UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Aproximación al biomimetismo mediante lenguas y narices electrónicas en medios complejos: detección de explosivos, agentes nerviosos y control de alimentos

TESIS DOCTORAL

Doctorando: Cristian Olguín Pinatti

Directores: Dr. Eduardo García Breijo

Dr. Rafael Masot Peris

Julio 2017

A mi hijo, mi mujer y mis padres

La erudición y la búsqueda de la verdad y el conocimiento puede ser una aventura personal y solitaria, pero también es un posible objetivo social. Parece ser que los tiempos actuales empujan más a mirar por la seguridad económica en una especie de "coge el dinero y corre" que a buscar ideales y mejorar el conjunto de la sociedad. Pero es indiscutible que nadie puede valorar lo que no conoce. Si la sociedad no conoce el ***significado de un doctorado****, difícilmente lo valorará. Parece que el sistema académico en nuestro país ha fallado comunicando el sentido de una tesis. Los científicos no hemos sabido aclarar a la sociedad cosas básicas de nuestra formación como es la tesis doctoral. El doctorado es una pieza esencial, necesaria pero no suficiente, para el desarrollo de un tejido cultural y científico que conecte y enriquezca los distintos actores y objetivos sociales.* ***Si la sociedad no vislumbra las implicaciones de un doctorado, tendrá difícil estimar qué importancia le concede a la investigación y le faltará criterio para situar el nivel de la inversión pública en la exploración del conocimiento****. Es decir, sin comprender la esencia y las implicaciones de la investigación, representadas por una tesis doctoral como primer paso, la estrategia de I+D+i de su país podrá tomar el rumbo errático de un pollo sin cabeza. Así pues, los científicos de esta España del siglo XXI tenemos una tarea adicional al quehacer investigador, la de mostrar para qué sirve un doctorado. Y a esta tarea le sumamos el desafío, o casi más bien la provocación personal, de* ***lograr que los ciudadanos se sientan más orgullosos de los doctorados a los que contribuye con sus impuestos que de los futbolistas que apoya comprando entradas para los eventos correspondientes.***

Fernando Valladares, Joaquín Hortal, Jordi Moya, Adrián Escudero.

AGRADECIMIENTOS

Algo tan simple como los agradecimientos, saben a poco cuando tienes claro que sin la ayuda de todas y cada una de las personas con las que te has ido encontrando, en el devenir de los quehaceres diarios, hubiera sido imposible la consecución de esta tesis.

Destacar sin duda, los directores de la tesis: Eduardo García y Rafael Masot. Sin su inestimable ayuda, comprensión y sobretodo su gran paciencia a la hora de encauzar y dirigir la tesis hubiera sido imposible ni siquiera el comienzo. Agradecer a Eduardo personalmente la confianza depositada en mí desde el comienzo, ya que si no fuera por la beca FPI concedida en el plan nacional que dirigía, jamás hubiera sido posible esta tesis. A lo largo de los años Eduardo ha estado para cualquier cosa que requiriese, ya fuera por motivo de la tesis o por cualquier otro trabajo en el que me implicase. Es por esto por lo que estoy en deuda con él y será difícil devolver toda la ayuda que me ha brindado, cualquier agradecimiento se queda corto. Agradecimientos también a Rafa, pues sus consejos siempre han ido encaminados a la mejora tanto en el ámbito académico como en lo personal. Agradecer también la paciencia y el consejo durante todo el proceso de desarrollo de la tesis, pues me alumbraba el camino para obtener unos buenos resultados, así como poner todo el interés en el detalle en todo lo que se hacía. También me siento en deuda con él, pues sin su tutela, no se habría podido realizar esta tesis. En conjunto quiero agradeceros la ayuda recibida por parte de los dos y como finalmente habéis conseguido que esta tesis finalizase. Sin duda quiero haceros participes de esta tesis puesto que sin vosotros como directores no creo que hubiera podido finalizarla del mismo modo.

Por otro lado, también quería agradecer la ayuda recibida por Juan Soto, ya que gracias a su ayuda he comprendido algo del complicado mundo de la electroquímica. Juan hace sencillo comprender muchos de los complicados conceptos que necesité para realizar algunos de los estudios abordados en esta tesis. Además de fomentar y animarme para no rendirme y seguir trabajando cuando las cosas parecían no salir bien.

También agradecer a Ramón Martinez su disposición y ayuda en todo aquello que necesité. Ramón siempre estaba a disposición para solventar cualquier problema. Siendo además muy objetivo en sus consejos, algo que agradezco.

No quisiera dejar de agradecer también, a todos aquellos que siempre que lo he necesitado me han ayudado, sin poner ninguna pega, a los integrantes del Lab 2.6 del IDM, que siempre que llegaba el *electrónico*, estaban a disposición para ayudarme en todo aquello que necesitase. De manera más directa también agradecer a Román, Inma y Lluís, por la paciencia que han tenido cuando llegaba con cualquier duda y con prisas para resolverlas. Se me hace difícil recordar a todas las personas del grupo de químicos del IDM que de alguna manera u otra han sido parte de la consecución de la tesis con sus explicaciones a la hora de preparar las disoluciones, o como trabajar con las muestras de manera segura. Me vienen a la mente Yoli o María, por ejemplo, también Carmen y otros muchos más. Por parte del laboratorio de alimentos también agradecer la colaboración de Carolina, Raúl, J. M. Barat y otros tantos que de igual modo me han ayudado de una manera u otra.

Quisiera agradecer de una manera especial a todos y cada uno de los integrantes del grupo de investigación al que pertenezco, puesto que gracias al apoyo de todos ellos, en cada etapa del desarrollo de esta tesis, me han ayudado siempre, confiando en mí, haciéndome parte del grupo y teniendo en cuenta las opiniones acerca de decisiones importantes en lo que atañe a diversos trabajos. Mis más sinceros agradecimientos a Nico y Claudia, puesto que me han apoyado y ayudado siempre que lo he necesitado en cualquier tarea de investigación. Hacer extensivo este agradecimiento también a Luis, Javier y Miguel puesto que siempre me han aconsejado sabiamente en todo aquello que les he consultado siendo siempre muy buenos consejos. Agradecer también a mis compañeros de laboratorio Javi, Roberto y Edwin toda la ayuda recibida durante todos estos años y por hacer que todos los días sean más llevaderos con el compañerismo demostrado.

No quisiera dejarme a nadie en el olvido, en lo que respecta a los agradecimientos pero también agradecer a todos y cada uno de las personas que de alguna forma, por pequeña que sea han contribuido a esta tesis y otros trabajos realizados todos estos años.

En el plano personal, me guastaría agradecer todo el apoyo y la confianza a mis padres, no solo desde que emprendí este viaje llamado Tesis, sino también desde mucho antes, concretamente en el momento que comencé a estudiar. Sin ellos todo este plan de vida no podría haber sido posible, con su sacrificio personal fueron los que comenzaron a forjar esta historia personal. Agradecer a mi compañera y mujer en la misma historia personal, pues gracias a ella he podido darle sentido a cada uno de los logros obtenidos, por pequeños que fueran, y continuar trabajando día a día. Sin ella esto tampoco hubiera sido posible, resultando un aliento en los momentos complicados y además siendo parte del mayor hito que tendremos nunca en esta historia personal, tener un hijo del que estamos profundamente enamorados. También agradecer a toda la familia que ha estado siempre pendiente de los logros obtenidos y me ha apoyado a lo largo de los años.

RESUMEN

En la presente tesis doctoral se han utilizado las llamadas técnicas biomiméticas, dentro de las cuales se encuentran las lenguas y narices electrónicas. Basándose en la utilización de estos dispositivos se ha procedido a la detección, clasificación y/o cuantificación de muestras de diversa naturaleza, desde sustancias explosivas y agentes de guerra química hasta productos alimenticios, tanto en disoluciones como en muestras gaseosas.

Mediante el uso del sistema de lengua electrónica y la técnica electroquímica de voltametría de pulsos, se ha realizado el estudio de la sustancia explosiva 2,4,6-Trinitrotolueno (TNT). Dicho estudio ha consistido en el análisis de los datos mediante técnicas multivariantes como el análisis de componentes principales (PCA), la regresión por mínimos cuadrados parciales (PLS) o el análisis mediante redes neuronales artificiales (ANN). Los óptimos resultados obtenidos del procesamiento de la matriz de datos, convierten el algoritmo propuesto en una gran herramienta de compresión de datos para futuros ensayos.

Por otra parte, mediante el uso del sistema de nariz electrónica, se ha realizado el estudio de simulantes de agentes de guerra, en concreto los simulantes de los gases: Sarín, Somán, y Tabún. Además se ha realizado el estudio de posibles interferentes con dichos gases. Se ha realizado un estudio de los datos obtenidos mediante técnicas de análisis multivariante como el análisis de componentes principales (PCA), la regresión por mínimos cuadrados parciales (PLS) o el análisis mediante redes neuronales artificiales (ANN). Los resultados han arrojado buenos modelos de clasificación y predicción, alcanzando un relevante coeficiente de determinación y un notable límite de detección.

Por último, y también empleando la nariz electrónica, se ha realizado el estudio de clasificación de distintas variedades de caqui, obteniendo una clara clasificación de las muestras estudiadas. Posteriormente se han procesado los datos mediante una red neuronal, extrayéndose una correcta distribución de los resultados en función de los días de almacenamiento de la fruta o, lo que es lo mismo, del grado de maduración. Se ha logrado reducir el número de los sensores de gases que componen la nariz, mediante el estudio del grado de contribución al modelo de predicción, logrando así un sistema portable y específico para cada aplicación.

RESUM

En la present tesi doctoral s'han utilitzat les anomenades tècniques biomimètiques, dins de les quals es troben les llengües i nassos electrònics. Basant-se en la utilització d'aquests dispositius s'ha procedit a la detecció, classificació y/o quantificació de mostres de diversa naturalesa, des de substàncies explosives i agents de guerra química fins a productes alimentaris, tant en dissolucions com en mostres gasoses.

Mitjançant l'ús del sistema de llengua electrònica i la tècnica electroquímica de voltametría de polsos, s'ha realitzat l'estudi de la substància explosiva 2,4,6-Trinitrotoluè (TNT). Aquest estudi ha consistit en l'anàlisi de les dades per mitjà de tècniques multivariants com ara l'anàlisi de components principals (PCA), la regressió per mínims quadrats parcials (PLS) o l'anàlisi mitjançant xarxes neuronals artificials (ANN). Els òptims resultats obtinguts del processament de la matriu de dades, converteixen l'algoritme proposat en una gran ferramenta de compressió de dades per a futurs assajos.

D'altra banda, per mitjà de l'ús del sistema de nas electrònic, s'ha realitzat l'estudi de simulantes d'agents de guerra, en concret els simulantes dels gasos: Sarin, Soman i Tabun. A més, s'ha realitzat l'estudi de possibles interferents amb aquests gasos. S'ha realitzat un estudi de les dades obtingudes per mitjà de tècniques d'anàlisi multivariant com l'anàlisi de components principals (PCA), la regressió per mínims quadrats parcials (PLS) o l'anàlisi per mitjà de xarxes neuronals artificials (ANN). Els resultats han permès obtindre bons models de classificació i predicció, aconseguint un rellevant coeficient de determinació i un notable límit de detecció.

Finalment, i també emprant el nas electrònic, s'ha realitzat l'estudi de classificació de distintes varietats de caqui, obtenint una clara classificació de les mostres estudiades. Posteriorment s'han processat les dades per mitjà d'una xarxa neuronal, obtenint una correcta distribució dels resultats en funció dels dies d'emmagatzemament de la fruita o, el que és el mateix, del grau de maduració. S'ha aconseguit reduir el nombre dels sensors de gasos que componen el nas per mitjà de l'estudi del grau de contribució al model de predicció, aconseguint així un sistema portable i específic per cada aplicació.

ABSTRACT

In this thesis, the so-called biomimetic techniques such as electronic tongues and noses have been used. Based on the use of these devices, the detection, classification and/or quantification of samples have been carried out. These studied samples were different in nature including explosive substances, chemical warfare agents and food products, both in liquid and gaseous forms.

An electronic tongue system and the electrochemical pulse voltammetry technique have been used to study the explosive substance 2,4,6-Trinitrotoluene (TNT). This study has consisted in the analysis of data using multivariate analysis such as principal component analysis (PCA), partial least squares regression (PLS) or artificial neural network (ANN) analysis. The optimal results obtained from data matrix processing make the proposed algorithm a great data compression tool for future trials.

The study of simulants for war agents as Sarin, Soman and Tabun, has been carried out by using the electronic nose system. Furthermore, the study of potential interferents with these gases was also carried out. Next, the obtained data was studied by using multivariate analysis techniques such as principal component analysis (PCA), partial least squares regression (PLS) or artificial neural network (ANN) analysis. The results have yielded good classification and prediction models with relevant coefficients of determination and remarkable limits of detection.

Finally, the electronic nose was used to conduct a classification study of different varieties of persimmon obtaining a clear classification of the samples. Subsequently, the obtained data was processed using a neural network. As a result, a correct distribution of the results was obtained depending on the days of storage of the fruit or the degree of ripeness. It has been possible to reduce the number of gas sensors in the electronic nose by studying the degree of contribution to the prediction model, thus achieving a portable and specific system for each application.

INDICE

Introducción

Los sistemas biomiméticos son los que en su diseño, estructura o funcionamiento imitan a la naturaleza y para su realización se utilizan tecnologías como la ingeniería, el diseño, la informática, etc. Es el caso de las lenguas para substancias líquidas y las narices electrónicas para substancias gaseosas, las cuales mediante un sistema de captura de señales y un procesado posterior de las mismas se pueden considerar "sentidos artificiales". Los principios de lengua o nariz electrónica son análogos al sentido del gusto u olfato. Estos sistemas están formados por un conjunto de electrodos no específicos[1] con diferentes grados de sensibilidad a múltiples propiedades. Esta *sensibilidad cruzada* significa que el sensor no responde sólo a un analito[2] sino a diferentes sustancias presentes en el medio. La respuesta de este conjunto de electrodos ofrece una huella electrónica característica de cada especie química[3]. La suma de todas las huellas permite establecer un patrón de reconocimiento. Con el adecuado tratamiento estadístico de los datos se obtiene un análisis tanto cualitativo como cuantitativo.

El hecho de que puedan emplearse como herramientas de análisis en la industria, favoreciendo el control de procesos y la evaluación de la calidad de los productos, las convierte en una excelente alternativa a las técnicas clásicas de control. Otros factores a valorar muy positivamente son que no requieren el empleo de mano de obra cualificada, lo que se traduce en una reducción de costes y que proporciona determinaciones instantáneas, eliminando el tiempo empleado en la realización de los análisis. El desarrollo de sensores basados en estas técnicas permite disponer de métodos de medida portables, fiables, rápidos y económicos.

Hoy en día, estos sistemas de medida tienen una amplia aplicación en diversos campos de la ciencia y de la tecnología, aunque en muchos aspectos están en fase de investigación y optimización. Su utilización en la detección selectiva de agentes nerviosos o explosivos es todavía muy limitada por lo que existe un gran abanico de posibilidades y desarrollo en este campo.

Estos sistemas de medida electrónicos sensoriales son sistemas de "aprendizaje supervisado" en el que primeramente, en la fase de entrenamiento o calibración, el sistema ha de aprender de un conjunto de medidas en las que el usuario ha de proveer al sistema la respuesta correcta. De esta manera el sistema electrónico deduce la relación existente entre la respuesta de los sensores y las características de las muestras generando un modelo matemático. Posteriormente, en la fase de evaluación o validación, el usuario introduce nuevas muestras y el sistema electrónico realiza la clasificación y/o cuantificación de acuerdo con las reglas inferidas en la etapa de aprendizaje.

[1] Electrodos diseñados para responder a los cambios de actividad de múltiples analitos.
[2] Componente (elemento, compuesto o ion) de interés analítico de una muestra.
[3] Se usa comúnmente para referirse de forma genérica a átomos, moléculas, iones, radicales, etc. que sean el objeto de consideración o estudio.

Los ensayos realizados en esta tesis doctoral se enmarcan dentro del proyecto nacional MAT2009-14564-C04-02 cuyo título es *Aproximación al biomimetismo usando lenguas electrónicas y narices para la detección de explosivos y agentes nerviosos*. Se centran, principalmente, en la detección de sustancias peligrosas tanto para la salud pública como para el medio ambiente, como los gases de guerra y explosivos. Además, se ha realizado un tercer ensayo en el ámbito alimentario, concretamente en la detección de la vida útil del caqui.

A continuación se hace una breve introducción de las aplicaciones de estos sistemas de medida en las diferentes áreas relacionadas con la tesis.

1. Medioambiente

Hoy en día una de las preocupaciones que tiene la sociedad, es el medioambiente. Esto se observa en los diferentes proyectos que se llevan a cabo a nivel mundial en los que tratan, de una manera u otra, de minimizar los efectos de una civilización que no siempre vela por el medioambiente. En el ámbito de la protección medioambiental, existen distintos organismos de referencia en el mundo como son la Agencia de Protección Medioambiental en Estados Unidos (Environmental Protection Agency-EPA) y la Agencia Europea de Medio Ambiente (European Environment Agency-EEA), que elaboran documentos e informes que sirven de referencia para el control de determinadas sustancias y velan por el cumplimiento de dichas normas.

Para llevar a cabo los distintos controles medioambientales, se hace necesario el uso de metodologías de detección y/o cuantificación de las sustancias bajo estudio. Un ejemplo relacionado con esta tesis, es la cuantificación del 2,4,6-Trinitrotolueno establecida en el método 8330 (EPA, 2017). Bajos límites de detección y una alta precisión hacen que estos sistemas de medida suelan ser complejos y costosos.

1.1. Gases de guerra y explosivos

Los agentes de guerra química son definidos como substancias gaseosas, liquidas o sólidas que son capaces de herir y matar a animales o personas puesto que atacan al sistema nervioso (Sugendran, et al., 1998), (Kaplan, 1999). Los agentes de guerra química son extremadamente tóxicos y tienen efectos severos en la salud humana y animal. El envenenamiento puede ocurrir por inhalación de gas, contacto con la piel o por consumo de alimentos contaminados.

Naciones Unidas, en la Conferencia de Desarme de Ginebra, aprobó la Convención sobre Armas Químicas (Chemical Weapons Convenion - CWC) el 3 de septiembre de 1992. La CWC es el primer acuerdo de desarme negociado dentro de un marco multilateral que prevé la eliminación de toda una categoría de armas de destrucción masiva en virtud de normas internacionales para su control y aplicación en un ámbito internacional.

1.1.1. Detección de gases de guerra

En la literatura científica se encuentran artículos sobre el control y la detección temprana de estos agentes químicos. A continuación se enumeran algunos de los estudios:

- Inmunoensayos para la detección de agentes químicos de guerra (Chemical Warfare Agents, CWA) (Lenz, et al., 1997): Desarrollo de anticuerpos para la detección y cuantificación del Somán[4], agente de guerra química, en el medioambiente.
- Sensor luminiscente para detección de la hidrólisis del Somán en agua (Jenkins, et al., 1999): Las técnicas de impresión molecular y luminiscencia de lantánidos sensibilizados se han combinado para crear la base para un sensor que puede medir selectivamente el producto de hidrólisis del Somán en agua.
- Métodos analíticos para la medida de CWA (Hill & Martin, 2002): Se utilizan métodos analíticos para la detección e identificación de agentes de guerra química.
- Sensores quimiresistivos mediante nanotubos de carbono para detección de CWA (Wang, et al., 2008): Se presenta un sensor quimiresistivo fabricado mediante dispersión estable de nanotubos de carbono (CNT) y presenta una alta sensibilidad al dimetilmetilfosfonato (DMMP).
- Sensor de onda acústica superficial (SAW) para detección de explosivos y CWA (Nimal, et al., 2009): Desarrollo de sensores de onda acústica recubiertos de diversos polímeros selectivos para la detección y cuantificación de explosivos y CWA.
- Detección de simulantes de agentes nerviosos mediante lengua electrónica (Campos, et al., 2010): Utilizan una lengua electrónica basada en voltamperometría de pulsos para predecir la presencia de simulantes[5] de agentes nerviosos en ambientes acuosos.
- Detección cromogénica mediante nanopartículas funcionalizadas (Climent, et al., 2010): Utilizan nanopartículas de sílice funcionalizadas con alcoholes y tioles para la detección cromofluorogénica de simulantes de agentes nerviosos.

[4] Es una sustancia química extremadamente tóxica empleada como arma química y clasificada como agente nervioso.

[5] Presentan una estructura y reactividad similares como agentes nerviosos pero tienen mucha menos toxicidad para los estudios en el laboratorio.

- Detección y cuantificación de CWA mediante cristales líquidos (VanTreeck, et al., 2011): Presentan las características de rendimiento de los sensores a base de cristal líquido para la detección del dimetilmetilfosfonato (DMMP), un simulante del gas Sarín en la guerra química.
- Detección cromogénica específica para el simulante del Tabún[6] el Cianofosfato de dietilo (DCNP) (Royo, et al., 2011): Se utiliza un colorante azoico con restos de piridina y anilina para detectar selectivamente el dietilcianofosfonato (DCNP) mediante cambios de color.

Estudios que hacen uso de un sistema de nariz electrónica:

- Clasificación de agentes de guerra química utilizando un conjunto de sensores de gas de capa gruesa (Choi, et al., 2005): Fabricación de semiconductores de capa gruesa a base de óxido de estaño como sensores de gas y caracterización de su respuesta ante gases simulantes de agente de guerra química.
- Fabricación de matriz de sensores SAW para clasificar agentes de guerra (Byung-Su, et al., 2007): Se utiliza una matriz con diferentes transductores interdigitales recubiertos con diferentes tipos de polímeros para detectar agentes químicos analizando sus respuestas.
- Nariz electrónica basada en la matriz de sensores SAW recubierta de polímero para la clasificación de simulantes de agentes de guerra (Alizadeha & Zeynali, 2008): Se emplean tres sensores de onda acústica superficial (SAW) recubiertos de distintos polímeros para la detección de simulantes de agentes de guerra química.
- Semiconductores de óxido de estaño y película delgada para la detección de CWA (Sberveglieri, et al., 2009): Preparación y caracterización estructural de nanocables de óxido de estaño como materiales funcionales para el desarrollo de sensores en la detección de simulantes de agentes de guerra química.
- Detección de agentes de guerra con SAW optimizados (Matatagui, et al., 2011): Desarrollo de sensores SAW recubiertos de diferentes polímeros optimizados para detectar simulantes de agentes de guerra química.
- SAW´s funcionalizados con distintos óxidos metálicos (Raj, et al., 2013): Desarrollo de una matriz de cuatro sensores SAW con diferentes recubrimientos de óxidos metálicos para la detección de CWA.
- Óxido de grafeno como capa sensible en los sensores SAW para la detección de simulantes de agentes de guerra química (Sayago, et al., 2016): Desarrollo de un dispositivo de ondas superficiales horizontalmente polarizadas (love-

[6] Es una sustancia química extremadamente tóxica empleada como arma química y clasificada como agente nervioso.

wave sensor) con óxido de grafeno aerografiado como capa sensible para la detección de simulantes de agentes de guerra química.

En la presente tesis se ha realizado un estudio de clasificación y cuantificación sobre los gases de guerra Sarín, Somán y Tabún. Debido a la peligrosidad de las muestras y al no poseer los medios ni el equipamiento necesario para trabajar con este tipo de agentes nerviosos, el estudio se ha hecho concretamente sobre sus *simulantes*. La gran ventaja es que éstos presentan las mismas propiedades que los gases reales pero tienen mucha menor toxicidad. También se ha realizado el estudio de los distintos derivados organofosforados de los simulantes y de posibles interferentes como amoniaco, acetona y otros.

1.1.2. Detección de explosivos: 2,4,6-Tiritrotolueno (TNT)

Los explosivos, a diferencia de los gases de guerra, no están limitados solo al ámbito militar ya que pueden ser habituales en la industria al forman parte de algunos procesos productivos. En este sentido es especialmente importante el control de la contaminación producida por estas sustancias, como el 2,4,6-Trinitrotolueno (TNT) que es el más empleado. El uso y la eliminación de estos explosivos provocan contaminación en suelos y aguas subterráneas.

A continuación se relacionan algunos de los distintos métodos de medida empleados para la detección de TNT, indicando el tipo de sensor utilizado:

- Detección de TNT mediante polímeros impresos (Holthoff, et al., 2011): Desarrollo de sensores que integran polímeros impresos molecularmente (MIPs) y mediante espectrometría Raman de superficie mejorada (SERS) se realiza la detección del TNT.
- Detección de TNT mediante sensores fluorescentes (Li, et al., 2011): Demostración de un método de amplificación de fluorescencia basado en el cristal fotónico para la detección del TNT.
- Detección mediante nanopartículas funcionalizadas con oro (Demeritte, et al., 2012): Desarrollo de un sustrato de espectroscopía Raman (SERS) a gran escala a partir de nanopartículas de oro en forma de palomitas de maíz y en nanotubos de carbono híbridos de capa delgada de una sola pared para la detección de TNT.
- Detección de TNT por un sensor de resonancia de plasmón (Bao, et al., 2012): Desarrollo de un sensor de resonancia de plasmón superficial (SPR) utilizando una película de polímero con impresión molecular (MIP) para la detección de TNT.
- Detección ultrasensible de TNT mediante nanopartículas de grafeno (Liu & Chen, 2013): Desarrollo de un sensor, basado en nanopartículas de plata

funcionalizadas de p-aminotiofenol (PATP) soportadas en grafeno nanosheets (Ag / GNs), que mediante el uso de la espectroscopía Raman de superficie mejorada es posible la detección de TNT.

- Detección de TNT mediante luminiscencia selectiva (Ma, et al., 2013): Desarrollo de sensores luminiscentes preparando nanoesferas multifuncionales codopadas con nanopartículas mediante autoensamblaje de los bloques de construcción. La luminiscencia de estos nanocompuestos decrece mediante la adición de TNT.
- Detección electroquimioluminiscente de TNT (Qi, et al., 2014): Estudio de la detección de TNT basada en electroquimioluminiscencia (ECL) a través de la formación de un complejo de TNT-amina y detección de TNT a través de la transferencia de energía de resonancia de electroquimioluminiscencia (ECRET).
- Detección rápida de TNT en medio acuoso mediante espectroscopía Raman (Jamil, et al., 2015): Desarrollo de un sistema de detección rápido y ultra sensible para el TNT utilizando nanopartículas de oro sin modificar y espectroscopía Raman de superficie mejorada (SERS).
- Detección visual ultrarrápida de TNT (Senthamizhan, et al., 2015): Detección in situ de TNT, por debajo de partes por trillón, utilizando un sistema hibrido con un único grupo de oro fluorescente incrustado en una nanofibra.
- Detección fotoluminiscente mediante funcionalización con anticuerpos (Zhen, et al., 2016): Desarrollo de un biosensor fotoluminiscente selectivo para la detección del TNT en disolución acuosa utilizando un fragmento de anticuerpo anti-TNT.
- Detección de TNT mediante capa delgada funcionalizada con nanocompuesto (Yang, et al., 2016): Preparación electroquímica de un nanocompuesto de capa fina para la detección de TNT.

En esta tesis se plantea la detección de este compuesto en disolución mediante lengua electrónica voltamétrica[7], ya que los métodos anteriormente mencionados son complejos o requieren de equipos caros y complicados de operar.

2. Alimentación

El desarrollo de equipos y técnicas de medida para la detección de gases de guerra y explosivos han permitido al grupo de investigación al que pertenezco trabajar en áreas muy diferentes a las propuestas como primer objetivo de la tesis. Así surgió la

[7] En las técnicas voltamétricas se mide la intensidad de corriente que atraviesa un electrodo por aplicación de una diferencia de potencial.

posibilidad de realizar estudios sobre calidad en alimentos empleando estos mismos equipos y técnicas de detección.

Hoy en día existe una concienciación muy clara sobre la calidad en la alimentación. Todos los estados miembros de la Unión Europea (UE) deben cumplir los principios generales de la Ley de Alimentos y Piensos que se inscribe en la Ley General de Alimentos, Reglamento (CE) nº 178/2002. Esta ley cubre todas las fases de la producción, transformación y distribución de alimentos y piensos. Los objetivos generales de la legislación alimentaria comunitaria son: garantizar un nivel elevado de protección de la salud humana y de los intereses de los consumidores, garantizar también prácticas equitativas en el comercio de alimentos y la libre circulación de alimentos y piensos fabricados y comercializados en la UE.

La calidad (Fernandez, 2009) en las propiedades organolépticas[8] (Flath, et al., 1967) de los alimentos es un valor a tener en cuenta, ya que aun cumpliendo las normas de higiene y seguridad, es posible que no todos los productos alimenticios se encuentren en las mejores condiciones organolépticas. Por ello, es necesario llevar un control, no solo higiénico-sanitario, sino también de las distintas propiedades organolépticas (Dornelles & Tatsuo, 2002) (Hang, et al., 2002). Existen numerosos estudios acerca del control y medida de distintas propiedades cualitativas en alimentos (Pliquett, et al., 2003). Un ejemplo de ello es el trabajo de Olssona, donde utiliza un cromatógrafo de gases acoplado a un espectrómetro de masas capaz de medir los volátiles presentes tras la evolución de un alimento específico como la cebada (Olssona, et al., 2000).

2.1. Medida de la calidad de alimentos: fruta

En este tesis se estudiarán frutas climatéricas[9], en concreto Diospyros kaki T. (caqui), capaces de seguir madurando una vez cosechadas, siempre y cuando hayan alcanzado un nivel de desarrollo total y cuya maduración está regulada por el etileno (Wills et al., 1998).

Es sabido que el caqui se originó en China (Luo & Wang 2008) y posteriormente se extendió a otras regiones del mundo. En la actualidad, es bastante común en los países mediterráneos. El color del caqui generalmente oscila entre amarillo claro-naranja a naranja-naranja oscuro. Dependiendo de la variedad, los caquis varían en tamaño de 1,5 a 9 cm de diámetro y pueden ser esféricos, de bellota o de calabaza. Los frutos del caqui tienen alto contenido en glucosa, con un perfil equilibrado de proteínas, y presentan diversos usos medicinales y químicos (Ragazzini, 1995).

[8] Las propiedades organolépticas de los alimentos son las características físicas que pueden percibir de ellos los distintos sentidos, como el sabor, el olor, la textura y el color.

[9] Se caracterizan porque maduran después de la cosecha y, como parte del proceso de maduración, aumentan la producción de etileno.

Como repaso a los estudios realizados en el ámbito de la industria alimentaria y en concreto en las frutas, se enumerarán los distintos estudios a lo largo de los años y que utilizan técnicas de narices electrónicas o similares:

- Calidad micológica de la cebada mediante nariz electrónica y cromatografía de gases acoplado a un espectrómetro de masas (Olssona, et al., 2000): Uso de la cromatografía de gases combinada con espectrometría de masas (GC-MS) para cuantificar ergosterol y unidades formadoras de colonias (UFC) de muestras de cebada naturalmente contaminadas.
- Monitorización de la madurez utilizando una nariz electrónica (Brezmes, et al., 2000): Desarrollo de una nariz electrónica basada en una matriz de sensores químicos de óxido de estaño capaz de clasificar muestras de fruta en tres estados diferentes de madurez.
- Discriminación de distintos tipos de aceite de oliva mediante nariz electrónica con sensores poliméricos (Guadarrama, et al., 2001): Desarrollo de una nariz electrónica, utilizando una serie de sensores basados en polímeros conductores electrodepositados para la caracterización organoléptica del aceite de oliva.
- Estudio de maduración de manzanas pinklady (Brezmes, et al., 2001): Estudio mediante una nariz electrónica para evaluar el estado de madurez de las manzanas pinklady a través de su vida útil.
- Detección temprana de contaminación microbiológica en tomates procesados (Concina, et al., 2009): Estudio por medio de una nariz electrónica, basada en sensores de gas de óxido metálico de película delgada, para la monitorización de la flora microbiana añadida a tomates pelados enlatados.
- Nariz electrónica para la calidad de los alimentos (Falasconi, et al., 2012): Revisión de cuatro estudios concretos relacionados con el diagnóstico de la contaminación microbiana en el control de la calidad de los alimentos.
- Método de predicción del tiempo de almacenamiento de la manzana Fuji con nariz electrónica (Guohua, et al., 2013): Desarrollo de una nariz electrónica basada en sensores de gas de semiconductores de óxido metálico como método de predicción del tiempo de almacenamiento de las manzanas Fuji almacenadas a temperatura ambiente.
- Estudio de la madurez y el grado de calidad de la fruta (Baietto & Wilson, 2015): Sistema de nariz electrónica, capaz de discriminar mezclas complejas de volátiles de frutas, para análisis más eficiente de aromas de frutas y reemplazar métodos costosos convencionales usados en evaluación de aromas en frutas.
- Aplicación de una nariz con sensores MOS para la predicción de la calidad de la banana (Sanaeifar, et al., 2016): Estudio de la capacidad de una nariz electrónica de bajo coste para predecir los índices de calidad del plátano, tales

como los sólidos solubles totales (TSS), la acidez titulable (TA), el pH y la firmeza en diferentes etapas de vida útil.

- Revisión de las técnicas de nariz electrónica dentro del ámbito de la alimentación (Loutfia, et al., 2015): Este estudio presenta una revisión de los trabajos más recientes en narices electrónicas utilizadas en la industria alimentaria.

3. Técnicas de medida

3.1. Lenguas electrónicas

Las lenguas electrónicas se han empleado extensamente desde hace años (Legin, et al., 1996). En el ámbito de aplicación en medios líquidos, se puede destacar el uso de esta tecnología para el análisis de aguas residuales (Di Natale, et al., 1997) o para determinar el gusto de distintas bebidas (Legin, et al., 1997). La definición de lengua electrónica como tal, parte de la base de la semejanza del sistema en cuestión con el sentido del gusto biológico (Winquist, et al., 2000).

Dos de los tipos más habituales de lenguas electrónicas son, en primer lugar, las lenguas electrónicas potenciométricas, las cuales usan un par de electrodos encargados de medir el potencial de equilibrio (Martínez-Máñez, 2005) y en segundo lugar las lenguas electrónicas voltamétricas, las cuales aplican potenciales de distinta morfología a los electrodos, para ver cómo se comporta la corriente que atraviesa la muestra con el objetivo de caracterizar los procesos que ocurren en el interfaz electrodo-muestra (Ivarsson, 2005). Otro tipo de lengua electrónica, que no cumple de manera estricta la esencia de lo que se conoce como lengua electrónica (Gutés, et al., 2007), es la lengua electrónica basada en espectroscopía de impedancias, la cual realiza un barrido en frecuencia de la impedancia del analito a medir a través de un único elemento sensor.

A continuación, se realiza una síntesis acerca de los diferentes estudios de aplicación de las lenguas electrónicas, realizados en la actualidad.

En 2001, Krantz realiza una revisión del uso de la lengua electrónica en la monitorización de parámetros medioambientales (Krantz-Rülcker, et al., 2001). Posteriormente en 2004, Apetrei en sendas publicaciones estudia diferentes tipos de disoluciones empleando la filosofía de las lenguas electrónicas, aunque no las cite como tal en sus estudios (Apetrei, et al., 2004), (Apetrei, et al., 2004).

Destacar también el uso de las lenguas electrónicas potenciométricas para la clasificación de aguas minerales. Este estudio es de gran ayuda para corroborar que es posible detectar distintos compuestos en el agua presente en el medioambiente y más en concreto, de acuíferos que podrían ser objeto de contaminación (Martínez-Máñez, et al., 2005). Este sistema de lengua electrónica también se puede emplear para la detección de diferentes tipos de alcoholes (Legin, et al., 2005). El estudio llevado a cabo por Lvova en 2006, es de especial interés por el tipo de sensor y técnica electroquímica empleada, ya que hace uso de sensores metálicos potenciométricos (Lvova, et al., 2006). Más información sobre el uso de estos sensores metálicos se puede encontrar en la tesis de L. Gil (Gil, 2007). En otra publicación basada en lengua electrónica potenciométrica Kirsanov realiza un estudio acerca de la evaluación y seguimiento de la calidad del agua y detección de la toxicidad del agua en base a tres organismos vivos presentes en el agua (Kirsanov, et al., 2013).

En cuanto a la tipología de lenguas electrónicas voltamétricas, se pueden encontrar en la bibliografía estudios como el diseño de una metodología de lengua electrónica para sistemas de medida de inyección de flujo (Gutés, et al., 2006) o el estudio del medio acuático con lengua electrónica de Vlasov (Vlasov, et al., 2008), en donde además, se explica una de las fundamentales ventajas de los sensores de sensibilidad cruzada usados en las lenguas.

Uno de los grandes investigadores de las lenguas voltamétricas es F. Winquist, que en sus trabajos iniciales (Winquist, et al., 2000) usaba como elementos sensores electrodos metálicos y en el artículo del 2008 (Winquist, 2008) realiza una revisión de los principios básicos y sus aplicaciones en la industria alimentaria, en el análisis medioambiental y en la industria del papel.

Basándose en el trabajo de Winquist, el Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) de la Universitat Politècnica de València (UPV) implementó una lengua electrónica con las técnicas de medida espectroscopía de impedancia y voltametría para la detección y clasificación de agentes nerviosos, llevando a la consecución de dos tesis doctorales (Alcañiz, 2011) (Campos, 2013).

Otro de los campos importantes de las lenguas electrónicas es en la industria farmacéutica donde también es posible la contaminación medioambiental por posibles vertidos no deseados o a la hora de desechar medicamentos caducados. Dos estudios relacionados con este ámbito fueron desarrollados en 2011. Un trabajo sobre el control mediante lengua electrónica para aplicaciones farmacéuticas (Woertz, et al., 2011a) y otro en el que se hace un estudio comparativo donde se evalúan dos tipos de lenguas electrónicas comerciales (Woertz, et al., 2011b).

Ivarssona y Olsson han trabajado en el estudio de detección de distintas aguas de enjuague procedente de la colada de la ropa (Ivarssona, et al., 2005) (Olsson, et al., 2008). Por otro lado, Winquist ha realizado un estudio de agua potable de la red de distribución (Winquist, et al., 2011). Otro tipo de estudio es el realizado por Zhao, donde se estudia la proliferación de distintos tipos de moho (Zhao, et al., 2011).

Destacar también otro campo de investigación importante como es el alimentario, en donde las lenguas electrónicas también tienen cabida. Por ejemplo, en 2014 Rodriguez-Mendez realizó un estudio para analizar el contenido fenólico de vinos tintos empleando un sistema de lengua electrónica conjuntamente con un sistema de nariz electrónica (Rodriguez-Mendez, et al., 2014). Por otro lado, en otro estudio realizado en 2015, Ha estudió un sistema de lengua electrónica como sensor de sabores empleando una lengua electrónica potenciométrica además de comparar los resultados con una lengua bioelectrónica (Ha, et al., 2015). Otro ejemplo de aplicación de lengua electrónica en el ámbito alimentario lo llevó a cabo Veloso en 2016 realizando un estudio de la intensidad del aceite de oliva, para clasificarlo como intenso medio o ligero (Veloso, et al., 2016).

3.2. Narices electrónicas

En 1994 Gardner y Barlett revisan en su estudio el concepto de nariz electrónica (Gardner & Barlett, 1994) (Gardner & Barlett, 1999), el cual se basa en su similitud con el sentido del olfato biológico (Persaud & Dood, 1982).

Existen numerosas aplicaciones de las narices electrónicas como por ejemplo: Discriminación de distintos olores (Shurmer & Gardner, 1992); Discriminación de distintos compuestos en una fragancia (Branca, et al., 2003); Detección de fenoles en agua (Diz, et al., 2006); Seguimiento de la feromona de la polilla de la manzana (Negri & Bernik, 2008). A su vez también existen distintos tipos de tecnología de sensores para las narices electrónicas y sus aplicaciones como se observa en los siguientes trabajos: Transistores orgánicos de película delgada (Liao, et al., 2005); Sensores de óxido metálico (Berna, 2010); Polietileno impreso (Changa, et al., 2006); Nanotubos de carbono (Wongchoosuk, et al., 2010). Y en relación a los distintos tipos de procesamiento de los datos para las narices electrónicas: Análisis de componentes principales (PCA) y perceptrón multicapa MLP (Penza & Cassano, 2003); Algoritmos genéticos (Boilot, et al., 2003).

Por lo que respecta al ámbito alimentario en general, se enumerarán los siguientes estudios: Estudio de perfiles fenólicos en purés de membrillo, pera y manzana (Andrade, et al., 1998); Medida de calidad en estudios de frutas y vegetales (Abbot, 1999); Calidad micológica de la cebada (Olssona, et al., 2000); Monitorización de la madurez utilizando una nariz electrónica (Brezmes, et al., 2000); Discriminación de distintos tipos de aceite de oliva mediante nariz electrónica con sensores poliméricos (Guadarrama, et al., 2001); Estudio de maduración de manzanas pinklady (Brezmes, et al., 2001); Evaluación de la calidad de los filetes de salmón en diversas condiciones de conservación (Du, et al., 2002); Determinación no destructiva de la frescura del huevo (Dutta, et al., 2003); Degradación de la leche (Labreche, et al., 2005); Clasificación de vinos con matriz de sensores de onda acústica (Lozano, et al., 2006); Evaluación del olor de los camarones tratados con diferentes productos químicos (Luzuriaga, et al., 2007); monitorización de la auto oxidación del aceite de colza (Mildner-Szkudlarz, et al., 2008); Detección temprana de contaminación microbiológica en tomates procesados (Concina, et al., 2009); Detección de la bacteria Alicyclobacillus en refrescos (Concina, et al., 2010); Discriminación de la calidad del té verde (Chen, et al., 2011).

Otros estudios nombrados anteriormente son: Nariz electrónica para la calidad de los alimentos (Falasconi, et al., 2012); Método de predicción del tiempo de almacenamiento de la manzana Fuji con nariz electrónica (Guohua, et al., 2013); Estudio de la madurez y el grado de calidad de la fruta (Baietto & Wilson, 2015); Aplicación de una nariz con sensores MOS para la predicción de la calidad de la banana (Sanaeifar, et al., 2016).

REFERENCIAS

Abbot, J., 1999. Quality measurement of fruits and vegetables. *Postharvest Biology and Technology,* pp. 207-225.

Alcañiz, M., 2011. *Tesis doctoral: Diseño de un sistema de lengua electrónica basado en técnicas electroquímicas voltamétricas y su aplicación de el ámbito agroalimentario.* s.l.:UPV.

Alizadeha, T. & Zeynali, S., 2008. Electronic nose based on the polymer coated SAW sensors array for the warfare agent simulants classification. *Sensors and Actuators B: Chemical,* pp. 412-423.

Andrade, P., Carvalho, A., Seabra, R. & Ferreira, M., 1998. A previous study of phenolic profiles of quince, pear, and apple purees by HPLC diode array detection for the evaluation of quince puree genuineness. *Journal of Agricultural and Food Chemistry,* pp. 968-972.

Apetrei, A. y otros, 2004. Array of voltammetric sensors for the discrimination of bitter solutions. *Sensors and Actuators B: Chemical,* pp. 145-152.

Apetrei, A., Rodríguez, M. & Mendez, J. d. S., 2004. Voltammetric sensor array based on conducting polymer-modified electrodes for the discrimination of liquids. *Electrochimica Acta,* pp. 4543-4551.

Baietto, M. & Wilson, A., 2015. Electronic-Nose Applications for Fruit Identification, Ripeness and Quality Grading. *Sensors,* pp. 899-931.

Bao, H. y otros, 2012. Detection of TNT by a molecularly imprinted polymer film-based surface plasmon resonance sensor. *Chinese Science Bulletin,* pp. 2102-2105.

Berna, A., 2010. Metal Oxide Sensors for Electronic Noses and Their Application to Food Analysis. *Sensors,* pp. 3882-3910.

Boilot, P., Hines, E., Gongora, M. & Folland, R., 2003. Electronic noses inter-comparison, data fusion and sensor selection in discrimination of standard fruit solutions. *Sensors and Actuators B: Chemical,* pp. 80-88.

Branca, A. y otros, 2003. Electronic nose based discrimination of a perfumery compound in a fragrance. *Sensors and Actuators B: Chemical,* pp. 222-227.

Brezmes, J. y otros, 2001. Correlation between electronic nose signals and fruit quality indicators on shelf-life measurements with pinklady apples. *Sensors and Actuators B: Chemical,* pp. 41-50.

Brezmes, J. y otros, 2000. Fruit ripeness monitoring using an Electronic Nose. *Sensors and Actuators B: Chemical,* pp. 223-229.

Byung-Su, J., Jeung-Soo, H. & Duk-Dong, L., 2007. Fabrication of polymer SAW sensor array to classify chemical warfare agents. *Sensors and Actuators B: Chemical*, pp. 47-53.

Campos, I., 2013. *Tesis Doctoral: Sensores electroquímicos tipo lengua electrónica voltamétrica aplicados al control medioambiental y a la industria alimentaria.* s.l.:UPV.

Campos, I. y otros, 2010. Use of a Voltammetric Electronic Tongue for Detection and Classification of Nerve Agent Mimics. *Electroanalysis,* pp. 1643-1649.

Changa, J., Liu, V. & Subramanian, V., 2006. Printable polythiophene gas sensor array for low-cost electronic noses. *Journal of Applied Physics.*

Chen, Q. y otros, 2011. Discrimination of green tea quality using the electronic nose technique and the human panel test, comparison of linear and nonlinear classification tools. *Sensors and Actuators B: Chemical,* pp. 294-300.

Choi, N.-J.y otros, 2005. Classification of chemical warfare agents using thick film gas sensor array. *Sensors and Actuators B: Chemical,* pp. 298-304.

Climent, E. y otros, 2010. Chromogenic Detection of Nerve Agent Mimics by Mass Transport Control at the Surface of Bifunctionalized Silica Nanoparticles. *Angewandte Chemie*, pp. 5945-5948.

Concina, I. y otros, 2010. Alicyclobacillus spp.: detection in soft drinks by electronic nose.. *Food Research International,* pp. 2108-2114.

Concina, I. y otros, 2009. Early detection of microbial contamination in processed tomatoes by electronic nose. *Food Control,* pp. 873-880.

Demeritte, T., Kanchanapally, R., Fan, Z. & Singh, A., 2012. Highly efficient SERS substrate for direct detection of explosive TNT using popcorn-shaped gold nanoparticle-functionalized SWCNT hybrid. *Analyst*, pp. 5041-5045.

Di Natale, C. y otros, 1997. Multicomponent analysis on polluted waters by means of an electronic tongue. *Sensors and Actuators B: Chemical,* pp. 423-428.

Diz, V., Cassanello, M. & Negri, R., 2006. Detection and discrimination of phenol and primary alcohols in water using electronic noses. *Environmental Science and Technology,* pp. 6058-6063.

Dornelles, L. & Tatsuo, L., 2002. Review of the use of biosensors as analytical tools in the food and drink industries. *Food Chemistry,* pp. 237-256.

Dutta, R. y otros, 2003. Non-destructive egg freshness determination: an electronic nose based approach. *Measurement Science and Technology,* pp. 190-198.

Du, W. y otros, 2002. Potential application of the Electronic Nose for quality assessment of Salmon fillets under various storage conditions. *Journal of Food: Microbiology, Safety and Hygiene,* pp. 307-313.

EPA, 2017. *Method 8330.* [En línea] Available at: http://www.caslab.com/EPA-Methods/PDF/EPA-Method-8330.pdf [Último acceso: 2014].

Falasconi, M. y otros, 2012. Electronic Nose for Microbiological Quality Control of Food Products. *International Journal of Electrochemistry,* pp. 1-12.

Fernandez, M., 2009. Pautas de calidad en el sistema agroalimentario español. *Agroalimentaria,* pp. 59-65.

Flath, R. y otros, 1967. Identification and organoleptic evaluation of compounds in Delicious apple essence. *Journal of Agricultural Food Chemistry,* pp. 29-35.

Gardner, J. & Barlett, P., 1994. A brief history of electronic noses. *Sensors and Actuators B: Chemical,* pp. 211-220.

Gardner, J. & Barlett, P., 1999. Electronic noses. Principles and applications. *Oxford University.*

Gil, L., 2007. *Tesis Doctoral: Diseño de lenguas electrónicas potenciométricas.* s.l.:UPV.

Guadarrama, A. y otros, 2001. Electronic nose based on conducting polymers for the quality control of the olive oil aroma.. *Analytica Chimica Acta,* pp. 283-292.

Guohua, H., Yuling, W., Dandan, Y. & Wenwen, D., 2013. Fuji Apple Storage Time Predictive Method Using Electronic Nose. *Food Analytical Methods,* pp. 82-88.

Gutés, A., Cespedes, F. & del Valle, M., 2007. Electronic tongues in flow analysis. *Analytica chimica acta,* Volumen 600, pp. 90-96.

Gutés, A. y otros, 2006. A flow injection voltammetric electronic tongue applied to paper mill industrial waters. *Sensors and Actuators B: Chemical,* pp. 390-395.

Ha, D. y otros, 2015. Recent achievements in electronic tongue and bioelectronic tongue as taste sensors. *Sensors and Actuators B: Chemical,* Volumen 207, pp. 1136-1146.

Hang, Y. y otros, 2002. Nondestructive prediction of moisture and sodium chloride in cold smoked atlantic salmon. *Journal of Food Science,* p. 7.

Hill, H. & Martin, S., 2002. Conventional analytical methods for chemical warfare agents. *Pure and Applied Chemistry,* pp. 2281-2291.

Holthoff, E., Stratis-Cullum, D. & Hankus, M., 2011. A Nanosensor for TNT Detection Based on Molecularly Imprinted Polymers and Surface Enhanced Raman Scattering. *Sensors,* pp. 2700-2714.

Ivarssona, P. y otros, 2005. Supervision of rinses in a washing machine by a voltammetric electronic tongue. *Sensors and Actuators B: Chemical,* pp. 851-857.

Jamil, A., Izake, E., Sivanesan, A. & Fredericks, P., 2015. Rapid detection of TNT in aqueous media by selective label free surface enhanced Raman spectroscopy. *Talanta*, pp. 732-738.

Jenkins, A., Uy, O. & Murray, G., 1999. Polymer-based lanthanide luminiscent sensor for detection of the hydrolysis product of the nerve agent Soman in water. *Analytical Chemistry,* pp. 373-378.

Kaplan, M., 1999. The efforts of WHO and Pugwash to eliminate. *Bulettin of the World Health Organization,* Volumen 77, pp. 149-155.

Kirsanov, D. y otros, 2013. Water toxicity evaluation in terms of bioassay with an Electronic Tongue. *Sensors and Actuators B: Chemical,* Volumen 179, pp. 282-286.

Krantz-Rülcker, C., Stengberg, M., Winquist, F. & Ludström, I., 2001. Electronic tongues for environmental monitoring based on sensor arrays and pattern recognition: a review. *Analytica Chimica Acta,* pp. 217-226.

Labreche, S., Bazzo, S., Cade, S. & Chanie, E., 2005. Shelf life determination by electronic nose: application to milk. *Sensors and Actuators B: Chemical,* pp. 199-206.

Legin, A. y otros, 1997. Tasting of beverages using an electronic tongue. *Sensors and Actuators B: Chemical,* pp. 291-296.

Legin, A., Ruudnistkaya, A. .., Seleznev, B. & Vlasov, Y., 2005. Electronic tongue for quality assesment of ethanol, vodka and eau-de-vie. *Analytica Chimica Acta,* pp. 129-135.

Legin, A., Vlasov, Y., Rudnitskaya, A. & Bychkov, E., 1996. Cross-sensitivity of chalcogenide glass sensors in solutions of heavy metals ions. *Sensors ans Actuators B: Chemical,* pp. 456-461.

Lenz, D., Brimfield, A. & Cook, L., 1997. Development of immunoassays for detection of chemical warfare agents. *American Chemical Society,* pp. 77-86.

Liao, F., Chen, C. & Subramanian, V., 2005. Organic TFTs as gas sensors for electronic nose applications. *Sensors and Actuators B: Chemical,* pp. 849-855.

Li, H. y otros, 2011. Amplifying fluorescence sensing based on inverse opal photonic crystal toward trace TNT detection. *Journal of Materials Chemistry,* pp. 1730-1735.

Liu, M. & Chen, W., 2013. Graphene nanosheets-supported Ag nanoparticles for ultrasensitive detection of TNT by surface-enhanced Raman spectroscopy. *Biosensors and Bioelectronics,* pp. 68-73.

Loutfia, A. y otros, 2015. Electronic noses for food quality: A review. *Journal of Food Engineering,* pp. 103-111.

Lozano, J. y otros, 2006. Wine classification with a zinc oxide SAW sensor array. *Sensors and Actuators B: Chemical,* pp. 166-171.

Luzuriaga, D., Kore, F. & Balaban, M., 2007. Odor evaluation of shrimp treated with different chemicals using an electronic nose and a sensory panel. *Journal of Aquatic Food Product Technology,* pp. 57-75.

Lvova, L. y otros, 2006. Electronic tongue based on an array of metallic potentiometric sensors. *Talanta,* pp. 833-839.

Martínez-Máñez, R., 2005. A multisensor in thick-film technology for water quality control. *Sensors and Actuators A: Physical,* Issue 120, p. 589–595.

Martínez-Máñez, R. y otros, 2005. An “electronic tongue” design for the qualitative analysis of natural waters. *Sensors and Actuators B: Chemical,* Issue 104, p. 302–307.

Matatagui, D. y otros, 2011. Chemical warfare agents simulants detection with an optimized SAW sensor array. *Sensors and Actuators B: Chemical,* pp. 199-205.

Ma, Y., Huang, S. & Wang, L., 2013. Multifunctional inorganic–organic hybrid nanospheres for rapid and selective luminescence detection of TNT in mixed nitroaromatics via magnetic separation. *Talanta,* pp. 535-540.

Mildner-Szkudlarz, S., Jelen, H. & Zawirska-Wojtasiak, R., 2008. The use of electronic and human nose for monitoring rapeseed oil autoxidation. *European Journal of Lipid Science and Technology,* pp. 61-72.

Negri, R. & Bernik, D., 2008. Tracking the sex pheromone of codling moth against a background of host volatiles with an electronic nose. *Crop Protection,* pp. 1295-1302.

Nimal, A. y otros, 2009. Development of handled SAW vapor sensor for explosives and CW agents. *Sensors and Actuators B: Chemical,* pp. 399-410.

Olssona, J., Börjensson, T., Lundstedt, T. & Schürer, J., 2000. Volatiles for mycological quality grading of barley grains: determinations using GC-MS and electronic nose. *International Journal of Food Microbiology,* pp. 167-178.

Olsson, J., Ivarsson, P. & Winquist, F., 2008. Determination of detergents in washing machine wastewater with a voltammetric electronic tongue. *Talanta,* pp. 91-95.

Penza, M. & Cassano, G., 2003. Application of principal component analysis and artificial neural networks to recognize the individual VOCs of methanol/2-propanol in a binary mixture by SAW multi-sensor array. *Sensors and Actuators B: Chemical*, pp. 269-284.

Persaud, K. & Dood, G., 1982. Analysis of discrimination mechanism in the mammalian olfactory system using a model nose. *Nature*, pp. 352-355.

Pliquett, U., Altmann, M., Pliquett, F. & Schöberlein, L., 2003. Py- a parameter for meat quality. *Meat Science*, pp. 1429-1437.

Qi, W. y otros, 2014. Electrochemiluminescence Detection of TNT by Resonance Energy Transfer through the Formation of a TNT–Amine Complex. *Chemistry*, pp. 4829-4835.

Raj, V. y otros, 2013. Oxide thin films (ZnO, TeO2, SnO2, and TiO2) based surface acoustic wave (SAW) E-nose for the detection of chemical warfare agents. *Sensors and Actuators B: Chemical*, pp. 636-647.

Rodriguez-Mendez, M. y otros, 2014. Evaluation of oxygen exposure levels and polyphenolic content of red wines using an electronic panel formed by an electronic nose and an electronic tongue. *Food Chemistry*, Volumen 155, pp. 91-97.

Royo, S. y otros, 2011. Chromogenic, Specific Detection of the Nerve-Agent Mimic DCNP (a Tabun Mimic). *Chemistry - A European Journal*, pp. 6931-6934.

Sanaeifar, A., Mohtasebi, S., Ghasemi-Varnamkhasti, M. & Ahmadi, H., 2016. Application of MOS based electronic nose for the prediction of banana quality properties. *Measurement*, pp. 105-114.

Sayago, I. y otros, 2016. Graphene oxide as sensitive layer in Love-wave surface acoustic wave sensors for the detection of chemical warfare agent simulants. *Talanta*, pp. 393-400.

Sberveglieri, G. y otros, 2009. Semiconducting tin oxide nanowires and thin films for Chemical Warfare Agents detection. *Thin Solid Films*, pp. 6156-6160.

Senthamizhan, A., Celebioglu, A. & Uyar, T., 2015. Ultrafast on-site selective visual detection of TNT at sub-ppt level using fluorescent gold cluster incorporated single nanofiber. *Chemical Communications*, pp. 5590-5593.

Shurmer, H. & Gardner, J., 1992. Odour discrimination with an electronic nose. *Sensors and Actuators B: Chemical*, pp. 1-11.

Sugendran, K., Kumar, P. & Vijayaraghavan, R., 1998. Military Law Review. *Defence Science Journal*, pp. 155-155.

VanTreeck, H., Most, D., Grinwald, B. & Kupcho, K., 2011. Quantitative detection of a simulant of organophosphonate CWA using liquid crystals. *Sensors and Actuators B: Chemical,* pp. 104-110.

Veloso, A. y otros, 2016. Sensory intensity assessment of olive oils using an electronic tongue. *Talanta,* Volumen 146, pp. 585-593.

Vlasov, Y., Legin, A. & Rudnitskaya, M., 2008. Electronic tongue: Chemical sensor systems for analysis of aquatic media. *Russian Journal of General Chemistry,* pp. 2532-2544.

Wang, F., Gu, H. & Swanger, T., 2008. Carbon Nanotube/Polythiophene Chemiresistive Sensors for CWA. *Journal of the American Chemical Society,* pp. 5392-5393.

Winquist, F., 2008. Voltammetric electronic tongues – basic principles and applications. *Microchimica Acta,* pp. 3-10.

Winquist, F. y otros, 2000. A hybrid electronic tongue. *Analytica Chimica Acta,* Issue 406, p. 147–157.

Winquist, F., Olsson, J. & Eriksson, M., 2011. Multicomponent analysis of drinking water by a voltammetric electronic tongue. *Analytica Chimica Acta,* pp. 192-197.

Woertz, K., Tissen, C., Kleinebudde, P. & Breitkeutz, J., 2011a. Taste sensing systems (electronic tongues) for pharmaceutical applications. *International Journal of Pharmaceutics*, pp. 256-271.

Woertz, K., Tissen, C., Kleinebudde, P. & Breitkeutz, J., 2011b. A comparative study on two electronic tongues for pharmaceutical formulation development. *Journal of Pharmaceutical and Biomedical Analysis,* pp. 272-281.

Wongchoosuk, C., Wisitsoraat, A., Tuantranont, A. & Kerdcharoen, T., 2010. Portable electronic nose based on carbon nanotube-SnO2 gas sensors and its application for detection of methanol contamination in whiskeys. *Sensors and Actuators B: Chemical*, pp. 392-399.

Yang, T. y otros, 2016. Electrochemical preparation of thin-layered molybdenum disulfide-poly(m-aminobenzenesulfonic acid) nanocomposite for TNT detection. *Journal of Electroanalytical Chemistry,* pp. 70-75.

Zhao, G. y otros, 2011. Use of the smart tongue to monitor mold growth and discriminate between four mold species grown in liquid media. *Analytica Chimica Acta,* pp. 240-247.

Zhen, L. y otros, 2016. Photoluminescence detection of 2,4,6-trinitrotoluene (TNT) binding on diatom frustule biosilica functionalized with an anti-TNT monoclonal antibody fragment. *Biosensors and Bioelectronics,* pp. 742-748.

Objetivos

La presente tesis está enmarcada en los trabajos que se desarrollaron en el proyecto del Plan Nacional MAT2009-14564-C04-02 "*Aproximación al biomimetismo usando lenguas electrónicas y narices para la detección de explosivos y agentes nerviosos*". El principal objetivo de este proyecto era crear nuevos sistemas de detección basados en lenguas y narices electrónicas para su aplicación en diversos campos. En esta tesis en concreto se estudia su viabilidad en la determinación de explosivos y gases nerviosos.

Se desarrollaron varias lenguas electrónicas con diferentes técnicas de medida electroquímica (potenciometría, voltametría y espectroscopía de impedancia) y una nariz electrónica basada en una matriz de sensores de semiconductores de óxidos metálicos (MOS, *Metallic Oxide Semiconductor*). Se llevaron a cabo multitud de estudios para validar estos sistemas sensoriales, tales como la detección de gases de guerra y explosivos, pesticidas, fármacos, valoración de la calidad de alimentos, calidad del agua, etc. La aplicación de técnicas de análisis multivariante en combinación con las redes neuronales permitió obtener modelos de predicción satisfactorios.

En la presente tesis, fruto de aquel extenso trabajo que me permitió desarrollar mi formación como investigador FPI, se proponen tres grandes objetivos:

Detección y cuantificación de sustancias explosivas en medio acuoso

Se propone la detección y cuantificación de sustancias explosivas y otros productos utilizados en su síntesis en medio acuoso, empleando como sistema de medida una lengua electrónica voltamétrica con electrodos metálicos.

Detección de gases de guerra e interferentes

Se sugiere la discriminación y cuantificación de simulantes de agentes nerviosos (gases de guerra Sarín, Somán y Tabún), sus derivados organofosforados y substancias interferentes mediante un sistema de nariz electrónica.

Determinación del grado de maduración en fruta por reconocimiento de aromas

Se plantea la clasificación de los aromas que desprende la fruta con distintos niveles de maduración para determinar su variedad, calidad y vida útil mediante un sistema de nariz electrónica de parámetros configurables.

Chapter 1: TNT detection using a voltammetric electronic tongue based on neural networks

García Breijo, Eduardo; Olguín Pinatti, Cristian Ariel; Masot Peris, Rafael; Alcañiz Fillol, Miguel; Martínez Mañez, Ramón; Soto Camino, Juan. *TNT detection using a voltammetric electronic tongue based on neural networks.* Sensors and Actuators A: Physical. 192, pp. 1 - 8. 2013.

https://doi.org/10.1016/j.sna.2012.11.038

Abstract

We report here the use of a voltammetric electronic tongue based in simple metallic electrodes for the detection and discrimination of different concentrations of 2,4,6-trinitrotoluene (TNT) in acetonitrile: water 1:1 v/v mixtures. The tongue consisted of noble working electrodes made of iridium, rhodium, platinum and gold and non-noble electrodes including silver, copper, cobalt and nickel. Both, the Self Organizing Map (SOM) and Multi-Layer Feed-Forward Network (MLFN) neural networks were applied to the data obtained from the electronic tongue and TNT solutions. From SOM analysis it was established that a suitable response in terms of a correct classification of the TNT concentration was observed when using only noble metal electrodes and only 5 selected pulses. Similar good classifications were found when using MLFN. Moreover, the algorithm of neural network MLFN was embedded in a microcontroller in order to obtain a smart portable system for discrimination of TNT. In this case a R squared of 0.993 was obtained for predicted vs observed graphs of concentrations of TNT concentrations.

Keywords: TNT, explosives, Electronic Tongue, Neural Networks.

1. Introduction

Current international public awareness on terrorist attacks using explosives has resulted in the particular interest of developing quick and reliable methods to detect these chemicals. Moreover, given the widespread use of explosive formulations, the analysis of explosives is also of interest in landmine detection, forensic research, and to study environmental problems associated with explosive residues. Among the different chemical explosives, nitroaromatics are perhaps the most commonly used and from them probably 2,4,6-trinitrotoluene TNT is the most representative. TNT is well known not only for its use for military purposes (Jenkins, et al. 2001) but it also has a great industrial interest (Irving, et al. 1987). With the production, storage and use of TNT, an environmental problem has been created because this mutagenic, toxic, and persistent compound has been reported to leach from soils and to accumulate in the food chain (Hilmi, et al. 1999), (Rieger, et al. 1995), (Walker & Kaplan, 1992), (Won, et al. 1976). Current methodologies employed for the detection of nitroaromatic explosives such as TNT are enzymatic assays (Smith, et al. 2008), gas and liquid chromatography (Moore, 2004), mass spectrometry (Hakansson, et al. 2000), ion-mobility spectroscopy (Wallis, et al. 2005), optodes (Germain & Knapp, 2009), electrochemical procedures (Forzani, et al. 2009) and fluorescent and colorimetric probes (Salinas, et al. 2012). Some portable systems have also been reported (Sanoita, et al. 2009).

In recent years, electronics tongues have appeared as an excellent alternative to traditional analysis methods in different areas as food, pharmaceutical industries, environment and others (Alcañiz, et al. 2012), (Toko, 2000), (Cosio, et al. 2007). These systems combine electrochemical techniques such as potentiommetry, voltammetry or impedance spectroscopy with multivariate analysis tools (neural networks, Principal Component Analysis (PCA), Partial Least Squares (PLS), fuzzy logic, etc.) in order to classify samples or quantify their physicochemical properties (Vlasov, et al. 2000). Their main advantage compared to traditional methods is that they allow the implementation of fast and low-cost measurements systems avoiding pre-processing of the samples and the need of qualified personnel to carry out the analyses.

Traditional electronic tongue systems have been mainly designed using potentiometric and voltammetric techniques (Martínez-Máñez, et al. 2005), (Lvova, et al. 2006), (Winquist, et al. 2005). In particular voltammetric procedures have recently become popular for the design of electronic tongues using arrays of electrodes suitable for voltammetric experiments with very fine results (Winquist, et al. 2005), (Ivarsson, et al. 2001), (Martina, et al. 2007), (Gil-Sánchez, et al. 2011), (Gil-Sánchez, et al. 2008). Voltammetry covers a group of electro-analytical methods in which the information of the analyte is derived from the measurement of the current versus the applied potential under conditions that helps the polarization of a working electrode.

In this particular work we report the design of a voltammetric electronic tongue based in simple metallic electrodes and the study of its use as a suitable system for the detection of TNT in aqueous samples. Moreover we were also interested in testing the use of neural networks to study the electrochemical response of the electrodes in the presence of this chemical. In particular, neural networks allows a facile implementation of algorithms in microprogrammable system such as Field-Programmable Gate Array (FPGA), Digital Signal Processor (DSP) or microcontrollers, and this is the basis for the further design of easy-to-use portable systems for in situ or at site detection applications (Alcañiz, et al. 2012),(Gil-Sánchez, et al. 2008), (Labrador, et al. 2010), (Campos, et al. 2010), (Campos, et al. 2012a), (Campos, et al. 2012b), (Gil-Sánchez, et al. 2011), (García-Breijo, et al. 2011a), (Ibáñez, et al. 2011), (García-Breijo, et al. 2011b).

2. *Materials and methods*

2.1. Electronic System

The electronic system used in the electronic tongue was developed in the IDM Research Institute at the Polytechnic University of Valencia (Spain). The system consisted of a software application, which runs on a PC, and electronic equipment. The electronic system allows to work with three electrochemical techniques: impedance spectroscopy, cyclic voltammetry and pulse voltammetry. For impedance spectroscopy the system generates sinusoidal signals with frequencies in the range 1Hz to 200kHz and an amplitude up to 500mV. For cyclic voltammetry the amplitude of the triangular potential waveforms can be configured in the range of -2V to +2V and the scan rate can be established form 2mV/s to 10V/s. Finally for pulse voltammetry up to 50 pulses can be programmed with amplitude from -2V to +2V and a pulse width from 1ms to 800ms. The system carries out measurements on up to ten multiplexed electrodes for voltammetric techniques and on one electrode for impedance spectroscopy. A potentiostat controls the voltage applied to the electrodes and measures the resulting current. The potentiostat can be configured to work in two-electrodes mode (counter electrode and working electrode) or in three-electrodes mode (counter electrode, reference electrode and working electrode). The user configures the test in the software application where the data required to carry out the measurement are prepared and this is sent to the electronic equipment through a USB bus. The electronic equipment applies the voltage signals to the electrodes and measures the generated current signals. The digitalized values of the signals from the samples are then sent to the PC where the software application stores them in a file for further processing.

2.2. Electrodes

The electronic tongue system is designed to work with different electrodes configuration. Voltammetric studies can be carried out in up to ten working electrodes. Saturated calomel or silver chloride electrodes are normally used as reference electrodes. The configuration of the counter and working electrodes depends on the applied technique. For voltammetric techniques the electrodes used are based on the Voltammetric Electronic Tongue (VET) described by Winquist et al. in (Winquist, et al. 2005). Two types of electrodes have been used in this work; noble working electrodes made of iridium, rhodium, platinum and gold and non-noble electrodes including silver, copper, cobalt and nickel (see Fig. 1).

Fig. 1. Electrodes.

2.3. Implementation of MLFN in a microcontroller

Most of the systems of electronic tongues remain in the laboratory version, which requires the presence of a computer and, especially above all, two separate processes, one for taking measurements and another for data processing. If it is desired for these systems to have industrial application however, it is necessary to unify these two phases into a single system. The best method for achieving this goal is the use of microcontrollers in systems which, in addition to the measurement process, are able to perform the analysis of relevant data using a software program implemented in the microcontroller memory. Thus portable electronic tongues are becoming popular as they offer simplicity, reliability and use in situ. Pattern recognition algorithms have become a critical component in the implementation of electronic tongues and noses, and have been used successfully in these applications. For implementation in portable equipment the algorithm must be transferable to a microcontroller which has a limited amount of memory. Thus the perfect pattern recognition algorithms will require high accuracy, to be fast, work in real-time and have low memory requirements in order to be implemented in a microcontroller.

The embedded system was built around a Microchip PIC24FJ256 microcontroller. The PIC24FJ256 is a PIC24/16-bit family microcontroller and has 16KB of RAM and 256KB of reprogrammable flash memory. The software was coded in C language for the microcontroller and consists of two main routines: (i) overall system control and (ii) implementation of the pattern recognition algorithm. In the implementation of the pattern recognition algorithm, weights (Wji) and biases (Bj) of trained Multi-Layer Feed-Forward Network (MLFN) were used in order to program the MLFN into the microcontroller memory. Using the normalized input values, weights and biases, the microcontroller calculated the output for each hidden node by using a sigmoid transfer function. With these outputs the microcontroller calculated the output by using a linear transfer function in fitting case and a sigmoid transfer functions in classification case. This routine was coded in C language and was converted to HEX code using the cross compiler. The HEX file is downloaded into the flash memory of the microcontroller.

2.4. Experimental

The designed electronic tongue system was applied to the detection and quantification of 2,4,6-trinitrotoluene (TNT). The reactives used for samples preparation were a commercial TNT solution 0.13M in acetonitrile and potassium nitrate (KNO_3). A background electrolyte was prepared with 50% of potassium nitrate KNO_3 0.01M in distilled water and 50% of acetonitrile. This background electrolyte was used for the preparation of the TNT samples. Different solutions having different concentrations of TNT were prepared in acetonitrile (3 ml). The final TNT samples were prepared by mixing these 3 ml with 22 ml of the background electrolyte. The final concentrations for the tested TNT solutions were $3.9x10^{-7}$M, $6.0x10^{-7}$M, $1.2x10^{-6}$M, $6.0x10^{-6}$M, $1.2x10^{-5}$M, $6.0x10^{-5}$M, $1.2x10^{-4}$M and $5.9x10^{-4}$M. Fifteen samples were prepared for each concentration and three cycles of Pulse Voltammetry Measurements were carried out on each sample.

Two sets of electrodes were used: one with noble metals (Ir, Rh, Pt and Au) and one with non-noble metals (Ag, Co, Cu and Ni). Different pulses arrays were applied to the working electrodes depending on their nature. The applied pulses array for Ir, Rh, Pt, Au, Ag and Ni is shown in Fig. 2. The pulse sequence used for Cu and Co is similar but the pulses P2, P8 and P12 have -300mV of amplitude. The pulse arrays were designed according to a method recently described by us for voltammetric electronic tongues. (Campos, et al. 2012b). As example, Fig. 3 shows the response of the Cu electrode for each of the concentrations.

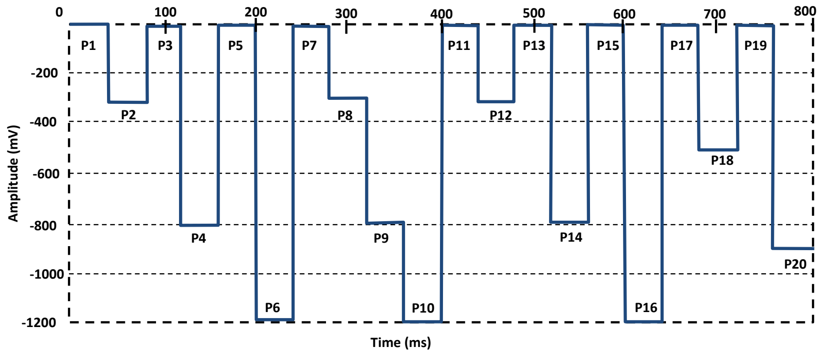


Fig. 2. Pulses array for Ir, Rh, Pt, Au, Ag and Ni.

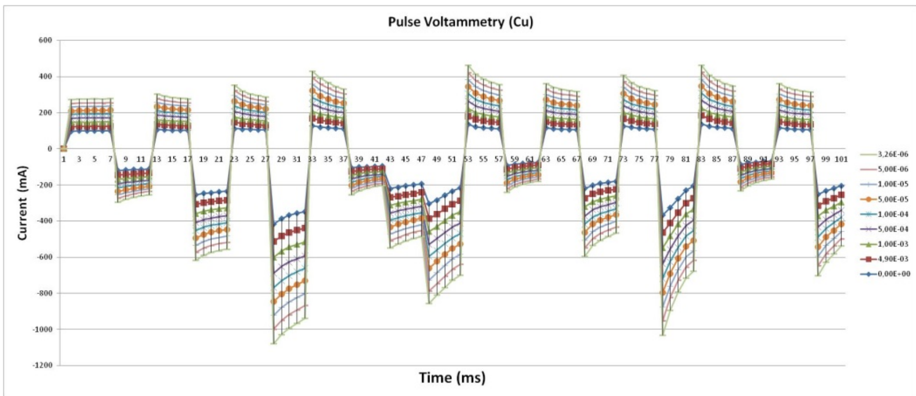


Fig. 3. The response of the Cu electrode for each of the concentrations.

A saturated calomel electrode was used as a reference electrode. Before each measurement the sample was bubbled with argon for 5 minutes. The measurement process was carried out at a temperature of 25ºC.

2.5. Data Preprocessing

In pulse voltammetry the measured current is related with the nature and concentration of the species present in the solution (Bard & Faulkner, 2001). The electronic tongue system collects the samples corresponding to the temporal evolution of the current circulating through the working electrodes. Neural networks were then used to establish a correlation between the collected data and properties the corresponding sample. For each electrode the electronic tongue system collected 1000 data points of the current signal. Considering that in most of the experiments 8 electrodes were used, neural networks tools have to deal with a large amount of data. In order to reduce the number of data to be processed, a compression algorithm was developed. This algorithm applied a 4th order polynomial approximation to the current samples for each pulse resulting in the final measurement of the corresponding area. The effect of the applied algorithm was not only the compression of the data but also the filtering of the current signals. Finally for each electrode 20 data (areas) were obtained for each experiment (Fig. 4). The input data were 20 values of pulse areas (considering each area to be the only input). Thus, with 8 electrodes and 20 pulses applied to each electrode, 160 input data were obtained. With 15 measures for each of 3 cycles (45 measures) for each of the 8 concentrations, a total of 360 measures (with 160 input data each measure) were obtained.

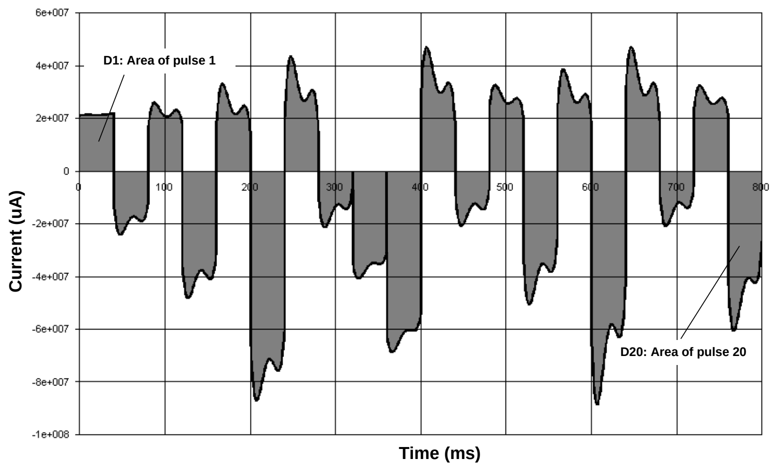


Fig. 4. Set of data for Cu electrode (D1 to D20 corresponding to the area of the each one of the pulses obtained from the 4^{th} order polynomial approximation).

In order to validate this data compression algorithm a Principal Component Analysis (PCA) was carried out. A total of 24 samples (3 measures for each concentration) were selected for this study. PCA results are shown in Fig. 5 and Fig.6. For the first PCA plot (Fig. 3) raw data (i.e. the values corresponding to the temporal evolution of the current signal) were used while the second PCA plot (Fig. 6) was generated using the coefficients of the 4th order polynomial approximation to the current samples for each pulse.

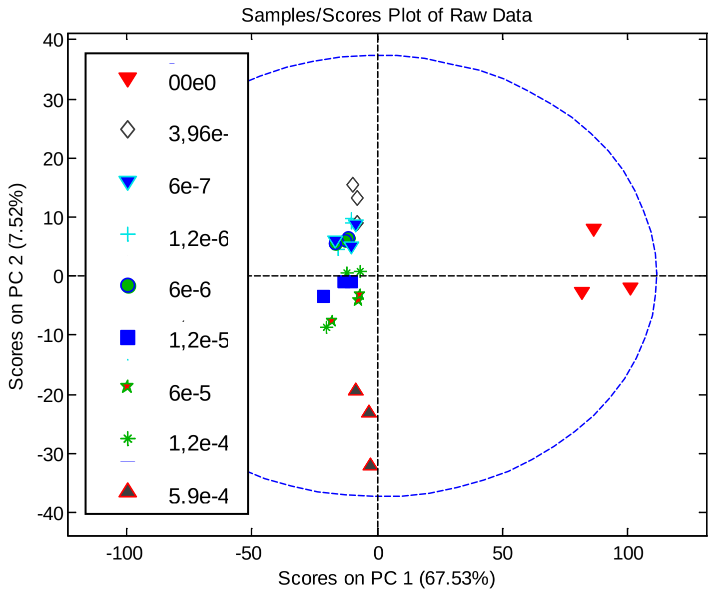


Fig. 5. PCA plot from raw data.

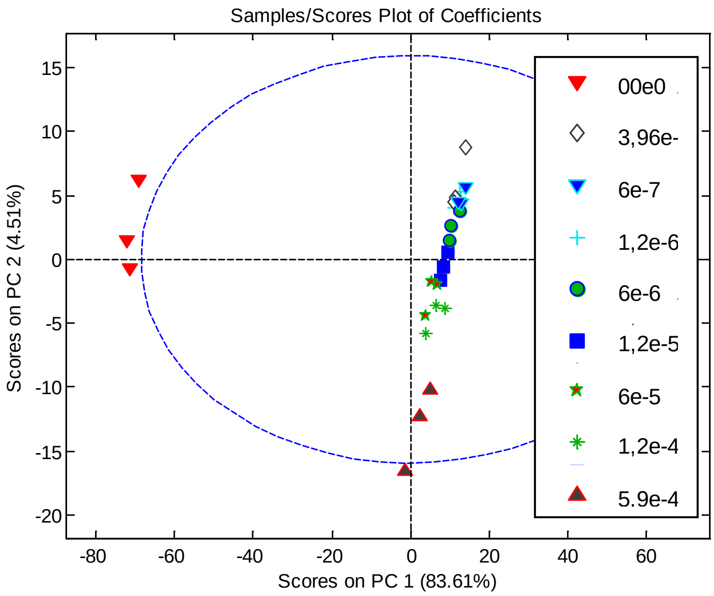


Fig. 6. PCA plot from the coefficients of the 4th order polynomial approximation to the current samples for each pulse.

Both PCA plots present a different distribution of the samples, but their relative position in both graphs is very similar. In both cases the samples corresponding to the background electrolyte are clearly separated from the TNT samples. Besides in both plots TNT samples are organized in the same way: lower TNT concentration solutions are located in the upper area while higher concentration solutions are in lower area. We can then conclude that the discrimination capabilities of this technique are maintained when using the data compression algorithm.

2.6. Neural Networks

Neural networks are constituted of simple components operating in parallel simulating a biological nervous system. As in nature, the connections between components largely determine the network function and the neural network can be trained to perform a particular function by adjusting the values of the connections between the elements. Neural networks have been used to perform complex functions in various fields, including pattern recognition, identification or classification. Data matrices generated by electronic tongue systems have generally a considerable size and neural networks tools are suitable to process them. Two types of neural networks, very commonly used, are supervised and unsupervised neural networks. There are several types of both neural networks but among them, SOM (unsupervised) and MLFN (supervised) have been used in this work.

MLFN has proved to be a very useful tool for pattern recognition and classification problems and has been used to investigate problems that cannot be easily solved by traditional methods in several fields. This network is particularly powerful for pattern classification and function approximation. An advantage of MLFN is that once a network is well trained, it can retain excellent performance even if degraded, noisy, or missing data are used (Yang & Griffiths, 1999). An elementary MLFN with R inputs is shown in Fig. 7. Each input is weighted with an appropriate W. The sum of the weighted inputs and the bias forms the input to the transfer function f. Neurons can use any differentiable transfer function f to generate their output.

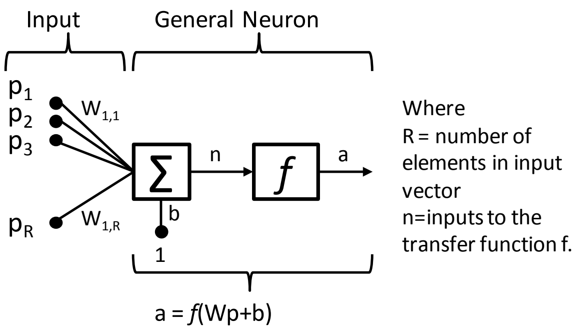


Fig. 7. Multilayer Neural Network Architecture.

The SOM, also known as the Kohonen Map, is an unsupervised neural model of widespread use in areas such as pattern recognition. The SOM is a neural network model that projects a high dimensional input space usually onto a one or two dimensional output space. Because of its typical two-dimensional shape, it is also easy to visualize. This architecture (Fig.8) is similar to that of a competitive network[10], except no bias is used here. Each neuron in the output layer is a cell containing a template against which input patterns are matched. All cells are presented with the same input pattern in parallel and compute the distance between their template and the input in parallel. Also all cells compete so that only the node with the closest match between the input and its template produces an active output.

[10] Competitive networks are based on algorithms of unsupervised learning, in which the output neurons of a neural network compete among themselves to become active.

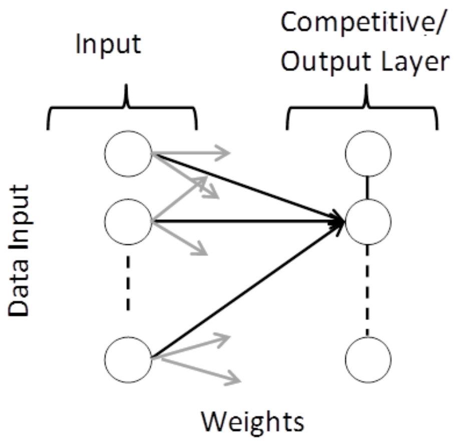


Fig. 8. Self-Organizing Map Architecture.

3. Results and discussions

3.1. Classification

The electronic tongue, using both non-noble and noble metals, was applied to a total of 8 concentrations of TNT employing the pulse sequences shown in Fig. 2. Table I shows the relation between the concentrations and the name of the assigned class; see also experimental section for details. As a result of the application of the set of pulses the corresponding pulse-signal diagrams were obtained. This corresponded to a total of 1000 data points that were reduced using a compression algorithm that allowed calculating the corresponding area for each current-time curve related with the pulses. Using this approach, a total of 20 areas were calculated for each electrode and each sample (see experimental section for details). The solutions of TNT were prepared in acetonitrile:water 1:1 v/v mixtures containing potassium nitrate 0.01 M as supporting electrolyte. Both the SOM and MLFN neural networks were applied to the data obtained from the electronic tongue.

Class	1	2	3	4	5	6	7	8
Concentration [M]	**$3,9{\cdot}10^{-7}$**	**$6{\cdot}10^{-7}$**	**$1,2{\cdot}10^{-6}$**	**$6{\cdot}10^{-6}$**	**$1,2{\cdot}10^{-5}$**	**$6{\cdot}10^{-5}$**	**$1,2{\cdot}10^{-4}$**	**$5,9{\cdot}10^{-4}$**

Table I. Concentrations named as classes.

Study with SOM

The program SOMmine5 from Viscovery Software GmbH was employed to carry out the study using SOM. The number of nodes used in all the studied cases was 1000. The program calculates a neighbourhood of at least 50 nodes with a linear neighbourhood weight function. As output the program divides a map into regions, called clusters.

The SOM neural network allows carrying out a relatively simple study of the contribution that the different pulses have on the response of a selected electrode and the weight that a certain electrode has in the overall response obtained in the electronic tongue. In relation to the first issue it was found that for most electrodes the pulses corresponding to a potential of 0V (i.e. P1, P3, P5, P7, P11, P15, P17 and P19) gave practically no information. This poor contribution of the 0V pulses means that the response observed for these pulses does not depend significantly on the previous pulse applied to the electrode. In fact for most of the electrodes it was observed that a worse classification of the TNT concentration was obtained considering all the pulses that when some selected pulsed were applied. These studies also allow concluding that the set of pulses could be reduced. For a more detailed study it was found that pulses P1, P2, P4, P6 and P20 have a significant contribution for all the electrodes. Although some of the remaining pulses also contributed to the classification it was found that similar accurate classifications were reached when only using pulses P1, P2, P4, P6 and P20 or when those are combined with others. As a conclusion it was obvious from the study that a significant reduction in the train of pulses can be carried out.

Once the set of pulses was selected, a study of the contribution of different electrodes to the response of the electronic tongue was also carried out. In a first study nobles and non- nobles have been studied separately and Fig. 9 shows the SOM obtained (only pulses P1, P2, P4, P6 and P20 were used). It can be observed, a correct classification in both cases, due to the position with a distribution from above to below and from left to right in concentration. The concentrations 3 ($1.2x10^{-6}$M) and 7 ($1.2x10^{-4}$M) are the worst classified.

From these studies it was established that a better response in terms of a correct classification of the TNT concentration was observed when using only noble metal electrodes and the pulses P1, P2, P4, P6 and P20.

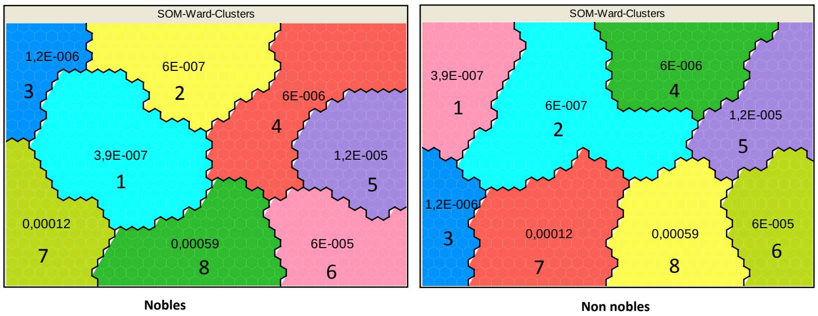


Fig. 9. Comparison between SOM for nobles and not nobles.

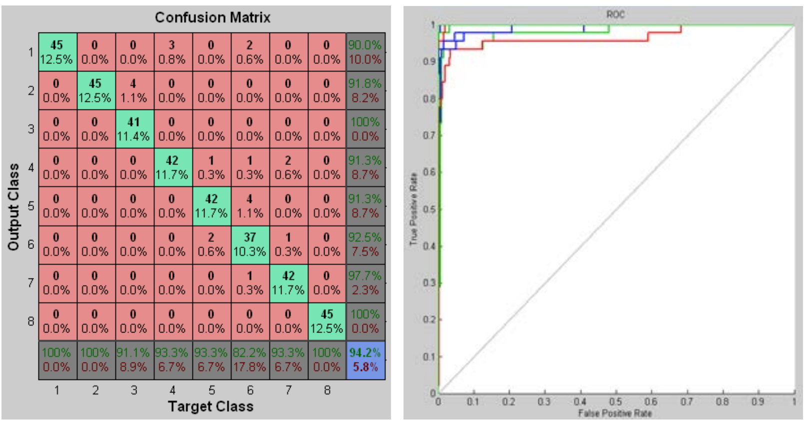
Study with MLFN

A study of the classification of the TNT's samples using a MLFN was also carried out. The network had 160 nodes in the input layer (8 concentrations x 5 pulses x 4 electrodes), and the exit layer was formed by 8 neurons corresponding to the 8 concentrations used. Studies with different numbers of hidden neurons were done, obtaining an optimum result with 8 secret neurons. The study was performed using the toolbox MATLAB's NPRTOOL, the standard network that was used for pattern recognition was a two-layer feedforward network, with sigmoid transfer functions in both the hidden layer and the output layer.

A network has been built up using only noble metals and with only the most significant pulses (i.e. P1, P2, P4, P6 and P20). Using these parameters, Table II shows the value of Mean Squared Error (MSE) and the percentage of error (%E) of the study. Whereas Fig. 10 shows the Confusion matrix (Plot Confusion) and the Receiver Operating Characteristic (Plot ROC), where it is observed that all the samples are classified between 82.2 % and 100 %. In general the recognition rate was 94.2 %.

	Samples	MSE	%E
Training	**252**	**$5.2E^{-3}$**	**1,6**
Validation	**54**	**$4.03E^{-3}$**	**14.8**
Testing	**54**	**$2.87E^{-3}$**	**16,7**

Table II. MSE y %E for training, validation and testing of all metal nobles samples.



a) PlotConfusion **b) PlotRoc**

Fig. 10. Plot Confusion and Plot Roc of the concentrations with noble metal.

From the studies with neuronal networks SOM and MFLN it has been deduced the conclusion that it is not necessary to carry out the measure with all the types of electrodes and pulses. By using only five of the pulses and together with the noble metal electrodes a satisfactory measure is obtained, and therefore the circuit and the electrode could be simplified in future applications.

3.2. Fitting

A MLFN has been used for determining the relation between the electrodes signal and the concentration of TNT. The used program has been the NeuralTools from Palisade Corporation, carrying out the comparative for different number of neurons in the hidden layer. The network has 160 nodes in the input layer and the exit layer is formed by 1 neuron corresponding to the estimation of concentration. Studies with different number of secret neurons have been done and the ideal case was for 8 neurons in the hidden layer. The standard network that was used for function fitting was a two-layer feedforward network, with a sigmoid transfer function in the hidden layer and a linear transfer function in the output layer.

The study was done with only noble metals and considering only the relevant P1, P2, P4, P6 and P20 pulses. In this case the optimal number of hidden neurons calculated by the program was 9 and the number of input nodes was 24 (3 repetitions x 8 concentrations). Training and validation have been carried out using 70 % (252) and 30 % (108) of the samples respectively. Samples for training and validation were chosen randomly. For the 252 training samples a R^2 of 0.996 was obtained (see Fig. 11) with a MSE of $1.1362.10^{-5}$. In the case of the 108 validation samples a R^2 of 0.974 was found with a MSE of $3.0069.10^{-5}$.

With that study, the weights and biases matrices of the MLFN were obtained and a similar MFLN was implemented in a microcontroller. In order to test the implementation of this MLFN in a microcontroller, a new array of samples were measured. In this case a R^2 of 0.993 was obtained (see Fig. 12) which was a value quite similar to that obtained using NeuralTools in a PC.

Both Fig. 11 and 12 show a quite large spread in the actual vs predicted data especially at low concentration, whereas the prediction is much more accurate when the concentration increases. Data dispersion at low concentrations is most likely related with the limit at which the electronic tongue was able to detect TNT.

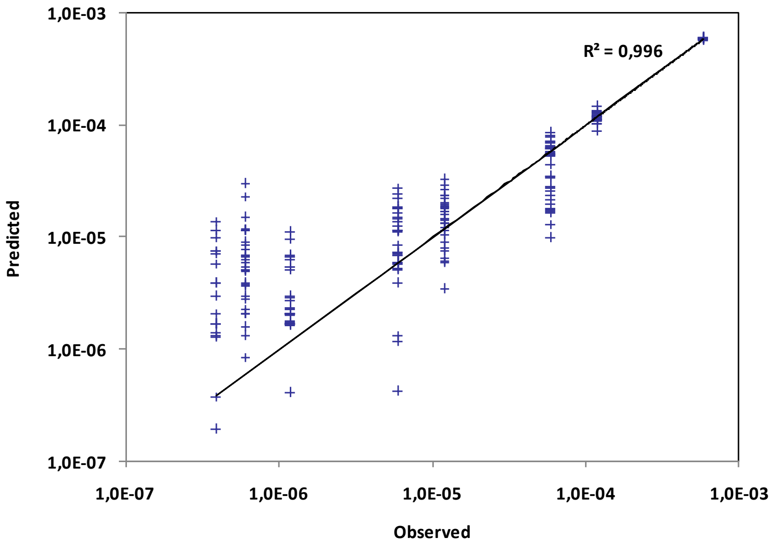


Fig. 11. Predicted vs Observed graph of concentrations using noble metals electrodes and pulses P1, P2, P4, P6 and P20 (training set).

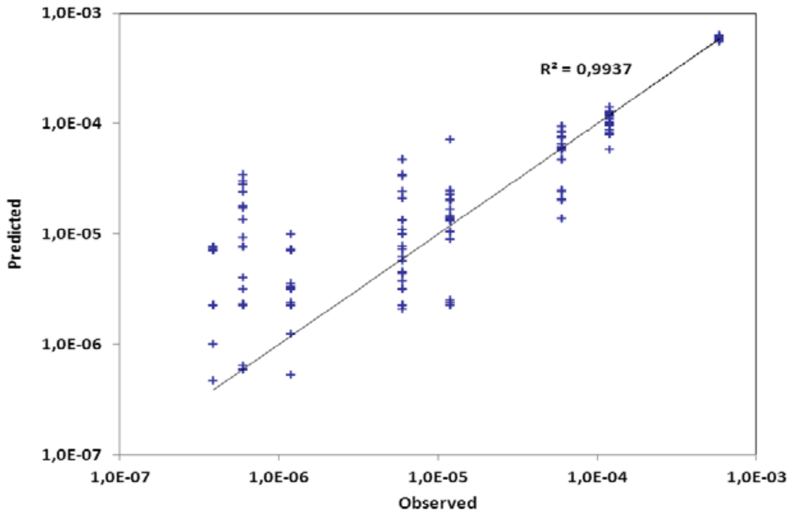


Fig. 12. Predicted vs Observed graph of concentrations with data gathered by microcontroller.

4. Conclusion

A voltammetric electronic tongue that used simple metallic electrodes has been designed for the detection and discrimination of different concentrations of TNT in acetonitrile:water 1:1 v/v mixtures. The tongue was built with noble (iridium, rhodium, platinum and gold) and non-noble electrodes (silver, copper, cobalt and nickel) and its response in the presence of TNT was measured. Both, the SOM and MLFN neural networks were applied to the data obtained from the electronic tongue in order to select pulses and electrodes which gave a suitable response and to obtain models for classification applications. From SOM analysis it was found a correct classification of the TNT concentration when using only noble metal electrodes and only 5 selected pulses. Similar good classifications were found when using MLFN. The algorithm of neural network MLFN was embedded in a microcontroller in order to obtain a smart portable system for discrimination of TNT. Although we are aware that some additional studies should be carried out in order to detect TNT at very low concentrations, we believe that our results suggest that it might be possible to develop simple and easy-to-use portable equipment for the detection of TNT in real samples. Moreover we are currently designing further studies directed to the detections and discrimination of other nitrated explosives.

Acknowledgements

We gratefully acknowledge financial support from the Spanish Government project MAT2009-14564-C04.

References

Alcañiz, M. y otros, Design of an electronic system and its application to electronic tongues using variable amplitude pulse voltammetry and impedance spectroscopy, Journal of Food Engineering,(2012).

Bard, A., Faulkner, L., Electrochemical Methods: Fundamentals and Applications. John Wiley & Sons, Inc. (2001).

Campos, I., Alcañiz, M., Aguado, D., Barat, R., Ferrer, J. Gil-Sánchez, Marrakchi, L., Martínez-Mañez, R., Soto, J., Vivancos, J., A voltammetric electronic tongue as tool for water quality monitoring in wastewater treatment plants, Water Research 46 (2012a) 2605-2614.

Campos, I., Alcañiz, M., Masot, R., Soto, J., Martínez-Máñez, R., Vivancos, J., Gil-Sánchez, L., A method of pulse array design for voltammetric electronic tongues, Sensors and Actuators B: Chemical, 161 (2012b) 556-563.

Campos, I. y otros, Accurate concentration determination of anions nitrate, nitrite and chloride in minced meat using a voltammetric electronic tongue, Sensors and Actuators B: Chemical 149 (2010) 71-78.

Cosio, M. y otros, Evaluation of different storage conditions of extra virgin olive oils with an innovative recognition tool built by means of electronic nose and electronic tongue, Food Chemistry, 101 (2007) 485–491.

Forzani, E. y otros, A Hybrid Electrochemical-Colorimetric Sensing Platform for Detection of Explosives, J. Am. Chem. Soc., 131 (2009) 1390–1391.

García-Breijo, E. y otros, An electronic tongue for monitoring drinking waters using a fuzzy ARTMAP neural network implemented on a microcontroller, IEEE International Symposium on Industrial Electronics (ISIE), (2011a) 1270-1275.

García-Breijo, E. y otros, A comparison study of pattern recognition algorithms implemented on a microcontroller for use in an electronic tongue for monitoring drinking waters, Sensors and Actuators A: Physical 172 (2011b) 570-582.

Germain, M., Knapp, M.,Optical explosives detection: from color changes to fluorescence turn-on, Chem. Soc. Rev. 38 (2009) 2543-2555.

Gil-Sánchez, L. y otros, A novel humid electronic nose combined with an electronic tongue for assessing deterioration of wine, Sensors and Actuators A: Physical 171 (2011), 152–158.

Gil-Sánchez, L. y otros, Classification of honeys of different floral origins by artificial neural networks, IEEE Sensors, (2011) 1780-1783.

Gil-Sánchez, L. y otros, Fish freshness analysis using metallic potentiometric electrodes Sensors and Actuators B: Chemical 131 (2008) 362–370.

Hakansson, K. y otros, Low-mass ions observed in plasma desorption mass spectrometry of high explosives, Mass Spectrom. 35(2000) 337-346.

Hilmi, A. y otros, Determination of explosives in soil and ground water by liquid chromatography–amperometric detection, Journal of Chromatography A 844 (1999) 97–110.

Ibáñez, J. y otros, Artificial neural network onto eight bit microcontroller for Secchi depth calculation, Sensors and Actuators B: Chemical 156 (2011) 132-139.

Irving, N., Lewis, R., Hawley's Condensed Chemical Dictionary, 11th ed., Van Nostrand, New York, 1987.

Ivarsson, P. y otros, Discrimination of tea by means of a voltammetric electronic tongue and different applied waveforms, Sens Actuators B Chem 76 (2001) 449-454.

Jenkins, T. y otros, Characterization of Explosives Contamination at Military Firing Ranges, TechnicalReport ERDC-TR-01-5, 2001.

Labrador, R. y otros, Prediction of NaCl, nitrate and nitrite contents in minced meat by using a voltammetric electronic tongue and an impedimetric sensor, Food Chemistry 122 (2010) 864-870.

Lvova, L. y otros, Electronic tongue based on an array of metallic potentiometric sensors, Talanta 70 (2006) 833–839.

Martina, V. y otros, Development of an electronic tongue based on a PEDOT-modified voltammetric sensor, Anal Bioanal Chem 387 (2007) 2101–2110.

Martínez-Máñez, R. y otros, An “electronic tongue” design for the qualitative analysis of natural waters, Sensors and Actuators B 104 (2005) 302–307.

Moore, D., Instrumentation for trace detection of high explosives, Rev. Sci. Instrum. 75 (2004) 2499-2513.

Rieger, P. y otros, Biodegra dation of Nitroaromatic Compounds, Plenum Press, New York, (1995) 1–18.

Salinas, Y., y otros, Chem. Optical chemosensors and reagents to detect explosives, Soc. Rev. 41 (2012) 1261-1296.

Sanoita, J. de, y otros , Electrochemical diamond sensors for TNT detection in water, Electrochimica Acta 54 (2009) 5688–5693.

Smith, R. y otros, A review of biosensors and biologically-inspired systems for explosives detection, Analyst 133 (2008) 571-584.

Toko, K., Taste sensor, Sensors and Actuators B 64 (2000)205–215.

Vlasov, Y. y otros, «Electronic tongue» — new analytical tool for liquid analysis on the basis of non-specific sensors and methods of pattern recognition, Sensors and Actuators B 65 (2000) 235–236.

Walker, J., Kaplan, D., Biological degradation of explosives and chemical agents, Biodegradation 3 (1992) 369-385.

Wallis, E. y otros,. Instrument response measurements of ion mobility spectrometers in situ: maintaining optimal system performance of fielded systems, Proc. SPIE–Int. Soc.Opt. Eng. 5795 (2005) 54-64.

Winquist, F. y otros, An electronic tongue in the dairy industry, Sensors and Actuators B 111–112 (2005) 299–304.

Won, W. y otros, Toxicity and mutagenicity of 2,4,-6-trinitrotoluene and its microbial metabolites, Appl. Env. Microbiol. 31 (1976) 576-580.

Yang, H., Griffiths, R., Application of Multilayer Feed-Forward Neural Networks to Automated Compound Identification in Low-Resolution Open-Path FT-IR Spectrometry. Anal. Chem. 71 (1999) 751–761.

Chapter 2: An electronic nose for the detection of Sarin, Soman and Tabun mimics and interfering agents

Olguín Pinatti, Cristian Ariel; Laguarda Miró, Nicolás; Pascual, Lluís; García Breijo, Eduardo; Martínez Mañez, Ramón; Soto Camino, Juan. *An electronic nose for the detection of Sarin, Soman and Tabun mimics and interfering agents*. Sensors and Actuators B: Chemical. 202, pp. 31 - 37. 2014.

https://doi.org/10.1016/j.snb.2014.05.060

Abstract

An electronic nose system (E-nose) with metal oxide semiconductor sensors (MOS) has been designed to discriminate and quantify different chemical warfare agents (CWA) mimics. The E-nose consists of an array of commercial MOS sensors for different gases, two sensors for temperature sensing, a sample handling system, a data acquisition system and a laptop with the data acquisition system control.

With this device, discrimination studies have been carried out to detect specific CWA simulants (diethyl chlorophosphonate (DCP), diethyl cyanophosphate (DCNP), diisipropyl fluoride (DFP)), their derivatives (diethyl 1-phenylethyl phosphonate (OP-1), diethyl (2- cyanoethyl)phosphonate (OP-2), dimethyl methyl phosphonate (OP-3) and diethyl (2-oxopropyl)phosphonate (OP-4)) and some potential interfering substances (sulfuric acid, ammonia, ethanol and acetone). Principal Componets Analyses (PCA) show that it is possible to discriminate the studied organophosphorous CWA mimics (DCP, DCNP and DFP) from the other studied derivatives and potential interfering agents. In addition, DCNP quantification studies have been done by using Partial Least Squares (PLS) and a mathematical model has been obtained to predict DCNP concentrations in air. In this model, the coefficient of determination (R^2) is 0.9567, the RMSEP[11] is 30 and the Limit of Detection (LOD) is 5 ppm, so the model is considered statistically valid. These results suggest that this E-nose system is capable to discriminate and quantify CWA mimics and it would be a feasible system to be used in a real scenario.

1. Introduction

The term “Electronic Nose” was first used in 1988 by Gardner and Bartlett, who defined it as “an instrument which comprises an array of electronic chemical sensors with partial specificity and appropriate pattern recognition system, capable of recognizing simple or complex odours” (Gardner & Bartlett, 1994), (Gardner & Bartlett, 1999). Due to the characteristic response pattern provided by the array of unspecific sensors, this Electronic Nose System is capable to give information about the surrounding environment. So, it is possible to identify and quantify certain gaseous compounds using an appropriate data analysis technique.

The first electronic nose model was provided by Persaud and Dodd. Their system was based on three different metal oxide sensors and it was able to identify several gases by using the measured steady-state signals of these three sensors (Persaud & Dodd, 1982). Nowadays, Electronic Noses have evolved considerably and there are several technologies that can be applied in these devices such as acoustic wave sensors (SAW, BAW) (Barié, et al. 2006) (Yadava & Chaudhary, 2006), metal oxide semiconductor field effect transistors (MOSFETs) (Kalman, et al. 2000), conducting polymers (CP) (Guadarrama, et al. 2001), optical sensors (Di Natale, et al. 2000), gas chromatography (Olssona, et al. 2000), ion mobility spectroscopy(Vautz, et al. 2006), infrared

[11] Root mean square error of prediction

spectroscopy (Cozzolino, et al. 2006), a review of different technologies (Rock, et al. 2008).

Due to the Biological and Toxic Weapons Conventions on the prohibition of the development, production and stockpiling of bacteriological and toxin weapons and on their destruction signed at London, Moscow and Washington on 10th April 1972 and revised in 1993, chemical warfare agents shouldn´t be in use. But nowadays, the chemical warfare is still a real problem. The threat of exposure to chemical warfare agents (CWAs) has been considered a military issue. However, several recent events have demonstrated that civilians may also be exposed to these agents. Chemical warfare agents are defined as "chemical substances, gaseous, liquid or solid, which might injure humans, animals and plants, and they are known as nerve agents" (Chemical Warfare Agents, 1997), (Sugendran, et al. 1998). CWA are extremely toxic and have severe effects on human and animal health, either as a gas or liquid and poisoning may occur by gas inhalation, contact with skin or polluted liquid/food consumption (Campos, et al. 2010a).

Our investigation reported herein focuses on G-type organophossphorous nerve agents as Sarin (GB), Soman (GD) and Tabun (GA) whose effects in the organism are due to their ability to inhibit the action of acetylcholinesterase (S. Somani, 1992). Given the high toxicity of nerve gases, organophossphorous model compounds, such as diethyl chlorophosphonate (DCP), diethyl cyanophosphate (DCNP), diisipropyl fluoride (DFP), which a similar structure and reactivity as a nerve agents but displays less toxicity, are generally used in studies in the laboratory (Royo, et al. 2011), (Costero, et al. 2008). The close reactivity is related with the presence of "similar" leaving groups (i.e. F, Cl and CN) in DFP, DCP and DCNP to those found in Sarin, Soman and Tabun (i.e. F and CN). Moreover DFP, DCP and DCNP are less toxic and in fact are not viable nerve agents because are readily hydrolysed (poorly persistent) when compared with Sarin, Soman and Tabun.

Nowadays, there are several equipment and analytical methods that have been approved by the Chemical Weapons Convention in 1993 for CWA in-situ detection and quantification (Somani, et al. 1992), (Hill & Martin, 2002). Air monitoring systems for nerve agents are mainly based on Ion Mobility Spectroscopy (IMS) or gas chromatography coupled with mass spectrometry (GC/MS). However, these systems commonly present several difficulties, for instance: Analyses have to be carried out in a laboratory, qualified personnel is required to operate these devices, instrumental and chemicals are complex, the equipment is expensive and the analyses are time consuming. Due to these disadvantages, some alternative methods have been rising such as Surface Acoustic Wave (SAW) devices (Nimal, et al. 2009), electrochemistry (Hammond, et al. 2006), spectrophotometric sensors (Jenkins, et al. 1999), immunochemical sensors (Lenz, et al. 1997), capillary electrophoresis (Kientz, et al. 1997), enzymatic assays (Wheelis, 2004), chromo-fluorogenic probes (Mohr, 2006), (Climent, et al. 2010), chemiresistive sensors (Wang, et al. 2008), and liquid crystals

(VanTreeck, et al. 2011). It would be necessary to include the electronic nose with MOS sensors in dynamic zone, which is studied in this thesis.

2. Theory

2.1. Principle of operation

Despite MOS sensors have problems with humidity (as water is an interfering compound for this kind of sensors) we have used them in our system because MOS are robust in terms of aging, common and easy to buy everywhere. Therefore, in order to compensate the potential interference of water, an humidity sensor has been included in the system. On the other hand, as temperature is another potential interfering factor, two temperature sensors have also been included in the system. A detailed explanation of the MOS sensors operation is provided next (Metal Oxide Semiconductor, AppliedSensor).

2.1.1. Chemical Principle

MOS sensors use metal oxide-based sensing thick films deposited onto a silica substrate. The substrate contains electrodes that measure the resistance of the sensing layer and a heater to desorb any volatile compound remaining in the sensing layer by increasing the temperature of the sensor. The sensing layer is a porous thick film made of polycrystalline SnO_2. So the gases to be measured are adsorbed in this surface.

In a clean atmosphere, both oxygen and water vapor-related species are adsorbed on the surface of the SnO_2 grains but, when other pollutant gasses are present, a series of reactions take place in the sensor’s surface. In case of having reducing gases such as CO or H_2, a reaction takes place with the pre-adsorbed oxygen and water vapor-related species which decreases the resistance of the sensor. Instead, when oxidizing gases such as NO_2 and O_3 are present, the resistance increases. The magnitude of the changes depends on the microstructure and the composition/doping of the base material, on the morphology and the geometrical characteristics of the sensing layer and substrate, as well as on the working temperature at which the sensing takes places (Metal Oxide Semiconductor, AppliedSensor).

In order to explain the resistance change in the sensor when measuring a CWA sample, the reaction mechanisms for DMMP sample are shown (Brunol, et al. 2006), (these mechanisms can be extrapolated to other components and they help the understanding of how the MOS sensors work). DMMP is quite thermally stable at temperatures between 300ºC and 600ºC Its degradation generates two compounds: carbon dioxide and methylphosphonic acid as shown in Fig. 1.

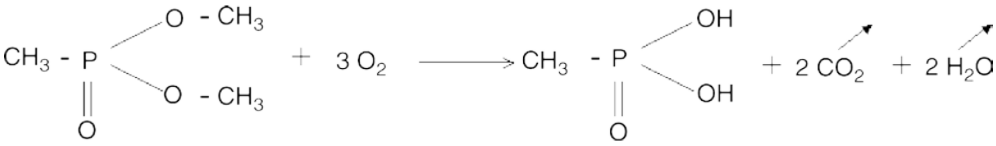


Fig. 1. Reaction mechanism for the thermal degradation of DMMP at 300-600ºC.

The decrease in resistance obtained when DMMP is detected, takes place in two stages. First, DMMP is adsorbed onto the SnO_2 surface, allowing it to react with an oxygen species (O^-). This reaction leads to the formation of methylphosphonic acid, which remains adsorbed onto the SnO_2, CO_2 which does not react with the sensor, and H_2O. At the same time, electrons can be released to the conduction band leading to a decrease in the SnO_2 resistance as shown in Fig. 2.

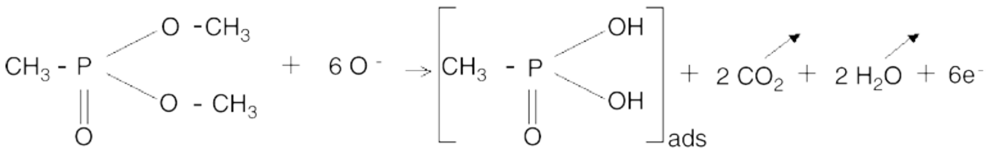


Fig. 2. Reaction mechanism for the reaction of DMMP and SnO_2.

Finally, the last stage of this mechanism leads to the formation of an ionic phosphorous compound, which is adsorbed onto the SnO_2 (Fig. 3).

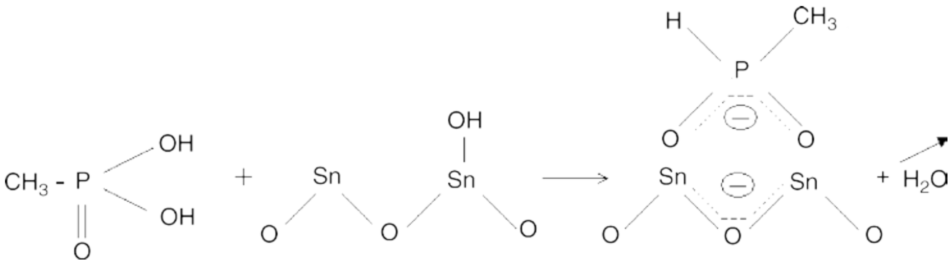


Fig. 3. Reaction mechanism for the reaction of methylphosphonic acid and SnO_2.

2.1.2. *Transducer Principle*

Composition changes in the environment will determine changes in resistance on the sensing layers. The relationship between sensor resistance and the concentration of the target gas (the gas or gases to which the sensor is designed) usually follows a power law describes below:

$$R \approx K \times c^{\pm n}$$

Where ‘c’ is the concentration of the target gas, ‘K’ is a measurement constant and ‘n’ is a value in the range [0.3-0.8]. The positive sign is used for oxidizing gases and the negative sign for the reducing ones.

3. Materials and methods

3.1. Chemicals

All the chemical compounds have been purchased from Sigma-Aldrich company. Discrimination studies have been carried out at their respective saturated vapour concentrations. In addition, a quantification study has been developed in the range [0-208] ppm for the DCNP as shown in Table I.

3.2. Experimental

In this paper, two types of experiments have been done: First, a study has been carried out to discriminate among the different mimic agent samples and some potential interfering substances. Secondly, a quantification study of one of the mimics has been done in order to be able to assess the concentration of this compound in a sample and obtain the Limit of Detection (LOD).

First of all, discrimination studies have been carried out by using the selected nerve agent simulants (diethyl chlorophosphonate (DCP), diethyl cyanophosphate (DCNP), diisipropyl fluoride (DFP)), and a set of four similar organophosphorous derivatives (diethyl 1-phenylethyl phosphonate (OP-1), diethyl (2- cyanoethyl)phosphonate (OP-2), dimethyl methyl phosphonate (OP-3), and diethyl (2-oxopropyl)phosphonate (OP-4)). On the other hand, a set of different potential interfering samples were studied: Sulfuric acid and ammonia samples were prepared and measured to analyze the system’s response when acid and basic vapors are present. The influence of ethanol and acetone was also studied using them as a reference of the system’s response to the presence of volatile solvents.

Product	Vapour pressure (mmHg,25°C)	Saturated vapour concentration (ppm v/v)
Sarin[a]	2.90	3816
Soman[a]	0.40	526
Tabun[a]	0.070	92
DFP[a]	0.58*	762
DCP[a]	0.10	132
DCNP[a]	0.16	208
OP-1[b]	<0.01	<5

OP-2[c]	0.03	38
OP-3	<0.1*	<130
OP-4[a]	0.01	13
H_2SO_4 [a]	<0.01	<5
NH_3 [a]	6.65*	8743
EtOH [a]	44.60*	58682
C_3H_6O[a]	185*	243420

Table I. Table of vapour pressure for all compounds.

*Data at 20 ºC
[a]Experimental value
[b]Predicted value (EPI Suite modified Grain method)
[c]Predicted value (ACD/Labs)

The samples shown in Table I were measured under real conditions in order to study the hardness of the system. The Evaporation chamber was the only part of the device that was under control. In this way, it is possible to ensure that changes in the response signal are only due to changes in the measured sample. The gas to be measured has been mixed with normal air and not with a pattern gas. Next, measurements of the samples were carried out in different seasons (winter, spring and summer) throughout the year in order to develop assays in a wide range of ambiance conditions. Every gas sample has been measured three times one for season. The repetitions were made at random checking that the samples were not measured consecutively.

Quantification studies have been carried out using DCNP due to its higher response and selectivity observed in the discrimination studies previously conducted. A simple dosage system was used to introduce different known concentrations of DCNP into the system (Table II).

DCNP (500 µL) were deposited and evaporated in a 500 mL thermostated balloon at 25ºC and vacuum until gas saturation was reached. Then, a controlled volume was extracted with a syringe and injected into the measurement chamber. First, an equal volume of air must be extracted from the measurement chamber before the sample injection in order to avoid overpressure. The injected volumes used in this study are those shown in Table II.

Volume injected (mL)*	ppm(μL/m^3)
0	0.00
0.5	0.08
1	0.16
5	0.82
10	1.64
30	4.91
100	16.37
250	40.92
500	109.13
800	130.95
1100	180.06
1270**	207.89

Table II. Volumes of sample injected and concentration obtained in e-nose system.

*Volume in syringe from 500mL balloon
**Corresponds to gas saturation so we used an excess of liquid (100μL drop) and no dosage system was required

3.3. Equipment

The equipment has been designed, developed and manufactured by the Group of Electronic Development and Printed Sensors member of the Center of Molecular Recognition and Technological Development (IDM) at the Polytechnic University of Valencia (UPV) and it was previously used to detect maturation on fruit (García-Breijo, et al. 2013).

The equipment (named E-nose system) consists of an array of commercial MOS sensors (FIGARO Engineering Inc., Japan) for different gases (hydrogen, carbon monoxide, butane, methane, etc.), two LM35DZ sensors for temperature sensing, a sample handling system, a data acquisition system and a laptop with the data acquisition system control. The complete E-Nose System is shown in Fig. 4.

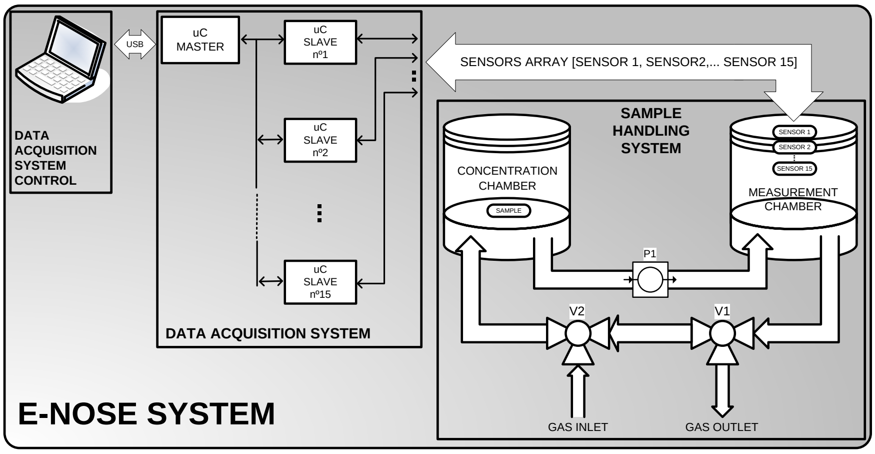


Fig. 4. A scheme of the implemented E-Nose System.

3.3.1. Sensors array

This E-nose is capable to handle an array of 15 sensors. The main advantage of the system is that all the sensors can be configured independently, so their configuration can be totally different. The idea is to use the nonspecific behaviour of the sensor to recognize patterns. Therefore, the system has been designed to be flexible and use the sensors as a complex array and not exclusively to detect their corresponding specific gas.

Concerning the used sensors, Table III shows the list of the specific sensors in the E-Nose.

Sensor Slot	Sensor Code	Specificity from Figaro Inc, Japan
S2	TGS 2201	Gasoline and Diesel Exhaust Gas
S3	TGS 823	Organic Solvent Vapours
S4	TGS 2106	Diesel Engine Exhaust Gas
S5	LM 35	Temperature
S7	TGS 2611	Methane
S8	TGS 3870	Methane and Carbon Monoxide
S9	TGS 2602	Air Contaminants
S10	TGS 826	Ammonia
S11	TGS 203	Carbon Monoxide
S12	TGS 2620	Volatile Organic Compound
S13	TGS 2610	LP gas (propane and butane)
S14	TGS 2180	Water Vapour Detection
S18	TGS 2442	Carbon Monoxide
S19	TGS 2612	Methane and LP Gas
S20	LM 35	Temperature

Table III. MOS Sensors array configuration.

3.3.2. Sample handling system

The sample handling system includes two chambers: the concentration chamber (where samples are placed) and the measurement chamber (were the sensors array is placed). The concentration chamber has a cylindrical shape (12 cm i.d x 16 cm h.) and is connected to the measurement chamber (12 cm i.d x 14 cm h.) through a BTC diaphragm pump (Brushless Motor model H054B-11 from Hargraves) especially designed for gas flow and it has a diaphragm that is compatible with this type of dangerous gasses. The sample handling system also includes two stopcocks to control the gas flow. In this way, the sample handling system is flexible in configuration.

When a measurement has finished, the heating process ensures desorption of all the remaining molecules in the sensors. Vacuum is also applied to the system in order to assure the removal of every volatile compound from the sample handling system. Therefore, the sensors become ready to be used again.

3.3.3. Data acquisition system

The data acquisition system includes the control for each sensor and the measuring electronic system. It has a master–slave structure (see Fig. 4). All slave boards are controlled by the master board that gathers the data of the 15 slaves and send them to the PC. Each slave controls several parameters of the sensor such as the supply voltage

(V_C), heating voltage (V_H), load resistance (R_L) and polarization pulses. These parameters can be configured through the PC by using a proprietary software. The slave is based on a PIC18F2580 microcontroller, a 12-bits analogical digital converter (AD7237A) and a 10-bit digital potentiometer (MAX5481). The master is based on a PIC18F4550 microcontroller that controls the communication between the PC and the slaves; furthermore it controls the whole gas flow system.

3.3.4. Data acquisition system control

In order to handle the entire system, a software interface has been designed. This software let the user configure the sensors and control the parameters of the experiment. The parameters are sent to the master through a serial port. The master sends the configuration data to every slave by using an I2C bus. Next, the slave-microcontroller configures the DAC to supply V_C and V_H to the sensor. If pulses are required, the slave-microcontroller also configures these voltages temporarily. Then, the microcontroller modifies the value of R_L through the digital potentiometer by a SPI protocol. The implemented software has three main parts.

The first one is the data acquisition control application, in which we can control the parameters of every assay such as the time of the probe, the cleaning process, the diagnosis test, etc.

The second part of the implemented software is a display showing the result of the measuring.

The last part of the software is the sensors configuration application. It makes the system versatile and let the user configure all sensors separately and control the number of sensors involved in our system. In addition, this application let the user define all the operating point parameters: heater supply voltages, sensor supply voltages, heater heating/cooling times, sensor connection/disconnection times, measurement time, test establishment time, as well as the assignation of the sockets to the sensors. In fact, this important advantage let the user chose among different configurations. Moreover, it supplies information of the manufacturer about the nominal performance and security values of the different sensors.

4. Results and discussion

As a preliminary way to detect and discriminate CWA and interfering substances, a principal components analysis (PCA) has been done with the obtained electrochemical data of the studied samples. Next, an experiment to predict the concentration of DCNP was performed by using the partial least square technique (PLS). All statistical analyses were performed using the Solo (version 7.0.3, Eigenvector Research, Inc) software application.

4.1. PCA Classification

As PCA is an efficient approach to show a dataset in two dimensions, principal component 1 (PC1) and principal component 2 (PC2), with the maximum representativity, the responses of different organophosphorous CWA agent simulants were analysed by this linear unsupervised method. In addition, a set of potential environmental interfering such as solvents, acid and basic compounds were also analysed in order to determine the hardiness of our system in a non-ideal environment.

Fig. 5 shows a PCA analysis developed using data from all the measured samples. This PCA is an approach of how the system might work in real conditions. It can be seen that there is an effective discrimination among types of samples.

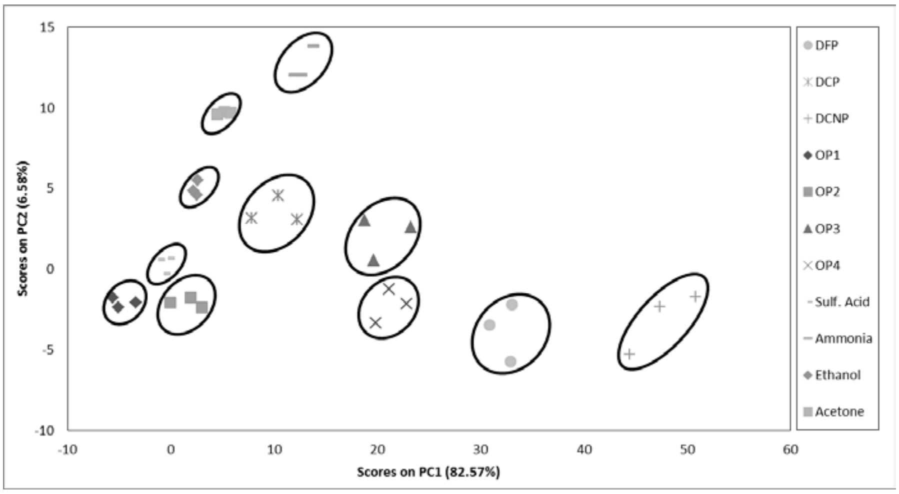


Fig. 5. PCA all samples.

Fig. 6 shows a second PCA model developed including only organophosphorous compounds in order to analyse the response of the system just with chemically similar samples. As shown, there is a clear discrimination among types of samples.

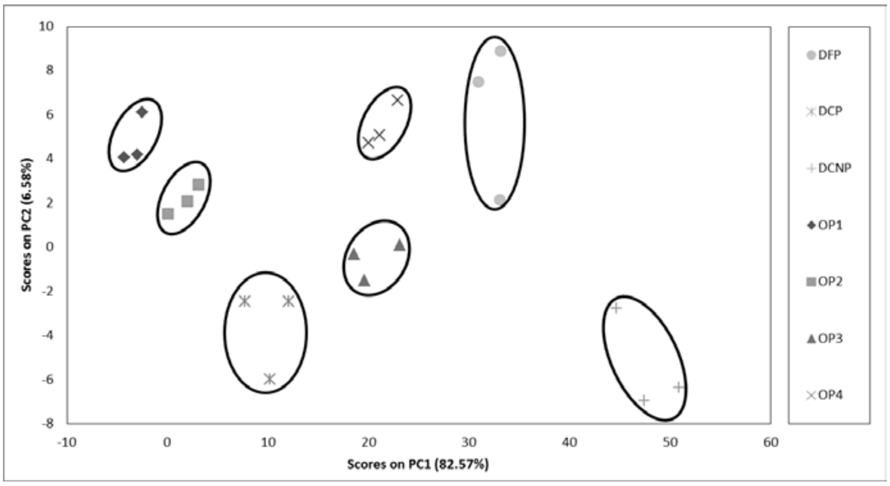


Fig. 6. PCA for the analyzed organophosphorous compounds.

According to the obtained results our system is able to discriminate well among DCP, DFP, DCNP, typical organophosphorous interfering agents with similar structure, and some potential environmental interfering agents. Principally, DCNP is the easiest discriminated compound as DCP and DFP are also easily detected but their discrimination is not as selective as DCNP's discrimination is. Another reason to study the DCNP has to do with the operability of the volumes necessary to reach the desired concentrations.

4.2. PLS Quantification

A quantification study using PLS was carried out (Laguarda-Miró, et al. 2012), (Martínez, et al. 2013), (Campos, et al. 2010b) in order to evaluate the performance of the system and determine the Limit of Detection for CWA simulants. According to the obtained results in previous classification studies, DCNP was selected as quantification analito due to its high response.

The data collected was divided into two subsets; the first one, includes two replicates, and was used to calibrate the model. The second one, includes one replicates to test it with independent data.

According to the cross-validation variance studies, 6 latent variables have been used to build the model. In order to create this model, 33 samples of DCNP were measured in the concentration range of 0-208 ppm and then analysed by PLS. The calibration of the model was performed using 22 samples so the remaining 11 samples were used to test the model. Fig. 7 shows the predicted values versus the real ones for DCNP. In this model, the coefficient of determination (R^2) is 0.9567 and the RMSEP is 30 so the model is considered statistically valid. In addition, the estimated limit of detection

(LOD) is 5 ppm. These values let us affirm that it is feasible to quantify warfare gas mimics by combining an electronic nose and this kind of mathematical models.

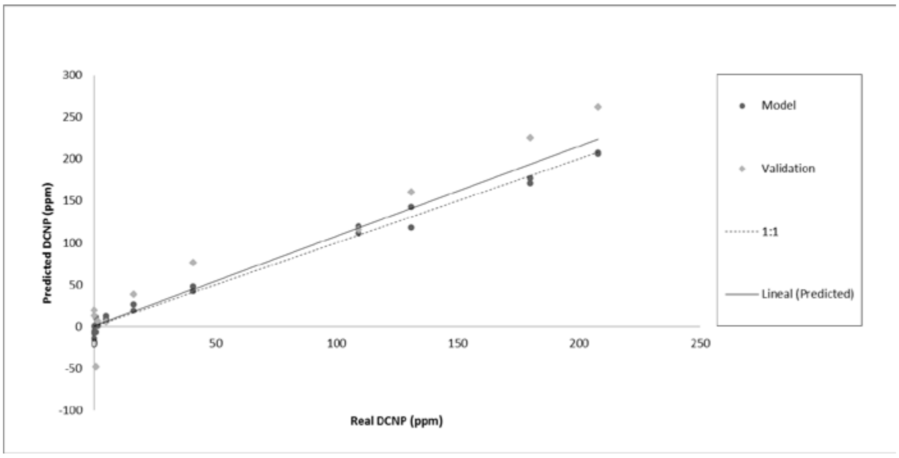


Fig. 7. PLS of DMMP samples.

5. Conclusion

A new method for nerve agents' mimics detection is introduced using a new device (E-Nose) developed by the Group of Electronic Development and Printed Sensors. This group is a member of the Center of Molecular Recognition and Technological Development (IDM) at the Polytechnic University of Valencia (UPV).

Classification studies by PCA analyses show that the E-Nose system is able to discriminate the mimics of the main G-type nerve agents (DCP, DFP and DCNP) from typical organophosphorous interfering agents and some environmental interfering compounds such as acids, bases and solvents. These assays reveal that DCNP is the compound that shows a higher response. So, it was selected to carry out quantification studies. These determinations were performed by using PLS analyses and they showed statistically valid models. For the best of the obtained models, the coefficient of determination is 0.9567, RMSEP is 30 and the Limit of Detection for DCNP is 5 ppm.

Finally, according to these preliminary obtained results, the introduced E-Nose seems to be a reliable system to detect and quantify CWA mimics in complex samples with some potential interfering substances. This system provides a selective and statistically valid response, in short measurement times; it is easy to use and cheap compared to other solutions. These results give rise to begin the development of specific equipment for early detection and easy to use of CWA's.

Acknowledgements

Financial support from the Spanish Government (MAT2009-14564-C04-02), the grant BES-2010-034031 from the FPI-MINECO Subprogram and the European FEDER funds (MAT2012-38429-C04-04) are gratefully acknowledged.

References

Barié, N. y otros, A novel electronic nose based on miniaturized SAW sensor arrays coupled with SPME enhanced headspace-analysis and its use for rapid determination of volatile organic compounds in food quality monitoring, Sensors and Actuators B 114 (2006) 482–488.

Brunol, E. y otros, Detection of dimethyl methylphosphonate (DMMP) by tin dioxide-based gas sensor: Response curve and understanding of the reactional mechanism, Sensors and Actuators B 120-1 (2006) 35-41.

Campos, I. y otros, Use of a Voltammetric Electronic Tongue for Detection and Classification of Nerve Agent Mimics, Electroanalysis 22-14 (2010a) 1643-1649.

Campos, I. y otros, Accurate concentration determination of anions nitrate, nitrite and chloride in minced meat using a voltammetric electronic tongue, Sensors and Actuators B 149 (2010b) 71–78.

Chemical Warfare Agents: An Overview of Chemicals defined as Chemical Weapons. Organization for the Prohibition of Chemical Weapons, April 29, 1997, Assessed 11/20/2001.

Climent, E. y otros, Chromogenic Detection of Nerve Agent Mimics by Mass Transport Control at the Surface of Bifunctionalized Silica Nanoparticles, Angew. Chem. Int. Ed. 49 (2010) 5945–5948.

Costero, A. y otros, Chromogenic detection of nerve agent mimics, Chem. Commun (2008) 6002-6004.

Cozzolino, D. y otros, Combining mass spectrometry based electronic nose, visible–near infrared spectroscopy and chemometrics to assess the sensory properties of Australian Riesling wines, Analytica Chimica Acta 563 (2006) 319–324.

Di Natale, C. y otros, Porphyrins-based opto-electronic nose for volatile compounds detection, Sensors and Actuators B 65 (2000) 220–226.

García-Breijo, E. y otros, Odour sampling system with modifiable parameters applied to fruit classification, Journal of Food Engineering 116-2 (2013) 277–285.

Gardner, J., Bartlett, P., A brief history of electronic noses, Sensors and Actuators B 18-19 (1994) 211-220.

Gardner, J., Bartlett, P., Electronic Noses. Principles and Applications, Oxford University, 1999.

Guadarrama, A. y otros, Electronic nose based on conducting polymers for the quality control of the olive oil aroma. Discrimination of quality, variety of olive and geographic origin, Analytica Chimica Acta 432 (2001) 283–292.

Hammond, M. y otros, A novel chemical detector using cermet sensors and pattern recognition methods for toxic industrial chemicals, Sensors and Actuators B 116 (2006) 135-144.

Hill, H., Martin, S., Conventional analytical methods for chemical warfare agents, Pure Appl. Chem 74-12 (2002) 2281-2291.

Jenkins, A. y otros, Murray, Polymer-based lanthanide luminescent sensor for detection of the hydrolysis product of the nerve agent Soman in water, Anal. Chem. 71 (1999) 373–378.

Kalman, E. y otros, Classification of complex gas mixtures from automotive leather using an electronic nose, Analytica Chimica Acta 403 (2000) 31–38.

Kientz, C. y otros, Capillary electrophoresis coupled online with flame photometric detection: Determination of alkylphosphonic acids, J. Micro. 9 (1997) 253–259.

Laguarda-Miró, N. y otros, Glyphosate detection by voltammetric techniques. A comparison between statistical methods and an artificial neural network, Sensors and Actuators B 171-172 (2012) 528-536.

Lenz, D. y otros, Development of immunoassays for detection of chemical warfare agents, in D. A. Aga and E. M. Thurman (Eds.), Immunochemical Technology for Environmental Applications, American Chemical Society, Washington, 1997, pp. 77–86.

Martínez, P. y otros, Glyphosate detection with ammonium nitrate and humic acids as potential interfering substances by pulsed voltammetry technique, Talanta 115 (2013) 702-705.

Metal Oxide Semiconductor (MOS) Sensors, AppliedSensor, www.appliedsensor.com.

Mohr, G., New chromogenic and fluorogenic reagents and sensors for neutral and ionic analytes based on covalent bond formation–a review of recent developments, Anal Bioanal Chem 386 (2006) 1201–1214.

Nimal, A. y otros, Development of handheld SAW vapor sensors for explosives and CW agents, Sensors and Actuators B 135 (2009) 399-410.

Olssona, J. y otros, Volatiles for mycological quality grading of barley grains: determinations using gas chromatography–mass spectrometry and electronic nose, International Journal of Food Microbiology 59 (2000) 167–178.

Persaud, K., Dodd, G., Analysis of discrimination mechanism in the mammalian olfactory system using a model nose, Nature 299 (1982) 352-355.

Rock, F. y otros, Electronic nose: Current status and future trends, Chem. Rev. 108 (2008) 705–725.

Royo, S. y otros, Crhomogenic, Specific Detection of Nerve-Agent Mimic DCNP(a Tabun Mimic), Chem. Eur. J. 17 (2011) 6931-6934.

Somani, S. y otros, Toxicodynamics of nerve agents, Chemical warfare agents 68 (1992).

Sugendran, K., Kumar, P. y Vijayaraghavan, R., 1998. Military Law Review. *Defence Science Journal*, pp. 155-155.

VanTreeck, H. y otros, Quantitative detection of a simulant of organophosphonate chemical warfare agents using liquid crystals, Sensors and Actuators B 158 (2011) 104-110.

Vautz, W. y otros, Beer Fermentation Control Using Ion Mobility Spectrometry – Results of a Pilot Study, Journal of the Institute of Brewing 112-2 (2006) 157-164.

Wang, F. y otros, Carbon Nanotube/Polythiophene Chemiresistive Sensors for Chemical Warfare Agents, JACS 130 (2008) 5392-5393.

Wheelis, M., Biotechnology and chemical weapons control, Pure Appl. Chem 74 (2004) 2247–2251.

Yadava, RDS., Chaudhary, R., Solvation, transduction and independent component analysis for pattern recognition in SAW electronic nose, Sensors and Actuators B 113 (2006) 1-21.

Chapter 3: Odour sampling system with modifiable parameters applied to fruit classification

García Breijo, Eduardo; Guarrasi, Valeria; Masot Peris, Rafael; Alcañiz Fillol, Miguel; Olguín Pinatti, Cristian Ariel. *Odour sampling system with modifiable parameters applied to fruit classification*. Journal of food engineering. 116, pp. 277 - 285. 2013.

https://doi.org/10.1016/j.jfoodeng.2012.11.033

Abstract

The aroma emanating from fruits can indicate the maturity level and thus the quality and shelf life of the products. In this work it is presented an odour sampling system (called also electronic nose), to classify the aroma of Diospyros kaki, whose working parameters can have variable configuration making the system flexible. The system can be appropriately reconfigured for each type of sampling. It is based on a metal oxide semiconductor (MOS) commercial sensors matrix, which are individually controlled, being able to modify by software several parameters of their operating point. Working this way there is not a limited number of sensors but a very high number of virtual sensors. The system shows the ability to discriminate aroma of two Diospyros kaki cultivars. Moreover the instrument is capable to distinguish the fruits, inside the persimmon cultivar cluster, according to the ripe state after few days of storage.

Keywords: Electronic Nose Tongue, Pattern recognition, Microcontroller, food classification.

1. Introduction

The analytical technique as well as the sensorial analysis used to analysed the fruits volatile composition are very time consuming, so it could be advantageous to utilize systems called electronic nose to monitor fruit freshness and shelf life in a rapid way. The electronic noses are based on a tool consisting of an array of sensors with partial specificity, sensors control and signal acquisition electronic, and a control and data treatment software able to recognize patterns of gases or odours. Currently there is a large number of this type of systems both commercial and laboratory ones. In 2009 Wilson published a table summarising the commercial electronic noses (Wilson & Baietto 2009).

Sensors can be very varied as it is shown in Table I (Wilson & Baietto 2009). [1], both commercial and own development ones. These sensors are usually polarized for an only operating point, being specific for some concrete volatile compound but unspecific for the rest, or can even not give an answer to that compound. So if the system has N sensors, N signals will be obtained to be processed, or fewer in case some of the sensors do not answer. This way of working can be very useful with systems working for specific fields such a as food, military industry, etc, but are difficult to adapt and reconfigure.

Sensor Type	Sensitive Material	Detection principle
Acoustics sensors: Quartz crystal microbalance (QMB); surface & bulk acoustic wave (SAW, BAW)	Organic or inorganic film layers	Mass change (frequency shift)
Calorimetric; catalytic bead (CB)	Pellistor	Temperature or heat change (from chemical reactions)
Catalytic field-effect sensors (MOSFET)	Catalytic metals	Electric field change
Colorimetric sensors	Organic dyes	Color changes, absorbance
Conducting polymer sensors	Modified conducting polymers	Resistance change
Electrochemical sensors	Solid or liquid electrolytes	Current or voltage change
Fluorescence sensors	Fluorescence-sensitive detector	Fluorescent-light emissions
Infrared sensors	IR-sensitive detector	Infrared-radiation absorption
Metal oxides semi-conducting (MOS, Taguchi)	Doped semi-conducting metal oxides (SnO2,GaO)	Resistance change
Optical sensors	Photodiode, light-sensitive	Light modulation, optical changes

Table I. Types and mechanisms of common electronic-nose gas sensors.

In this work it is presented a gases detection system based on metal oxide semiconductor sensors (MOS) but with some differences in comparison to other similar systems. In this system it is possible to reconfigure the operating point of each of the sensors. So multiple operating points for each sensor can be obtained. This is counterproductive when the sensor array must detect the compound for which it is developed, but it is an advantage in its use in electronic noses as it generates nonspecificity, base of the functioning of the electronic noses (Baldwin, et al 2011).

In order to obtain different operating points, its polarization potential, heating temperature and signal times are changed. So a sensor becomes different types of sensors, as many as possible variations in its operating point are obtained. With N sensors a nearly infinite number of processing signals would be obtained. This way of working allows to configure the sensors matrix for an application, to store the data for that application and to configure the sensors again for another completely different application. Thus a flexible and optimized system is obtained. The disadvantage is that it needs more training time as the most appropriate configuration for each sensor must be found.

The developed system has a matrix of up to 15 sensors. All of them are metal oxide semiconductor (MOS) and commercial ones. The innovation of the system lies in the fact that each of the sensors has its own configuration stage (slave) and a general control stage (master). During the system training stage the different parameters of the sensor operating point can be changed through an own software in PC.

The system was tested on two *Diospyros kaki* T. cultivars. Persimmon (*Diospyros kaki* T.) is believed to be originated in China (Luo & Wang, 2008), subsequently spread to Korea and Japan (Sugiura, 1997)0, where it is a traditional crop, and then to other regions of the world. It gained popularity in Europe and in particular in the Mediterranean countries few years ago (Spain and Italy).

The persimmon colour generally ranges from light yellow-orange to dark red-orange. Depending on the species, persimmons vary in size from 1.5 to 9 cm diameter, and may be spherical, acorn-, or pumpkin shaped. The calyx often remains attached to the fruit after harvesting, but becomes easier to be removed upon ripening. Persimmon fruits have high content of glucose, with a balanced protein profile, and present various medicinal and chemical uses (Ragazzini, 1995). These fruits are of great interest due to their content of biologically active important compounds (different types of carotenoids and vitamin C).

Persimmon cultivars are usually classified into two groups, astringent and non-astringent type, depending on the degree of astringency at the mature stage (Ragazzini, 1995; Arnal & Del Rio, 2003; Matsuo, 1998). When an astringent persimmon is eaten, the tannin cells in the flesh are crushed and soluble tannins are released, giving a strong astringent sensation (Taira, 1996). The astringency disappears when soluble tannins become insoluble (Matsuo, 1998; Taira, et al. 1997), at the mature stage. Persimmons are climacteric fruits whose ripening is regulated by ethylene. They are at their best quality at the end of the preclimacteric stage, when the sugar content reaches its maximum value and the required orange colour of the fruit has developed just before the onset of the respiratory climacteric and the induction of ethylene. When astringent persimmons with firm texture are harvested, the fruit has not suffered the overripening that naturally removes their astringency. For this reason, astringent varieties of persimmon have a limited consumption as a fresh product and their features must be improved by technological processes (Arnal & Del Rio, 2003; Agustí, et al 2003; Hernándiz, 1999). Persimmon production is now increasing due to the application of techniques to remove astringency. This allows to commercialize and transport fruits still having a firm consistency (Arnal & Del Rio, 2003; Llácer & Badenes, 2002).

Little literature exists on the aroma characteristic of persimmon fruits, whereas the marketing of many products derived from persimmon flavour is continuously growing. We used the equipment test for its ability to discriminate the odour fingerprint of two different cultivars of *D. kaki* produced in Spain.

2. *Electronic Nose Description*

2.1. Introduction

The variation parameter in the metal oxide semiconductor (MOS) sensors is resistance. The relationship between the sensor resistance R_S and the gas concentration [C] can be expressed in a certain margin of the gas concentration, with the equation: $R_S=K[C]^{-a}$, where **K** is a constant and **a,** the slope of the curve R_S. For example, Fig. 1.a shows the answer of a typical MOS sensor (the TGS 826 ammonia sensor of the Figaro Inc.). It is shown that the sensor is sensitive to ammonia but also to some other combustible gases (scarce selectivity). As real resistance varies from one sensor to another, characteristics are expressed as the relation of the sensor resistance referred to the resistance for a specific ammonia concentration. The axis Y shows the ratio between the sensor resistance in several gases (R_S) and the sensor resistance in 50ppm of ammonia (R_O).

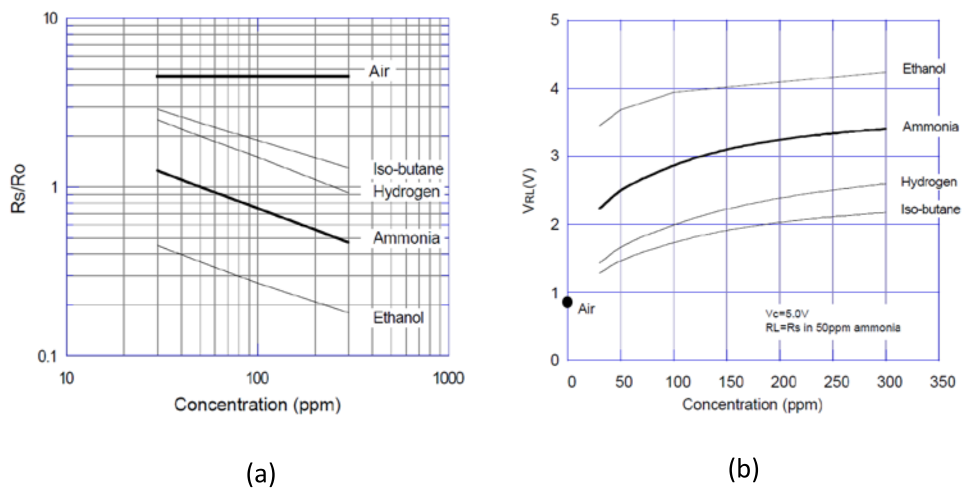


Fig. 1. Sensitivity to various gases: a) Rs/Ro and b) V_{RL}.

The polarization basic circuit is shown in Fig. 2. It is necessary a V_C supply tension, a V_H heating voltage and a R_L load resistance.

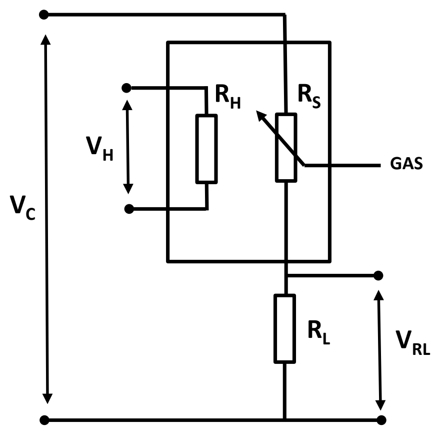


Fig. 2. Diagram of basic measuring circuit.

The sensor resistance is calculated according to the Equation (1).

$$\mathbf{R_S = \left(\frac{V_C}{V_{R_L}} - 1\right) \cdot R_L} \qquad \textbf{(1)}$$

Using the basic circuit shown in Fig. 2, choosing a value of R_L equivalent to R_S in 50 ppm of ammonia, the V_{RL} output voltage is shown in Figure 1.b.

The manufacturer advises very specific working values for each application. In the case of the TGS 826:

- V_C = 5.0±0.1V (DC)
- V_H = 5.0±0.05V (DC)
- R_L = 33kΩ±1%

Some of these sensors need polarization pulses instead of fixed tensions. For example the TGS2442 carbon monoxide sensor has to be polarized according to the diagram of Fig. 3.a, using the pulses shown in Fig. 3.b.

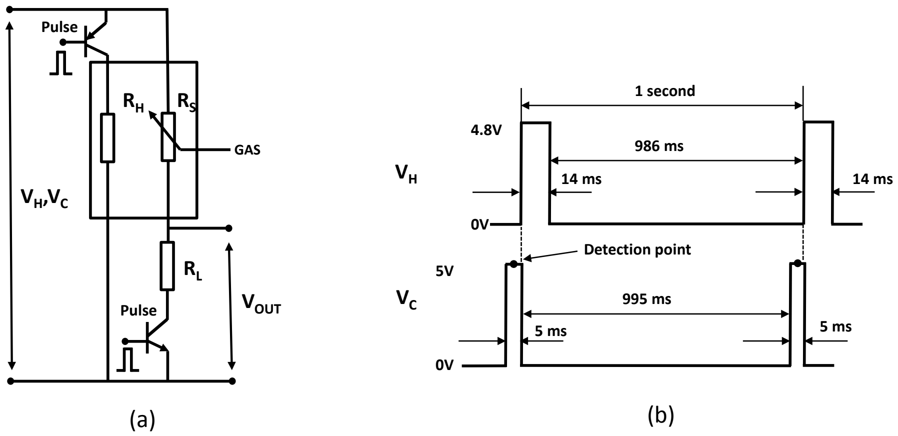


Fig. 3. Diagram of basic measuring circuit (a) and Circuit voltage and heater voltage cycles (b).

The parameters that can be modified to establish the operating point are:

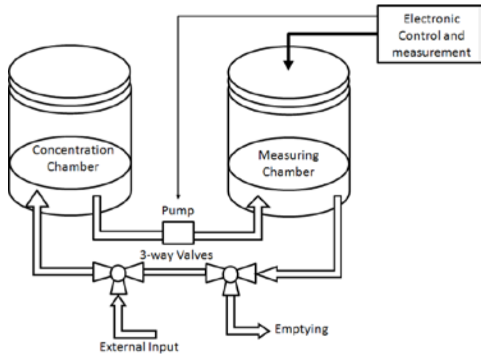
- Supply voltage (V_C).
- Heating tension (V_H).
- Load resistance (R_L).
- Periods of polarization pulse.

2.2. Electronic System

The 15 sensors were selected among commercial sensors for different gases (Table II). The sensors are presented on a same board in a measuring chamber. The general structure of the system is presented in Fig. 4. It has been designed including two chambers: the measuring chamber where the sensors are put and the concentration chamber where the samples are put. Both chambers are cylindrical; the measuring chamber is 12x14 cm and the concentration chamber is 12x16 cm. The system includes a pump (HARGRAVES, model B.1F32E1.A24Vdc) that allows the flow between the two chambers. There is the possibility of introducing samples or pattern gases from outside thanks to the three way valve. Through the configuration of these valves several working ways arise: closed cycle, cleaning cycle or external measurement. The system is completed with the implementation of measuring and control software.

Model	Control	V_{HH} (V)	V_{HL} (V)	V_{CH} (V)	V_{CL} (V)	Tgas (s)	T heater (s)	Time test (s)	Range VH	Range VC	Rs (KOhm)	Function
TGS-3870	pulses	0,9	0,2	5	0	n/d	n/d	20	Fixed	Fixed	0,35-40	Methane and Carbon Monoxide
TGS-2442	pulses	5	0	5	0	1	60	1	4,3-5,3 and 0-15ms	Fixed	10-1000	Carbon Monoxide
TGS-203	pulses	0,8	0,25	5	5	300	1200	150	0 a 1,1	Fixed	1-1000	Carbon Monoxide
TGS-2612	normal	5	5	5	5	n/d	n/d	cont			0,68-30	Methane and LP Gas
TGS-2180	normal	5	5	5	5	n/d	n/d	cont			23-145	Water Vapour Detection
TGS-2201	normal	7	7	15	15	n/d	n/d	cont			25-250	Gasoline and Diesel Exhaust Gas
TGS-826	normal	5	5	5	5	6	1200	6	4,5-5,5	0 a 24	20-450	Ammonia
TGS-2610	normal	5	5	5	5	120	90	120	4,5 - 5,5	Fixed	0,68-30	LP gas
TGS-2611	normal	5	5	5	5	120	300	120	4,6-5,3	Fixed	0,68-30	Methane
TGS-823	normal	5	5	10	10	n/d	n/d	cont			1-10	Organic Solvent Vapours
TGS-2620	normal	5	5	5	5	240	n/d	240	4,5-5,5	Fixed	1-100	Volatile Organic Compound
TGS-2106	normal	6,2	6,2	6,2	6,2	180	n/d	180			may-50	Diesel Engine Exhaust Gas
TGS-2602	normal	5	5	5	5	60	600	60	4-6	Fixed	10-100	Air Contaminants
TGS-821	normal	5	5	10	10	n/d	n/d	cont			1-10	Hydrogen
TGS-813	normal	5	5	10	10	20	180	20	4,5-5,5	0 a 24	0,1-1000	Combustible Gases

Table II. Sensors used.



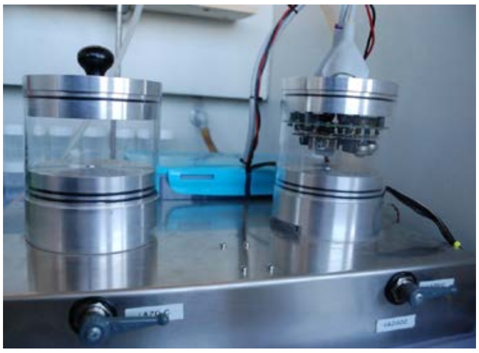


Fig. 4. Diagram of pneumatic system and photography.

The control and measuring electronics has been designed for each of the sensors obtaining a master-slave structure (Fig. 5). Each slave controls a different sensor measuring cycle and the master compiles the data from the 15 slaves and sends them to the PC.

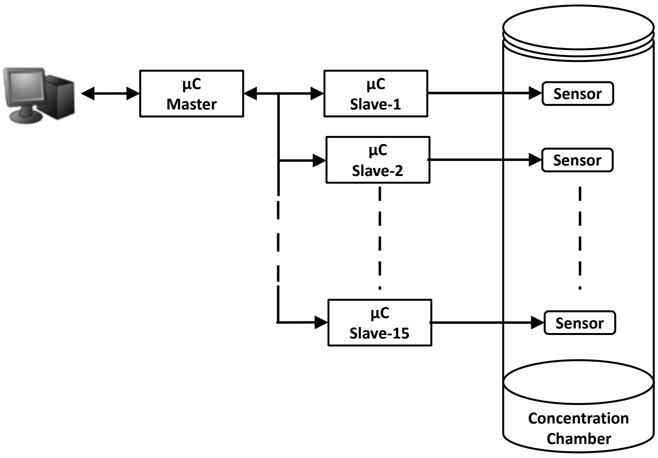


Fig. 5. Block diagram of electronic system.

Each slave can configure several parameters of the sensor such as the supply voltage(V_C), heating voltage (V_H), load resistance (R_L) and polarization pulses. Each of these parameters can be configured through the PC using a dedicated software. The slave is based on a PIC18F2580 microcontroller, a 12-bit analogical digital converter (AD7237A) and a 10-bit digital potentiometer (MAX5481). The circuit of a slave is shown in Fig. 6. The master is based on a PIC18F4550 microcontroller that controls the communications between the PC and the slaves; furthermore it controls the whole gas flow system.

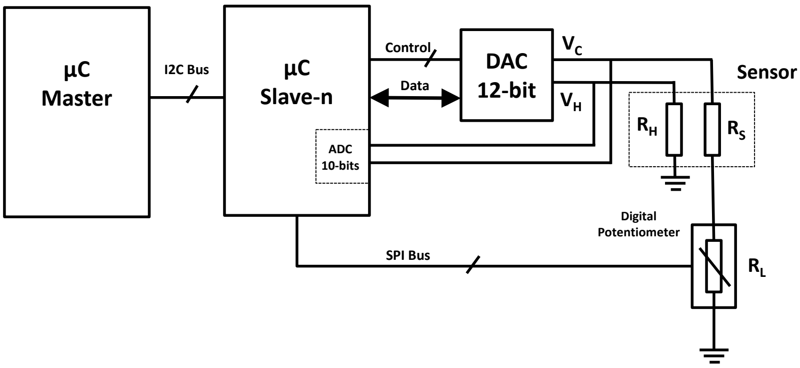


Fig. 6. Diagram of measuring circuit.

The parameters are configured by software by the user and sent by a serial port to the master. The master sends to every slave the configuration data using an I2C bus. The slave-microcontroller configures the DAC to supply the V_C and V_H to the sensor. If working with pulses is necessary, the slave-microcontroller also configures these voltages temporarily. Through the digital potentiometer, the microcontroller modifies the value of R_L through a SPI protocol.

Once the operating point has been established, the slave-microcontroller acquires, through an internal ADC of 10 bits, the output signal of the sensor (V_{RL}); the value of this signal is sent back to the master and then to the working software.

Apart from the 15 gas sensors, the data acquisition system incorporates a temperature sensor and a humidity one.

In Fig. 7, the whole measurement and control system is shown.

Fig. 7. Photography of the electronic system.

2.3. Software

The control and acquisition system allows the user to establish the following parameters: heater supply voltages, sensor supply tensions, heating/cooling times of the heater, connection/disconnection times of the sensor, measurement time, and test establishment time. The software is only for acquisition and control, in order to carry out the data processing a different kind of program must be used.

Furthermore, the program will have to send the configuration data to the electronic equipment through the RS-232 port. When the test establishment time has passed, the software asks to the electronic equipment the result of the test. That result is displayed on the screen and stored in a database.

The main window of the programme allows the user:

- To configure the sensors (Fig. 8).
- To configure the system.
- To ask help.

The sensors configuration screen allows the user to define the operating point parameters: heater supply voltages, sensor supply voltages, heater heating/cooling times, sensor connection/disconnection times, measurement time, test establishment time, as well as the assignation of the sockets to the sensors for possible changes. Moreover it supplies information of the manufacturer about the nominal performance and security values of the different sensors.

The system configuration screen allows to determine the parameters of the communication with the system, apart from establishing the configuration of the files that will be generated in the assays.

The user support screen allows the user to know the modifications of the slave board colours legend, description of the sensors performance modes, and in this screen there is also help to register new types of sensors.

The actions that can be carried out from the main programme are:

- The graphic representation of the assays. Several sensors data analysis and temporal representation. Detailed data analysis of a single sensor.
- Starting up or stopping the flow recirculation pump.
- Data sampling.
- Communications I2C bus test.
- Sensors cleaning through the activation of the heaters with the aim of desabsorbing any substance.

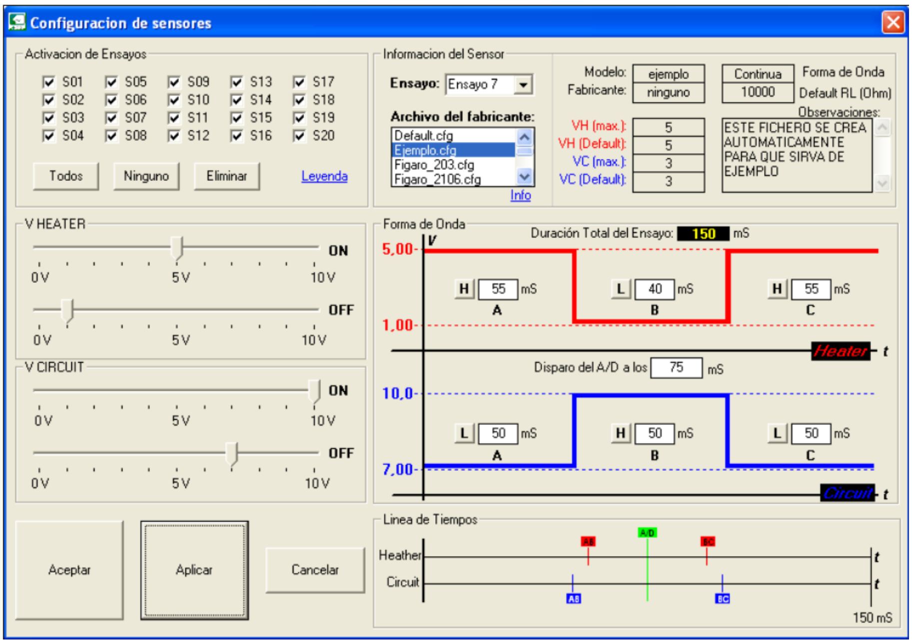


Fig. 8. Graphical User Interface for sensors configuration.

3. Materials and methods

Two cultivars of persimmon (*D. kaki*) were used for the experiments. Samples of 'Rojo Brillante' (Fig. 9.a) and 'Triumph' (Fig. 9.a) were obtained from an experimental orchard (near the Polytechnic University of Valencia). Twenty fruits for each cultivar were collected in October 2011. The fruits were ripened for commercial picking (Stage 8. 85 – BBCH scale; (García-Carbonell, et al. 2002)). The fruits were selected with the aim of having high homogeneity in samples thus reducing the variability of the results. The Electronic nose, whose detailed description is given above, was used.

Fig. 9. a) D. Kaki cv. 'Rojo Brillante'.and b) D. Kaki cv. 'Triumph'.

The measurement was carried out by putting half of cut persimmon fruit (40 g, pulp and skin) inside the chamber, closing the top and starting to record data from sensors. Each measurement was stopped after 20 min, when all sensors reached a steady-state (stable maximum value).

4. Results and discussions

As a first step of electronic nose data pre-processing, the significant features were extracted from the sensors' response curves. In the present study, the feature considered was R_S, that is the maximum of sensor resistance during the exposure to sample headspace[12]. Explorative data analysis was firstly performed by Principal Component Analysis (PCA). The raw data matrix was a Covariance Matrix.

The PCA score plot (Fig. 10) showed not only a distinct separation of fruit's aroma from atmospheric air (used as control), but also the ability to distinguish between the two different cultivars of *D.kaki*. The first separation was on PC1 with 95.20% of variance and the second one on PC2 with 2.97% of variance. In this case it was not necessary to use more principal components. Within cultivar's cluster it is possible to note two sub-clusters, according to the ripe state of fruit. The electronic nose seems to be able to distinguish also the fruits according to the ripe state as it's shown in Fig 10.

[12] Headspace sampling is a separation technique in which volatile material may be extracted from a heavier sample and measured.

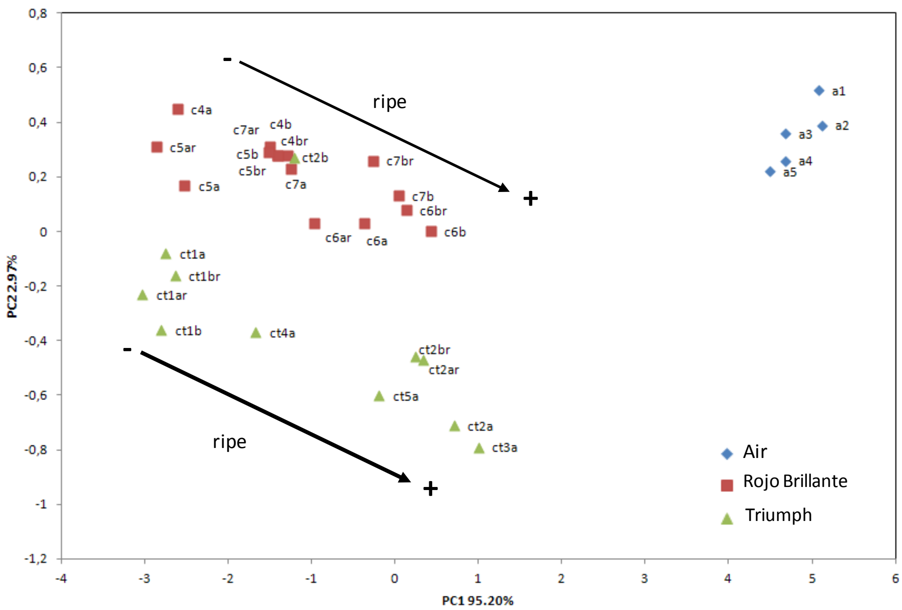


Fig. 10. PCA score plot of E-nose data for two cultivars Diospyros kaki T.: 'Rojo Brillante' and 'Triumph' cultivar according to different ripe state.

SOMmine5 Viscovery programme has been used to carry out a Self-Organized Maps (SOM) study. The number of nodes in all the cases studied has been 1000, the programme calculates a neighbourhood of at least 50 nodes with a linear neighbourhood weight function. Viscovery SOMine divides a map into regions, called clusters. The nodes of a cluster are similar in the sense that they are neighbours in the data space. In Fig 11 it is clearly observed the distribution of the "Triumph" variety according to different ripe state. In the case of "Rojo Brillante" more clusters have been created due to the fact that the distribution according to the ripe state is not as clear; this has also been observed in the PCA.

If we analyse the different sensors maps (Fig. 12) the S8 (Methane and Carbon Monoxide), S18 (Carbon Monoxide) y S20 (Temperature) sensors contribute very little to the classification so the number of sensors could be reduced to carry out new studies, whereas the others sensors are sensitive to the persimmon's aroma.

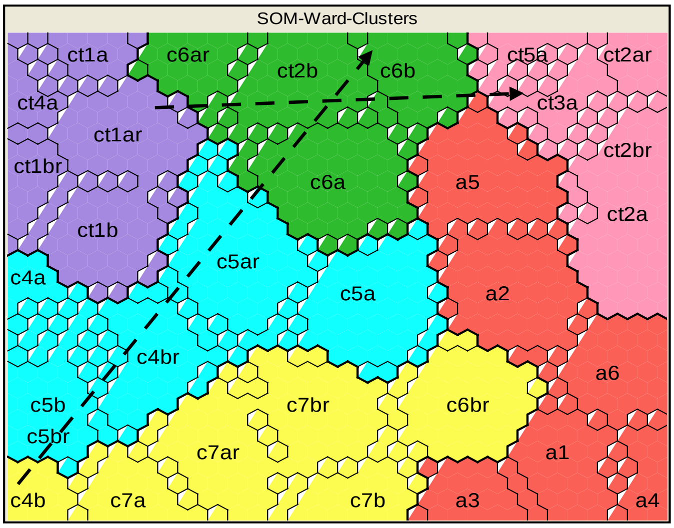


Fig. 11. SOM of E-nose data for two cultivars Diospyros kaki T.: ‘Rojo Brillante’ and ‘Triumph’ cultivar according to different ripe state.

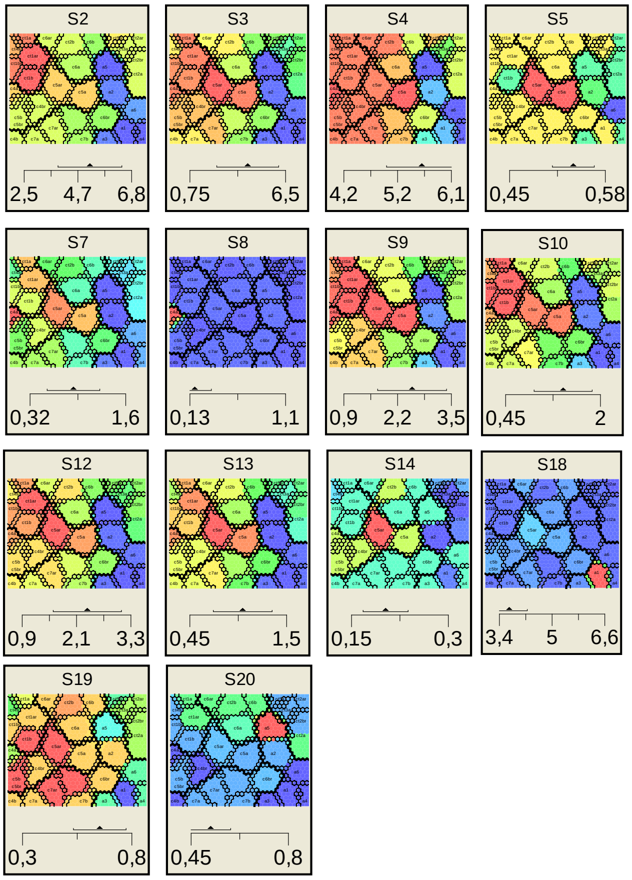


Fig. 12. Maps of cluster for sensors of E-nose.

5. Conclusion

An odour detection system based on MOS sensors has been developed, to monitor and discriminate the aroma of fruit in a rapid way. The advantage to use these array of sensors is that their operating point can be modified by software. This allows to have more flexibility in measures and thus to create measuring patterns for each sample using the same tool. To validate this device, the discrimination of fruit ripeness has been used, specifically kakis. It has been observed in this study that only some of the sensors have a good response what it might facilitate the design of a portable specific system for this application.

Acknowledgements

We gratefully acknowledge financial support from the Spanish Government project MAT2009-14564-C04-02.

References

Agustí, M. y otros, Treatments to delay the kaki (Diospyrus kaki L.) fruit maturation, Comunitat Valenciana Agraria 24 (2003) 27-33.

Arnal, L., Del Rìo, M., Removing astringency by carbon dioxide and nitrogen-enriched atmospheres in persimmon fruit cv. Rojo Brillante, Journal of Food Science, 68-4 (2003) 1516–1518.

Baldwin, E. y otros, Electronic Noses and Tongues: Applications for the Food and Pharmaceutical Industries, Sensors 11 (2011) 4744-4766.

García-Carbonell, S. y otros, Phenological growth stages of the persimmon tree (Diospyros kaki), Annals of Applied Science 141 (2002) 73–76.

Hernándiz, B., El cultivo de Kaki en la Comunidad Valenciana. Cuadernos de Tecnología Agraria, 3. Serie Fruticultura. Ed. Conselleria de Agricultura, Pesca y Alimentaciòn. Generalitat Valenciana, Valencia, España, (1999).

Llácer, G., Badenes, M. Situaciòn actual de la producciòn de caquis en el mundo, Agricola Vergel 242 (2002) 64–70.

Luo, Z., Wang, R., Persimmon in China: Domestication and traditional utilizations of genetic resources. Advances in Horticultural Science, 22-4 (2008) 239−243.

Matsuo, T., Chemistry, metabolism and biological functions of fruit tannins and relative compounds, Recent Research Developments in Phytochemistry 2 (1998) 269–285.

Ragazzini, D., Caracteres culturales, third ed. In: El kaki. Mundi Prensa Eds., Madrid, Spain, (1995) 59–132.

Sugiura, A., Proceedings of the First International Persimmon Symposium, Keynote. Acta Horticulturae 436 (1997) 15−19.

Taira, S., Astringency in persimmon. In: Linskens, H.-F. and Jackson, J.F. (Eds.). Modern Methods of Plant Analysis, Fruit Analysis. Springer-Verlag, Berlin, Heidelberg, (1996) 97–110.

Taira, S. y otros., Reduction of persimmon astringency by complex formation between pectin and tannins, Postharvest Biology and Technology 12- 3 (1997) 265–271.

Wilson A., Baietto M., Applications and Advances in Electronic-Nose Technologies, Sensors 9-7 (2009) 5099-5148.

Discusión general de resultados

Detección y cuantificación de TNT mediante lengua electrónica voltamétrica

Se ha diseñado un sistema de lengua electrónica voltamétrica con electrodos metálicos para detectar muestras de TNT en acetonitrilo. Los sensores constan de cuatro metales nobles (iridio, rodio, platino y oro) y otros cuatro no nobles (plata, cobre, cobalto y níquel).

Se han empleado ocho diferentes concentraciones de TNT, que varían en un rango de tres órdenes de magnitud, desde $3.9 \cdot 10^{-7}$M hasta $5.9 \cdot 10^{-4}$M. Cada una de las muestras se ha sometido a un tren de pulsos eléctricos con amplitudes de voltaje que oscilan desde -1.2V hasta 0V, con una anchura de pulso de 1ms.

Se han realizado diferentes estudios con las respuestas electroquímicas de los electrodos. En primer lugar y debido a la gran cantidad de datos obtenidos (mil valores por cada electrodo), se ha desarrollado un algoritmo de compresión de datos que consiste en aproximar un polinomio de cuarto orden a la curva de corriente de cada pulso reduciendo considerablemente la matriz de datos a veinte valores por electrodo. Los análisis por componentes principales de los datos comprimidos y sin comprimir han demostrado que el algoritmo de compresión mantiene la capacidad de discriminación.

En segundo lugar se han empleado redes neuronales no supervisadas SOM y supervisadas MLFN para verificar la existencia de una correlación significativa entre los datos obtenidos con la lengua y las diferentes propiedades de las muestras. Se han conseguido excelentes modelos de clasificación con ambos tipos de redes. Mención especial merece el modelo obtenido de la red MLFN, que fue embebido en un microcontrolador PIC24FJ256 de 16 bits para desarrollar un sistema de lengua electrónica portable, rápido y de análisis en tiempo real de cuantificación, alcanzando un relevante R^2=0.993 en el modelo de predicción de concentraciones de TNT.

La red MLFN se compone de 160 nodos en la capa de entrada, y 8 neuronas en la capa de salida correspondientes a las concentraciones de TNT. Si bien se han realizado estudios con diferentes números de neuronas, los óptimos resultados logrados situando 8 neuronas ocultas han sido decisivos para adoptar su configuración definitiva. El estudio se ha realizado con una red feedforward de dos capas, con funciones de transferencia sigmoide tanto en la capa oculta como en la capa de salida.

De los estudios realizados con redes SOM se ha evidenciado, por un lado, que los pulsos eléctricos que contienen un nivel nulo de voltaje apenas aportan información al modelo de clasificación, por lo que su exclusión permite reducir el número de pulsos manteniendo la capacidad de clasificación del modelo. Por otro lado se ha observado que los mejores modelos de clasificación se han obtenido utilizando solo metales nobles. Por consiguiente, disminuyendo el número de pulsos y el número de electrodos se puede reducir el tamaño de la matriz de datos sin alterar la sensibilidad del modelo, al mantenerse unas altas tasas de reconocimiento.

Nariz electrónica para la detección de simulantes de los gases Sarín, Somán y Tabún y agentes interferentes

Se ha desarrollado un sistema de medida basado en nariz electrónica para la detección y cuantificación de simulantes de gases de guerra, además de sus derivados organofosforados y agentes interferentes.

Se han realizado dos estudios mediante análisis de componentes principales. Por una parte, se han analizado simulantes de gases de guerra como son Sarín, Somán y Tabún y compuestos organofosforados y por otro lado agentes interferentes como el ácido sulfúrico, etanol, acetona, etc. Los resultados obtenidos han mostrado una gran discriminación efectiva entre las muestras en ambos casos. Por tanto se puede considerar el sistema de medida como una herramienta válida para establecer relevantes modelos de clasificación en este campo.

Además, con objeto de obtener un modelo predictivo de la concentración de uno de los compuestos estudiados, el simulante del Tabún, determinar su bondad predictiva y conocer su límite de detección, se ha realizado un análisis por mínimos cuadrados parciales. Se sometieron al sistema de medida diferentes disoluciones de concentraciones comprendidas en un rango de 0 a 208 ppm. Con un modelo de seis variables latentes el coeficiente de determinación ha sido R^2=0.9567 y el límite de detección LOD=5 ppm. Estos valores confirman que es factible cuantificar simulantes de gases de guerra combinando un sistema de nariz electrónica con técnicas de reconocimiento de patrones.

Sistema de muestreo de olores con parámetros modificables aplicados a la clasificación de fruta

Se ha desarrollado un sistema de detección de aromas basado en nariz electrónica con diferentes sensores semiconductores de óxidos metálicos de baja selectividad para la clasificación de distintas variedades de caqui (Diospyros kaki).

La primera parte del estudio ha consistido en realizar una puesta a punto del equipo de medida, optimizando los parámetros de trabajo (potencial de polarización, voltaje de calentamiento, temporizaciones, etc.) de cada uno de los quince sensores de gases hasta alcanzar una correcta clasificación. De esta forma se puede configurar la sensibilidad cruzada del sistema, almacenarla y volver a establecer nuevos parámetros para otra aplicación totalmente distinta obteniendo un sistema flexible y optimizado y permitiendo su uso en múltiples aplicaciones, lo que sin duda es un valor añadido muy a tener en cuenta.

Los resultados de los análisis por componentes principales muestran una clara discriminación entre las variedades del caqui y muestras de aire puro utilizadas como control. Se puede observar también cómo las muestras evolucionan a lo largo de la primera componente a medida que aumenta su grado de maduración, por lo que se puede concluir que la nariz electrónica es capaz de detectar también los días de almacenamiento del producto.

La segunda parte del estudio ha consistido en aplicar la nariz electrónica para determinar el grado de maduración en función de los días de almacenamiento. Para ello se ha utilizado una red neuronal tipo SOM observando la distribución de los clusters de las diferentes muestras según el grado de maduración. El número de nodos en todos los casos estudiados ha sido de mil y el programa ha calculado una vecindad de al menos cincuenta nodos con una función de peso vecinal lineal. Los resultados son muy similares a los obtenidos por componentes principales, tanto a nivel de clasificación como a nivel de contribución de los sensores.

Por último, tras el análisis de los resultados obtenidos con la red neuronal se evidencia que los sensores de metano y monóxido de carbono así como el de temperatura contribuyen muy poco al modelo de clasificación, por lo que se podrían obviar y reducir los datos a procesar sin alterar la sensibilidad del modelo.

Conclusiones

Esta tesis doctoral es fruto de muchos años de investigación y esfuerzo como investigador FPI en el Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) de la Universitat Politècnica de València. Ha sido realizada en el seno de un proyecto del Plan Nacional de I+D+i gestionado por el Ministerio de Ciencia e Innovación (MICINN). En ella se han desarrollado dos sistemas de medida sensoriales, de bajo coste y de fácil manejo, capaces de llevar a cabo la caracterización de propiedades químicas de matrices complejas. Frente a los lentos y costosos sistemas de medida tradicionales (cromatógrafos, espectrógrafos, etc.), las lenguas y narices electrónicas van adquiriendo cada vez más relevancia en la industria como métodos de análisis rápidos fiables y económicos.

Se ha evaluado la capacidad de clasificación y de cuantificación de la nariz y de la lengua electrónica a través de tres ensayos:

Detección y cuantificación de sustancias explosivas en medio acuoso

La lengua electrónica puede considerarse un excelente sistema de detección y cuantificación del explosivo TNT en medio acuoso. Se ha desarrollado un sistema portable y de bajo coste capaz de analizar muestras in-situ y que, tras un procesado de reconocimiento de patrones mediante redes neuronales, establece una clasificación y un modelo de predicción de forma rápida y fiable. Los resultados obtenidos del procesamiento de la matriz de datos reducida, convierten el algoritmo propuesto en una gran herramienta de compresión de datos para futuros ensayos. Además, el límite de detección obtenido y la estabilidad temporal de los sensores ponen de manifiesto que la lengua electrónica es un sistema de detección rápido, flexible y de bajo coste capaz de estar a la altura de los costosos equipos utilizados en los diferentes grupos de investigación internacionales.

Detección de gases de guerra e interferentes

Se ha desarrollado una nariz electrónica parametrizable con el objeto de diseñar un sistema de medida de aromas flexible y optimizado para cada caso. En este ensayo en concreto la nariz ha sido utilizada para la detección y cuantificación de simulantes de gases de guerra (como Sarín, Somán y Tabún), derivados organofosforados y agentes interferentes, todos ellos en muestras complejas. Los resultados han arrojado buenos modelos de clasificación y predicción, alcanzando un relevante coeficiente de determinación y un notable límite de detección. La aplicabilidad de los sistemas de nariz electrónica se postula como una gran herramienta válida, fiable y económica en la detección de agentes de guerra química.

Determinación del grado de maduración en fruta por reconocimiento de aromas

La capacidad de configuración de los parámetros fundamentales de los sensores de la nariz electrónica hace que se pueda adaptar a un gran abanico de aplicaciones. Por lo que en segundo lugar se ha aplicado a la clasificación de distintas variedades de caqui, obteniendo una clara clasificación de las muestras estudiadas. Posteriormente se han procesado los datos mediante una red neuronal, extrayéndose una correcta distribución de los resultados en función de los días de almacenamiento de la fruta o, lo que es lo mismo, del grado de maduración. Con el objetivo de reducir el número de los sensores de gases que componen la nariz, también se ha estudiado el grado con el que contribuyen al modelo de predicción, con la intención de eliminar los sensores que tengan una aportación nula o prácticamente nula y, de esta manera, crear un sistema portable y específico para cada aplicación.

Producción científica

Publicaciones SCI

Acosta, Carolina; Olguín Pinatti, Cristian; García-Breijo, Eduardo; Martínez-Máñez, Ramón; Bernardos, Andrea; Barat, Jose M.; *Masking garlic odour enhanced by gate-like systems on mesoporous silica supports.* Food Chemistry. Enviado.

Pascual Vidal, Lluís; Campos Sánchez, Inmaculada; Bataller Prats, Román; Olguín Pinatti, Cristian Ariel; Martínez Mañez, Ramón; García Breijo, Eduardo; Soto Camino, Juan. *A humid electronic nose for the detection of nerve agent mimics; a case of selective sensing of DCNP (a Tabun mimic)*. Sensors and Actuators B: Chemical. 192, pp. 134 - 142. 2014.

Olguín Pinatti, Cristian Ariel; Laguarda Miró, Nicolás; Pascual, Lluís; García Breijo, Eduardo; Martínez Mañez, Ramón; Soto Camino, Juan. *An electronic nose for the detection of Sarin, Soman and Tabun mimics and interfering agents*. Sensors and Actuators B: Chemical. 202, pp. 31 - 37. 2014.

García Breijo, Eduardo; Masot Peris, Rafael; Olguín Pinatti, Cristian Ariel; Alcañiz Fillol, Miguel; Ibáñez Civera, Francisco Javier; Bataller Prats, Román. *Low-cost electronic tongue system and its application to explosive detection*. IEEE transactions on instrumentation and measurement. 62, pp. 424 - 431. 2013.

García Breijo, Eduardo; Guarrasi, Valeria; Masot Peris, Rafael; Alcañiz Fillol, Miguel; Olguín Pinatti, Cristian Ariel. *Odour sampling system with modifiable parameters applied to fruit classification*. Journal of food engineering. 116, pp. 277 - 285. 2013.

García Breijo, Eduardo; Olguín Pinatti, Cristian Ariel; Masot Peris, Rafael; Alcañiz Fillol, Miguel; Martínez Mañez, Ramón; Soto Camino, Juan. *TNT detection using a voltammetric electronic tongue based on neural networks*. Sensors and Actuators A: Physical. 192, pp. 1 - 8. 2013.

Masot Peris, Rafael; Alcañiz Fillol, Miguel; García Breijo, Eduardo; Olguín Pinatti, Cristian Ariel; Ibáñez Civera, Francisco Javier; Gil Sánchez, Luis. *Portable measurement system for voltammetry and impedance spectroscopy. Application for TNT detection*. Procedia Engineering. 47, pp. 1129 - 1132. 2012.

García Breijo, Eduardo; Atkinson, J.K; Gil Sánchez, Luis; Masot Peris, Rafael; Ibáñez Civera, Francisco Javier; Garrigues Baixauli, José; Glanc, M.; Laguarda Miró, Nicolás; Olguín Pinatti, Cristian Ariel. *A comparison study of pattern recognition algorithms implemented on a microcontroller for use in an* *electronic tongue for monitoring drinking waters*. Sensors and Actuators A: Physical. 172, pp. 570 - 582. 2011.

Congresos

Olguín Pinatti, Cristian Ariel; Barrera Bermeo, Oscar; Laguarda-Miro, Nicolás; Gutiérrez Guzmán, Nelson; Barat Baviera, José Manuel; Grau Meló, Raúl. *Clasificación de diferentes tipos de café mediante nariz electrónica*. X International Workshop on Sensors and Molecular Recognition. "Proceedings" .pp. 174 - 180. 2016.

Gil Sánchez, Luís; Grau Meló, Raúl; Barat Baviera, José Manuel; Ibáñez Civera, Francisco Javier; Olguín Pinatti, Cristian Ariel; Garcia-Breijo, Eduardo; Antequera, Teresa. *Combinación de medidas de lengua electrónica y de imagen sobre grasas de cerdos con diferentes tipos de alimentación*. X International Workshop on Sensors and Molecular Recognition. "Proceedings" .pp. 96 - 100. 2016.

Gil Sánchez, Luís; Baigts Allende, Diana Karina; Garcia-Breijo, Eduardo; Olguín Pinatti, Cristian Ariel; Ibáñez Civera, Francisco Javier; Barat Baviera, José Manuel. *Detección de la calidad del pescado fresco y descongelado con lengua electrónica potenciométrica*. X International Workshop on Sensors and Molecular Recognition. "Proceedings " .pp. 91 - 95. 2016.

Olguín Pinatti, Cristian Ariel; Acosta, Carolina; Ordoñez, Roberto; Barat Baviera, José Manuel; Laguarda Miró, Nicolás. *Electronic nose for diallyl disulphide (DADS) detection*. IX International Workshop on Sensors and Molecular Recognition “Proceedings” .pp. 182 - 186. 2015.

Olguín Pinatti, Cristian Ariel; Laguarda Miró, Nicolás; Conesa Domínguez, Claudia; Ibáñez Civera, Francisco Javier; Loeff, Edwin. *Estudio voltamétrico para el control de biocidas no oxidantes en torres de refrigeración*. VIII International Workshop on Sensors and Molecular Recognition. “Proceedings” .pp. 193 - 197. 2014.

Olguín Pinatti, Cristian Ariel; Laguarda Miró, Nicolás; Masot Peris, Rafael; Alcañiz Fillol, Miguel; Ibáñez Civera, Francisco Javier; Gil Sánchez, Luís. *Electronic nose for chemical warfare detection*. VII International Workshop on Sensors and Molecular Recognition. “Proceedings” .pp. 90 - 90. 2013.

Masot Peris, Rafael; Alcañiz Fillol, Miguel; García Breijo, Eduardo; Olguín Pinatti, Cristian Ariel; Ibáñez Civera, Francisco Javier; Gil Sánchez, Luís. *Portable Measurement System for Voltammetry and Impedance Spectroscopy.*

Application for TNT Detection. Eurosensors 2012: 26th Conference on Solid-State Transducer. “Proceedings” .pp. 1 - 2. 2012.

Valera Anchel, Marta; Laguarda Miró, Nicolás; Ibáñez Civera, Francisco Javier; García Breijo, Eduardo; Gil Sánchez, Luís; Masot Peris, Rafael; Alcañiz Fillol, Miguel; Olguín Pinatti, Cristian Ariel. *Estudio voltamétrico de la leche envasada en brik. La correlación del ph y conductividad con su degradación acelerada*. VI Workshop on Sensors and Molecular Recognition. “Proceedings”.pp. 37 - 37. 2012.

Olguín Pinatti, Cristian Ariel; Masot Peris, Rafael; Alcañiz Fillol, Miguel; García Breijo, Eduardo; Gil Sánchez, Luís. *Sistema de medición portátil para la detección de tnt*. VI Workshop on Sensors and Molecular Recognition. “Proceedings” .pp. 26 - 26. 2012.

Garrigues Baixauli, José; García Breijo, Eduardo; Gil Sánchez, Luís; Ibáñez Civera, Francisco Javier; Laguarda Miró, Nicolás; Olguín Pinatti, Cristian Ariel. *Implementación de redes neuronales artificiales en microcontrolador*. V Workshop on Sensors and Molecular Recognition. "Proceedings" .pp. 29 - 36. 2011.

García Breijo, Eduardo; Atkinson, J.K; Garrigues Baixauli, José; Gil Sánchez, Luís; Ibáñez Civera, Francisco Javier; Glanc, M.; Olguín Pinatti, Cristian Ariel. *An electronic tongue for monitoring drinking waters using a fuzzy ARTMAP neural network implemented on a microcontroller*. 20th IEEE International Symposium on Industrial Electronics. " Proceedings" .pp. 1270 - 1275. 2011.

Alcañiz Fillol, Miguel; Olguín Pinatti, Cristian Ariel; Bataller Prats, Román; Masot Peris, Rafael; Gil Sánchez, Luís. *Desarrollo de un equipo de medida con diferentes técnicas electroquímicas*. IV Workshop on Sensors and Molecular Recognition. "Proceedings" .pp. 1 - 9. 2010.