Effects Evaluation of UHF RFID systems on the molecular structure of biological drugs

Raffaele Acierno, Elisabetta Carata, Michele Maffia, Luca Mainetti, and Luigi Patrono
University of Salento
Lecce, Italy

***Abstract*—The growing counterfeiting problem and the significant fragmentation of the pharmaceutical market are resulting on an increase of difficulty to trace medicines. In these scenarios, where an item-level traceability is crucial, the Radio Frequency Identification (RFID) technology holds the promise to eliminate many of the previous problems. Unfortunately, there are still some technical and economic barriers that are retarding the deployment of these innovative technologies in large-scale application scenarios. For the pharmaceutical supply chain, there have been concerns raised regarding the potential effects on the quality of drugs due to exposure to electromagnetic fields. In this paper, some results, obtained by a recent experimental study focused on the evaluation of potential effects on biological drugs, have been reported. This work aimed to evaluate potential effects of tracing RFID systems on the molecular structure of biological drugs. In particular, some samples of a commercial human insulin preparation have been exposed for different periods to electromagnetic fields generated by RFID devices. In order to evaluate possible alterations on the molecular structure, the following diagnostic techniques have been used: High Pressure Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR). The experimental results have shown that the electromagnetic field generated by UHF RFID readers does not cause any damage on the structure of the insulin molecule.**

Keywords- biological drugs, effects on molecular structure, HPLC, item-level, RFID, UHF, traceability, supply chain.

I. INTRODUCTION

Radio Frequency Identification (RFID) [1] is a very powerful technology to guarantee the ability to trace and track individual object in a global scenario controlled by pervasive computing systems. This auto-identification technology has recently seen growing interest from a wide range of application sectors such as retail, logistics, and pharmaceutical. Among these, the pharmaceutical supply chain is a very interesting scenario, in which an item-level traceability is crucial to guarantee transparency and safety in the drugs flow. Recently, the counterfeiting attempts are growing more and more so to force the government of many countries to define effective strategies to face this serious problem [2]. Indeed, the RFID technology will play a very important role in development of item-level tracing systems for the pharmaceutical supply chain because it does not require line-of-sight alignment, multiple tags can be identified simultaneously, high read rates can be guaranteed, and the read range between reader and tag can be also several meters. Among the different types (i.e. passive, semi-passive, and active) of RFID transponders, often called "tags", that passive and in Ultra High Frequency (UHF) band is used in most tracing systems since it is battery less and very cheap.

Recently, there have been several encouraging signals that offer the prospective to attend a diffusion of this technology in large-scale. Some international institutions (e.g. FDA, EMEA, and EFPIA) are recommending the adoption of serialization procedures of drugs by using standardized coding as GS1 (Global Standard 1) and EPC (Electronic Product Code). Furthermore, some FDA recommendations [3] invite the main actors of the pharmaceutical supply chain to experiment, through pilot projects, the use of the RFID technologies to create an "e-pedigree", a secure record documenting that the drug was manufactured and distributed under safe and secure conditions.

Unfortunately, there are still some technical and economic barriers that are retarding this complex revolution in the pharmaceutical sector. Some challenges proposed recently to RFID research community are: high reliability of RFID tags in presence of liquids and metals, high scalability of the tracing services, evaluation of potential effects of the RFID systems on quality of drugs, etc.

This paper reports a research work carried out in collaboration with some actors of the pharmaceutical supply chain. Taking into account some recent works [4, 5] that have excluded destructive effects of RFID systems on synthetic or semisynthetic drugs, we have focused on the evaluation of potential impacts of the RFID systems on biological drugs. In [4] is described an approach that uses particular test environments able to overexpose samples of drugs to electric and magnetic fields whose intensities are several times those generated by commercial RFID readers in order to evaluate mainly the thermal effects. The results of these works showed damage only on thermo-fleeting molecules but not on other drugs.

Unlike this type of approach, we have evaluated potential impact of RF exposure on biological and protein drugs analyzing the effects on the structure of the molecule. Among biological drugs, we have chosen to test the insulin in our experimental analysis because it is a biological drug used in enormous quantities for the treatment of insulin dependent diabetes mellitus.

The experimental results have demonstrated that also long periods of exposure to electromagnetic field in UHF band does not cause breakage in the structure of the insulin molecule. The rest of the paper is organized as follows. The Section II

978-1-4244-4713-8/10/$25.00 ©2010 IEEE

reports a short related work on the previous topic. Instead, the section III describes main features of the test environment used to perform this study. Finally, the last section reports main results obtained by experimental campaign.

II. Related Works

The use of RFID devices to guarantee the item-level traceability of drugs has stimulated very interesting discussion areas related to potential effects of electromagnetic fields on the integrity of drugs, especially those biological based. [5] analyzes the main risks associate to RF exposure and reports a classification in thermal or non-thermal effects. Further, a recent paper from FDA's Center for Devices and Radiological Health [4] simulated exposure of liquid drugs in vials to RF under worst case conditions and observed a rise in temperature less than 2 ºC. [6] and [7] analyzed the RF exposure problem by using an theoretical and experimental approach respectively. Let us observe that both works are characterized from unrealistic reference scenario. In addition, the effects reported in the previous two papers are understandable as extremely localized thermal effects. In order to extrapolate the previous effects for a realistic environment, characterized by tracing systems based on RFID, a drug that is sufficiently thermally labile and concentrated so that these potential highly localized effects could propagate through the sample to a sufficient extent to create a measurable impact would be required. Unfortunately, a realistic pharmaceutical supply chain presents characteristics most unlikely.

III. Methods and Materials

A. RFID Devices Description

In order to conduct effective experimental campaigns to evaluate the potential effects of UHF RFID technologies on biological drugs, that flow through the whole supply chain, a particular test environment reproducing the main steps (i.e. manufacturer, wholesaler, retailer) of the supply chain is needed. Therefore, a controlled laboratory environment was created, enabling an unbiased and repeatable comparison among the technologies. The laboratory is equipped with an "items line", a "cases line", and a "border gate" in order to simulate the main steps of the supply chain. Among these, our attention for this study focused on the "cases line" because it is characterized to operating conditions considerably stressed; in fact, the values of electric and magnetic fields are higher than those measured in the others steps. These conditions are needed to guarantee high performance in presence of multiple reading of tags and no line-of-sight condition.

The "cases line" consists of a conveyor belt, equipped with one UHF RFID reader, the Impinj's Speedway. The Speedway is a high-performance reader designed to support the EPCglobal UHF Gen 2 standard in its entirety. Its operating frequencies range is 865-956 MHz. Its maximum transmission RF power is equal to +30 dBm (1 Watt) respecting the regulations of most countries. A sensitivity equal to -80 dBm characterizes this reader. Furthermore, the line is characterized by a metallic tunnel, shown in detail in Fig. 1, that is equipped with four near field UHF antennas of the same type: the Impinj's CS-777 Brickyard. Each reader antenna is in the centre of each tunnel

Figure 1. Main components of the "cases line".

side. The distance between the two opposite sides of the tunnel is equal to 0.6 m. The material of the external cover of the tunnel is metallic and shielding. The reader antennas are suitable for the frequency range 865-868 MHz. The antenna diameter is equal to 0.30 m. Further characteristics are: 50 Ω of impedance, 6 dBi as maximum far field gain and -15 db as Return Loss.

In order to obtain some indications about the intensity of the electric field E in the zone inside the tunnel, where the sample of drug is located to be irradiated, an analyzer able to perform wideband measurements was used. In particular, the PMM 8053A with an electric field probe PMM EP-330 able to work in the frequency range from 100 KHz to 3 GHz were used.

Let us observe that some measurements performed in laboratory by using the previous analyzer confirmed that the step of the supply chain more critical for drugs in terms of irradiated electric field is the "cases line" step. This controlled test environment has was used to carry out the first part of the experimental study that aimed to exposure of biological drugs to particular RF electromagnetic fields in UHF band for several different periods. After the exposure phase, the samples of drug were analyzed in a biology laboratory in order to evaluate potential effects on the structure of the molecule.

B. Biological Materials

As representative of biological drugs potentially susceptible of degradation an injectable preparation of human insulin (ActrapidTM - Novo Nordisk, 10 I.U. ml-1, corresponding to 0.35 mg ml-1) was used. As shown in Fig. 2, the insulin molecule consists of two peptide subunits, A and B, linked by disulfide bonds; albeit the disulfide bonds are quite strong, with a dissociation energy of 60 kcal/mol, they are the weakest bonds in the molecule, then the most likely to be affected by energy irradiation. In order to evaluate potential damages on the molecular structure of the commercial insulin, structural

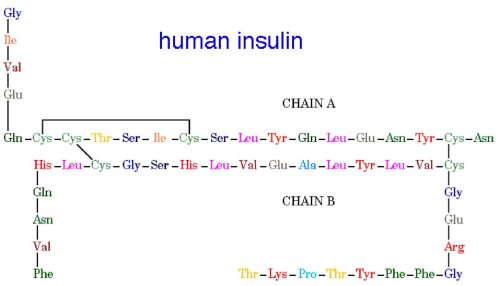

Figure 2. Structure of the human insulin molecule.

analyses of the molecule after radiofrequency exposition were carried out by NMR and Reverse Phase HPLC.

C. NMR Technique

The Nuclear Magnetic Resonance (NMR) spectroscopy is a technique, which allows detailed structural analyses of many compounds, among which peptides and proteins. The technique allows determining molecular structures by analysing the nuclear spin of specific atoms (typically 1H and 13C). These nuclei possess specific magnetic moments, which can be aligned by an external magnetic field. Practically, the analyte is positioned in a strong magnetic field and excited via radio frequency pulsation. When the frequency of the electromagnetic radiation equals that of a specific nuclear magnetic moment (resonance), part of the radiation energy is transferred to the nuclei, with a consequent inversion of their alignment in the magnetic field. When the pulsating RF is interrupted, the excited nuclei emit a weak RF signal, which is detected by a specific circuit and used to generate the output signal (i.e.: the NMR spectrum) that allows to establish the structure of the analyte. The types of information accessible via high resolution NMR include: functional group analysis, bonding connectivity and orientation, molecular conformations and chemical dynamics.

D. HPLC Technique

The analysis of the possible structural modifications of the insulin molecule arisen from the exposition to Radio Frequency was made by Reversed Phase High Performance Liquid Chromatography, using a Vydac 218TP54 column fitted on an Agilent 1100 HPLC system. Owing to the specific interactions of different molecules with the stationary and mobile phases in the column, this kind of chromatography allows the separation and the quantization of different molecules, even when these differences are very small. An UV detector placed at the exit of the column allows the quantitation of the analytes by measurement of the absorbance at specific wavelengths. Isocratic separation with 0.05M KH2PO4-CH3CN (65:35,v/v; pH 2.4) mobile phase was carried out at 1.0 ml min-1 flow, 25 °C, UV detection at 230 nm [8]. A standard curve obtained by injecting in the column different, known amounts of insulin allowed to calculate the amount of the sample passed through the column from the area of its peak of UV absorbance.

IV. EXPERIMENTAL RESULTS

In order to evaluate potential effects of a tracing RFID system on the structure of the insulin, some samples of Actrapid 10 I.U. ml-1 human insulin were exposed for different periods to an Electric field (E), generated by devices previously described. In particular, we considered as exposition times the following values: 3600 s, 10800 s, and 21600 s. During the exposition phase, by using the previous analyzer, both the average value and the peak value of the E field were measured, obtaining respectively 33 V/m and 45 V/m. For all periods, the transmission power of the RFID reader was set to 30 dBm (i.e. 1 W). Furthermore, let us observe that during the exposition the temperature was constant as room temperature (25°C). Afterwards, the samples were analyzed through the previous diagnostic techniques.

As first test, the effects of the radiofrequency exposition on the molecular structure of insulin were analysed by using the RP-HPLC. A comparison of the chromatograms of untreated and treated samples of Actrapid® was performed. Under the same chromatographic conditions, any possible structural difference between the two samples would result in appreciable differences in the time occurred to pass through the column (RT – Retention Time) and/or in the area of the peak of UV absorbance which is proportional to the quantity of analyte. The accuracy of the HPLC assay is good throughout the range tested. The percent difference in mean concentrations does not exceed 0.5% for any concentration. The qualitative analysis of molecules detected by this chromatography is carried out according to the time of retention. As in all other chromatographic techniques the greater affinity for the stationary phase results in an increased retention time. The size of the molecules and their charge are indeed factors that influence the equilibrium of analyses between mobile phase and stationary phase.

Fig. 3, that was referred to control, shows the presence of two peaks, the first characterized by a shorter retention time as 3.556 minutes and a value of area as $2.60901e^4$mAU*s, and the second peak characterized by a retention time as 5.834 minutes and a value of area as $2.78440e^4$ mAU*s. In order to determine which of the two peaks was referring to insulin, the single fractions were recovered and analyzed with Ellmann's reagent; the Ellmann reaction consisting in a colorimetric assay. Each fraction was incubated in the presence of 5.5'-dithio-bis-(2-nitrobenzoic acid), also known as DNTB, as a versatile water-soluble compound for quantitating free sulfhydryl groups in solution. A solution of this compound produces a measurable yellow-colored product when it reacts with sulfhydryls. At the end of Ellmann reaction could be identified peak 1 as metacresol, a substance maintenance of insulin, and peak 2 as insulin. Fig. 4 shows the chromatogram of the denatured insulin, obtained by exposition to 2M NaOH. A decrement of insulin peak and the presence of new peaks referred a possible fragment of insulin are highlighted in this chromatogram. Furthermore, it is also possible to observe the variation of retention time, probably attributable at formation of new molecules as a consequence of denaturation. The last chromatogram (Fig. 5), relate to insulin irradiated, shows how the values are maintained constants both of the areas ($2.59886e^4$ mAU*s/$2.78351e^4$ mAU*s) that the retention times. The results related to HPLC, reported in this work, show no significant differences in RT and peak area of insulin before

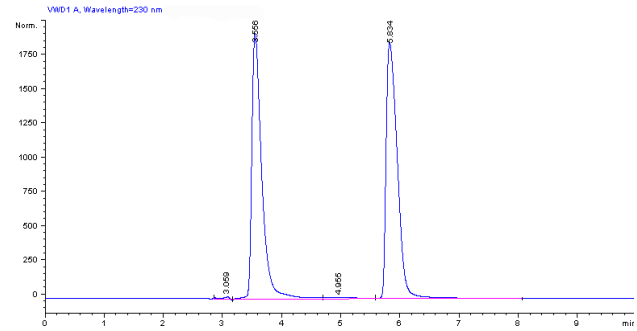

Figure 3. Typical RP-HPLC chromatogram of ActrapidTM showing two main peaks of UV absorbance at 230 nm.

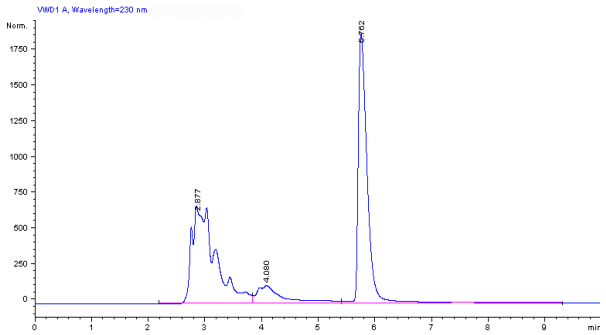

Figure 4. Typical RP-HPLC chromatogram of ActrapidTM after alkaline degradation.

and after exposition, until 6 hours, to RF, while very evident modifications of RT and peak area were observed after alkaline treatment, suggesting the apparent safety of the RF exposure toward in relation with the structural integrity of the molecule.
Finally, the same samples of insulin were analyzed by the NMR technique, in order to validate and complete the investigation phase on potential effects of RFID systems on the structure of the Actrapid preparation. The NMR spectrum of the four samples (i.e. control, exposed 1 hour, 3 hours and 6 hours) was calculated. The spectra comparison showed no significant differences between control sample and irradiated samples. Fig. 6 reports the NMR spectrum of the irradiated sample. This preliminary analysis, performed by the NMR technique, allows us again to assert that the irradiated insulin preparation did not suffer substantial damages in its structure.

V. CONCLUSION

The chromatographic analysis showed no damages to the tertiary structure of the molecule of insulin; therefore, it is assumed that this molecule is still able to bind to the receptor through that key-lock mechanism, previously described. As you can see from structural analysis, carried out by the HPLC technique, of the peak area corresponding to the amount of this molecule integrates into the suspension, remains unchanged by the assumed absence of alterations in the structure of the molecule. A preliminary analysis by NMR technique highlighted also no significant damages.
In conclusion, the results obtained in this study support the asserting that exposure to electromagnetic field generated by tracing RFID systems, even for very long periods (6 hours), does not generate any damage to the structure of the insulin molecule.

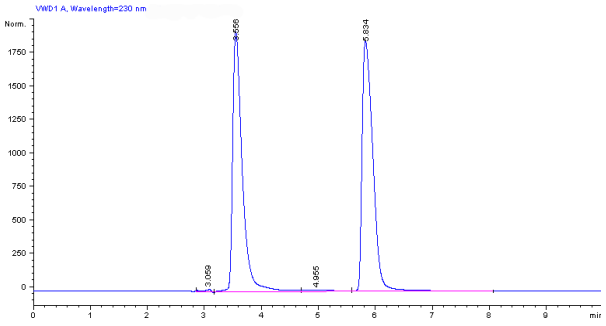

Figure 5. Typical RP-HPLC chromatogram of ActrapidTM after RF irradiation.

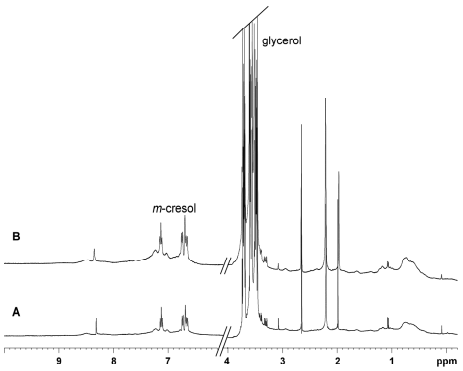

Figure 6. 1H NMR water presaturation spectra (watergate) of ACTRAPID (A) and ACTRAPID after 6 h of irradiation (B) at 0.1 mM concentration in D2O/H2O 10/90, 298 K.

As next step, these results, related to molecular structure, will be further validated by experiments conducted in vitro functional analysis and faces of the molecule, which will evaluate the ability of the molecule to bind to the receptor cell. This analysis is very important because if the molecule is even slightly damaged, for example against weak ties as hydrogen bonds, it will lose the binding affinity to the receptor.

ACKNOWLEDGMENT

The authors wish to thank Prof. F.P. Fanizzi, Dr. S.A. De Pascali and Dr. P. Papadia of the General and Inorganic Chemistry laboratory of the Di.S.Te.B.A. of University of Salento for their useful support related to NMR technique. Furthermore, deep gratitude is also expressed to Dr. F. Bini of CTP System (Italy), Dr. V. Novielli president of the pharmaceutical wholesaler Farpas (Italy), and Eng. G. Risola of Merck Serono (Italy), without whose knowledge and assistance this study would not have been successful.

REFERENCES

[1] K. Finkenzeller, “RFID Handbook: Fundamentals and Applications in Contact-less Smart Cards and Identification”, Wiley & Sons, 2003.

[2] FDA (2004-1), “Combating Counterfeit Drugs - A Report of the Food and Drug Administration”, U. S. Department of Health and Human Services, Food and Drug Administration Rockville, February 2004.

[3] FDA (2004-2), “Radiofrequency Identification Feasibility Studies and Pilot Programs for Drugs, Guidance for FDA Staff and Industry, Compliance Policy Guides, Sec. 400.210, Radiofrequency Identification Feasibility Studies and Pilot Programs for Drugs”, November 2004.

[4] H. Bassen, “Liquid Pharmaceuticals and 915 MHz Radio Frequency Identification Systems, Worst-Case Heating and Induced Electric Fields”, RFID Journal, Oct. 31, 2005.

[5] Robert H. Seevers, “Report to FDA of PQRI RFID Working Group”, December 1, 2005.

[6] JA Laurence, et al., “Biological Effects of Electromagnetic Fields-Mechanisms for the Effects of Pulsed Microwave Radiation on Protein Conformation”, Journal of Theoretical Biology, 206 (2000), 291-298.

[7] DI de Pomerai, et al., “Microwave Radiation Can Alter Protein Conformation Without Bulk Heating”, FEBS Letters 543 (2003) 93-97.

[8] L. Hoyer, et al., “Selective stability-indicating high-performance liquid chromatographic assay for recombinant human regular insulin”, Journal of Chromatography A 699 (1995), pp 383-388.