Electronic Supplementary Information (ESI)

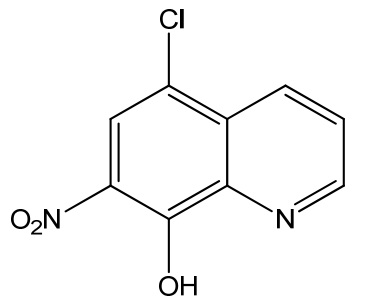
Solid sensory polymer substrates for the quantification of iron in blood, wine and water by a scalable RGB technique.

Saúl Vallejos, Asunción Muñoz, Saturnino Ibeas, Felipe Serna, Félix Clemente García, José Miguel García.*

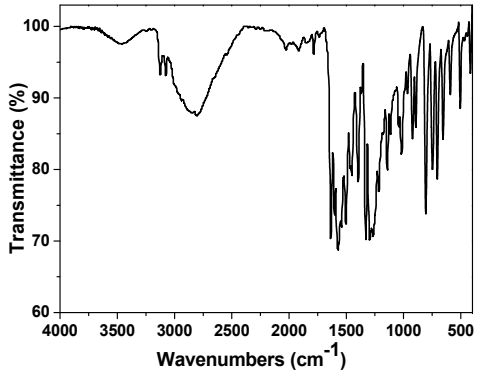
Departamento de Química, Facultad de Ciencias, Universidad de Burgos, Plaza de Misael Bañuelos s/n, 09001 Burgos, Spain. Email: jmiguel@ubu.es

Table of contents:

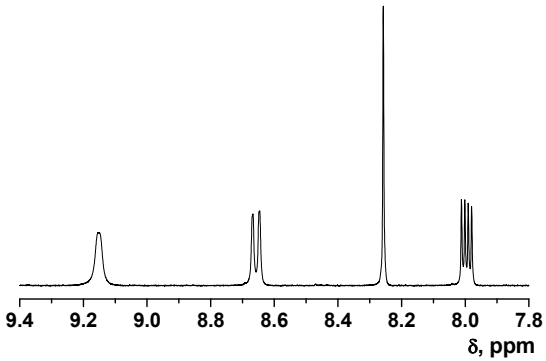
S1.- NMR and FT-IR of intermediates and monomer



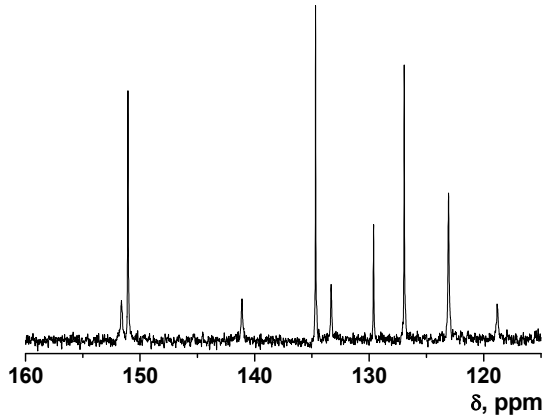
a) 5-chloro-7-nitroquinolin-8-ol



b)

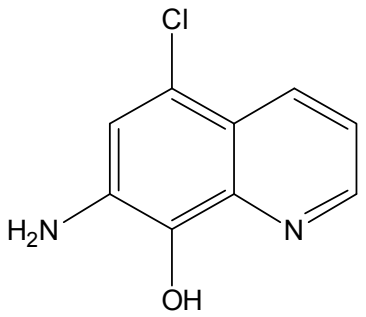


c)

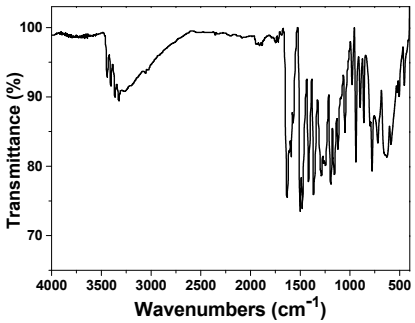


d)

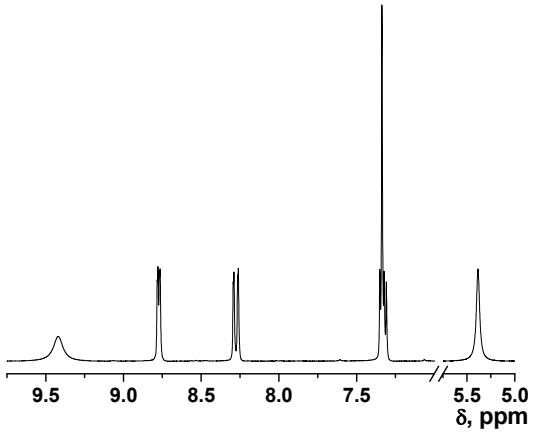
Figure S1. Characterization of 5-chloro-7-nitroquinolin-8-ol (**1**): (a) chemical structure; (b) FT-IR; (c) ^{1}H NMR; (d) ^{13}C NMR (NMR solvent: DMSO-d_6).



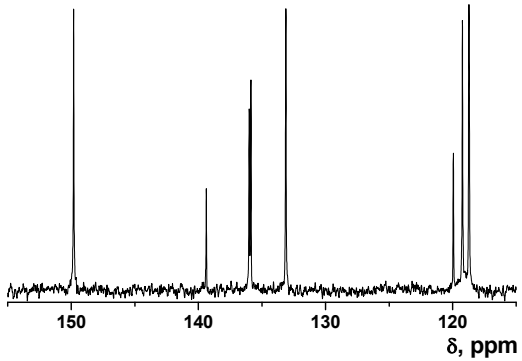
a) 7-amino-5-chloroquinolin-8-ol



b)

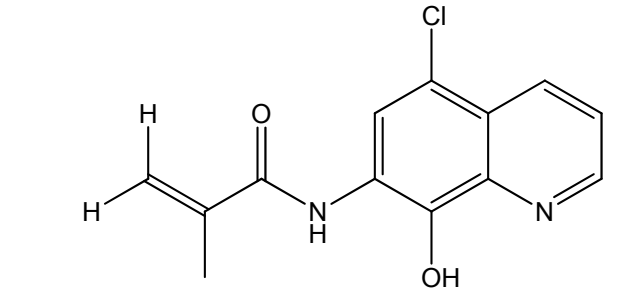


c)

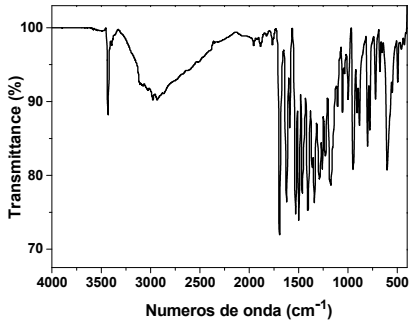


d)

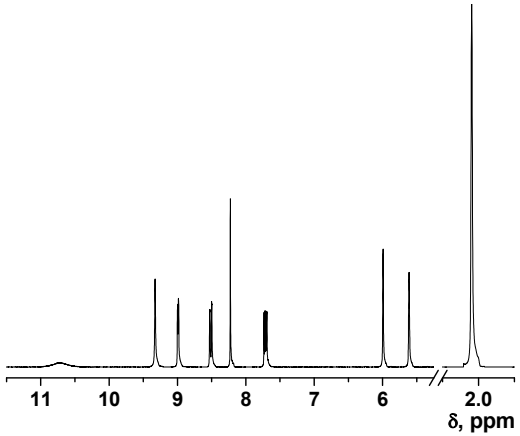
Figure S2. Characterization of 7-amino-5-chloroquinolin-8-ol (**2**): (a) chemical structure; (b) FT-IR; (c) ^{1}H NMR; (d) ^{13}C NMR (NMR solvent: DMSO-d_6).



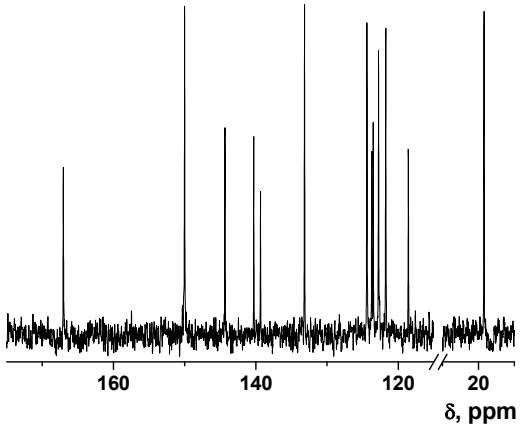
a) *N*-(5-chloro-8-hydroxyquinolin-7-yl)methacrylamide



b)



c)



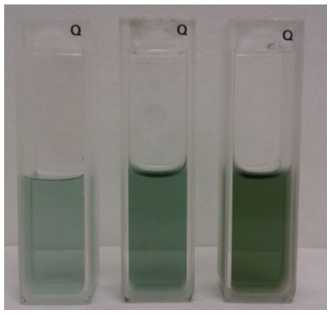
d)

Figure S3. Characterization of *N*-(5-chloro-8-hydroxyquinolin-7-yl)methacrylamide (**HOxMMA**): (a) chemical structure; (b) FT-IR; (c) ^{1}H NMR; (d) ^{13}C NMR (NMR solvent: DMSO-d_6).

S2.- Structure and stability constants of Fe(III)-8-hydroxyquinoline complexes (Turnquist and Sandell),[1] as well as a crystal structure of Fe(8-hydroxyquinoline)3 (Pech et al.)[2]

Table S1 depicts the species corresponding to the interaction between the Fe(III) and 8-hydroxyquinoline complexes.

Table S1. Stability constants of Fe(III):8-hydroxyquinoline complexes (1:1, 1:2 and 1:3)

Complexes	Stability constants
$Fe(III) + Ox^- \rightleftharpoons FeOx^{2+}$	$K_1 = \frac{[FeOx^{2+}]}{[Fe(III)][Ox^-]} = 4.9 \times 10^{13}$
$FeOx^{2+} + Ox^- \rightleftharpoons FeOx_2^+$	$K_2 = \frac{[FeOx_2^+]}{[FeOx^{2+}][Ox^-]} = 4.2 \times 10^{12}$
$FeOx^+ + Ox^- \rightleftharpoons FeOx_3$	$K_3 = \frac{[FeOx_3]}{[FeOx_2^+][Ox^-]} = 3.9 \times 10^{10}$
 Color development upon increase of the HOx concentration relative to a Fe(III) concentration of 2.5×10^{-4} M in EtOH:H_2O (1/1, v/v; pH = 2). From left to right, dominant species 1:1, 2:1, 3:1 ($Fe:Ox^-$).	Overall stability constant (K_s): $\log K_s = \log K_1 K_2 K_3 = 36.9$

The complex Fe(8-hydroxyquinoline)$_3$ was found to crystallize with an ethanol molecule, which was not included in the coordination sphere of the iron. The O and N atoms of the bidentate ligands form three five-membered chelated rings, as shown in Figure S4.

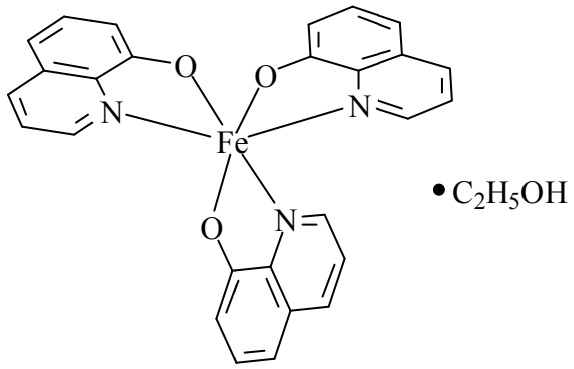


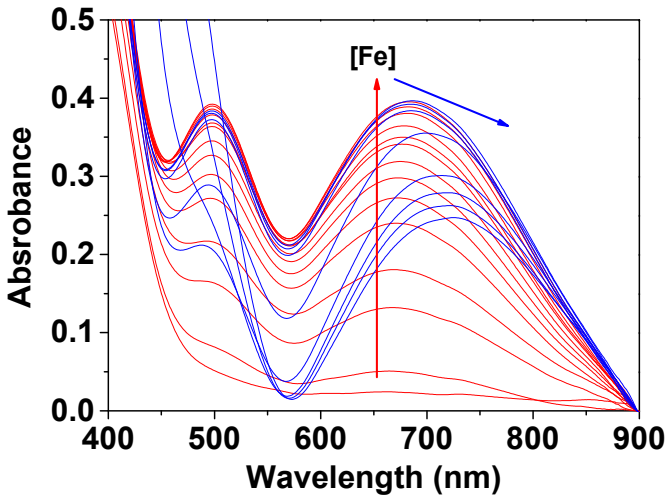
Figure S4. Representation of the crystal structure of the Fe(8-hydroxyquinoline)$_3$ complex, which was crystallized from $CHCl_3$/EtOH, as reported by Pech *et al*.[2]

[1] T.D. Turnquist, E.B. Sandell, Anal. Chim. Acta 1968, 42, 239-245.

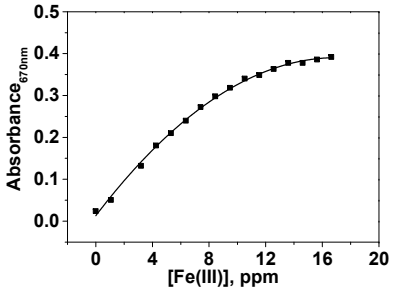
[2] L. Pech, Y.A. Bankovsky, A. Kemme, J. Lejejs, Acta Cryst. 1997, C53, 1045-1047.

S3.- Titration of Fe(III) with the monomer HOxMMA, copolymer LCp, and membrane Mem by means of UV/Vis spectroscopy

a)



b)



c)

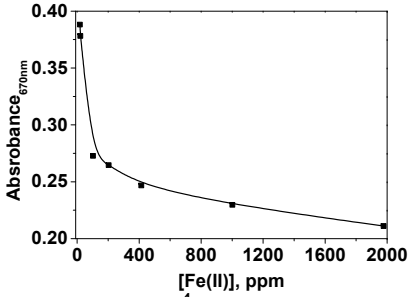
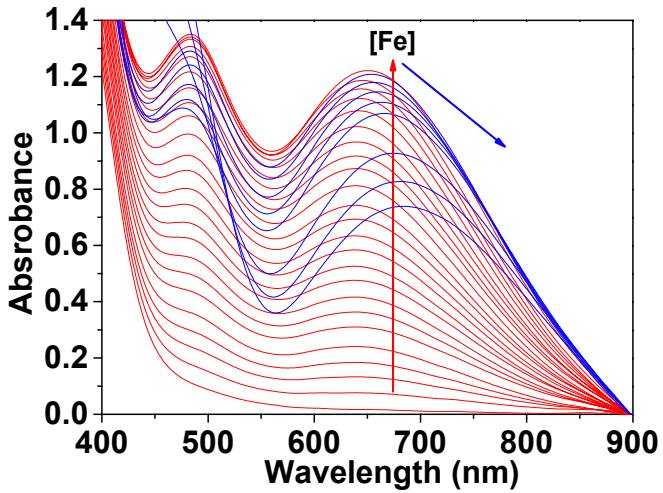
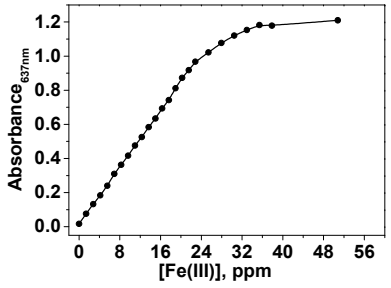


Figure S5. UV/Vis spectra taken during the titration of Fe(III) with **HOxMMA**, 3.82×10^{-4} M, with Fe(III) in ethanol:water (50/50 v/v) (pH = 2, KCl–HCl). The concentration of Fe(III) ranged from 1.91×10^{-5} to 3.53×10^{-2} M. a) UV/Vis spectra. Right: absorbance at 670 nm corresponding to lower -b)- and higher –c)- ferric iron concentrations (higher than 16 ppm). The top curve -b)- indicates the formation of ligand:metal 3:1 and 2:1 complexes, while the bottom curve -c)- indicates the displacement of the complex equilibrium toward the formation of a 1:1 complex. The titration was carried out in a quartz cuvette by adding increasing quantities of $Fe(NO_3)_3 \cdot 9H_2O$ in water solution.

a)



b)



c)

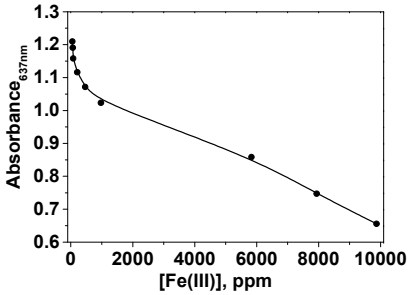
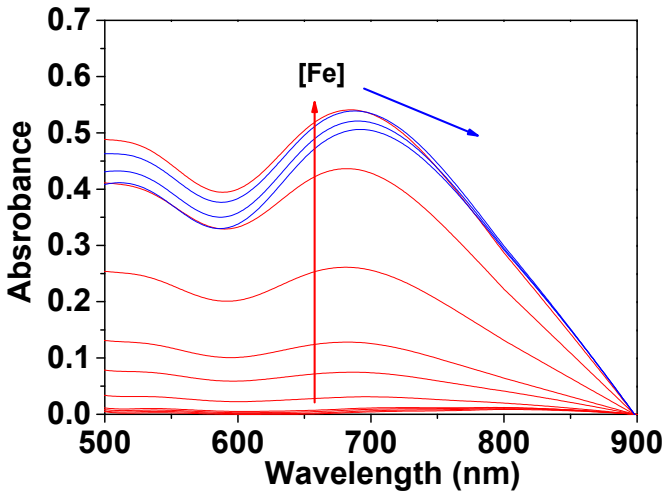
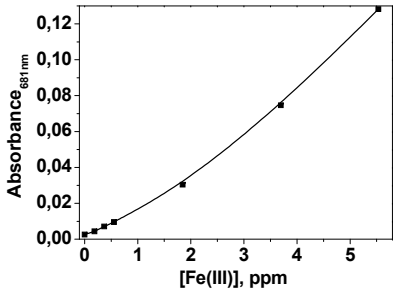


Figure S6. UV/Vis spectra taken during the titration of Fe(III) with **LCp** in water (pH = 2, KCl–HCl). The concentration of the **LCp** was 17.64 g/L, which corresponded to 22.3 milliequivalents of sensory motifs (HOx derivative) per liter. The concentration of Fe(III) ranged from 2.49×10^{-5} to 0.18 M. a): UV/Vis spectra. Right: absorbance at 637 nm corresponding to lower –b)- and higher –c)- ferric iron concentration (the former lower than 50 ppm and the later until 10000 ppm). The top curve -b)- indicates the formation of ligand:metal 3:1 and 2:1 complexes, while the bottom curve -c)- indicates the displacement of the complex equilibrium toward the formation of a 1:1 complex. The titration was performed in a quartz cuvette by adding increasing quantities of $Fe(NO_3)_3 \cdot 9H_2O$.

a)



b)



c)

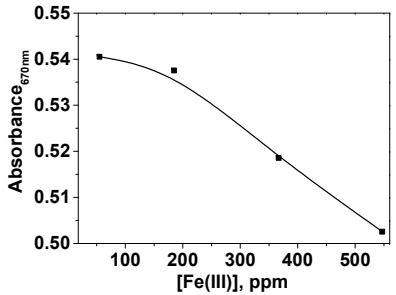
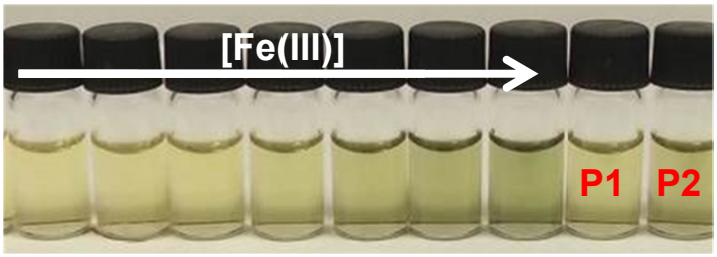


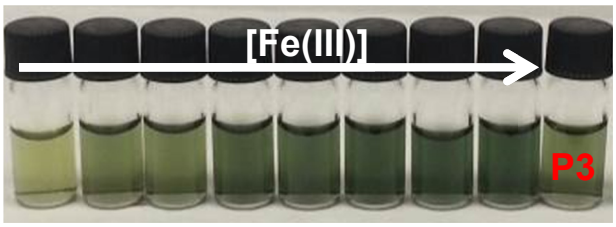
Figure S7. UV/Vis spectra taken during the titration of Fe(III) with **Mem** in water (pH = 2, KCl–HCl). The concentration of Fe(III) ranged from 3.30×10^{-6} to 9.77×10^{-3} M. a): UV/Vis spectra. Right: absorbance at 681 nm, which corresponded to lower -b)- and higher -c)- ferric iron concentrations (the former lower than 20 ppm and the later until 550 ppm). The top curve -b)- indicates the formation of ligand:metal 3:1 and 2:1 complexes, while the bottom curve -c)- indicates the displacement of the complex equilibrium toward the formation of 1:1 complex. The titration was performed by immersing the membrane in the water inside of the quartz cuvette and adding increasing quantities of $Fe(NO_3)_3 \cdot 9H_2O$.

S4.- Titration of Fe(III) in water with copolymer LCp via the analysis of the RGB parameters from each vial in a digital picture



[Fe (III)], ppm	R	G	B	PC
0.002	211	201	142	2.26
0.282	208	199	138	1.956
0.566	204	196	130	1.45
1.411	193	187	123	0.54
2.822	181	176	109	-0.72
4.234	169	165	102	-1.732
5.656	157	157	93	-2.71
P1	191	185	119	0.261
P2	175	169	105	-1.30

Standardized; eigenvalue = 2.99; % variance = 99.5%



[Fe(III)], ppm	Log [Fe(III)]	R	G	B	PC
5.66	0.753	154	156	99	3.66
14.11	1.150	104	112	70	1.06
42.34	1.627	70	81	53	-0.64
55.89	1.747	67	79	53	-0.73
184.80	2.267	57	70	49	-1.19
369.04	2.567	56	68	48	-1.28
559.44	2.748	52	66	46	-1.45
18.648 (P3)	1.271	95	105	64	0.58

Standardized; eigenvalue = 2.99; % variance = 99.8%

Figure S8. Principal component analysis (PCA) of the RGB data from the pictures of **LCp** solutions in water (pH = 2, KCl–HCl) that contained different quantities of Fe (III). The concentration of the **LCp** was 17.61 g/L, which corresponds to 22.3 milliequivalents of sensory motifs per litre. The titration was performed in the quartz cuvette by adding increasing quantities of $Fe(NO_3)_3 \cdot 9H_2O$. The test samples are labelled in red as P1, P2 and P3.

S5.- Sensory membrane or film Mem

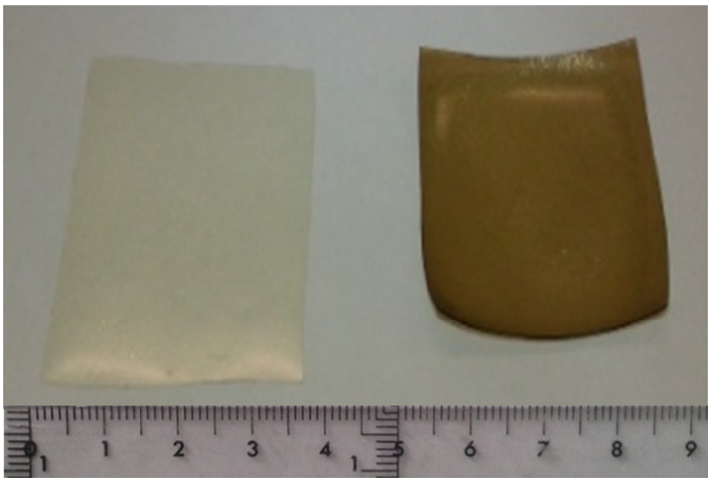


Figure S9. Dry sensory film, **Mem,** before (left) and after immersing into water containing 0.1 M of Fe(III).

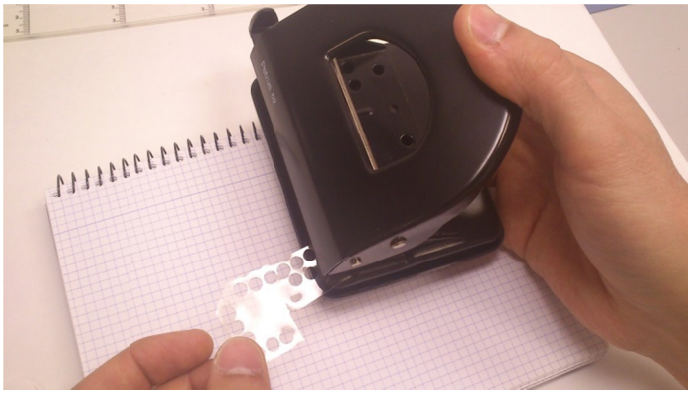


Figure S10. Preparation of the sensory discs by cutting 5 mm diameter circles from the sensory membrane or film **Mem** with an office paper punch.

S6.- Schematic procedure followed to quantify Fe(III) in water media by a scalable RGB technique

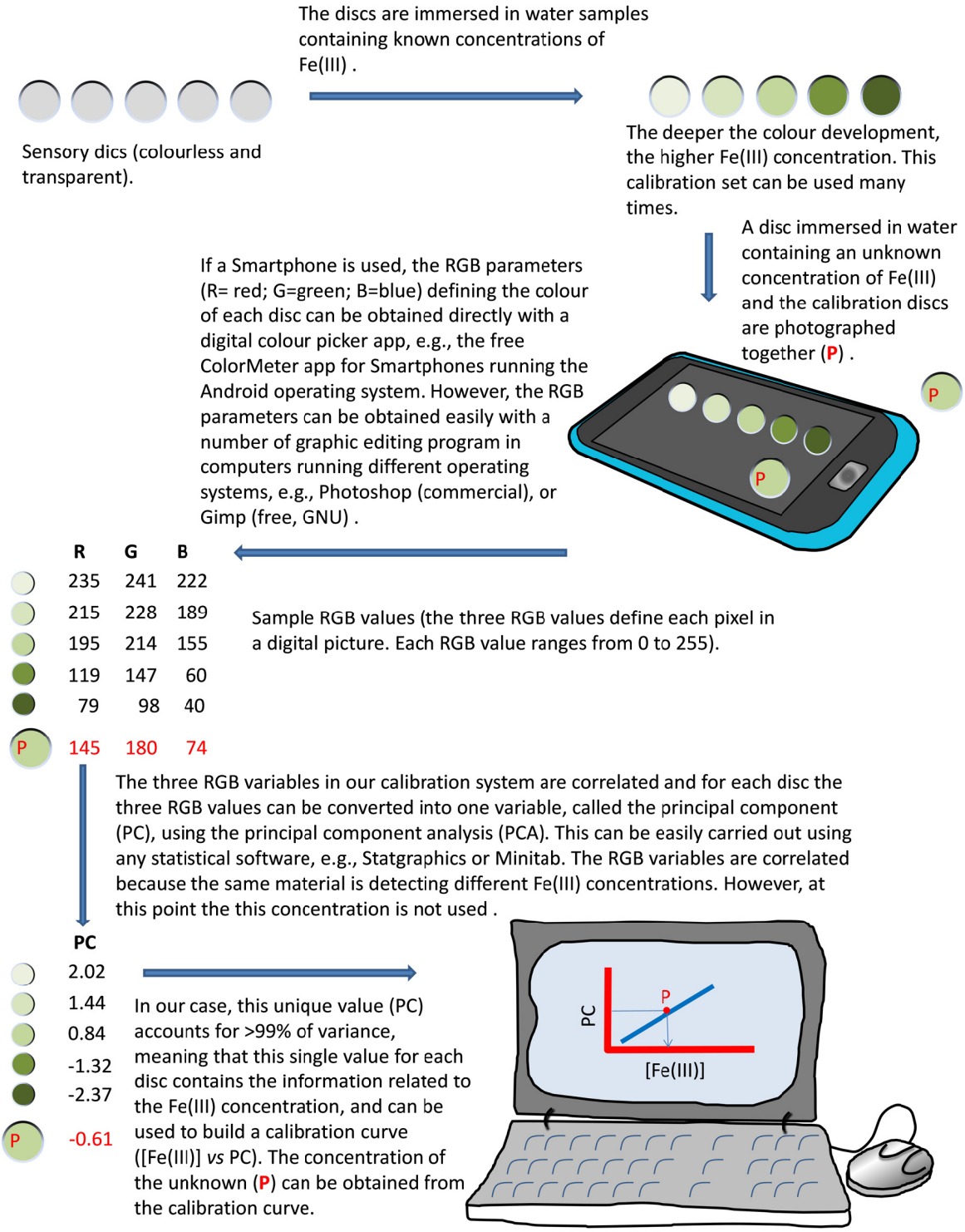
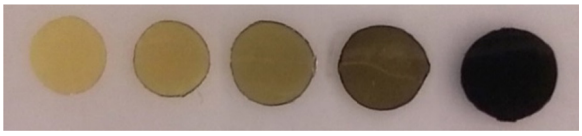


Figure S11. Schematic procedure followed to quantify Fe(III) in water media by a scalable RGB technique.

S7.- Titration of Fe(III) in water with the discs that were cut from sensory membrane Mem via the analysis of the RGB parameters for each vial within a digital picture



[Fe(III)], ppm	Log [Fe(III)]	R	G	B	PC
$5.60x10^{-2}$	-1.252	36	48	70	-2.01
$2.80x10^{-1}$	-0.553	46	57	77	-1.06
1.40	0.146	58	66	81	-0.25
5.60	0.748	73	79	87	0.88
56.00	1.748	96	96	95	2.44

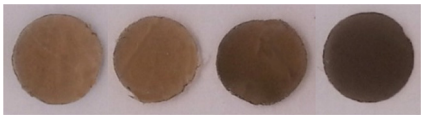
Standardized; eigenvalue = 2.99; % variance = 99.7%

Figure S12. Principal component analysis (PCA) of the RGB data from pictures of the **Mem** sensory discs that were treated with aqueous solutions (pH = 2, KCl–HCl) containing different quantities of Fe (III).

S8.- Titration of Fe(III) in wine with discs that were cut from sensory membrane Mem via the analysis of the RGB parameters for each vial within a digital picture

Brand name	Winery	Appellation of origin	Fe, ppm
Charquiño, 2011	Albariño	Rías Baixas	1.002
Albarín, 2011	Pardevalles	Tierra de León	3.436
Viura, 2009	Faustino	Rioja	1.157
Don García Blanco	JGC	Plonk, no appellation	2.320

Figure S13. White wine used for the Fe(III) measurements. The iron content was measured with flame atomic absorption spectrometry.

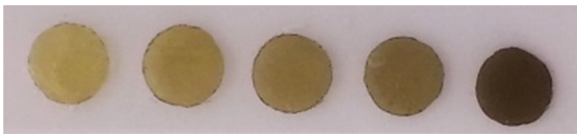


[Fe(III)], ppm	Log [Fe(III)]	R	G	B	PC
1.002	0.001	57	70	78	-1.68
3.446	0.536	78	84	87	1.89
1.157	0.063	59	71	80	-1.22
2.320	0.366	71	80	86	1.01

Standardized; eigenvalue = 2.97; % variance = 99.0%

Figure S14. Principal component analysis (PCA) of the RGB data from pictures of the **Mem** sensory discs after being immersing in white wine without further treatment. The concentration of iron in wine was determined with flame atomic absorption spectroscopy.

S9.- Titration of Fe(III) in blood serum with discs cut from sensory membrane Mem via the analysis of the RGB parameters for each vial within a digital picture



[Fe(III)], ppm	Log [Fe(III)]	R	G	B	PC
1.10	0.041	42	51	72	-1.87
1.86	0.270	49	58	76	-0.94
2.60	0.415	54	63	79	-0.27
3.32	0.521	60	68	81	0.36
10.60	1.025	79	85	91	2.72

Standardized; eigenvalue = 3.00; % variance = 99.9%

Figure S15. Principal component analysis (PCA) of the RGB data from pictures of **Mem** sensory discs that were immersed in reconstituted human blood serum without further treatment. Commercial lyophilized human sera with constituents added as required to obtain desired component levels were used (PreciControl ClinChem Multi 1 and 2, Cobas®, Roche).

Table S2. Concentration of selected components of the PreciControl ClinChem Multi 1 and 2 (Cobas®, Productos Roche).[#]

Short name/Component	MULTI 1		MULTI 2	
	Value	Unit	Value	Unit
GLUH2/Glucose	1020	ppm	2340	ppm
GLDH/Glutamate dehydrogenase	260	U/L	443	U/L
GGT/Glutamyltransferase gamma	538	U/L	2370	U/L
HDLC3/HDL-Cholesterol	288	ppm	615	ppm
HGLOB/Haptoglobin	873	ppm	1240	ppm
HBDH/Hydroxybutyrate dehydrogenase alpha	1700	U/L	3320	U/L
IGA/Immunoglobulin A	1470	ppm	2290	ppm
IGG/Immunoglobulin G	7470	ppm	11900	ppm
IGM/Immunoglobulin M	729	ppm	1090	ppm
Fe/Iron	1.1	ppm	2.45	ppm
KAPPA/KAPPA	1840	ppm	3030	ppm
LDLC2/LDL-Cholesterol	557	ppm	963	ppm
LAC/Lactate	145	ppm	308	ppm
LDH/Lactate dehydrogenase	1740	U/L	3260	U/L
LAMBD/Lambda	1020	ppm	1690	ppm
LIP/Lipase	46.5	U/L	1030	U/L
MG/Magnesium	19.8	ppm	32.1	ppm
PHOS/Phosphate (inorganic)	38.1	ppm	64.5	ppm
K/Potassium	143	ppm	251	ppm
PALB2/Prealbumin	187	ppm	285	ppm
Na/Sodium	2620	ppm	3170	ppm
TP/Total protein	48800	ppm	75400	ppm
TRSF2/Transferrin	2020	ppm	3520	ppm
TG/Triglycerides	1090	ppm	2060	ppm
UREA/Urea	417	ppm	1210	ppm
UA/Uric acid	54.6	ppm	109	Ppm

Food and Drug administration decision summary report - PreciControl ClinChem Multi 1 and 2, device description section:[3] "The PreciControl ClinChem Multi 1 and 2 consist of lyophilized human sera with constituents added as required to obtain desired component levels. Concentrations of the components in the controls have been adjusted to represent normal and pathological levels. The concentrations of the components in the controls are lot-specific.
All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were FDA-approved".

[3] FDA Decision on novel multi-analyte control device, document downloaded from FDA web page http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pmn&id=K102016, document link: http://www.accessdata.fda.gov/cdrh_docs/reviews/K102016.pdf. Date prepared: July 16, 2010.