ARTÍCULO ORIGINAL

Revista de Investigación de Física **24(3)**, (Set-Dic 2021)
Doi: 10.15381/rif.v24i3.20521

eISSN:1728-2977

Application of *Accu-Chek®* Test Strips as inexpensive microelectrodes for BIA research

Christian Lexequías *[1,3], Bryan Urcia[1], Adriano Peña[1], Oscar Baltuano[1,2] and Galo Patiño[1]

[1] *Universidad Nacional Mayor de San Marcos, Grupo de investigación INFISA, Lima, Perú*
[2] *Instituto Peruano de Energía Nuclear, Dirección de Investigación y Desarrollo, Lima, Perú.*
[3] *Universidad Nacional Mayor de San Marcos, Museo Histórico de Ciencias Físicas, Lima, Perú.*

Recibido 06 Jun 2021 – Aceptado 01 Nov 2021 – Publicado 12 Dic 2021

Abstract
In the midst of an investigation to perform bioelectrical impedance analysis (BIA), we were faced with the need to search for optimally adjusted electrodes to perform reading in small biological samples. The best option to carry out these readings is the well-known gold microelectrodes; however, these are very expensive for our research purposes. For this reason, we found an alternative using Accu-Chek Performa test strips as reading microelectrodes due to their low cost and ease of disposal. This article contains an in-depth detail of the components of the Accu-Chek Performa test strip and the process that was used so that they could be suitable for taking measurements on biological material. In addition, a measurement scheme is shown in conclusion to the operation of the test strip as a microelectrode and the possible problems to consider if it is to be used for future research.

Keywords: Microelectrode, Test strip, BIA, Accu-Chek Performa, impedance.

Aplicación de Tiras Reactivas *Accu-Chek®* como microelectrodos económicos para investigación BIA

Resumen
Durante la investigación para realizar el análisis de impedancia bioeléctrica (BIA), nos vimos con la necesidad de buscar electrodos que se ajusten de forma óptima para realizar lectura en muestras biológicas de tamaño pequeño. La mejor opción para realizar dichas lecturas son los conocidos microelectrodos de oro; sin embargo, estos resultan muy costosos para los objetivos de la investigación. Debido a ello es que encontramos como alternativa usar las tiras reactivas Accu-Chek Performa como microelectrodos de lectura por su bajo costo y facilidad para desecharse. Este artículo contiene un detalle profundo de las componentes de la tira reactiva Accu-Check Performa, como también el proceso que se utilizó para que estas pudieran estar aptas para realizar medidas en material biológico. Además, se muestra un esquema de medida en conclusión al funcionamiento de la tira reactiva como un microelectrodo y los posibles problemas a considerar si se desea hacer uso de esta para investigaciones futuras.

Palabras clave: Microelectrodo, tira reactiva, BIA, Accu-Check Performa, impedancia.

Introduction

Bioelectric impedance analysis (BIA) is a method that allows to characterize the electrical behavior of biological material [1]; however, to develop this method, it is necessary to use an electrode that is adjusted to the size of the sample to be studied. The microelectrodes or interdigitated electrodes are those that are commonly used to be able to perform readings using EIS (Electrochemical Impedance Spectroscopy) equipment [2]. But for the BIA

*christian.lexequias@unmsm.edu.pe

research carried out in the INFISA (Physical Instrumentation and Applications) laboratory [3], these electrodes have not got the requirements of bioelectrical analysis, since they must be immersed in the sample accompanied by an aqueous solution or they use samples larger than what is required to use. In addition, due to the fact that the biological sample contaminates the electrode, these must be disposed of safely according to the sanitary regulations of the MINSA [4], and to carry out the study in a large number of samples respecting the health protocols, it would be too expensive to research by the price of the same electrodes.

Given this problem, it was possible to see in the *Accu-Chek®* test strips an opportunity to be used as a microelectrode to fulfill impedance studies. The test strip chosen for this work is Performa [5] because inside it hides an electrode arrangement based on a gold foil. These strips fit perfectly into the parameters that are raised for the development of BIA research due to the amount of sample used and the ease of being able to trash, also added to the cost that is much cheaper compared to standard microelectrodes and easy accessibility to purchase this product.

This article aims to present the features of this test strip as an inexpensive microelectrode option for bioelectric impedance analysis since although we can observe the use of test strips as electrodes in some foreign investigations [6,7], these do not show the internal features that must be taken into account for a biological investigation and the possible problems to consider in the readings.

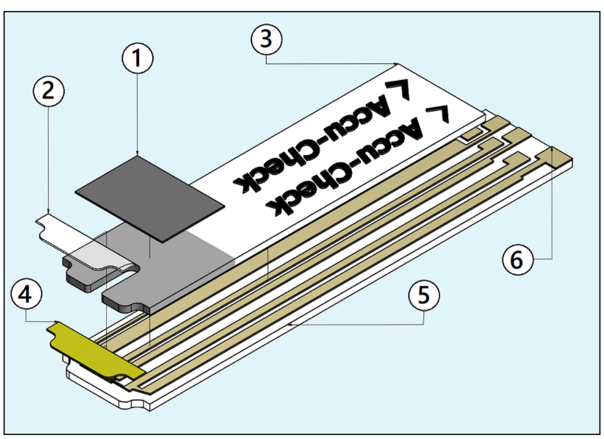

Figure 1: Graphic diagram of the parts of the *Accu-Chek®* test strip, elaborated in the INVENTOR program [8]
(1) First adhesive, for greater rigidity when connecting it to the equipment. (2) Capillarity adhesive. (3) Base adhesive, protect the body of the strip. (4) Glucose Dehydrogenase. (5) Rigid test strip base.

Materials and Methods

The test strip used in the BIA research is the Accu-chek Performa PK50, it contains 6 gold contacts which are joined in an arrangement as a microelectrode (Figure 1), and can take readings on samples of size 0.6 uL. However, to use this strip as a microelectrode, some modifications must be made, since among the components of the test strip it has a reagent, which can improve or worsen the reading depending on the biological sample to be analyzed.

Disassembling the test strip, the first object found is a slightly rigid adhesive, which provides greater protection to the head of the strip when it is connected to the equipment. Along with this first adhesive, there is another transparent adhesive that serves to carry out the capillary action by encapsulating an exact amount of 0.6 uL of the sample. Under these first two adhesives, there is a larger but thinner one that is responsible for protecting the body of the test strip from possible impurities or static that could be produced by holding it with your fingers. It should be mentioned that during the extraction of these first parts of the strip, the adhesives use a glue that is totally soluble in alcohol ($\geq 70°$), which with the help of tweezers and a swab comes out easily (Figure 2). It is not advisable to use water or alcohol with lower graduation ($< 70°$) than we mentioned because these do not evaporate nor can they be completely cleaned causing errors when making measurements.

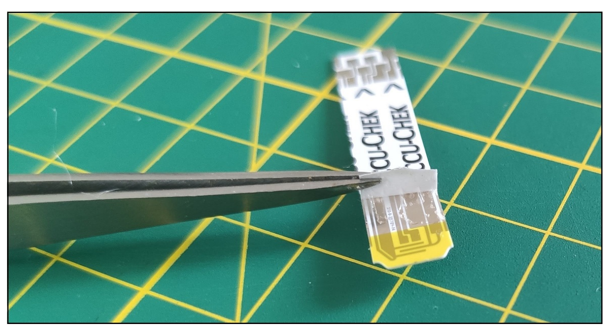

Figure 2: In this image, we can see a slight viscosity caused by the glue of the adhesive when it is removed.

Once all the adhesive parts have been removed, we can observe a yellow coloration in the extreme part where the sample is placed, which corresponds to the recombinant protein known as Glucose Oxidase (Figure 3). This protein is synthesized by microorganisms in the midst of their growth or metabolism and is used in a large number of test strips to detect blood glucose. The strip used for research contains *β-D-glucose*, this enzyme is characterized as a glucose dehydrogenase; however, by diffusion term, its name is still valid as Glucose Oxidase (GOx) [9].

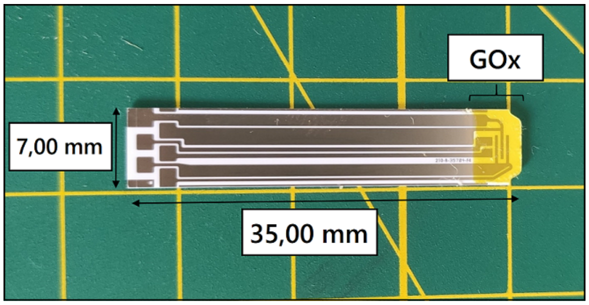

Figure 3: In this image, you can see the dimensions of the test strip and the GOx arranged at the reading end.

Compound	Relative velocity (s)
β-D-glucose	100
α-D-glucose	0.64
2-*Deoxy-D-glucose*	3.3
3-*Deoxy-D-glucose*	1
4-*Deoxy-D-glucose*	2
5-*Deoxy-D-glucose*	0.05
6-*Deoxy-D-glucose*	10

Table 1: In this table, glucose dehydrogenase (*β-D-Glucose*) can be observed in comparison with the reaction rate with different substrates GOx known in electrochemistry. *Source: Data obtained from research published by Claudio Enrique Voge, UNP, Argentina.* [9]

To clean the glucose oxidase, it was diluted with alcohol and cleaned with a swab until the surface of the resulting electrodes stopped showing the characteristic yellow color of GOx (Figure 4).

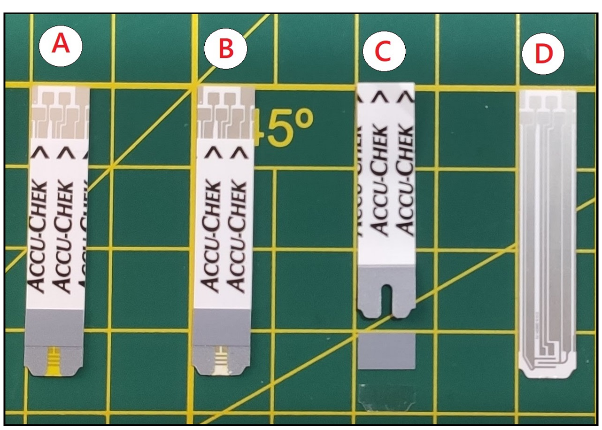

Figure 4: This image shows the comparison of test strips in the cleaning process. (A) Test strip without any modification. (B)Test strip without GOx in the reading capillary. (C)The adhesive parts of the test strip were removed. (D) Clean microelectrode obtained from the test strip.

For future research purposes, it is mentioned that recombinant proteins (PR) are produced mainly by bacterial hosts (*E. Coli, Bacillus subtilis, and Bacillus megaterium*) and these currently make a great contribution in the areas of biotechnology [10]. The recombinant "GOx" protein contained in the Accu-Chek © Performa test strip is one produced specifically by the Escherichia coli bacterium, which belongs to a glucose dehydrogenase mutant classified as Mutant 31 [11], it is highly soluble and its characteristics can be observed in Table 2.

Enzyme	Stability, 30 min, 64°C	Amino Acid Exchanges
Mutant 31	80%	D87R+N122K+S124K+ S146G+L169F+ Y171G+E245D+Q246H+ V298L+M341V+ T341V+T348S+L386F+ ins429P+V436P

Table 2: This table shows the characteristic of the Mutant 31 enzyme in response to the impact of stability in relation to thermostability and the amino acid that represents *Source: Data obtained from the patent published by ROCHE DIAGNOSTICS GMBH* [11].

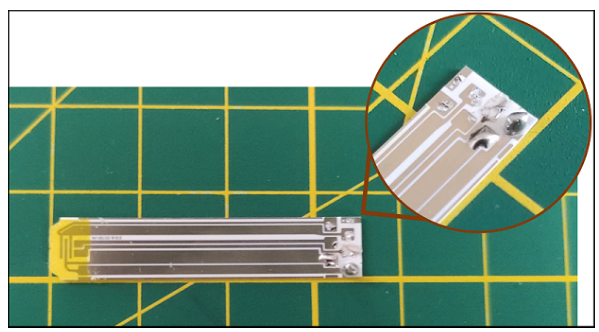

Figure 5: This image shows the test strip used to experiment with tin solder, along with a zoom of the best photograph that could be obtained from the connector side of the strip.

Results and Discussions

During the investigation, each connection point of the test strip was welded with a fine-tipped soldering iron, to observe the reaction to the heat of the gold filaments and if it would resist the melted tin. The result was that the heat that the soldering iron emanated as it approached melted the connectors; however, with a lower temperature controlled by the same welding equipment and adding a light layer of solder paste it was possible to form solder points without melting it (Figure 5). Despite this achievement, it should be noted that the soldering point to be able to insert an extra connection cable ends up melting the connector again and the amount of tin could exert an extra resistance when making the measurements, causing alterations in the readings due to what is recommended to use a female connector to avoid making use of solders.

In the middle of the stage of eliminating all traces of GOx on the test strip, a drop of alcohol was applied with a syringe in the extreme side where the reading is made. We waited approximately 5 seconds for the reagent to dissolve and it was cleaned with a swab, then (Figure 6), alcohol was added again to the entire strip to make sure it was completely clean. As a result, a microelectrode was obtained on which, impedance readings were made.

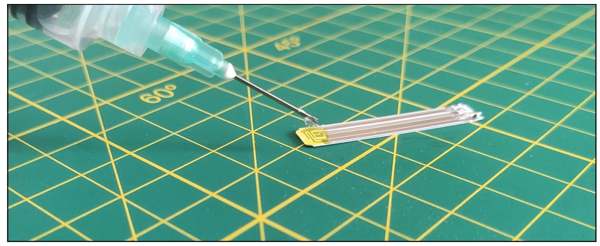

Figure 6: We notice how the alcohol is applied to the test strip to remove the GOx layer.

Below there are two characteristic Nyquist diagram (Figure 7 & 8) results from the BIA investigations that were performed using the test strip as a microelectrode. The first graph shows the impedance spectrum obtained in 0.6 uL samples of blood tissue (Figure 7) and the second graph shows the impedance spectrum in 0.6 uL samples of water (Figure 8).

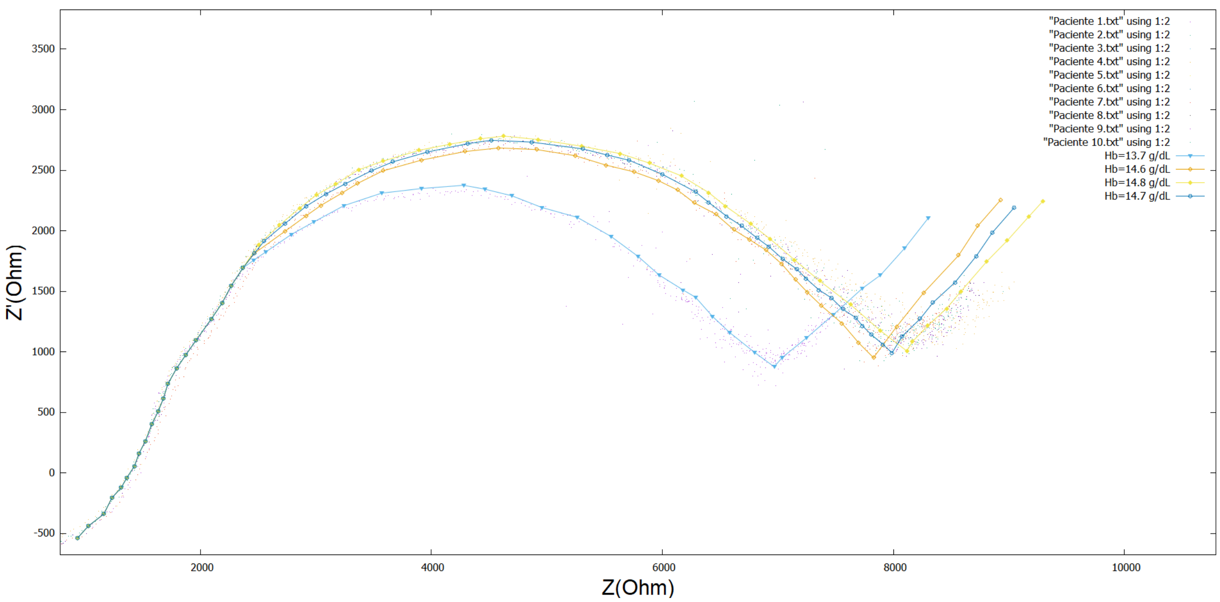

Figure 7: Impedance spectrum corresponding to blood tissue in different amounts of hemoglobin. The electrodes will be used in these readings without eliminating the GOx protein due to its direct relationship with hemoglobin [3].

Conclusions

Based on the BIA investigations that were carried out in the INFISA biophysics laboratory, it can be concluded that the *Accu-Chek*® Performa test strip can be used as a more economical microelectrode option, especially if the samples are biological material where the electrode should be disposed of after use. This microelectrode obtained from the modification of the test strip can be used with specific samples ($\geq 0.6\ uL$), it can even be immersed in an appropriate amount of sample that requires it. If it is decided to carry out measurements without making modifications to take advantage of the GOx protein, the samples are exactly 0.6 uL.

The exact composition of the reagent attached to the electrode is shown below to be considered in future investigations (Table 3).

The electrical diagram compared to that of the interdigitated electrodes or microelectrodes (IDEs) [15] is shown in Figure 9, where the sub-electrodes that fulfill the function of reference, measurement aid, and work can be identified.

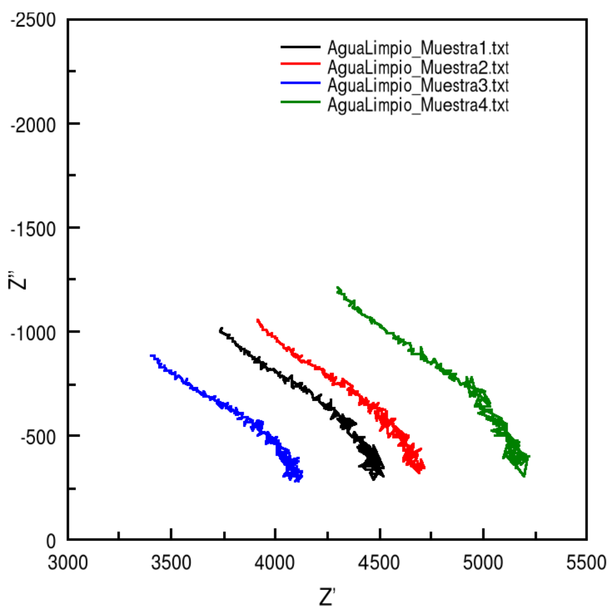

Figure 8: Impedance spectrum corresponding to samples plotted in the Zview program [12]. Electrodes without GOx were used in these readings [13].

Mediator	6.72%
Quinoprotein glucose dehydrogenase	15.27%
Pyrroloquinoline quinone	0.14%
Buffer	34.66%
Stabilizer	0.54%
Non-reactive ingredients	42.66%

Table 3: Percentage data of the internal composition of the reagent compared to that of the non-reactive components. *Source: Information obtained from the technical data sheet provided by Roche Diabetes Care, Germany* [14].

It is important to mention that if you plan to use the electrode with the protein as a reading aid for BIA analysis, it is necessary to wait 5 seconds to be able to take a coherent measurement, because this is the time it takes for the enzyme to react with the biological material in its entirety. This article, focused only on the electrode, is considered important since it greatly facilitates the development of BIA research and the results that are obtained satisfy its comparison with the literature and the objectives set. We also ended up mentioning that with the test strip it was possible to obtain impedance readings in water, blood tissue, GOx, Rhinovirus in the nasal sample, and SARS-CoV-2 coronavirus (COVID19) in blood. It is intended to publish about these last biological samples before the end of the year 2021.

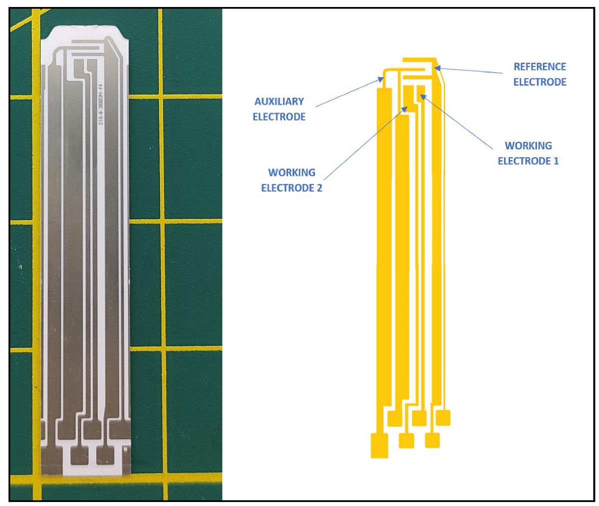

Figure 9: Electrode scheme posed for the test strip.

Acknowledgment

We are grateful for the support of Dr. Sthy W. Flores D. in the development of the research, as well as Sara Vanessa Diaz Arrue, Manuel Jesús Torres Calla and Lisbeth Lucero Cisneros Huarcaya, who provided their knowledge at the completion of the project.

This article is part of the research in biophysics, which is developed in the INFISA laboratory of the Faculty of Physical Sciences, thanks to the support of the Vice-Rector for Research of the Universidad Nacional Mayor de San Marcos.

References

[1] A. Malik, Aplicaciones clínicas del método de impedancia bioeléctrica (BIA). ReNut, **4**(12), 624-628. https://revistarenut.org/index.php/revista/article/view/132 (2010).

[2] Metrohm DropSens. *Electrodos interdigitados (IDEs) / Microelectrodos.* http://www.dropsens.com/electrodos_interdigitados.html (2021).

[3] C. Lexequías, B. Urcia, B. Franco, O. Baltuano, G. Patiño Desarrollo de un espectrómetro de impedancia eléctrica portátil para análisis y caracterización del tejido sanguíneo. Rev. Inv. Fis. **24**(1), 9-16. Doi: https://doi.org/10.15381/rif.v24i1.20241 (2021)

[4] MINSA, Midori de Habich. *Manual de procedimientos de laboratorio: laboratorios locales I: laboratorios*

locales II. Lima, Perú. https://repositorio.ins.gob.pe/handle/INS/153 (2013).

[5] Accu-Chek®. *Tira reactiva Performa* Roche Diabetes Care https://www.accu-chek.com.pe/tiras-reactivas/tiras-accu-chekr-performa (2019).

[6] J. Bernal, C. Guerrero y F. Hernández, *Determinación de las propiedades eléctricas en tejido sanguíneo*. Universidad Autónoma de Nuevo León, 510-515. http://ingenierias.uanl.mx/29/index.html (2005).

[7] M. Bernal, J. Sosa, M. Bernaln, C. Jiménez, G. Hernández, M. Gutiérrez, J. Huerta, C. Villagómez, and P. Palomares. Magnetic field influence on electrical properties of human blood measured by impedance spectroscopy. Bioelectromagnetics, **26**(7), 564-570. Doi: https://doi.org/10.1002/bem.20132 (2005)

[8] Inventor Professional. Autodesk. *Software de diseño asistido por computadora*. Windows. https://www.autodesk.com/products/inventor/free-trial (2021).

[9] C. Voget. *Producción de glucosa oxidasa*. Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Argentina. http://sedici.unlp.edu.ar/bitstream/handle/10915/2533/Documento_completo.pdf?sequence=1&isAllowed=y (1985)

[10] A.R. Lara. Producción de proteínas recombinantes en Escherichia coli. Rev. Mex. Ing. Quím. **10**(2), 209-223. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1665-27382011000200006 (2011).

[11] M. Boenitz-Dulat, D. Beck, P. Kratzsch, R. Schmuck, H. Von Der Eltz. *WO2007118647 - Improved Mutants Of Pyrroloquinoline Quinone Dependent Soluble Glucose Dehydrogenase*. Roche Diagnostics GMBH. Penzberg, DE. https://patentscope.wipo.int/search/en/detail.jsf;jsessionid=A8494B86E7410FF4C244A77912EF8C36.wapp1nC?docId=WO2007118647&tab=PCTBIBLIO (2006).

[12] Scribner. *ZView®DataSheet*. Windows, V3.2. https://www.scribner.com/software/ (2020).

[13] C. Lexequías, B. Urcia, L. Cisneros, S. Diaz, A. Peña, O. Baltuano y G. Patiño. Análisis de impedancia Bioeléctrica en diferentes concentraciones de Glucosa Oxidasa (GOx) Rev. Inv. Fis. **24**(2), 54-62 Doi: https://doi.org/10.15381/rif.v24i2.20427 (2021)

[14] Roche Diabetes Care Gmbh. *Accu-Chek Performa Nano* Sandhofer Strasse 116. 68305 Mannheim, Germany. www.accu-chek.com (2019).

[15] DropSens. *InterDigitated Electrodes*. Metrohm. ISO 13485 Parque Tecnológico de Asturias - Edif. CEEI. 33428 LLanera (Asturias). Spain. http://www.dropsens.com/pdfs_productos/new_brochures/g-ide222_g-ide555.pdf (2008).