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Catalysis, Complexation, and Photolysis

JENS T. CARSTENSEN

Madison, Wisconsin

1. CATALYSIS

General and specific acid and base catalyses have been discussed in Chapter 3, and are one example of catalysis. When discussing catalysis, however, the metal induced decomposition is what comes to the pharmaceutical investigator's mind. In parenterals especially, great care is taken to exclude metals, because only slight decomposition caused by trace metals may cause sufficient discoloration to render the product unsatisfactory. Examples of this are thiamine hydrochloride injectables and ascorbic acid injectables.

Metals are most detrimental in oxidations, as shown in the previous chapter. Examples of metal catalyzed oxidation in pharmaceutical systems are cyanocobalamine (which is stabilized at very low concentrations, but destabilized at higher concentrations of ferrous ion), erythromycin (which is stabilized by such ions as mercuric, magnesium calcium, ferric, and aluminum, and destabilized by cobaltous, plumbic, zinc, and nickel) and (Kassem et al., 1969) ascorbic acid (which, in general, is destabilized by metal ions).

Figure 1 shows data by Kassem et al. (1969). Barcza and Lenner (1988) have shown that chloral hydrate forms hydrogen bonded complexes with halide ions

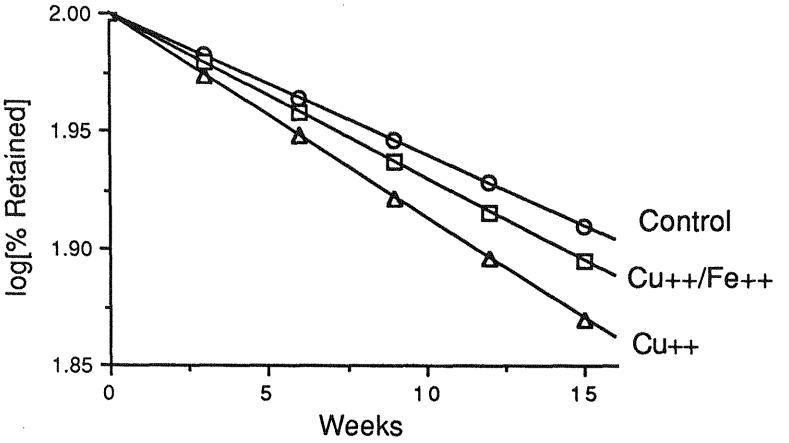

Fig. 1 Effect of metal ions on the decomposition of ascorbic acid. Least squares fit lines are (Control) $y = 2 - 0.006x$, (with copper and iron) $y = 2 - 0.007x$, (with copper alone) $y = 2 - 0.0087x$. (Figure constructed from data published by Kassem, 1969.)

in aqueous solutions. They are 1 : 1 complexes with relatively high stability constants. Upadrashta and Wurster (1988) used ethylene diamine tetraacetic acid to protect anthralin solutions from metal catalyzed oxidation. Tomida et al. (1987) showed that zinc ion increased the degradation of cephalosporins in tromethamine solution. The second-order rate constant, divided by $[Zn_o]$ plotted versus pH has unity slope from pH 7.5 to 8.5. They suggest the formation of a ternary complex (penicillin-Zn^{2+}-tromethamine).

Substances such as vitamin A and D are also prone to metal ion catalyzed decomposition.

Of other works in this area, McCrossin et al. (1998) reported on the effect of guanidine HCl on degradation of recombinant porcine growth hormone at alkaline pH and different concentrations and found it to be first order.

Fredholt et al. (1999) studied the catalytic effect of α-chymotrypsin on desmopressin decomposition and reported on the influence of concentration, pH, and cyclodextrin. The reaction is presumably A-B-C and the disappearance rate of the compound is first order. The pH profile is type AHJD with a maximum at pH 7.7.

2. COMPLEXATION

It is obvious that in many cases, drugs may complex with one or more of the ingredients in a solution dosage form. Sometimes this is intentional, e.g. bioavailability in certain instances may be improved (Levy and Reuning, 1964; Newmark et al., 1970). In other instances the stability of a drug is favorably affected by complexation, although in many cases the opposite is the case.

The basic principles of complex formation have been reviewed by Connors and Mollica (1964) and demonstrated by them as well. Only the formation and stability of 1 : 1 complexes will be covered here. For coverage of 1 : 2 and 2 : 1 complexes,

the reader is referred to the work by Connors and coworkers (Rosanske and Connors, 1980; Connors and Rosanske, 1980; Pendergast and Connors, 1984).

In the following, A will denote drug (substrate) and B will denote complexing agent (ligand). (It should be noted that either could be called either, and that there is no generally accepted nomenclature).

If A complexes with B by the scheme

$$A + B \leftrightarrow AC \tag{5.1}$$

then the complex is denoted 1 : 1. The terminology 1 : 2 or 2 : 1 is then obvious. The equilibrium (stability) constant of the complex AB is given by

$$K = \frac{[AB]}{[A][B]} \tag{5.2}$$

In a 1 : 1 complex (and other types as well), it would be fortuitous if the stability of both the drug and the complex were identical. In other words, in degrading, there will be two decomposition reactions:

$$A \rightarrow \text{products (rate constant } k) \tag{5.3}$$

and

$$AB \rightarrow \text{products (rate constant } k^*) \tag{5.4}$$

The analytically measured quantity is

$$C = [A] + [AB] \tag{5.5}$$

except if the study is carried out by other than chemical means, e.g., if one species is charged, then conductimetry might elucidate the concentration of one of the species.

The analytically measured rate is

$$-\frac{dC}{dt} = k[A][B] + k^*[AB][B] = \{k + k^*K[B]\}[A][B] \tag{5.6}$$

where use has been made of Eq. (5.2) for the last step.

The apparent rate with which the reaction proceeds is given by

$$\begin{aligned} -\frac{dC}{dt} &= k_{obs}[B]C = k_{obs}[B]\{[A] + [AB]\} \\ &= k_{obs}[B][A]\{1 + K[B]\} \end{aligned} \tag{5.7}$$

where Eq. (5.2) has been used for the last step. Equating Eqs. (5.6) and (5.7) then gives

$$\{k + k^*K[B]\}[A][B] = k_{obs}[B][A]\{1 + K[B]\} \tag{5.8}$$

Dividing through by [A][B] gives

$$k + k^*K[B] = k_{obs}\{1 + K[B]\} \tag{5.9}$$

Table 5.1 Effect of β-Cyclodextrin on Benzocaine Stability

Concentration of cyclodextrin (%)	k_{obs} (h^{-1} M^{-1})
0	0.666
0.25	0.358
0.5	0.299
1	0.129

Source: Data from Lach and Chin (1964a).

from which we obtain

$$k_{obs} = \frac{k + k^*K[B]}{\{1 + K[B]\}} = k + \frac{(k^* - k)K[B]}{1 + K[B]} \tag{5.10}$$

which may be expressed reciprocally as

$$\frac{1}{k_{obs} - k} = \frac{1}{k^* - k} + \frac{1}{K(k^* - k)} \cdot \frac{1}{[B]} \tag{5.11}$$

If the amount of [B] complexed in small, then [B] is synonymous with the amount of [B] added. Hence if kinetic studies were carried out in a series of systems with different concentrations of [B], then a reciprocal plot should be linear.

Example 5.1.

Lach and Chin (1964a,b) reported kinetic data for benzocaine, complexed with betacyclodextrin (Table 1). Calculate the equilibrium (stability) constant between benzocaine and betacyclodextrin, assuming a 1 : 1 complex.

Answer.

First of all the concentration units have to be consistent. Beta-cyclodextrine is taken to have a molecular weight of 1700, so that e.g. 0.25% = 2.5 g/L = $2.5/1700 = 1.47 \times 10^{-3}$ molar. This is shown in column 2 of Table 2. The sixth column lists the reciprocal of these figures, e.g., $1/0.00147 \times 680$ M^{-1}.

The values for $(k_{obs} - k)$ are listed in the fourth column, e.g., for the second entry, $0.229 - 0.666 = -0.43$.

The fifth column then lists $1/(k_{obs} - k)$, e.g. $1/(-0.537) = -1.862$.

The fifth column, $(k - k_{obs})$ is then plotted versus the sixth column, 1/[B]. This is shown in Fig. 2.

It is seen that the slope $= -0.0027$ (M^2h) and that the intercept is -1.3822 (Mh). Equation (5.11) predicts that the slope-to-intercept ratio is K, i.e.,

$$K = \frac{\text{slope}}{\text{intecept}} = \frac{-0.0027}{-1.3822} = 2 \times 10^{-3} M^{-1} \tag{5.12}$$

Table 5.2. Treatment of Data in Table 1. Effect of β-Cyclodextrin on the Decomposition of Benzocaine in Solution

Concentration of cyclodextrin					
%	$10^3 \times$ Molar	k_{obs} $h^{-1}M^{-1}$	$(k_{obs} - k)$	$1/(k - k_{obs})$	1/[B]
0	0	0.666	0		
0.25	1.47	0.368	−0.298	−3.247	680
0.5	2.94	0.299	−0.467	−2.288	340
1	5.88	0.129	−0.537	−1.862	170

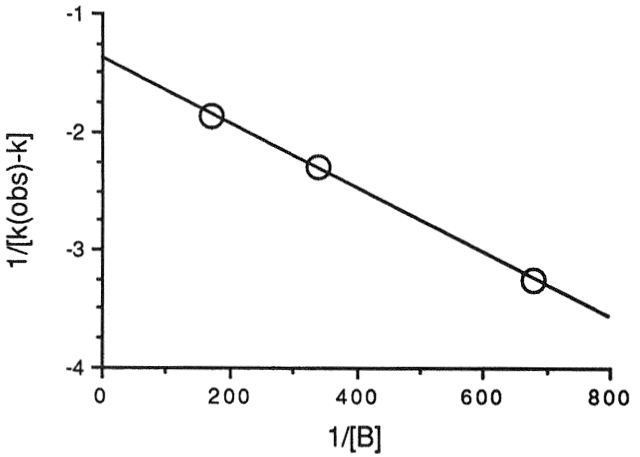

Fig. 2 Data from Table 2. (Graph constructed from data published by Lach and Chin, 1964.)

Complexation constants can also be deduced by determining the solubility of A in aqueous (or other) solutions of different concentrations of B. An example of this is the work by Chen et al. (1994).

Chen et al. (1994) have studied the complexation of adenine with a series of ligands. Their data from using caffeine as a ligand are shown in Fig. 3. The complexation constant is obtained by Eq. (5.13) by which

$$K = \frac{\text{slope}}{\text{intercept} \times (1 - \text{slope})} = \frac{0.26}{(0.0076 \times 0.74)} = 46.2\ (M^{-1}) \tag{5.13}$$

The pharmaceutical literature dealing with complexes is not abundant. One interesting example is urea including compounds, which were in vogue in the 1960s (and probably still are, although they are not as frequently published on), an example being the thiourea and urea inclusion compounds of alpha-lipoic acid methyl ester reported by Mima and Nishiwasha (1964). Other, presently quite researched, com-

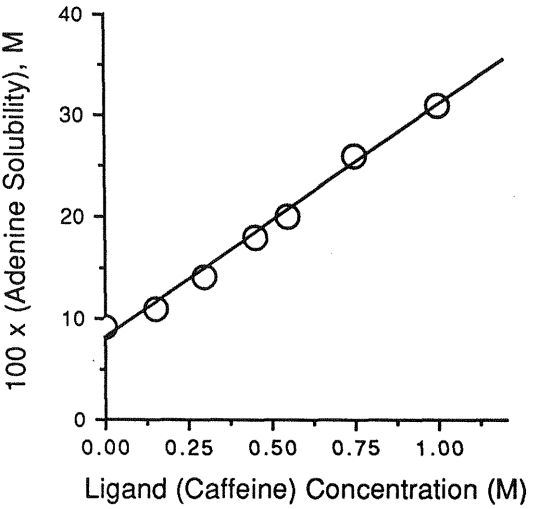

Fig. 3 Least squares fit: $y = 0.26x + 0.0076$, where x is solubility (rather than $100 \times$ solubility, as used in the figure). (Graph constructed from data published by Chen et al., 1994.)

plexes are those with cyclodextrins: for instance Connors and coworkers have published substantially in this area (Pendergast and Connors, 1984; Connors et al., 1982). Duchene et al. (1986) have reviewed the effect of cyclodextrin complexes on drug stability. Lach and Chin (1964a) have studied the complexes of cyclodextrins with benzocaine and found them to be 1 : 1 complexes. Higuchi and Lachman showed that benzocaine complexed with caffeine (1955). Lach and Chin (1964b) showed that a series of substituted benzoic acids complexed with cyclodextrin. Cyclodextrins are unique (alpha, beta, and gamma) in that they are doughnut shaped molecules that allow inclusion of the drug molecule. Other (non-macrocyclic) carbohydrate molecules also complex, for instance Gupta (1983) has shown that procainamide complexes with glucose, lactose, and maltose. All of these have a hemiacetal group, as opposed to sucrose and fructose. The complex formation was pH dependent.

Complexation constants may be obtained by spectrophotometric means as well. The complex can exhibit a specific maximum in the ultraviolet spectral range, so its concentration can be distinguished from that of the parent species such as in the case of the alendronate/Cu^{++} complex and the case of metal complexes of anhydrotetracycline (Siqueira et al., 1994).

2.1. Complexing Agents

Caffeine and polyvinylpyrrolidone were the most common complexing agents used in pharmaceutics for a series of years. In recent years the cyclodextrins have become of importance.

An example of this is the work by Van Der Houwen et al. (1994) dealing with the kinetics of 7-*N*-(*p*-hydroxyphenyl)mitomycin C (M-83) in the presence of γ-cyclodextrin. The pH profile of this is V shaped with a couple of extensions of

lesser slope and with a minimum at pH 7. Cyclodextrins have also been used in stabilization of monoclonal antibodies (Ressing et al., 1992).

Cyclodextrins (CDs) are powerful complexing agents, and much work has centered about their use in drug solubilization and stabilization. They are the subject of a fair amount of pharmaceutical research. For instance, Scalia et al. (1998) have described the complexation of butyl-methoxydibenzoilmethane with hydroxypropyl-β-cyclodextrin. Veiga and Ahsan have described the interaction between tolbutamide and β-cyclodextrins. The tolbutamide phase solubility diagram, in this case, shows a maximum in solubility at a concentration of β-CD of 0.008 M. Such maxima are usually ascribed to the limit of solubility of the complex. Ventura et al. (1998) have described the interaction between papaverine and modified and natural β-cyclodextrins. The phase-solubility diagram is such that the papaverin solubility increases monotonically (in a straight line or curved) with β-CD concentration. Ventura et al. (1997) have shown the increase in aqueous solubility of ursodesoxycholic and chenodesoxycholic acids by complexation with β-CDs.

Miyake et al. (1999) have demonstrated the inclusion compounds of itraconazolone with 2-hydroxypropyl-β-cyclodextrin in propylene glycol solution. Antoniadou-Vyza et al. (1997) have reported that the hydrolysis rate constant of methocarbamol is reduced almost 50% when complexed with hydroxypropyl-β-cyclodextrin. Vianna (1998) et al. showed that complexes of dexamethasone acetate with cyclodextrin showed marked improvement in aqueous solution stability and gave rise to first-order decomposition. Másson et al. (1998) have shown that chlorambucil and indomethacin have greatly improved (first-order) aqueous stability when complexed with a variety of cyclodextrins. Diazepam behaved in different manners depending on the particular cyclodextrin used.

Less common ligands such as dextran stabilize porcine pancreatic elastase (Chang et al., 1993).

3. PHOTOLYSIS

Attention is given to light stability in the 1993 ICH guidelines, although at the time of this writing, testing methods are not finalized. This is exemplified in lines 471–473:

> Light testing should be an integral part of stress testing. [The standard conditions for light testing are still under discussion and will be considered in a further ICH document.] (471–473).

In photolysis, light ($h\nu$) is absorbed by the solution and activates a species in it. ($h\nu$) here represents a quantum of light, where h is Planck's constant ($h = 6.626 \times 10^{-27}$ erg s) and ν (in units of s^{-1}) is the frequency of the light. The activated species (denoted by [*]) then returns to ground state, it either

1. Emits light (of a different frequency ν'); this is referred to as fluorescence or phosphorescence
2. Causes the activated species to decompose, in which case one deals with photolysis. The simplest sequences in photolysis would, therefore, be

$$A + (h\nu) \rightarrow [A^*] \tag{5.14}$$

$$[A^*] \rightarrow \text{products} \tag{5.15}$$

$$[A^*] + A \rightarrow 2A \tag{5.16}$$

At times a second component of the system, B, may preferentially absorb light, in which case photosensitization may occur:

$$B + (h\nu) \rightarrow [B^*] \tag{5.17}$$

$$[B^*] + A \rightarrow B + [A^*] \tag{5.18}$$

If only (5.17) predominates, and is followed by

$$[B^*] \rightarrow \text{products} \tag{5.19}$$

or

$$[B^*] \rightarrow B + (h\nu'') \tag{5.20}$$

then B is referred to as a screening agent. In the last case, [B] will not decrease with time and hence will protect the other photosensitive compounds in the preparation during the shelf life of the product.

When a photolysis experiment is carried out, one should know the quantum yield, i.e., the number of molecules decomposed as a function of the number of quanta absorbed. To measure the latter, use is made of an actinometer (which is a photosensitive system with known quantum yield). A simple and reliable actinometer is the chloroacetic acid actinometer, where the reaction is

$$ClCH_2COOH + H_2O + (h\nu) \rightarrow HOCH_2COOH + HCl \tag{5.21}$$

This has a quantum yield of 1.0 at concentrations of 0.3–0.5 molar. The use is simple. A product is placed in a container, e.g., a clear ampul, or a specially designed apparatus; then, directly before or after, a chloroacetic acid solution is studied under the same circumstances, for a given length of time, t, (e.g., 5 minutes). The hydrochloric acid formed is then titrated and the number of moles calculated, and this is then converted to number of molecules, N. If the drug substance subsequently is irradiated for t^* minutes, then the number of quanta absorbed (provided the absorbency of preparation and actinometer is the same) is given by

$$(h\nu) = \frac{t^* N}{t} \tag{5.22}$$

If the UV spectrum of the substance is known, and if the spectral distribution of the light source is known, then the absorbency (fraction f) of the drug substance solution in relation to the chloroacetic acid solution can be calculated, and the number of quanta are in this case

$$(h\nu) = \frac{ft^* N}{t} \tag{5.23}$$

The 1993 ICH Stability Guidelines promote the general philosophy of attempting to establish a reaction order.

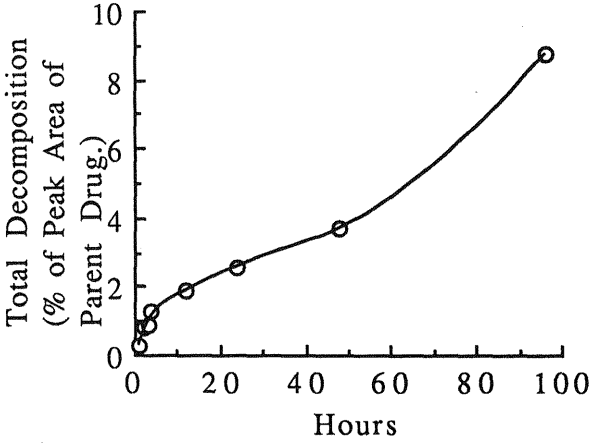

Fig. 4 Photolysis of ciprofloxacin. Irradiation with natural/artificial light. (Figure constructed by averaging of data from Table 2 of the publication by Tievenbacher et al. 1994.)

> The nature of the degradation relationship will determine the need for transformation of the data for linear regression analysis. Usually the relationship can be represented by a linear, quadratic or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit on all batches and combined batches (where appropriate) to the assumed degradation line or curve (303–308).

In pharmaceutical systems, most reported photolysis has been first order. Examples of this are cefatoxime photolysis (Lerner et al., 1988) and the work by Mizuno et al. (1994). They point out that the wavelength of the irradiating light plays an important part in photodecomposition. They showed the wavelength influence on the photodegradation of ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate in solution.

Another example of photodegradation is the work by Tievenbacher et al. (1994) dealing with a series of antimicrobial quinolones. Their data are shown in Fig. 4.

It is noted that here the reaction is not first order. More rarely is the reaction zero order except when it is an oxidation, in which case it often is zero order. The work by Asker et al. (1985) and Asker and Larose (1987), and the oxidation of chlorpromazine (Ravin et al., 1978; Felmeister et al., 1965) are examples of this.

In general the time frame in photolysis is different from that in usual kinetics, and this is also addressed in the 1993 ICH Stability Guidelines:

> Frequency of testing should be sufficient to establish the stability characteristic of the drug product. Testing will normally be every three months over the first year, every six months over the second year and then annually (271–273).

It is obvious that photolysis is a stress situation and that trimonthly time protocols would be unrealistic. For solution products, the testing should be carried out both in bulk, unprotected solution (and that is what elucidates the kinetic schemes and rates) and in the final package. This latter is more truly a package test. The 1993 ICH Stability Guidelines addresses this as follows:

The testing should be carried out in the final packaging proposed for marketing. Additional testing of unprotected drug product can form a useful part of the stress testing and pack evaluation, as can studies carried out in other related packaging materials in supporting the definitive pack(s) (276–279).

Some additional pharmaceutical examples of photolyses are the following: Fabre et al. (1993) have studied the photoisomerization kinetics of befurroxime axetil and found them to follow an A-B-C reaction. Matsuda and Masahara (1983) have shown that ubidecarenone is photochemically decomposed by a first-order process, and that the activation energy of a solution is different from that in solid state. Heat, light, and metal ions (e.g. copper) accelerate the oxidative decomposition of vitamin D3. This can be retarded and actually inhibited by the use of β-cyclodextrin (Szeijtli et al., 1980; Szejtli and Bollan, 1980). Vitamin A stability is also increased when complexed with cyclodextrins (Kyoshin, 1982). Asker and Larose (1987) have shown that uric acid increases the photostability of sulfathiazole sodium solutions, and Asker et al. (1985) have shown that dl-methionine increases the photostability of ascorbic acid in solution. Vandenbossche et al. (1993) have reported on the photostability of molsidomine in infusion fluids. Akimoto et al. (1985) have reported on the photostability of cianidanol in aqueous solution. In this case there is a leveling off, and the approach to the plateau is first order. Tønnesen et al. (1997) have reported on the photoreactivity of mefloquine hydrochloride in the solid state. Baertschi (1997) has discussed the quinine actinometry system embodied in the ICH guideline.

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