

A NON-LINEAR KINETIC APPROACH
FOR CHEMICALLY INTERACTING BIO-MOLECULES
AND SOME STABILITY-INSTABILITY PROBLEMS

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For a better understanding of metabolism in living systems under inappropriate diet and drug interaction a biochemical model is proposed and stability-instability problems are revealed, solving the associate equations analytically.

1. INTRODUCTION

As it is well known, the level of integration at which all life processes occur coordinately is the cell. The cell is the smallest unit which can perform metabolism, self-reproduction and mutability [1].

The metabolism, as one of the basic processes of the cell, is owing to the exchange of substance and energy between the living system and the environment. The principal way to have that exchange is to supply the cell with “food”, *i.e.*, by diet. However depending on the molecular composition of diet (“normal”, “rich” or “poor”), the diet can be one of the major risk factors for many diseases, like coronary heart disease, stroke, diabetes, cancer, etc. For example, atherosclerosis is the major cause of morbidity and mortality especially of people enjoying a Western life style [2–4]. Also it is a very good opportunity to be revealed the hamsters hypercholesterolemia produced by hyper-lipemic diet supply [5–7] and the similarities to the human aspects of atherosclerosis [8, 9].

To identify the cells and molecules involved in each stage of the mentioned (or any others) diseases, as well the environmental and genetic factors, or the drugs necessary for the treatment to restore the normal state of patients, is still an important task in spite of progress obtained in the last fifty years.

As we know the cell is an open system hence the living subjects are, in general, near or far away of thermodynamical equilibrium such that the kinetic phenomena are important to be studied both experimentally and theoretically.

In two previous papers [10, 11] we have studied the kinetic processes by using a linear approximation to get the time depending concentration of different

bio-molecules important for atherosclerosis (as total cholesterol – C, triglycerides – TG, low density lipoproteins – LDL, high density lipoproteins – HDL, etc) taking into account the coupling among these molecules and their interaction with the supplied diet and drug molecules (especially simvastatin) correspondingly. Because most of the processes in living systems are non linear the aim of the present paper is to study the kinetic of bio-molecules (or larger complex molecules formed by the former ones) interacting with the supplied diets or/and drugs in order to get more light on the metabolism and the stability – instability “dipole” which can arise.

2. THE THEORETICAL MODEL FOR INTERACTION WITH SUPPLIED DIET

To formulate a non-linear kinetic theory, we imagine a bio-chemical picture to take into account the coupling of different bio-molecules (or bio-complexes formed by these molecules) with the supplied diet – hoping that the time dependence of the concentration of the corresponding components will explain the connected experimental facts.

If at time $t = 0$ (initial time) a diet D is administered to a living subject (a human being or an animal) then there may be imagined a biochemical reaction of the following form:



where A is the symbol for the bio-molecules (or bio-complex) of interest, D is the symbol for diet, E is the necessary enzyme, C is the symbol for the decomposition product, n is the multiplication factor for A ($n \neq 0, 1$), k_1 and k_2 are the rates of chemical reaction. This autocatalytic reaction was considered possible when LDL is depleted from antioxidant [2] (p. 365), [12].

One could object that the above reaction is too simple to be valid for describing the metabolism. Actually, the real metabolic processes are much more complicated than the above autocatalytic reaction and more individual steps are necessary (about 100 different chemical reaction). But we have in mind that an experimental researcher measures, at $t = 0$, a value of concentration for oxidized LDL to say and after a time t another one larger or smaller. Everything in between seems to be a “black box” such that the “overall” kinetics will come close to (1) under many realistic conditions (see also [13], p. 884 in discussing the replication of RNA).

2.1. THE KINETIC EQUATION

Now, if X_A is the transformed quantity of A at the moment t and

$$\Delta X_{AD} = X_A(0) - X_{AD} \quad (2)$$

untransformed quantity, $X_A(0)$ being the initial concentration of A, then the kinetic equation for ΔX_A is:

$$-\frac{d}{dt}\Delta X_{AD} = k_1\Delta X_A X_D X_E - k_2\Delta X_{AD}^n X_C X_E \quad (3)$$

where X_D, X_C, X_E are the corresponding concentration of D, C and E.

a) The solution of kinetic equation (3)

To solve the equation (3) we suppose that X_D, X_C, X_E are constant parameters. This hypothesis may be true in the following situations: 1) the concentration of D and C are very much in excess with respect to the “normal values”, such that any variation of them can be neglected and the order of reaction is apparently reduced; 2) when the subject consumes the same amount from diet in the time unit (the cell keeps a part from diet as reserve); 3) when the diet is nothing else but perfusion and 4) the diet is a constant beam of radiation. In the above conditions the equation (3) becomes:

$$\frac{d}{dt}\Delta X_{AD} = -k_{1D}\Delta X_{AD} + k_{2C}\Delta X_{AD}^n \quad (4)$$

where $k_{1D} = k_1 X_D X_E$, $k_{2C} = k_2 X_C X_E$. The equation (4) is a Bernoulli equation and the solution for $n \neq 0, 1$ is:

$$\Delta X_{AD} = e^{-k_{1D}t} \left[X_A^{1-n}(0) + \frac{k_{2C}}{k_{1D}} (e^{(1-n)k_{1D}t} - 1) \right]^{\frac{1}{1-n}} \quad (5)$$

b) The limit conditions for the solution (5)

- For $t \rightarrow 0$: $\Delta X_{AD} = X_A(0)$ for $n \neq 0, 1$
- For $t \rightarrow \infty$: $\Delta X_{AD} = 0$ when $n > 1$, which is quite normal
- For $t \rightarrow \infty$: $\Delta X_{AD} = \left[\frac{k_{2C}}{k_{1D}} \right]^{\frac{1}{1-n}}$ when $n < 1$, also an expected result, which obviously coincides with the “stationary state” obtained from equation (4) when $\frac{d}{dt}\Delta X_{AD} = 0$, *i.e.*

$$\Delta X_{AD}(s) = \left[\frac{k_{2C}}{k_{1D}} \right]^{\frac{1}{1-n}} = \left[\frac{k_{1D}}{k_{2C}} \right]^{\frac{1}{n-1}} \quad (6)$$

From (2), (5) and (6) one obtains the solution for transformed quantity of A:

$$X_{AD}(t) = X_A(0) \left\{ 1 - e^{-k_{1D}t} \left[1 + \left(\frac{\Delta X_{AD}(s)}{X_A(0)} \right)^{1-n} (e^{(1-n)k_{1D}t} - 1) \right]^{\frac{1}{1-n}} \right\} \quad (7)$$

c) *The thermodynamic flux and force*

By using the solution (2) in the equation (4) one gets:

$$\frac{d\Delta X_{AD}}{dt} = k_{1D}[X_A(0) - X_{AD}(t)] - k_{2C}[X_A(0) - X_{AD}(t)]^n \quad (8)$$

and the thermodynamic flux is:

$$J_{AD} = \frac{dX_{AD}}{dt} \quad (9)$$

The derivative of (9) with respect to X_{AD} gives:

$$\frac{dJ_{AD}}{dX_{AD}} = -k_{1D} + nk_{2C}[X_A(0) - X_{AD}(t)]^{n-1} \quad (10)$$

which becomes zero for a critical concentration $X_{AD}(cr)$ given by:

$$X_{AD}(cr) = X_A(0) - \left[\frac{k_{1D}}{nk_{2C}} \right]^{\frac{1}{n-1}} = X_A(0) - \left[\frac{nk_{2C}}{k_{1D}} \right]^{\frac{1}{1-n}} \quad (11)$$

In terms of critical concentration the derivative of the flux J_A can be written:

$$\frac{dJ_{AD}}{dX_{AD}} = nk_{2C} \left[[X_A(0) - X_{AD}(t)]^{n-1} - [X_A(0) - X_{AD}(cr)]^{n-1} \right] \quad (12)$$

The corresponding thermodynamic force is:

$$F_{AD} = R \left\{ \ln k_{1D}[X_A(0) - X_{AD}(t)] - \ln k_{2C}[X_A(0) - X_{AD}(t)]^n \right\} \quad (13)$$

and its derivative:

$$\frac{dF_{AD}}{dX_{AD}} = R(n-1) \frac{1}{X_A(0) - X_{AD}(t)} \quad (14)$$

R being the ideal gas constant.

d) *The stability-instability dipole*

- For $n > 1$ and $X_{AD}(t) < X_{AD}(cr)$ then $\frac{dF_{AD}}{dX_{AD}} > 0$, $\frac{dJ_{AD}}{dX_{AD}} > 0$, our living system is “thermodynamic stable”.
- When $n > 1$ and $X_{AD}(t) > X_{AD}(cr)$ then, $\frac{dF_{AD}}{dX_{AD}} > 0$, $\frac{dJ_{AD}}{dX_{AD}} < 0$, the system is “thermodynamic unstable”, the entropy is growing up, the regulatory system is perturbed and the integrity of cell and organism as a whole may be affected.

- For $n < 1$ and $X_{AD}(t) < X_{AD}(cr)$ then $\frac{dF_{AD}}{dX_{AD}} < 0$, $\frac{dJ_{AD}}{dX_{AD}} < 0$, the living system is “thermodynamic stable”.
- When $n < 1$ and $X_{AD}(t) > X_{AD}(cr)$ then $\frac{dF_{AD}}{dX_{AD}} < 0$, $\frac{dJ_{AD}}{dX_{AD}} > 0$, there is again “instability”.

We have to note that in the above analysis the following inequalities are always satisfied:

$$0 \leq X_{AD}(t) \leq X_A(0) \quad (15)$$

Also, from the same analysis it can be seen that our interest is to have, for a given n a critical concentration $X_{AD}(cr) = X_A(0) - \left[\frac{k_{1D}}{k_{2C}} \right]^{\frac{1}{n-1}}$ as big as possible, *i.e.*, k_{2C} to be large enough, what means that studied molecules (or complexes) contain inside them some other molecules that have reversed action with respect diet or the diet itself contains some favorable substances - which, in general, it is the case – “Low vitamin E content is a greater risk factor than high cholesterol and blood pressure” [2] and $k_2 \approx 0$! If we return to $\Delta X_{AD}(t)$ then the corresponding critical concentration is:

$$\Delta X_{AD}(cr) = \left[\frac{k_{1D}}{nk_{2C}} \right]^{\frac{1}{n-1}}, \quad X_{AD}(cr) + \Delta X_{AD}(cr) = X_A(0) \quad (16)$$

hence and $X_{AD}(cr)$ and $\Delta X_{AD}(cr)$ are complementary parameters and the ratio of critical concentration (16) to stationary state (6) is:

$$R_D \equiv \frac{\Delta X_{AD}(cr)}{\Delta X_{AD}(s)} = \left[\frac{1}{n} \right]^{\frac{1}{n-1}} < 1 \quad (17)$$

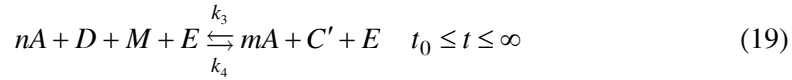
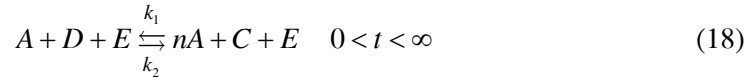
For $1 < n < \infty$, $0.368 < R_D < 1$, and for $0 < n < 1$, $0 < R_D < 0.368$. For example from (17) it results that the use of $n = 3$ ($R_D \approx 0.577$) is more dangerous than $n = 2$ ($R_D \approx 0.500$) since the interest is to have $\Delta X_A(cr)$ as small as possible to reduce the region of instability (it was supposed that $\Delta X_A(s)$ is the same in the two cases). This is in agreement with the experimental facts of intracellular accumulation of ester-cholesterol when the macrophages are incubated with auto-oxidized LDL ($n = 2$) and LDL oxidized by Cu^{2+} ($n = 3$) [14–15].

The existence of a critical concentration $X_A(cr)$ or $\Delta X_A(cr)$ is also confirmed by some experimental data that TBARS must reach a threshold value in order to make LDL cyto- toxic (see p. 359 of [2]).

From (17) it results that for $n > 1$ the lowest value of $\Delta X_{AD}(cr)$ is $\Delta X_{AD}(cr) = 0.386X_A(s)$ and the highest is $\Delta X_{AD}(cr) = \Delta X_{AD}(s)$.

3. THE DRUG ACTION

When a drug M is administered we suppose a “chemical reaction” in two steps:



where M is the supplied drug, D , C , C' , E have the same meaning as before and the corresponding concentrations are supposed to be constant (including for M) and t_0 is the time when treatment with M starts. We have to mention that M may be also some convenient additional diet.

In the following one more supplementary hypothesis is that $m = 1$ (to make the calculations easier) that means the efficiency of M is one hundred percents (the total reversibility is assured).

By analogy to (4) the new kinetic equation is:

$$\frac{d}{dt} \Delta X_{AM} = -(k_{1D} + k_{4C}) \Delta X_{AM} + (k_{2C} + k_{3M}) \Delta X_{AM}^n \quad (20)$$

where $k_{4C} = k_4 X_C' X_E$ and $k_{3M} = k_3 X_D X_M X_E$. Comparing equation (4) with (20) the only difference is that now the k_{1D} is replaced by $k_{1D} + k_{4C}$ and k_{2C} by $k_{2C} + k_{3M}$.

The solution of (20) is for $t \geq t_0$

$$\Delta X_{AM}(t) = X_A(0) e^{-(k_{1D} + k_{4C})(t-t_0)} \cdot \left[1 + \frac{k_{2C} + k_{3M}}{(k_{1D} + k_{4C}) X_A^{n-1}(0)} (e^{(1-n)(k_{1D} + k_{4C})(t-t_0)} - 1) \right]^{\frac{1}{n}} \quad (21)$$

or

$$\Delta X_{AM}(t) = X_A(0) e^{-(k_{1D} + k_{4C})(t-t_0)} \cdot \left[1 + \left(\frac{\Delta X_{AM}(s)}{X_A(0)} \right)^{1-n} (e^{(1-n)(k_{1D} + k_{4C})(t-t_0)} - 1) \right]^{\frac{1}{n}} \quad (22)$$

For the transformed parameter $X_{AM}(t)$ the solution is:

$$X_{AM}(t) = X_A(0) \left\{ 1 - e^{-(k_{1D} + K_{4C})(t-t_0)} \cdot \left[1 + \left(\frac{\Delta X_{AM}(s)}{X_A(0)} \right)^{1-n} (e^{(1-n)(k_{1D} + k_{4C})(t-t_0)} - 1) \right]^{\frac{1}{n}} \right\} \quad (23)$$

where $\Delta X_{AM}(s)$ is the new stationary state.

The solution (23) can be compared with different experimental data. If this is done, for instance for oxidized LDL by Cu^{2+} and then treated with different anti-oxidants like α -tocopherol (vitamin E), glutathione [2] and some others [16, 17], or the stimulation of intracellular coenzyme A (CoA) biosynthesis by pantothenate kinase (PanK) [18] it can be seen a qualitative agreement between experimental and theoretical dependence of the corresponding concentrations on time and t_0 may be somewhat assimilated with the lag time.

Repeating the calculations for thermodynamic flux J_{AM} and force F_{AM} a new critical concentration is obtained:

$$\Delta X_{AM}(cr) = \left[\frac{k_{1D} + k_{4C}}{n(k_{2C} + K_{3M})} \right]^{\frac{1}{n-1}} \quad (24)$$

or

$$X_{AM}(cr) = X_A - \left[\frac{k_{1D} + k_{4C}}{n(k_{2C} + K_{3M})} \right]^{\frac{1}{n-1}} \quad (25)$$

Formally, the ratio R_M , as a function of n is the same as in (17):

$$R_M \equiv \frac{X_{AM}(cr)}{X_{AM}(s)} = \left[\frac{1}{n} \right]^{\frac{1}{n-1}} < 1 \quad (26)$$

but n is different, closer to the unity, now.

However, by using (11) and (25) it can be defined another ratio:

$$R_0 \equiv \frac{X_{AM}(cr)}{X_{AD}(cr)} = \frac{X_A(0) - \left[\frac{k_{1D} + k_{4C}}{n(k_{2C} + K_{3M})} \right]^{\frac{1}{n-1}}}{X_A(0) - \left[\frac{k_{1D}}{nk_{2C}} \right]^{\frac{1}{n-1}}} \quad (27)$$

In general, for an efficient drug it is to be expected that $k_{4C} \ll k_{3M}$, such that for $n > 1$:

$$R_0 > 1, \quad k_{4C} \ll k_{3M}, \quad n > 1 \quad (28)$$

hence as a result of drug action the “stability” region is “larger” and the “unstable one” is “narrower”, what is a very important result of our model.

For $n < 1$

$$R_0 \equiv \frac{X_{AM}(cr)}{X_{AD}(cr)} = \frac{X_A(0) - \left[\frac{n(k_{2C} + K_{3M})}{k_{1D} + k_{4C}} \right]^{1-n}}{X_A(0) - \left[\frac{nk_{2C}}{k_{1D}} \right]^{1-n}} \quad (29)$$

and the analysis is more intricate.

Unfortunately from (29), $R_0 < 1$ which means that, in general, the same drug is not good for both “molecules”, to say LDL and HDL *i.e.*, both for $n > 1$ and $n < 1$ when the diet is acting respectively, even if sometimes the experience contradicts this [11].

4. DIET DEPENDING ON TIME

In the last paragraph we have mentioned that solution (23) is in agreement with many experimental data. On the other hand, there are other concentration measurements pointing out some minima and maxima, which are not described by (23).

To obtain an explanation for these extremes we believe it is necessary to consider that diet is varying in time. This fact can be taken into account in two ways. First is to consider the living system together with diet forming a closed system, to write the kinetic equation for all components and to use the conservation conditions. Secondly, more realistic, is to use diet in kinetic equations as an external field and then to find the solution for concentrations.

Here, we are following only the first way; the second will be done in a separate paper.

Now, it is proper to write (1) under the form:



where A_1 is the product “molecule” having essentially the same nature as A .

From the kinetic equations written for A , D , A_1 one gets the conservation conditions:

$$\begin{aligned}
X_A + X_{A_1} &= a = X_A(0) \\
X_A - X_D &= d = X_A(0) - X_D(0) \\
X_A + X_C &= c = X_A(0) - X_C(0) \\
X_A + X_{A_1} + X_D + X_C &= X_A(0) + X_D(0) + X_C(0)
\end{aligned} \tag{31}$$

In (31) to avoid confusion with the previous paragraph X_A means untransformed quantity and ΔX_{A_1} the transformed one, etc.

If one makes use of (31) the kinetic equation for X_A on the left side of (1) is:

$$\frac{dX_A}{dt} + PX_A^2 + QX_A + R = 0 \tag{32}$$

which is a Riccati equation, where $P = (k_1 - k_2)X_E$, $Q = -[k_1d - K_2(a + c)]X_E$, $R = -k_2acX_E$.

The equation (32) can be solved if it has some particular solutions. In our case these are:

$$X_{AD}^{1,2} = \frac{-Q \pm \sqrt{Q^2 + 4(k_1 - k_2)ac}}{2(k_1 - k_2)}, \quad k_1 \neq k_2 \tag{33}$$

and in general $k_1 > k_2$ otherwise the diet is not assimilated. As a matter of fact from (33) under realistic conditions we have only one possible particular solution (there is no bifurcation in the system) such that the equation (32) can be transformed in a Bernoulli equation with the following variable transformation:

$$X_{AD} = X_A^1 + \frac{1}{z} \tag{34}$$

and the solution for $z(t)$ can be found in the same way as above.

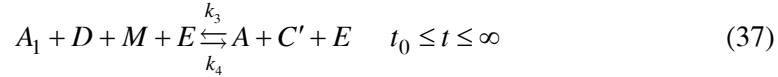
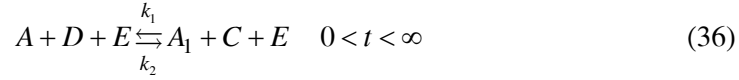
If the thermodynamic flux and force for X_{A_1} are written, again we obtain a critical concentration:

$$X_{AD}(cr) = \frac{k_1[X_A(0) + X_D(0)] + k_2X_C(0)}{2(k_1 - k_2)} \tag{35}$$

over which the system is thermodynamic “unstable”, *i.e.*, $\frac{dJ_{A_1}}{dX_{A_1}} > 0$, $\frac{dF_{A_1}}{dX_{A_1}} < 0$.

These results are bringing nothing new with respect to the constant diet and they are very similar to those obtained in [13].

In the case when a drug is administered then as before we can write a chemical reaction in two steps:



and the corresponding kinetic and conservation equations. However to be able to solve the kinetic equation we have to suppose that the concentration of drug X_M is very small and constant: $X_M \ll X_D$ (realized in general).

Following the same procedure as before the flux and force can be written and a new critical concentration is obtained and the ratio R_0 is:

$$R_0 = \frac{X_{A_1M}(cr)}{X_{A_1D}(cr)} > 1 \quad (38)$$

i.e. the stability region becomes larger and the unstable narrower.

Unfortunately no minima and maxima are obtained for X_{A_1} such that only hope is to prescribe from the start a diet as a function of time which is not a trivial problem, or to be content with linear approximation where strange enough they are almost naturally there (in which $X_D(t)$ is done as a result of interaction with all involved molecules) [10, 11].

5. DISCUSSION AND RESULTS

In order to get more light on the metabolism of cell in living systems and to explain the kinetic processes of the involved molecules, here is proposed a quasi-chemical model, with high order reactions taking into account the interaction of bio-molecules (or complexes formed by these) with administered diets and drugs.

The kinetic equations, for the concentration of bio-molecules, are written and solved in terms of time, analytically, both for unreacted and product molecules. Also the thermodynamic fluxes and forces are analyzed.

To resume, our main results are:

- For constant diet and drugs the corresponding solutions of kinetic equations are Bernoulli's type and the limit conditions are very well satisfied. The constant values for diet and drug are motivated.
- From the thermodynamic flux a critical concentration of bio-molecule product is obtained.
- For values above the critical concentration the living system is "thermodynamically unstable", the regulatory system is perturbed and the integrity of cell and organism may be affected. For example the

oxidation of LDL by Cu^{2+} is more dangerous than the auto-oxidation of LDL in agreement with the measured ester-cholesterol intra-cellular accumulated [14, 15]. Also TBARS must reach a threshold value to make LDL cytotoxic [2].

- The critical concentration is smaller than the stationary state, if the last one exists.
- After drug or additional convenient diet administration the stability region becomes larger and the instable one is narrowed, which is the most important result of our approach.
- Our theoretical results are compared with the experimental ones especially for oxidized LDL by Cu^{2+} and treated with different antioxidants [2, 16, 17]. Also with those of [18] regarding the stimulation of intra-cellular coenzyme A (CoA) biosynthesis by pantothenate kinase. The agreement between theoretical and experimental data is qualitatively good.
- If two different bio-molecules are studied under the same diet in which one is increasing (like LDL) and the other is decreasing (like HDL) the theoretical results show that the same drug is not good for both even the experiments with simvastatin seem to contradict that [11].
- The calculations repeated for time variable diet, resulting from conservation conditions (and considering that the living system and diet form a closed system) do not offer an explanation for minima and maxima experimentally obtained [2, 16, 18]. This negative result requires the consideration of the diet as an external field with time dependence or to use only the linear approximation with all involved molecules interactions (like Cholesterol, TG, LDL, HDL, etc). Of course our approach can be used for a large category of bio-molecules and diseases as atherosclerosis, diabetes, cancer, etc. and the diet can be food, radiation or any other environmental factors.

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