

Improvement of electrocatalytic effect in voltammetric sensors based on phthalocyanines

María L. Rodríguez-Méndez^{*a}, Cristina Medina-Plaza^a, Celia García-Hernández^a,
Silvia Rodríguez^a, Cristina García-Cabezón^b, David Paniagua^a,
Miguel A. Rodríguez-Pérez^c and José A. de Saja^c

^a Department of Inorganic Chemistry, Industrial Engineers School, Universidad de Valladolid, 47011 Valladolid, Spain

^b Department of Materials Science, Industrial Engineers School, Universidad de Valladolid, 47011 Valladolid, Spain

^c Department of Condensed Matter Physics, Faculty of Sciences, Universidad de Valladolid, 47011 Valladolid, Spain

Dedicated to Professor Kevin M. Smith on the occasion of his 70th birthday

Received 30 November 2015

Accepted 12 January 2016

ABSTRACT: Voltammetric sensors based on phthalocyanines have been used to detect a variety of compounds. In this paper, the state of the art of sensors prepared using classical techniques will be revised. Then, new strategies to improve the performance of the sensors will be described using as example sensors chemically modified with lutetium bisphthalocyanine (LuPc₂) dedicated to the detection of phenols of interest in the food industry. Classical LuPc₂ carbon paste electrodes can detect phenols such as catechol, caffeic acid or pyrogallol with limits of detection in the range of 10⁻⁴–10⁻⁵ M. The performance can be improved by using nanostructured Langmuir–Blodgett (LB) or Layer by Layer (LbL) films. The enhanced surface to volume ratio produce an increase in the sensitivity of the sensors. Limits of detection of 10⁻⁵–10⁻⁷ M are attained, which are one order of magnitude lower than those obtained using conventional carbon paste electrodes. Moreover, these techniques can be used to co-immobilize two electrocatalytic materials in the same device. The limits of detection obtained in LB sensors combining LuPc₂/AuNPs or LuPc₂/CNT are further improved. Finally, the LB technique has been used to prepare biosensors where a phenol oxydase (such as tyrosinase or lacasse) is immobilized in a biomimetic environment that preserves the enzymatic activity. Moreover, LuPc₂ can be co-immobilized with the enzyme in a lipidic film formed by arachidic acid (AA). LuPc₂ can act as an electron mediator facilitating the electron transfer. These biomimetic sensors formed by LuPc₂/AA/enzyme show Limits of detection of 10⁻⁸ M and an enhanced selectivity.

KEYWORDS: voltammetric sensor, phthalocyanine, bisphthalocyanine, phenol, nanoparticle, biosensor.

INTRODUCTION

Phthalocyanines have been extensively used as sensing materials in electrochemical sensors due to their remarkable electrochemical and electrocatalytic properties [1–5]. Electrochemical sensors chemically modified with phthalocyanines can work under different principles including potentiometry, amperometry, voltammetry or

impedance measurements and have been used for the determination of many organic and inorganic compounds [6, 7]. In amperometric or voltammetric sensors, the electrode surface is covered with a sensing material that facilitates the charge-transfer reactions between the electrode and the solution. The sensing layer can thus reduce the oxidation potential of the analyte while increasing the intensity of the response. Usually in amperometric sensors, measurements of the intensity are carried out at a fixed potential. Which corresponds with the value at which the analyte is oxidized. Voltammetric sensors are superior

*Correspondence to: María L. Rodríguez-Méndez, email: mluz@eii.uva.es, tel: +34 983-423-540, fax: +34 983-423-310

1 because the whole curve contains information about the
2 analyte and can be used to identify it. For this reason,
3 voltammetric sensors based on phthalocyanines have been
4 successfully used to detect a variety of compounds such
5 as phenols, amines or organic acids among many others.

6 Voltammetric sensors based on phthalocyanines can
7 be prepared using a range of classical techniques. They
8 can be deposited on the surfaces by physical adsorption,
9 casting, sping coating or electropolymerization [8, 9].
10 Phthalocyanines can also be incorporated into an
11 electrode matrix (*i.e.* carbon paste electrodes or screen
12 printing inks), entrapped in a polymeric or organic matrix
13 or attached covalently to a surface [10–12]. Sol–gel
14 technique is a valuable tool to disperse phthalocyanines
15 in a carbon matrix [13].

16 In the last years new developments in the field of
17 nanotechnology have helped to improve the sensitivity
18 of the electrochemical sensors [14]. Nanotechnology
19 provides new nanomaterials (nanoparticles, nanocarbons,
20 *etc.*) with improved electrocatalytic properties. Further-
21 more, traditional sensing materials such as phthalocy-
22 anines can be deposited as nanostructured films using
23 self-assembling [15], or by the Layer by Layer (LbL)
24 [16] or the Langmuir–Blodgett (LB) techniques [17–19].

25 In this paper, recent works in classical amperometric
26 and voltammetric sensors based on phthalocyanines will
27 be revised. Then, using a specific phthalocyanine, the
28 lutetium bisphthalocyanine (LuPc₂), examples will be
29 given to illustrate how nanostructured films can improve
30 the performance of voltammetric sensors.

31 32 33 **CLASSICAL ELECTROCATALYTIC** 34 **ELECTRODES BASED ON** 35 **PHTHALOCYANINES** 36

37 Electrodes modified with phthalocyanines have the
38 ability to catalyze the oxidation or reduction of solved
39 compounds by lowering the potential required for the
40 catalyzed redox systems, when compared with the
41 unmodified electrode. The number of papers published
42 in this field is quite large and include a variety of
43 phthalocyanine compounds, electrode designs, and target
44 molecules. From the large class of the phthalocyanine
45 molecules, metallophthalocyanines (MPc), where a
46 transition metal is coordinated with a phthalocyanines
47 ring have been the most widely studied derivatives. In
48 MPcs, the electrocatalytic process occurs through the
49 oxidation/reduction of the central metal ion. Cobalt
50 phthalocyanine (CoPc) and its derivatives are excellent
51 electrocatalytic materials due to their high reactivity
52 which, depending on the target, involves the couples
53 Co^{III}/Co^IPc²⁻ or Co^{II}/Co^IPc²⁻ [20]. Other MPcs such as
54 NiPc or FePc also present a good electrocatalytic activity
55 owing to the MII/MIII redox process [2].

56 Sandwich-type derivatives (LnPc₂) in which two Pc
57 rings are coordinated with a rare earth ion (Ln^{III}), have

1 attracted considerable interest as voltammetric sensors
2 [1, 5, 20]. The electrocatalytic effect shown by LnPc₂, is
3 not related to the oxidation/reduction of the central metal
4 ion, but to the oxidation/reduction of the phthalocyanine
5 ring [18, 21–23]. The catalytic activity of MPcs or LnPc₂
6 can be “tuned” by manipulating the E° formal potential,
7 using proper substituent groups on the macrocyclic
8 ligand. The chemical modification can also be used to
9 provide other interesting properties such as solubility in a
10 certain solvent, chemical or thermal stability, required to
11 prepare electrodes using a particular technique.

12 Sensors chemically modified with phthalocyanines
13 have been dedicated to the electrocatalytic determination
14 of many important compounds such as nitrite and cyanide,
15 hydrogen peroxide, nitric oxide (biological medium),
16 hydrazine, thiols and sulphurated organic compounds,
17 glucose and polysaccharides, aliphatic amines, ascorbic
18 acid, glutathione, phenols or serotonin. The applications
19 mentioned are only an illustration of the wide variety of
20 targets evaluated instead of an exhaustive list (see Ref. 2
21 for more details). Some examples are described in the next
22 paragraphs.

23 In classical works phthalocyanines are adsorbed
24 directly to the electrode surface [24]. For instance, a
25 graphite electrode modified with CoPc has been used
26 to detect thiols and disulfides [24] or lipoic acid [25].
27 Carbon Paste Electrodes (CPEs) prepared by mixing
28 graphite with a phthalocyanine and a mineral oil or an
29 epoxy resin have been successfully used to detect phenols
30 [26] amitrol [27] nitrites [28] or citric acid [29] among
31 many others. CPEs modified with phthalocyanines have
32 been used as sensing units in electronic tongues [30].
33 Drop casting on a bare graphite electrode or spin coating
34 are also popular techniques [31]. Electropolymerized
35 metal phthalocyanines possess excellent electrocatalytic
36 properties towards H₂O₂, thiols, S²⁻ or NO among many
37 other targets [32, 33]. MPcs can be encapsulated during
38 the surface preparation using the sol–gel method. A gluta-
39 thione sensor based on CoPc has been developed using
40 this method [32]. CoPc-modified screen-printed carbon
41 electrodes have been investigated as disposable sensors
42 for the measurement of many compounds such as nitric
43 acid [34], phenols [12] or TNT [35] and also as the
44 sensing units in electronic tongues [36].

45 46 47 **IMPROVEMENT OF THE** 48 **PERFORMANCE THROUGH THE** 49 **USE OF NANOMATERIALS AND** 50 **NANOSTRUCTURED FILMS** 51

52 Electrodes with improved characteristics have been
53 obtained by mixing phthalocyanines with nanomaterials
54 such as nanoparticles, nanotubes or graphene. Functionalization of nanomaterials with phthalocyanines
55 has also been widely used to prepare electrochemical
56 sensors. The number of papers in this field is quite
57

1 large and only some examples will be shown in next
2 paragraphs.

3 It has been demonstrated that the combination of
4 MPcs with gold nanoparticles (AuNPs) can improve
5 the catalytic properties of the electrodes [37–40].
6 Similarly, electrochemical sensors have been developed
7 by combining phthalocyanines with carbon nanotubes
8 on carbon electrodes [41–44]. CoPc has been dispersed
9 on graphene to detect glucose and hydrogen peroxide
10 [45]. Electrocatalytic behavior has also been found
11 in tetraamino-phthalocyanine in the presence of a
12 composite formed by nanotubes and reduced graphene
13 [46]. In a more sophisticated method, pre-grafted
14 screen-printed gold electrodes have been used to attach
15 functionalized single-walled carbon nanotubes and
16 metal tetra-amino phthalocyanines using Schiff-base
17 reactions [47]. A hybrid nanocomposite based on CoPc
18 immobilized on nitrogen-doped graphene has been used
19 to modify glassy carbon electrode and used to detect
20 thiols with improved sensitivity [48]. Nanocomposites
21 of graphene, tetrasulphonic phthalocyanine and
22 polyaniline have been used to modify screen printed
23 electrodes (SPE) for selective determination of ascorbic
24 acid [49]. By comparing the electrocatalytic effect
25 of conjugates formed by phthalocyanines–carbon
26 nanotubes or phthalocyanines–gold nanoparticles it has
27 been demonstrated that single walled carbon nanotube
28 complexes show better performances towards bisphenol
29 A than gold nanoparticle conjugates [50]. The sol–gel
30 technique or electrodeposition techniques can produce
31 mesoporous materials with an increased surface-to-
32 volume ratio that have been successfully used to detect
33 a variety of substances such as oxalic acid or phenols.
34 [9, 51, 52]. A soluble sulfonic phthalocyanine has been
35 immobilized in a mesoporous silica xerogel and used to
36 detect dopamine [53].

37 The organization of the structure at the nanometric
38 level causes the film properties to differ from those
39 obtained with the same materials in the form of thick films
40 [54, 55]. Self-assembled monolayers (SAM) of thiolic
41 or carboxylic phthalocyanine derivatives deposited onto
42 gold have demonstrated an enhanced catalytic activity
43 with very fast responses towards thiols [56] and nitrite
44 [57]. LbL sensors have been prepared by alternating
45 anionic phthalocyanines with a variety of polycations
46 [58]. For instance, electrochemical sensors made
47 from layered double hydroxide nanosheets and cobalt
48 phthalocyanines have been successfully used to detect
49 dopamine [59]. LbL electrodes alternating layers of
50 polyaniline (PANI) and tetrasulfonated phthalocyanines,
51 are also able to detect dopamine efficiently. Furthermore,
52 they can distinguish between dopamine and ascorbic
53 acid, a natural interfering molecule usually present in
54 biological samples [60]. Similarly, LbL films constructed
55 from layers of polyallilamine hydrochloride (PAH) and
56 iron phthalocyanine have been able to detect dopamine
57 with a detection limit of 10^{-6} M [61]. LB films of many

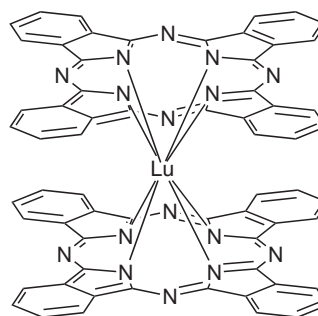
1 different phthalocyanines have been used to obtain
2 electrochemical sensors for the detection of many
3 compounds including amines, phenols, pesticides or
4 organic acids among others [17, 23, 62–65].

5 SAM, LbL and LB techniques are interesting methods
6 for fabrication of films combining phthalocyanines
7 with nanomaterials with electrocatalytic properties.
8 LbL films have been used to obtain mixed films of gold
9 nanoparticles and nickel phthalocyanine that show an
10 increased electrocatalytic activity towards hydrogen
11 peroxide [66]. Similarly, the introduction of silver
12 nanoparticles (AgNPs) in PAH/iron phthalocyanine LbL
13 films show increased sensitivity towards dopamine [61].
14 Nanocomposites formed by tetrasulfonic phthalocyanine
15 and functionalized graphene synthesized by electrostatic
16 self-assembly method, have also been used as sensors
17 to detect bisphenol A [67]. LB films combining
18 phthalocyanines and NPs have been rarely studied as
19 gas sensors [68] and only one attempt has been made to
20 analyze the role of the interactions of phthalocyanines
21 and AuNPs in electrochemical sensors [69].

22 CASE STUDY: ELECTROCHEMICAL 23 SENSORS BASED ON 24 BISPHTHALOCYANINES FOR THE 25 DETECTION OF PHENOLS WITH 26 IMPROVED PERFORMANCE 27 28 29

30 In previous sections, the state of the art of
31 amperometric and voltammetric sensors based on
32 phthalocyanines has been revised. In the present section,
33 the particular case of voltammetric sensors based on
34 lutetium bisphthalocyanine (LuPc_2) will be used to show
35 three strategies followed by our group to improve the
36 electrocatalytic properties towards phenols.

37 LuPc_2 are double decker derivatives in which two
38 Pc rings are coordinated with a rare earth ion (Fig. 1)
39 [5, 6, 70, 71]. In the range from -1.0 V to +1.0 V they
40 show two reversible redox pairs associated to the one
41 electron oxidation and the one electron reduction of
42 the phthalocyanine ring respectively [1]. A variety of
43 LnPc_2 have been investigated as the sensing material for
44 voltammetric electrodes [18, 19, 72].



51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

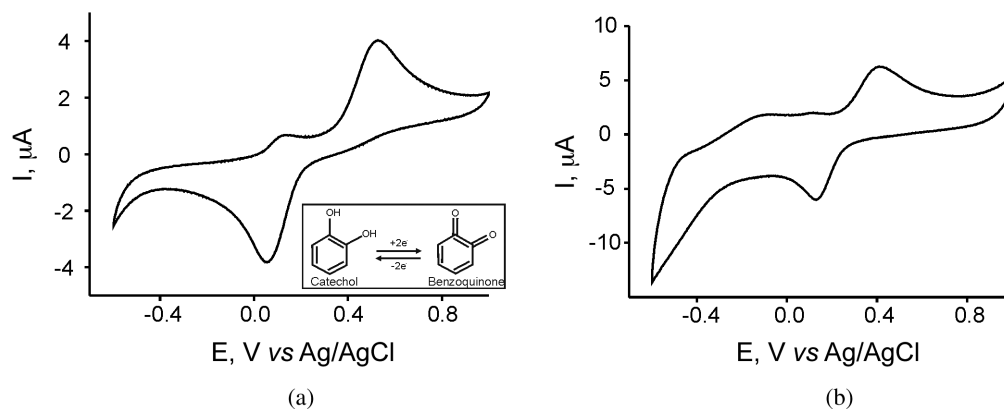


Fig. 2. Voltammetric response of (a) a bare CPE electrode and (b) a CPE modified with LuPc₂ towards 10⁻³ M catechol in 0.1 M KCL. Scan rate 100 mV/s

The lutetium derivative, LuPc₂ has been considered as the model to illustrate the electrochemical and sensing behavior of lanthanide bisphthalocyanines and has been the subject of many studies. LuPc₂ can be used to prepare cheap CPEs, in which the phthalocyanine is mixed (15% w/w) with graphite powder (Ultracarbon, Ultra F purity). Nujol oil is added until a paste is obtained. Paste is packed into the body of a 1 mL plastic syringe and compressed. A metallic wire is used as the contact [10, 73]. LuPc₂ has demonstrated to be an efficient electrocatalysts toward phenols. This is illustrated in Fig. 2, where the responses of a bare CPE electrode and an electrode modified with LuPc₂ immersed in 10⁻³ M catechol is shown. The curves show a redox pair caused by the reversible oxidation of the diphenol to the quinoid form. The presence of LuPc₂ not only increases the intensity of the response, but also decreases the separation between the anodic and the cathodic waves indicating that the reversibility of the process is facilitated. The intensity of the peaks is proportional to the concentration and a limit of detection (LOD) of 10⁻⁴ M is easily attained. LODs in the range of 10⁻⁴–10⁻⁵ are attained for other phenols such as pyrogallol or hydroquinone [18, 23].

As shown in the above paragraph LuPc₂ is an excellent electrocatalytic material for the detection of phenols. Nanotechnology has helped to develop new strategies to improve the performance of the LuPc₂ sensors. Three main strategies have been followed to improve their performance.

Strategy 1: Nanostructured sensors

Ultrathin nanostructured thin films can improve the sensitivity of chemical sensors due to several reasons. Ultra-thin films show high surface uniformity and an enhanced number of active sites that produce an increase in the intensity, and lower the detection limits.

Nanostructured LB films based on lanthanide bisphthalocyanines have been frequently used as voltammetric

sensors to analyze phenols. LB films are formed by spreading a chloroform solution of a mixture of LuPc₂ and an amphiphilic substance (1:10) onto the water surface of a Langmuir trough. The amphiphile is required to decrease the rigidity of the LB films and to facilitate the formation of the monolayer. It can be a fatty acid such as arachidic acid (AA) [74, 75] or a phospholipid such as DPPG acid (or dimyristoyl phosphatidic phospholipid acid) [62]. Upon compression, the molecules are oriented at the interface giving rise to an ordered monolayer. This floating monolayer can be transferred to a solid substrate by dipping an ITO substrate perpendicularly to the water sub-phase. Repeated dippings allow multilayers to be obtained where the thickness can be controlled by the number of dipping cycles [6]. Typically 10–20 monolayer LB films are used as voltammetric sensors.

The response of LuPc₂–LB electrodes is similar to that observed in CPEs, but LB films facilitate the electron transfer, the peaks are better resolved, and the repeatability is improved. The electrochemical studies also indicate that nanostructured films show faster kinetics because ultra-thin films with enhanced surface to volume ratio, can allow the analyte molecules to adsorb or desorb from the molecular sites more readily. LODs calculated for mono- di- and tri-phenols are one order of magnitude lower than those found in CPE sensors and can easily reach 10⁻⁵–10⁻⁶ M and 10⁻⁷ M has been reached in some cases [62]. The LbL technique has also demonstrated to be a good alternative for the preparation of LuPc₂ sensors. In the LbL technique multilayers are assembled by immersing the substrate in a cationic and an anionic aqueous solution alternatively. Thus, layers of different charges are deposited thanks to electrostatic interactions. A method has been developed to use the LbL technique to deposit alternate layers of a water soluble polycation, the poly(allylamine hydrochloride) (PAH) and a chloroform solution of LuPc₂. Such LbL films have been used to detect catechol with excellent limit of detection of 10⁻⁶ M [61] (Fig. 3).

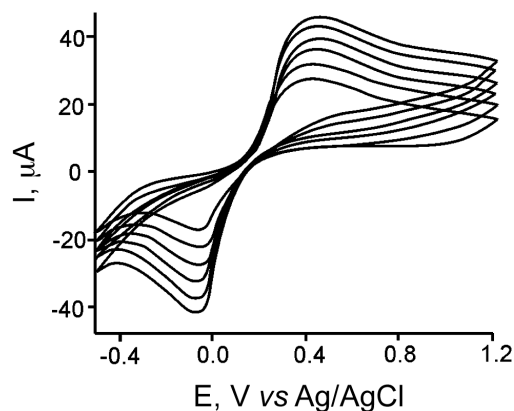


Fig. 3. Cyclic voltammograms recorded using a PAH/LuPc₂ LbL film immersed in catechol at concentrations ranging from 50 to 500 μM

Strategy 2: Nanostructured films combining electrocatalytic materials: synergy

The control of the molecular architectures afforded by the LB and LbL methods has led to a variety of devices where synergy is achieved by combining electroactive materials, including LuPc₂-inorganic hybrids. Combinations of electrocatalytic materials can improve the performance of the sensors. For instance, LuPc₂ has been combined with functionalized gold nanoparticles (11-mercaptoundecyl)tetra(ethylene glycol) (SAuNP) using a new method developed specially for this goal. In the method developed, a chloroform solution of AA and LuPc₂ (1:1) is spread onto the water subphase of an LB trough. AuNPs are injected underneath a floating film formed by the fatty acid (AA) and the LuPc₂. Then, the floating monolayer is compressed and transferred to ITO glass [69].

Voltammograms registered when LuPc₂/DODAB/SAuNPs (Fig. 4) are immersed in hydroquinone show more intense peaks than those obtained in LuPc₂/DODAB. LOD calculated from the slope of the calibration curve are one order of magnitude lower than the obtained with LuPc₂/DODAB. According to this, the interaction between phthalocyanines and gold nanoparticles facilitated by the LB technique induces a synergistic electrocatalytic effect towards phenols [69].

Similar electrocatalytic effect has been observed in LB films of LuPc₂ combined with carbon nanotubes. Such films show highly sensitive responses towards phenols or citric acid. In the latest case, the synergy is promoted by the π - π interactions between both electrocatalytic materials [76].

Strategy 3: Nanostructured biosensors

It is well-known that the incorporation of enzymes to build electrochemical biosensors can help to improve the selectivity of the sensing devices. During the reaction enzymes consume oxygen (*e.g.* the oxydases), or produce

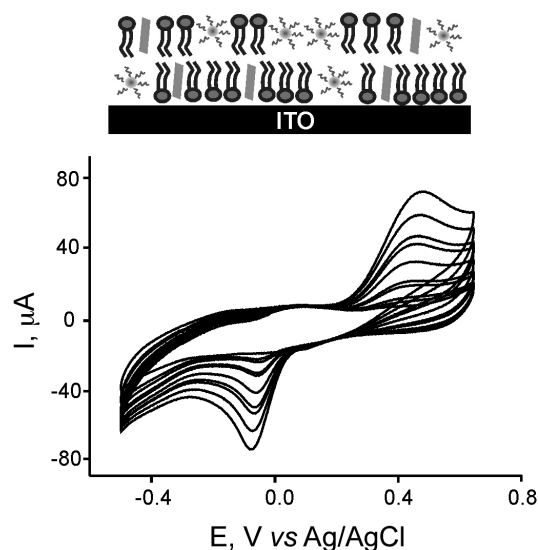


Fig. 4. Voltammetric Response of an LB film of LuPc₂/DODAB/SAuNP toward hydroquinone (10⁻⁴ to 10⁻⁶ M in Phosphate buffer 10⁻¹ M)

hydrogen peroxide or produce the reduced form of the NAD(P)H (*e.g.* dehydrogenases). These transformations can be measured by electrochemical methods. Sensors containing phenol oxidases such as tyrosinase, laccase or horseradish peroxidase can be used to detect phenols [77].

The immobilization of the enzyme at the electrode substrate is a key step in the development of biosensors with high enzymatic activity [78]. Enzymes can be immobilized using different methods and different electrode supports [79–85]. The incorporation of electron mediators facilitates the electron transfer between the enzyme and the electrode, improving the sensitivity of the electrochemical biosensors. Phthalocyanines are excellent electron mediators for the oxidase reactions [86, 87].

LB, LbL and SAM are of special interest for enzyme immobilization because they allow preparing biocompatible systems, where the enzyme is adsorbed in a lipidic layer mimicking the cellular membrane. This biomimetic environment can increase the enzymatic activity. With these techniques, film fabrication is performed under mild conditions, which is particularly important for preserving activity of biomolecules a wide diversity of materials may be employed [88, 89]. The LB technique can also be advantageous because the electron mediator can be co-immobilized with the enzyme in the same lipidic layer, facilitating the electron transfer process (Fig. 5). In particular, our group has demonstrated that tyrosinase or laccase co-immobilized with LuPc₂ in lipidic LB films show an excellent electrocatalytic and electron mediator behavior [90, 91]. The excellent functionality of the enzyme obtained using a biomimetic immobilisation method, the selectivity afforded by enzyme catalysis, the signal enhancement caused by the lutetium bisphthalocyanine mediator

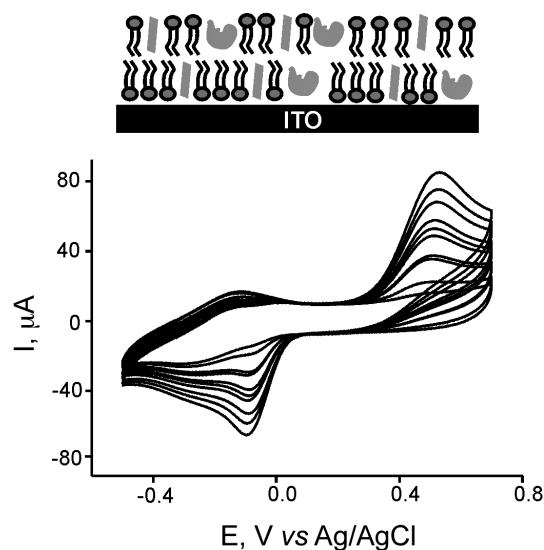


Fig. 5. Voltammetric response of an LB film of LuPc₂/AA/Laccase toward hydroquinone (10^{-4} to 10^{-6} M in Phosphate buffer 10^{-1} M)

and the increased selectivity of the curves due to the occurrence of two redox processes make these sensors exceptionally suitable for the detection of phenolic compounds [92]. Detection limits attained using these biomimetic biosensors depend on the type of phenol (mono-di- or tri-phenol) due to the different affinity of the enzymes to different compounds. Detection limits as low as 10^{-8} M have been attained [93, 94].

Biosensors based on LB films have been combined to form an array of sensors. Such an array combined with an appropriate pattern recognition software has been the basis of a bioelectronic tongue [95]. Such system combines the characteristics of electronic tongues (which provide global information about the composition of the sample) with the specificity of biosensors. These systems have been successfully used to discriminate grape juices according to their phenolic content [94].

CONCLUSION

Voltammetric sensors based on phthalocyanines have been widely applied for the analysis of a variety of compounds. Lutetium bisphthalocyanine is of particular interest due to their excellent electrochemical properties. This compound has been used to illustrate several strategies to improve the performance towards phenols.

Classical LuPc₂ CPE sensors prepared by mixing LuPc₂ with graphite, showed limits of detection in the range of 10^{-4} – 10^{-5} M. Their performance can be improved by preparing the sensing layers using the Langmuir–Blodgett or the Layer by Layer techniques which provide nanostructured surfaces with an improved surface to volume ratio. These techniques are also suitable to obtain mixed films formed by two electrocatalytic materials. Improvement of the performance of voltammetric

sensors by means of synergy between phthalocyanines and metallic nanoparticles or carbon nanotubes has been achieved.

Finally, the LB and LbL techniques are of particular interest for enzyme immobilization because they allow preparing biomimetic systems, where tyrosinase (or laccase) are adsorbed in a lipidic layer. When LuPc₂ is introduced simultaneously in the sensing film, it can act as electron mediator facilitating the electron transfer between the redox enzyme and the electrode. Using this biomimetic assembly, LODs of 10^{-8} M can be easily attained.

Acknowledgements

Financial support by the MINECO (grant CICYT-AGL2012-33535) and Junta Castilla y León (VA-032U13) is gratefully acknowledged. CMP and CGH also thanks the University of Valladolid and the JCyL for their FPI grant.

REFERENCES

- Rodríguez-Méndez ML, Gay M and de Saja JA. *J. Porphyrins Phthalocyanines* 2009; **13**: 1159–1167.
- Zagal JH, Griveau S, Silva JF, Nyokong T and Bedioui F. *Coord. Chem. Rev.* 2010; **254**: 2755–2791.
- The Porphyrin Handbook*, Vols. 1–20, Kadish K, Smith KM and Guillard R. (Eds.) Academic Press: New York, 2003.
- Lever ABP, Milaeva ER and Speier G. *Phthalocyanines: Properties and Applications, The redox chemistry of metallophthalocyanines in solution*, Vol. 3, VCH Publishers Inc.: New York, 1993.
- Jiang J. *Functional Phthalocyanine Molecular Materials, Structure and Bonding* (1st ed), Vol. 135, Springer: Heidelberg, 2010.
- Rodríguez-Méndez ML, Medina-Plaza C, de Saja JA, Apetrei C and Muñoz R. In *Sensor arrays based on phthalocyanines: New developments on nanostructured and biomimetic electrochemical sensors. Multisensor systems for chemical analysis: Materials and Sensors*, Lvova L, Kirsanov D, Di Natale C and Legin A. (Eds.) Pan Stanford Publishing: Singapore, 2012; pp. 70–109.
- Vlasov YG, Ermolenko YE, Legin AV, Rudnitskaya AM and Kolodnikov V. *J. Anal. Chem.* 2010; **65**: 880–890.
- Rella R, Spadavecchia J, Ciccarella G, Siciliano P, Vasapollo G and Valli L. *Sens. Actuators, B* 2003; **89**: 86–91.
- Gay M, Rodríguez-Méndez ML and de Saja JA. *Langmuir* 2010; **26**: 19217–19224.
- Arrieta A, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 2003; **95**: 357–365.

- 1 11. Apetrei C, Apetrei IM, Nevares I, Del Alamo M, Parra V, Rodriguez-Mendez ML and de Saja JA. *Electrochim. Acta* 2007; **52**: 2588–2594.
- 2
- 3
- 4 12. Matemadombo F, Apetrei C, Nyokong T, Rodriguez-Mendez ML and de Saja JA. *Sens. Actuators, B* 2012; **166**: 457–466.
- 5
- 6
- 7 13. Wang J, Pamidi PVA, Parrado C, Park DS and Pingarron J. *Electroanal.* 1997; **9**: 908–911.
- 8
- 9 14. *Sensors Based on Nanostructured Materials*, Arregui FJ (Ed.) Springer: Heidelberg, 2009.
- 10
- 11 15. Yang ZY, Gan LH, Lei SB, Wan LJ, Wang C and Jiang JZ. *Phys. Chem. Chem. Phys.* 2005; **109**: 19859–19865.
- 12
- 13
- 14 16. Santos AC, Zucolotto V, Constantino CJL, Cunha HN, Dos Santos JR Jr and Eiras C. *J. Solid State Electrochem.* 2007; **11**: 1505–1510.
- 15
- 16
- 17 17. Valli L. *Adv. Colloid Interface Sci.* 2005; **116**: 13–44.
- 18
- 19 18. Casilli S, De Luca M, Apetrei C, Parra V, Arrieta AA, Valli L, Jiang J, Rodriguez-Mendez ML and de Saja JA. *Appl. Surf. Sci.* 2005; **246**: 304–312.
- 20
- 21 19. Gorbunova Y, Rodriguez-Mendez ML, Tomilova L and de Saja JA. *Chem. Mater.* 1995; **7**: 1443–1448.
- 22
- 23 20. Kozub BR and Compton RG. *Sens. Actuators, B* 2010; **147**: 350–358.
- 24
- 25 21. Weiss R and Fischer J. In *Lanthanide phthalocyanine complexes. The Porphyrin Handbook*, Kadish KM, Smith KM and Guillard R. (Eds.) Academic Press: New York, 2003; pp. 171–246.
- 26
- 27 22. Yilmaz I, Nakanishi T, Gurek A and Kadish KM. *J. Porphyrins Phthalocyanines* 2003; **7**: 227–238.
- 28
- 29 23. Apetrei C, Casilli S, de Luca M, Valli L, Jiang J, Rodríguez-Méndez ML and de Saja JA. *Colloids Surf., A*. 2006; **284**: 574–582.
- 30
- 31 24. Zagal JH and Henriquez JJH. *Bol. Soc. Chil. Quím.* 2000; **45**: 237–242.
- 32
- 33
- 34 25. Ferreira APM, dos Santos Pereira LN, Santos da Silva I, Tanaka SMCN, Tanaka AA and Angnes L. *Electroanal.* 2014; **26**: 2138–2144.
- 35
- 36 26. Apetrei C, Apetrei I, de Saja JA and Rodríguez-Méndez ML. *Sensors*. 2011; **11**: 1328–1344.
- 37
- 38 27. Siswana M, Ozoemena KI and Nyokong T. *Talanta*. 2006; **69**: 1136–1142.
- 39
- 40 28. Parsaei M, Asadi Z and Khodadoust S. *Sens. Actuators, B* 2015; **220**: 1131–1138.
- 41
- 42 29. Apetrei C, Medina C, de Saja JA and Rodríguez-Méndez ML. *J. Porphyrins Phthalocyanines* 2013; **17**: 522–528.
- 43
- 44 30. Rodríguez-Méndez ML, Parra V, Apetrei C, Gay M, Prieto N and de Saja JA. *Microchim Acta*. 2008; **163**: 23–31.
- 45
- 46 31. Devasenathipathy R, Palanisamy S, Chen SM, Karuppiyah C, Mani V, Ramaraj SK, Ajmal-Ali M and Al-Hemaid FMA. *Electroanal.* 2015; **27**: 1403–1410.
- 47
- 48 32. Wem ZH and Kang TF. *Talanta*. 2004; **62**: 351–355.
- 49
- 50 33. Pereira-Rodrigues N, Albin V, Koudelka-Help V, Auger V, Pailleret A and Bedoui F. *Electrochem. Commun.* 2002; **4**: 922–927.
- 51
- 52 34. Guro Y and Guadalupe AR. *Sens. Actuators, B*. 1998; **46**: 213–219.
- 53
- 54 35. Caygill JS, Collyer SD, Holmes JL, Davis F and Higson SPJ. *Electroanal.* 2013; **25**: 2445–2452.
- 55
- 56 36. Blanco C, de la Fuente R, Caballero I and Rodríguez-Méndez ML. *J. Food Eng.* 2015; **157**: 57–62.
- 57
37. Chauke VP, Chidawanyika W and Nyokong T. *Electroanal.* 2011; **23**: 487–496.
38. Saeed AA, Singh B, Abbas MN, Issa YM and Dempsey E. *Electroanal.* 2015; **27**: 1086–1096.
39. Nada FA, Galal A, Abdel-Gawad FM and Mohamed EF. *Electroanal.* 2015; **27**: 415–428.
40. Chelladurai K, Devasenathipathy R, Chen S, Arulraj D, Palanisamy S, Mani V and Vasantha VS. *Electroanal.* 2015; **27**: 485–493.
41. Zagal JH, Griveau S, Ozoemena KI, Nyokong T and Bedioui F. *J. Nanosci. Nanotechnol.* 2009; **9**: 2201–2214.
42. Gooding JJ. *Electrochim. Acta.* 2005; **50**: 3049–3060.
43. Shi M, Chen ZM, Guo LX, Liang XH, Zhang JL, He CY, Wang B and Wu YQ. *J. Mat. Chem.* 2014; **2**: 4876–4882.
44. Zagal JH, Griveau S, Ozoemena KI, Nyokong T and Bedioui F. *J. Nanosci. Nanotechnol.* 2009; **9**: 2201–2214.
45. Wang H, Bu Y, Dai W, Li K, Wang H and Zu X. *Sens. Actuators, B* 2015; **216**: 298–306.
46. Nyoni S and Nyokong T. *Electrochim. Acta* 2014; **136**: 240–249.
47. Mashazi P, Mugadza T, Sosibo N, Mdluli P, Vilakazi S and Nyokong T. *Talanta* 2011; **85**: 2202–2211.
48. Xu HY, Xiao JJ, Liu BH, Griveau S and Bedioui F. *Biosens. Bioelectron.* 2015; **66**: 438–444.
49. Pakapongpan S, Mensing JP, Phokharatkul D, Lomas T and Tuantranont A. *Electrochim. Acta* 2014; **133**: 294–301.
50. Chauke VP, Antunes E and Nyokong T. *J. Electroanal. Chem.* 2001; **661**: 1–7.
51. Rahim A, Barros SBA, Arenas LT and Gushikem Y. *Electrochim. Acta* 2011; **56**: 1256–1261.
52. Wang K, Dai LN, Liu Q, Li HN, Ju C, Wu J and Li HM. *Analyst* 2011; **136**: 4344–4349.
53. Deon M, Muncinelli Caldas E, de Santos da Rosa D, Weber de Menezes E, Pereira Dias SL, Barbalho Pereira M, Haas Costa TM, Ticona Arenas L and Valmir Benvenuto E. *J. Solid State Electrochem.* 2015; **19**: 2095–2105.
54. Ferreira M, Zucolotto V, Oliveira ON Jr and Wohnrath K. *Encyclopedia of nanoscience and nanotechnology* 4, American Scientific Publishers: Los Angeles, 2003; pp. 441–460.
55. Foster CW, Pillay J, Metters JP and Banks CE. *Sensors* 2014; **14**: 21905–21922.
56. Ponce I, Silva JF, Oñate R, Rezende MC, Paez MA, Pavez J and Zagal JH. *Electrochem. Commun.* 2011; **13**: 1182–1186.
- 57

- 1 57. Matemadombo F, Durmus M, Togo C, Limson
2 J and Nyokong T. *Electrochim. Acta* 2009; **54**:
3 5557–5565.
- 4 58. Crespilho FN, Zucolotto V, Oliveira ON Jr and Nart
5 FC. *Int. J. Electrochem. Sci.* 2006; **1**: 194–214.
- 6 59. Han JB, Xu XY, Rao XY, Wei M, Evans DG and
7 Duan XJ. *J. Mater. Chem.* 2011; **21**: 2126–2130.
- 8 60. Zucolotto V, Ferreira M, Cordeiro MR, Constantino
9 CJL, Moreira WC and Oliveira ON Jr. *Sens.*
10 *Actuators, B* 2006; **113**: 809–815.
- 11 61. Alessio P, Rodríguez-Méndez ML, de Saja JA and
12 Constantino CJL. *Phys. Chem. Chem. Phys.* 2010;
13 **12**: 3972–3983.
- 14 62. Alessio P, Pavinatto F, Oliveira O, de Saja JA,
15 Constantino JC and Rodríguez-Méndez ML.
16 *Analyst* 2010; **135**: 2591–2599.
- 17 63. Arrieta A, Rodríguez-Méndez ML and de Saja JA.
18 *Sens. Actuators, B* 2003; **95**: 357–365.
- 19 64. Alessio P, Apetrei C, Rubira RJG, Constantino
20 CJL, Medina-Plaza C, de Saja JA and Rodríguez-
21 Méndez ML. *J. Nanosci. Nanotechnol.* 2014, **14**:
22 6754–6763.
- 23 65. İpek Y, Şener MK and Koca A. *J. Porphyrins*
24 *Phthalocyanines* 2015; **19**: 708–713.
- 25 66. Alencar WS, Crespilho FN, Martins MVA,
26 Zucolotto V, Oliveira ON Jr and Silva WC. *Phys.*
27 *Chem. Chem. Phys.* 2009; **11**: 5086–5091.
- 28 67. Keyu H, Lei H, Yongbo Q, Caixia H, Haibo P and
29 Min D. *Mater. Sci. Eng. C* 2015; **49**: 640–647.
- 30 68. Kotiaho A, Lahtinen R and Lemmetyinen H. *Pure*
31 *Appl. Chem.* 2011; **83**: 813–821.
- 32 69. Medina-Plaza C, Furini LN, Constantino CJL, de
33 Saja JA and Rodríguez-Méndez ML. *Anal. Chim.*
34 *Acta* 2014; **851**: 95–102.
- 35 70. Bouvet M. In *Radical Phthalocyanines and Intrinsic*
36 *semiconduction. The Porphyrin Handbook*, Vol.
37 19, Kadish KM, Smith KM and Guillard R. (Eds.)
38 Academic Press: New York, 2003; pp 37–104.
- 39 71. Njanja E, Nassi A, Ngameni E, Elleouet C, Quentel
40 F and L'Her M. *Electrochem. Commun.* 2007; **9**:
41 1695–1699.
- 42 72. Rodríguez-Méndez ML. In *Sensing properties*
43 *of phthalocyanines. Encyclopedia of Sensors 9*,
44 Grimes CS, Dickey EC and Pishko MV (Eds.)
45 America Scientific Publishers: California, 2006;
46 pp. 111–134.
- 47 73. Parra V, Hernando T, Rodríguez-Méndez ML and
48 de Saja JA. *Electrochim. Acta* 2004; **49**: 5177–5185.
- 49 74. Gorbunova Y, Rodríguez-Méndez ML, Kalashnikova
50 IP, Tomilova L and de Saja JA. *Langmuir* 2001; **17**:
51 5004–5010.
- 52 75. Gorbunova Y, Rodríguez-Méndez ML, Tomilova L
53 and de Saja JA. *Chem. Mater.* 1995; **7**: 1443–1448.
- 54
55
56
57
76. Apetrei C, Nieto M, Rodríguez-Méndez ML and de
Saja JA. *J. Porphyrins Phthalocyanines* 2011; **15**:
1–10.
77. Fahmida K and Fakhruddin ANM. *Rev. Environ.*
Sci. Biotech. 2012; **11**: 261–274.
78. Goto TE, Lopez RF, Oliveira ON and Caseli L.
Langmuir 2010; **26**: 11135–11139.
79. Moyo M and Okonkwo J. *Sens. Actuators, B* 2014;
193: 515–521.
80. Cetó X, Capdevilla J, Mínguez S and del Valle M.
Food Res. Int. 2014; **55**: 455–461.
81. Apetrei IM, Rodríguez-Méndez ML, Apetrei C
and de Saja JA. *Sens. Actuators, B* 2013; **177**:
138–144.
82. Dong J, Liu T, Meng X, Zhu J, Hang K, Ai S
and Cui S. *J. Solid State Electrochem.* 2012; **16**:
3783–3790.
83. Mariani AM, Natoli ME and Kofinas P. *Biotechnol.*
Bioeng. 2013; **10**: 2994–3002.
84. Vicentini FC, Janegitz BC, Brett CMA and
Fatibello-Filho O. *Sens. Actuators, B* 2013; **188**:
1101–1108.
85. Guan H, Liu X and Wang W. *J. Solid State*
Electrochem. 2013; **17**: 2887–2893.
86. Wang K, Xu J and Chen H. *Biosens. Bioelectron.*
2005; **20**: 1388–1396.
87. Medina C, Revilla G, Muñoz R, Fernández-Escudero
JA, Barajas E, Medrano G, de Saja JA and Rodríguez-
Méndez ML. *J. Porphyrins Phthalocyanines* 2014;
18: 76–86.
88. Cajab J, Soloduch J, Chyla A, Bryjak J and Zynek
K. *Sens. Actuators, B* 2009; **136**: 425–431.
89. Caseli L, Perinotto AC, Vitala T, Zucolotto V and
Oliveira ON. *Langmuir* 2009; **25**: 3057–3061.
90. Apetrei C, Alessio P, Constantino CJL, de Saja JA,
Rodríguez-Méndez ML, Pavinatto FJ, Fernandes
EG, Zucolotto V and Oliveira ON Jr. *Biosens.*
Bioelectron. 2011; **26**: 2513–2519.
91. Fernandes EGR, Brazaca LC, Rodríguez-Méndez
ML, de Saja JA and Zucolotto V. *Biosens.*
Bioelectron. 2011; **26**: 4715–4719.
92. Apetrei C, de Saja JA, Zurro J and Rodríguez-
Méndez ML. *Catalysts.* 2012; **2**: 517–531.
93. Pavinatto F, Fernandez EGR, Alessio P, Constantino
JC, de Saja JA, Zucolotto V, Oliveira ON, Apetrei C
and Rodriguez-Mendez ML. *J. Mat. Chem.* 2011;
21: 4995–5003.
94. Medina C, de Saja JA and Rodríguez-Méndez ML.
Biosens. Bioelectron. 2014; **57**: 276–286.
95. Sliwinska M, Wisniewska P, Dymerski T, Namiesnik
J and Wardenci W. *J. Agric. Food. Chem.* 2014; **62**:
1423–1448.
- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57