{[\mathrm{CD}]_T}{k_o - k_c}. \quad (4)$$

Experimental data from Figure 4 are plotted according to Equation 4 in Figure 5. A linear relationship is observed confirming the formation of a 1:1 complex [3, 4, 21]. From the intercept and slope, values of 517 M^{-1} and 8.22×10^{-4} s^{-1} were obtained for $K_{1:1}$ and k_c, respectively. The value for the equilibrium constant obtained from the kinetic approach is higher than that established by the solubility method. This can be explained considering that (i) the products of the hydrolysis of diloxanide furoate (furoic acid and diloxanide) compete with diloxanide furoate for the complexation by β-CD, and (ii) the actual concentration of diloxanide furoate is lower than that used in solubility calculations because of its decomposition.

Increasing the concentration of inorganic salts can either increase or decrease the stability constant of complex formation [7]. In order to evaluate the effect of ionic strength in the formation of FD–CD, complexation was carried out under the previous experimental conditions but in the absence of KCl. The results suggested that the ionic strength did not affect the measured value of the constant ($K_{1:1} = 509$ M^{-1}).

Conclusions

Measurements of the stability or equilibrium constant for the formation of the diloxanide furoate–β-cyclodextrin complex required approximately 7 days according to the solubility method proposed by Higuchi and Connors [18]. Kinetic data revealed that some hydrolysis of diloxanide furoate occurred after 7 days at 37 °C. The products of the hydrolysis competed with diloxanide furoate for complexation with β-cyclodextrin.

Therefore, the solubility method is subjected to considerable uncertainty. By taking into account that the concentration of diloxanide furoate decreased with time, the value of the constant ($K_{1:1}$) obtained by the solubility method (427 M^{-1}) was lower that resulting from the kinetic approach (517 M^{-1}). It was not possible to establish the formation of the complex by ^{1}H or ^{13}C NMR or fluorescence. Therefore, the kinetic approach is the most reliable way to determine the formation constant of the diloxanide furoate (FD) and β-cyclodextrin (β-CD) complex and, it can be the choice method for compounds unstable in aqueous media.

Acknowledgements

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References

1. J. Szejtli: *Chem. Rev.* **98**, 1743 (1998).
2. D. Duchêne and D. Wouessidjewe: *Acta Pharm. Technol.* **36**, 1 (1990).
3. T. Loftsson: *Drug Stability* **1**, 22 (1995).
4. T. Loftsson and M.E. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).
5. V.J. Stella and R.A. Rajewski: *Pharm. Res.* **14**, 556 (1997).
6. D.O. Thompson: *Therapeutic Drug Carrier Systems* **14**, 1 (1997).
7. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988), p. 78.
8. K.H. Fromming and J. Szejtli: *Cyclodextrins in Pharmacy*, Kluwer Academic Publischers, Dordrecht (1994), p. 83.
9. J.A. Castro Hermida, E. Ares Mázas, L. Nieto Reyes, F. Otero Espinar and J. Blanco Méndez: 9th Int. Symp. on Cyclodextrins, Santiago de Compostela, Spain (1998), 3-P-65.
10. J.A. Castro Hermida, E. Ares Mázas, L. Nieto Reyes, F. Otero Espinar and J. Blanco Méndez: *Parasitol. Res.* **87**, 449 (2001).
11. P.H. Felix, J. Joly, V. Fort and M. Fromantin: *Bull. Soc. Path. Exot.* **55**, 370 (1962).
12. E.C. Wilmshurst and E.E. Cliffe: *Absorpt. Distrib. Drugs* 191 (1963).
13. M.S. Wolfe: *Med. Clin. North Am.* **66**, 707 (1982).
14. W.A. Petri: *Crit. Rev. Clin. Lab. Sci.* **33**, 1 (1996).
15. H. Dutta, N.K. Mehta, M.S. Gupta, D.K. Pal, C.S. Gupta and A.U. De: *Indian J. Pharm. Sci.* **50**, 328 (1988).
16. Martindale. *The Extra Pharmacopoeia*, The Pharmaceutical Press, London, 29th, ed. (1989) pp. 662–663.
17. *British Pharmacopoeia* HMSO, London, (1993) pp. 227–228.
18. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
19. L. Nieto Reyes: Ph.D. Thesis, University of Santiago de Compostela, Spain, 1999.
20. M.B. Zughul and A.A. Badwan: *J. Incl. Phenom. Mol. Recognit. Chem.* **31**, 243 (1998).
21. D.Q. MA, R.A. Rajewski, D.V. Velde and V.J. Stella: *J. Pharm. Sci.* **89**, 275 (2000).
22. K.A. Connors: *Comprehensive Supramolecular Chemistry*, Jerry Atwood, USA (1996), p. 205.
23. P. Ramos Cabrer, E. Alvarez Parrilla, F. Meijide, J.A. Seijas, E. Rodríguez Núnez and J. Vázquez Tato: *Langmuir* **15**, 5489 (1999).
24. E. Alvarez Parrilla, P. Ramos Cabrer, A. Pal Singh, F. Meijide, E. Rodríguez Núnez and J. Vázquez Tato: 10th Int. Cyclodextrin Symp. CD 2000, University of Michigan, Ann Arbor, MI, USA (2000), 2–p27.
25. I. Perdomo López, M. Echezarreta López, T. Esclusa Díaz, B. Pose Vilarnovo, J.L.Vilajato and J.J. Torres Labandeira: 10th Int. Cyclodextrin Symp. CD 2000, University of Michigan, Ann. Arbor, MI, USA (2000), 3–p30.
26. B. Pose Vilarnovo, P. Schroth Pardo, M. Echezarreta López, C. Glemet, B. Perez Marcos and J.J. Torres Labandeira: 10th Int. Cyclodextrin Symp. CD 2000, University of Michigan, Ann Arbor, MI, USA (2000), 3–p29.
27. P. Ramos Cabrer, E. Alvarez Parrilla, F. Meijide, E. Rodríguez Núnez and J. Vázquez Tato: 10th Int. Cyclodextrin Symp. CD 2000, University of Michigan, Ann Arbor, MI, USA (2000), 2–p3.
28. T. Backensfeld, B.W. Müller, M. Wiese and J.K. Seydel: *Pharm. Res.* **7**, **484** (1990).
29. S. Choudhury and A.K. Mitra: *Pharm. Res.* **10**, 156 (1993).
30. M.L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag, Berlin (1978).
31. W.P. Jencks: *Catalysis in Chemistry and Enzymology*, Dover Publications Inc., New York (1969).