FUNDAMENTAL RESEARCH

Modeling Physical-Chemical Properties of High Dilutions: an electrical conductivity study*

1Carla Holandino, 1Rafael Harduim, 2Venício Feo da Veiga, 3Sheila Garcia, 4Carlos Renato Zacharias

1. UFRJ – Rio de Janeiro Federal University – Laboratory of Pharmaceutical Sciences
2. UFRJ – Rio de Janeiro Federal University – Microbiology Institute
3. UFRJ – Rio de Janeiro Federal University – Control Quality Laboratory
4. UNESP - São Paulo State University – Chemistry and Physics Department

The most common way to perform succussions is to place a liquid preparation inside a glass vessel and beat it vigorously against a hard elastic surface, either manually or using a mechanical apparatus. This procedure has been assumed able to transfer mechanical energy to the molecular level, where it becomes available to perform chemical work. Such interpretation has been enforced by observed changes in the electrical conductivity (EC) of High Dilutions (HD) due to succussion. In order to address this question, we compared the electrical conductivity changes of HD prepared from Vincristine sulfate (VCR) samples with those of an inert solvent. Samples were produced through manual and mechanical succussions in order to observe the influence of bubbles production. The results confirmed the timing of EC changes but these were equivalent for VCR and solvent, except for VCR 1cH samples. Also, the production of bubbles does not affect the EC in an extent able to distinguish succussion procedures. We concluded that the physical-chemical properties of HD can be modeled by chemical and diffusive mechanisms typical of distilled water.

Key words: succussion; electrical conductivity; high dilutions; distilled water; modeling

Introduction

High Dilutions (HD) have been prepared all over the world through a biphasic serial process (named dynamization) consisting of dilution and succussion (shaking). As the homeopathic therapy is the most successful application of HD, its Pharmacopeia has been adopted by the HD scientific and technological community. The dilution is usually performed on a decimal or centesimal scale (per unit of volume) while succussion can be made by manual, mechanical, or vortex procedures, among others [1-2].

The preference for the various succussion methods is cultural technological commercial or philosophical in reasoning. However, experimental models and clinical trials have demonstrated the efficacy of all these procedures, validating the different succussion techniques [3-7].

The most common method of succussion is to place a liquid preparation inside a glass vessel and beat it vigorously against a hard elastic surface, either manually or using a mechanical apparatus.

Succussions were initially proposed by Hahnemann [8], probably inspired on alchemist techniques [9]. Mechanical succussion had probably originated during the Industrial Revolution, with the desire to produce higher potencies faster, cheaper and more standardized [10]. An important feature of mechanical succussion is the intense production of bubbles in the liquid phase [11], as compared with those produced by manual succussion. Physical chemistry leads us to assume that bubbles increase the surface area of and increase gasification, in some way augment the chemical degradation process. However, it is not known if and how this affects the physical structure and biological action of high dilutions [12-13]. Also, succussion has been interpreted as a mechanism that allows transfer of mechanical energy down to the molecular level, where it becomes available to perform chemical work [14-16].

Some literature suggests unexpected physical chemistry properties and biological implications of high dilutions [17-25] after dynamization. Electrical conductivity measurement is one technique able to observe the dependence of HD on preparation protocols and aging [18-20, 23, 26].
The electrical conductivity of liquid samples measures its ability to conduct electricity and is influenced by chemical species which tend to ionize in the solution. Therefore chemical degradation and diffusion play an important role in the process. Also, aqueous systems have their own natural ionic species [27] other than outsider contaminants.

In this paper, we measured the electrical conductivity (EC) of manually and mechanically succussed samples of highly diluted Vincristine sulfate (VCR). The main objective was to quantify the EC dependence on time and potency following different succussion procedures, with the hope of adding to our physical-chemical understanding of these procedures.

**Material and Methods**

The starting solution was Vincristine sulfate – VCR – (Zodiac®) in purified water. VCR is a chemotherapeutic agent metabolized by the human body at 37 Celsius degree, despite its low storage temperature (-20ºC) [28]. The samples were prepared and stored at room temperature. VCR was chosen due our interest treating neoplastic cell cultures with multidrug resistance (MDR) to VCR, [29], according to the principles of isotherapy.

The starting solution consisted of 1.0 mg/ml VCR diluted in 7.5ml of distilled water, placed in a 10 ml test-tube. Centesimal Hahnemannian potencies were produced to 15cH (potentized VCR). Mechanical succussions (Vm group) were performed with one hundred succussions (Denise 10-50, Autic®) over 33 seconds (approximately 3 hertz), while handmade ones (Vh group) were performed with similar frequency. In order to register the chemical effect of the VCR presence or systematic experimental errors on measurements, equivalent sets of control samples (1 to 15cH) were prepared with distilled water only, by mechanical (Wm) and manual (Wh) succussions.

In order to reduce eventual chemical release and contaminations, ambar borosilicate (USP type I) test-tubes were used, composed mainly of silicon dioxide and boric oxide, with low levels of the non-network-forming oxides [30], [31]. Furthermore, all samples were analyzed in a differential way, comparing the active sample with its control prepared with solvent only, but processed in the same manner. All test-tubes and plastic stoppers were washed three times with distilled water (Millipore®) before initiating sample preparation. Test-tubes (10 ml) were filled up to 2/3 volume. Samples were monitored for microbiological contamination.

Electrical conductivity (EC) measurements were performed on all samples at 25°C with systematic calibration and temperature compensation using a Mettler-Toledo MPC 227 apparatus. Intrinsic experimental errors were 0.5%. The potencies 1cH and 4cH were selected to evaluate the extent of effects due to the presence of VCR molecules; 7 and 9cH as examples of pre-Avogadro’s dilutions and 12 to 15cH as post-Avogadro samples. The Vh and Vm groups were formed only by potencies at or above 4cH. The data from the samples were collected immediately after sample preparation (time zero) and after 7, 14, 21 and 35 days. The samples were kept at rest in the interval and no succussions were performed before measurements. Each sample was measured 4 (four) times to evaluate the averaged value and standard deviation.

**Results**

Figures 1 to 4 shows the EC time dependence, for VCR and water (control) samples. All electrical conductivity values are on μS/cm and time in days. The data are presented as averaged value and standard deviation.

![Figure 1: Water control samples – mechanical succussions (Wm). Averaged curves with standard deviation bars are shown for all potencies (n cH). The bold line is the averaged curve over all potencies.](image1)

![Figure 2: Water control samples – handmade succussions (Wh). Averaged curves with standard deviation bars are shown for all potencies (n cH). The bold line is the averaged curve over all potencies.](image2)
day one can observe a slight increase in EC, similar to those observed to Vm and Vh groups, as well to control (Wm and Wh). However, these curves will not be interpreted in this article. They were collected only to verify up to what extent the presence of VCR molecules affects the EC. One can realize from figures 3 and 4 that for VCR 4cH and higher, EC results reveal a non-measurable presence of VCR molecules and theirs derivatives.

In figures 1 and 2, all curves show the same time dependence: an initial rapid increase of EC up to day 7th, starting from EC value about 6.0 μS/cm, followed by an asymptotical convergence, similar for all potencies (within standard deviations) and independent of the succussion technique. Thus, one can calculate an averaged curve representative of Wm and Wh groups (figure 5).

Wm and Wh groups have similar behaviors, despite the different succussion procedures and potencies. Thus, one can assume that this is the behavior for the control dynamized distilled water. The EC values for distilled water used on preparations, while kept at rest, were (2.76 ± 0.16) μS/cm, typical of values for distilled water [1]. According to figure 5, the initial value for dynamized water (EC for t = 0) was about 6.5 μS/cm. This value means that the Dynamization procedure, particularly the succussions, alters the physical-chemical properties of water, probably due to gasification and bubbling.

The time dependence can be attributed to typical water dynamics [27,32] as distilled water is a non-

Analysis

Observing figures 1 to 4, some common aspects (except for VCR 1cH curves) can be noted: a time dependent increase similar for all potencies and a common starting EC value. Regarding 1cH samples, the EC time evolution shows two behaviors. The first, up to 7th day, shows a decrease in EC values (from 24 to 18 μS/cm), followed by a gradual increase. The initial decrease can probably be attributed to a chemical degradation. As noted earlier, VCR must be stored at -20ºC, despite its clinical use at 37ºC. The samples were prepared and stored, during the measurement period, at room temperature (about 25ºC).

A second effect may be attributed to distilled water dynamics, as will be discussed latter. From 7th to 35th day one can observe a slight increasing in EC, similar to those observed to Vm and Vh groups, as well to control (Wm and Wh). However, these curves will not be interpreted in this article. They were collected only to verify up to what extent the presence of VCR molecules affects the EC. One can realize from figures 3 and 4 that for VCR 4cH and higher, EC results reveal a non-measurable presence of VCR molecules and theirs derivatives.

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![Figure 3](http://www.ijrh.org/)

**Figure 3:** VCR samples – mechanical succussions (Vm). Averaged curves with standard deviation bars are shown for all potencies (n ≤ 4). The bold line is the averaged curve over all potencies, except 1cH (n ≥ 4).

![Figure 4](http://www.ijrh.org/)

**Figure 4:** VCR samples – handmade succussions (Vh). Averaged curves with standard deviation bars are shown for all potencies (n ≤ 4). The bold line is the averaged curve over all potencies, except 1cH (n ≥ 4).

![Figure 5](http://www.ijrh.org/)

**Figure 5:** Wm and Wh data. Experimental points (average and standard deviation) are shown in squares (Wm) and circles (Wh). The averaged curve is shown (triangles). The bold curve is the logarithm fitting (see details in text and table 1). The fitted function and determination coefficient (r²) are also shown.
equilibrium system. The natural and more stable water state is reached by the equilibrium of molecular species, ionic contaminants, dissolved gases and solutes, aerosols, and silica among others. As the samples were prepared using special test-tubes and distilled water, the molecular dynamics might be driven mainly by the intrinsic water ion formation and dissolved gases and aerosols. Ion formation and other chemical reactions generally are very rapid processes, while gasification or diffusive mechanisms are slower. Wm and Wh groups exhibited similar behaviors for all measured time and for all potencies, indicating that if gasification really happens, it might be important only during the first hours after succussions, but would not strongly influence the chemical stabilization of the solutions. One can visualize this rapid dynamic period in figure 5: up to the first four days after succussion the EC values change significantly. After 35 days the system reach an EC values about 14.4 μS/cm, an intermediated value between fresh and aged distilled water [33-34].

The EC time dependence of Wm and Wh groups (figure 5) suggests a rapid dynamic in the first 7 days, followed by a slow convergence to a stable value (aged water). Such behavior can be approximated by many mathematical functions. One convenient function can be proposed from the natural logarithm, defined as

\[ x(t) = A + B \ln(t + tr) \]  

(eq. 1)

where the coefficients A, B and tr can be adjusted numerically. Physically, this function describes an increasing logarithmic behavior weighted by B, time shifted by a factor tr with a baseline given by A.

The fit of this function to experimental data is shown in figure 5 and the fitted coefficients are reported in table 1:

**Table 1: Logarithm fitting for water groups (averaged curve)**

| \( X(t) = A + B \ln(t + tr) \) | Numerical fitting |
|--------------------------------|-------------------|
| A                             | 7.72 [μS/cm]      |
| B                             | 1.88 [μS/cm.day]  |
| tr                            | 0.53 [day]        |
| \( r^2 \)                     | 0.972             |

For VCR groups, the analyses can be done in two steps: one related to 1cH samples only and the other, to all other potencies. Figures 3 and 4 refer to VCR groups (Vm and Vh). At a first glance, one may note that, except for 1cH samples, the EC time dependence is similar for all potencies and are independent from the succussion procedure. The exception of 1cH samples can be understood by the presence of molecules of VCR (10.0 μg/ml), able to affect the EC values. For 4cH samples, the concentration reaches a mathematical value of 1.0 ng/ml, and the EC remains similar for all higher potencies (within experimental deviations). Thus, we assumed that after 4cH the EC time dependence is governed only by solvent effects other than the presence of VCR molecules or theirs derivatives. These curves can be analyzed through averaged curves (figure 6) as well fitted by a logarithm function, as reported in table 2:

**Table 2: Logarithm fitting for VCR groups (averaged curve)**

| \( X(t) = A + B \ln(t+tr) \) | Numerical fitting |
|-------------------------------|-------------------|
| A                             | 7.31 [μS/cm]      |
| B                             | 1.74 [μS/cm.day]  |
| tr                            | 0.51 [day]        |
| \( r^2 \)                     | 0.929             |

Comparing both fitted curves (figure 7) as well their fitted parameters (tables 1 and 2) one can note that they show the same general behavior, meaning the chemical process involved might be similar for both groups. However, one can note that VCR groups show a slower convergence (smaller B coefficient) with a slight smaller baseline value (A coefficient), with equivalent time shift (tr).
from 1cH to 15cH requires some time in itself. Further, considering the EC changes occur by different chemical channels, the elimination of some of them can reduce, but not eliminate, the EC temporal changes.

Mathematical Modeling

The EC time dependence showed in figure 7 and fitted by equation 1 by values reported on table 1 and 2 suggests a slow chemical process, probably due to gasification or diffusion.

Let’s consider a general chemical reaction (eq. 2) where some reagents ($R_i$, $i=1,L$) produce two sets of products ($P_j$, $j=1,M$) and ($P_k$, $j=1,N$) in such way that only products $P_k$ are involved in the electrical conductivity time dependence.

$$\sum_{i=1}^{L} c_i R_i \rightarrow \sum_{j=1}^{M} c_j P_j + \sum_{k=1}^{N} c_k P_k$$

(eq. 2)

For simplicity, all products $P_k$ will be treated as one and the effective molar concentration will be named $[P]$. As the chemical reaction proceeds, $[P]$ changes over time. Let’s suppose that such time dependence can be described by a power law (eq. 3)

$$[P](t) = P_0 \alpha(t)(t + t_0)^c$$

(eq. 3)

where $[P](t)$ is the $[P]$ value at the instant $t$ (mol/l), $t$ is the time (day), $P_0$ is the $[P]$ for $t = 0$, $\alpha(t)$ is a function related to the reaction speed (day -1), $c$ is the exponent that governs the $[P]$ time dependence (reaction order) and $t_0$ is a reference time indicating that the reaction has already started before $t=0$ (day).

The use of a power law for fitting is convenient because by adjusting the exponent $c$ one can model different time dependences as described in table 3.

Table 3: Time dependence accordingly exponent $c$.

| $c$ value | Interpretation                                    |
|-----------|--------------------------------------------------|
| $c<0$     | $[P]$ decrease in time                           |
| $c=0$     | $[P]$ is constant                                |
| $0<c<1$   | $[P]$ increase accordingly a dissipative dynamics|
| $c=1$     | $[P]$ increase linearly in time                  |
| $c>1$     | $[P]$ increase accordingly a cooperative dynamics|
The assumption of speed reaction time dependent \([\alpha(t)]\) is important because as \(t \to \infty\) the reaction must stop (dynamical equilibrium) due to the consumption of reagents or inversion in the reaction direction. However, as we have measured the EC time dependence only during the first 35 days, and figure 7 indicates that the reaction continues for longer times, we will assume that \(\alpha(t)\) is a constant, named only as \(\alpha\).

So, eq. 3 can be rewrite as

\[
[P](t) = P_0 \alpha(t + t_0)c \quad (eq. 4)
\]

The values of \(P_0\), \(\alpha\), \(t_0\) and \(c\) can be determined by fitting with averaged experimental values (figure 7). As the obtained experimental data refers to EC values, one has to establish a correlation between \([P]\) and EC. As far as the authors know, such correlation seems to be non-trivial. However, it is well known that the measurement of pH and EC are technically similar, based on ion transport. The relation between pH and molar concentration is defined through a logarithm function. Thus, one can propose an equivalent relation to EC and \([P]\) as defined in eq. 5.

\[
\frac{X(t)}{X_0} = \ln \left( \frac{[P](t)}{P_0} \right) \quad (eq. 5)
\]

where, \(X(t)\) and \([P](t)\) are, respectively, the EC and molar concentration of \(P\) at any time \(t\) and \(X_0\) and \(P_0\) are the respective initial values. The hypothesis proposed in eq. 5 involves relative quantities to simplification and dimensional adjustment.

Starting from eq. 4, one can calculate the EC time dependence as bellow:

\[
\frac{[P](t)}{P_0} = \alpha(t + t_0)c
\]

\[
\ln \left( \frac{[P](t)}{P_0} \right) = \ln(\alpha(t + t_0)c)
\]

\[
\ln \left( \frac{[P](t)}{P_0} \right) = \ln(\alpha) + c \ln(t + t_0)
\]

The left term can be substituted accordingly eq. 5 to obtain

\[
\frac{X(t)}{X_0} = \ln(\alpha) + c \ln(t + t_0)
\]

Finally we can state,

\[
X(t) = X_0 \ln(\alpha) + X_0c \ln(t + t_0) \quad (eq. 6)
\]

Equation 6 has the same mathematical structure as the eq. 1, used to fit experimental data. Comparing both equations we can identify parameters \(A\), \(B\) and \(t_r\) as:

\[
A = X_0 \ln(\alpha) \quad (eq. 8)
\]

\[
B = X_0c \quad (eq. 9)
\]

\[
t_r = t_0 \quad (eq. 10)
\]

**Numerical Fittings**

In order to compare eqs. 8 to 10 with numerical data reported in tables 1 and 2, one first needs to determine \(X^0\) values for water (W) and VCR (V) averaged curves. These can be calculated from data on tables 1 and 2, imposing \(t=0\) in eq. 1. Thus one obtains \(X^0 = 6.5\) and \(6.1\) \(\mu\text{S/cm}\) for water and VCR groups, respectively. Then, the parameters \(A\), \(B\) and \(t_r\) can be calculated, as reported on table 4.

**Table 4: Numerical fitted parameters**

| Parameter | Water | VCR | average |
|-----------|-------|-----|---------|
| \(a\) (day\(^{-1}\)) | 3.28  | 3.31 | 3.30    |
| \(c\)    | 0.289 | 0.284 | 0.287   |
| \(t_0\) (day) | 0.53  | 0.51 | 0.52    |

The values reported on table 4 indicate that the chemical temporal dynamics for water and VCR are similar. As discussed before, the differences observed on figure 7 might be explained as a consequence of the initial VCR degradation, while the samples were being prepared or during the first hours after preparation. The similar values reported on table 4 come to reinforce this hypothesis. So, it is convenient calculate the averaged values for these parameters.

The parameter \(c\) reveals a dissipative dynamics where the continuous formation of the product \(P_k\) inhibits the chemical reaction and thus, the increase of the EC. From eq. 4 and table 4 one can determine the relative molar concentration (\(M_p\)) as well its formation speed (\(S_p\)) as given below:

\[
M_p(t) = \frac{[P](t)}{P_0} = 3.30 (t + 0.52)^{0.287}
\]

\[
S_p(t) = \frac{d}{dt} \left( \frac{[P](t)}{P_0} \right) = 0.95(t + 0.52)^{-0.71}
\]

\(M_p(t)\) and \(S_p(t)\) can be visualized in figure 8.
Figure 8 shows that the reaction speed reduces to 50% in 1 day and 25% after 7 days, when the second set of measurements were collected. Thus, the details of this fast dynamics could not be directly observed. After the 7th day, the speed decreases slowly, as the relative molar concentration increase almost linearly. As discussed before, the reaction must reach a steady state represented by stable concentrations and speed values. However, this condition was not observed within the first 35 days.

Conclusions

The first objective of this work was compare mechanically and manually succussed highly diluted samples regarding a physical chemical parameter. The selection of electrical conductivity was based on recently published articles suggesting the existence of a complex dynamic of the water able to exhibit memory and time dependent effects.

As the initial solution contained molecules of VCR and was prepared at room temperature, chemical degradation was expected. Thus, we analyzed VCR data only for potencies 4cH or higher. The control samples were prepared using the same protocol using only distilled water.

Mechanical succussions are known to produce intense bubbling when compared to handmade ones. Bubbles, beyond being a mechanical agent, increase the contact air-liquid area generally affecting the speed of reactions. Thus, a chemically instable substance submitted to bubbling would reveal differences when compared to water control samples.

The experimental results showed differences between VCR and control samples (figure 7) but these were systematic (figure 7, lower graphic). These differences could be explained by chemical effects in the first days after samples preparation. Such a hypothesis was reinforced by the calculation of the model parameter, reported on table 4, similarly valued for both groups of samples, as well for the similarity between VCR and control samples in all potencies (n cH).

Mathematical modeling requires the assumption of a relationship between EC and [P] (eq. 5). Despite its similarity with the concept of pH, this assumption must be studied further to be confirmed. The use of a power law to fit EC time dependence is not critical, because this function can describe many natural phenomena and the parameters were adjusted from experimental data, obtaining a correlation factor almost unitary (see tables 1 and 2).

The main conclusion is that water shows a slow dynamics, probably driven mainly by diffusive mechanisms without relation with any special property of the dynamization procedure beyond than mechanical agitation. Memory effect can be understood as a time shift in such dynamics. Bubbling represents an important process only when an instable substance is present. For samples prepared with distilled water only, no difference was observed between 1cH and higher potencies. However, all potencies for both groups showed an increase of EC even at the first measurements, when compared with distilled water kept at rest. Our findings are in agreement with the well known fact that distilled water is an out-of-equilibrium state and naturally goes to maximum entropy, especially when perturbed by bubbling and mechanical agitation.

All these conclusions are strongly limited to electrical conductivity measurements. No biological implication can be extracted directly from these results until further biological experiments are performed, in order to correlate EC with biological effects [35].

Despite the findings reported in this article indicate that dynamized Vincristine is not different from water (in terms of EC), it does not means that HDs do not have biological action. One cannot invalidate the biological activity evidences based on isolated EC results or any other physical-chemical parameter. HD biological activity is not well understood and some evidences seem to indicate that a biological sensor is required to observe the responses. If this hypothesis is true, non-biological studies should be secondary to characterize a HD. Much research work is required to clear this point.
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