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Protein Aggregation Measurement through Electrical Impedance Spectroscopy

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Abstract. The paper presents a novel methodology to measure the fibril formation in protein solutions. We designed a bench consisting of a sensor having interdigitated electrodes, a PDMS hermetic reservoir and an impedance meter automatically driven by calculator. The impedance data are interpolated with a lumped elements model and their change over time can provide information on the aggregation process. Encouraging results have been obtained by testing the methodology on K-casein, a protein of milk, with and without the addition of a drug inhibiting the aggregation. The amount of sample needed to perform this measurement is by far lower than the amount needed by fluorescence analysis.

1. Introduction

Protein aggregation leading to amyloid fibrillation is responsible for several serious human diseases. Neurodegenerative diseases such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), Amyotrophic Lateral Sclerosis (ALS) are some of the important diseases associated to the presence of protein amyloid deposits. Amyloid aggregates consist of fibers containing misfolded proteins with substantial extent of beta-sheet conformation, arranged in the so-called cross-beta structure. The complexity and dynamics of protein folding present unique challenges for elucidating the molecular mechanisms involved in protein aggregation and designing effective amyloid inhibitors. Continuous development of creative approaches to identify an ultimate solution for controlling protein amyloid aggregation in biopharmaceuticals and clinical pathology is clearly required.

K-casein is arguably the best characterized milk protein and constitutes over 70–80% of total bovine milk protein. Recently it has been demonstrated that the reduced and carboxymethylate K-casein (RCM-k-CN) can be used as first trial for screen compounds with putative inhibitor or aggregation blocker activity [1]. RCM-k-CN is an amyloidogenic protein that readily undergoes nucleation-dependent aggregation and amyloid fibril formation via a similar pathway to disease-specific amyloidogenic peptides like Amyloid β ($A\beta$), which is associated with Alzheimer's disease [2].

Currently the measurement of RCM-k-CN fibrillar aggregation is performed by means of optical fluorescence. A thioflavin-T (ThT) fluorescence binding assay is used. When ThT binds to amyloid



aggregates, it exhibit fluorescence at 485 nm following excitation at 440 nm. By monitoring the time-course of this ThT fluorescence, the fibril formation kinetics can be assessed [3].

In the present paper we investigate a new method, based on the Electrical Impedance Spectroscopy, for monitoring the aggregation of proteins into fibrils. We developed a bench composed by a sensor with interdigitated gold electrodes, a Poly Dimethyl Siloxane (PDMS) hermetic and removable reservoir, a LCR meter and a control panel to perform automatic measurements. Since the protein and the ThT are dissolved in a buffer whose electrical properties are mainly conductive, when the fibrils of the protein drop on the electrode surface there is a local change of charge distribution, that modifies the impedance spectroscopy response. The preliminary results suggest that electric impedance can be a new valuable method for analyzing the fibrillar aggregation of proteins for screening fibrillar aggregation inhibitors without the presence of ThT molecule as marker.

2. Bench Description and Measurements Setup

The developed system is represented in Figure 1. A fully programmable control panel (described in detail in [4]), has been developed in order to automatically acquire impedance data from the Agilent E4980A LCR meter via USB interface.

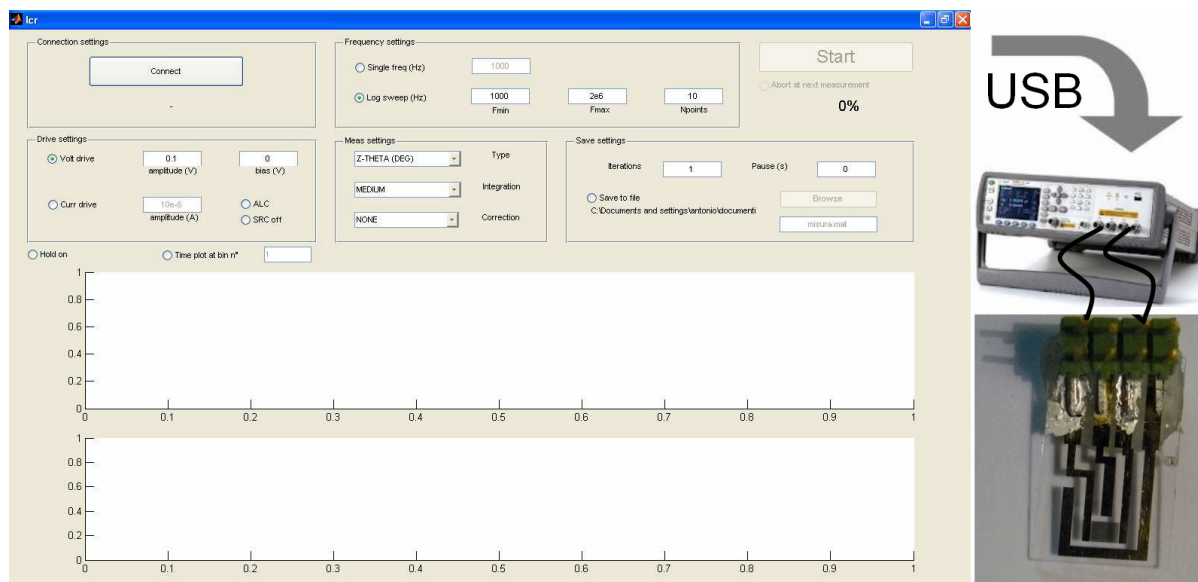


Figure 1: Developed bench is composed by a control panel, a LCR meter and an interdigitated electrodes sensor.

To the LCR meter it is connected a sensor having gold interdigitated electrodes sputtered on quartz; on the sensing electrodes we posed a removable PDMS reservoir which is topped by a removable polycarbonate slide. The three layers are held together with a clamp.

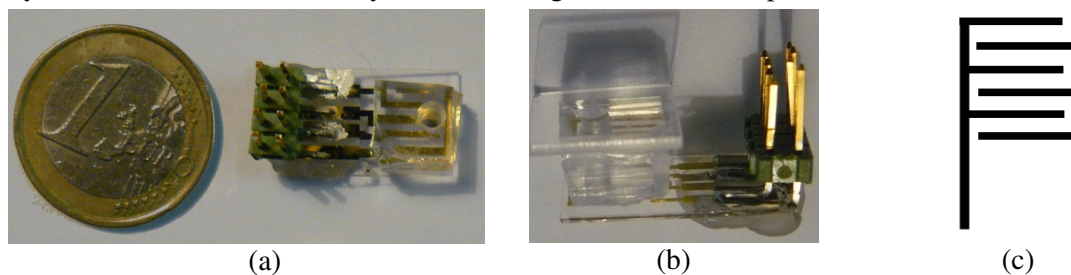


Figure 2: (a) Top view and (b) side view of the sensor with reservoir; (c) electrodes arrangement.

The sensor is composed by 65 couples of interdigitated electrodes; the dimensions of each finger are $10\ \mu\text{m} \times 2\ \text{mm}$ and the space between fingers is $5\ \mu\text{m}$. The PDMS cylindric reservoir is $3\ \text{mm}$ in diameter and $8\ \text{mm}$ in height, yielding to a sample volume of $60\ \mu\text{l}$, the assembly of the sensor is shown in Figure 2 (a) and (b).

2.1. Measurements Setup

The solution of RCM-k-CN freshly dissolved in $50\ \text{mM}$ sodium phosphate buffer pH 7.2 $10\ \mu\text{M}$ ThT at final concentration of $0.5\ \text{mg/ml}$ ($27\ \mu\text{M}$) is poured in the reservoir and hermetically closed with the top of the sensor; the sensor is then posed at controlled temperature in a thermostated chamber at $37\ ^\circ\text{C}$.

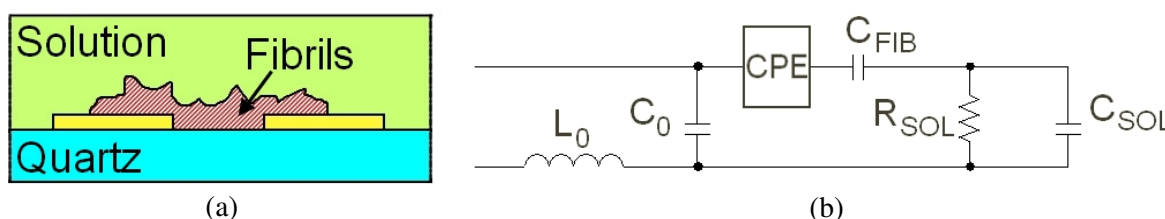


Figure 3: (a) Fibrils precipitation on gold electrodes; (b) equivalent circuit

Figure 3 (a) shows the precipitation of protein fibrils on the gold electrodes and Figure 3 (b) shows the equivalent circuit considered for assessment of fibril deposition. The elements C_0 and L_0 have been compensated performing the “open” and “short” compensations before measuring the sample properties. The bench has been set to perform impedance measurements with a drive voltage of $100\ \text{mV}$ (to avoid redox reactions) with 24 points frequencies in the range $[20, 2 \cdot 10^6]$ Hz logarithmically spaced. Sweeps are performed every 5 minutes for a total duration of 18 hours. The Constant Phase Element CPE in Figure 3 (b) represents the effect of double layer due to interaction between the solution and the electrode [5], its impedance is

$$Z_{CPE} = \frac{1}{(j\omega)^\alpha C_{DL}} \tag{1}$$

being C_{DL} the double layer capacitance and $0 \leq \alpha \leq 1$ its phase shift. The quantities R_{SOL} and C_{SOL} represent the impedance behaviour of the solution while the measurand is represented by the change over time of the capacitance C_{FIB} due to the fibril precipitation on the electrodes.

3. Experimental results

Figures 4 (a) and (b) show, respectively, the absolute value and the phase of measured impedance (after the open-short compensations) during a 18 hours experiment assaying fibril precipitation on the

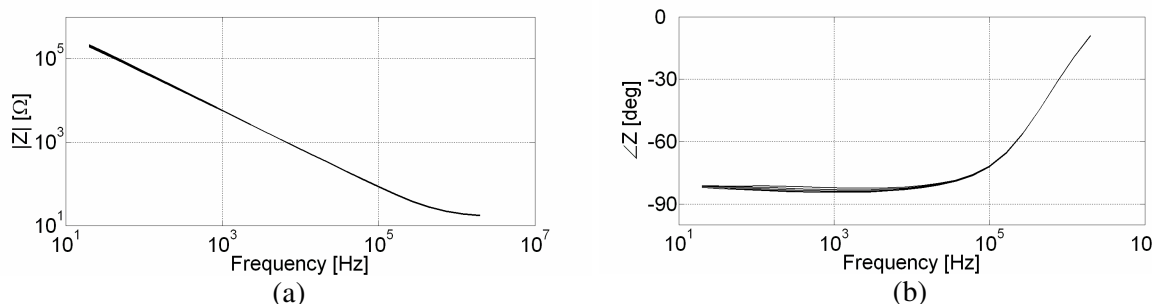


Figure 4: (a) Impedance magnitude and (b) phase behaviour.

electrodes. The accuracy of acquired impedance data after “open” and “short” compensations is 0.1% [6].

As a first comment it is clear that the double layer (CPE) plays a significant role and it strongly masks the impedance changes over time; it is also possible to see that the quantity C_{SOL} is too small and does not intervene in this frequency range.

In order to better distinguish the parameters of the model which are varying over time, the spectroscopy impedance data have been interpolated with an iterative fitting software [7]. The interpolated data provided $R_{SOL} \approx 10 \Omega$, $C_{DL} \approx 70 \text{ nF}$, $\alpha \approx 0.9$, the accuracy of interpolated data is 5%. The quantity C_{FIB} cannot be directly estimated by the fitting software since in the model it is in series to a CPE having a high value of α . For this reason we performed the impedance measurement of the buffer solution in the same conditions, with data acquired every 5 minutes over 18 hours.

The C_{FIB} value can be derived considering the double layer changes over time of the protein with respect to the double layer changes of the buffer:

$$C_{FIB,PROTEIN} = \left(\frac{(j\omega)^{1-\alpha}}{C_{DL}} \Big|_{PROTEIN} - \frac{(j\omega)^{1-\alpha}}{C_{DL}} \Big|_{BUFFER} \right)^{-1} \quad (2)$$

In (2) we considered the double layer impedance for protein as the sum of the double layer contribution plus the C_{FIB} contribution and we subtracted the double layer contribution of the buffer; to verify our data we repeated the experiment three times in the same conditions.

In order to validate our approach we added to the buffer blank and to the protein solution the drug doxycycline at concentration of 81 μM . This doxycycline concentration is known to abolish A β fibril formation. As in (2) we evaluated the quantity

$$C_{FIB,PROTEIN+DRUG} = \left(\frac{(j\omega)^{1-\alpha}}{C_{DL}} \Big|_{PROTEIN+DRUG} - \frac{(j\omega)^{1-\alpha}}{C_{DL}} \Big|_{BUFFER+DRUG} \right)^{-1} \quad (3)$$

The experiments with and without drug were repeated three times in the same conditions and results proved encouraging.

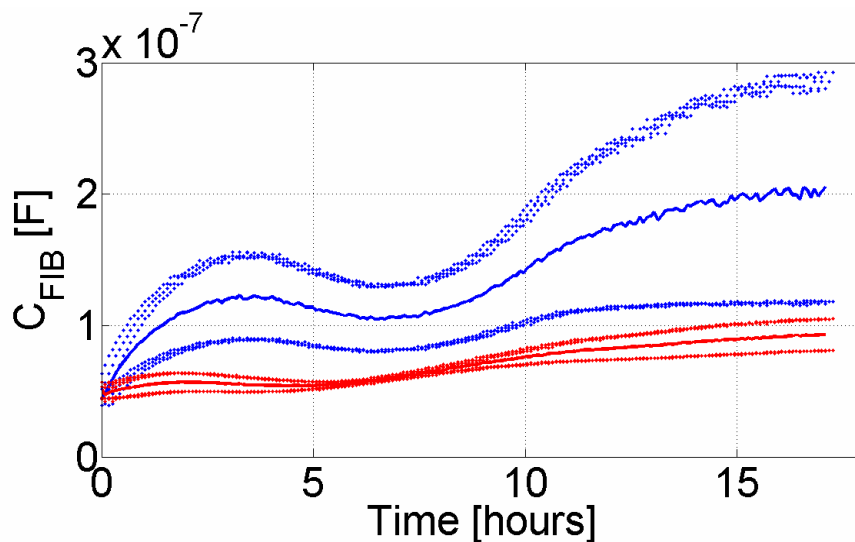


Figure 5: C_{FIB} estimation with (red line) doxycycline inhibitor addition and without (blue line) doxycycline inhibitor addition. The points above and below the curves represent the experimental standard deviation of the mean that was extracted from three experiments.

Figure 5 shows the estimation of C_{FIB} when the protein solution fibrillates (blue line) and when doxycycline is added (red line). The points surrounding the curves represent the accuracy of the measurements estimated as standard deviation of the mean of the three experiments performed.

4. Discussion and Conclusions

Figure 5 shows that the behaviours of the protein with and without the tested drug are quite different, especially in the first eight hours of the experiments. This is in good agreement with the doxycycline principle of operation; this drug in fact mainly inhibits the initial formation of oligomers which can lead to long organized protein fibrils.

Doxycycline does not necessarily prevent the amorphous precipitation of the protein that could be related to the residual C_{FIB} increase observed over long times. It is expected, however, that the impedance characteristics of the solution could be sensitive to the type of precipitating aggregates, thereby enabling the assessment of the actual process undergoing in solution. This issue, along with the very relevance of the precipitation, compared to the formation of the very anisotropic soluble adducts that occurs in solution upon at the early stages of fibrillogenesis, requires further investigation. In addition the effects on amyloidogenic protein solutions of other perturbations such as agitation or ultrasound application could be interestingly monitored by impedance change measurements.

The results that have been reported in this first preliminary work show that the Electrical Impedance Spectroscopy could be a good method for measuring fibril formation for evaluating new drug efficiency. With this method the experiments can be carried out on the protein solution without the addition of any contrast medium (like ThT) thus providing information on the real aggregation kinetics; the addition of ThT in fact can modify the fibril kinetics since ThT binds to the fibrils and may therefore compete with or even interact the tested drug. Moreover, with the presented device, measurements can be performed with a very limited amount of solution and therefore the method efficiency is coupled to sensitivity advantage.

References

- [1] Carver, J. A., Duggan, P. J., Ecroyd, H., Liu, Y., Meyer, A. G. & Tranberg, C. E. "Carboxymethylated-k-casein: A convenient tool for the identification of polyphenolic inhibitors of amyloid fibril formation", *Bioorganic & Medicinal Chemistry*, 18 (1), 2010, 222-228.
- [2] Ecroyd, H., Koudelka, T., Thorn, D. C., Williams, D. M., Devlin, G., Hoffmann, P. & Carver, J. A. "Dissociation from the oligomeric state is the ratelimiting step in amyloid fibril formation by k-casein.", *J. Biol. Chem.* 283, 2008, 9012–9022.
- [3] H. LeVine III, "Thioflavine T interaction with synthetic Alzheimer's disease β -amyloid peptides: Detection of amyloid aggregation in solution", *Protein Science* 2 (3), 1993, 404-410
- [4] A. Affanni, R. Specogna, F. Trevisan "Measurement Bench for Impedance Tomography during Hemostasis Process in whole Blood", *IEEE International Symposium on on Medical Measurements and Applications (MeMeA 2011) Proceedings, Bari (Italy), 30-31 May 2011*, pp. 80-83
- [5] E. Barsoukov, J. R. Macdonald, "Impedance Spectroscopy Theory, Experiment, and Applications", 2nd ed. J. Wiley and Sons, 2005, 30 – 90
- [6] Agilent E4980A Precision LCR Meter Users Guide, 7th Edition, Agilent Technology, Santa Clara, CA, Manufacturing No. E4980-90070, Jun. 2008.
- [7] <http://www.jrossmacdonald.com/levminfo.html>
- [8] K.S. Cole, R.H. Cole, "Dispersion and absorption in dielectrics: 1. Alternating current characteristics", *J. Chem. Phys.* vol. 9 pp. 341-351,1941.
- [9] J. R. Macdonald, "Impedance Spectroscopy", *Annals of Bionedical Engineering*, vol. 20, pp. 289-305, 1992