

Table of Contents

I Introduction	3
1.1 Water contamination.....	3
1.2 Gold standard methods used for water monitoring.....	10
1.3 Monitoring and screening systems.....	12
1.4 Rapid assays for detection of pollutant residues in water.....	17
1.5 Multiplex bioanalytical methods for environmental monitoring.....	19
1.5.1 Planar arrays.....	21
1.5.2 Suspension arrays.....	23
1.5.3 Automated multi-analyte systems.....	25
1.6 Biosensing.....	29
1.6.1 Biorecognition elements.....	30
1.6.2 Signal tags.....	32
1.6.3 Assay formats.....	40
1.6.4 Supports.....	44
1.6.5 Techniques for detection of the immunointeractions events.....	47
1.7 Compact Disk technology.....	50
1.7.1 Analytical applications of disks as analytical platform.....	53
1.7.1.1 Lab-on-a-CD.....	53
1.7.1.2 Compact disk technology as analytical tool.....	54
II Objectives.....	63
III Materials and Methods.....	77
3.1 Reagents.....	77
3.2 Instruments.....	78
3.3 Antibody purification.....	79
3.4 Preparation of coating conjugates.....	79
3.5 Gold colloid titration procedure.....	80
3.6 Preparation of antibody-nanogold conjugates (Ab-AuNPs).....	81
3.7 Preparation of antibody-gold nanorod conjugates (Ab-AuNRs).....	82
3.8 Preparation of antigen-nanogold tracers (OVA-H-AuNPs).....	84
3.9 Preparation of hapten-nanogold tracers (H-AuNPs).....	85
3.10 Microarraying.....	86
3.11 Microimmunoassay protocols.....	88
3.11.1 Two-step antigen-coated microimmunoassay using antibody-nanogold conjugates.....	88
3.11.2 One-step antigen-coated microimmunoassay using antibody-nanogold conjugates.....	89
3.11.3 One-step antigen-coated microimmunoassay using antibody-gold nanorod conjugates.....	89
3.11.4 Two-step antibody-coated microimmunoassay using antigen-nanogold tracers.....	90
3.11.5 Two-step antibody-coated microimmunoassay using hapten-nanogold tracers.....	91
3.11.6 Immunocapture microimmunoassay.....	92
3.12 Analysis of water samples.....	92
3.13 Compact disk scanning and data acquisition.....	93
IV Results and Discussion.....	97
4.1 Purification of antibodies.....	97
4.2 Characterization of gold nanoparticles.....	99
4.3 Optimization of antibody-gold nanoparticles conjugation.....	101

4.4 Selection of the nanoparticle size.....	108
4.4.1 Two-step antigen-coated microimmunoassay using antibody-nanogold.....	108
4.4.2 One-step antigen-coated microimmunoassay using antibody-nanogold.....	110
4.5 Two-step antibody-coated microimmunoassay using antigen – nanogold tracers.....	112
4.6 One-step microimmunoassay using antibody-gold nanorods.....	115
4.6.1 Titration experiments of the antibody-gold nanorods conjugates.....	118
4.6.2 Competitive immunoassays using antibody-gold nanorod conjugates.....	119
4.7 Two-step antigen-coated microimmunoassay using hapten nanogold tracers.....	122
4.8 Optimization of the performances of microimmunoassay.....	125
4.9 Individual calibration curves.....	133
4.10 Selection of the antibodies for the development of a multiplex assay.....	138
4.11 Multiplex calibration curves.....	150
4.12 Study of the assay controls.....	155
4.13 Study of the antibody-gold nanoparticles stability.....	158
4.14 Immunocapture microimmunoassay.....	160
4.14.1 Selection of the optimal conditions for homogeneous immunocapture step.....	160
4.14.2 Competitive assays using immunocapture step.....	164
4.15 Determination of the potential of the developed methodology. Analysis of water samples.....	170
V Conclusions.....	179
VI References.....	185