

ENHANCING VOCS SELECTIVITY BY DESIGNING NOVEL GAS SENSITIVE SOFT MATERIALS

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Master in Biotechnology

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To my parents, my sister and my partner.

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“You can do anything, if you want it enough.” – Effy Stonem

ABSTRACT

Artificial olfaction aims to mimic the biological sense of smell, using electronic-nose devices that result from the combination of (bio)chemical sensors, electronics, and artificial intelligence tools. These devices can detect and identify individual or mixtures of gas analytes, such as Volatile Organic Compounds (VOCs).

This thesis explores the design of novel gas sensing materials based on ionogels and hybrid gels materials, with the goal of increasing their selectivity and discrimination towards different VOCs. Sensing materials based on the entrapment of ionic liquids within a biopolymeric matrix (ionogel), or on liquid crystal-ionic liquid droplet systems within a biopolymeric matrix (hybrid gels) were created and coupled with artificial intelligence algorithms to develop a custom-made electronic nose, for gas sensing purposes. The ionic liquid and liquid crystal components involved in this type of sensing materials provide complementary measurable signals (electric and optical), that can help tune the sensor selectivity, and due to their dynamic self-assembling nature allows the creation of dual responsive materials in the presence of VOCs.

The tunability of ionogels and different hybrid gels towards VOCs discrimination capability was first assessed by changing the materials composition. Changing components such as the ionic liquid cation, and the hydrophobicity of the chosen polymeric matrix, influenced morphological properties, VOC sensing capability, and sensors performance and storage-stability under room ambient.

The addition of simple biological molecules to the gel's formulation, such as peptides and small affinity ligand molecules, was also explored. In one study, three peptides were incorporated in the ionogels and hybrid gels composition. In a second approach, a library of fourteen different small ligands were chemically immobilized onto the glass surface of hybrid gel sensors. These materials were explored in a tailor-made electronic nose showing their potential to discriminate between a set of distinct volatiles under environmental conditions.

Overall, this work demonstrates the versatility of sustainable and stimuli-responsive novel gas sensing materials for use in electro-optical devices, capable of performing under environmental conditions, exhibiting promising results in artificial olfaction and gas sensing applications.

Keywords: electronic nose, gas sensing, volatile organic compounds, ionogel, hybrid gel, liquid crystal, ionic liquid, polymeric matrix, peptides, ligands.

RESUMO

Narizes eletrônicos tem como objetivo imitar o sentido biológico do olfato, através de dispositivos que resultam da combinação de sensores (bio)químicos, eletrônica, e de ferramentas de inteligência artificial. Estes dispositivos conseguem detetar e identificar analitos gasosos, tais como Compostos Orgânicos Voláteis (COV).

Nesta dissertação é explorado o desenho de novos sensores de deteção de gases em narizes eletrônicos, baseados em géis iónicos e em géis híbridos, com o objetivo de aumentar a sua seletividade e discriminação para com diferentes COVs.

Os materiais de deteção podem ser de dois tipos. Num sistema há encapsulamento de líquidos iónicos numa matriz bio-polimérica (géis iónicos) e num outro gotas de cristal líquido-líquido iónico são encapsulados numa matriz bio-polimérica (géis híbridos). Estes dois tipos de materiais foram desenvolvidos, caracterizados e acoplados com algoritmos de inteligência artificial, com o propósito de desenvolver um nariz eletrónico, como tecnologia de deteção de gases.

Os componentes envolvidos neste tipo de sensores, tais como líquidos iónicos e cristais líquidos, originam sinais complementares (tanto eléctricos, como óticos), que conseguem melhorar a seletividade do sensor, e dado à sua natureza dinâmica de auto-organização, permitem a origem de sensores com dupla resposta na presença de COVs.

O aumento da capacidade discriminatória deste tipo de sensores, tanto géis iónicos como géis híbridos, foi explorado neste trabalho através do estudo dos seus componentes. O uso de diferentes componentes, tais como líquidos iónicos, ou a hidrofobicidade da matriz bio-polimérica que é escolhida, influenciam não só a capacidade de deteção de analitos gasosos, como também o desempenho do sensor, as suas propriedades morfológicas, bem como a sua estabilidade face ao armazenamento em condições ambientais.

O efeito da adição de moléculas biológicas (tais como péptidos e pequenos ligandos de afinidade) à formulação dos sensores foi também estudado. Num dos estudos, três péptidos foram incorporados na composição de ambos os géis iónicos e os géis híbridos. Numa segunda abordagem, catorze ligandos de afinidade foram quimicamente imobilizados na superfície de vidro que constitui a base dos sensores que derivam dos géis híbridos. Estes novos sensores foram testados num nariz eletrónico, onde se verificou o potencial na deteção discriminatória de vários analitos gasosos em condições ambientais.

Este trabalho vem elucidar a versatilidade de novos materiais biológicos sustentáveis e responsivos a diversos estímulos, que atuam como detetores de COVs no desenvolvimento de dispositivos eletro-óticos, capazes de funcionar em condições ambientais. Estes são materiais promissores, com resultados inovadores, no que toca à área de detecção de gases e narizes eletrônicos.

Palavras-chave: nariz eletrônico, sensor de gás, compostos orgânicos voláteis (COVs), géis iônicos, géis híbridos, cristal líquido, líquido iónico, matriz polimérica, péptidos, ligandos.

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ABBREVIATIONS, ACRONYMS, SYMBOLS AND CONSTANTS

3D	Three dimensional
5CB	4-cyano-4'-pentylbiphenyl
APTES	3-aminopropyltriethoxysilane
[BMIM][Cl]	1-butyl-3-methylimidazolium chloride
[BMIM][DCA]	1-butyl-3-methylimidazolium dicyanamide
[BMPip][Cl]	1-Butyl-1-methylpiperidinium chloride
[BMPyr][Cl]	1-Butyl-1-methylpyrrolidinium chloride
[BuPy][Cl]	1-Butylpyridinium chloride
[Bu₂Pic][Cl]	1-Butyl-2-methylpyridinium chloride
[Bu₄Pic][Cl]	Butyl-4-methylpyridinium chloride
BF	Bright field
CM	Confusion Matrix
CNT	Carbon nanotube
CP	Conducting polymer
DMF	N,N-Dimethyl Formamide
E-nose	Electronic nose
FET	Field effect transistor
H₂O₂	Hydrogen peroxide
IL	Ionic Liquid
L0	1-pyrenemethylamine
L1	Ethanolamine
L2	Benzylamine
L3	4-Aminobutan-2-one
L4	Ethyl-4-aminobenzoate
L5	Agmatine

L6	Aminopyrazine
L7	Iminodiacetic acid
L8	N,N-Dimethyl-4,4'-azodianiline
L9	4-aminobenzamide
L10	Leucine
L11	Histamine
L12	Pararosaniline
L13	Tryptamine
LC	Liquid crystal
LED	Light emitting diode
LOD	Limit of detection
MFC	Mass flow controller
MOS	Metal oxide semiconductors
mVOC	Microbial Volatile Organic Compound
P1	Peptide 1
P2	Peptide 2
P3	Peptide 3
PCA	Principal component analysis
PDLC	Polymer dispersed liquid crystal
PDMS	polydimethylsiloxane
POM	Polarized optical microscopy
QCM	Quartz crystal microbalance
RH	Relative humidity
RTILs	Room temperature ionic liquids
SAW	Surface acoustic wave
SEM	Scanning electron microscopy
SVM	Support vector machine
TEM	Transmission electron microscopy
UK	United Kingdom
USA	United States of America
USD	United States Dollar
VOC	Volatile Organic Compound
Å	Angstrom
μm	micrometer
%(v/v)	Percentage (volume / volume)

Hz	Hertz
kHz	kilohertz
mm	millimeter
nm	nanometers
mM	milimolar
M	molar
ml	milliliter
mV	millivolts
s	seconds
wt %	weight percentage

Amino acid abbreviations

Alanine	Ala	A
Aspartic acid	Asp	D
Biphenilalanine	Bip	
Isoleucine	Ile	I
Proline	Pro	P
Serine	Ser	S
Tryptophan	Trp	W
Norleucine	Nle	

BACKGROUND AND OBJECTIVES

Artificial olfaction aims to mimic the biological sense of smell, through the combination of artificial intelligence tools and biological components. These devices are called electronic-noses, and they are able to detect gas analytes, such as Volatile Organic Compounds (VOCs)^{1,2}.

The detection of VOCs is of significant interest, not only to understand biological processes, but also in several scientific and technological areas. For instance, VOC sensors hold great promise in the early diagnosis of diseases^{3–8}, food quality control^{9–11}, security¹², agriculture¹³, environmental monitoring¹⁴, and insect supervision^{15,16}.

Usually, e-nose devices are comprised of an array of chemical sensors with partial selectivity and appropriate pattern recognition systems, capable of recognizing simple or complex odors. The field of these sensors has been widely explored over the last decades, and current they include, include mostly metal oxide semiconductors and conductive polymers. Nevertheless, there are still some remaining challenges linked to the optimized performance of these devices, such as the low stability and high promiscuity towards VOC molecules, resulting in low selectivity. As such, incorporating sensing components derived from the actual biological olfaction systems, into these gas-sensing materials, can increase the VOC selectivity from the devices^{15–20}.

Hybrid gels and ionogels are a class of sensing materials that present versatility and unique stimuli-responsive properties, and when combined with other biological molecules, they can help to tune the so-desired selectivity. By modifying these materials intrinsic composition – like changing the polymeric matrix or the used conductive ionic liquid, as well adding small biological molecules (e.g. peptides and other small affinity ligands) – we can advance into achieving this purpose, ultimately creating arrays of novel gas-sensitive soft materials to accurately discriminate numerous VOCs.

This thesis focusses on exploring the field of gas sensing materials and the design of novel gas sensing materials based on ionogels and hybrid gels materials. The ionic liquid and liquid crystal main components, provides complementary measurable signals (electric and optical), which allows the creation of dual responsive materials in the presence of VOCs. These

responses are analyzed through complex Supervised Machine Learning algorithms, which enables accessing the accuracy displayed by the sensors to discriminate/predict the volatiles.

The dissertation novelty is based on:

(i) The implementation of Supervised Machine Learning algorithms in the developed optical and electrical e-nose devices, to study the accurate prediction of VOCs when using ionogels and hybrid gels as gas sensing materials.

(ii) Uncovering the influence of different ionic liquid cations in gelatin ionogels and hybrid gels sensing materials to tune the discrimination of volatiles in e-nose gas sensing devices.

(iii) Exploring the use of polymeric matrices with different polarities, used as matrices to encapsulate ionic liquid and liquid crystal droplets systems, and their influence to discriminate volatiles, as well as their humidity responsive profile.

(iv) The use of peptide sequences and small affinity ligands as additional components in the sensing materials, to enhance the discrimination of gas analytes, by develop new sensors to be used in e-nose devices.

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STRUCTURE OF DISSERTATION

This dissertation is composed by six chapters.

The first chapter comprises a literature review introducing general concepts on gas sensors and e-nose systems. Special emphasis is given to gas sensing materials focusing on the use of peptides, Olfactory Receptors (ORs) and Odorant Binding Proteins (OBPs), as well as ionic liquids (ILs) and liquid crystal (LC) materials to tune the selectivity of the sensors towards Volatile Organic Compounds (VOCs).

The following four chapters present original research. Chapter two and three are focused on the use of different ionic liquids and polymeric matrices in gas sensing materials, while the fourth and fifth chapters report the use of small peptide sequences and small ligand molecules as new components introduced to the sensing materials formulation.

In the second chapter, gelatin-based ionogels and hybrid gels containing ionic liquids, from six different families, were designed, produced and further exposed to different VOCs using an electrical and an optical tailor-made electronic nose devices. The resulting signals were analyzed through Supervised Machine Learning algorithms to assess VOC classification accuracy. Ionogels conductance was analyzed through conductivity assays, while the hybrid gels air-stability and morphology were characterized through polarized optical microscopy (POM), and Scanning Electron Microscopy (SEM), which allowed to know how the gel components are organized within the matrix. In this chapter it is explored the importance of the ionic liquid, specifically the ionic liquid cation, in the gas sensing performance of ionogels and hybrid gels, regarding their accuracy to discriminate different volatiles.

Chapter three reports the use of different polymeric matrices in the hybrid gel formulations, by comparing an hydrophilic polymeric matrix like gelatin, or a hydrophobic polymeric matrix such as PDMS, in the presence of two different ionic liquids. The morphology of the resulting sensing materials was characterized by POM and SEM, and sensors were also characterized regarding their air-stability. The effect of humidity and the sensitivity of the sensing materials with different polymeric matrices was also assessed, by testing them against four different humidity environments and to four model VOCs under humidified conditions. Accuracies to predict the different volatiles was also procured in optical electronic nose experiment, and data analysis regarding classification of the VOCs was the same as implemented in chapter two.

Chapter four and five explore the addition of small molecules to the sensing material formulations, with the intent of enhancing the discrimination of different volatiles.

In chapter four, the addition of three small peptide sequences to the ionogels and hybrid gels sensing materials was conducted. Peptide 1 (P1) was used as the original sequence, and two modified versions of it were designed (Peptide 2 (P2) and Peptide 3 (P3)), through the addition of two non-natural amino acids to the sequence. Modification of the original P1 structure was done to further explore the interaction of the peptides with the ionic liquid and liquid crystal droplet system, as well as to explore the different discrimination of VOCs that would arrive from it.

In chapter five, inspired by chapter four, several ligands from distinct chemical functional classes were introduced to the sensing materials compositions, by chemically immobilizing them on the surface of glass substrates for the hybrid gels deposition. The VOCs accuracy discrimination system developed in the second and third chapter of this dissertation was also applied in the data analysis of these two chapters, as well as the morphological characterization through POM of the produced hybrid gels, and their use in the tailor-made electronic nose devices.

Finally, chapter six presents the general conclusions of the work performed within this dissertation, giving considerations on its possible applications for future work with regards to the field of electronic noses and gas sensing.

LITERATURE REVIEW

Sections 1.1 and 1.2 are part of a publication in peer-review paper

Barbosa, AJM, **Oliveira AR**, Roque, ACA. Protein- and Peptide-Based Biosensors in Artificial Olfaction. Trends in Biotechnology (2018). 36(12):1244-1258.
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Authors contributions:

The manuscript was written with contributions of all authors.

1.1 Biosensors in Artificial Olfaction

The possibility of mimicking nature, building artificial intelligent systems to perform complex tasks, and dealing with large sets of data is gaining increasing relevance in all areas, including biological sciences¹. The mysteries of olfaction, in particular human olfaction, still intrigue scientists and, not surprisingly, it is considered the least understood sense^{2,3}. Generally, in the human olfactory system, the detection of odors derives from a combinatorial response, in which a library of olfactory receptors (ORs) and odorant-binding proteins (OBPs) generates a unique fingerprint of each odorant⁴. Artificial olfaction systems aim to mimic the sense of smell and typically consist of electronic nose devices (e-noses) that include an array of gas sensors associated with signal-processing tools⁵⁻⁷. Classically, in an e-nose, a sample enters the system through an inlet that guides the gas molecules to a chamber where the sensing material is deposited. The interaction of the volatile organic compounds (VOCs) with the sensing material generates a signal (for example, electric, optical, or gravimetric) that is transduced and further processed by a signal-processing computer. Odors are typically composed of a set of VOCs. The detection of VOCs is of significant interest, not only to understand biological processes, but also in several scientific and technological areas. For instance, VOC sensors hold great promise in the early diagnosis of diseases⁸⁻¹³, food quality control¹⁴⁻¹⁶, security¹⁷, agriculture¹⁸, environmental monitoring¹⁹, and insect supervision^{20,21}. The broad range of application areas for e-noses has been accelerating the progress of gas-sensing technologies, with an extensive research and market space for developing new sensing materials and devices². Current gas-sensing materials, including those commercially available, include mostly metal oxide semiconductors and conductive polymers. The main drawbacks of these systems are the low stability and high promiscuity towards VOC molecules, resulting in low selectivity. As such, incorporating the sensing components of biological olfaction systems into gas-sensing materials can increase the VOC selectivity of the resultant gas sensors and bio-electronic noses^{5,7,20-23}.

Also, these types of sensors tend to be highly expensive and time-consuming, involving non-portable and complex instrumentation that eventually culminates in the need of trained personnel. Therefore, there is high interest in the development of devices that are low cost, portable and user friendly to serve as gas sensing methodologies, such as e-noses devices and novel sensing materials. In 2022, gas sensors market was valued at USD 655.5 million, and expected to reach USD 883.6 million by 2026, with an expected growth of Compound Annual Growth Rate (CAGR) of 5.1% from 2021-2026²⁴. E-noses systems represent a smaller but increasing market evaluated in USD 17.8 million in 2020, and it is expected to reach USD 34.78 million by 2026, with a CAGR of 12.44% from 2021-2026²⁵.

Essentially, a gas sensor is composed by a sensing material with the purpose of detecting and quantifying as gas analyte²⁶, and the e-nose device is a system built to incorporate an array of these sensors, leading to the identification and discrimination of a set of gas analytes, the odors. More specifically, the process includes implementation sophisticated tools and algorithms for features extraction a patterning recognition, which works in a similar way to the human olfactory system (Figure 1.1)²⁷.

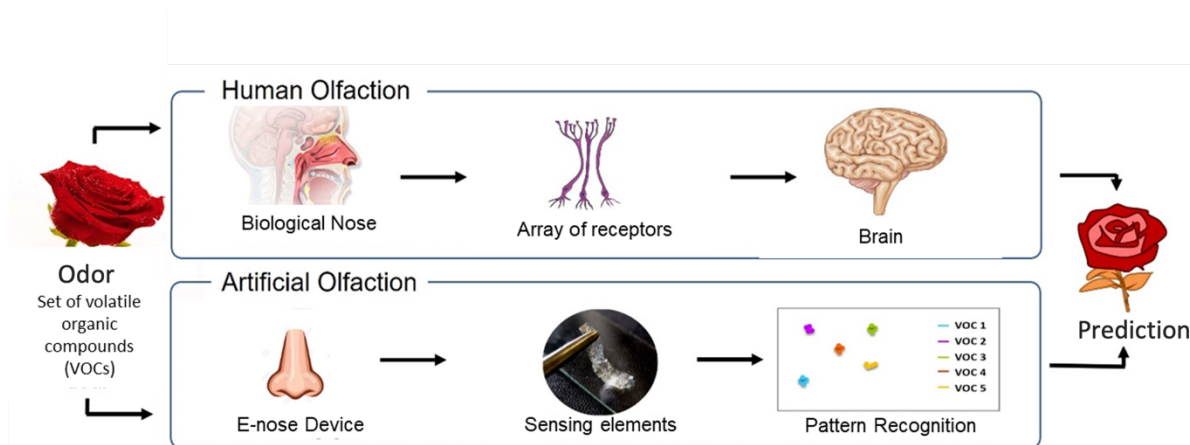


Figure 1.1 – Comparison of the human olfaction system and electronic nose system.

As mentioned, metal-oxides semiconductors (MOS) and conductive polymers have been for many years the most common sensing materials used as components of gas sensors for e-nose devices, in which the variation in resistance or conductance that occurs in the material upon interaction with the target analytes with the oxide surface, is being measured^{28,29}, through redox reactions³⁰. Between the most common MOS, TiO_2 and SnO_2 are one of the ones being extensively studied³¹, and as for conducting polymers, polyaniline, polypyrrole and polythiophene³², which generates an electrical signal upon interaction with the volatiles³³.

The use of non-conducting polymers is mainly conducted when coating surfaces such as quartz crystal microbalance (QCM) acoustic sensors, or surface acoustic wave (SAW), in which the changing in frequency and resonance derived from the mass changes upon absorption and desorption of gas analytes is being measured²⁹. Other type of sensing materials includes nanoporous pigments or dyes, applied in colorimetric and fluorescence sensors³⁴, or optical sensors employing liquid crystal (LC)-based films, based on optical changes due to the birefringence of the material upon interaction with the gas analytes³⁵. Rehman and Zeng³⁶ have also reviewed ionic liquid-based gas sensitive materials, and its use as Electrochemical, QCM, SAW and optical sensors has been explored as well³⁶.

Other systems have also been described by Peng et al³⁷, using an array of sensors designed by employing chemiresistors and gold nanoparticles, deposited in interdigitated gold electrodes, aiming to identify a set of lung cancer biomarker VOCs, through the combination of solid-phase microextraction, gas chromatography and mass spectrometry.

1.2 Biological components in gas sensing

Animals' olfactory systems rely on proteins, olfactory receptors (ORs) and odorant-binding proteins (OBPs), as their native sensing units to detect odors. Recent advances demonstrate that these proteins can also be employed as molecular recognition units in gas-phase biosensors. In addition, the interactions between odorant molecules and ORs or OBPs are a source of inspiration for designing peptides with tunable odorant selectivity. Progress can be achieved

in gas biosensors employing biological units (ORs, OBPs, and peptides) in artificial olfaction to detect gas-phase analytes (Figure 1.2 and 1.3).

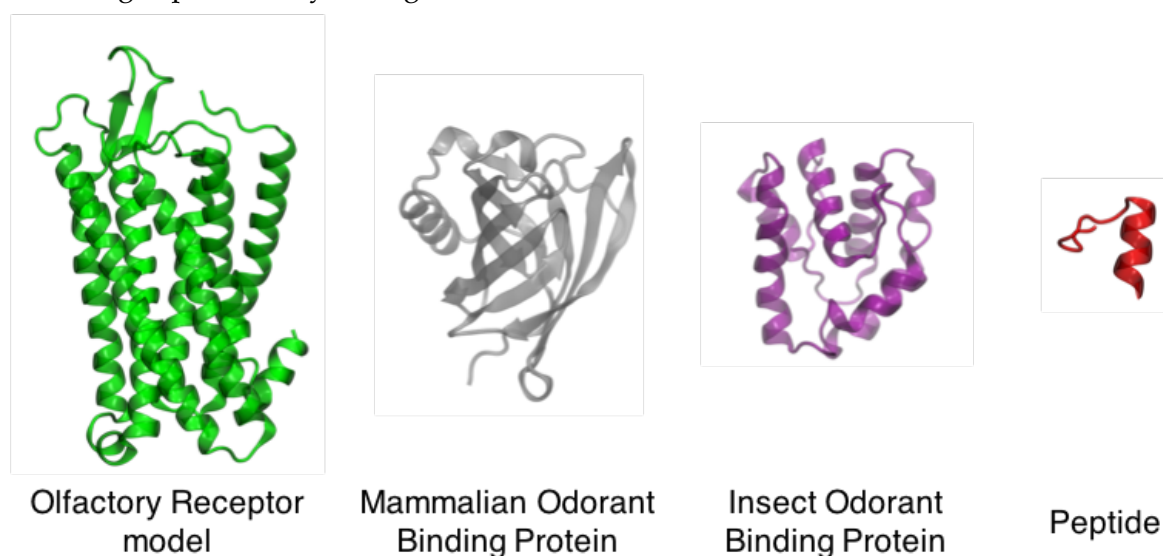


Figure 1. 2 – Structural Features of Odor-Sensing Proteins and Peptides.

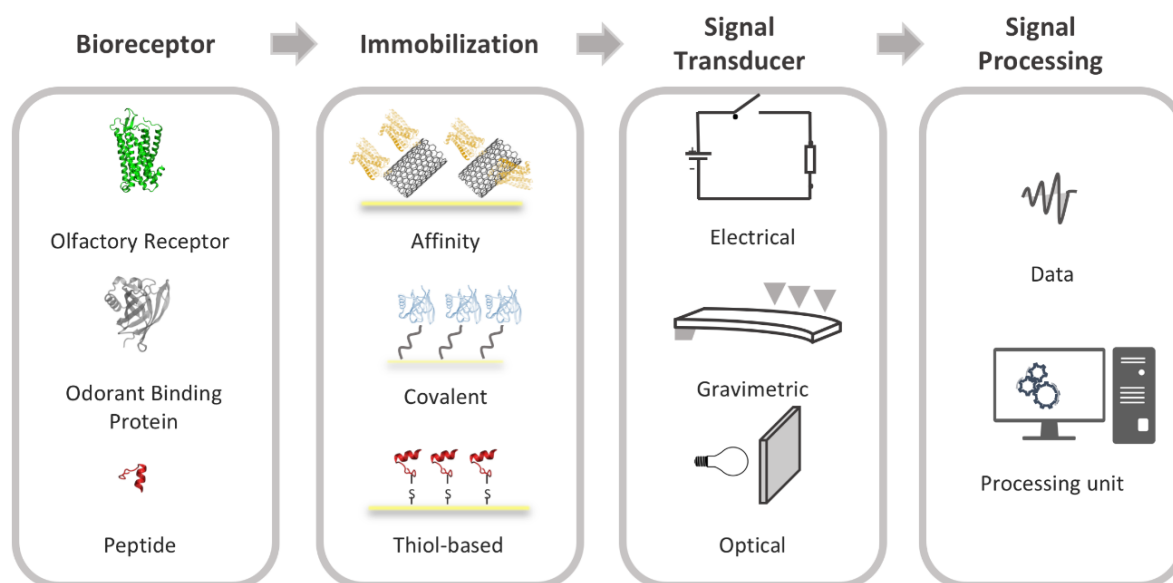


Figure 1. 3 – Scheme of Main Constituents of Protein and Peptide-Based Biosensors for Artificial Olfaction. A bio-receptor known to bind to volatile organic compounds (VOCs) – olfactory receptors, odorant binding proteins or VOC affinity peptides – is selected for immobilization. Surface immobilization can be made through different methods including affinity, covalent spacers, and thiol-based chemistry. The biosensor is then applied in a signal transducing system which may be, for example, electrical, gravimetric, or optical. The collected data is finally processed to yield qualitative or quantitative information about target VOCs.

Olfactory receptors (ORs) are believed to recognize different odorants, so odorant recognition depends on the OR being activated and on the extent of its activation^{38,39}. ORs are members of the G protein-coupled receptor family: they are structurally defined by seven trans-membrane α -helices, about 320 amino acid residues in length³⁹. These proteins, upon volatile

binding, generate a signal that is transmitted from the olfactory sensory neurons, where ORs are located, to the brain in a recognized pattern that culminates in olfactory perception.

Identifying which odorants specifically bind to a certain OR is called deorphanization^{40,41}. The deorphanization of ORs is a crucial step to understand several aspects of the smelling sensory process, including the complete knowledge of an OR's activity, the impact of the physicochemical properties of odorant molecules in an OR's selectivity, and the way in which information is accurately processed to generate the recognized pattern that culminates in the final olfactory perception⁴¹. These studies must take into account the ability of an odorant to be recognized by multiple ORs, and the fact that a specific OR can recognize several odorants. Some ORs have already been isolated and applied to gas-sensing biosensors.

OBPs are an essential component of the olfactory system. They are in the first line of olfaction, located in the nasal epithelium of mammals (nose) and in the sensillar lymph of insects (e.g., antennae)^{47,48}. Less soluble VOCs are believed to first bind to OBPs that carry them to the ORs in the olfactory sensory neurons. More hydrophilic VOCs are able to cross the mucosa or lymph to bind directly to ORs. Furthermore, OBPs are believed to act as scavengers when VOCs are present in high amounts.

Mammalian OBPs belong to the lipocalin superfamily of proteins, transport proteins^{42–44} that are 150–160 residues in length⁴⁵ and have typical lipocalin-fold of eight anti-parallel b-sheets and a short a-helix close to the C terminal. The b-sheets form an antiparallel b-barrel shaping a central pocket for ligand binding⁴⁶. Vertebrate and insect classes of OBPs differ in their 3D structure. Insect OBPs present well-conserved folding with protein chains of 130–150 amino acids⁴⁷. The tertiary protein structure comprises a compact set of six a-helices with a hydrophobic cavity. Additionally, the folding is stabilized by the presence of three disulfide bonds^{47,48}. These disulfide bonds are considered the fingerprint of insect OBPs. Notwithstanding amino acid variability, the overall structure of insect OBPs is highly conserved, even among members of different orders of insects^{47–49}.

OBPs can withstand high temperatures before experiencing denaturation, and after unfolding they frequently refold, restoring their initial structure⁴⁴. Interestingly, when binding high-affinity ligands, the stability of pig OBP (pOBP) increases at higher temperatures⁵¹. Insect OBPs are even more resistant, as the three interlocked disulfide bonds prevent proteolytic degradation and thermal denaturation^{44,47}.

It is important to merge structural information on OBP–VOC interaction for protein design and engineering towards target VOCs of interest, which currently still remains a challenge due to the limited structural information available for a wide variety of VOC structures (Figure 1.4).

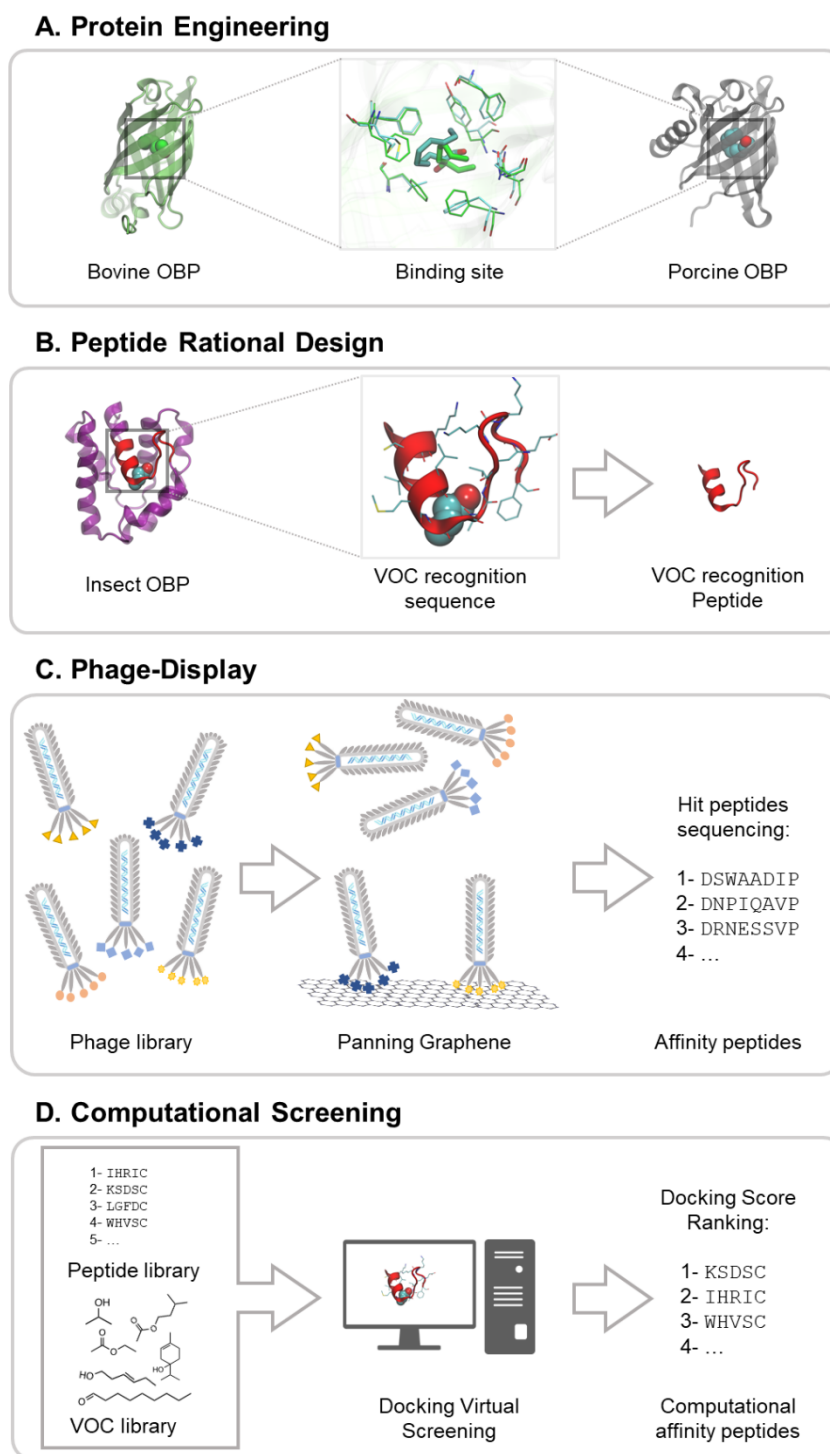


Figure 1. 4 – Superimposition of the binding pockets of bovine and porcine odorant-binding proteins (OBPs) reveals how small differences in residue composition lead to different volatile organic compound (VOC) affinities. (B) The extraction of volatile binding sequence from the fruit fly OBP LUSH, as a way to rationally develop peptides for VOC detection. (C) Phage-display panning screens a multitude of peptide sequences against a graphitic surface mimicking benzene. (D) Computational techniques predict the best peptide–VOC pairs to be experimentally tested.

To tailor proteins and peptides for binding target VOCs, several protein engineering techniques may be applied (Figure 1.4). Predominant techniques involve site-specific protein mutations (Figure 1.4A), the design of peptides inspired by the VOC recognition sites of

olfactory receptors and odorant-binding proteins (Figure 1.4B), and screening of peptide libraries by phage display and virtual screening approaches (Figure 1.4C,D).

A detailed analysis of the VOC-binding pocket from OBPs reveals a close similarity among the binding site of OBPs from different organisms, such as pig and bovine OBPs (Figure 1.4A), even though the target VOC is different. These properties led to the development of sensor arrays including OBPs from different organisms and mutated OBPs, in order to better discriminate detected VOCs^{52,53}. An example mutation is the bovine double mutant described to increase the sensitivity of bOBP to octenol and carvone⁵².

Peptides are promising alternatives to ORs and OBPs as molecular recognition units in VOC sensing. Due to their smaller size (ranging from approximately 5 to 15 protein residues), they can be synthetically or biologically produced, and represent a simple and low-cost option for biosensors, that is usually easier to immobilize in an oriented and predefined way onto sensor surfaces. Additionally, the selectivity towards target VOCs is easier to tune^{21,50}. Peptides are also somewhat more stable than ORs and more resilient to reusability and shelf-life. Another approach consists of a peptide that was rationally designed by extracting the sequences responsible for VOC recognition in ORs and OBPs. Successful examples have been reported for peptides derived from human OR1E1^{54–56} and dog OR-P30955^{57,58}. Three different peptides derived from insect OBPs were successfully produced and implemented in gas sensors to detect 3-methyl-butanol^{59,60}, 1-hexanol⁵⁹, and trinitrotoluene⁶¹ (Figure 1.4B).

Combinatorial screening techniques, such as phage display and virtual screening, can broaden peptide sequence variability and find sequences for specific VOCs. Phage display is implemented when a VOC analog is immobilized onto a surface or a VOC-analog surface is used to screen randomized peptides for binding^{62–65} (Figure 1.4C).

Computational virtual screening of peptide libraries is also used in the discovery of VOC-binding peptides^{66–68} (Figure 1.4D). The top-performing peptides are usually synthesized and experimentally tested in gas sensing, with satisfactory correlation between *in silico* and experimental data^{66,68}.

VOC-sensing peptides have been developed by different approaches (Figure 1.4), and applied in several substrates and sensing devices, as summarized in Table 1.1.

Table 1. 1 – Peptide-based sensors in gas sensing.

Peptide	VOCs	Support	Transducer	LDL/ Meas- ured*	Development	Ref
NQLS- NLSFSDL C (dORp61) Dog OR - P30955	Trimethylamine (TMA); ammo- nia; acetic acid; ethyl acetate; methanol	Gold Surface	Piezoelectric multi-array analyzer	-	OR-based De- sign	⁵⁷

ACSDAQ VNE (dORp188) Dog OR - P30955	Trimethylamine (TMA); ammonia; acetic acid; ethyl acetate; methanol; benzene			-		
FLS-NLSFSDLC (hORp61) human OR1E1	Trimethylamine (TMA)	Gold Surface	Piezoelectric multi-array analyzer	6.7 Hz	OR-based Design	54
FLLFGC (hORp103) human OR1E1	O-xylene			5.1 Hz		
DLESFLC (hORp109) human OR1E1	Ammonia	Gold Surface / Silicon (SiNW)	Piezoelectric multi-array analyzer / Conductance	11 Hz / 100ppm [*]		54,70
RVNEWVIC (hORp193) human OR1E1	Acetic acid	Gold Surface / Gold Surface / Silicon (SiNW)	Piezoelectric multi-array analyzer / QCM / Conductance	28 Hz / 3.1±1.2 ppm / 100ppm [*]		54,55, 70
LHYTTIC (PAC1) Dog OR - P30955	Acetic acid; butyric acid	Gold Surface	QCM (Multi-array)	-	OR-based Design	58
TIMSPKL C (PAC2) Dog OR - P30955	Acetic acid; butyric acid					
DLESC (PAM1) Human OR1E1	Dimethyl amine					
ELPLGCG (PAM2)	Acetic acid; dimethylamine					
SLMAGTV NKKGEFC	3-methyl-1-butanol; 1-hexanol	Gold Surface	QCM	1 – 3 ppm	OBP-based Design	71

(LUSH OBP)						
TKCVSL-MAGTVN KKGEFF F (LUSH OBP)	3-methyl-1-butanol	CNT	Field-Effect transistor	1 fM	OBP-based Design	59
HSSYWYA FNNKTG GGGWFVI (P1-ASP1C peptide) (Honeybee Antenna OBP)	Trinitrotoluene (TNT)	SWCNT	Field-Effect transistor	12 ppb	OBP-based Design	72
WHYQRP LMPVSI (TNT-BP)	Trinitrotoluene (TNT)	Gold Surface	Thermal desorption GC-MS	-	Phage Display	62
HPNF-SKY-ILMPVSI (DNT-BP)	2,4-Dinitrotoluene (DNT)					
CH ₃ ONH-KMHTAS LSQPLMG C-CONH ₂ (DNT-BP1C)	2,4-Dinitrotoluene (DNT)	Gold Surface	QCM liquid phase/ microcantilever	- /431ppt	Phage display	63,65
DSWAADIP (GP1)	Benzene	Gold Surface	Microcantilever	121 ppb	Phage Display	64
DNPIQAVP (GP2)	Toluene; xylene; hexane			2.2 ppm (toluene); 28 ppm (xylene); 1 ppm (hexane)		
DRNESSVP (BP1)	Benzene; toluene			-		
AYSSGAP PMPPF (A3)	Acetonitrile; dichloromethane; methyl salicylate	Gold Nano Particles	inductor-capacitor-resistor resonators	-	Phage Display	73
NFQGI			QCM			67

NFGGQ	2,3,7,8-TCDD (2,3,7,8-tetrachlorinated dibenzo- <i>p</i> -dioxin)	Gold Surfaces		10-20ppb*	Rational Design (Virtual Screening)	
NFQGF						
CG	2-propanol; acetone; acetonitrile; butane-2,3-dione; ethanol; ethyl acetate; ethyl butanoate; ethyl octanoate; hex-3-en-1-ol; hexane; isopentylacetate; nonanal; octanal; terpin-4-ol	Gold Nano Particles	QCM	Sensor array (discriminates all tested VOCs)	Rational Design (Virtual Screening)	45/46
ECG (Glutathione)						
CIHNP						
CIQPV						
CRQVF						
IHRIC	Terpinen-4-ol>hexane>octanal>nonanal	Gold Nano Particles	QCM	Sensor array (discriminates all tested VOCs)	Rational Design (Virtual Screening)	74
KSDSC	Small alcohols (2-propanol; ethanol)					
LGFD	Ethanol, 2-propanol; acetone; butane-2,3-dione; ethyl acetate					
TGKFC	Alcohols (hex-3-en-1-ol; terpinen-4-ol) and aldehydes (nonanal; octanal)					
WHVSC	Esters (ethyl acetate; ethyl butanoate; ethyl octanoate; isopentyl acetate) and hexane					
IHRIC	Alcohols (1-butanol; 1-hexanol; 2-methyl-1-propanol; ethanol; hexen-3-en-1-ol) and esters (ethyl acetate; ethyl-methyl-2-butyrate; isopentyl acetate)	Zinc oxide nanoparticles	QCM	Sensor array (discriminates all tested VOCs)	Rational Design (Virtual Screening)	75
LAWHC						
TGKFC						
WHVSC						

In artificial olfaction, though, the combination of biological recognition units has not yet reached its full potential, in some cases due to the difficulty in establishing VOC markers from complex mixtures⁶⁹. However, adapting protein engineering, phage display, and computational tools to address the molecular recognition between biological binders and analytes in gas phases has made tremendous progress and has matured over the last 15 years. As such, the tools to develop VOC binders are in place, as well as the methodologies to interface the bioreceptors with gas-sensing devices².

An investment in the expansion of tested VOCs for the development of gas biosensors is required, as recent examples report a very limited number and chemical diversity of VOC targets. In almost all areas of VOC sensing, gas samples are rich in the variety of chemical entities, and it is essential to address molecular discrimination in current and future research. The increasing improvements in protein and peptide engineering may be very useful to increase the stability of biomolecules (for example, through the use of nanodiscs) or to expand the variability in generating binders against less common VOCs (for example, through the molecular modeling and in vitro evolution techniques).

With the developments in volatolomics, we expect that the implementation of gas-phase bio-sensors to address biological questions will become a reality in the near future. High-impact social and economic areas will be the central application of this technology. Early disease diagnostics and health monitoring, pollutant and fire hazards in the environment, spoilage and quality monitoring in food and agriculture, explosive and chemical/biological weaponry detection in safety, and odor identification in prosthetics are just some potential examples for gas biosensing. Overall, the development of protein and peptide VOC biosensors will be important to attain the desired sensitivity and selectivity in VOC detection, either in single-gas sensors for a defined analyte or in arrays of gas-sensing materials coupled to artificial intelligence tools for signal processing.

1.3 Ionic Liquids and derived materials in gas sensing

Other than adding biological units – based on the human olfactory system – other components that built the sensing materials can be manipulated, in order to tune selectivity towards gas-phase analytes.

Ionic liquids (ILs) are organic salts that are liquid below 100°C, and possess low melting temperatures. They consist only of ions, a positive (cation) and a negative (anion) component bound together by electrostatic interactions, which actually allows a freedom of movement^{76,77}. Some important features of ILs include high electrochemical and thermal stability, negligible vapor pressure and ionic conductivity. One of the most attractive aspect of ionic liquids is that their physicochemical properties can be tuned by modifying the anion-cation pair, leading to innumerable possible structures, tailored for almost any electrochemical application^{77,78}. Representative structures combine organic cations such as imidazolium, pyridinium, pyrrolidinium, piperdinium, ammonium, phosphonium and organic/inorganic anions e.g.

carboxylate, azolate, phenoxide, proline, thionate, halide, tetrafluoroborate, hexafluorophosphate⁷⁹.

These unique aspects exhibited by ILs are promising for the development of gas sensitive sensors⁸⁰. Ionic liquids are commonly used in the detection of gas analytes such as oxygen^{81–85}, carbon dioxide^{86–88}, sulfur dioxide^{89,90}, hydrogen chloride⁹¹ amongst other volatiles^{92,93}.

Usually the use of Ionic Liquids as part of sensing materials, requires their stabilization onto sensors' surface, due to their natural liquid state⁹⁴. Typically, the IL can be immobilized in a polymeric matrix. The net result is a gel material defined as ionogel, similar to the term for hydrogels which refers to a gel in which the entrapped liquid is water. Ionogels combine the properties of both the polymer matrix and the corresponding IL^{76,77}. These ionogels can serve as sensors for gas sensing devices, by measuring the changes in conductance of the gel due to interaction with the gas analyte (electrical signal), and they are generally deposited in planar substrates as previously mention, like platinum electrodes by drop casting⁹⁵ or onto gold interdigitated electrodes (IDE) either by drop casting⁹⁶ or spin coating^{97,98}. Furthermore, the use of ionogels for gas sensing purposes has been proved to provide higher sensitivity when compared to the use of the pure ionic liquids^{96,99}.

Several studies report the chemoselective gas sensing potential of ILs^{100–102} and especially ionogels^{103–105}. Ion jelly, a stable and fairly conductive gel (up to 10^{-3} S cm⁻¹) resulting from the combination of an ionic liquid component with gelatin, successfully detected and classified eight different volatiles when tested in an electrical e-nose (that is a device that continuously monitors the conductance of the sensors)^{106,107}. Zhu et al.⁹⁹ studied imidazolium-based ionogels with a silica gel matrix. The gels produced a fast and reversible response when exposed to seven different VOCs, due to the change in ion mobility. Also, on the field of exploring ILs' electric conductance in gas sensing devices, some designs comprising a membrane-free micro-electrode modified with layers of ILs to study the transport-limited currents and response times of different types of amperometric gas sensor have been explored⁸¹. The incorporation of other functional molecules in the ionogel matrix can endow the resulting gel with additional properties and features, leading to numerous possibilities in the design of new sensing materials⁷⁷. The existing reports concerning the use of ionogels in gas sensing applications are summarized in Table 1.2.

Table 1. 2 – Examples of ionogels in gas sensors and electronic nose systems.

Ionic Liquid	Polymer	VOCs	Transduction mechanism	Ref.
[EMIM][EtSO4]	pNIPAAm	Benzene, Formaldehyde	Conductimetric (electrical e-nose)	96
[EMIM][NTf2]	PMMA	Oxygen	Voltammetry	94
[EMIM][NTf2]	PVDF	Nitrogen dioxide	Amperometric	108

[BMIM][CF ₃ SO ₃] [EMIM][BF ₄] [HMIM][FAP] [BMPyr][NTf ₂]				
[EMIM][BF ₄]	PVDF-HFP	Carbon dioxide	Impedance	95
[EMIM][DCA]	Agar Gelatin Sodium alginate	Methanol, Ethanol, Acetone, Ethyl acetate, Hexane	Conductimetric (electrical e-nose)	98
[EMIM][DCA] [BMIM][DCA] [BMPyr][DCA] [BMIM][Br]	Gelatin	Organic vapors (ethyl acetate, acetone, chloroform, ethanol, hexane, methanol, toluene and water)	Conductimetric (electrical e-nose)	109
BmimNTf ₂ BmimOTf BmimClO ₄ VOImPF ₆ P _{6,6,6,14} Triz BmimPF ₆ BmimCF ₃ CO ₂ BmimBF ₄ EPL	TEOS MTMS	Dichloromethane, ethanol, ethyl acetate, hexane, methyl ethylketone, toluene, trichloroethylene and butanone	Conductimetric (electrical e-nose)	99

[EMIM][EtSO₄], 1-ethyl-3-methylimidazolium ethyl sulfate; [EMIM][NTf₂], 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, [BMIM][CF₃SO₃], 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [EMIM][BF₄], 1-ethyl-3-methylimidazolium tetrafluoroborate; [HMIM][FAP], 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate; [BMPyr][NTf₂], 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide; [EMIM][DCA], 1-ethyl-3-butylimidazolium dicyanamide; [BMIM][DCA], 1-butyl-3-methylimidazolium dicyanamide; [BMPyr][DCA], 1-butyl-1-methylpyrrolidinium dicyanamide; [BMIM][Br], 1-butyl-3-methylimidazolium bromide; BmimNTf₂ (1-Butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide); BmimPF₆ (1-Butyl-3-methylimidazolium hexafluorophosphate); BmimOTf (1-Butyl-3-methylimidazolium trifluoromethanesulfonate); BmimClO₄ (1-Butyl-3-methylimidazolium perchlorate); VOImPF₆ (3-vinyl-1-octyl-1H-imidazol-3-ium hexafluorophosphate); BmimCF₃CO₂, (1-Butyl-3-methylimidazolium trifluoroacetate); BmimBF₄, (1-Butyl-3-methylimidazolium tetrafluoroborate); EPL (N-ethyl piperazinium lactate); P_{6,6,6,14}Triz (Trihexyl(tetradecyl)phosphonium triazole); pNIPAAm, poly(N-isopropyl acrylamide); PMMA, poly(methyl methacrylate); PVDF, Polyvinylidene fluoride; PVDF-HFP, polyvinylidene fluoride-co-hexafluoro propylene; TEOS (Tetraethyl orthosilicate); MTMS (methyltrimethoxysilane).

1.4 Liquid Crystals and derived materials in gas sensing

Liquid crystals (LC) are a state of matter intermediate between that of a crystalline solid and a conventional liquid. When in the liquid crystal state, the molecules are mobile, much like an isotropic liquid but also have significant orientational order and in some cases positional order. These materials are probably best known for their ability to manipulate the polarization of light. Their molecular organization can be easily perturbed by external influences such as electric or magnetic fields, surface effects and chemical analytes. Several researches focused on exploring the use of Liquid crystals-based systems in gas sensing applications have been emerging over the last years^{110–114}. In sensors-based LC devices, using Cyanobiphenyl-based LCs (such as 5CB) deposited on surfaces, the LC assumes a homeotropic orientation due to coordination interactions between the nitrile group of 5CB and the cation of the metal salt (serving as a binding receptor)³⁵. When an analyte with affinity toward the substrate¹¹⁵ is introduced in this environment, it will interact and adsorb to the surface of the substrate. Subsequently, the adsorption will disrupt the coordination interactions between the LC and the functionalized substrate, triggering an orientational transition within the LC system³⁵. A summary of liquid crystal-based gas sensing devices to detected volatiles is presented in Table 1.3.

The potential of using combined systems of Ionic liquids and Liquid Crystals to be explored as gas sensing materials was already reported, where a novel formulation for a gas sensitive multicomponent gel – hybrid gels – was developed^{116–118}. The gels are procured by self-assembled liquid crystal (LC) droplets confined in ionic liquid interfaces and dispersed within a gelatin matrix, a non-conductive polymer. The overall composition promotes physical compartmentalization within the gel. The IL promotes the anchoring of the LC in a spherical geometry, due to the dynamic balance of hydrophilic and hydrophobic intermolecular interactions. It also helps in the dissolution of the biopolymer through ionic, electrostatic and hydrogen bonding with the polymer chains^{117,118}. On the other hand, the gelatin stabilizes the droplets. Each component has an integral role in the final product, which exhibits ionic conductance due to the IL and also interesting optical properties stemming from the presence of the LC component. When the gas analytes adsorbs to the hybrid gels, the interaction leads to the disordering of the LC molecules (isotropization), which culminates in optical changes that can be seen through Polarized Atomic Microscopy. These interactions are usually very fast and reversible, and upon the interaction of the analyte with the gel, the LC molecules undergo reorganization, leading either to a director reorientation or a phase transition.

Table 1. 3 – Liquid Crystal-based gas sensing devices using solid functionalized surfaces.

Liquid crystal	Sensing Material	VOCs	Transduction method	Ref.
5CB	LC film deposited onto chemically modified gold coated glass substrate with SAMs of MUA, covered with metal salts with mixed anions	DMMP	Optical (POM)	119

Liquid crystal	Sensing Material	VOCs	Transduction method	Ref.
5CB	LC film deposited onto gold coated glass substrate chemically modified with MUA and aluminium perchlorate	DMMP	Optical (POM)	120,121
5CB	LC thin film supported on a micro-pillar array [†] modified with copper perchlorate	DMMP	Optical (POM)	122
5CB	LC film assembled on top of micro-groove patterned amino-terminated organic substrate chemically modified with copper perchlorate salts	DMMP	Optical (POM)	123
5CB	LC layer supported on a thiol sensitive layer containing metal ions	Thiol vapors	Optical (POM)	124
5CB	LC thin film supported on a micro-pillar array [‡] modified with copper perchlorate	DMMP	Optical (POM)	125
5CB	Thin layer of LC on an AEAPS functionalized DMOAP-coated glass slide	Glutaraldehyde	Optical (POM)	115
5CB	LC film deposited onto chemically modified gold coated glass substrate with SAMs of MUA, covered with different metal perchlorate salts	DMMP	Optical (POM)	126
5CB	LC film between gold substrates modified with mixed SAMs containing –COOH functionalities	n-Hexylamine	Optical (POM)	127
5CB	LC film between gold substrates modified with mixed SAMs containing –COOH functionalities (1.) or deposited on chemically modified gold surfaces treated with copper perchlorate (2.)	Hexylamine (1.) DMMP (2.)	Optical (POM)	128
5CB	LC film on top of chemically modified glass substrate with chitosan-copper perchlorate	Ammonia	Optical (POM)	129
5CB	LC film on glass substrates cover with manganese perchlorate	Chlorine gas	Optical (POM)	130
5CB	LC thin film between glass substrates covered with metal salts	DMMP	Optical (POM)	131
5CB	LC film on the bottom of polymeric wells modified with aluminium perchlorate	DMMP water	Optical (POM)	132
5CB	LC film on the bottom of polymeric wells modified with metal perchlorates	DMMP water	Optical (POM)	133
5CB	Thin films of LC supported on chemically patterned micro-wells in glass, gold and polystyrene substrates	Toluene	Optical (POM)	134

Liquid crystal	Sensing Material	VOCs	Transduction method	Ref.
5CB	LC functionalized PVP fiber mats	Toluene	Optical (POM and Naked eye)	135

#Copper perchlorate chemically functionalized gold coated nickel micro-pillar glass slides.

\$Nickel micro-pillar on top of aluminium perchlorate chemically functionalized gold coated glass slides.

Overall, these gel formulations exhibit a unique functionality. Gas analyte detection can be achieved either via electrical or optical means, depending on the e-nose platform. More importantly, due to their combinatorial nature diverse gel compositions can be obtained, facilitating VOC selectivity.

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THE IMPACT OF IONIC LIQUID CATIONIC MOIETY ON CHEMOSELECTIVE ARTIFICIAL OLFACTION

Abstract

Ionogels and derived materials are assemblies of polymers and ionic liquids characterized by high stability and ionic conductivity, making them interesting choices as gas sensors. In this work, we assessed the effect of the ionic liquid moiety to generate iono- and hybrid gels as electrical and optical gas sensors. Six ionic liquids consisting of a constant anion (chloride) and distinct cationic head-groups were used to generate iono- and hybrid gels, and further tested as gas sensors in customized electronic nose devices. In general, ionogel-based sensors yielded higher classification accuracies of standard volatile organic compounds, when compared to the hybrid materials-based sensors. In addition, the high chemical diversity of ionic liquids is further translated to high functional diversity in analyte molecular recognition and sensing.

2.1 Introduction

Ionic liquids (ILs) are organic salts that consist only of ions, a positive (cation) and a negative (anion) bound together via electrostatic interactions. Some important features of ILs include high electrochemical and thermal stability, negligible vapor pressure and high ionic conductivity. One of the most attractive aspects of ILs is that their physicochemical properties can be tuned by modifying the anion-cation pair, leading to innumerable possible structures, tailored for different applications. ILs can be immobilized in polymeric matrices, giving rise to iono-based materials as ionogels, that combine the properties of both the polymer matrix and the corresponding IL¹.

Several studies report the chemoselective gas sensing potential of ILs²⁻⁴ and especially ionogels^{5,6}. Typically, ionogel-based gas sensors yield fast and reversible response when

exposed to different VOCs, which is mainly attributed to changes in ion mobility. The incorporation of other functional molecules in the ionogel matrix, as for example liquid crystal (LC) molecules, can endow the resulting gel with additional properties and features, leading to numerous possibilities in the design of new sensing hybrid materials and additional optical transduction mechanisms⁷.

In this study, we explore the potential of six chloride-based ILs with distinct cationic moieties to generate sensors for VOCs discrimination. We produced gelatin-based ionogels, which yield an electrical signal; and hybrid materials which additionally have a liquid crystal (5CB), and that yield an optical signal. The chloride-based ILs studied were 1-Butyl-1-methylpiperidinium (BMPip), 1-Butyl-1-methylpyrrolidinium (BMPyrr), 1-Butylpyridinium, 1-Butyl-2-methylpyridinium and 1-Butyl-4-methylpyridinium (BuPy, Bu₂Pic and Bu₄Pic respectively), and 1-Butyl-3-methylimidazolium (BMIM). The BMIM cation was chosen due to the fact that it has already been reported as gelatin-based ionogels and hybrid gels materials, which generate both electrical and optical responses in the presence of gas analytes^{8–10}. The 4-carbon chain of the cation was maintained constant – when combined with the cation's aromaticity, contribute to low viscosities and high conductivity in IL materials¹¹, and it is also a key element in the stabilization of self-assembled spherical interfaces encapsulating LC droplets¹². The gas sensing capabilities of the materials were tested in tailor-made electrical and optical electronic noses (e-noses), and the VOC classification capabilities were evaluated using an automatic classifier based on Support Vector Machines (SVM). We show that the production and use of sensing materials with distinct compositions and transduction mechanisms lead to increased selectivity and discrimination of different VOCs, which can be combined in an array to further advance the field of artificial olfaction¹³.

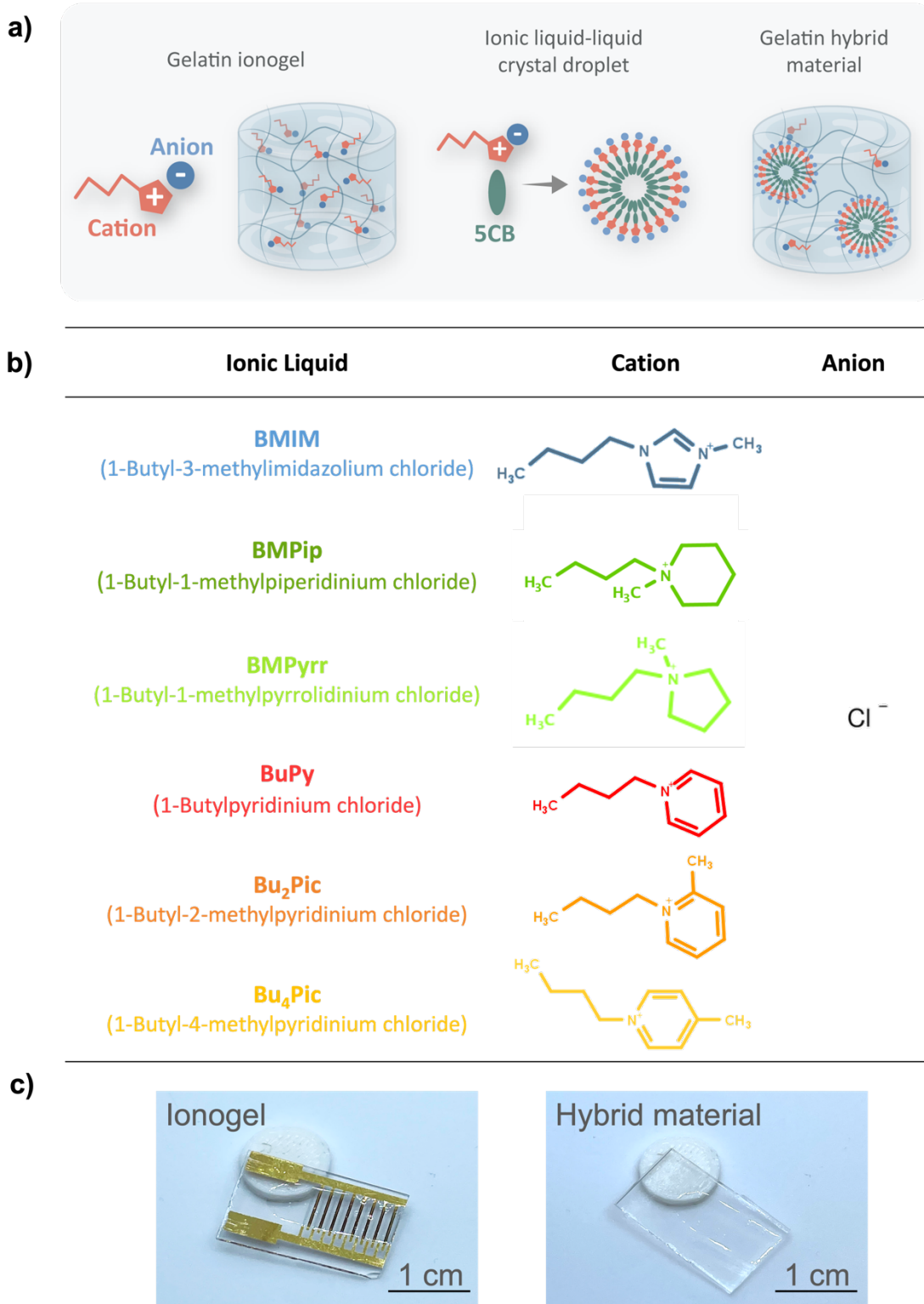


Figure 2. 1 – a) Schematic representation of the materials used in this study; 5CB stands for the liquid crystal 4-cyano-4'-pentylbiphenyl. (b) Chemical structures of the six different ionic liquids used in this study. (c) Demonstrative picture of the gelatin ionogels and hybrid materials used as electrical and optical gas sensors, respectively.

2.2 Materials and Methods

2.2.1 Materials and reagents

Gelatin from bovine skin (gel strength ≈ 225 g; Bloom, Type B), was purchased from SigmaAldrich. The liquid crystal 4-cyano-4'-pentylbiphenyl (5CB), was acquired from TCI Europe, and the ionic liquids 1-Butyl-3-methylimidazolium chloride ([BMIM][Cl], $>98\%$), 1-Butyl-1-methylpiperidinium chloride ([BMPip][Cl], 99%), 1-Butyl-1-methylpyrrolidinium chloride ([BMPyrr][Cl], 99%), 1-Butylpyridinium chloride ([BuPy][Cl], 99%), 1-Butyl-2-methylpyridinium chloride ([Bu₂Pic][Cl], 99%) and 1-Butyl-4-methylpyridinium chloride ([Bu₄Pic][Cl], 99%) were purchased from IoLiTec. Dichloromethane (DCM) and hexane were purchased from VWR. Ethanol and acetic acid (purity 99.8%) was purchased from Sigma-Aldrich. Acetonitrile (purity 99.9%), chloroform, diethyl ether (HPLC grade), ethyl acetate, heptane, methanol (HPLC grade), and toluene were supplied by Fisher Scientific. Acetone (purity 99.5%) was purchased from Honeywell, and isopropanol (purity 99.5%) from ROTH. MilliQ water was used.

2.2.2 Production of electrical and optical sensors

For the production of both ionogels and hybrid gels, the protocol described in previous works^{8,10} was followed. The mixture was spread onto gold interdigitated electrodes deposited on glass slide, using an automatic film applicator equipped with a heated bed and a quadruplex with a predefined thickness of $30\text{ }\mu\text{m}$ (TQC Sheen)¹⁰. Hybrid gel sensors were prepared by spreading the gel on an untreated glass slide using an automatic film applicator equipped with a heated bed and a quadruplex with a predefined thickness of $30\text{ }\mu\text{m}$ (TQC Sheen). After production of the both the ionogels and hybrid materials, the sensors were left resting for 24h before use.

2.2.3 Characterization of electrical and optical sensors

The ionic conductivity of the ionogels was determined by Electrochemical Impedance Spectroscopy using a potentiostat Gamry Instruments – Reference 3000 (measurement conditions: frequency range from 100 kHz – 0.1 Hz and V_{pp} 100 mV (AC)) as previously described⁹. The conductivity was calculated according to the equation $\sigma = (1/R) * (l/A)$, where σ represents the conductivity, R is the resistance; l is the thickness of the sample and A the area of the sample. Experimental data were collected in duplicates.

The morphology of the hybrid gels was firstly assessed after production by polarized optical microscopy (POM) in a Zeiss Axio Observer.Z1/7 microscope equipped with an Axio-cam 503 color camera and ZEN 2.3 software for image acquisition and processing. Images were taken under crossed (at 90°) polarizers, as well as without polarizers (bright field). The optical textures of the hybrid materials were further monitored over one month to assess the stability to storage at ambient conditions. An area of interest was defined in each of the hybrid gels and

POM photos using crossed polarizers were taken right after production, and then every week. The hybrid materials were also characterized by scanning electron microscopy with a Carl Zeiss AURIGA CrossBeam (FIB-SEM) workstation coupled with energy dispersive X-ray spectroscopy (EDS). The gels were previously coated with an Ir and Au/Pd conductive film to avoid charge effects.

2.2.4 VOC discrimination using electrical and optical e-nose devices

Both ionogels and hybrid materials were used as gas sensors in tailor-made e-noses. The electrical and optical e-nose versions have a common design and operation. Detailed information on the e-nose setup have been described in previous publications^{6,9,10}. In the electrical version, the output of the signal acquisition module is the voltage across each sensor, which is inversely proportional to their conductance. In the optical device, the sensors are placed between crossed polarizers and paired with a light emitting diode and a light-dependent resistor (LDR) to monitor light transmittance. In both versions, the sensor films are placed in the detection chamber which has 12 or 6 slots in the electrical and optical e-nose device, respectively. These are then sequentially exposed to the vapors of 12 different VOCs in the same order (heptane, hexane, toluene, chloroform, dichloromethane, diethylether, ethylacetate, acetone, acetone-trile, ethanol, methanol, acetic acid), by placing the solvents in a water bath and heated at 37° C. For each VOC experiment the tested films are exposed to analyte vapors for 45 consecutive cycles, each cycle consisting of 5 sec of VOC exposure, followed by 10 sec of recovery with ambient air. For each studied material composition three batches were produced, and triplicates of each batch were tested to ensure reproducibility of results.

The signals obtained from both electrical and optical e-nose experiments were further processed using data analysis tools based on Python libraries (SciPy, sklearn and novanistrumentation). Subsequently, feature selection and classification algorithms were implemented using Support Vector Machine (SVM), following the procedures previously detailed^{6,8-10,14-16}, and the classification results for all the tested sensors formulations were presented in normalized confusion matrices and accuracy plots.

2.3 Results and Discussion

2.3.1 Ionogels and hybrid materials composed by distinct ionic liquids

In this work, chloride-based ionic liquids (ILs) with cation groups from distinct families were evaluated to produce self-assembled gelatin materials (Figure 2.1). Firstly, gelatin was dissolved and gelled with the studied ILs yielding a set of six distinct ionogels. Secondly, the formulation included the presence of the liquid crystal 4-cyano-4'-pentylbiphenyl (5CB) yielding hybrid materials with compartmentalized droplets containing organized liquid crystal molecules.

The ionic conductivity of the IL component is maintained in ionogels. Typically, a threshold of 1 mS cm^{-1} is required in order to implement an IL-containing material into an electrical device^{17,18}. Frequency-dependent ionic conductivity experiments were performed for all ionogel formulations, indicating that all act as ionic conductive materials, especially at 2000 Hz, which is the conductivity that the electrical e-nose performs the measurements during the VOC discrimination assays (Figure 2.2a and Table S2.1). For values higher than 10^3 Hz the ionic conductivity of the ionogels stabilizes. At 2 KHz the determined conductivity values followed the decreasing order $\text{BuPy} > \text{BMPip} > \text{Bu}_4\text{Pic} > \text{BMPyrr} > \text{BMIM} > \text{Bu}_2\text{Pic}$. Cations containing aromatic moieties, as those from pyridinium ILs, are known to turn materials less viscous, and therefore lead to great conductivity results¹⁹ – exception is observed for the Bu_2Pic member of the pyridinium family and for BMIM from the imidazolium family. Bu_2Pic has a methyl group at the ortho position, conferring a more hydrophilic character to the IL (confirmed by logP in Table S2.1), which can explain a slightly different conductivity when compared to the remaining members of the pyridinium family²⁰. In addition, the presence of the polymeric matrix can change conductivity of the materials, due to a less rigid entanglement of the ring within the polymer, therefore the multicomponent assembly apparently leads to higher conductivity in the non-aromatic cation of the piperidium IL¹⁹. A similar justification can be found to a lower conductivity in the case of the imidazolium IL, BMIM. These results are in accordance with the literature, where is stated that the presence of rings – whether they are aromatic or cyclic – contributes to different viscosities in the materials, and therefore different ionic mobility and ionic conductivity^{19,21,22}. Also, it is known that existence of methyl groups in the rings, placed in different positions, can also confer different hydrophobicity to the ILs²³.

With regards to the hybrid materials, and considering the optical transduction mechanism given by the presence of IL-LC droplets, we started by studying the morphological aspects of all the produced compositions via POM. In general, the gels present heterogeneous and polydispersed IL-LC droplets, which were stable over a week after production (Figure S2.1) and visible at the surface of the materials (Figure S2.2). Hybrid gels containing BMIM and BMPip displayed the largest droplets, over a wider range of diameter sizes, but predominantly between $15\text{--}20 \text{ }\mu\text{m}$ and $40\text{--}50 \text{ }\mu\text{m}$, respectively, followed by Bu_2Pic ($5\text{--}15 \text{ }\mu\text{m}$), and finally BMPyrr, BuPy and Bu_4Pic showing the smallest droplets ($5\text{--}10 \text{ }\mu\text{m}$) (Figure S2.3). It was observed that the LC inside the droplets has assumed a radial configuration, which is revealed by the Maltese cross pattern seen in POM images under crossed polarizers⁷. In Bright Field photos the characteristic core defect, named hedgehog, can be seen. The radial director profile is due to the homeotropic alignment provided essentially by the interactions of the LC with the cation of the IL. The presence of radial configuration of LCs is actually quite relevant, since it is showed to provide a fast and dynamic optical response upon exposure to VOCs¹⁰.

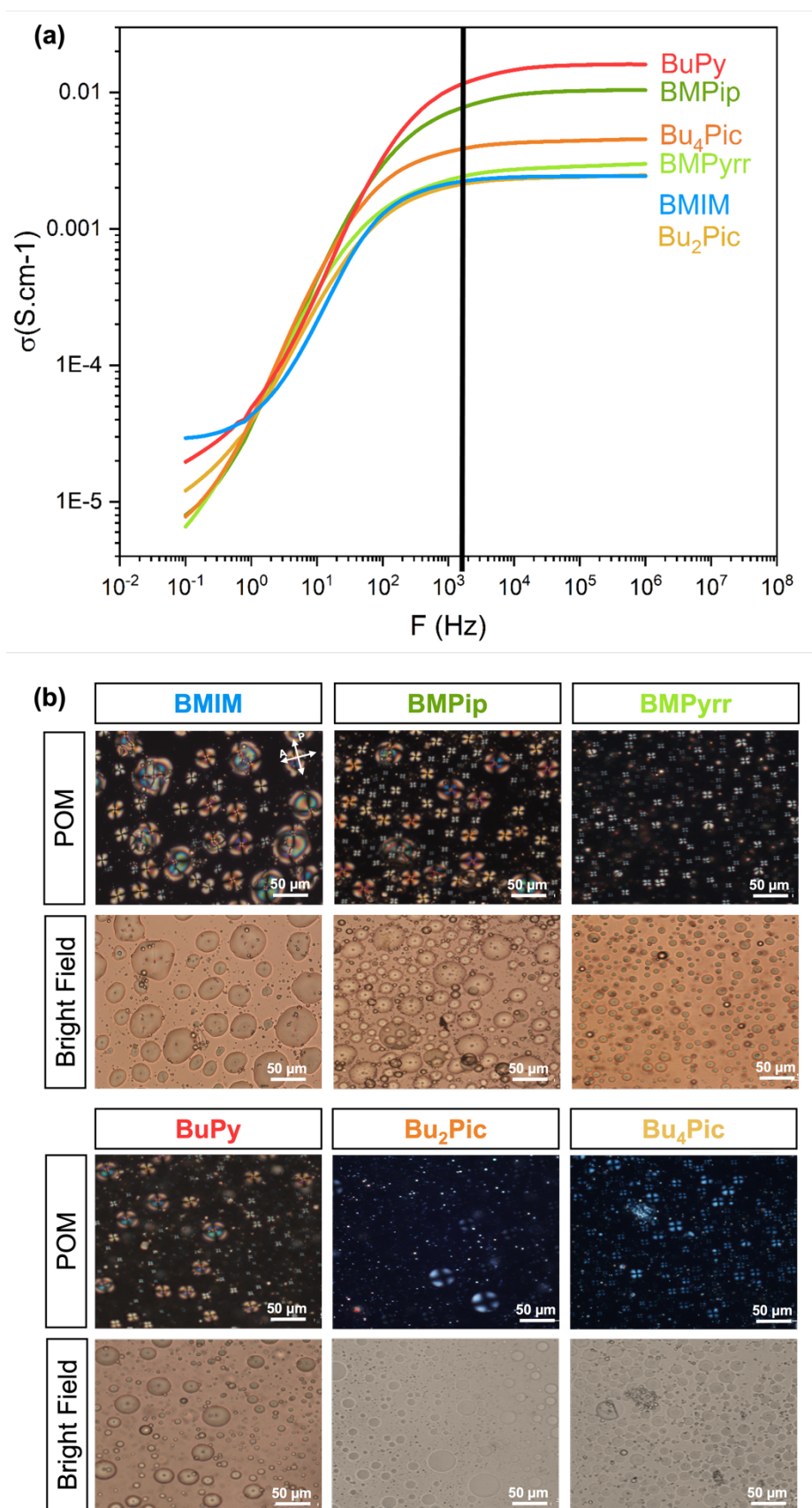


Figure 2. 2 – Characterization of the gelatin sensors produced in this work. (a) Conductivity results for all electrical ionogels. The conductivity at which the electrical e-nose performs the measurements during the experiments is

represented by the black vertical line. (b) Morphological characterization of the optical hybrid gels sensors: through POM and Bright Field for BMIM, BMPip, BMPyrr, BuPy, Bu₂Pic and Bu₄Pic sensors.

2.3.2 Ionogels as VOC-electrical sensors

In the electrical e-nose, films of ionogels spread on interdigitated electrodes are used as VOC sensors (Figure 3.1). When VOCs adsorb onto an ionogel sensor, there will be an increase or a decrease in the mobility of the IL ions present in the gel composition due to interactions between the VOC and the gelatin and IL moieties. As a result, the gels admittance change, the conductance for a fixed frequency can be measured, and an electrical response is obtained. This process is reversible upon VOC desorption¹⁵. The six different ionogel compositions were sequentially exposed to a set of VOCs from distinct chemical classes, generating a set of electrical signals characteristic of each ionogel composition – it should be noted that the signals deriving from these interactions are a collaborative response of all the individual components of each formulation. Representative cycles acquired for the tested VOCs on all the ionogel sensors are presented in Figure 2.3. All sensor responses were reversible and reproducible upon consecutive exposures, being that each VOC yielded typical and different signals (Figure 2.3). The aliphatic VOCs such as hexane and heptane, as well as methanol and acetic acid, yielded electrical signals with the lowest amplitudes. Typically, the sensors with the highest ionic conductivity values yield signals with higher amplitude (Table S2.1 and Figure 2.2). After signal collection, the data was analyzed using machine learning tools. Morphological features and parameters of curve fitting models¹⁶ were selected and treated by computational methods and used as input to train and validate an SVM-based classification algorithm. Through this SVM training method the algorithm was able to make predictions on unknown inputs. A Confusion Matrix is generated, representing the accuracy in which every volatile was predicted by each sensor during the experiment – for further and detailed study on the accuracy performance of the algorithm for each VOC please see Supplementary Information (Figure S2.4). To discuss further how accurately the sensors can classify and identify the volatiles, a summarized plot of accuracies (in percentage) can be seen in Figure 2.3. The highest overall accuracies provided by the ionogels sensors to predict the studied volatiles were registered for BMPip, BMPyrr and BuPy (88%, 83% and 81% respectively), followed by BMIM (69%), and the lowest ones were observed by Bu₄Pic and Bu₂Pic (46% and 14% respectively), both from the pyridinium family. Interestingly, the cyclic cation families of ILs (BMPip and BMPyrr) have similar behaviors in predicting VOCs, with high scores of accuracies. BMPip and BMPyrr contain cyclic moieties in their cationic part, and those tend to be more hydrophilic especially when combined with the presence of the Cl⁻ anion^{23–25}. BMIM, BuPy, Bu₂Pic and Bu₄Pic ILs contain aromatic rings in their cation part which confer a more hydrophobic character to the cation. For Bu₂Pic, the methyl group, placed in the ortho position within the cation structure²³, could form a second ring with the 4-carbon side chain, minimizing interactions with VOCs and yielding weaker signals. An exception to this was observed for methanol exposure. According to the literature the presence of the methyl group within the ortho position grants a more hydrophilic

character to the IL²³, which alongside with the small size of the (methanol) molecule, could explain the gel response.

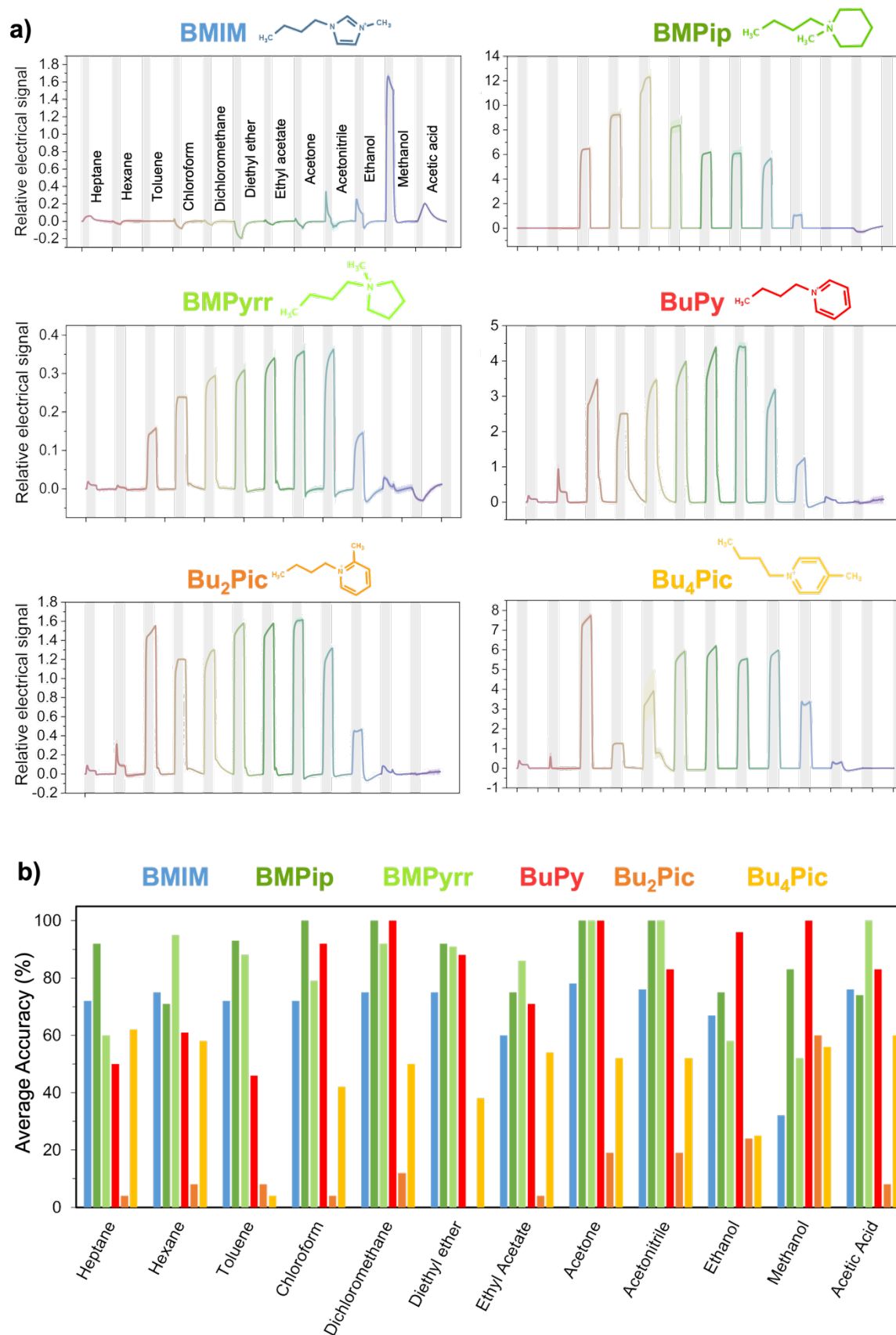


Figure 2. 3 – (a) Representative signals of the electrical amplitude responses of all gelatin ionogels to distinct VOCs. Each curve represents the average and standard deviation of at least 19 replicate cycles from the same sensor. VOC

exposure periods (5 s) are highlighted in grey, and each cycle corresponds in total to 15 s. The plots were generated by python. (b) Average accuracy scores from a Confusion Matrix analysis regarding the ability of each sensor to accurately predict each volatile being tested, during the electrical e-nose experiments.

2.3.3 Hybrid gels as VOC-optical sensors

The principle of VOC-analyte detection using the liquid crystal-based sensors is that the presence of the analyte influences the LC self-assembly triggering an orientational transition^{26–28}, a change in the helical pitch of short-pitch chiral systems^{29–31}, or even a complete loss of long-range-ordering^{10,32–34}. In the case of the hybrid gels the latter is typically observed as the adsorption of gas analytes onto the hybrid materials triggers 5CB transition to the isotropic phase. This is a reversible process since 5CB can recover its initial configuration upon removal of the volatile with ambient air, as it has been verified via POM observations¹⁰. In hybrid materials, the observed optical signal is a combined result of the VOC effect on the different gel components, namely the gelatin matrix, the ionic liquid, and the IL-LC droplets⁸.

The optical e-nose is designed to monitor the light transmittance of the hybrid ionogel sensors. Any event disrupting the LC molecular order will result in different light intensity, yielding a variation in the optical signal. These signals are essentially the output voltage of the photodiode that monitors the light exiting the system. Consequently, when the signal amplitude increases, which corresponds to the VOCs exposure period, is due the sensor's brightness decreasing. The experimental protocol for optical gas sensing was identical to the one followed for the electrical e-nose, and after signal's collection, the data was analyzed through the same machine learning algorithm as explained previously for the electrical e-nose process. Overall, all sensor responses were reversible, exhibiting an upwards trend upon gas analyte exposure, as well as a fast response profile to the volatiles. The representative cycles regarding the optical signals generated by the hybrid materials sensors for the set of 12 tested VOCs can be seen in Figure 2.4a, as well as the overall classification accuracy provided by each IL-sensor (Figure 2.4b). The obtained confusion matrices can be found in Supplementary Information (Figure S2.5). As already observed for the ionogel-based sensors, the ILs structural differences give distinct properties to the gels, contributing to different discrimination potential towards the various VOCs.

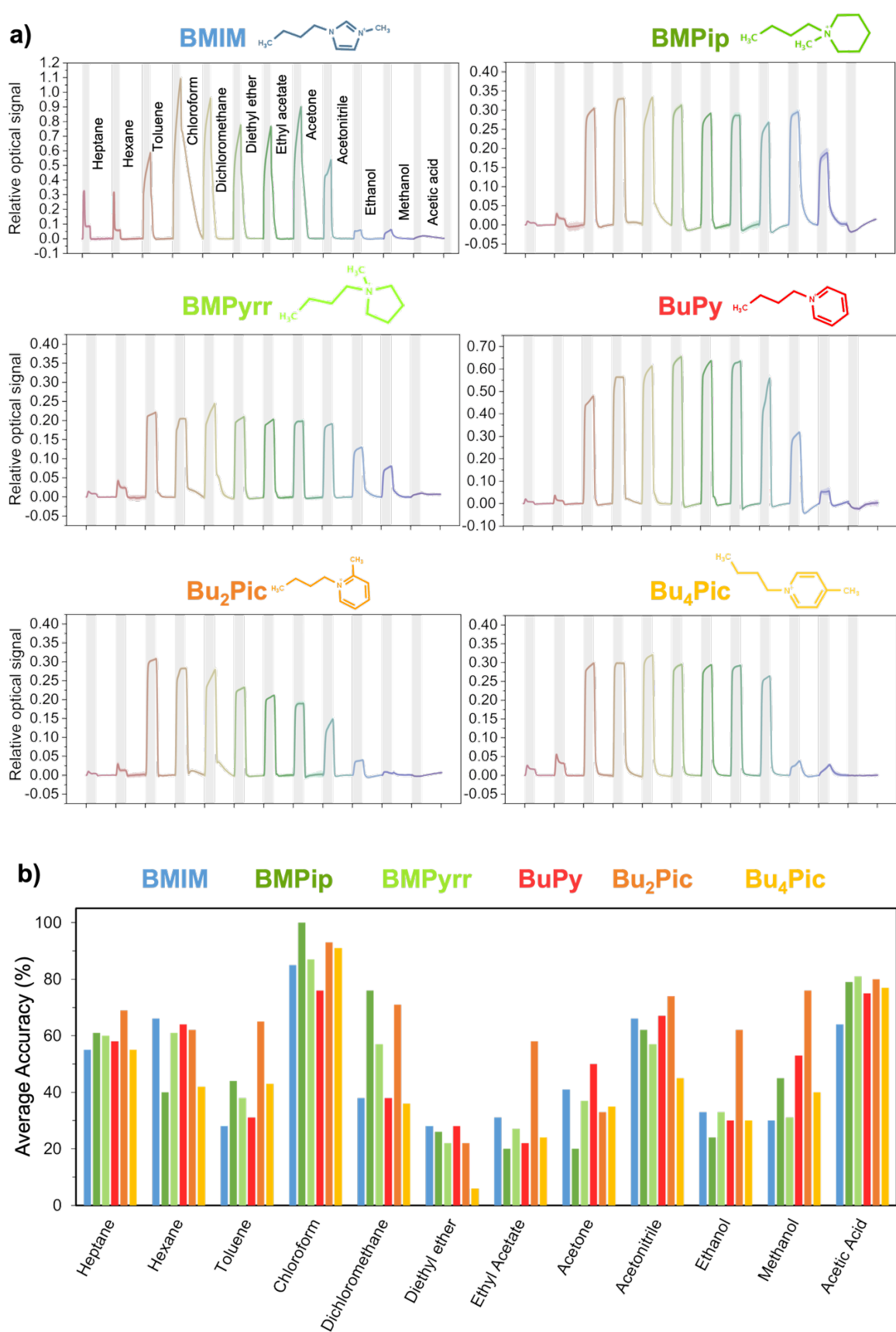


Figure 2. 4 – (a) Representative signals of the optical amplitude responses of all gelatin hybrid gels to distinct VOCs. Each curve represents the average and standard deviation of at least 19 replicate cycles from the same sensor. VOC

exposure periods (5 s) are highlighted in grey, and each cycle corresponds in total to 15 s. The plots were generated by python. (b) Average accuracy scores from a Confusion Matrix analysis regarding the ability of each sensor to accurately predict each volatile being tested, during the optical e-nose experiments.

The highest overall accuracy to classify volatiles (Figure 2.4b) was registered for the Bu₂Pic sensors, achieving a 63% score of overall accuracy discrimination of VOCs), and the lowest accuracies were found for the Bu₄Pic sensor (43%). It is interesting to notice that the BMPip sensors reached a complete discrimination of chloroform (Figure 2.4 and Figure S2.5). In general, hydrophobic VOCs, such as hexane and heptane, are likely to interact mainly with the oil phase formed by LC molecules inside the droplets, which can cause the different pattern of response as seen in all gels (Figure 2.4). The protic VOCs prone to form hydrogen bonds (such as diethyl ether, methanol, ethanol, acetic acid), tend to interact both with the IL-LC droplets and the gelatin matrix yielding more complex and varied signals. Interestingly, some sensors possessing members of the pyridinium family of ILs (i.g. BuPy and Bu₂Pic sensors) presented distinctive responsive signals for both alcohols, ethanol and methanol. Bu₂Pic sensor presented the best classification for both alcohols, comparing to the remaining sensors. This could be due to the fact that it has been showed that the ortho position confers a more hydrophilic character to that specific IL²³, when compared to the meta and para positions of the same aromatic compound.

2.4 Conclusions

Ionic liquids and derived materials present high chemical diversity that can be explored to yield distinct performances in gas sensing. In this work, we varied the cation group of chloride-based ILs to obtain ionogels and hybrid materials with distinct VOC discrimination potential. The different ionogels studied yielded versatile electrical sensors, not only providing good overall classification of VOCs, but also successfully discriminating several volatiles, such as acetic acid (BMPyrr sensors), acetone (BMPip, BMPyrr and Bupy sensors), acetonitrile (BMPip and BMPyrr sensors), chloroform (BMPip sensors), DCM (BMPip and BuPy sensors) and methanol (Bupy sensors) with 100% accuracy. It is also interesting to note that all cyclic cation families of ILs (BMPip and BMPyrr) showed similar behaviors in predicting VOCs, with high accuracies and very similar signature responses for the hydrophobic VOCs, as well as for the hydrophilic VOCs. The Bu₂Pic ionogel sensors presented the weakest overall capacity to discriminate volatiles, whereas the opposite was observed for the Bu₂Pic optical sensors that presented the highest general accuracy to discriminate VOCs in the optical e-nose experiment. This was an exception, as in general ionogel-based electrical sensors yielded higher accuracies in VOC classification, when compared with the optical hybrid sensors (Table 3.1). This may be related with the fact that in hybrid gels, the mechanism of VOC partitioning and sensing is much more complex and dependent on the several compartments created within the material. Thus, different ILs create different the interfaces at the IL-LC droplets and also influence LC molecule alignment in distinct ways. Our results further strength the role of ionic liquids as

extremely versatile molecules in which their chemical diversity can be translated into functional diversity.

Table 2. 1 – Overall VOC classification for each ionogel and hybrid gels with the six different ILs, for both electrical and optical e-nose experiments.

Ionic Liquid Sensor	Overall accuracy in Electrical E-nose	Overall accuracy in Op- tical E-nose
BMIM	69%	47%
BMPip	88%	49%
BMPyrr	83%	49%
BuPy	81%	49%
Bu ₂ Pic	14%	63%
Bu ₄ Pic	46%	43%

Supplementary Information

Table S2. 1 – Properties of the Ionic Liquids used in the study. Log P values were extracted from PubChem; the conductivity values were determined experimentally in this work.

Ionic Liquid	Log p (cation)	Conductivity at 2 kHz
BMIM (1-Butyl-3-methylimidazolium chloride)	1.3	2.41 mScm ⁻¹
BMPip (1-Butyl-1-methylpiperidinium chloride)	2.4	9.90 mScm ⁻¹
BMPyrr (1-Butyl-1-methylpyrrolidinium chloride)	2	2.78 mScm ⁻¹
BuPy (1-Butylpyridinium chloride)	2.2	15.26 mScm ⁻¹
Bu₂Pic (1-Butyl-2-methylpyridinium chloride)	2.6	2.35 mScm ⁻¹
Bu₄Pic (1-Butyl-4-methylpyridinium chloride)	2.5	4.33 mScm ⁻¹

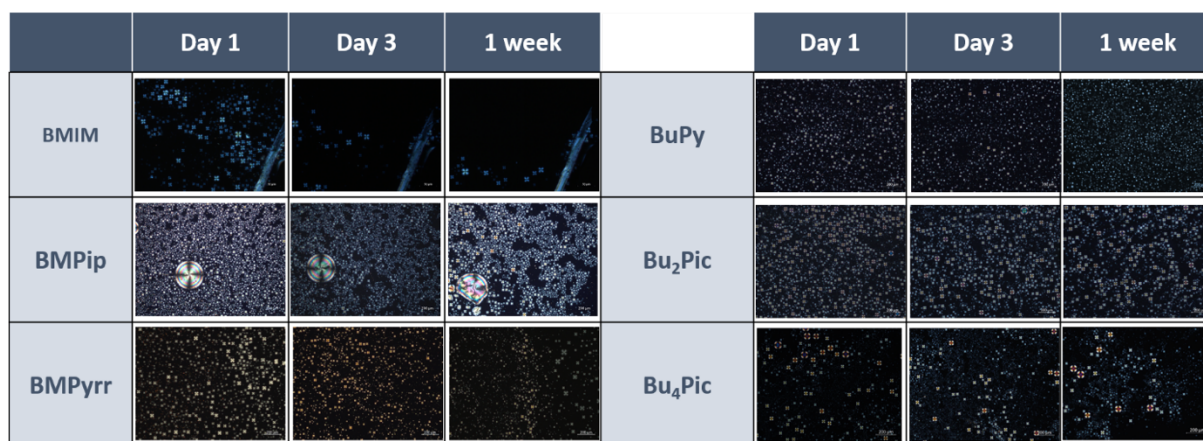


Figure S2. 1– Stability to long term storage of gelatin optical sensors. POM images were obtained by Zeiss Polarized Optical Microscope (Zen 2.3 software), with 90° polarizers, at 10x magnifying objectives.

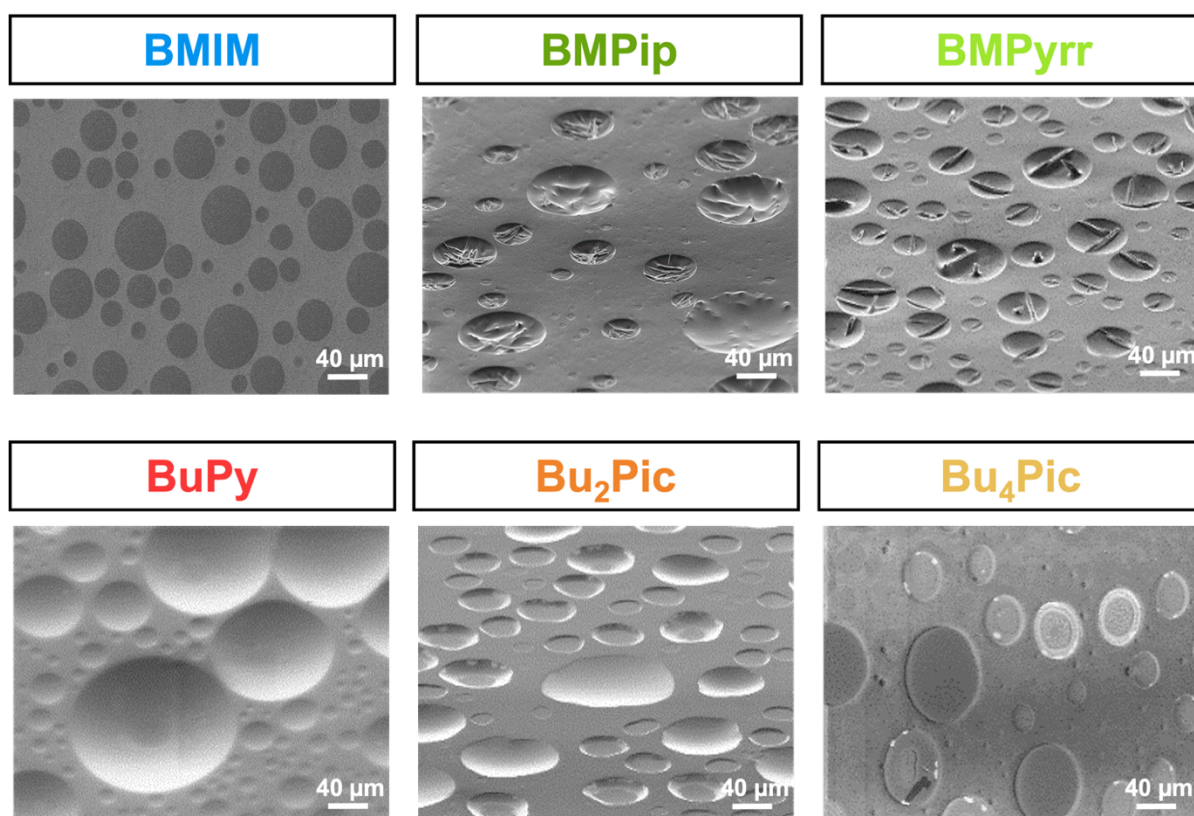


Figure S2. 2 – SEM images showing the surface morphology of hybrid materials, where droplets of IL-LC can be observed.

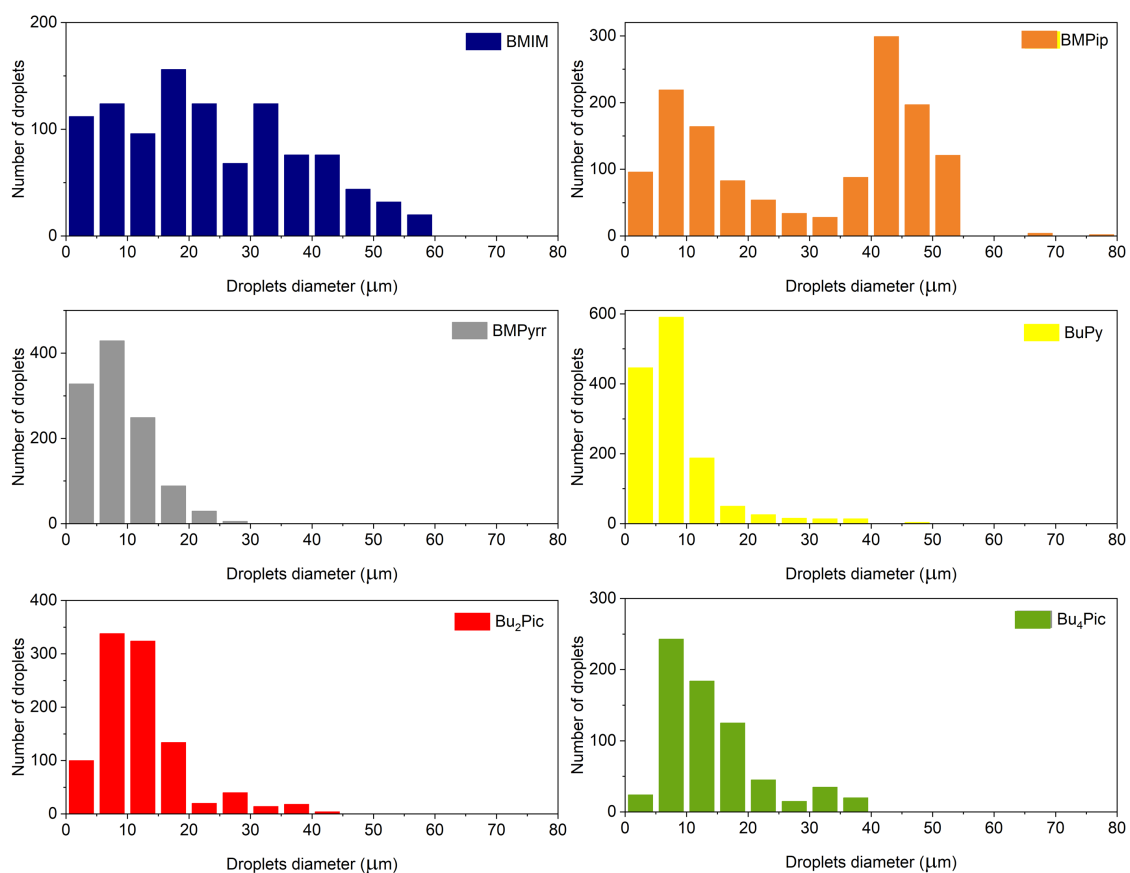


Figure S2. 3 – Histogram of droplets diameter distribution for the hybrid gels produced with ionic liquids BMIM, BMPip, BMPyr, BuPy, Bu₂Pic and Bu₄Pic.

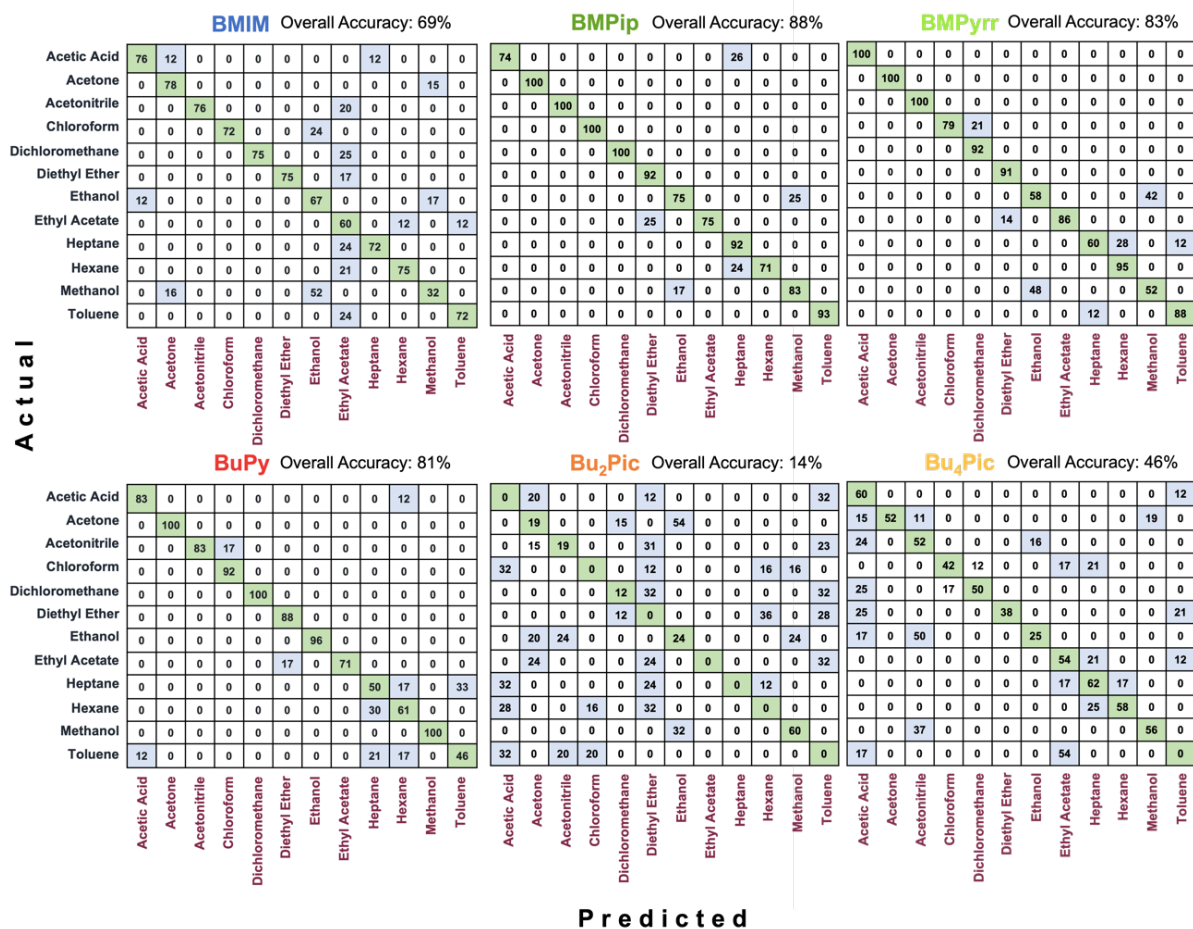


Figure S2. 4 – Classification accuracies for the 12 distinct volatiles given by each ionogel used in the work. The green squares in the diagonal represent the correct predictions made by the classifier, and the lighter blue squares outside of the diagonal represent the incorrect prediction of a VOC by being confused with other VOC.

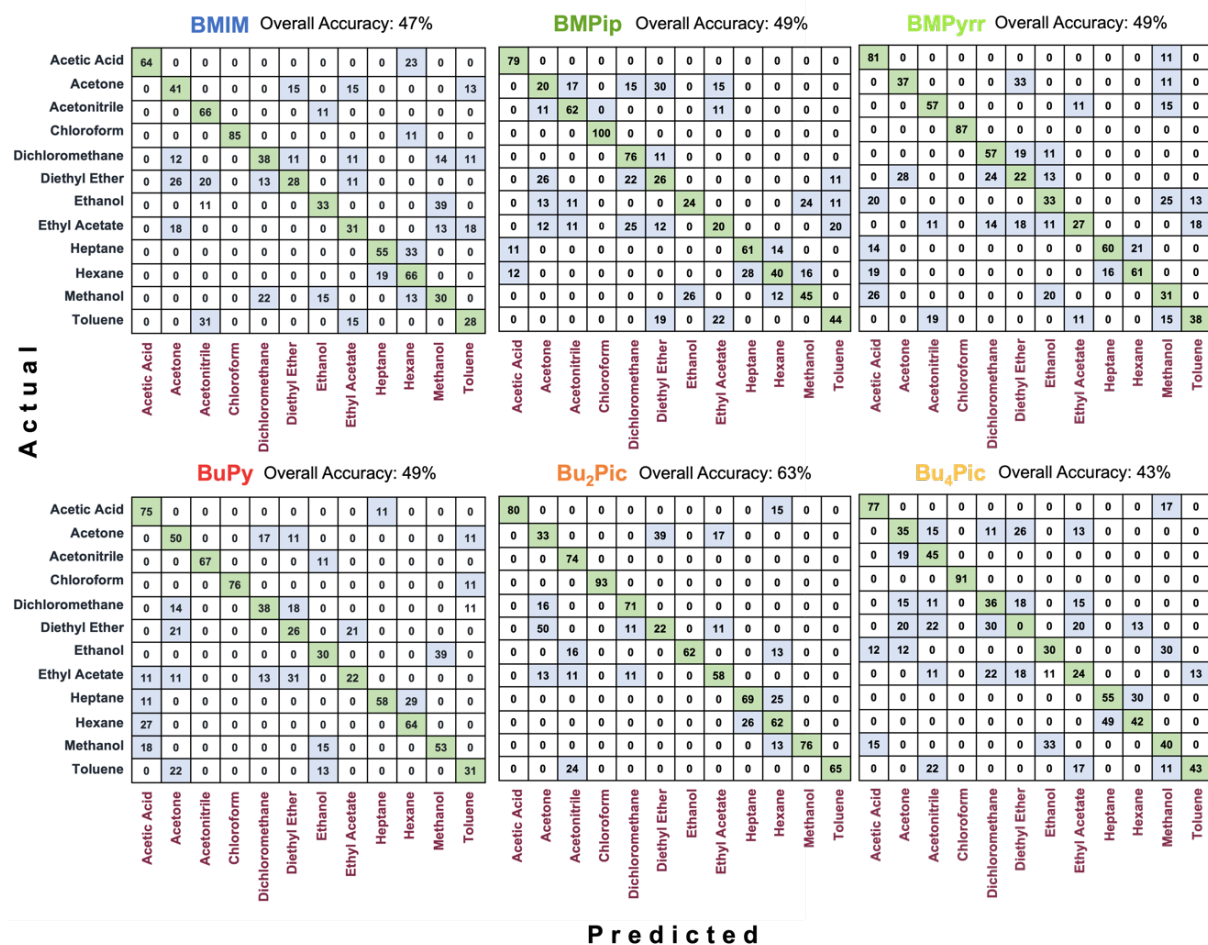


Figure S2. 5 – Classification accuracies for the 12 distinct volatiles given by each hybrid material used in the work. The green squares in the diagonal represent the correct predictions made by the classifier, and the lighter blue squares outside of the diagonal represent the incorrect prediction of a VOC by being confused with other VOC.

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EFFECT OF POLYMER HYDROPHOBICITY IN THE PERFORMANCE OF HYBRID GEL GAS SENSORS

Authors contributions:

Ana Rita Oliveira and Henrique Costa contributed equally for the work involved in this chapter. Ana Rita Oliveira and Henrique Costa planned and performed the work, analyzed the data and wrote the manuscript. Ana Rita Oliveira performed the work correspondent to the development, characterization, and use of sensing materials for VOCs discrimination in the electronic nose gas sensing experiments. Henrique Costa produced the sensing materials and performed the humidity and sensitivity studies. Ana C. A. Roque conceived and initiated the project. Susana I. C. J. Palma developed the python scripts used in Supervised Machine Learning for data analysis. Carina Esteves contributed for the optimization of the sensing films' formulation. Efthymia Ramou and Ana C. A. Roque reviewed the article. Ana C. A. Roque supervised the project.

Abstract

Hybrid gel materials used as gas sensing materials in electronic noses contain self-assembled droplets of ionic liquid and liquid crystal molecules encapsulated in a polymeric matrix. In this work, we assessed the effect of the matrix hydrophobic properties in the performance of hybrid gel materials for VOC sensing in humid conditions. We used two different polymers – the hydrophobic PDMS and the hydrophilic bovine Gelatin – as polymeric matrices in hybrid gel materials containing imidazolium-based ionic liquids – [BMIM][Cl] and [BMIM][DCA] and the thermotropic liquid crystal 5CB. Better accuracy of VOC prediction is obtained for the hybrid gels composed of a PDMS matrix combined with the [BMIM][Cl] ionic liquid, and the use of this hydrophobic matrix reduces the effect of humidity on the sensing performance when compared to the gelatin counterpart. VOCs interact with all the moieties of the hybrid gel multicomponent system, thus VOC correct classification depends not only on the polymeric matrix used, but also on the IL selected, which seems to be key to achieve distinct VOCs discrimination.

3.1 Introduction

Electronic-noses (e-noses) are devices that mimic the biological olfactory system, by combining an array of independent semi-selective gas sensors with a signal transduction unit and a pattern-recognition system, allowing the differentiation of distinct odors. Gas sensors typically alter their physical or chemical properties in the presence of volatile organic compound (VOC) molecules. The most common gas sensors are metal oxide semiconductors (MOS) or conductive polymers, which typically present low VOC-selectivity and are susceptible to fluctuations in surrounding temperature and humidity¹. Other gas sensing materials can be used including, for example those incorporating biological receptors² or liquid crystal molecules³.

Humidity is a common interferent in gas sensors and it can seriously affect sensors performance. Different approaches are adopted to correct the effect of humidity. One approach is to lower or even remove the humidity content before samples contact with gas sensors, therefore operating the system in dry conditions. Another alternative is to correct the humidity signal background by signal processing and machine learning approaches. Lastly, gas sensors composition can be altered to decrease humidity interference. This can be achieved by coating sensors with hydrophobic polymers such as Polydimethylsiloxane (PDMS)⁴, or by tuning the chemical components of the gas sensor itself⁵. In the latter, the anion moiety of the ionic liquids from ionogels and hybrid gels (optical ionogels) used as gas sensors, can tune the sensors sensitivity to humidity. In these hybrid gels, a hydrophilic gelatin polymer was previously used as the matrix to encapsulate liquid crystal-ionic liquid droplets that act as optical probes. Here, we aimed at understanding the effect of the polymer hydrophobicity in hybrid gels gas sensors response to different humidity levels, and to further assess their performance to classify different VOCs under humid atmospheres. Thus, we studied hydrophobic (PDMS) and hydrophilic (Bovine Gelatin) as the matrices to incorporate liquid crystal-ionic liquid droplets. We

employed the well-studied ionic liquids from the imidazolium family – [BMIM][DCA] and [BMIM][Cl]⁵ – and the thermotropic liquid crystal 4-cyano-4'-pentylbiphenyl (5CB)⁶. The resultant hybrid gels were used as gas sensors in tailor-made optical e-noses. The sensors response to humidity was firstly evaluated, and then their ability to operate under humidified conditions for VOC discrimination. Better VOC classification accuracy in humid conditions were obtained when using the PDMS matrix combined with the [BMIM][Cl] ionic liquid.

3.2 Materials and Methods

3.2.1 Materials

The polymer polydimethylsiloxane (PDMS, SylgardTM 184 Silicone Elastomer Kit) was purchased from The Dow Chemical Company (Germany), and Gelatin from bovine skin (gel strength ≈ 225 g; Bloom, Type B), was purchased from SigmaAldrich. The liquid crystal 4-cyano-4'-pentylbiphenyl (5CB, $>98.0\%$) was purchased from TCI Europe (Belgium). The ionic liquids 1-butyl-3-methylimidazolium dicyanamide ([BMIM][DCA], $>98.0\%$) and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl], $>98.0\%$) were purchased from IoLiTec (Germany). The anhydrous binary salts magnesium chloride ($\geq 98.0\%$), potassium carbonate (99.9%) and sodium bromide ($\geq 99.0\%$) were purchased from Sigma-Aldrich (Portugal), sodium chloride ($>99.5\%$) was purchased from VWR Chemicals (Portugal). The solvents dichloromethane and hexane were purchased from VWR, and both ethanol and acetic acid (purity 99.8%) was purchased from Sigma-Aldrich. Acetonitrile (purity 99.9%), chloroform, diethyl ether (HPLC grade), ethyl acetate, heptane, methanol (HPLC grade), and toluene were supplied by Fisher Scientific. Acetone (purity 99.5%) was purchased from Honeywell, and isopropanol (purity 99.5%) from ROTH.

3.2.2 Methods

3.2.2.1 Preparation and characterization of hybrid gel sensing films

The gelatin hybrid sensors containing the ionic liquids (ILs) [BMIM][DCA] or [BMIM][Cl] and the liquid crystal 5CB were prepared as previously described^{7,8}. To produce the sensing films with PDMS, the polymer solution was firstly prepared by adding the curing agent in a 1:10 ratio and leaving the mixture in the desiccator for 20 minutes. Then, the prepared PDMS solution was added to a heated glass vial containing the IL and the liquid crystal, and finally milliQ water was added. The final formulation presented two separated phases, which were manually stirred with a tip and finally collected and deposited on a clean glass slide. The spreading of the deposited material alongside the whole glass slide was conducted using a quadruplex with a predefined thickness of 30 μm (TQC Sheen). For control sensors, the ionic liquid was removed from the formulation.

The films were left at room temperature and controlled humidity (50% relative humidity) before further use. For the morphological characterization of the prepared films, we used

polarised optical microscopy (POM) and scanning electron microscopy (SEM). For the POM analysis, images were taken under crossed (at 90°), semi crossed (at 45°) polarizers, as well as under the absence of polarizers (bright field) in a Zeiss Axio Observer.Z1/7 microscope equipped with an Axiocam 503 colour camera and ZEN 2.3 software for image acquisition and processing. As for the Scanning electron microscopy (SEM), observations were conducted on a Carl Zeiss AURIGA CrossBeam (FIB-SEM) workstation coupled with energy dispersive X-ray spectroscopy (EDS). The dried hybrid gel materials were previously coated with an Ir and Au/Pd conductive film to avoid charge effects. Stability to long-time storage was also evaluated by following a selected optically active region of interest by POM, for a period of a month.

3.2.2.2 Response of hybrid gels gas sensors to distinct humidity environments

Humidity reference sensors (HTU21D-F, Adafruit, New York, USA, with accuracy of $\pm 2\%$ RH and response time between 5 - 10 s at 63% of the signal) were installed the outlet of the detection chamber of the optical e-nose devices to record the variations of relative humidity in the chamber (Figure S3.1). The measurements of the RH sensor in the saturated salt solution vial were synchronized with the measurement of the optical signal using an Arduino due, which controls the signal transducer device operation. To generate controlled levels of relative humidity, the sample delivery system depicted in Figure S3.1 was assembled and adapted to the optical e-nose device^{5,7}. Two mass flow controllers (MFC1, MC-5SLPM-D/5M, and MFC2, MC-2SLPM-D/5M, Alicat Scientific Inc.) were fed with nitrogen as a carrier gas. A glass vial containing a supersaturated salt solution at room temperature ($22 \pm 2^\circ\text{C}$) was installed at the outlet of the MFC2. Distinct RH levels were generated in the detection chambers by bubbling the nitrogen stream in supersaturated solutions of different salts⁷ (Table S3.1) before entering the detection chamber.

Triplicates of sensing films were placed in the detection chamber and exposed to five humidification/ drying periods, for each RH level. The optical signals were acquired with the associated in-house developed signal transducer⁷⁻¹¹. To establish the humidification and drying periods, MFC1 flow rate was constant at 1.5 slpm and MFC2 was programmed to alternately switch the flow rate between 0 and 1.5 slpm: i) 120 seconds at 1.5 slpm: Humidification period; ii) 120 seconds at 0.0 slpm: Drying period. During the humidification period, the films were exposed to humidified nitrogen. During the drying period, dry nitrogen purged the detection chamber to ensure 0% RH. Independent experiments were made for each RH level, with 3 replicates of each sensing film type.

3.2.2.3 Performance of PDMS and gelatin hybrid gels as sensors for VOC discrimination

To assess the performance of the hybrid gels as sensors to classify 12 distinct VOCs with small structural differences, a tailor-made optical e-nose was used^{5,7}. The device contains a detection chamber with six sensor slots and employs two pumps that function alternately to expose the sensors either to VOCs, or to ambient air for recovery inside the chamber. The detection chamber is connected to a signal transduction unit that digitalizes the optical sensing signal and sends it to a computer, where it is presented in real time and stored for further

processing. The acquisition of signals is possible because liquid crystal molecules act as optical probes, providing the measurable optical signals that are being analyzed. Due to the presence of the ionic liquid and its induced anchoring, liquid crystal molecules adopt a radial configuration, which exhibits typical optical textures observable under POM with crossed polarizers. This corresponds to baseline levels in the optical e-nose experiment – the baseline levels correspond to the total amount of polarized light being transmitted and reaching the detector. However, when VOCs interact with hybrid gels, the liquid crystal molecules lose their ability to rotate polarized light, which culminates in a significant decrease of the initially transmitted polarised light, and an increase of the optical signal from baseline levels. This process is reversible once the volatiles are desorbed from the gels, by pumping ambient air. Throughout the optical experiment, changes in light intensity are read by the photodetector, during both exposure and recovery cycles, so they can be used for subsequent analysis. This phenomenon is the basis for the optical VOC sensing experiment⁷⁻¹⁰.

The sensors were positioned in the six sensor slots of the detection chamber, including 3 films of each PDMS and gelatin matrix gels – PDMS with [BMIM][DCA], PDMS with [BMIM][Cl], gelatin with [BMIM][DCA], gelatin with [BMIM][Cl], and the two control sensors. In the in-house developed e-nose the gels were positioned between two crossed polarizers. Their responses were tested against a set of 12 saturated atmospheres of VOCs: heptane, hexane, chloroform, toluene, dichloromethane, diethyl ether, ethyl acetate, acetonitrile, acetone, ethanol, methanol, and acetic acid (VOCs listed in order of exposure). The solvents were kept in a thermostated bath at 37°C, for at least 15 min prior to exposure. The VOCs were pumped for 5 sec into the detection chamber, followed by 15 sec of ambient air for recovery (RH between 42-68%), completing 1 cycle. A total of 45 cycles were performed, totalizing 15 min of duration per test. The optical signals of all sensing films were acquired at a sampling rate of 90 Hz, and assays were performed in triplicates. The signals were then analysed using an automatic classifier algorithm based on support vector machines, and python programming tools previously developed^{5,7-12}.

3.2.2.4 VOCs limit of detection using PDMS and gelatin hybrid gels as sensors

To sample known concentrations of VOC (ethanol, acetone, toluene and hexane; Table S3.2) under controlled RH levels in the detection chamber, the apparatus depicted in Figure S3.2 was assembled. The dilution MFC (MC-5SLPM-D/5M, Alicat Scientific Inc.) was fed with nitrogen gas, which was bubbled through distilled water to generate a RH of 50% in the detection chamber or sent directly to the detection chamber to ensure a RH of 0%. The carrier MFC (MC-2SLPM-D/5M, Alicat Scientific Inc.) was also fed with nitrogen gas, which was bubbled through a known volume of pure solvent to generate a known flow rate of VOC vapor in the nitrogen carrier stream. The bubbler system is composed of a dip tube attached to a porous filter. The flow rate of VOC vapor and water vapor at the output of the bubbler flask were calculated as detailed in Supplementary Information. The dilution MFC was set at a fixed flow rate (1.5 slpm, for ethanol, toluene and hexane or 5.0 slpm, for acetone). The carrier MFC was programmed to vary the flow rate in increments of 0.05 slpm (between 0.1 and 1.5 slpm) and

repeat 5 times each flow rate step. In this way, 5 replicates of each VOC concentration were sampled to the detection chamber of the optical e-nose devices during exposure/recovery cycles. To generate the exposure/recovery cycles, a two-way solenoid valve was used. The valve was programmed to alternately direct the nitrogen carrier stream (Figure S3.2) to the exposure or the recovery path using an automated temporized switching: i) 5 seconds ON – Exposure; ii) 15 seconds OFF – Recovery. During the exposure period, the nitrogen carrier stream bubbles through the solvent, is mixed with the dilution nitrogen stream (dry or humid) and, finally, introduced in the detection chamber. During the recovery period, the carrier stream is directed to an exhaust line and the detection chamber is purged with dry or humid nitrogen gas.

A custom-made python script (Python 3.7, using alicat library 0.2.2) was used to program the carrier MFC and solenoid valve operation, as well as to synchronize them with the optical e-nose device and the readings of the reference RH sensor at the outlet of the detection chamber⁵.

3.2.2.5 Signal processing

The raw optical signals yielded by the hybrid sensors were firstly smoothed and filtered with a median filter to remove unwanted noise. Then, using a custom-made Python 3.7 script, the resulting signals were divided in cycles. The *Relative optical signal* (R_s) of each cycle was determined by taking its offset and normalising it to its baseline, according to equation 1:

$$\text{Relative optical signal } (R_s) = \frac{\text{signal} - \text{baseline}}{\text{baseline}} \quad (\text{Equation 1})$$

Where *signal* is the smoothed and filtered cycle signal, and *baseline* is the average of the *signal* immediately before (10 sample points) the exposure period starts.

Regarding the humidity assays, for each cycle, the following features were extracted from the relative optical signal using custom-made Python 3.7. scripts: *maximum derivative*, *minimum derivative*, and *relative amplitude* (R_a), defined as the maximum variation of the relative optical signal (it can be either a maximum or a minimum, depending on the shape of the signal).

The minimum and maximum derivatives were used to calculate the slope of the signals in the humidification and drying periods, which correspond to the response speed to relative humidity step changes. The *response time* (t_{res}), defined as the time required to reach the maximum variation of the relative optical signal (R_{Or}) after the start of a humidity step change (t_0), was estimated from the response speed (Equation 2) both for the humidification and for the drying periods of the experiments.

$$\text{response speed } (s) = \frac{R_{Or}}{t_{res} - t_0} \quad (\text{Equation 2})$$

The relative amplitude of the signals in the presence of VOCs were corrected by subtracting the relative amplitudes of the responses to control tests without VOC in order to eliminate the effect of changes in RH and electrical noise inherent to the design of the MFC and e-nose system.

3.2.2.6 Automatic VOC discrimination

To assess the ability of each sensing material to identify VOCs based on the different shapes of the optical signals, these were first normalized (relative optical signal), so their features could be extracted, and used as input variables to build an automatic classifier algorithm based on Support Vector Machine (SVM), as reported previously^{7–10,12}. For each sensor, the classifier was used to make predictions of how accurately they were able to identify the set of all tested VOCs. The performance of the classifier was represented in the form of confusion matrices, which demonstrates the rate of correct and incorrect predictions given by the sensors, upon exposure to the volatiles. The VOC identification ability of each sensor was given by the rate of correct predictions for that VOC^{7–10,12}.

3.3 Results and Discussion

3.3.1 Gelatin and PDMS hybrid gels

Liquid crystal molecules are appealing for sensing purposes due to their high molecular order and intrinsic stimuli-responsive nature, turning them into fast optical probes. In particular, the confinement of liquid crystal molecules into spherical arrangements increases the available surface area and yields a variety of optical textures that can be used as input signals^{13,14}. The possibility to encapsulate ordered droplets of liquid crystal molecules within macroscopic matrices is an ideal approach for accurate sensing. In particular, the entrapment of liquid crystal droplets stabilized by ionic liquid molecules into gelatin ionogel matrices has proven suitable to yield robust thin films for gas and VOC-sensing^{8,15}. To better understand the effect of the polymeric matrix on the sensing performance of these materials in humid conditions, both hydrophilic and hydrophobic polymeric matrices were selected for this study, while maintaining the ionic liquid ([BMIM][DCA] or [BMIM][Cl]) and liquid crystal (5CB) moieties constant. Type A gelatin from porcine skin (Gelatin bovine) was selected as the hydrophilic matrix for comparison against the hydrophobic polydimethylsiloxane (PDMS) polymer (Figure 3.1). PDMS is an organosilicon polymer, optically clear that has been explored in gas sensing as a matrix to encapsulate liquid crystal molecules (Polymer-dispersed liquid crystals)^{16,17} or as a hydrophobic coating to reduce humidity interference in gas sensors⁴.

The produced gelatin and PDMS-based optical hybrid gels were firstly characterized by SEM and polarised optical microscopy (POM) to observe morphological and optical textures, and to infer on liquid crystal molecular arrangements within the materials (Figure S3.3, S3.4 and Figure 3.2).

In gelatin-based films SEM images confirmed that the flexible, and yet robust, gelatin matrix contains droplets at its surface as previously reported⁸. For the PDMS-based films, a more uniform surface was observed, with no visible droplets, which indicates that these are likely placed inside the matrix or that the matrix was deteriorated during sample preparation (Figure S3.3).

Regarding optical textures observed by POM, the PDMS control films (with no ionic liquid) exhibited almost monodispersed liquid crystal droplets, where the majority of them feature a radial configuration due to a perpendicular orientation of the liquid crystal molecules at the droplet interface¹⁸. This is evidenced by the Maltese cross pattern seen in POM with crossed polarizers. According to Bronnikov et al.^{19,20} the semi-flexible nature of the PDMS matrix (due to the presence of the curing agent) coupled with the ratio of the liquid crystal:PDMS content used (1:10 v/v) allows for better phase separation between the liquid crystal and the polymeric matrix. This effect prevents droplet coalescence, leading to the formation of small radial droplets (on average of 10-15 μm diameter, Figure S3.4)^{18,21-23}. On the other hand, this is not the case for the gelatin-based control formulation⁵. Poor phase separation is observed between the liquid crystal and the gelatin matrix, which leads to droplet coalescence. Furthermore, since the liquid crystal:gelatin ratio is different (1:5 v/v) when compared to the PDMS control gel, polydisperse and occasionally irregularly shaped droplets are detected as a result. The droplets are very polydisperse in size and in most cases are significantly larger than those observed in the PDMS control gels. This induces weaker anchoring conditions between the liquid crystal and the polymer, leading to formations which feature a variety of configurations, typically bipolar²².

Interestingly, the ionic liquid presence in hybrid gels leads to distinct optical textures and droplets size. For the PDMS-based hybrid gels, radial droplets are formed exhibiting on average a smaller diameter than the control gels, namely 5-10 μm (with [BMIM][DCA]) and 1-5 μm (with [BMIM][Cl]) (Figure S3.4). In the case of gelatin, the presence of ionic liquids alters droplet size and morphology. Both [BMIM][DCA] and [BMIM][Cl] exhibit a surfactant-like effect that provides a stable perpendicular anchoring to the liquid crystal molecules, and also stabilizes the spherical shape of the droplets⁶⁻⁸. The formed liquid crystal droplets assume a radial profile with an average diameter of 15-20 μm in the case of [BMIM][DCA] and 5-10 μm in the case of [BMIM][Cl] (Figure S3.4). It should be noted that the presence of PDMS in hybrid gels restricts the growth of the nematic phase, resulting in smaller liquid crystal droplets when compared to gelatin-based hybrid gels. Also, it is evident that the ionic liquid anion influences droplet diameter. According to Esteves et al.⁷, [DCA⁻] and [Cl⁻] anions give rise to different physical constraints upon interaction with the imidazolium headgroup [BMIM⁺]. Since both nitrile and chloride anions exhibit a similar ionic radius (191 and 181 pm, respectively)²⁴ and considering that [DCA⁻] contains two nitrile groups, it is likely that steric hindrance at the droplet interface the [DCA⁻] anion facilitates the formation of larger droplets when compared to those containing [Cl⁻].

To evaluate the stability of the hybrid gels to storage at room temperature and controlled humidity conditions (50% RH), we monitored the films by POM during one-month. In general, droplets in [BMIM][DCA] materials – using either PDMS or gelatin – lead to more stable to storage optical sensors when compared to those bearing [BMIM][Cl] (Figure S3.5).

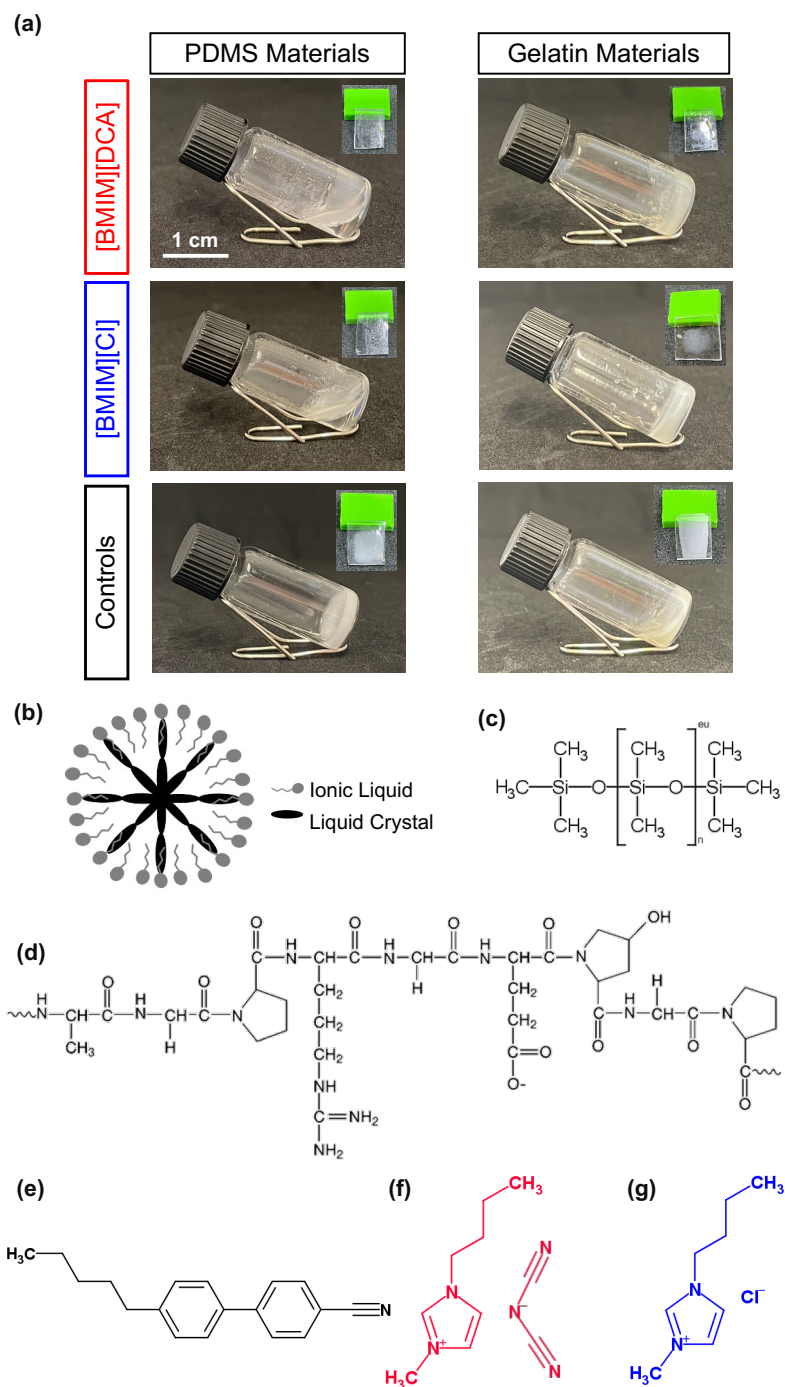


Figure 3. 1 – Summary of the different materials used to assemble hybrid gels. (a) Macroscopic images showing the different hybrid gel formulations; the inset photo shows the films produced with each formulation and used as gas sensors. (b) Schematic representation of ionic liquid-liquid crystal-droplets. (c) Chemical structure of PDMS, (d) gelatin, (e) liquid crystal 5CB and the two ionic liquids (f) [BMIM][DCA] and (g) [BMIM][Cl].

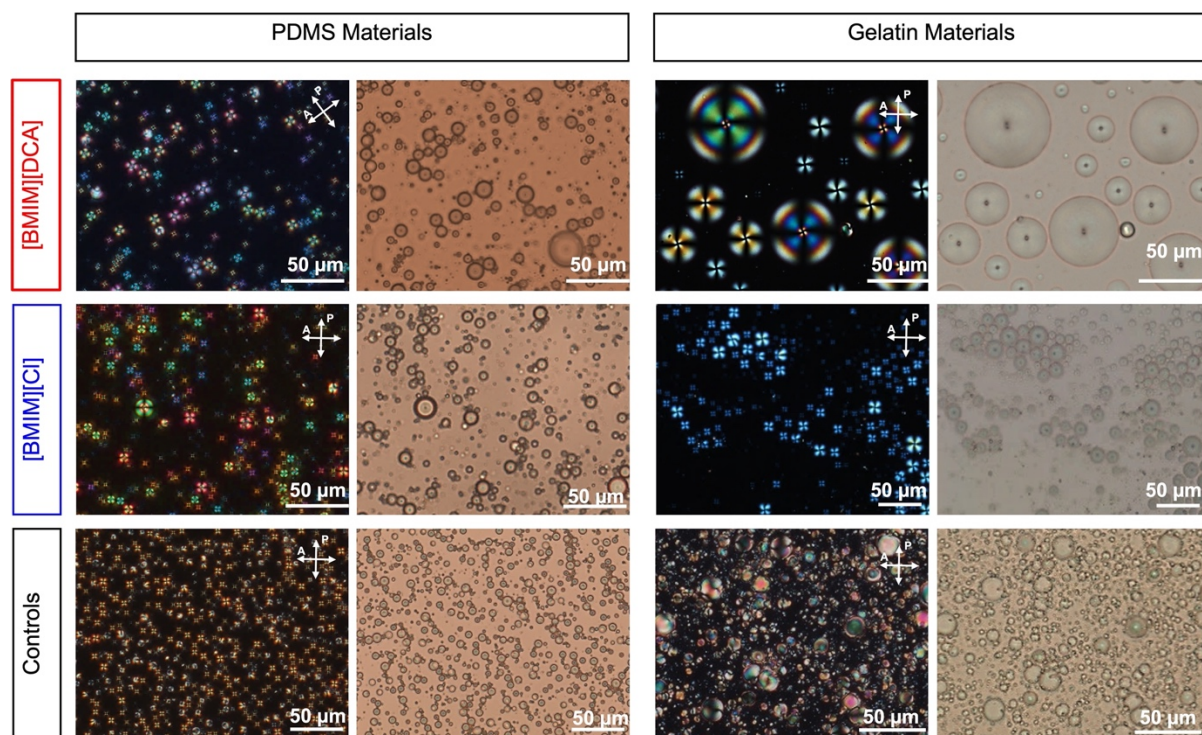


Figure 3. 2 – Examples of polarized optical microscopy (POM) images of optically active areas obtained for the sensing films under crossed polarizers (left columns) and bright field (right columns).

3.3.2 Optical responses of sensing films to humidity

The presence of liquid crystal molecules encapsulated within droplets in the PDMS and gelatin matrices makes it possible to study the changes in optical textures derived from the interaction of chemical analytes with the sensing films. In particular, the liquid crystal molecules inside the droplets can manipulate the polarization of light, allowing the transmission of light in optical devices under crossed polarizers. Based on previous works, the disruption in the orientation of liquid crystal molecules within the droplet, leads the molecular ordering to reversibly change from radial (points 1 and 3) to isotropic configuration (point 2; dark image) depending on absorption and desorption of volatile analytes (Figure 3.3a). The corresponding changes in transmission of light thus works as a sensing signal for the adsorption and desorption of analytes. This optical signal is transduced by a custom-made e-nose device described in previous works^{5,7–12}, where the optical signal is inversely proportional to the light transmitted through the sensing film.

To study the effect of humidity on the optical signal of the hybrid gel thin films, the e-nose device was coupled to a humidity delivery setup (Figure S3.1). The hybrid thin films were exposed to alternated cycles of dry (0% RH) and humid nitrogen (between 25 and 85% RH) (Table S3.1).

The relative optical response to increasingly higher levels of relative humidity is represented in Figure 3.3 for the hybrid thin films and further analyzed in Figure 3.4. The control films (without ionic liquid) are nearly unaffected by humidity, yielding raw optical signals

with extremely low amplitudes, even when exposed to the largest RH variation (0% - 80%) (Figure 3.3.d and g).

Considering now the hybrid films, the sensors response to humidity is mostly governed by the nature of the ionic liquid used.

The sorption and desorption of water molecules has a similar effect on the optical signal of films containing [BMIM][DCA] in PDMS and gelatin (Figure 3.3b,e), as in general the reduction of humidity content leads to a reduction on the amount of light transmitted (i.e. an increase in the relative optical signal). In [BMIM][DCA]-gelatin materials, the drying of the films is associated with a liquid crystal transition to a complete isotropic state, visualized by the transition of the optical signal to the maximum scale voltage (Figure 3.3e). The relative optical response varies linearly with humidity and response times vary between 20-60s (responses are faster as %RH increases) (Figure 3.4c,d). For [BMIM][DCA]-PDMS materials the optical signal does not fully transition to the maximum scale voltage when dried (optical signal amplitude is lower than for gelatin films), indicating that the liquid crystal is kept in a nematic phase although with a reduction on the total amount of light transmitted (Figure 3.3b). The variation of the relative optical response as a function of %RH follows a polynomial fit and response times vary between 1-10s (Figure 3.4a,b).

Considering now the hybrid gels containing [BMIM][Cl], it is observed that the amplitude of the relative optical signal is very low when compared to the same hybrid gels but with [BMIM][DCA] (Figure 3.3c,f). This is mostly attributed to the fact that [BMIM][Cl] holds strongly onto water, and as such the films are not so altered by variations in relative humidity as the anion is already equilibrated with water molecules⁵. For PDMS-based gels the reduction of humidity leads to a decrease in transmitted light, whereas for gelatin-based gels the increase of humidity leads to an increase in transmitted light.

Overall, our results indicate that in the absence of ionic liquid in the sensors composition (control films) the influence of humidity in the optical sensors response is governed by the hydrophobic properties of the polymeric matrix. For PDMS control sensors, the higher hydrophobicity of the matrix repels water molecules and therefore the liquid crystal molecules are barely affected by the presence of water molecules. When adding the ionic liquid into the composition, the measured optical signal is mostly affected by the nature of the anion from the ionic liquid, with a higher humidity effect for [BMIM][DCA]-containing hybrid gels. Still, the amplitude of the optical response for PDMS-based hybrid gels is lower than for gelatin-based gels, indicating a lower water interference.

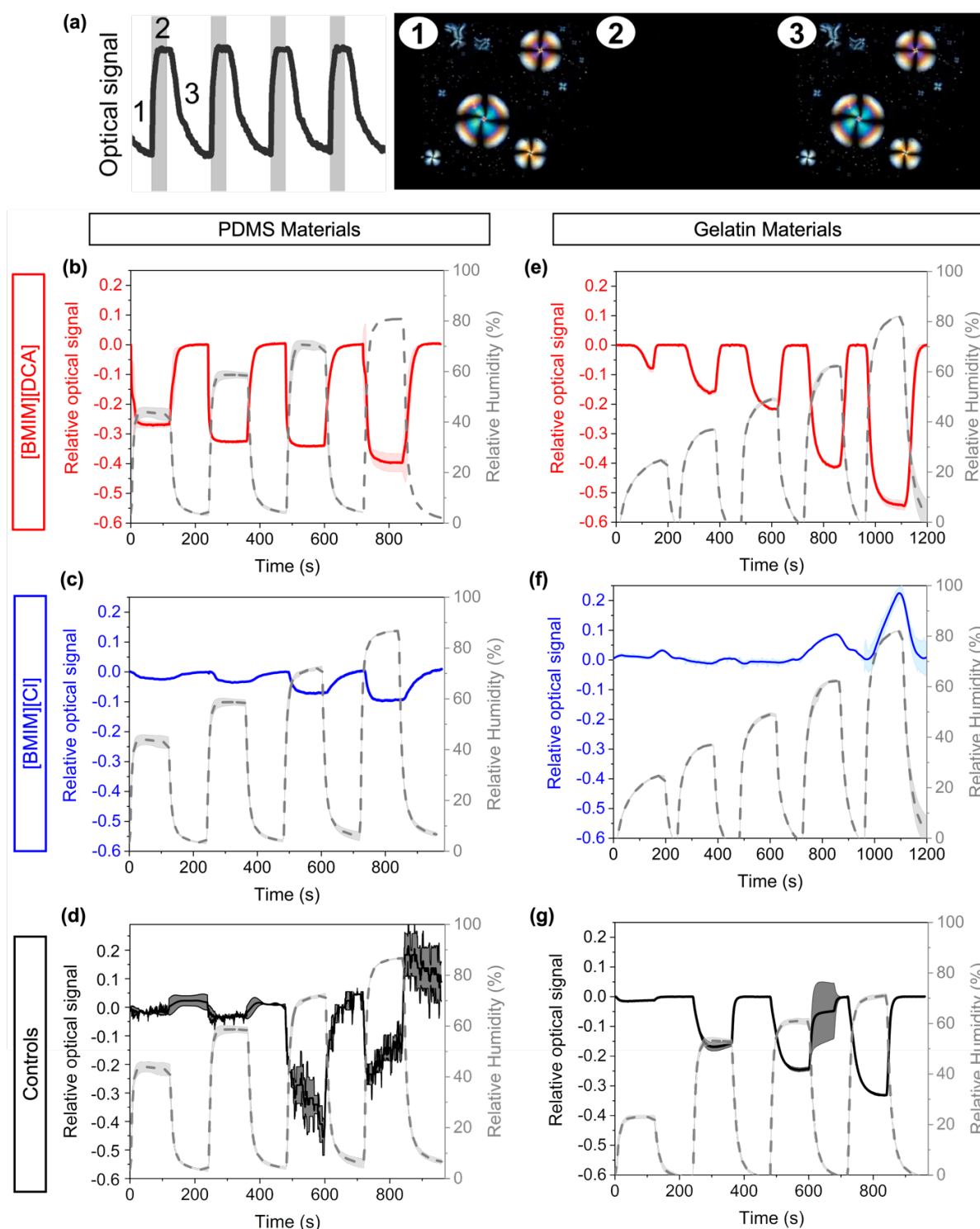


Figure 3.3 – Optical Response of sensing films to humidity. (a) Typical profile of the relative optical signals obtained during VOC-exposure and recovery cycles, as well as the corresponding optical morphological changes observed by POM in the hybrid gels during those cycles. Optical signals of [BMIM][DCA] and [BMIM][Cl]-based hybrid films upon exposure to cycles of humidification and drying. (b) Relative optical signal from hybrid films composed by PDMS, [BMIM][DCA], 5CB; (c) Relative optical signal from hybrid films composed by PDMS, [BMIM][Cl], 5CB; (d) Relative optical signal from PDMS control films; (e) Relative optical signal from hybrid films composed by gelatin, [BMIM][DCA], 5CB; (f) Relative optical signal from hybrid films composed by gelatin, [BMIM][Cl], 5CB; (g) Relative optical signal from gelatin control films.

(g) Relative optical signal from gelatin control films. The optical signals were represented averaging 3 independent sensors for each exposed RH.

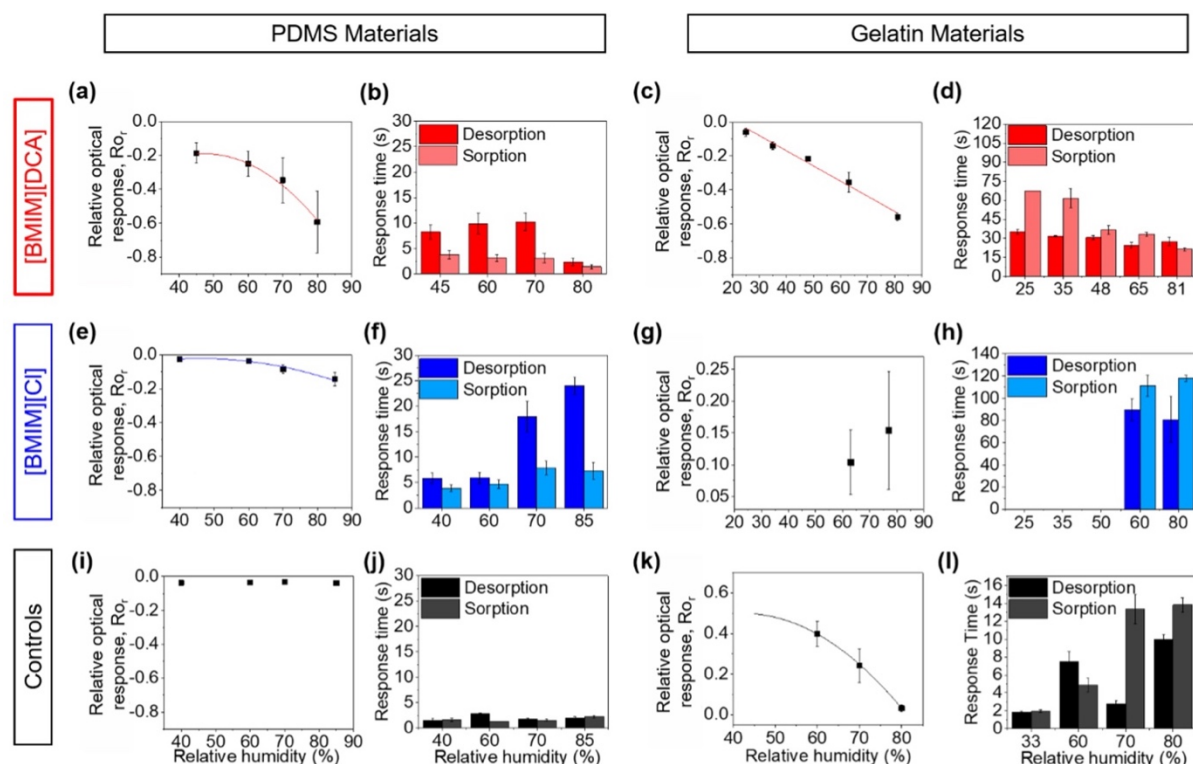


Figure 3. 4 – Analysis of the relative optical response and response time to humidity of hybrid films made with PDMS or gelatin, the ionic liquids [BMIM][DCA] (a-d), [BMIM][Cl] (e-h) or no IL (i-l). (a), (c), (e), (g), (i), (k) Variation of relative amplitude of the optical response of hybrid and control films as a function of % RH. (b), (d), (f), (h), (j), (l) Response time of the optical signal of hybrid and control films as a function of % RH, representing the required time to reach a stable-state response to humidification from 0% to different RH levels (Sorption) and to drying from those RH levels to 0% RH (Desorption). (a) Polynomial fit of the variations of the relative amplitude of the optical response of [BMIM][DCA]-based hybrid films made with PDMS as a function of % RH (Optical signal relative amplitude = $-0.0004 \times \%RH^2 + 0.0421 \times \%RH - 1.2208$, $R^2 = 0.99$). (c) Linear fit of the variations of the relative amplitude of the optical response of [BMIM][DCA]-based hybrid films made with gelatin as a function of % RH (Optical signal relative amplitude = $-0.009 \times \%RH + 0.188$, $R^2 = 0.98$). (e) Polynomial fit of the variations of the relative amplitude of the optical response of [BMIM][Cl]-based hybrid films made with PDMS as a function of % RH (Optical signal relative amplitude = $-7 \times 10^{-5} \times \%RH^2 + 0.0057 \times \%RH - 0.1475$, $R^2 = 0.98$). (k) Polynomial fit of the variations of the relative amplitude of the optical response of gelatin control films as a function of % RH (Optical signal relative amplitude = $-0.09995 + 0.02819 \times \%RH - 3.31729 \times 10^{-4} \times \%RH^2$, $R^2 = 0.99$) ($n = 3$ independent replicates of each hybrid film were used to measure the optical responses to humidity).

3.3.3 Hybrid gels sensors to discriminate between distinct VOCs

After studying how hybrid gels based on PDMS and gelatin behaved to the presence of humidity, we set to understand how these materials performed as VOC sensors in ambient humid environments (room temperature and RH between 40-60%). The hybrid gels and respective controls were sequentially exposed to twelve VOCs from distinct chemical classes and small structural differences. The sensors were exposed to saturated atmospheres of the following compounds: heptane, hexane, chloroform, toluene, dichloromethane, diethyl ether, ethyl acetate, acetonitrile, acetone, ethanol, methanol, and acetic acid (VOCs listed in order of

exposure). The representative relative optical signals obtained for each sensor and VOC are shown in Figure 3.5.

Considering the [BMIM][DCA]-based sensors, gelatin materials yielded signals with higher relative amplitudes and distinct responses to VOCs when compared to PDMS. For example, gelatin materials were unresponsive to acetic acid but more responsive to hexane and heptane. For the [BMIM][Cl]-based sensors, those containing PDMS yielded relative signals with higher amplitude and sharper signals with fast response and recovery. In the case of the control sensors, the PDMS controls gave very low signals to VOCs except for acetone that yielded a relative optical signal 10 times higher than the IL containing sensors. In the case of gelatin controls, relative signal amplitudes were also higher than the IL containing sensors, in particular the response to acetic acid which was extremely for IL-gelatin sensors.

After signals collection, the data was analysed through machine learning tools, using several features and parameters of curve fitting models as inputs for support vector machine-based classification algorithm. This resulted in plots summarizing mean normalized wave signals deriving from each VOC-gel interaction yielding typical signals, and also confusion matrices (Figure S3.6), where the accuracy and precision scores of the classifier towards the VOC being tested is evaluated. A summarized plot of the classification and prediction results are presented in Figure 3.6. The bars represent the percentage of correct prediction scores for each VOC. Above 50% it is considered a good classification of the volatile from the sensor. In general, the [BMIM][DCA]-based sensors yield classification systems lower than those obtained for [BMIM][Cl]-based sensors.

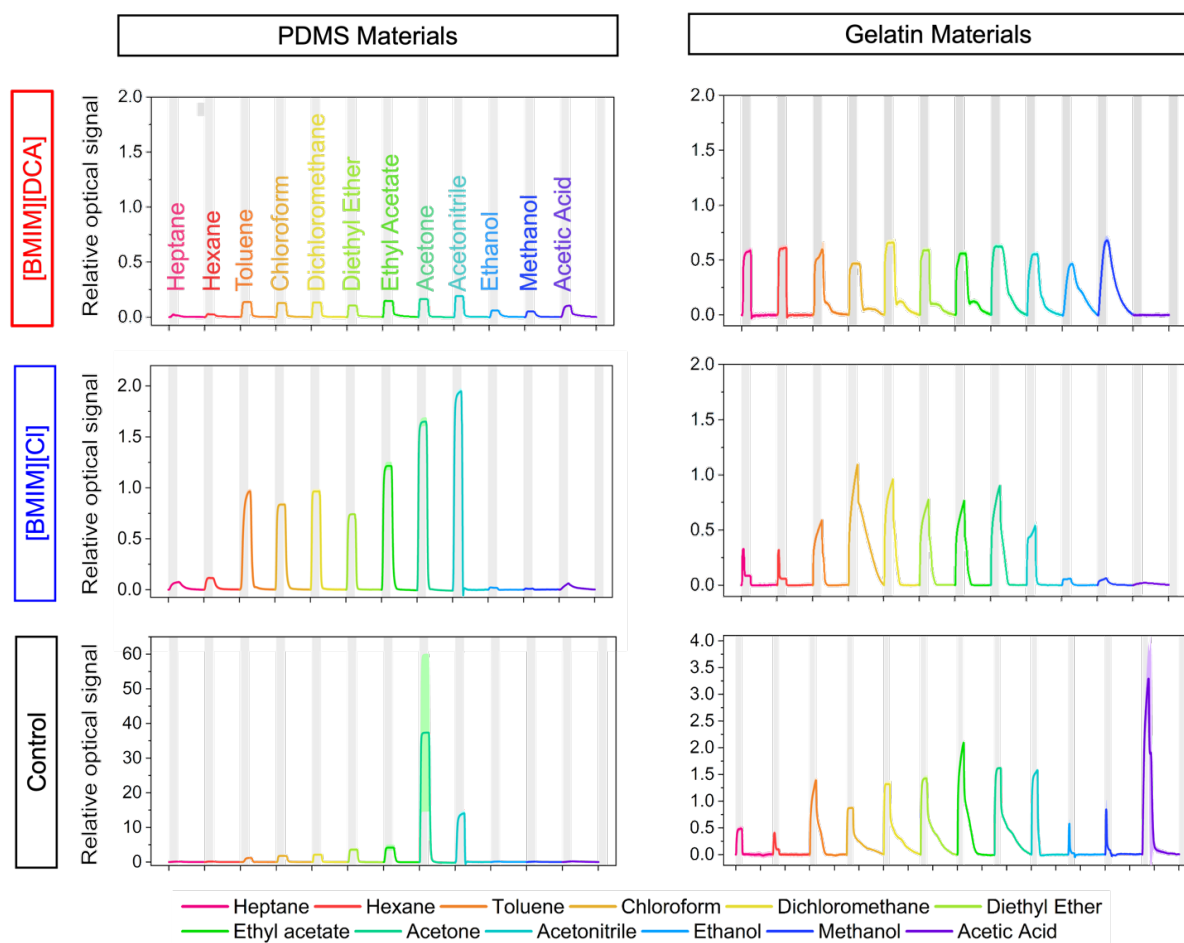


Figure 3. 5 – Representative signals of the optical amplitude responses of all hybrid gels and control gels studied. The typical profile of the signals obtained during exposure and recovery cycles in the optical e-nose. The plots were generated by python.

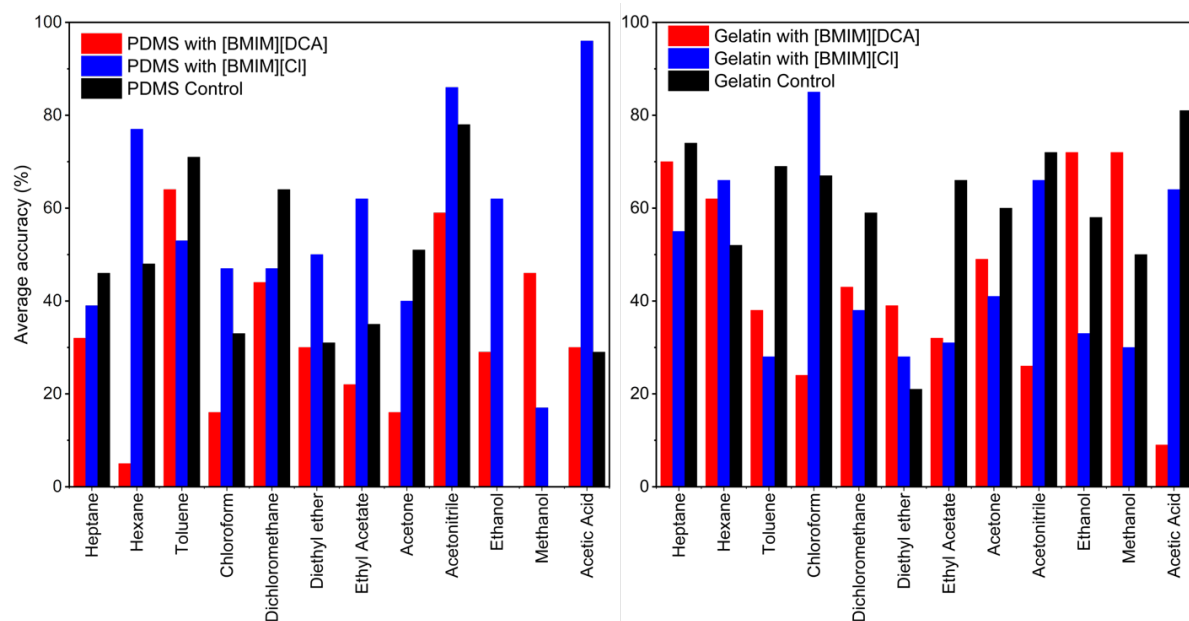


Figure 3. 6 – Average accuracy scores from a Confusion Matrix analysis regarding the ability of each sensor to accurately predict each volatile being tested, during the optical e-nose experiments. Above 50% it is considered a good classification of the volatile from the sensor. Sensors containing PDMS with [BMIM][DCA], [BMIM][Cl] and without ionic liquid (Control), as well as sensors containing gelatin with [BMIM][DCA], [BMIM][Cl] and without ionic liquid (Control) are represented.

The accuracy to predict volatiles in general was obtained with higher scores for the PDMS with [BMIM][Cl] ionic liquid, achieving a 56% of accuracy, highlighting the best results for acetic acid (96%), acetonitrile (86%) and hexane (77%), and the worst for methanol (17%). On the other hand, the lowest general accuracy predictions scores were obtained for the same matrix using the [BMIM][DCA] ionic liquid, achieving only a 33% of general accuracy predicting volatiles, being clear that overall all volatiles failed to be correctly identified by the sensing film, scoring only good values for acetonitrile (59%) and toluene (64%).

The [BMIM][DCA]-gelatin matrices presented better scores for ethanol (72%), methanol (72%), heptane (70%) and hexane (62%) prediction, when compared to [BMIM][DCA] in PDMS, obtaining a total general score of 45% of accuracy. For [BMIM][Cl] in Gelatin matrix, also good scores were found in general for volatiles like acetic acid (64%), acetonitrile (66%) and hexane (66%), similar to [BMIM][Cl] in PDMS matrix, which points for the critical role of ionic liquid in VOCs discrimination. Also, [BMIM][Cl] in gelatin presented the best discriminating for chloroform (85%) of all gels.

The presence of PDMS in the formulation of the sensing films favoured the distinction of both toluene and acetonitrile, while gelatin matrices performed better for heptane, hexane and chloroform discrimination. Overall, the results corroborate the joint effect of all the moieties of the multicomponent hybrid gels during VOC interaction, and also point for the important impact of the IL on VOCs discrimination. Furthermore, the diversity in sensors composition and the complementary accuracy results obtained, open the possibility to combine the results from several sensors to improve VOC discrimination.

3.3.4 VOCs limit of detection using PDMS and gelatin hybrid gels as sensors

After assessing the effect of humidity and the potential for VOC sensing in humid conditions using PDMS and gelatin hybrid gels, we assessed their limit of detection for selected VOCs under humid conditions. Ethanol, acetone, toluene and hexane were chosen as representative of chemical groups with distinct polarity, hydrophilicity and affinity to the components of the studied materials^{8,25}. The optical e-nose device employed in the previous section was coupled to a VOC delivery system to monitor the films optical responses upon exposure to increasing concentrations of VOCs diluted in humid (50% RH) nitrogen. VOC concentrations were controlled by manipulating the samples temperature and the flow rates of the carrier and dilution nitrogen streams in the VOC delivery system. To generate the humid stream, a water bubbling system was installed in the dilution stream (Figure S3.2).

To ensure a constant baseline for the VOC experiments, the films were first allowed to stabilize for 15 minutes in 50% RH. In addition, a calibration blank assay under the same humidity and nitrogen flow rates but without VOC was performed, to discount the interference of noise in the signal, where it was previously seen that the optical signal from noise causes a minimal interference⁵.

The relative optical signals to increasing concentrations of VOCs under a controlled RH of the hybrid films containing [BMIM][DCA] or [BMIM][Cl] in a PDMS or gelatin matrix are represented in Figure 3.7 and Figure S3.7. The estimated limit of detection (LOD), the triggering concentration, and the saturated response can also be found in Supplementary Information (Tables S3.3, S3.4). The triggering concentration is understood as the concentration of VOC molecules that is enough to reach the droplets surface and trigger a heightened phase transition of the LC droplets. Below this concentration, the variation in the relative optical response is minimal. Above, the variation is significantly larger until it reaches the saturated response.

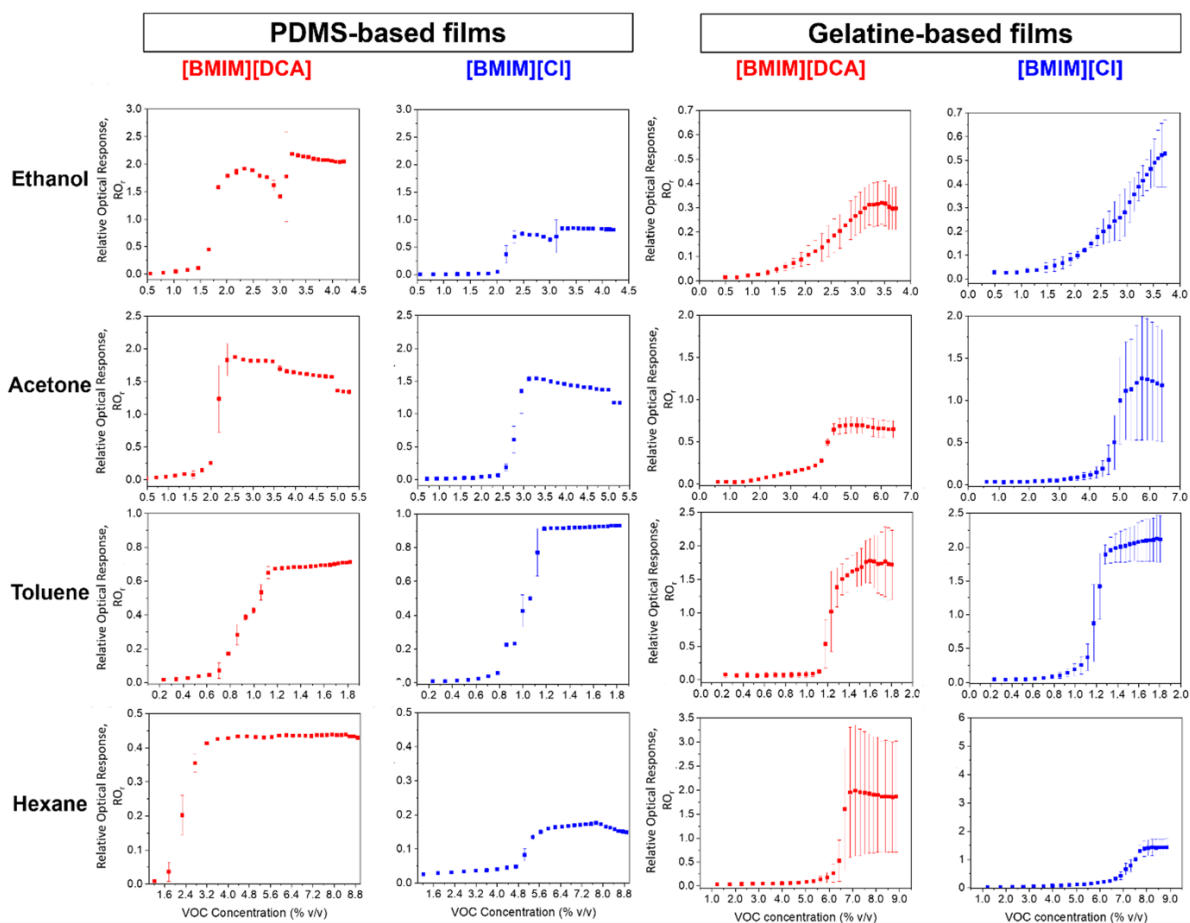


Figure 3.7 – Variation of the relative optical response of hybrid films with the increase in concentration of ethanol, acetone, toluene, and hexane, dissolved in humid (50% RH) nitrogen ($n = 5$ exposure cycles to VOC for each concentration).

The polarity of the VOCs influences the optical response of the films. More polar VOCs, like ethanol and acetone, cause a baseline drift on the signal throughout time, while the less polar VOCs, like toluene and hexane, provide a more stable and reproducible optical response (Figure 3.7). Non-polar VOCs tend to interact directly with the LC component of the hybrid films, thus conserving the configuration of the ionic liquid droplets, while polar VOCs likely interact mostly with the amphiphilic ionic liquids and, in case of gelatin, with the polymeric matrix. Therefore, over time polar VOCs alter the initial morphology of the IL-LC droplets, prompting a drift in the baseline of the optical signal. Changing the anion of the IL from [DCA⁻] to [Cl⁻] does not cause an evident alteration in terms of VOC-sensitivity performance. The optical response of the films has very similar waveforms, but the limits of detection (LOD) towards each VOC differ for PDMS and gelatin-based films.

In both PDMS and gelatin-based films, the optical response to ethanol of films containing the [Cl⁻] counterion is slightly weaker than the [DCA⁻] counterpart, likely because ethanol being very polar interacts strongly with surrounding water molecules, and films containing [BMIM][Cl] are less responsive to humidity than [BMIM][DCA]-based films. Additionally, the LOD in PDMS and gelatin-based films for [BMIM][DCA] is slightly lower than for [BMIM][Cl] (Table S3.3, S3.4).

It is also noted for ethanol, that gelatin films do not contain a triggering concentration. It is thought that in gelatin-based films, ethanol interacts preferentially with the matrix, the water, and the anion of the IL⁵. In PDMS films, ethanol would interact preferentially with the droplets, because the matrix being hydrophobic²⁶, the only component to interact with ethanol is the amphiphilic IL, thus the response mechanism functions similar to other VOCs (*i.e.* there is a triggering concentration that escalates the optical response).

For the remaining VOCs, it is observed that the triggering concentrations are lower for PDMS films than for gelatin (Table S3.3, S3.4). In gelatin films, the VOCs tend to perform H-bond and π - π interactions with the matrix⁵. PDMS is less prone to these interactions due to its chemical structure²⁷; instead, VOCs penetrate the film at voids, and because PDMS is cross-linked, added to the lower polarity of the VOCs, the rate of diffusion is also affected²⁶. Thus, the VOCs may preferentially interact with the droplets at the surface of the film⁷.

The response to hexane is significantly different between PDMS and gelatin-based films. Also, the LODs vary by changing the counter-ion of the IL (Table S3.3, S3.4). The detection limit is not detectable for [BMIM][DCA], and in [BMIM][Cl], PDMS-based films have lower LOD (1.76 % v/v) than what is observed for gelatin (2.27 % v/v).

The more hydrophobic PDMS polymer easily interacts with the less polar VOCs²⁶, while gelatin likely has a greater affinity towards more polar VOCs. In the case of hexane, perhaps due to its greater hydrophobicity, it must penetrate the IL droplets to interact with the LC and hydrophobic moiety of the IL, while the other VOCs are able to interact with the surface of the droplets⁵.

In summary, the change in the polymeric matrix between PDMS and gelatin of the hybrid films does not produce an evident improvement in VOC-sensitivity sensing-performance. Yet, due to the opposing nature of the two polymers, the films complement well one another, in terms of sensitivity to distinct VOCs.

3.4 Conclusions

Hybrid gel sensors were successfully produced using a PDMS matrix with 5CB liquid crystal and two different ionic liquids ([BMIM][Cl] and [BMIM][DCA]) and tested in an in-house built optical electronic nose device against a set of 12 different volatile organic compounds.

The presence of PDMS in the formulation of the sensing films seems to favour in general the distinction of both toluene and acetonitrile volatiles in general, as opposed to the presence of gelatin, which seems to do the same for heptane, hexane and chloroform instead. In this work, however, we conclude that the classification of VOCs through these experiments are consequence of the interactions with the entire multicomponent system, and not just dependent on the matrix individual component, but also that choice of the IL seems to be key in to achieve different VOCs' discrimination.

The highest accuracy results to generally predict volatiles was obtained for the PDMS with [BMIM][Cl] ionic liquid, achieving a 56% of accuracy, followed by PDLC control (scoring

41%), and the worst performance was given by PDMS with the [BMIM][DCA] ionic liquid, achieving only a 33% of general accuracy predicting volatiles.

When comparing the PDMS gels with the traditional gelatin sensing films with two different ionic liquids, in terms of morphology they diverge a lot in both droplets' visual appearance and quantity. As for stability to long-time storage, sensing materials containing [BMIM][DCA] (especially PDMS), proved to be the most stable gels, exhibiting radial stable droplets for over a month-period, against the ones where [BMIM][Cl] ionic liquid is used, that proved to be very unstable (especially the gelatin films).

Studies regarding the optical response of the sensing films to different humidity levels were also conducted, and the results indicate that, overall, the absence of ionic liquid in the sensor's composition (control films) the influence of humidity in the optical sensor's response derives essentially from the hydrophobic properties of the polymeric matrix. The use of PDMS as supporting matrix for the thin films changes the effect of humidity on the sensing performance compared to gelatin, but it does not entirely optimize it, due to the presence of the amphiphilic IL – when adding the ionic liquid into the composition, the measured optical signal is mostly affected by the nature of the anion from the ionic liquid. A higher humidity effect is clearly observed for [BMIM][DCA]-containing hybrid gels, and still, the amplitude of the optical response for PDMS-based hybrid gels is lower than for gelatin-based gels, indicating a lower water interference.

Generally, sensors containing [BMIM][DCA] are more responsive to humidity variations, while films containing [BMIM][Cl] remain very unresponsive in comparison.

VOC-sensitivity sensing-performance was also explored in this work, and conclusions can be drawn that neither gelatin nor PDMS-based films outperform one another, but the two distinct film recipes complement well each other when used in combination for VOC-sensing in low concentrations.

Supplementary Information

Materials and Methods

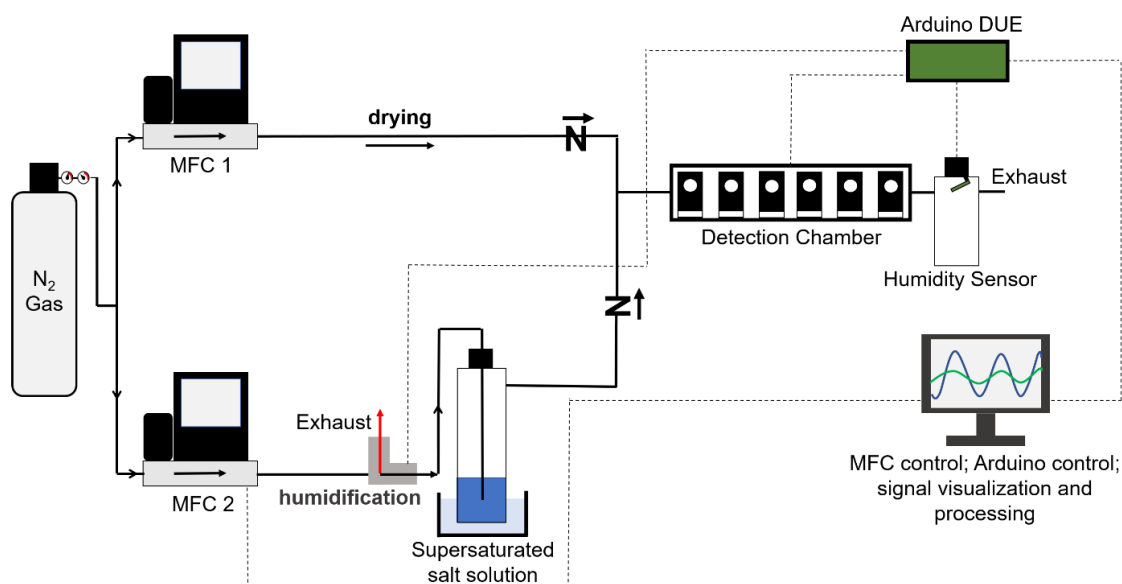


Figure S3. 1 – Experimental setup for humidity delivery coupled with the signal acquisition device. The sensors in the detection chamber are alternately exposed to dry (0% RH) and humid nitrogen (between 25-85% RH), by sampling nitrogen directly to the detection chamber or by bubbling nitrogen through supersaturated salt solutions at ambient temperature, respectively.

Table S3. 1 – Examples of supersaturated salt solutions and corresponding maximum relative humidity (RH) levels measured at ~20° C at the outlet of the detection chamber. Distilled water generates the maximum relative humidity.

Supersaturated salt solution	Maximum RH at outlet of the detection chamber (%)
Magnesium Chloride	45
Sodium Bromide	60
Sodium Chloride	70
Distilled Water	85

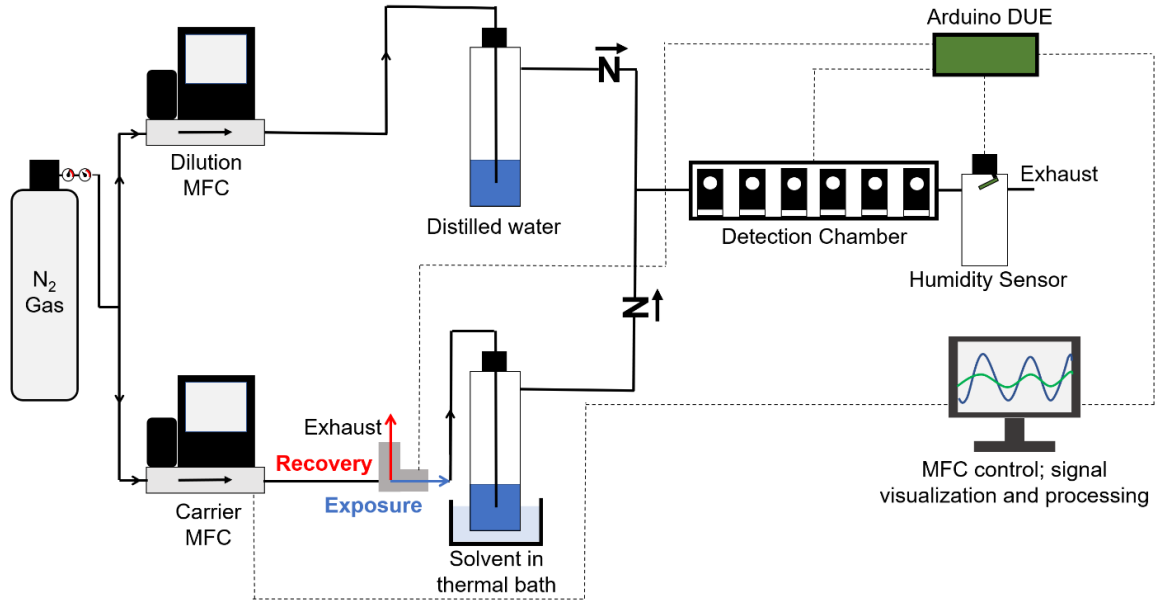


Figure S3. 2 – Experimental setup for delivery of known VOC concentrations under dry and humid conditions coupled with the signal acquisition device, for the evaluation of PDMS-based films containing [BMIM][DCA] or [BMIM][Cl] ILs as gas sensitive optical sensors. Distinct VOC concentrations in dry and humid nitrogen are generated and sent to the detection chamber by manipulating the temperature of sample in the thermal bath and the flow rates of the dilution and carrier mass flow controllers (MFC).

The flow rate of VOC vapor and water vapor at the output of the bubbler flask, F_n , was calculated using the bubbler equation (equation S1).

$$F_n = \frac{P_n^*}{P_i} \times F_c, \quad n = v \text{ (VOC)}; w \text{ (water)} \quad (\text{equation S1})$$

where P_n^* is the saturated vapor pressure of the solvent or of water at a given temperature, calculated by the Antoine Equation, or corresponding to the registered RH, respectively; P_i is the pressure of the inlet stream, given by the carrier MFC; and F_c is the flow rate of the nitrogen carrier stream.

Different VOC concentrations in the detection chamber were generated by varying the temperature of the solvent (20° - 28° C) and by mixing the carrier flow rates (nitrogen flow rate (F_c) and VOC flow rate (F_v)) generated at the output of the bubbler flask with the dilution flow rates (nitrogen flow rate (F_d) and water vapor flow rate, if present (F_w)) at different ratios. The resulting VOC concentration, in % (v/v), (Table S3.2) were calculated from F_v and the total mixed flow rates (equation S2).

$$C_{VOC} \text{ (}\% \text{ (v/v))} = \frac{F_v \times 10^6}{F_c + F_v + F_d + F_w} \text{ (ppm)} \times 10^{-4} \left(\frac{\% \text{ (v/v)}}{\text{ppm}} \right) \quad (\text{equation S2})$$

Table S3. 2 – VOC concentrations to which gelatin and PDMS-based sensing films containing either the ionic liquids [BMIM][DCA] or [BMIM][Cl] were exposed.

VOC	Range of concentrations sampled to the detection chamber (% (v/v)) ^(a)
Ethanol	0.50 – 4.30
Acetone	0.50 – 5.00
Hexane	1.20 – 9.00
Toluene	0.20 – 1.90

Results and Discussion

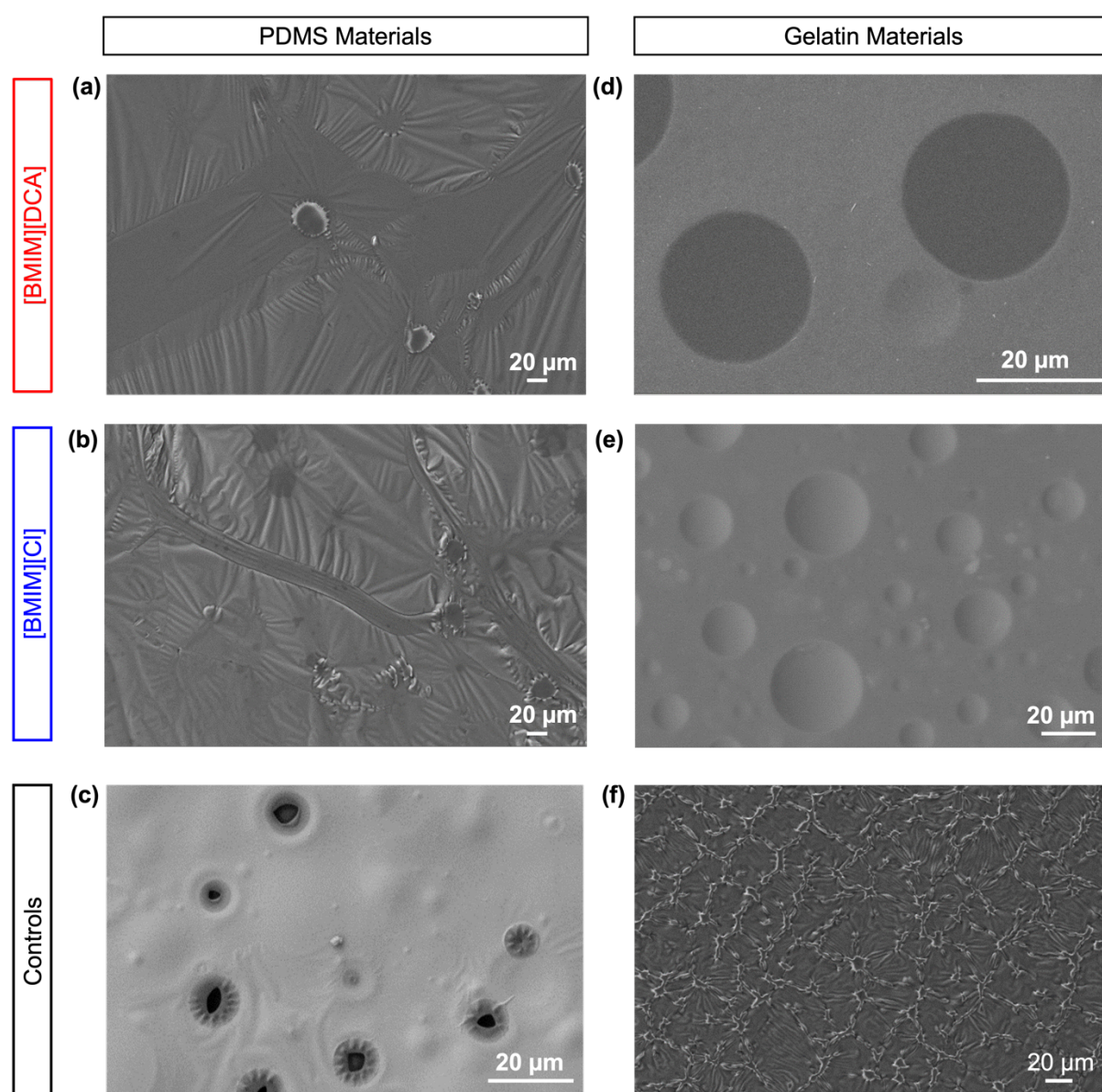


Figure S3. 3 – Scanning electron microscopy (SEM) images of Au/Pd coated films. a) PDMS matrix with liquid crystal 5CB and ionic liquid [BMIM][DCA]; b) PDMS matrix with 5CB and [BMIM][Cl]; c) PDMS matrix with 5CB (control); d) gelatin matrix with 5CB and [BMIM][DCA]; e) gelatin matrix with 5CB and [BMIM][Cl]; f) gelatin matrix with 5CB (control). Images were obtained by a Carl Zeiss AURIGA CrossBeam (FIB-SEM).

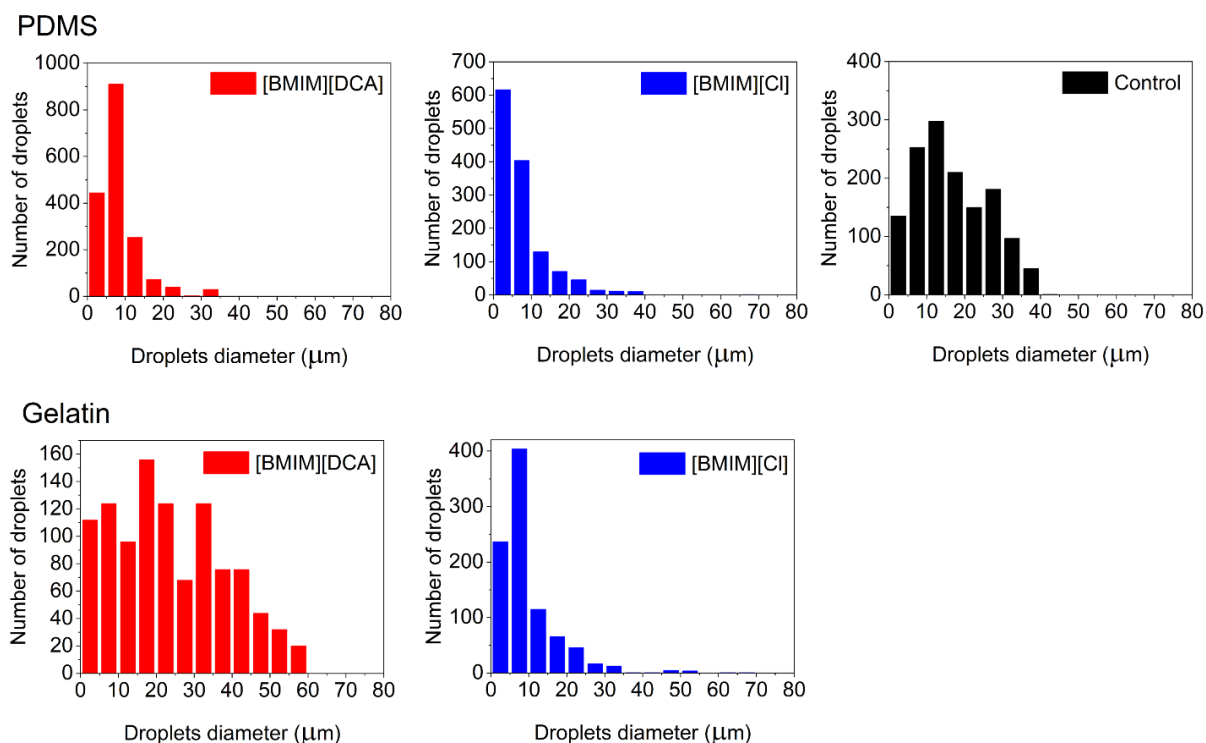


Figure S3. 4 – Histogram of droplets diameter distribution for the films produced with PDMS or gelatin, 5CB liquid crystal and both ionic liquids [BMIM][DCA] and [BMIM][Cl], as well as PDMS control.

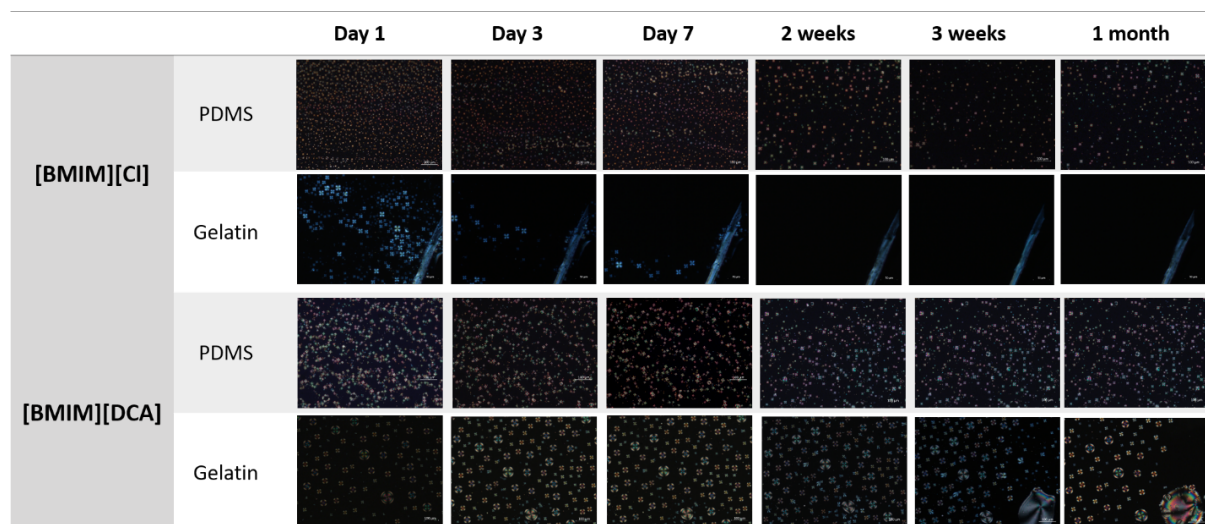


Figure S3. 5 – POM images of constant optically active areas obtained for the PDMS and gelatin sensing films produced from day 1 till 1 month after production. Images were obtained by Zeiss Polarized Optical Microscope (Zen 2.3 software), with 90° polarizers at 10x magnification.

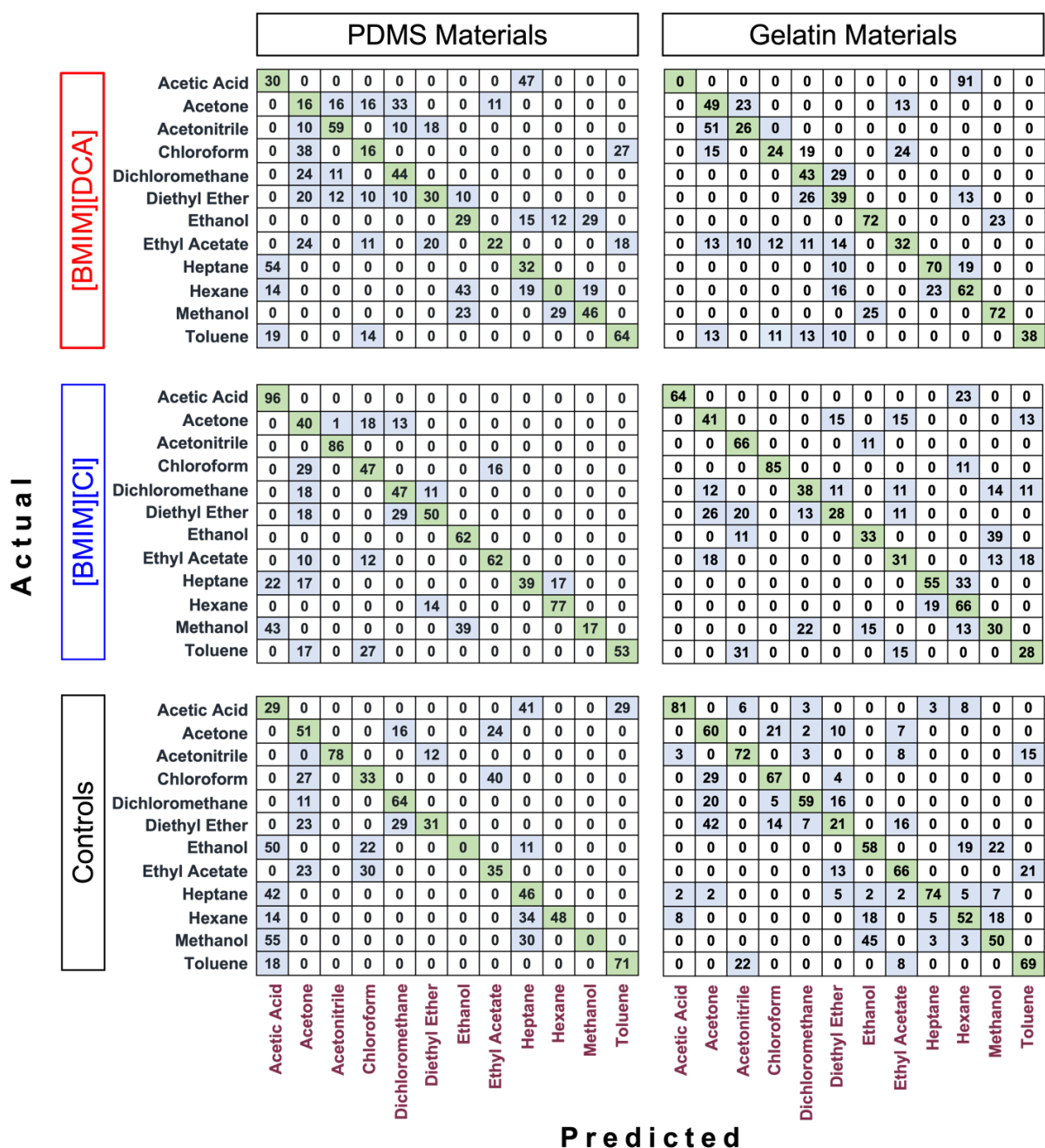


Figure S3. 6 – Optical e-nose confusion matrices obtained, illustrating the prediction results regarding 12 VOCs based on the signals obtained from the hybrid gel sensors with both PDMS and gelatin matrix hybrid sensors with two different ionic liquids ([BMIM][DCA] and [BMIM][Cl]). The green squares in the diagonal represent the correct predictions made by the classifier, and the lighter blue squares outside of the diagonal represent the incorrect prediction of a VOC.

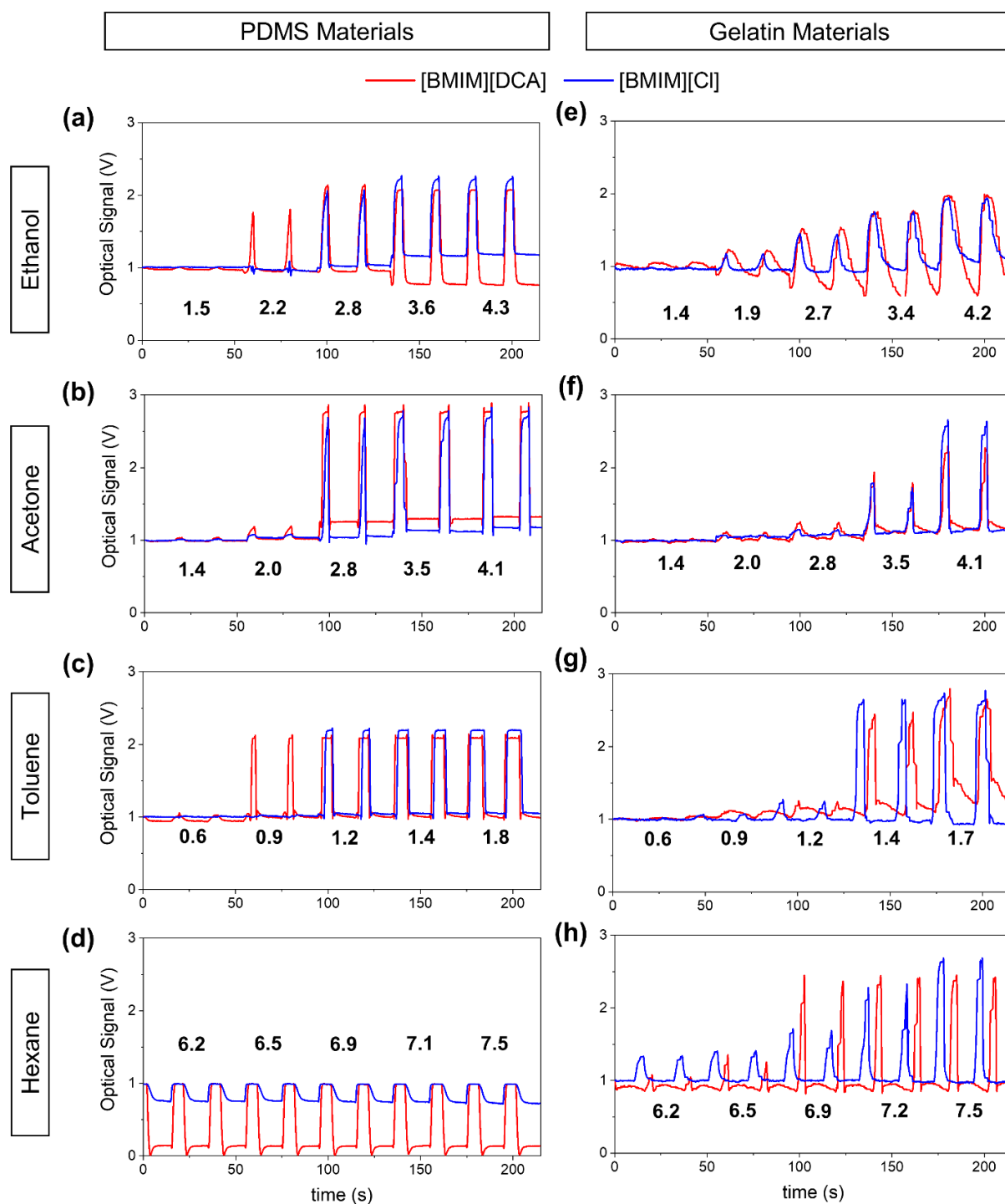


Figure S3.7 – Variation of the amplitude of the optical response of hybrid films with the increase in concentration (indicated in % (v/v) for each plot), of ethanol, acetone, toluene, and hexane dissolved in humid (50% RH) nitrogen. (a), (b), (c), (d) Response amplitude of hybrid films composed by PDMS, the ionic liquid [BMIM][DCA] or [BMIM][Cl], 5CB and water. (e), (f), (g), (h) Response amplitude of hybrid films composed by gelatin, the ionic liquid [BMIM][DCA] or [BMIM][Cl], 5CB and water. (n = 2 exposure cycles to VOC for each concentration).

Table S3. 3 – Optical sensing performance of [BMIM][DCA] hybrid films of PDMS or gelatin at 50% RH.

Polymer matrix	VOC	LOD (% v/v)	Triggering concentration (% v/v)	Saturation concentration (% v/v)
PDMS	Ethanol	1.03	1.65	3.24
	Acetone	1.16	1.99	2.57
	Toluene	0.44	0.70	1.20
	Hexane	n.d.	1.76	3.60
Gelatin	Ethanol	1.00	-	3.13
	Acetone	1.70	4.03	5.02
	Toluene	1.00	1.06	1.60
	Hexane	n.d.	6.17	7.11

Table S3. 4 – Optical sensing performance of [BMIM][Cl] hybrid films of PDMS or gelatin at 50% RH.

Polymer matrix	VOC	LOD (% v/v)	Triggering concentration (% v/v)	Saturation concentration (% v/v)
PDMS	Ethanol	1.84	2.01	3.24
	Acetone	1.79	2.57	3.12
	Toluene	0.53	0.80	1.20
	Hexane	1.76	4.71	5.92
Gelatin	Ethanol	1.10	-	n.d.
	Acetone	2.20	4.63	5.74
	Toluene	0.53	1.12	1.77
	Hexane	2.27	6.17	8.23

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INCORPORATION OF VOC-SELECTIVE PEPTIDES IN GAS SENSING MATERIALS

Publication in peer-review paper:

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Authors contributions:

Ana Rita Oliveira planned and performed the work, analyzed the data and wrote the manuscript. Ana C. A. Roque conceived and initiated the project. Gonalo D. G. Teixeira performed some of the MP-SPR experiments. Susana I. C. J. Palma developed the python scripts used in Supervised Machine Learning for data analysis. Efthymia Ramou and Ana C. A. Roque reviewed the article. Ana C. A. Roque supervised the project.

Communications

Oral communications

Rita Oliveira, Efthymia Ramou, Gonalo D. G. Teixeira, Susana I. C. J. Palma, and Ana C. A. Roque, 2022. “Incorporation of VOC-selective peptides in gas sensing materials”. BIOSTEC, BioDevices Conference 2022 (*presenting author*)

Poster presentation

Rita Oliveira, Gonalo Teixeira, Gonalo Santos, Cludia Alves and Ceclia Roque, “Enhancing VOCs selectivity by designing new e-nose sensing materials for gas sensing” – Affinity Conference, 2021, Lisbon, Portugal (22-24 June, 2021) (*presenting author*)

Rita Oliveira, Gonalo Teixeira , Gonalo Santos , Cludia Alves, Carina Esteves and Ceclia Roque, “Incorporating peptides in gas sensing gels: a case study for benzene selectivity” – Affinity Conference 2019, Stockholm, Sweden (26 - 28 June 2019) (*presenting author*)

Media coverage

Youtube Video

SCENT.IR: SCENT Intelligent Research video, Youtube (2020)

Incorporation of VOC-selective Peptides in gas sensing materials

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Keywords: Ionogels, Hybrid Gels, Peptides, Gas Sensing, Electronic Nose.

Abstract

Enhancing the selectivity of gas sensing materials towards specific volatile organic compounds (VOCs) is challenging due to the chemical simplicity of VOCs as well as the difficulty in interfacing VOC selective biological elements with electronic components used in the transduction process. We aimed to tune the selectivity of gas sensing materials through the incorporation of VOC-selective peptides into gel-like gas sensing materials. Specifically, a peptide (P1) known to discriminate single carbon deviations among benzene and derivatives, along with two modified versions (P2 and P3), were integrated with gel compositions containing gelatin, ionic liquid and without or with a liquid crystal component (ionogels and hybrid gels respectively). These formulations change their electrical or optical properties upon VOC exposure, and were tested as sensors in an in-house developed e-nose. Their ability to distinct and identify VOCs was evaluated via a supervised machine learning classifier. Enhanced discrimination of benzene and hexane was detected for the P1-based hybrid gel. Additionally, complementarity of the electrical and optical sensors was observed considering that a combination of both their accuracy predictions yielded the best classification results for the tested VOCs. This indicates that a combinatorial array in a dual-mode e-nose could provide optimal performance and enhanced selectivity.

4.1 Introduction

Gas sensing is currently emerging as a critically important technology related to a broad range of applications such as medicine¹, and early diagnosis of disease²⁻⁷. Indeed, volatile organic compounds (VOCs) are becoming increasingly recognized as potential biomarkers associated with disease. Artificial olfaction is the automated simulation of the sense of smell through the

use of electronic nose devices (e-noses), comprised by an array of chemical sensors with partial selectivity coupled with signal-processing and pattern recognition tools. The most common and commercially available gas sensing materials include metal oxide semiconductors⁸ or conducting polymers⁹. However, their main drawbacks include low long-term sensor stability, high maintenance, cumbersome and complex instrumentation, and most importantly low selectivity. Thus, it is of high interest to develop better and competitive alternatives, by adapting the components of the sensors in order to enhance their selectivity, reliability and portability¹⁰. In biological olfaction, odorant binding proteins and olfactory receptors are the main tools used to address the difficult problem of selectivity. In artificial olfaction, these find limitations as they are difficult to produce, as well as to stabilize and interface with electronic systems for signal transduction, long-term storage and repeated use¹¹.

Among the alternative options to tune selectivity, peptides are one of the most attractive choices due to their robustness, chemical diversity, compact size, and their adaptability to extreme environments and safely long-term storage^{12,13}. Furthermore, they can be developed to bind distinct targets through rational design or discovery by panning of phage display libraries¹⁴.

In this work, we investigated the incorporation of a previously reported peptide (peptide P1), and two designed derivatives (peptides P2 and P3) able to discriminate single carbon deviations among benzene and derivatives such as toluene and xylene¹⁵, into gel-like sensing materials yielding electrical and optical signals in the presence of VOCs. Two gel formulations were studied: a gelatin matrix gelled in an ionic liquid environment ([BMIM][DCA]) (ionogels yielding an electrical signal by monitoring the changes in ionic conductance of the formulation) and the same matrix with a liquid crystal component (hybrid gels yielding an optical signal due to their birefringence). The modified versions of P1 were obtained through the addition of non-natural amino acids at the C-terminal – norleucine (Nle - P2 peptide) and biphenylalanine (Bip, - P3 peptide) – with the purpose to facilitate their binding in the ionic liquid-liquid crystal interface, due to their structural resemblance with 5CB (Figure 4.1). These materials offer the possibility to add the peptide selective moiety with unprecedented simple procedures that take advantage of self-assembly and autonomous compartmentalization, avoiding harsh chemical reactions and peptide covalent attachment onto surfaces.

4.2 Materials and Methods

4.2.1 Materials and Reagents

Gelatin from bovine skin (gel strength ≈ 225 g; Bloom, Type B), was purchased from SigmaAldrich. The liquid crystal 4-cyano-4'-pentylbiphenyl (5CB), was acquired from TCI Europe, and the Ionic liquid 1-Butyl-3-methylimidazolium chloride ([BMIM][DCA], $>98\%$) was purchased from IoLiTec. Peptides 1, 2 and 3 were purchased from Genecust (purity $>95,9\%$). Ethanol (purity 99.8%) and Fluorescein isothiocyanate (FITC) Isomer I (99% purity) were purchased

from Sigma-Aldrich, while benzene, hexane, xylene and toluene were supplied by Fisher Scientific, and acetone (purity 99.5%) was purchased from Honeywell.

4.2.2 Confirmation of peptide P1 binding Multi-Parametric Surface Plasmon Resonance (MP-SPR)

For the MP-SPR studies, an Au-glass slide was modified with 3-mercaptopropionic acid (3-MPA), a linker with a hydroxyl group and a sulfhydryl group, which binds to the Au surface thiols. For the immobilization of the P1 peptide (10 μ M in 20 mM sodium phosphate solution, at pH 7) on the surface EDC/NHS chemistry was used, and Ethanolamine was used as a blocking agent for extremities where P1 was not bound. This method was used to produce the sample placed in the peptide chamber, whereas the control chamber contained an Au-glass slide with all the previously described functionalization, minus the immobilization of the peptide step. The MP-SPR signal variation within time was measured by using two wavelengths, 670 nm and 785 nm (mDeg), being the average of the signal measured at the two wavelengths defined as Δ mDeg.

4.2.3 Incorporation of the peptides into ionogels

For the production of ionogel sensors we used the same protocol as previously described with the addition of peptide solutions¹⁶. The final formulation was pipetted onto interdigitated golden electrodes deposited on untreated glass slides and spread into a thin film using a TQC film applicator (Automatic Film Applicator Standard, TQC) with a 30 μ m thickness. After production, the peptide ionogels were left to dry on a sealed clean petri dish, in a humidity box with environmental control. Control ionogels (without the incorporation of a peptide component) were also prepared, for comparison purposes.

4.2.4 Incorporation of the peptides into hybrid gels

Peptide hybrid gels were formulated by mixing all components plus the peptide solution following protocols previously described^{16,17}. When gelation occurs, the gel compositions were deposited on top of untreated glass slides and spread into thin films using a TQC film applicator (Automatic Film Applicator Standard, TQC) with a 30 μ m thickness. After production, the gels were stored within an environment of controlled humidity. Control hybrid gels (without a peptide component) were also prepared for comparison purposes.

4.2.5 Hybrid gel characterization

The formation of ionic liquid droplets and the morphological characteristics and differences between the hybrid gels containing the P1, P2 and P3 peptides were assessed via a ZEISS, Observer.Z1 Polarized Optical Microscope (POM) equipped with an Axiocam 503 color camera. Pictures were taken with crossed polarizers and in bright field and were processed with the ZEN 203 software.

Regarding the morphological stability of the hybrid gels upon storage over time, POM photos with crossed polarizers of the same region of interest were taken for a 30-day period. A custom-made python script (Python 3.6.2) was used to analyze the photos. The script uses binary images to calculate the differences between them (see Supplementary Information, Figure S4.3).

Fluorescence microscopy was also used to verify the binding of the P1 peptide to the ionic liquid interface, and its presence in the hybrid gels formulation (see Supplementary Information, Figure S4.4). To label the P1 hybrid gels with a fluorophore, 30 mg/ml stock solution of peptide P1 in carbonate-bicarbonate buffer (0.1M pH = 9.0) was prepared and 0.44 mg of FITC was diluted in 1 ml of DMSO. Then, 300 μ L of the FITC solution was added, in aliquots of 10 μ L, slowly while agitating, to 100 μ L of P1 solution. The final mixture was left to incubate in the dark overnight. P1-FITC conjugate was recovered by HPLC. Since the resulting sample was very diluted, it had to be concentrated through the use of a rotary evaporator. The labelled P1 was then added to the hybrid gels formulation, and observed under fluorescence microscopy, using the same a ZEISS, Observer.Z1 Polarized Optical Microscope device, equipped with a filter 38 HE Green Fluorescence filter (Ex/Em: 450nm-490nm/500nm-550nm).

4.2.6 VOC sensing using ionogels

All the prepared ionogels (peptide-based and controls) were assessed in an in-house tailor-made electrical e-nose device¹⁶. The device detects changes in the conductance upon VOC adsorption and desorption. The sensors were placed in a hermetically sealed array chamber and exposed to a sequence of six volatiles – hexane, benzene, toluene, xylene, ethanol and acetone – similar to the investigation of Ju et al.¹⁵. The solvents were kept in a bath thermostated at 37°C and their respective vapors were pumped to the array chamber (exposure period) followed with ambient air via a second air pump (recovery period). The films were exposed to each gas analyte for 45 consecutive cycles, each cycle consisting of 5 sec of exposure followed by 10 sec of recovery, in total 11 min and 15 sec of test duration per VOC. The electrical signals of the sensors were acquired at a sampling rate of 90 Hz, and assays were performed in duplicates.

Processing and evaluation of the signals obtained was performed, using methods described in our previous publications^{17–20}. Python programming tools were used to process the signals retrieved by the e-nose device.

For the evaluation of the sensors' performance regarding VOC identification, machine learning methods were applied to the individual cycles. Briefly, twelve features representative of the signals morphology were extracted from each cycle and used as input variables in an automatic classifier based on Support Vector Machines (SVM) algorithm, using a radial basis kernel and hyperparameters $C = 100$ and $\gamma = 0.1$. The dataset for each sensor formulation was composed by 30 cycles per VOC. Two thirds of this dataset were used as training set and one third as validation set for the automatic classifier. Accuracy bar plots, which exhibit the

percentage of the correct predictions of the classifier were used to represent the sensors performance.

4.2.7 VOC sensing using hybrid gels

The optical e-nose device is designed to monitor the light transmitted by the hybrid sensors and convert it into voltage. The detailed setup has been described in our previous works^{16–19}. The sensors are placed in a hermetically sealed detection chamber and each one is paired with a polarizer and a corresponding analyzer. The VOC experiments, as well as the data analysis of the signals were conducted and processed as described in the previous section.

4.3 Results and Discussion

4.3.1 Assessing VOC selectivity in ionogels – Electrical E-nose

The affinity and selectivity of peptide P1 towards VOCs was firstly assessed by MP-SPR (the full procedure and detailed results can be seen in Supplementary Information: Table S4.1 and S4.2, Figure S4.1 and S4.2). Our results indicated a preferred binding of the peptide P1 towards VOCs following the order benzene>xylene>toluene>ethanol>acetone>hexane, as can be seen by the increase of the Δm_{Deg} values (see Table 4.1).

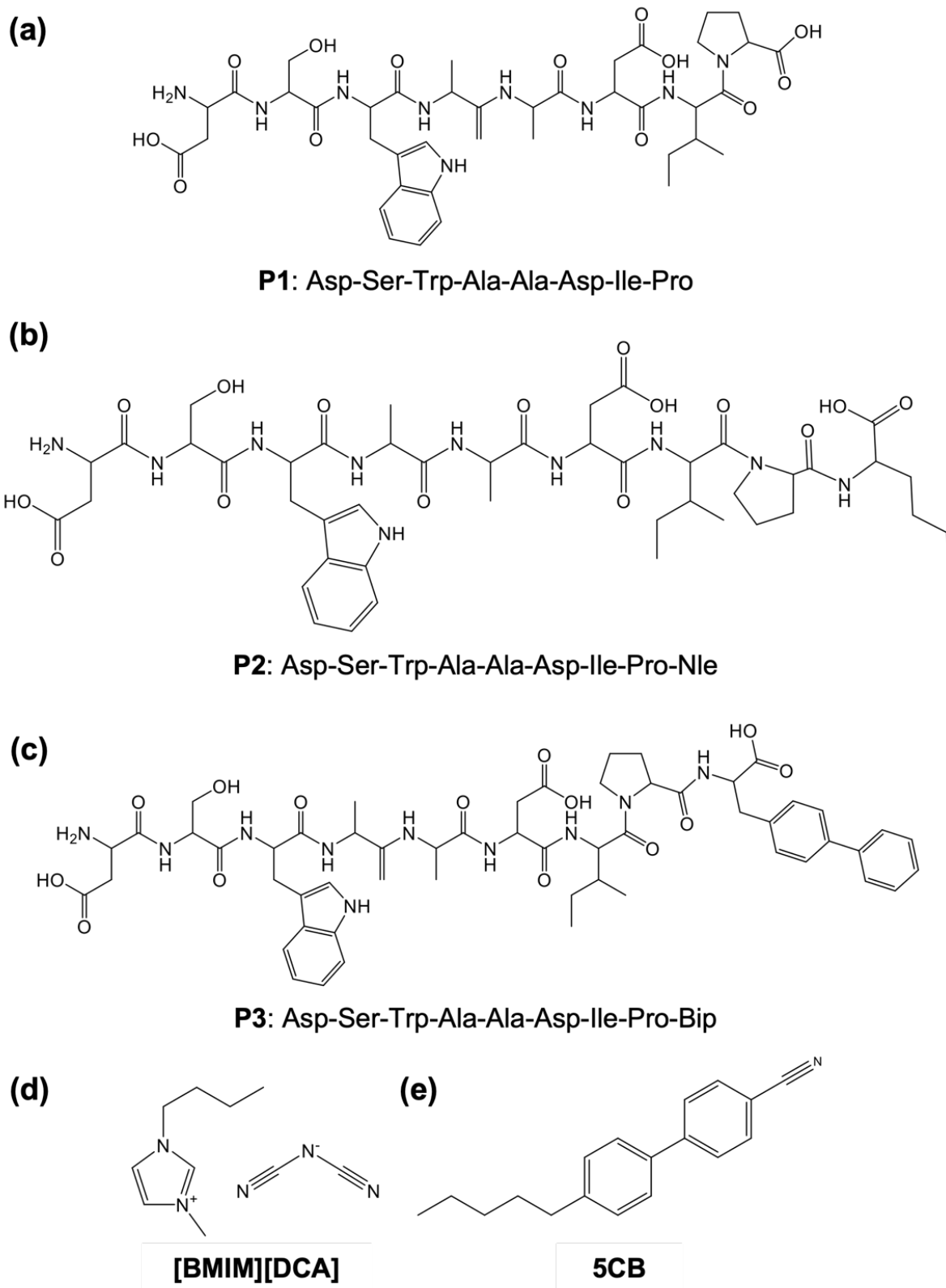


Figure 4. 1 – Chemical structure of (a) peptide 1, (b) peptide 2, (c) peptide 3, (d) ionic liquid 1-Butyl-3-methylimidazolium dicyanamide ([BMIM] [DCA]) and (e) liquid crystal 4-Cyano-4'-pentylbiphenyl (5CB).

Table 4. 1 – Summary-results of the interaction between the P1 functionalized Au surface and the VOCs. Δm_{Deg} (peptide-control) = Δm_{Deg} peptide - Δm_{Deg} control, where Δm_{Deg} peptide is the average of the signal measured at the 670 and 785 nm wavelengths in the peptide chamber, and Δm_{Deg} control is the average of the signal measured at the 670 and 785 nm wavelengths in the control chamber. Relative signal variation = the relative signal representing how much the signal increased on the peptide chamber, when compared to the control chamber.

VOCs	Δm_{Deg} (Peptide-Control)	Relative signal variation
Acetone	5.76 ± 2.16	0.21
Ethanol	2.20 ± 3.63	0.33
Benzene	32.54 ± 3.35	5.25
Xylene	14.69 ± 2.97	1.20
Toluene	5.61 ± 4.36	0.46
Hexane	6.19 ± 3.94	0.17

The three peptides were then incorporated into the ionogel gelatin matrices and spread as thin films onto interdigitated electrodes to be tested in an in-house developed electrical e-nose. VOCs adsorption to the ionogels affects the ion mobility within the materials. Therefore, the ionogels admittance changes and an electric response can be obtained. This is a reversible process upon VOC desorption and the basis for electrical VOC sensing with the electric E-nose¹⁸.

Through the sequential exposure of the peptide ionogel formulations to the 6 tested volatiles – hexane, benzene, toluene, xylene, ethanol and acetone – variations in the conductance of the sensors were monitored in real time and after signal processing (as described in Materials and Methods section) typical relative amplitude responses are presented in Figure 4.2a.

The sensor responses exhibit a variability in their profiles, characteristic of the corresponding gas analyte exposure. For example, all of the sensors during the linear 6C-alkyl chain hexane exposure respond with a downwards curve, while all other volatiles generate an upwards response signal. The majority of the sensors respond rather quickly (within seconds) upon VOCs exposure, during which most signals never reach a plateau, with the exception of xylene in control and P3 ionogels. Upon recovery, almost all sensor signals return to the baseline in a similar way.

After signal collection machine learning-based tools were implemented to analyze the data. The signals were divided into cycles and were then normalized. A set of features corresponding to the curve morphology^{17,18,20} was extracted and used as input for an SVM-based automatic classifier. The accuracy % of correct VOC prediction is presented in an accuracy bar plot seen in Figure 4.2b, depicting that the tested ionogel compositions exhibit distinct selectivity for the tested VOCs. More details and examples of the mean wave signals derived from the Electrical e-nose responses for all the different ionogel formulations, can be found in the Supplementary Information, in Figures S4.5-S4.8, alongside with the complete Confusion

Matrices containing the accuracy performance of all ionogels to predict and classify the tested volatiles (Supplementary Information, Figure S4.9).

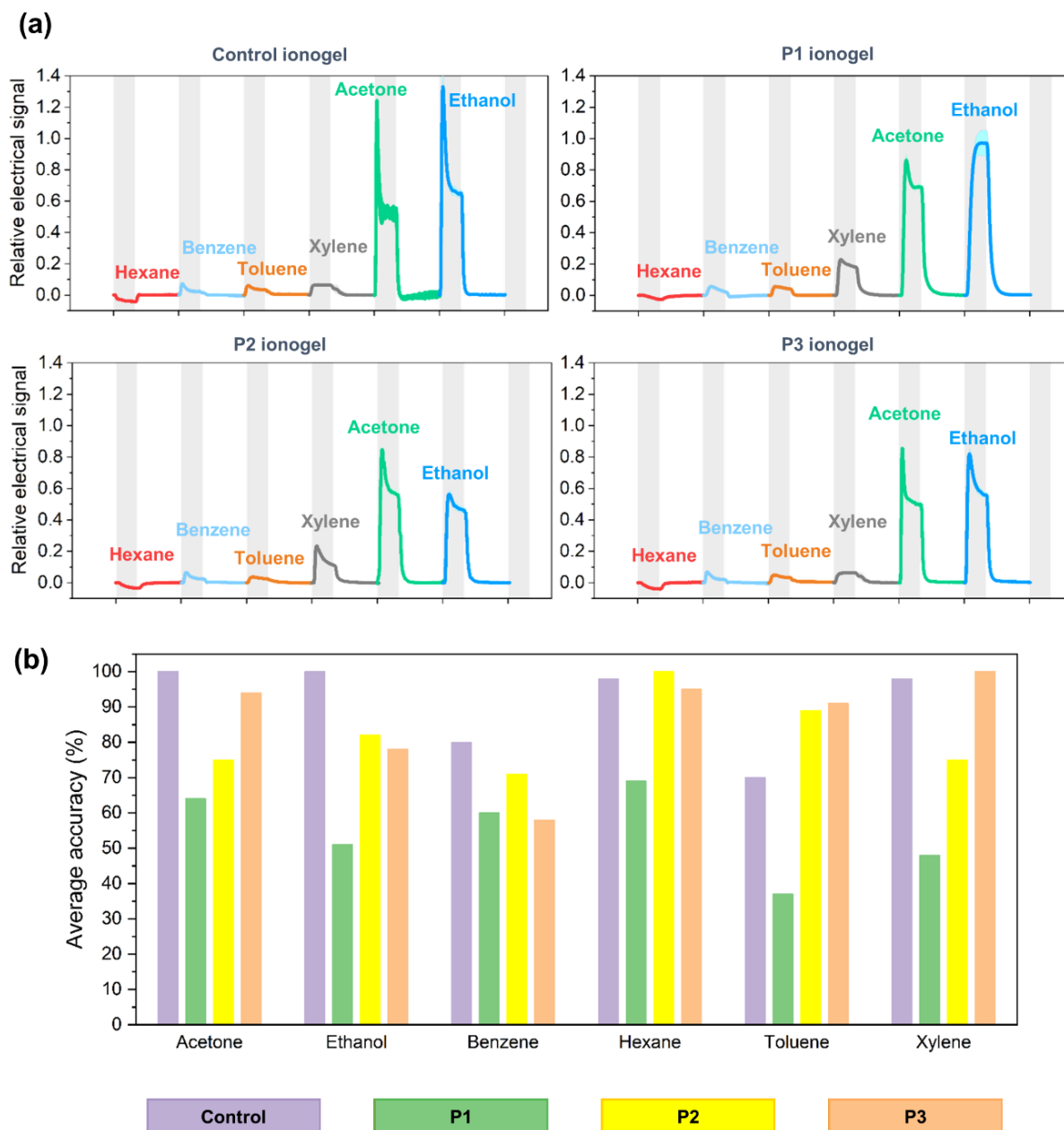


Figure 4. 2 – Electrical e-nose results for Control, P1, P2 and P3-based ionogels. (a) Typical cycle signals of all the sensors upon exposure to different VOCs. Each curve represents the average and standard deviation of at least 19 replicate cycles from the same sensor. VOC exposure periods (5 s) are highlighted in grey, and each cycle corresponds in total to 15 s. (b) Comparison of the VOC prediction accuracies obtained for all the ionogels tested in the electrical e-nose.

For example, P2 and P3-based sensors were able to discriminate and identify hexane and xylene, respectively, with an 100% accurate classification. This could be associated with the presence of the similar non-canonical aminoacid norleucine and biphenylalanine moieties in P2 and P3 peptides respectively and their similarity to the structure of the volatiles in question,

possibly enhancing the interaction between the sensors and the volatiles. On a similar vein, the control ionogel provides great classification results for acetone and ethanol, with a 100% accuracy. The accuracy scores for the P1 ionogel were not as high (e.g. for toluene and xylene) which suggests that certain signal profiles produced by the formulation exhibited similar characteristics, thus not allowing the distinction of the corresponding analytes. Overall, the control ionogel formulation achieved the best global accuracy score (91%) followed by the P3-based ionogel (86%).

These results indicate that the incorporation of the peptides onto the gelatin ionogels did not yield an improvement of selectivity towards benzene. This is mainly due to the fact that the control ionogel already displayed excellent discriminating behavior towards the particular VOCs tested, as conductance and selectivity are already driven by the ionic liquid component.

4.3.2 Assessing VOC selectivity in hybrid gels – Optical E-nose

The peptides P1, P2 and P3 were incorporated into hybrid gel formulations by simply adding them to the formulation and promoting autonomous self-assembly of the different moieties into the hybrid gel compartments. The formation of ionic liquid – liquid crystal droplets was observed by bright field and polarized optical microscopy (Figure 4.3).

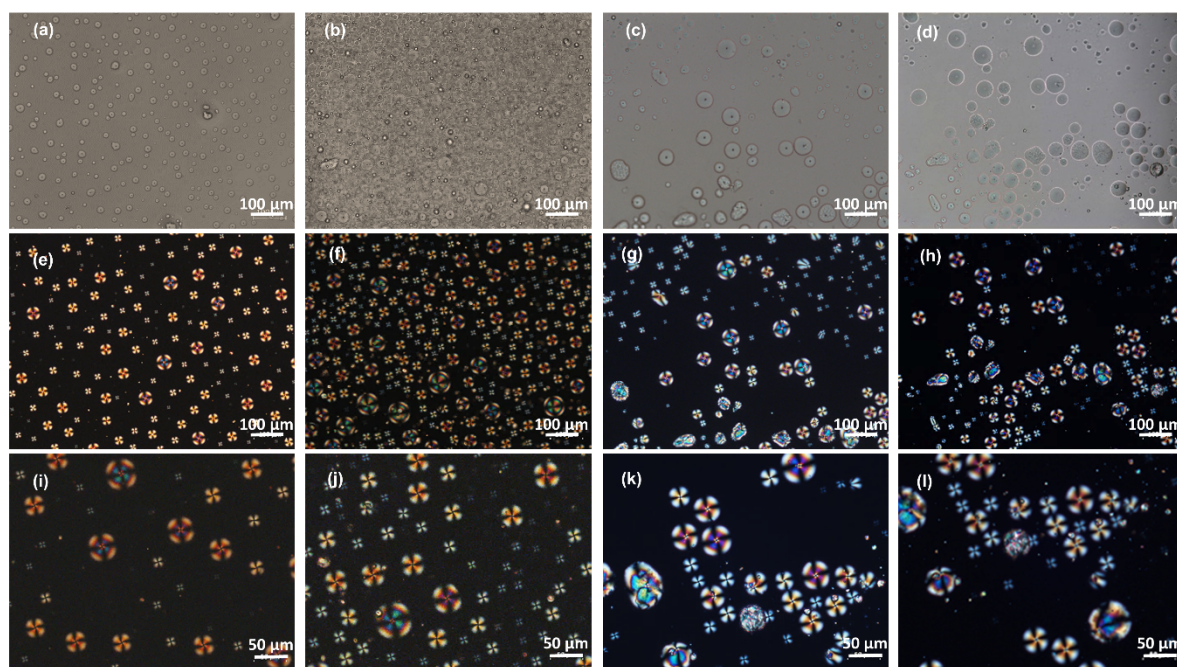


Figure 4. 3 – Representative POM images of control hybrid gel (a), (e) and (i), peptide 1 hybrid gel (b), (f) and (j), peptide 2 hybrid gel (c), (g) and (k), and peptide 3 hybrid gel (d), (h) and (l).

All the hybrid gels (control and peptide-based) exhibit polydisperse 5CB droplets as previously noted^{16–18}, featuring a radial configuration which under the POM gives rise to a distinctive Maltese cross pattern²¹. The characteristic core defect located in the center of the droplet can be observed in the bright field pictures^{16,17} – seen in Figure 4.3a,e and j. The radial droplet profile suggests that the liquid crystal molecules adopt a homeotropic alignment near the

ionic liquid interface, which is attributed to the interactions occurring between the alkyl chain of the ionic liquid and 5CB^{16,17,22}.

We need to point out that in the case of P2-based and P3-based hybrid compositions, apart from the radial droplets, some irregularly shaped and randomly oriented droplets were also observed. This finding suggests that both the Nle (in the case of the P2 peptide) and Bip (in the case of the P3 peptide) moieties interfere with the liquid crystal anchoring on the ionic liquid interface.

The peptide-based hybrid gels were tested in the optical e-nose. Exposure to the tested gas analytes results in a disorganization of the liquid crystal component, triggering a phase transition to the isotropic state. The signal responses are the collaborative responses of the individual compartments of the gel formulations to the corresponding analyte. For example, hydrophobic VOCs, such as hexane and the aromatic benzene, xylene and toluene, are more likely to interact mainly with the oil phase formed by the liquid crystal molecules inside the droplets. Protic VOCs, and those forming hydrogen bonds (e.g. ethanol), tend to interact not only with the LC droplets but also with the gelatin matrix itself, as previously reported^{16,17}. The sensor responses were repeatable and exhibited features (such as signal profile, response/recovery profile) characteristic of the tested volatiles.

Signal processing, analysis and presentation were conducted using the same tools as in the electrical e-nose results, described in the previous section. In Figure 4.4a relative amplitude responses for each tested hybrid peptide gel compositions and for all the studied gas analytes is shown. The baseline on each individual curve represents the initial light state of a sensor, due to the presence of the liquid crystal droplets. Upon VOC exposure the liquid crystal component becomes isotropic, thus it cannot alter the polarization of the transmitted light (which subsequently cannot pass through the analyzer). This generates an upwards response curve to a dark(er) state for the sensor.

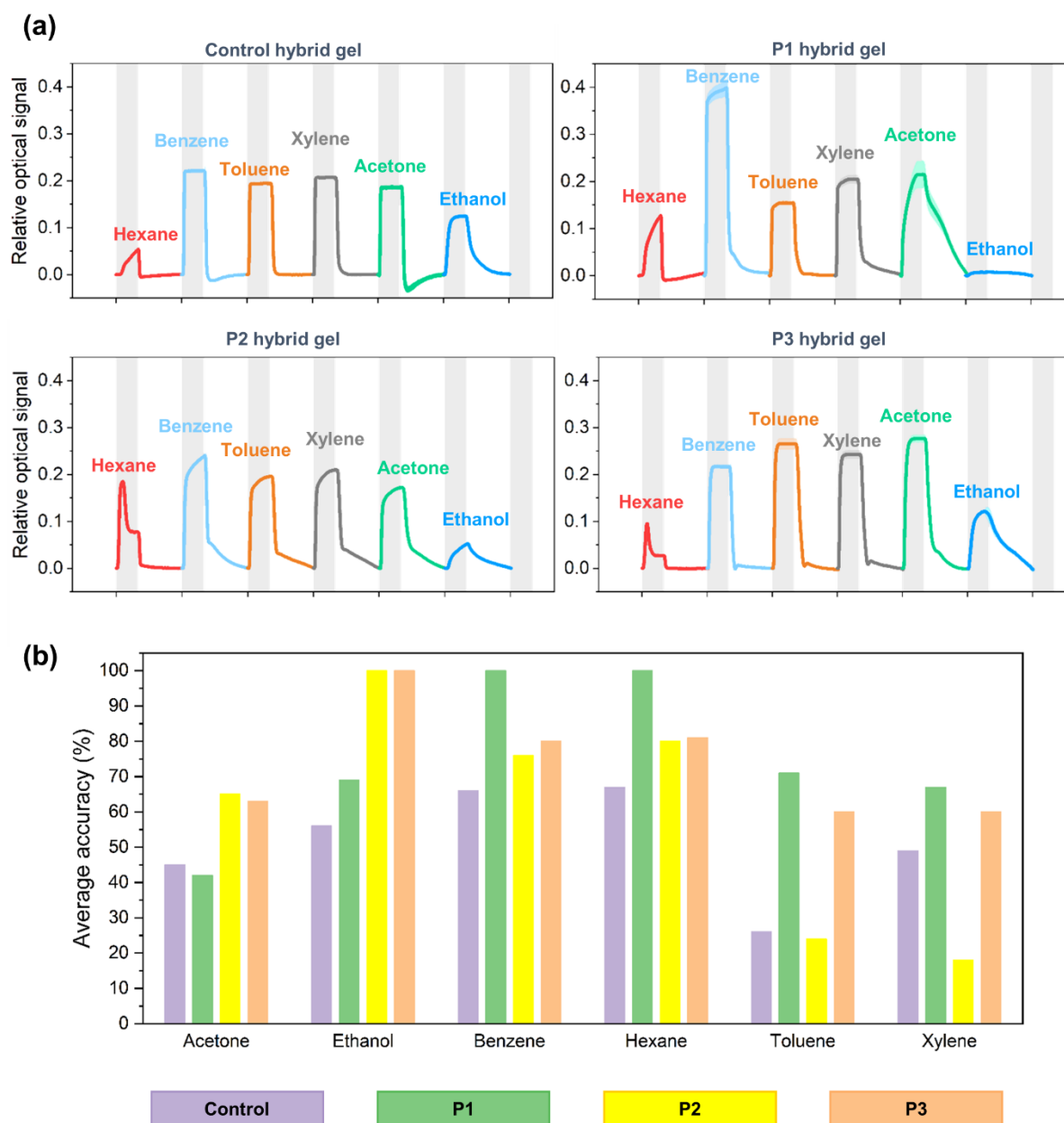


Figure 4. 4 – Optical e-nose results for the for Control, P1, P2 and P3-based hybrid gels. (a) Typical cycle signals of all the sensors upon exposure to different VOCs. Each curve represents the average and standard deviation of at least 19 replicate cycles from the same sensor. VOC exposure periods (5 s) are highlighted in grey, and each cycle corresponds in total to 15 s. (b) Comparison of the VOC prediction accuracies obtained for all the sensors tested in the optical e-nose.

It is possible to observe that each sensor holds a very characteristic amplitude signature signal, related to the different volatiles. For example, a delayed response upon analyte exposure is observed (e.g. hexane for control and P1-based hybrid gels), or the cases where a plateau in the response is reached (e.g. the control and P3-based sensors for all volatiles, with the exception of hexane and ethanol), and a very distinctive flat response from P1-based sensor towards ethanol.

Another interesting observation is that although the 10 s recovery period seems to be sufficient for the liquid crystal to completely recuperate from isotropisation in the control hybrid formulation (allowing the sensors signal to return to the initial baseline levels), the majority of the peptide-based sensors appear to take longer to completely recover within the 10 s period. This is evident in the case of P1 sensors with benzene, xylene and acetone, and both P2 and P3 sensors with all volatiles – with the exception of hexane. It should be noted that this recovery pattern was not detected for the ionogel sensors previously analyzed.

More details on the mean wave signal responses for all the different hybrid gel formulations can be found in the Supplementary Information, in Figures S4.10-S4.13.

When looking at the VOC prediction accuracy bar plot (seen in Figure 4.4b) it is noticeable that selectivity towards benzene and hexane from the P1-based sensor was achieved. Overall, the P1-based hybrid gel was able to distinguish and classify all the tested volatiles presenting a 74% score of global accuracy – See Supplementary Information, Figure S4.14, for the complete Confusion Matrices results.

4.4 Conclusions

In this work we studied the incorporation of peptides in two distinct groups of gas sensitive materials. Ionogels, comprised of gelatin and ionic liquid, tailored for electrical gas sensing and hybrid gels, containing gelatin, ionic liquid and liquid crystal, designed for optical gas sensing. A peptide (P1) known to discriminate single carbon deviations among benzene and derivatives was used as a model, along with two modified versions (P2 and P3). The set of ionogels were tested in an electrical e-nose, designed to monitor changes in the conductance of the sensors, and selectivity towards hexane and xylene was observed in the case of P2 and P3 ionogel sensors, respectively. Regarding the hybrid gels, the incorporation of peptide P1 did not disrupt the self-assembly of the ionic liquid crystal droplets (suggesting that peptide P1 is mainly distributed in the matrix), while the incorporation of peptides P2 and P3 disrupt some droplets, although the majority exhibit a radial configuration. The set of hybrid gels were tested in an optical e-nose, designed to monitor the light transmitted from the materials. An enhanced discrimination of benzene was observed for the P1-based gel and for ethanol in the case of both P2 and P3 hybrid gels. As a final note, we would like to highlight the complementarity of the two distinct gel formulations responses, since the combination of both optical and electrical prediction accuracies could provide the best classification accuracies for the tested VOCs. For example, the P2 ionogel was more selective towards hexane and toluene whereas the P2 hybrid gel was more selective towards ethanol, suggesting that additional information and enhanced selectivity can be obtained regarding discrimination of volatiles, by using a combinatorial sensors array in a dual-mode e-nose. An overall view of the delta accuracy results from all sensors, regarding discrimination of each VOC, can be seen in Table 4.2.

Table 4. 2 – Delta accuracy scores of VOC prediction from every ionogel and hybrid gel peptide sensors tested. The delta accuracy score is defined as the difference between the peptide-based accuracy score and the control accuracy score.

VOCs	Δ accuracy ionogels (%)			Δ accuracy hybrid gels (%)		
	P1	P2	P3	P1	P2	P3
Acetone	-36	-25	-6	-3	20	18
Ethanol	-49	-18	-22	13	44	44
Benzene	-20	-9	-22	34	10	14
Xylene	-29	2	-3	33	13	14
Toluene	-33	19	21	45	-2	34
Hexane	-50	-23	2	18	-31	11

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Supplementary Information

Selection of Affinity Ligands Against Volatile Biomarkers

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Materials and Methods

Confirmation of VOC selectivity by Multi-Parametric Surface Plasmon Resonance (MP-SPR)

For the MP-SPR studies, we followed the supplier's recommendations (Bionavis). In this strategy, the Au-glass slide was modified with 3-mercaptopropionic acid (3-MPA), which is a linker with a hydroxyl group, and sulfhydryl group, which binds to the Au surface thiols. EDC/NHS chemistry was used for the immobilization of the P1 peptide on the surface. EDC (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide) is a zero-length crosslinking agent used to couple carboxyl or phosphate groups to primary amines. In this case, it activates the carboxyl group of 3-MPA and forms an amine bond through 5' phosphate groups, creating amine-reactive NHS-esters when the peptide is added. Ethanolamine was used as a blocking agent for extremities where P1 was not bound. This method was applied in the peptide chamber.

A solution of 1 part of 30% ammonia (NH₄OH) and 1 part of 30% hydrogen peroxide (H₂O₂) in 5 parts of water was prepared and heated to 80-90°C. The temperature was adjusted to a value where the solution is only slightly bubbling. The Au-glass slide was placed in the boiling solution for 10 minutes. Immediately after removing the gold slide from the solution, it was rinsed with MilliQ water. Finally, the slide was dried by gently blowing a stream of nitrogen over it. The experiments were started as soon as possible after this treatment.

The cleaned Au-glass slide was immersed in a beaker, with 10 mL of 10 mM of 3-mercaptopropionic acid (3-MPA) in ethanol solution overnight. The solution was well covered in order to avoid evaporation. Next, the incubated Au slide was washed with ethanol (1x) and rinsed with MilliQ water to remove excess of 3-MPA. The slide was kept in MilliQ water until the MP-SPR assay.

The 3-MPA functionalized Au-glass slide was inserted into the MP-SPR equipment. First, phosphate buffer (20 mM, pH 7.2) was injected for 10 min, to perform the priming and the baseline. Then, 250 µL of freshly prepared mixed solution of 200 mM EDC and 50 mM

NHS, both dissolved in 20mM phosphate buffer solution pH 7.2, were injected in both chambers for 10 minutes. The EDC/NHS mixture was carefully degassed after preparation.

The control chamber serves as a control of MP-SPR signal in a non-functionalized Au-glass slide. All other conditions were reproduced in this chamber, besides the peptide. For peptide immobilization, a solution of peptide (10 μ M, in 20 mM sodium phosphate solution pH7) was injected in one of the chambers and incubated for 15 min. If not saturated, the injection is repeated. In the other chamber, 20 mM phosphate buffer solution was injected. This was followed by rinsing with phosphate buffer solution (20 mM sodium phosphate, pH 7.2) in both chambers (5 min). For the blocking, 250 μ L of 1 M solution of ethanolamine pH 8 were injected for 15 min, to deactivate the remaining NHS esters, in both chambers. To remove the excess of ethanolamine, rinse with buffer for 15 min. The slide was removed from the instrument and dried with N₂.

When testing VOCs interaction, between each VOC addition, a step with N₂ flow for 10 min was performed, for surface and chamber cleaning. i) Interaction with N₂ (baseline) 1 hour; ii) Interaction with acetone (5 mL) 10 min; iii) Interaction with ethanol (5 mL) 10 min; iv) Interaction with benzene (5 mL) 10 min; v) Interaction with toluene (5 mL) 10 min; vi) Interaction with xylene (5 mL) 10 min; vii) Interaction with hexane (5 mL) 10 min. The MP-SPR experiments were conducted using a flow rate of 20 μ L/min (liquid chamber) and 50 μ L/min (gas chamber), with a constant temperature of 23 $\pm$ 1°C, and controlled humidity.

Results and Discussion

Multi-Parametric Surface Plasmon Resonance (MP-SPR)

SPR signal variation within time, regarding the reactions of the peptide functionalization, is represented in Figure S4.1 in both affinity (red and green) and control (black and blue) chambers. The signal was measured with two wavelengths, 670 nm and 785 nm.

The Au modified slide functionalization with P1 presented 39.9 Δ mDeg in the peptide chamber and 0.47 Δ mDeg in the control chamber (Table S4.1 and Figure S4.2), using activation with EDC/NHS chemistry, and followed by ethanolamine as blocking agent. This variance may indicate some functionalization of P1. However, there is a significant difference in the Δ mDeg determined, in the activation with EDC/NHS, between the peptide and the control chamber (70.9 to 158.0 Δ mDeg, respectively). This cannot be explained since the same conditions were used to both cases.

Table S4. 1 – Functionalization of Au-glass-3-MPA modified slide results. * $\Delta m\text{Deg}$ (e.g. 670 nm) = $m\text{Deg}$ before injection (670 nm) – $m\text{Deg}$ after desorption (670 nm). $\Delta m\text{Deg}$ Peptide chamber = Average of both $\Delta m\text{Deg}$ (670 nm) and $\Delta m\text{Deg}$ (785 nm). $\Delta m\text{Deg}$ control chamber = Average of both $\Delta m\text{Deg}$ (670 nm) and $\Delta m\text{Deg}$ (785 nm).

	Peptide Chamber ($\Delta m\text{Deg}^*$)	Control Chamber ($\Delta m\text{Deg}^*$)
EDC/NHS	70.9 ± 22.4	158.0 ± 50.9
P1/buffer	39.9 ± 13.92	0.47 ± 0.72
Ethanolamine	-10.1 ± 4.8	-0.51 ± 0.1
Total experiment	92.8 ± 32.6	100.6 ± 30.0
Functionalization	Yes	Yes

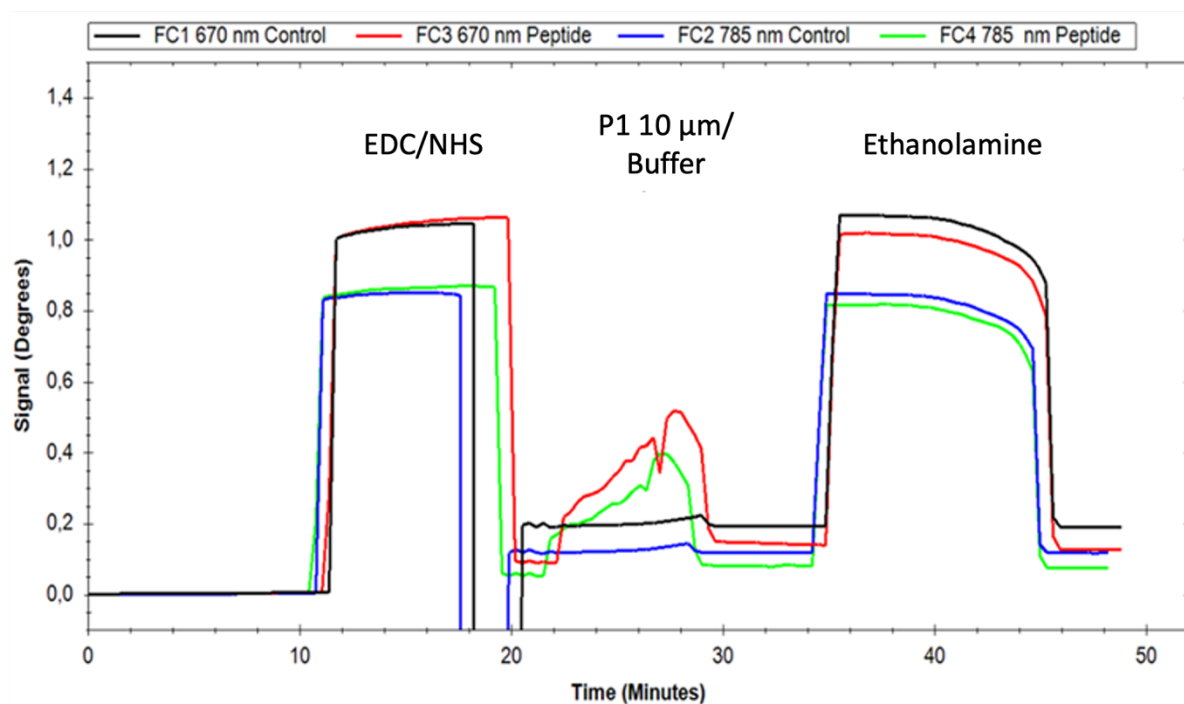


Figure S4. 1 – Functionalization with P1 of the modified Au-3-MPA, with EDC/NHS as activator and Ethanolamine as blocking agent, using the Liquid Chamber (MP-SPR).

In Figure S4.2, it is possible to observe the MP-SPR signal regarding the gas chamber, where the 6 VOCs were diffused in the P1-functionalized Au-glass slide. The signal's difference between benzene and the remaining VOCs is indicating that the functionalized Au-slide exhibits selectivity towards benzene – 38.74 ± 11.03 in the peptide chamber, against 6.20 ± 2.14 in the control chamber, that does not contain the peptide functionalized slide (Figure S4.2 and Table 4.2). However, selectivity towards xylene is also presented, although lower than benzene. With this, it is possible to conclude that, in MP-SPR conditions, P1 demonstrates selectivity towards benzene over the other similar volatiles and acetone and ethanol.

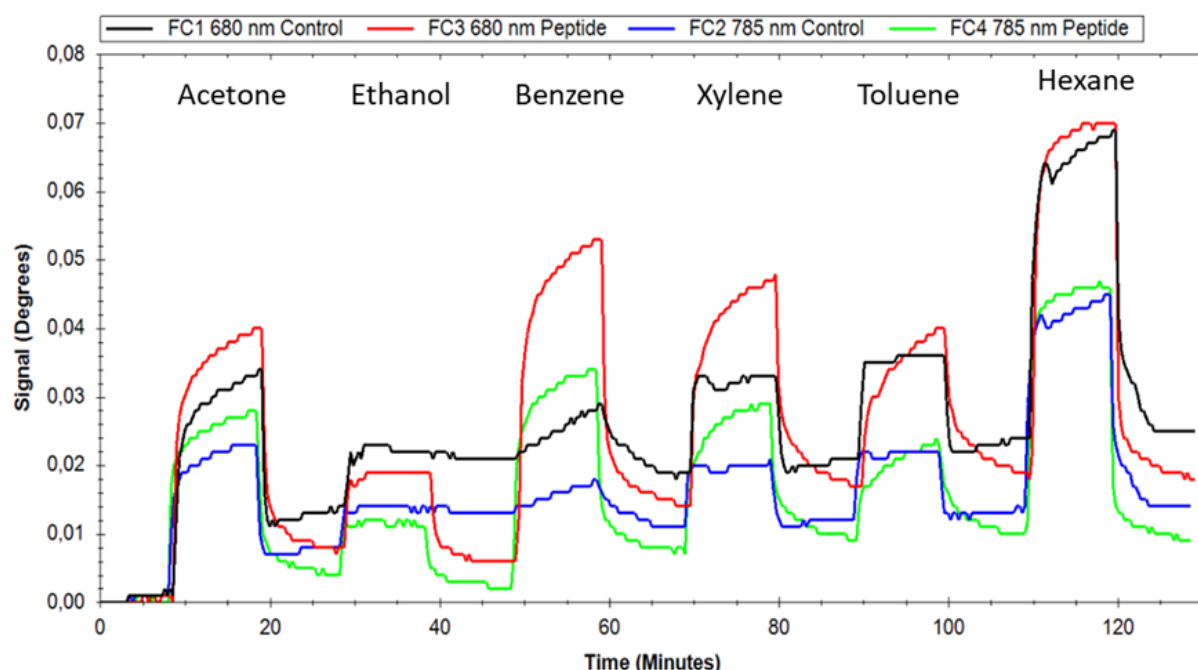


Figure S4. 2 – Interaction of the P1 functionalized Au-glass slide with the selected VOCs (acetone, ethanol, benzene, toluene, xylene and hexane), using the Gas Chamber.

Table S4. 2 – Summary-results of the interaction between the P1 functionalized Au-surface and the VOCs. $\Delta m\text{Deg}$ (e.g. 670 nm) = $m\text{Deg}$ after desorption (670 nm) – $m\text{Deg}$ before injection (670 nm). $\Delta m\text{Deg}$ Peptide chamber = Average of both $\Delta m\text{Deg}$ (670 nm) and $\Delta m\text{Deg}$ (785 nm). $\Delta m\text{Deg}$ control chamber = Average of both $\Delta m\text{Deg}$ (670 nm) and $\Delta m\text{Deg}$ (785 nm). Relative signal variation = the relative signal representing how much the signal increased on the peptide chamber, when compared to the control chamber.

VOCs	Control ($\Delta m\text{Deg}$)	Peptide ($\Delta m\text{Deg}$)	Relative signal variation ($\Delta m\text{Deg}$)
Acetone	27.33 ± 7.04	33.09 ± 8.53	5.76 ± 2.16
Ethanol	6.67 ± 1.90	8.87 ± 2.78	2.2 ± 3.63
Benzene	6.20 ± 2.14	38.74 ± 11.03	32.54 ± 3.35
Xylene	12.22 ± 3.25	26.91 ± 8.01	14.69 ± 2.97
Toluene	12.15 ± 3.43	17.76 ± 5.43	5.61 ± 4.36
Hexane	36.98 ± 9.24	43.17 ± 9.80	6.19 ± 3.94

Regarding these results, further experiments need to be conducted to fully understand the role of P1 regarding selectivity and affinity towards benzene, and for that, a different experiment was conducted.

Hybrid gels characterization: morphological stability

The impact of the incorporation of P1 in hybrid gels on the gel morphology upon storage conditions over time was studied over the course of 17 weeks. The study involved an initial treatment of the images using ImageJ software and a python code, written and customized in-house, in order to sequentially overlap the POM images of the same gel and monitor any droplet morphology variation with time.

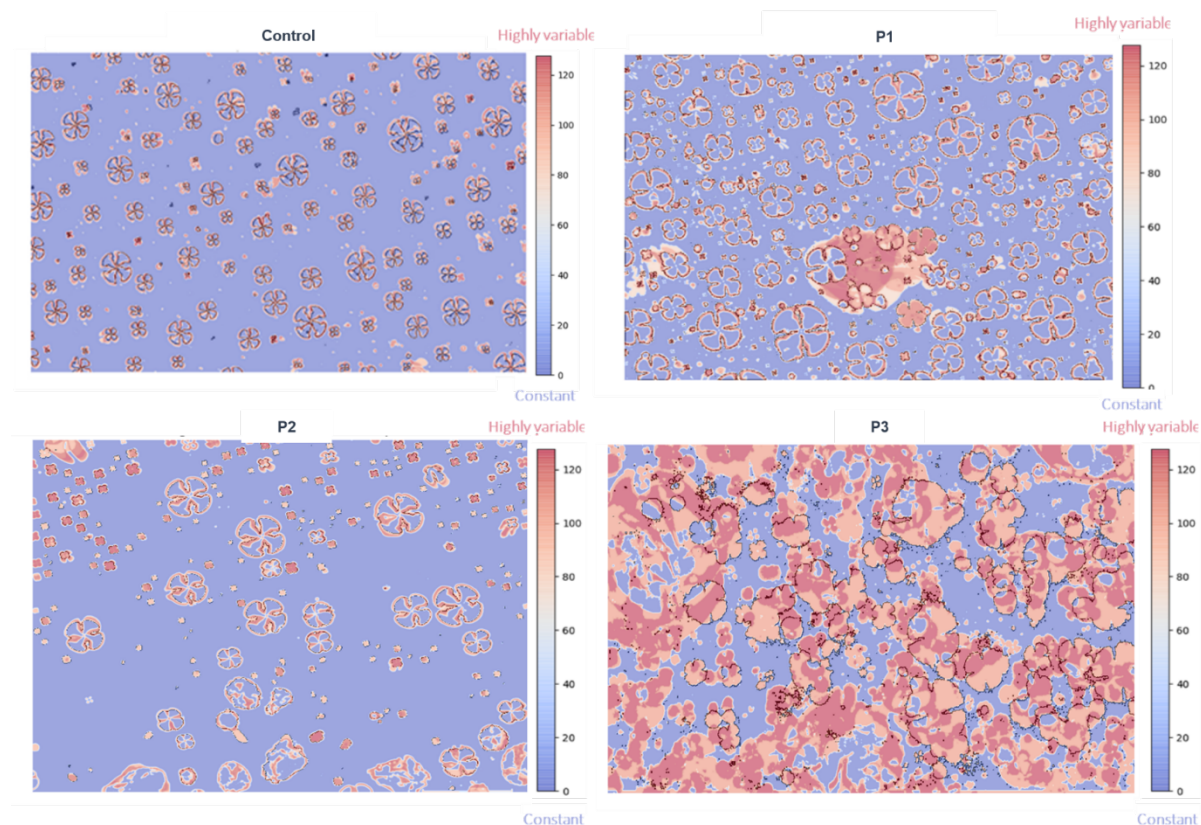


Figure S4. 3 – Optically active area of the hybrid gels followed over time (area of droplets) of all Control hybrid gels, and Peptide hybrid gels. Follow up images of 17 weeks obtained by Zeiss Polarized Optical Microscope (Zen 2.3 software), with 90° polarizers, 10x magnifying objectives. Image developed in Python by a customized code.

The morphological evolution of the chosen optically active areas of the studied peptide-based hybrid gels are depicted in Figure S4.3. The initial position of the droplets is outlined with a black color. The blue areas represent regions that are unchanged over time. Possible changes in the liquid crystal droplets morphology, such as droplet growth, shrinkage, translation, rupture, are shown in various shades of red.

P1 and P2 sensors appear to be very stable over time, as their corresponding liquid crystal droplets remained in the same positions, with just some fluctuations in size potentially due to humidity changes. However, this is not the case for the P3 sensors, as it can be clearly seen that the droplets were destabilized and ruptured or merged into larger inclusions during the course of the observation period.

Hybrid gels characterization: morphological characterization of the P1-hybrid gels through fluorescence by labelling P1 with FITC

It is important to understand how the addition of the peptide is going to change the regular biofilm structure, as well as exactly where the peptide was bound once it was added to the gel formula. For that, fluorescence assays were conducted, in which the C-terminal of the P1 was labeled with the fluorophore fluorescein isothiocyanate (FITC).

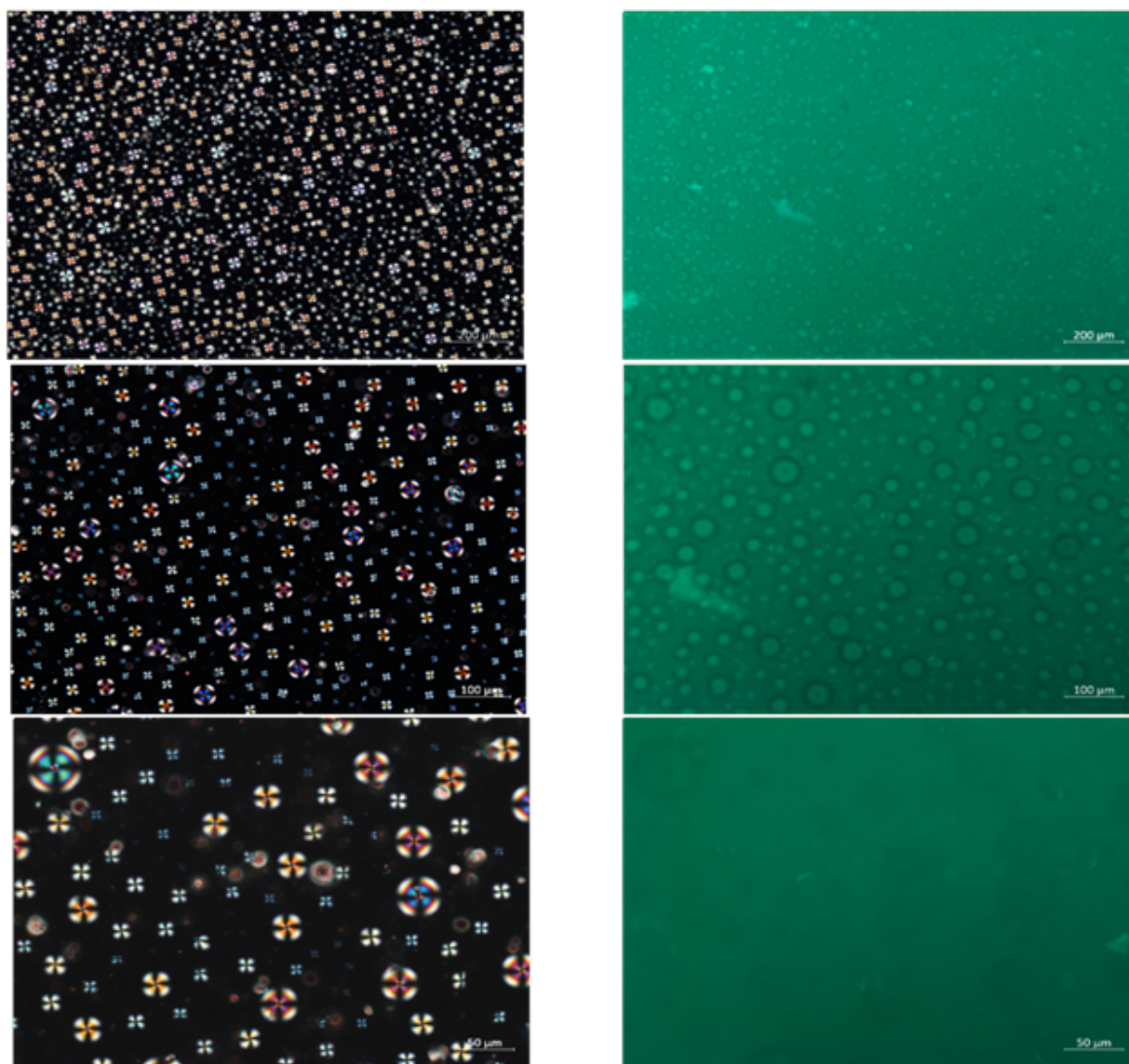


Figure S4. 4 – Comparison of POM photos taken with crossed polarizers (90°) and Fluorescence pictures of P1+FITC gels. Same optical region of interest is represented in all pictures, with increasing magnifying objectives (200 μm, 100 μm and 50 μm).

As it can be seen through the analysis of Figure S4.4, it appears that P1 is able to not only to infiltrate inside the droplets, but also to be spread within the matrix of the gelatin, indicating that the incorporation of P1 in the gel was achieved successfully.

Assessing VOC selectivity in ionogels – Electrical e-nose

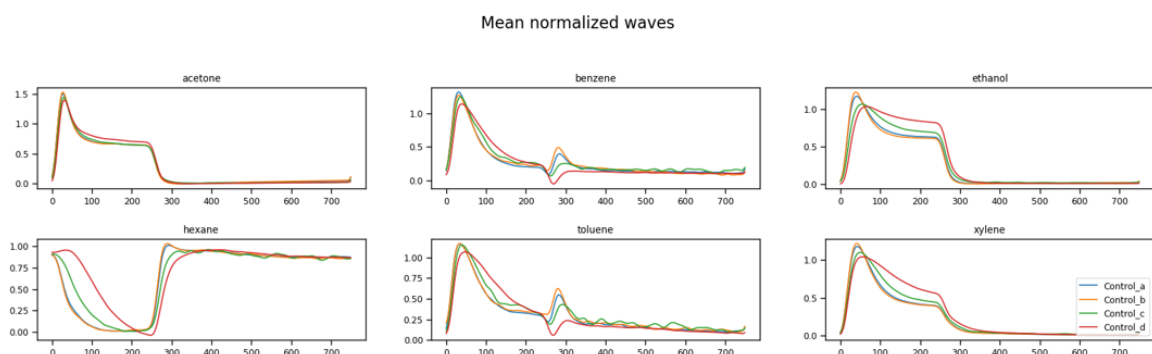


Figure S4. 5 – Examples of electrical e-nose mean wave signals of Control ionogels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

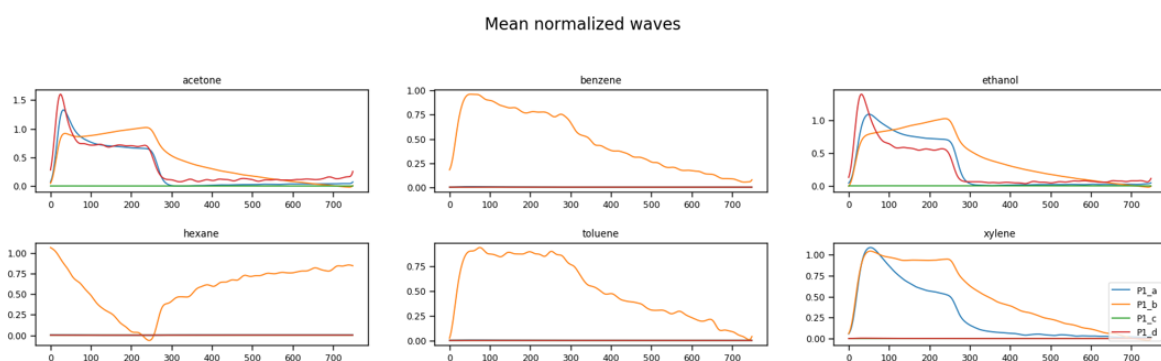


Figure S4. 6 – Examples of electrical e-nose mean wave signals of P1 ionogels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

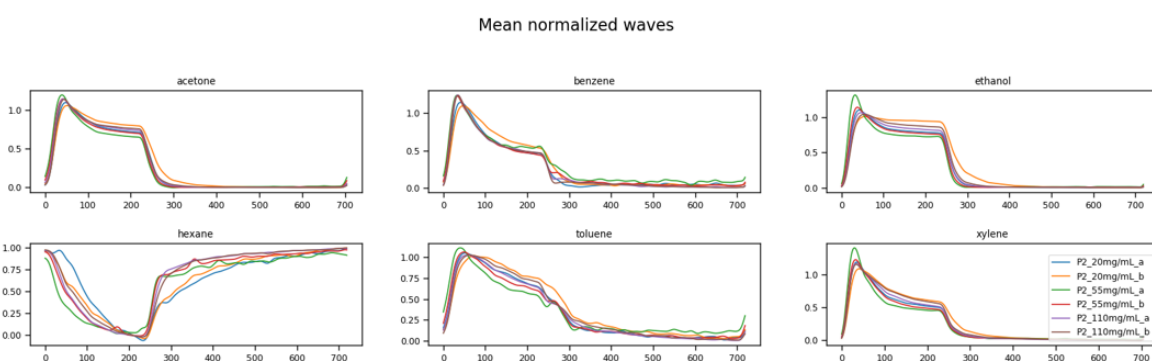


Figure S4. 7 – Examples of electrical e-nose mean wave signals of P2 ionogels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

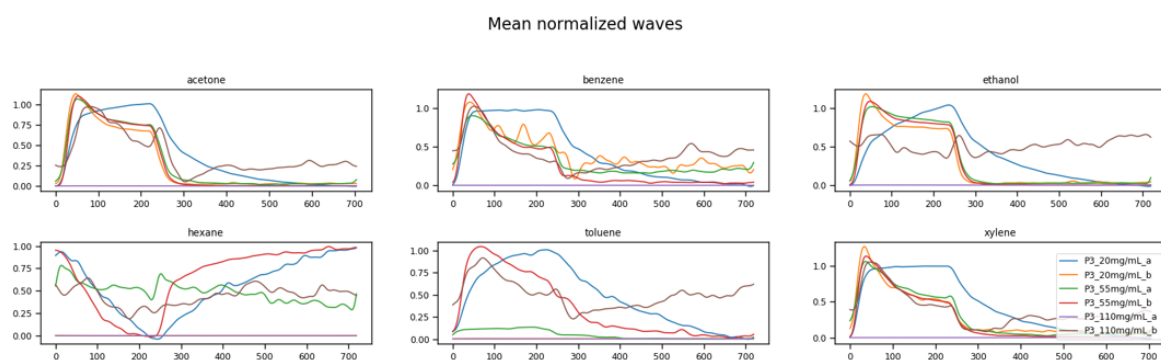


Figure S4. 8 – Examples of electrical e-nose mean wave signals of P3 ionogels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

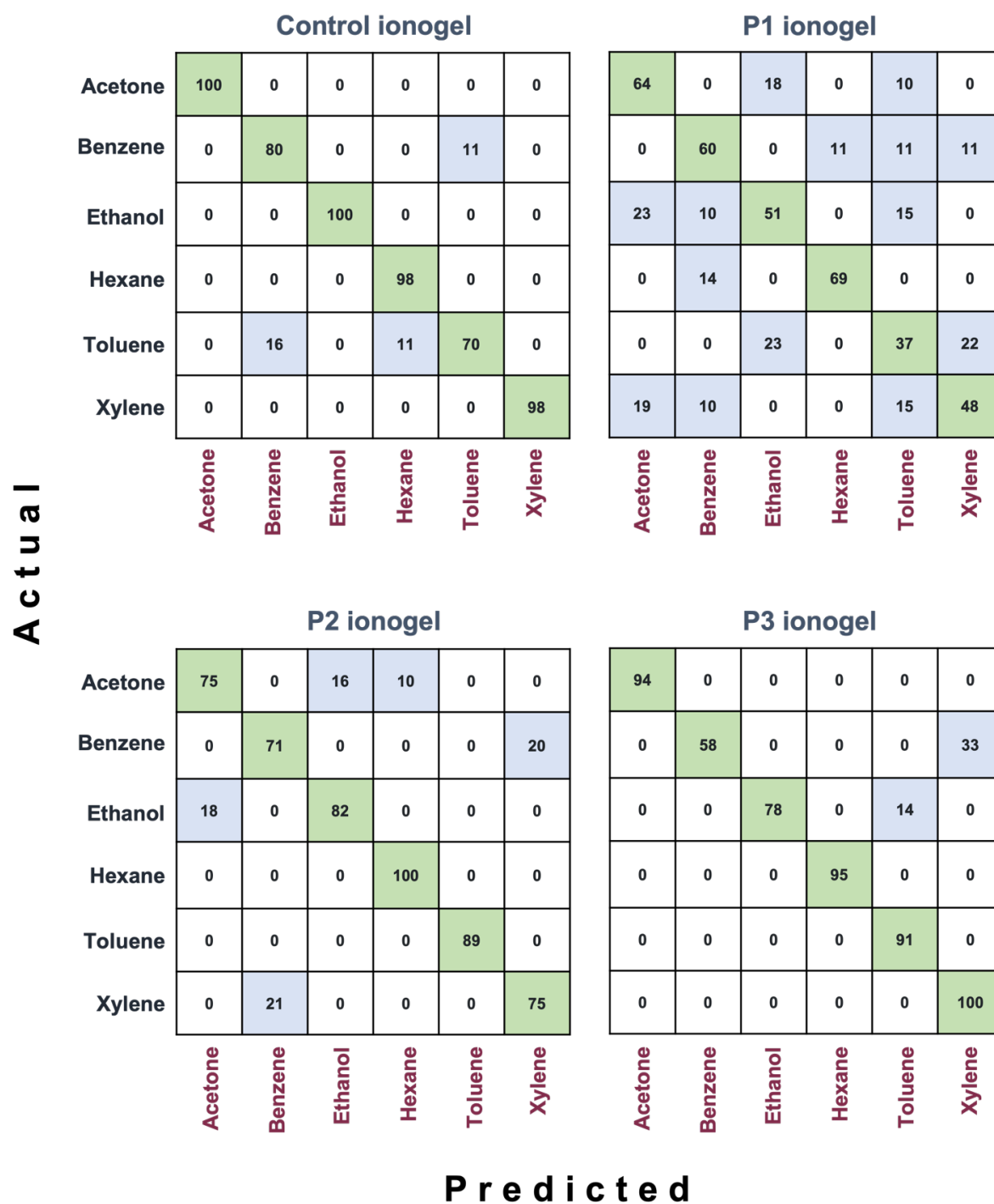


Figure S4. 9 – Confusion matrices obtained from the electrical e-nose experiments for all the sensors illustrating the prediction results of the tested VOCs. Y axis: the actual VOC being measured; X axis: the VOC that the gel identified as being measured. The squares represent the frequency (in percentage) of each prediction, with the diagonal green squares indicating the frequency of correct predictions of each VOC (accuracy). The lighter blue squares outside of the diagonal represent the incorrect prediction of a VOC by being confused with other VOC.

Assessing VOC selectivity in hybrid gels – Optical e-nose

Mean normalized waves

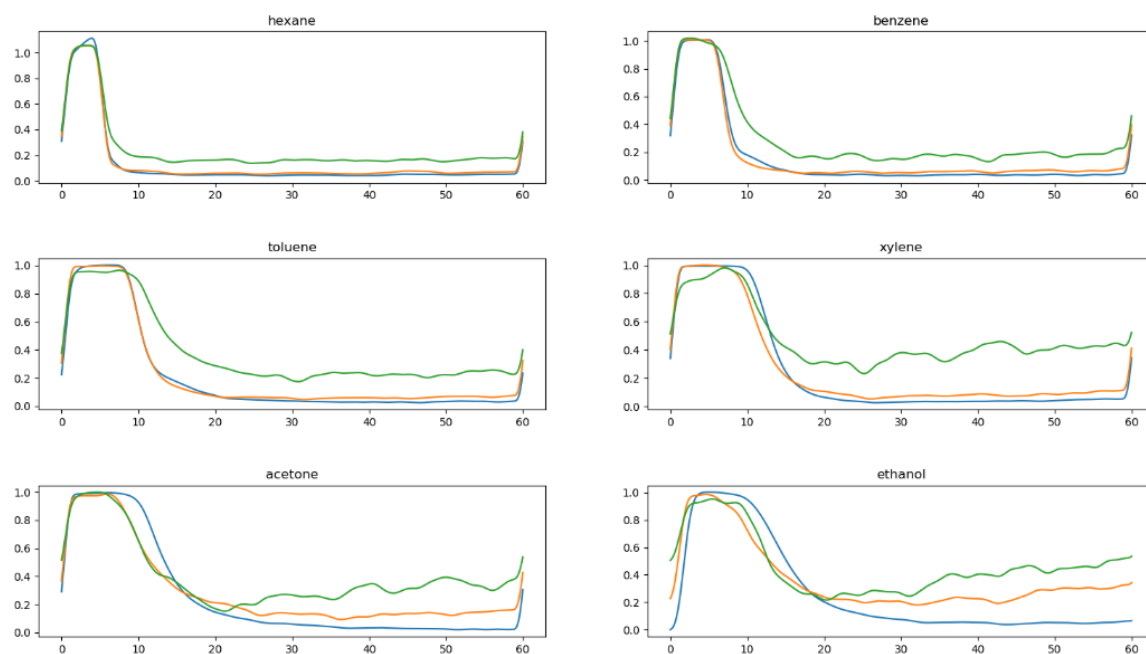


Figure S4. 10 – Examples of optical e-nose mean wave signals for the Control hybrid gels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

Mean normalized waves

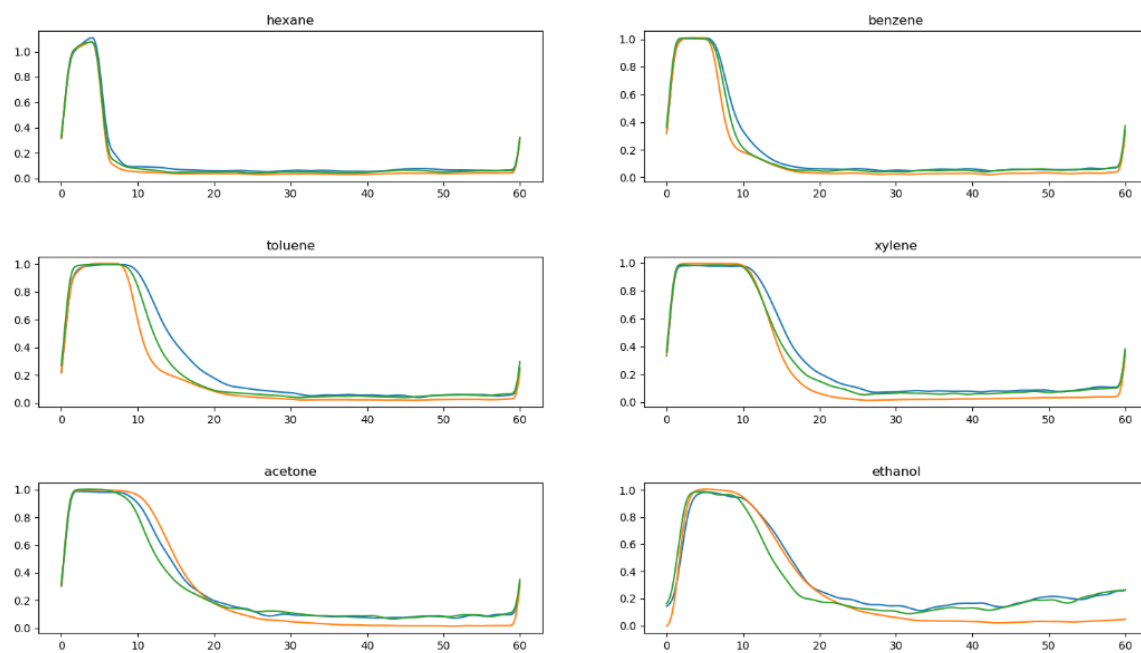


Figure S4. 11 – Examples of optical e-nose mean wave signals for the P1 hybrid gels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

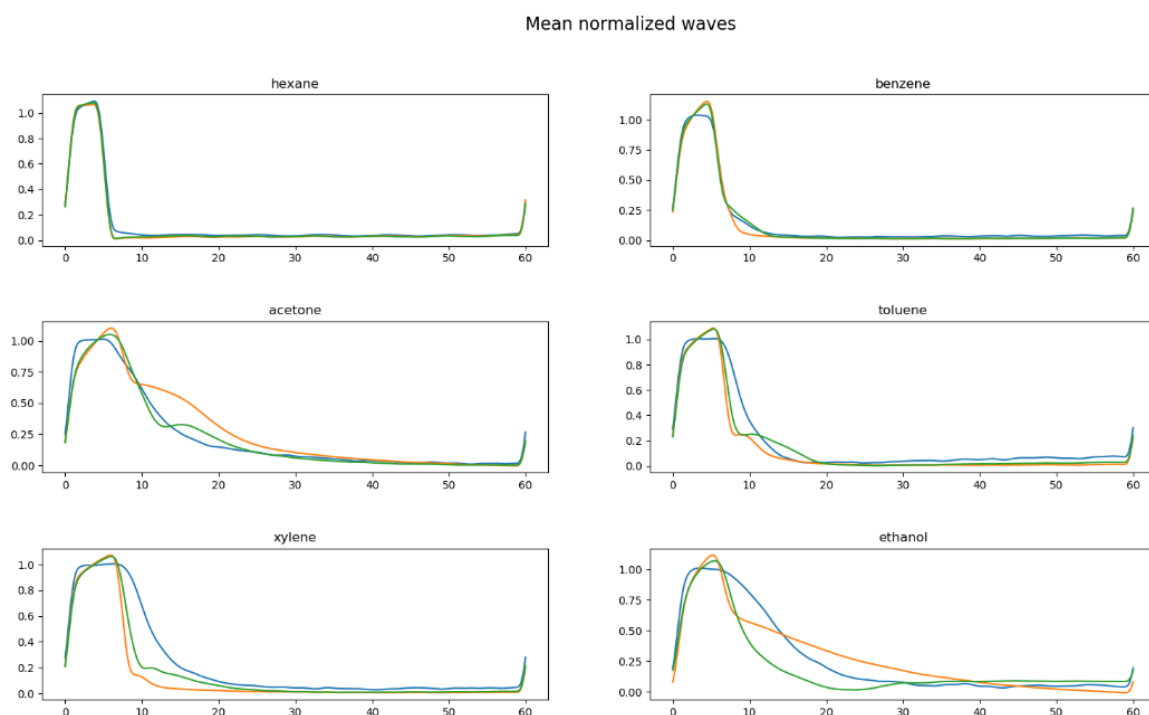


Figure S4. 12 – Examples of optical e-nose mean wave signals for the P2 hybrid gels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

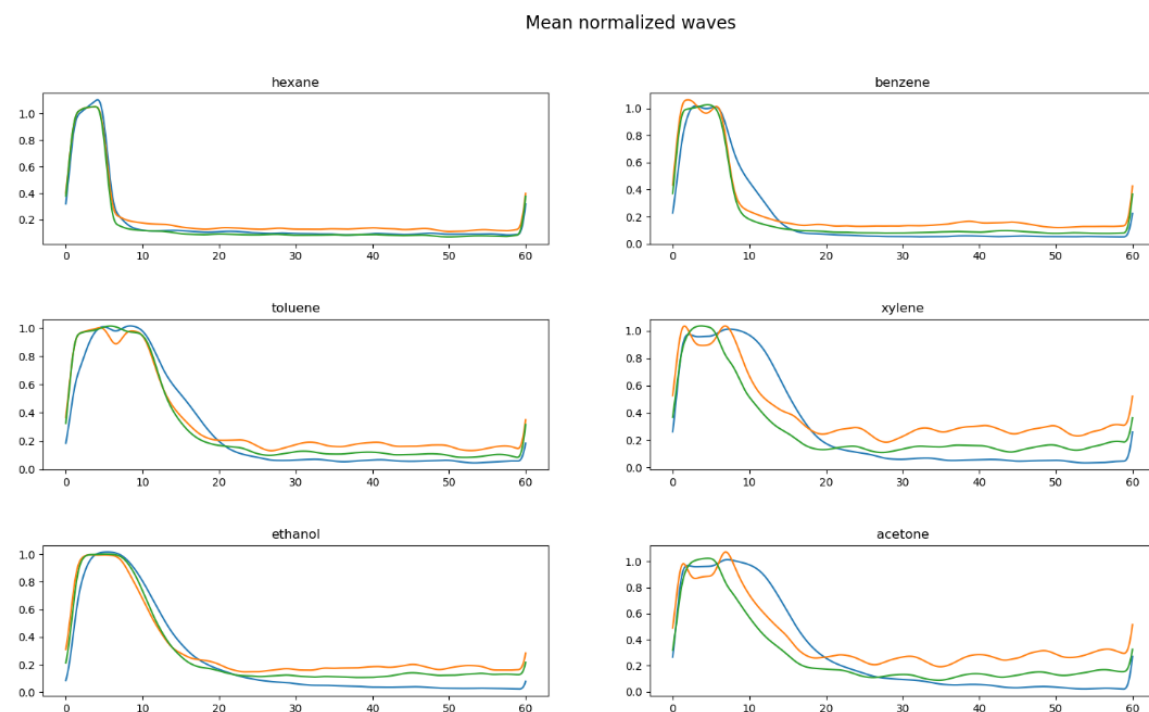


Figure S4. 13 – Examples of optical e-nose mean wave signals for the P3 hybrid gels. All VOCs are represented: acetone, ethanol, hexane, toluene, xylene and benzene. The plots were generated by python, and all replicas are represented.

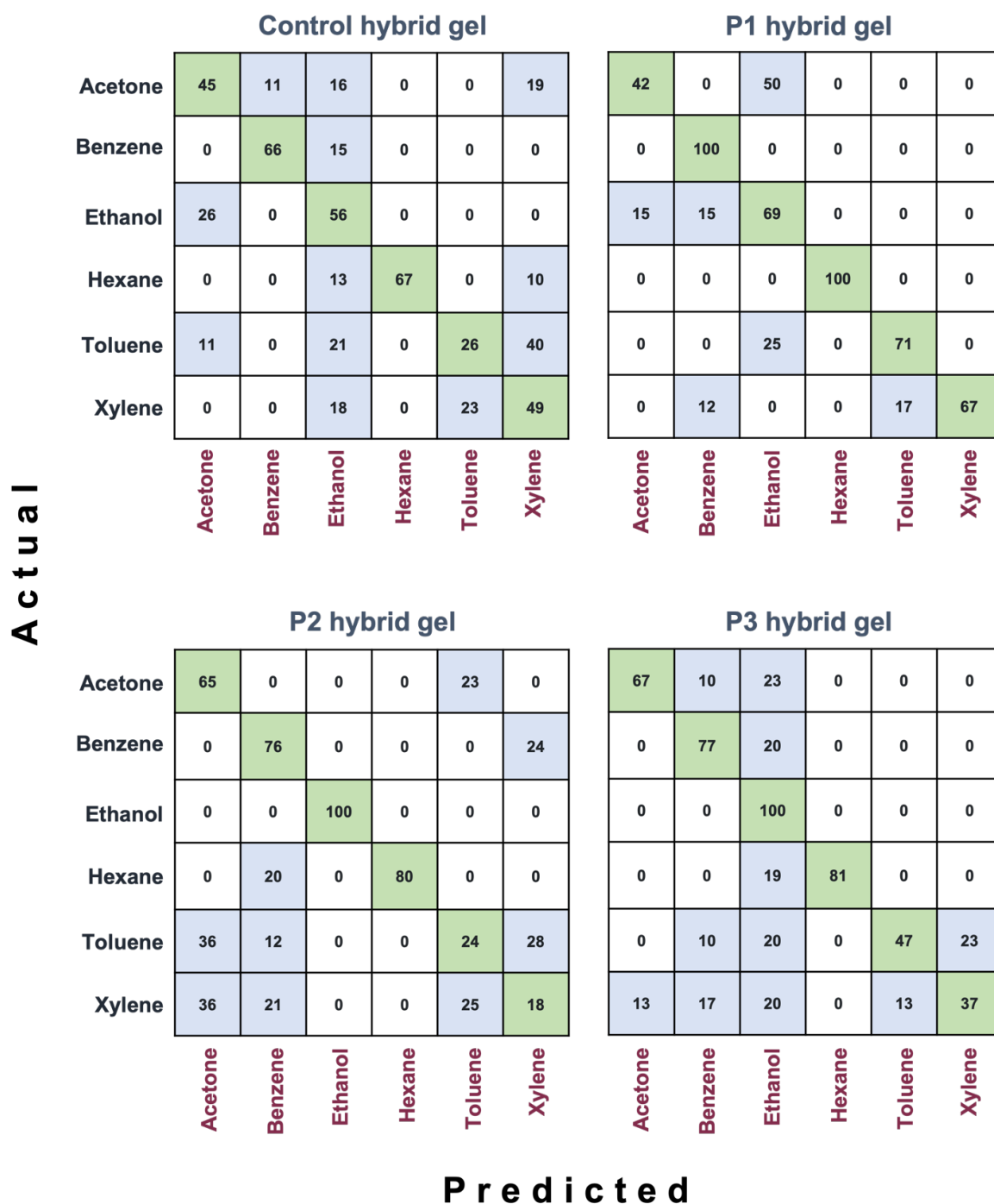


Figure S4. 14 – Confusion matrices for the six VOCs exposed in the optical e-nose experiments with Control, P1, P2, P3 hybrid gels. Y axis: the actual VOC being measured; X axis: the VOC that the gel identified as being measured. The squares represent the frequency (in percentage) of each prediction, with the diagonal green squares indicating the frequency of correct predictions of each VOC (accuracy). The lighter blue squares outside of the diagonal represent the incorrect prediction of a VOC by being confused with other VOC.

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LIGAND MODIFIED HYBRID GEL SENSORS TO TUNE THE CLASSIFICATION OF VOLATILE ORGANIC COMPOUNDS

Abstract

Volatile organic compounds (VOCs) that are found to be characteristic of bacteria (mVOCs) can serve as a tool for fast, early and non-invasive diagnostics. Their detection through electronic-nose devices can be achieved, by tuning the selectivity of the sensing materials used towards mVOCs. In this work we design an array of sensors to use in an optical e-nose device for mVOC discrimination. Optical gas sensors are modified with a library of ligands. Results demonstrate that discrimination of different mVOCs can be achieved by a combination of the tuned specific interactions between the Ligand-sensors and the volatiles, and the characteristic unique features derived from the optical signals originated from these interactions.

5.1 Introduction

Electronic noses (e-noses) are user-friendly and portable gas-sensing devices, designed to mimic the human olfactory system. They comprise of an array of semi-selective chemical sensors, which generate responses in the presence of volatile organic compounds (VOCs). The responses are transduced into measurable signals and are further processed by appropriate pattern recognition systems. One of the interesting potential applications for e-noses is in the early diagnostic of diseases¹⁻⁵.

Bacterial infections, particularly those caused by antimicrobial-resistant bacteria, pose a major threat to human health. In 2019 about 4.95 million deaths were associated to this cause⁶. Current methods for the diagnostics of bacterial infections are slow, taking from hours to days, and are conducted using invasive methods. Thus, over the last years, several solutions aiming at fast and non-invasive methods for pathogen identification have emerged⁷. E-noses that identify microbial VOC biomarkers (mVOCs) are considered one of the potential solutions. However, the complexity of microbial fingerprints emitted by biological sources is high, and

the use of more selective sensors in the e-nose array can prove useful. One way to increase the sensors performance towards specific VOCs is through the addition of VOC-selective elements in the sensors, namely peptides, odorant binding proteins or olfactory receptors⁸. Another alternative is to enhance selectivity by coating probe sensors, as gold nanoparticles, with small affinity ligands. With this approach it was possible to build an array of sensors capable of diagnosing lung cancer and other diseases in exhaled breath^{9,10}.

The purpose of this work was to assess whether the selectivity and discrimination of mVOCs using hybrid gel-based optical sensors could be increased by adding small mVOC-ligands. Hybrid gels are semi-selective gas sensitive multicomponent gels used in tailor-made e-nose devices^{11–15}. These compartmentalized materials consist of self-assembled liquid crystal (LC) droplets confined in ionic liquid (IL) interfaces, that are further immobilized within a gelatin matrix. The IL promotes the anchoring of the LC in a spherical geometry and helps in the dissolution of the biopolymer through ionic, electrostatic and hydrogen bonding with the polymer chains^{12,13}. To select the best mVOC ligands, we initially chose a list of microbial volatiles associated to several bacterial human pathogens¹⁶. With this information, we design 14 distinct ligands to increase the variety of potential interactions with the target mVOCs. These ligands were incorporated onto the hybrid-gel sensors and used in a tailor-made optical e-nose to assess the effect on increasing the discrimination between distinct mVOCs. In this work, results demonstrate that discrimination of different mVOCs can be achieved by using small affinity ligands from different chemical classes, based not only on their specific interactions with the volatiles, but also due to the interactions provided by the all the components that assembly the sensing materials.

5.2 Materials and Methods

5.2.1 Materials

The polymer Gelatin from bovine skin (gel strength ≈ 225 g; Bloom, Type B), was purchased from SigmaAldrich. The liquid crystal 4-cyano-4'-pentylbiphenyl (5CB, $>98.0\%$) was purchased from TCI Europe (Belgium). The ionic liquid 1-butyl-3-methylimidazolium dicyanamide ([BMIM][DCA], $>98.0\%$) was purchased from IoLiTec (Germany). The 3-aminopropyltriethoxysilane (APTES) 99%, Glutaraldehyde solution, sulfuric acid 97% and hydrogen peroxide solution 30% (w/w) were purchased from Sigma-Aldrich. N,N-Dimethyl Formamide was acquired by Fisher Scientific, and toluene by VWR. The ligands 1-pyrenemethylamine hydrochloride 95%, 4-aminobutan-2-one hydrochloride, iminodiacetic acid, N,N-Dimethyl-4,4'-azodianiline, 4-aminobenzamide, leucine, agmatine sulfate, ethanolamine, ethyl 4-aminobenzoate, aminopyrazine, benzylamine, tryptamine and histamine were purchased from Sigma-Aldrich (Portugal), and pararosaniline was purchased from Merck. As for the solvents 3-methyl-1-butanol (purity $>98\%$), acetaldehyde ($>99.5\%$), isovaleraldehyde (purity $>97\%$), 2,5-dimethylpyrazine, ethanol and acetic acid (purity 99.8%) were purchased from Sigma-Aldrich.

Hexane was purchased from VWR. Ethyl acetate and benzene were supplied by Fisher Scientific. Acetone (purity 99.5%) was purchased from Honeywell.

5.2.2 Chemical functionalization of the sensors surface

The glass slide surfaces were cleaned with Piranha solution (3 parts of concentrated sulfuric acid and 1 part of 30% hydrogen peroxide solution), rinsed with distilled water and then dried with nitrogen gas. The functionalization of the surfaces with primary amine groups was then followed by using immersing the clean substrates into a 2% APTES in toluene, overnight at room temperature and agitation. The silanized substrates were thoroughly rinsed with toluene, and dried with nitrogen gas. The determination of the extent of amination on the glass surfaces was conducted through Kaiser Test¹⁷, detailed in Supplementary Information, yielding an average value of 3.72 μmol of amines / glass (or 2.98 μmol of amines / cm^2).

Glutaraldehyde was used a linker to bind the ligands to the APTES molecules, and for that the substrates were incubated in a 5% Glutaraldehyde aqueous solution for 1h30min, under agitation and 30°C. After rinsing thoroughly with water, the substrates were left in ligand solutions (1:10 APTES:ligand ratio) overnight with agitation at room conditions. The ligands used were named as L0 (1-pyrenemethylamine), L1 (Ethanolamine), L2 (Benzylamine), L3 (4-Aminobutan-2-one), L4 (Ethyl-4-aminobenzoate), L5 (Agmatine), L6 (Aminopyrazine), L7 (Iminodiacetic acid), L8 (N,N-Dimethyl-4,4'-azodianiline), L9 (4-aminobenzamide), L10 (Leucine), L11 (Histamine), L12 (Pararosaniline), L13 (Tryptamine). After incubation, the substrates were washed with N,N-Dimethyl Formamide (DMF), followed by rinsing with water, and dried with nitrogen gas. Confirmation of the final step of ligand-functionalization was obtained by using the 1-pyrenemethylamine ligand (L0) as a reporter molecule and analyzing its intrinsic fluorescence (Figure S5.1). Fluorescence of the pyrene molecules immobilized in the glass surface was observed through fluorescence microscopy using a Microscope Zeiss Axio Observer.Z1/7 equipped with a filter 38 HE Green Fluorescent (Ex/Em: 450nm-490nm/500nm-550nm).

5.2.3 Preparation and characterization of hybrid gel sensing materials

Hybrid gels composed by gelatin, BMIM-DCA and the liquid crystal 5CB were prepared following protocols previously described^{13,15}. The gel compositions were deposited on top of untreated glass slides (control) or ligand-functionalised glass slides and spread into thin films using a TQC film applicator (Automatic Film Applicator Standard, TQC Sheen) with a 30 μm thickness. After production, the gels were stored at room temperature and controlled humidity (~50% RH). The morphological properties of the hybrid gels were evaluated by polarized optical microscopy (POM) through a ZEISS, Observer.Z1 Polarized Optical Microscope equipped with an Axiocam 503 color camera and ZEN 2.3 software for image acquisition and processing. Pictures were taken with crossed polarizers and in bright field. Also, histograms containing information regarding the droplets diameter size (μm) were measured using a python script developed in-house and the tools of FIJI distribution of ImageJ open software^{18,19}.

5.2.4 VOC discrimination using sensors modified with ligands

A customized optical e-nose device was used in the tests. It consists of a detection chamber with 12 available sensor slots (positioned between crossed polarizers) and uses two pumps that function alternately to expose the sensors either to VOCs, or to ambient air for recovery inside the chamber¹². The detection chamber is connected to a signal acquisition device to further analyze the obtained signals. The sensors were exposed to a set of 10 saturated atmospheres of the following mVOCs: isovaleric aldehyde, 3-methyl-1-butanol, acetaldehyde, ethyl acetate, acetone, ethanol, acetic acid, 2,5-dimethylpyrazine, hexane and benzene. The solvents were kept in a thermostated bath at 37°C, for at least 10 min prior to exposure. VOCs were pumped for 5 sec to the detection chamber, followed by 10 sec of ambient air for sensors recovery, in a total of 10 minutes (40 exposure-recovery cycles). The assays were performed using triplicates of each sensor, and the optical signals were acquired using a sampling rate of 100 Hz. The obtained signals were further analysed by methods previously described work^{11-15,20,21} using an automatic classifier algorithm based on SVM, and python programming tools.

5.3 Results and Discussion

5.3.1 Design and preparation of chemically functionalized sensors

The possibility to chemically functionalize gas sensors to increase selectivity towards volatile organic compounds (VOCs) is an interesting approach to facilitate the analysis of complex VOC fingerprints emitted from biological sources^{10,22}. We started by identifying the already reported microbial VOCs that could be used to distinguish between bacteria pathogenic to humans¹⁶. We selected a list of 8 different microbial VOCs – isovaleric aldehyde, 3-methyl-1-butanol, acetaldehyde, ethyl acetate, acetone, ethanol, acetic acid, and 2,5-dimethylpyrazine (Table S5.1) – and also included two hydrophobic molecules (hexane and benzene; aliphatic and aromatic) with high probability of binding to the reporter molecule 1-pyrenemethylamine (named as ligand L0). After VOC identification, we proceeded to the design of small ligands that could establish interactions – hydrophobic and H-bonds – with the selected mVOCs (Figure 5.1). A total of 14 ligands were considered, in which one (L0 - 1-pyrenemethylamine) was used as a reporter ligand to confirm the success of substrate functionalization (Figure S5.1 and Figure 5.1). Considering the rationale for ligand selection, we hypothesized that ligands containing aromatic groups (e.g. 1-pyrenemethylamine (L0), Benzylamine (L2), Histamine (L11) and Tryptamine (L13)) are likely to interact with VOCs such as 2,5-dimethyl pyrazine and benzene, through π - π stacking interactions. On the other hand, certain ligands possess chemical functionalities that can give other molecular recognition attributes, as for example ligand 4-aminobenzamide (L9) that possess an amide known for its interaction with carboxylic acid groups (e.g. acetic acid), esters (e.g. ethyl acetate), as well as to establish H-bonds. Other ligands as amines (Agmatine (L5)), anilines (Pararosaniline (L12)), alcohols (Ethanamine (L1)), pyrazines (Aminopyrazine (L6)) and hydrazines ((N,N-Dimethyl-4,4'-azodianiline (L8)) tend

to interact with H-bond acceptors, like aldehydes (acetaldehyde and isovaleric aldehyde), ketones (acetone), esters (ethyl acetate), pyrazines (2,5-dimethylpyrazine), and potentially alcohols (like ethanol and 3-methyl-1-butanol) set of volatiles. Ligands from the ketone and ester chemical classes (like 4-Aminobutan-2-one hydrochloride (L3) and Ethyl-4-aminobenzoate (L4) respectively), have known tendency to interact with H-bond donors, like alcohols (ethanol and 3-methyl-1-butanol) and acids (acetic acid).

After substrate functionalization with each ligand, the hybrid gel formulations were spread as thin films. For all tested ligand-functionalized surfaces, it was possible to obtain hybrid gel films with visible ionic liquid-liquid crystal droplets (Figure 5.1 and Figure S5.2), as observed for the control sensors in which the film is deposited onto non-functionalized glass surfaces^{11,13,15}. With regards to the hybrid gel materials produced upon spreading over the ligand-modified surfaces, we studied the morphological aspects via POM, using crossed polarizers and Bright Field analysis. All gels present polydisperse droplets within different ranges of sizes (Figure S5.2 and S5.3), in which the LC assumed predominantly radial configurations revealed by the Maltese cross pattern seen in POM images under crossed polarizers, which upon rotation does not change²³. In Bright Field Microscopy the characteristic point defect named hedgehog could also be observed. The radial director profile is due to the homeotropic alignment provided by the interactions of the LC with the cation of the IL. An exception to this is the case of N,N-Dimethyl-4,4'-azodianiline (L8) where the resulting gel presents a mixture of radial and escaped radial droplets. The presence of the ligands in the compositions led to certain morphological differences in the droplets, depending on the type of ligand used, as it can be seen in the colors and in some cases the droplet shape (Figure S5.2). For example, hybrid gels spread onto glass substrates modified with ligands like 1-pyrenemethylamine (L0), Ethanolamine (L1), Benzylamine (L2) and 4-Aminobutan-2-one hydrochloride (L3) present some misshaped radial droplets. In the case of ligand Aminopyrazine (L6), few radial droplets and IL interfaces were observed at room temperature (Figure S5.2). Thermal investigations of the L6-hybrid gel under POM with crossed polarizers, revealed that upon cooling from the room temperature the transition from the isotropic to the nematic states occurred at 16.7°C. This confirmed that the IL interfaces were filled with the liquid crystal component indicating that the L6 suppresses the nematic phase of the LC to lower temperatures (Figure S5.4). As an overall view, the presence of radial configuration of the LC component was confirmed, and it is actually quite relevant since it is showed to provide a fast and dynamic optical response upon exposure to VOCs¹³.

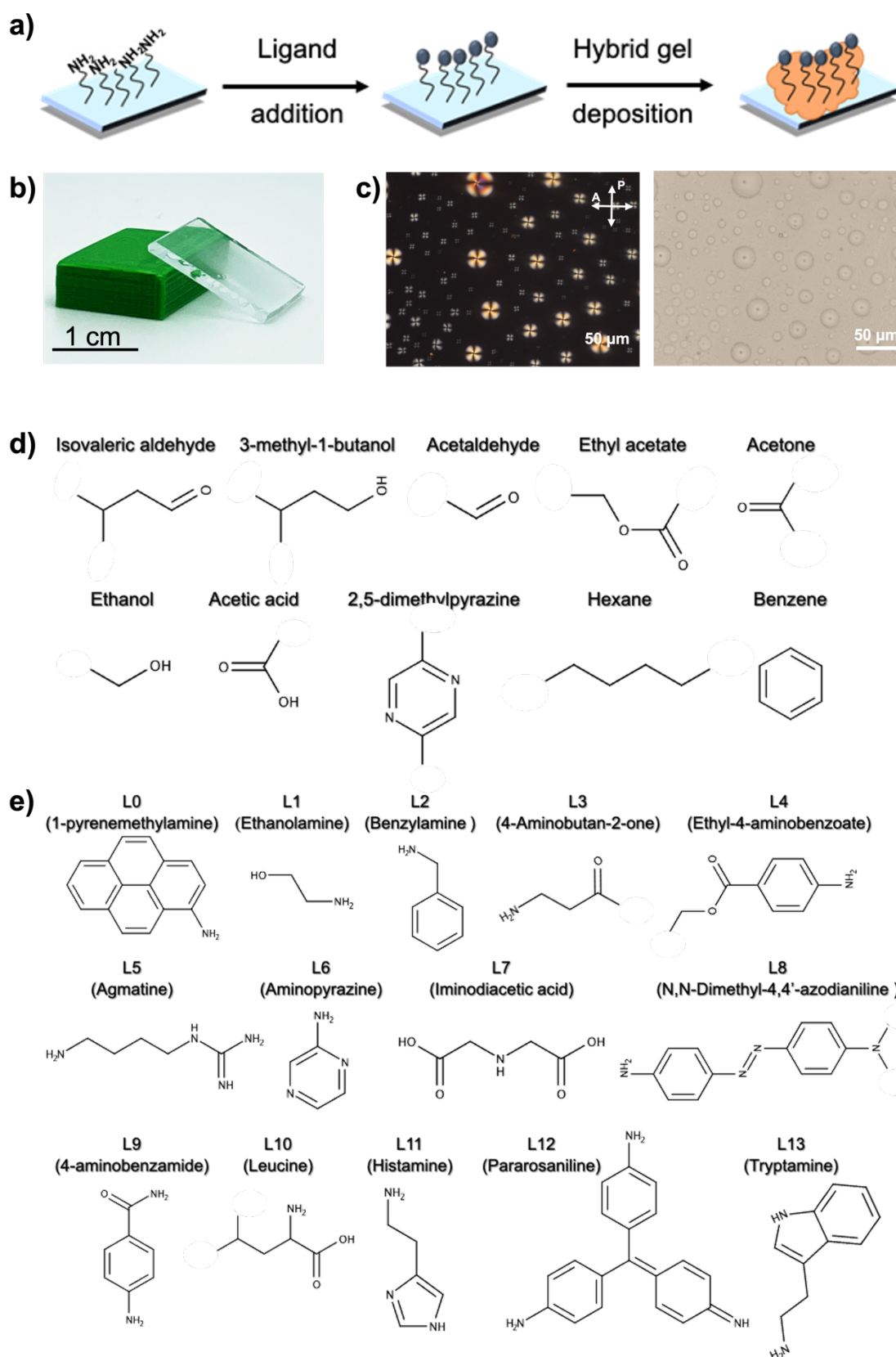


Figure 5. 1 – a) Schematic representation of the hybrid gel sensing materials general formulation process, from the silanization of the glass substrate, to the gel deposition stage. b) Macroscopic image of the produced hybrid gel. c)

Polarized optical microscopy (POM) images of optically active areas obtained for the sensing films under crossed polarizers (90°) and bright field (0°), respectively. Schematic representation of the names and structures of the chosen d) 10 mVOCs; and e) ligands.

5.3.2 Use of ligand-functionalized sensors for VOC classification

The hybrid gel sensors spread onto the ligand-modified substrates were further tested in the optical e-nose setup device, described in previous works¹¹⁻¹⁵. Exposure of the sensors to the selected VOC analytes results in the interaction of the VOC molecules with the different components of the sensors, namely the IL-LC droplets, the gelatin matrix and ultimately the ligands at the substrate surface. The optical signal that is picked up by the e-nose device results from any optical changes due to the molecular organization of the liquid crystal molecules inside the droplets. Thus, only VOC interactions that lead to a disruption of the LC order or arrangement at the molecular level are observed. Typically, the optical signal observed is a result of the disorganization of the liquid crystal component, triggering a phase transition to the isotropic state. The signal responses are the combined responses of the individual components of the gel formulations, and the immobilized ligands for each analyte. The derived signals were further processed and analyzed using feature selection and SVM tools, as described in previous works^{11-15,20,21}.

We verified that the sensors signals were reproducible and reversible (as can be seen in Figure 5.2, demonstrating representative optical signals for some of the tested VOCs) and exhibit a variability in their profiles depending on the sensor functionalization and on the VOC properties (Figure S5.5). For example, some signals present a spike-like downwards shaped response – for example hexane in L5, L8, L10-sensors or the majority of VOCs for L1 and L12-sensors, while others reach a plateau-response (majority of VOCs for L4, L7 and L8-sensors and acetic acid for L9-sensors). There are also distinct signal profiles during the recovery process, indicating distinct optical textures during the desorption of the VOC from the material. For example, ethanol signals in L0, L1, L7, L8 and L9-sensors, require a longer period to completely recover to baseline levels, when compared for example with ethanol for L10-sensors. It is also possible to observe other different properties of the signals, such as the different relative amplitudes of responses, that are distinguishable for every Ligand-sensors, like the case that Control, L4, L7, L8, L9, L10 L11 and L12-sensors, which are present higher amplitudes of response than sensors like L2, L3, L5 and L6, being that most of the signals appears to present a flat response.

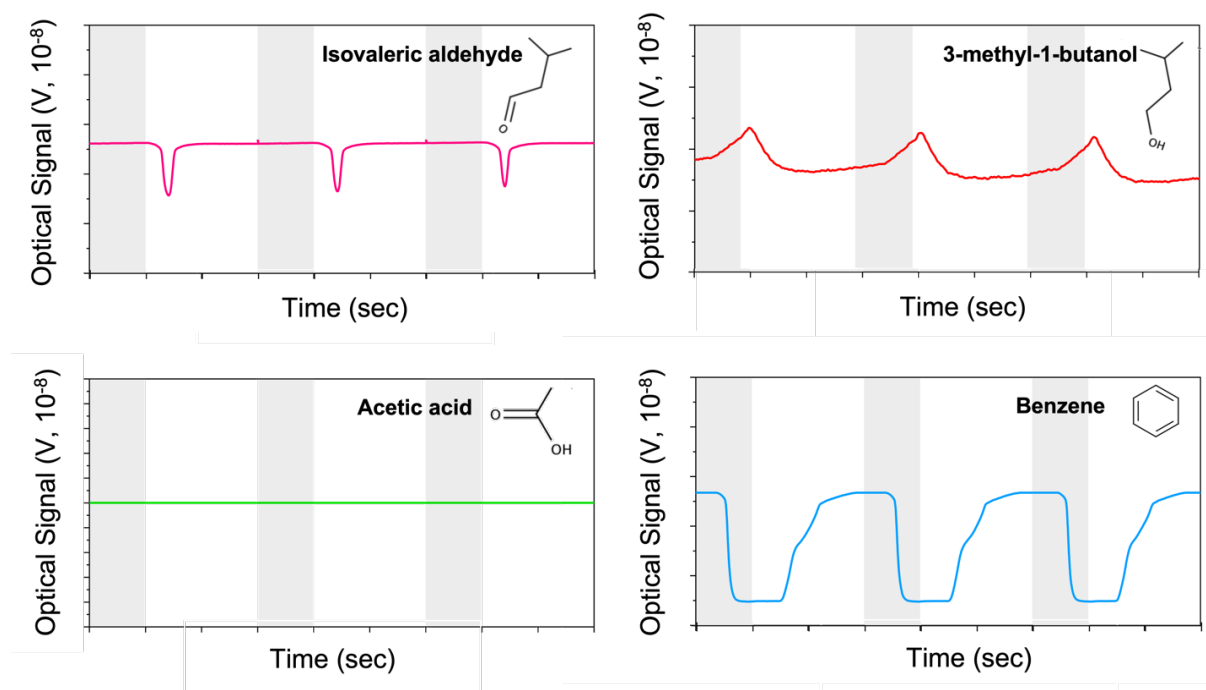


Figure 5. 2 – Representative examples of optical signals derived from the Optical e-nose experiment. The chosen signals are a result from the interaction of control sensors and four volatiles (isovaleric aldehyde, 3-methyl-1-butanol and benzene).

These individual differences in the signals will ultimately dictate how accurately the sensors are able to predict the VOCs that are being tested. Those results are obtained by analyzing the signals through a Supervised Machine Learning algorithm and are represented in detail in a Confusion Matrix (Figure S5.6). One way to analyze how well the several new Ligand-sensors were able to accurately discriminate and classify the volatiles that were being tested in the gas sensing optical device, as intended, is to compare their accuracy prediction scores to the performance of Control-sensors in the same experiment, can be seen in Figure 5.3 and Figure 5.4. It is possible to observe that the control sensors already provide a perfect discrimination for certain VOCs namely aldehydes (isovaleric aldehyde and acetaldehyde), esters (ethyl acetate) and aliphatic compounds (hexane) – which is actually matched by the performance of the majority of the Ligand-sensors, with the exception of the acetaldehyde and hexane volatiles. In the case of the alcohols (ethanol and 3-methyl-1-butanol), an enhanced discrimination was observed for most of the ligand modified sensors, except for L11 in the case of ethanol, and L6, L7 and L9 for 3-methyl-1-butanol. A better discrimination was also achieved by all ligand-sensors for acetone, while acetic acid, 2,5-dimethylpyrazine and benzene demonstrated having an average of half of the tested ligand modified sensors improving on the classification and discrimination of the respective volatiles.

The interactions between VOCs and the ligand-modified sensors are quite complex and difficult to rationalize, derived from the distinct components of the sensors – the modified glass surface with aminosilane groups, and the further functionalization with small ligands, creates a distinct environment for hybrid gel deposition that may also impact the encapsulated

IL-LC droplets. As such, the measured optical signal result from the complex contributions of the interaction between each VOC and the various sensor environments.

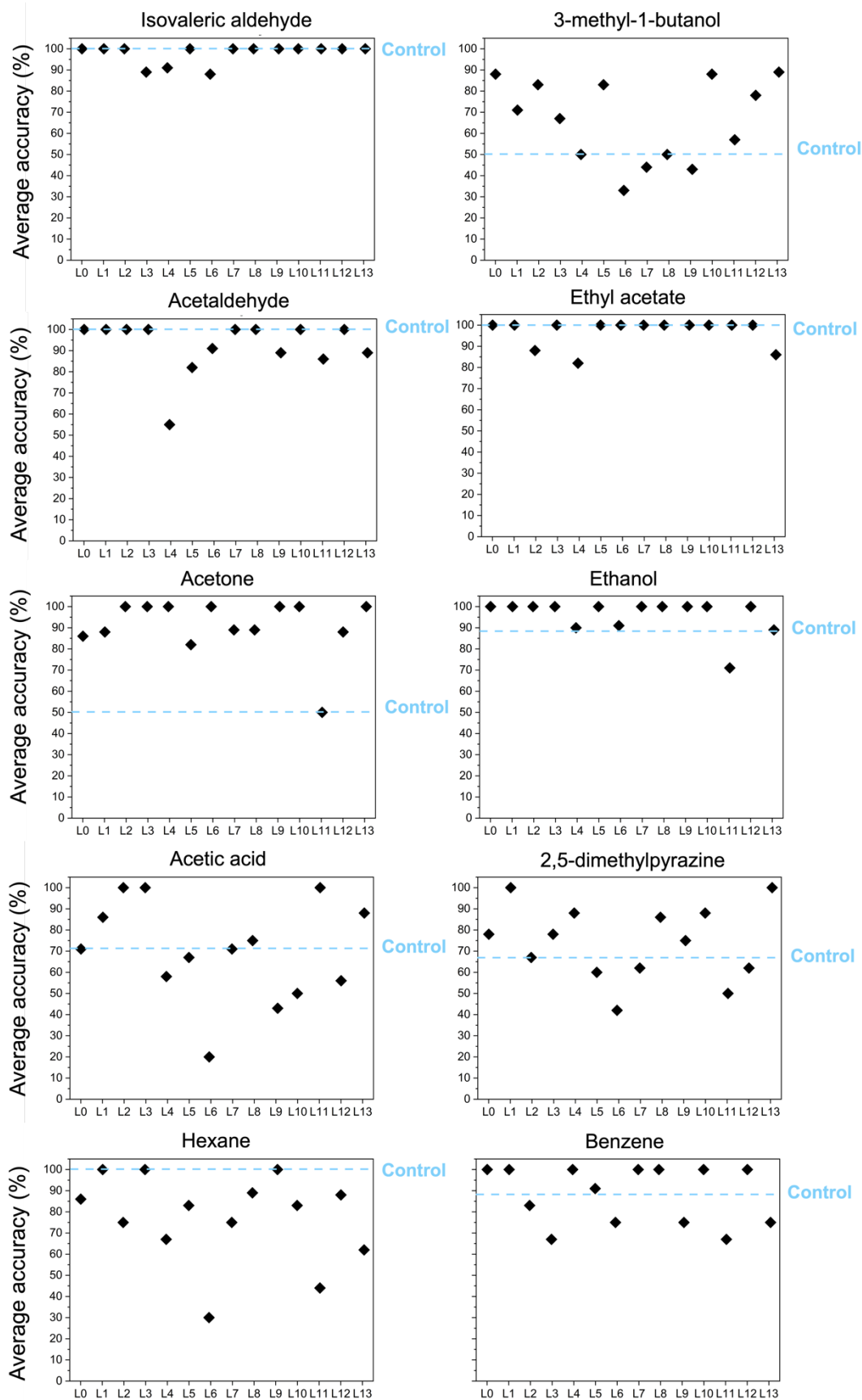


Figure 5. 3 – Accuracy scores for discrimination of the 10 set of mVOCs exposed in the optical e-nose experiments with all ligands-hybrid gels sensors, in comparison with Control-hybrid gel sensors (values are represented by the blue line). All ligands are represented (L0 (1-pyrenemethylamine), L1 (Ethanolamine), L2 (Benzylamine), L3 (4-Aminobutan-2-one), L4 (Ethyl-4-aminobenzoate), L5 (Agmatine), L6 (Aminopyrazine), L7 (Iminodiacetic acid), L8 (N,N-Dimethyl-4,4'-azodianiline), L9 (4-aminobenzamide), L10 (Leucine), L11 (Histamine), L12 (Pararosaniline), L13 (Tryptamine)).

		Isovaleric aldehyde	3-methyl- 1-butanol	Acetaldehyde	Ethyl acetate	Acetone	Ethanol	Acetic acid	2,5- dimethylpyrazine	Hexane	Benzene
L0		0	38	0	0	36	12	0	11	-14	12
L1		0	21	0	0	38	12	15	33	0	12
L2		0	33	0	-12	50	12	29	0	-25	-5
L3		-11	17	0	0	50	12	29	11	0	-21
L4		-9	0	-45	-18	50	2	-13	21	-33	12
L5		0	33	-18	0	32	12	-4	-7	-17	3
L6		-12	-17	-9	0	50	3	-51	-25	70	-13
L7		0	-6	0	0	39	12	0	-5	-25	12
L8		0	0	0	0	39	12	4	19	-11	12
L9		0	-7	-11	0	50	12	-28	8	0	-13
L10		0	38	0	0	50	12	-21	21	-17	12
L11		0	7	-14	0	0	-17	29	-17	-56	-21
L12		0	28	0	0	38	12	-15	-5	-12	12
L13		0	39	-11	-14	50	1	17	33	-38	-13

Figure 5. 4 – Summary of all prediction accuracy scores (in percentage (%)) for discrimination of the 10 set of mVOCs exposed in the optical e-nose experiments with Control and ligands-hybrid gel sensors. In green are represented the best predictions scores for mVOCs (improved accuracy to discriminate them, when compared to the scores provided by the control sensors); in yellow are represented the average predictions scores (same accuracy for discriminate of mVOCs, when compared to the scores provided by the control sensors); and in red are represented the poor predictions scores (inferior accuracies to discriminate volatiles, when compared to the scores provided by control sensors). All ligands are represented (L0 (1-pyrenemethylamine), L1 (Ethanolamine), L2 (Benzylamine), L3 (4-Aminobutan-2-one), L4 (Ethyl-4-aminobenzoate), L5 (Agmatine), L6 (Aminopyrazine), L7 (Iminodiacetic acid), L8 (N,N-Dimethyl-4,4'-azodianiline), L9 (4-aminobenzamide), L10 (Leucine), L11 (Histamine), L12 (Pararosaniline), L13 (Tryptamine)).

5.4 Conclusions

In this work, a chosen set of ligands, from different chemical classes were selected to be immobilized in a glass slide surface that is used as substrate for gas sensing materials, to which an optical sensitive multicomponent hybrid gel was then added.

The choice of ligands to be tested in a gas sensing e-nose device was made with the goal of enhancing the selectivity and discrimination of a set of 10 selected VOCs, who are known to be biomarkers for different bacterial infections. To do this, ligands from chemical classes like acids, amides, amines, anilines, alcohols, hydrazines, ketones, esters, pyrazines and aromatic compounds were tested, due to their affinity to interact with specific chemical classes of VOCs.

It was observed that the deposition of hybrid gel material on top of the chemically modified surfaces originated films with radial polydispersed droplets, as intended, for all Ligand-sensors, with the exception of N,N-Dimethyl-4,4'-azodianiline (L8), where it is possible to see a mixture of radial and escaped radial droplets. It was also notable, that overall, the presence of the ligands in the composition of the new sensors led to morphological differences in the droplets, which is visible by their different colors and, in some cases, even by their shape.

Upon testing the Ligand-sensors in the optical e-nose device, it became clear that Control sensors already provide great discrimination for aprotic polar VOCs, such as aldehydes (isovaleric aldehyde and acetaldehyde) and esters (ethyl acetate), as well as aliphatic compounds (hexane) – as expected by previous work results^{12,13,15}. An enhanced discrimination was achieved for polar aprotic VOCs like alcohols (ethanol and 3-methyl-1-butanol) and ketones (acetone) when using the majority of the Ligand-sensors, compared to Control. This could be a result of interactions between the OH groups of these VOCs and residual amine groups from the APTES molecules, used in the initial silanization step of all ligand-sensors.

Overall the prediction scores from the new sensors towards the volatiles, chosen based on their chemical classes and potential affinity, matched the intended results, having the majority of the volatiles been accurately predicted by the sensors. The accuracy to predict a volatile results from the combination of interactions between the sensor and the VOC molecules, resulting in unique signal features.

We observed that volatiles like 2,5-dimethylpyrazine and acetic acid generally tend to originate flat signals. This is mainly a result of the type of interactions between these VOCs and the sensing materials, that appears to generate no perturbation in the orientation of the optical LC probe. This behavior is followed by 3-methyl-1-butanol, and in the case of acetic acid is consistent with previous reported work^{12,13,15}.

Nevertheless, lower interactions can originate enhanced discriminations scores, just like higher interactions do (due to the unique features of these type of signals, as mentioned before) as can be observed multiple times through the entire optical e-nose experiment results (e.g. hexane in the case of L1-sensor, or acetic acid in the case of L11-sensor, acetone in the case of L13-sensor, amongst others).

Overall enhanced discrimination towards several mVOCs was achieved, by adding selected functional classes of small ligands to the previous described hybrid gel multicomponent materials, used in gas sensing optical experiments.

Supplementary Information

Materials and Methods

Determination of the extent of amination of the glass surfaces using Kaiser Test

A calibration line for standard glycine solutions (0-10 $\mu\text{mol}/\text{ml}$) versus their absorbance at 560 nm must be performed in order to obtain the parameters that allow to determine the concentration of primary amines in each sample. Dilutions for the calibration line (standards) and the samples were prepared in duplicates, and 50 μl of 80% crystalline phenol in ethanol (w/v), 50 μl of 2% 0.001M aqueous solution of potassium cyanide in pyridine (v/v) and 50 μl of 5% ninhydrin in ethanol (w/v) were added to each. The samples and standards were then placed in the water bath at 100°C for 5 minutes, and after that they were diluted by a factor of 20, and its absorbance read at 560 nm, in duplicates, using Microplate Reader TECAN Infinite 200 and Sarstedt 96 Flat Transparent Microplate. Average μmol of amines immobilized in the glass surfaces were determined by subtracting the μmol of amines present in the solution used to silanize the glass surface (calculated by calibration curve of the absorbance measured values; $y=0.2334+0.0773x$), to the initial concentration of amines used for the silanization process.

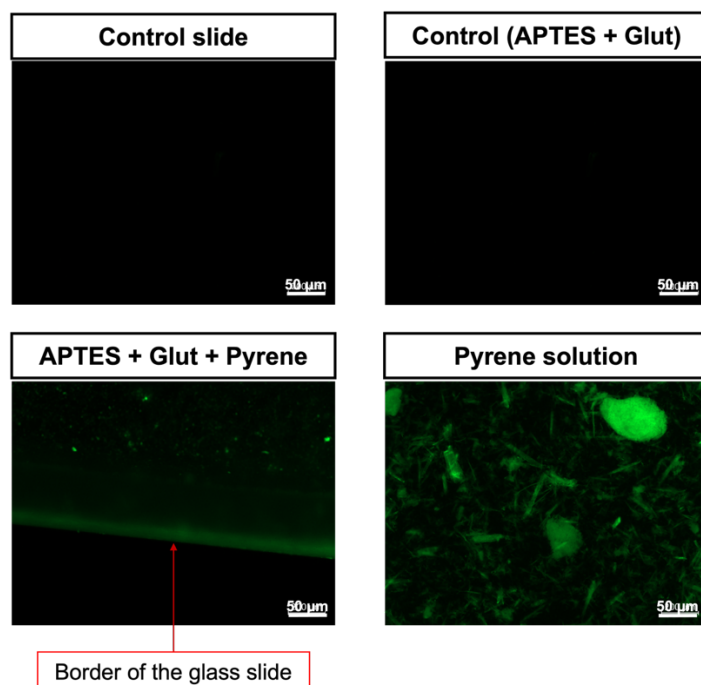
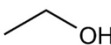
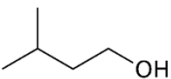
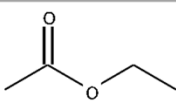
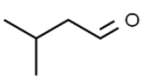
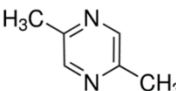


Figure S5. 1 - Fluorescence microscopy image of the pyrene ligand L0 immobilized glass substrate, using fluorescence microscopy with a filter 38 HE Green Fluorescent (Time of explosion=1357 ms).

Results and Discussion

Table S5. 1 – List of microbial VOCs selected for this work, respective structures and list of emitting microorganisms (according to Palma, S.I.C.J., Traguedo, A.P., Porteira, A.R. et al. Machine learning for the meta-analyses of microbial pathogens' volatile signatures. Sci Rep 8, 3360 (2018). <https://doi.org/10.1038/s41598-018-21544-1>).

Microbial VOC	Structure	Microbes emitting the VOC
Acetone		<i>P. aeruginosa</i>
Acetic Acid		<i>S. aureus</i> ; <i>P. aeruginosa</i> ; <i>S. epidermidis</i> ; <i>E. coli</i> ; <i>P. mirabilis</i> ; <i>H. Influenzae</i> ; <i>S. pneumonia</i> ; <i>K. pneumoniae</i> ; <i>C. difficile</i>
Ethanol		<i>P. mirabilis</i> <i>E. coli</i>
3-methyl-1-butanol		<i>P. aeruginosa</i> <i>S. aureus</i> <i>K. Pneumoniae</i>
Ethylacetate		<i>A. Niger</i> ; <i>S. aureus</i> ; <i>P. aeruginosa</i> ; <i>S. epidermidis</i> ; <i>E. coli</i> ; <i>P. mirabilis</i> ; <i>H. Influenzae</i> ; <i>S. pneumonia</i> ; <i>C. difficile</i>
Acetaldehyde		<i>K. Pneumoniae</i> <i>H. Influenzae</i>
3-methylbutanal		<i>K. Pneumoniae</i>
2,5-dimethylpyrazine		<i>S. aureus</i>

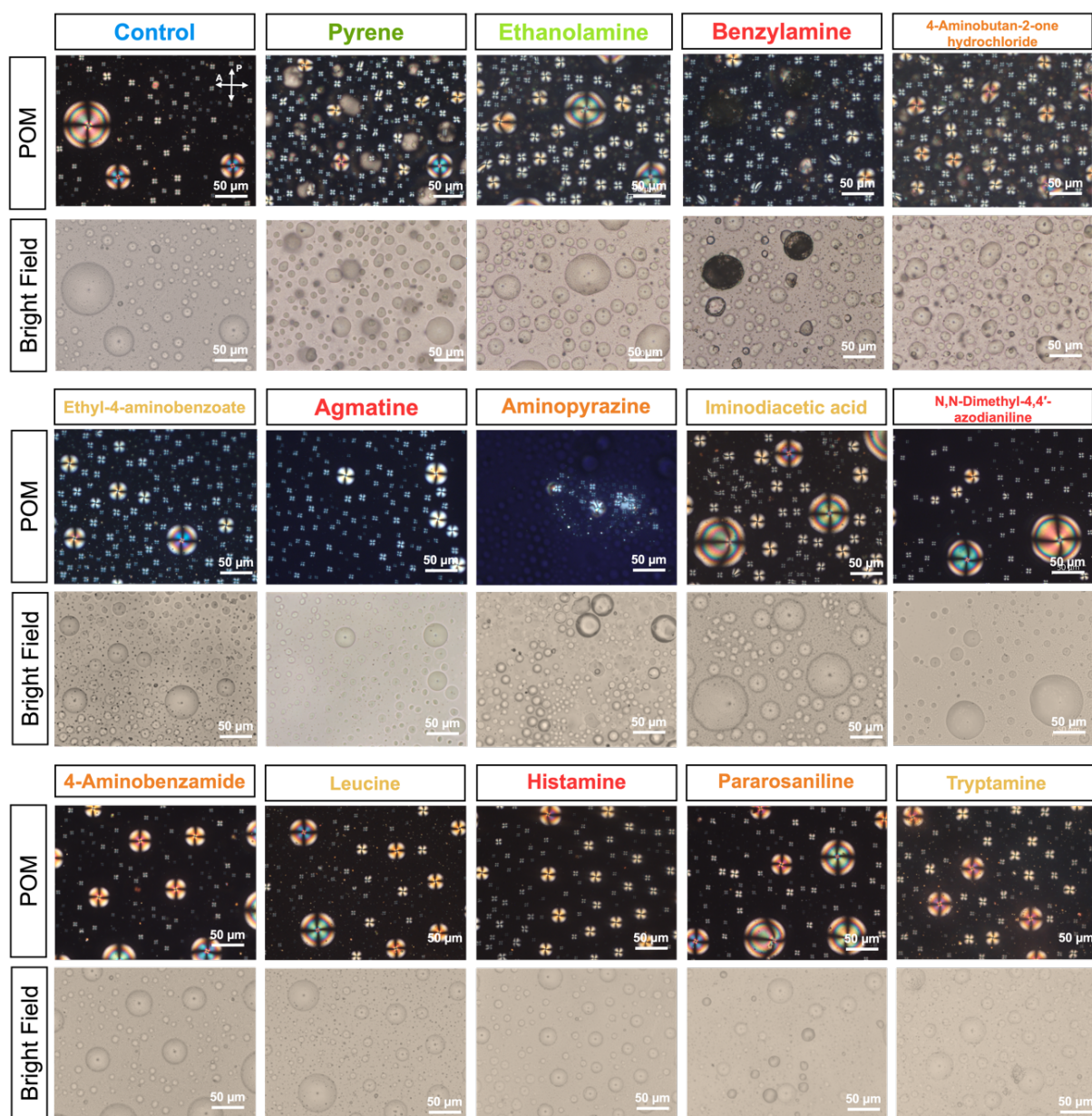


Figure S5. 2 – Morphological characterization of the optical ligand modified glass substrate hybrid gel sensors produced through POM and Bright Field, for all the ligands used and the control.

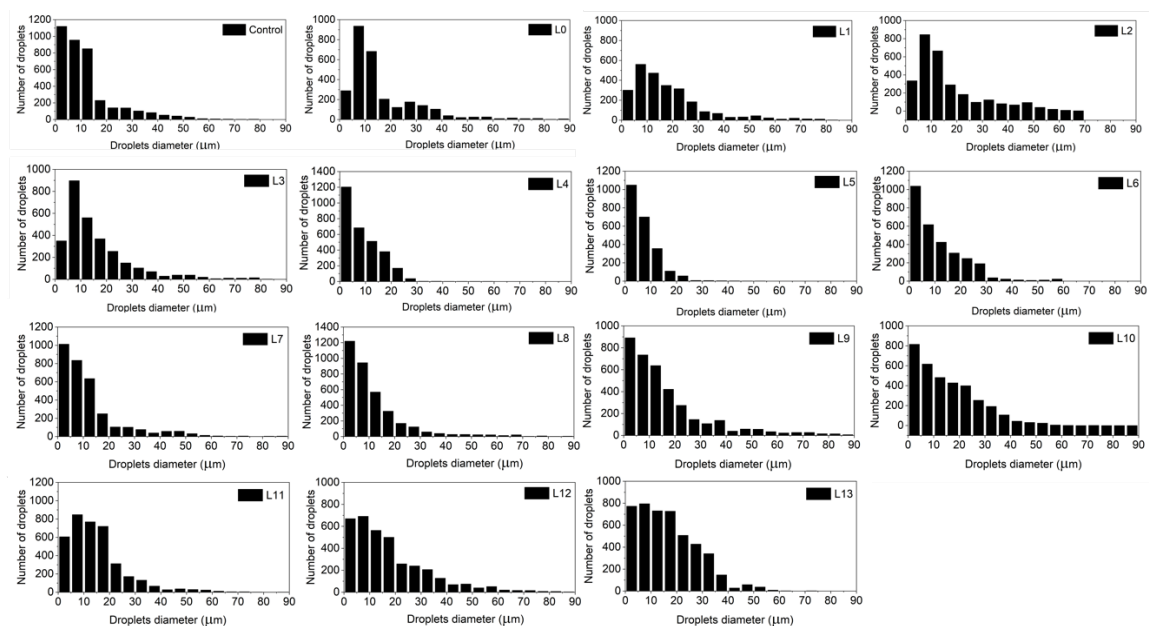


Figure S5. 3 – Histogram of the distribution of droplet's diameter, for the Control and Ligand-sensors produced. All ligands are represented (L0 (1-pyrenemethylamine), L1 (Ethanalamine), L2 (Benzylamine), L3 (4-Aminobutan-2-one), L4 (Ethyl-4-aminobenzoate), L5 (Agmatine), L6 (Aminopyrazine), L7 (Iminodiacetic acid), L8 (N,N-Dimethyl-4,4'-azodianiline), L9 (4-aminobenzamide), L10 (Leucine), L11 (Histamine), L12 (Pararosaniline), L13 (Tryptamine)).

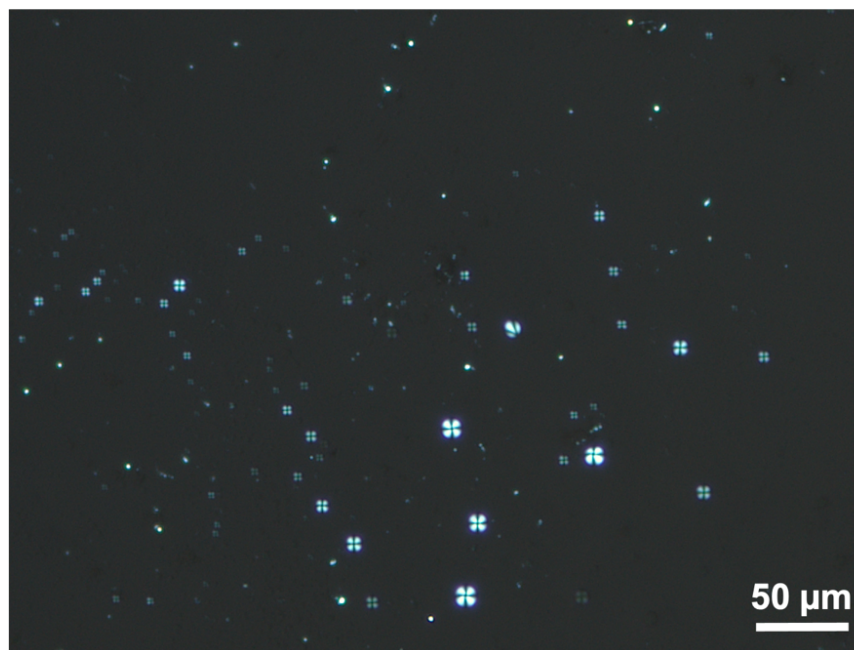


Figure S5. 4 – Thermal investigations regarding the morphological characterization of the L6-hybrid gel sensors under the POM with crossed polarizers, at 16.7°C.



Figure S5.5 – Representative signals of the optical amplitude responses in optical e-nose experiment, of control and all ligands-hybrid gels sensors, to the set of 10 distinct mVOCs tested. Each curve represents the average and standard deviation of at least 19 replicate cycles from the same sensor. VOC exposure periods (5 s) are highlighted in grey, and each cycle corresponds in total to 15 s. The plots were generated by python.

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GENERAL CONCLUSIONS

The detection of volatile organic compounds (VOCs) through gas sensing techniques using Electronic nose devices is a growing field, with applications in diverse areas including disease diagnostics^{1,2}. Nevertheless, some challenges remain, and tuning the materials' sensitivity, stability, environmental impact and the ability to operate under ambient conditions still requires optimization. Incorporating sensing components derived from biological olfaction systems into these gas-sensing materials can be an answer on how to increase the VOC selectivity when using these devices³⁻⁸.

In addition, the ionogels and hybrid gels studied in this work are bio-based opto-electrical materials, consisting of ionic liquids and liquid crystals, encapsuled in a biopolymeric matrix. Because these materials respond to different stimulus, and due to their dual optical and electrical properties and composition, they can be tuned and used to achieve gas analytes discrimination purposes, especially when combined with small affinity ligands⁹⁻¹⁴.

The novel bio-based gas sensing materials developed under the scope of this dissertation are summarized in Table 6.1 and Table 6.2.

Table 6. 1 – Summary of bio-based ionogels studied as gas sensing materials developed in this thesis.

	Polymer	Ionic liquid	Additives	Bio-based material	Thesis chapter
Study of the effect of the ionic liquid cation in ionogels	Gelatin	[BMIM][Cl]	-	Ionogel	2
	Gelatin	[BMPip][Cl]	-	Ionogel	2
	Gelatin	[BMPyrr][Cl]	-	Ionogel	2
	Gelatin	[BuPy][Cl]	-	Ionogel	2
	Gelatin	[Bu ₂ Pic][Cl]	-	Ionogel	2
	Gelatin	[Bu ₄ Pic][Cl]	-	Ionogel	2
Study of the incorporation of	Gelatin	[BMIM][DCA]	-	Ionogel	4

VOC-selective peptides in ionogels	Gelatin	[BMIM][DCA]	P1 (peptide)	Ionogel	4
	Gelatin	[BMIM][DCA]	P2 (peptide)	Ionogel	4
	Gelatin	[BMIM][DCA]	P3 (peptide)	Ionogel	4

Table 6. 2 – Summary of bio-based hybrid gels studied as gas sensing materials developed in this thesis.

	Polymer	Ionic liquid	Liquid crystal	Additives	Bio-based material	Thesis chapter
Study of the effect of the ionic liquid cation in hybrid gels	Gelatin	[BMIM][Cl]	5CB	-	Hybrid gel	2 and 3
	Gelatin	[BMPip][Cl]	5CB	-	Hybrid gel	2
	Gelatin	[BMPyrr][Cl]	5CB	-	Hybrid gel	2
	Gelatin	[BuPy][Cl]	5CB	-	Hybrid gel	2
	Gelatin	[Bu ₂ Pic][Cl]	5CB	-	Hybrid gel	2
	Gelatin	[Bu ₄ Pic][Cl]	5CB	-	Hybrid gel	2
Study of the effect of polymer hydrophobicity in the performance in hybrid gels	Gelatin	[BMIM][DCA]	5CB	-	Hybrid gel	3, 4 and 5
	PDMS	[BMIM][DCA]	5CB	-	Hybrid gel	3
	PDMS	[BMIM][Cl]	5CB	-	Hybrid gel	3
Study of the incorporation of VOC-selective peptides in hybrid gels	Gelatin	[BMIM][DCA]	5CB	-	Hybrid gel	4
	Gelatin	[BMIM][DCA]	5CB	P1 (peptide)	Hybrid gel	4
	Gelatin	[BMIM][DCA]	5CB	P2 (peptide)	Hybrid gel	4
	Gelatin	[BMIM][DCA]	5CB	P3 (peptide)	Hybrid gel	4

Study of Ligand-sensors to tune the classification of VOCs in hybrid gels	Gelatin	[BMIM] [DCA]	5CB	1-pyrenemethylamine (L0)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Ethanolamine (L1)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Benzylamine (L2)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	4-Aminobutan-2-one (L3)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Ethyl-4-aminobenzoate (L4)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Agmatine (L5)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Aminopyrazine (L6)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Iminodiacetic acid (L7)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	N,N-Dimethyl-4,4'-azodi-aniline (L8)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	4-aminobenzamide (L9)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Leucine (L10)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Histamine (L11)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Pararosaniline (L12)	Hybrid gel	5
	Gelatin	[BMIM] [DCA]	5CB	Tryptamine (L13)	Hybrid gel	5

The hybrid gel formulation composed by gelatin, the ionic liquid [BMIM][DCA] and the liquid crystal 5CB⁹⁻¹⁴, was used in the development of the first tailor-made optical electronic nose (e-nose), employing artificial intelligence algorithms^{11,12}. This formulation served as the basis for the development of the novel gas sensing materials described in this thesis.

Overall, the tunable ability of ionogels and hybrid gels is assessed on this work. Specifically, the impact of the ionic liquid cation in hybrid gels gas sensing capability is addressed in chapter 2. Six different cation-ionic liquids-based families (members of the piperidinium, pyrrolidinium, pyridinium and imidazolium families) were studied, maintaining constant the anion (chloride), selected due to its high hydrogen acceptor character.

Successful novel formulations were developed for both ionogels and hybrid gel-sensing materials, and their properties were evaluated through conductivity assays (ionogels), POM and SEM techniques (hybrid gels). The effects concerning the change of the ionic liquid cation were not exclusively related with gas sensing discrimination of volatiles, but also with regards to the properties of the produced sensing materials, in which morphological differences were observed.

The sensors were tested in electrical and optical e-nose experiments against a set of different VOCs, and a machine learning algorithm was used to assess the sensors performance. A set of features was extracted from the signals, and then used as input data for an SVM classifier method, that states the accuracy of the classifier towards a gel predicting a volatile.

Comparing accuracy results from the tested ILs sensors (both in electrical and optical experiment), a conclusion is reached that by using both electrical and optical approaches combined, we obtain additional complementary information as well as different discrimination of volatiles. As an overall, all the ionogels sensors present better accuracy prediction score values in the electrical e-nose, with the exception of Bu₂Pic.

The impact of the polymeric matrix in the hybrid gel formulations was analyzed in chapter 3. Reporting the use of two different polymeric matrixes in the hybrid gel formulations (a hydrophilic one like gelatin, or a hydrophobic such as PDMS), in the presence of two different ionic liquids ([BMIM][Cl] and [BMIM][DCA]) results regarding the achieved accuracies to discriminate accurately volatiles are compared.

Morphology of sensing materials is characterized through POM and SEM, and sensors were also monitored regarding their air-stability.

Immediate effects of the matrices are observed with either IL, being possible to see that PDMS materials present smaller droplets, due to an inhibitory effect of the nematic growth of the LC, inflicted by the presence of the hydrophobic PDMS matrix. As for stability to long-time storage, PDMS materials with [BMIM][DCA] proved to be the most stable gels, against gelatin with [BMIM][Cl] that was found to be the less stable.

Accuracies to predict the different volatiles was also procured in optical electronic nose experiment, and data analysis regarding classification of the VOCs was the same as implemented in chapter two. In the optical e-nose gas sensing experiment, it was possible to observed that, in general, the use alone of a more hydrophobic matrix (PDMS) in the formulation of the sensing films does not favor the isolate distinction of the more hydrophobic volatiles, since it is possible to see that by using both matrices (PDMS and gelatin), VOCs within different polarities are well classified by both sensing materials. This proves that the classification of VOCs through these experiments are consequence of the interactions with the entire

multicomponent system, and not just dependent on the matrix individual component – however, it seems that the choice of the IL plays a key role in it.

With regards to performance of the new sensing materials with both polymeric matrices, it was also possible to assess that when comparing the two ionic liquids [BMIM][DCA] and [BMIM][Cl], [BMIM][DCA] presents better optical e-nose performance overall within a gelatin matrix, while [BMIM][Cl] performs better within a PDMS matrix.

The use of biological small molecules to develop novel stimuli-responsive gas sensing bio-based materials, with tuned selectivity towards specific VOCs, is described in chapter 4 and chapter 5.

In chapter 4, Peptide (P1) known to discriminate single carbon deviations among benzene and derivatives was used as a model, along with two modified versions (P2 and P3). Novel sensing materials, both ionogels and hybrid gels were produced by incorporating the peptides into their formulation, and regarding the hybrid gels' morphology, it was observed by POM that the incorporation of peptide P1 did not disrupt the self-assembly of the ionic liquid crystal droplets (suggesting that peptide P1 is mainly distributed in the matrix), while the incorporation of peptides P2 and P3 disrupt some droplets, although the majority still exhibit the expected radial configuration.

The new sensors were tested in the house-built e-nose devices. The peptide-ionogels were tested in the electrical e-nose, designed to monitor changes in the conductance of the sensors, and selectivity towards hexane and xylene was observed in the case of P2 and P3-ionogel sensors, respectively. The set of peptide-hybrid gels tested in the optical e-nose, designed to monitor the light transmitted from the materials, an enhanced discrimination of benzene was observed for the P1- based gel, and for ethanol in the case of both P2 and P3-hybrid gels.

Once again, the results highlight the complementarity of the two distinct gel formulation' responses, since the combination of both optical and electrical prediction accuracies could provide the best classification accuracies for the tested VOCs, suggesting that additional information and enhanced selectivity can be obtained regarding discrimination of volatiles, by using a combinatorial sensors' array in a dual-mode e-nose.

The addition of small ligands to the hybrid gel formulation was evaluated under different conditions within chapter 5. A chosen set of ligands, from different chemical classes (like acids, amides, amines, anilines, alcohols, hydrazines, ketones, esters, pyrazines and aromatic compounds), were selected to be immobilized in a glass slide surface that is used as substrate for gas sensing materials, to which an optical sensitive multicomponent hybrid gel was then spread.

The choice of ligands was made with the goal of enhancing the selectivity and discrimination of a set of 10 selected VOCs, which are known to be biomarkers for different bacterial infections, through affinity interactions.

The addition of the hybrid gel material on top of the chemically modified surfaces originated films with radial polydispersed droplets. The presence of ligands in the substrates of sensors, leads to morphological differences in the droplets.

Upon testing the Ligand-sensors in the optical e-nose device, we observed that overall the prediction scores from the new sensors towards the volatiles, chosen based on their chemical classes and potential affinity for interaction, matched the intended results, having the majority of the volatiles been accurately predicted by the sensors.

Great results were delivered from the Control sensors towards aprotic polar VOCs (such as aldehydes and esters), as well as aliphatic compounds, and that an enhanced discrimination was achieved for polar aprotic VOCs like alcohols and ketones when using the majority of the Ligand-sensors.

Confirmation was obtained that the accuracy to predict a volatile is a result of combining interactions from the sensors and the VOCs, with the unique shape of the signal that is originated by these interactions playing a key role in defining the unique features which are analyzed through the algorithm.

In a general way, in this work the changes in bio-based material properties, either conductance (electrical) or optical, upon exposure to volatiles were independently analyzed. Nevertheless, both responses might be combined improving the selectivity and/or the sensitivity of our gas sensing bio-based materials technology, as observed in chapter 2 and 4. By changing the ionic liquids, the matrix and by adding different small molecules to the formulation of the sensing materials' compositions, different discriminations of volatiles were achieved (Table 6.3), which proves that these results are consequence of the interactions with the entire multi-component system, and not just dependent from an individual component. Nevertheless, modification and addition of them can serve changes and improvements on the accuracies to classify the volatiles, in combination with the use of the e-nose devices and the SVM algorithm.

Table 6. 3 – Summary of the gas sensing systems studied in the thesis and the VOC-enhanced discrimination achieved (100% accuracy predictions).

Bio-based sensing material	Detected VOC	Transduction method
BMPip-ionogel	Acetone, acetonitrile, chloroform, dichloromethane	Electrical e-nose
BMPyrr-ionogel	Acetic acid, acetone, acetonitrile	Electrical e-nose
BuPy-ionogel	Acetone, dichloromethane, methanol	Electrical e-nose
BMPip-hybrid gel	Chloroform	Optical e-nose
PDMS-[BMIM][DCA]-hybrid gel	Toluene and acetonitrile	Optical e-nose
PDMS-[BMIM][CL]-hybrid gel	Hexane, acetonitrile, ethyl acetate and acetic acid	Optical e-nose
PDMS Control-hybrid gel	Toluene and acetonitrile	Optical e-nose

Gelatin-[BMIM][DCA]-hybrid gel	Heptane, hexane, ethanol and methanol	Optical e-nose
Gelatin-[BMIM][CL]-hybrid gel	Heptane, hexane, chloroform and acetonitrile	Optical e-nose
Gelatin Control-hybrid gel	Heptane, toluene, chloroform, ethyl acetate, acetonitrile and acetic acid	Optical e-nose
Control-ionogel	Acetone, ethanol	Electrical e-nose
P2-ionogel	Hexane	Electrical e-nose
P3-ionogel	Xylene	Electrical e-nose
P1-hybrid gel	Benzene, hexane	Optical e-nose
P2-hybrid gel	Ethanol	Optical e-nose
P3-hybrid gel	Ethanol	Optical e-nose
Control-hybrid gel (modified glass slide)	Acetaldehyde, ethyl acetate, hexane, isovaleric aldehyde	Optical e-nose
L0-hybrid gel	Acetaldehyde, benzene, ethanol ethyl acetate, isovaleric aldehyde	Optical e-nose
L1-hybrid gel	2,5-dimethylpyrazine, acetaldehyde, benzene, ethanol, ethyl acetate, hexane, isovaleric aldehyde	Optical e-nose
L2-hybrid gel	Acetaldehyde, acetic acid, acetone, ethanol, isovaleric aldehyde	Optical e-nose
L3-hybrid gel	Acetaldehyde, acetic acid, acetone, ethanol, ethyl acetate, hexane	Optical e-nose
L4-hybrid gel	Acetone, benzene	Optical e-nose
L5-hybrid gel	Ethanol, ethyl acetate, isovaleric aldehyde	Optical e-nose
L6-hybrid gel	Acetone, ethyl acetate	Optical e-nose
L7-hybrid gel	Acetaldehyde, benzene, ethanol, ethyl acetate, isovaleric aldehyde	Optical e-nose
L8-hybrid gel	Acetaldehyde, benzene, ethanol, ethyl acetate, isovaleric aldehyde	Optical e-nose
L9-hybrid gel	Acetone, ethanol, ethyl acetate, hexane, isovaleric aldehyde	Optical e-nose
L10-hybrid gel	Acetaldehyde, acetone, benzene, ethanol, ethyl acetate, isovaleric aldehyde	Optical e-nose

L11-hybrid gel	Acetic acid, ethyl acetate, isovaleric aldehyde	Optical e-nose
L12-hybrid gel	Acetaldehyde, benzene, ethanol, ethyl acetate, isovaleric aldehyde	Optical e-nose
L13-hybrid gel	2,5-dimethylpyrazine, acetone, isovaleric aldehyde	Optical e-nose

Electrical e-nose: Changes in system's conductance due to interaction with VOCs; Optical e-nose: Phase transition or reorientation of the LC due to interaction with the VOCs.

The knowledge and tools presented in this thesis will contribute for the evaluation of the potential application of novel ionogels and hybrid gel sensing materials in the identification of VOCs that can be biomarkers for diseases. Furthermore, future studies attempting to elucidate how possible affinities and interactions can enhance the discrimination of VOCs, can be pursued on a more fundamental level. The information and results acquired in this dissertation will contribute to strengthen the position of bio-based sensing materials as the basis to design advance functional sensors with applications in artificial olfaction, especially in the field of diseases' diagnostics.



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ENHANCING VOCs SELECTIVITY BY DESIGNING NOVEL GAS
SENSITIVE SOFT MATERIALS

