Experimental Determination of ADMET Parameters in High Throughput, Using Colloidal Gold Composites and a Conductive Polymer as Reporting Reagents

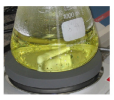
Roberto Martinez-Neira, Patricia De Pril, Anne Van Hoonacker, Patrick Englebienne
Pharmadiagnostics N.V., I.I.C.B. Z1 Research Park 310, B-1731 Zellik, Belgium

Summary

We developed and validated a high throughput in vitro setting to experimentally determine hSA and Orosomucoid affinities, fraction unbound in plasma, fraction absorbed, Log BBB, pKa and Redox potential of NCEs using gold nanoparticles functionalized with proteins, lipids or conductive polymers. Such automated ADME assays provide means for more objective decisions in early drug discovery.

1. The technology of PharmaDiagnostics NV is based on the pioneering work of Dr Patrick Englebienne in the fields of localized surface plasmon resonance (LSPR) with noble metal nanoparticles and their use in combination with other redox-responsive materials such as conductive polymers (P. Englebienne. Analyst. 123[1998] 1599-1603; P. Englebienne, A. Van Hoonacker & M. Verhas. Analyst. 126[2001] 1645-1651; P. Englebienne, A. Van Hoonacker, M. Verhas and N. G. Khlebtsov. Comb. Chem. High Throughput Screen . 6[2003] 777-787).

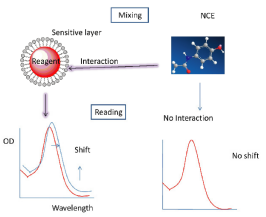
Gold Solution



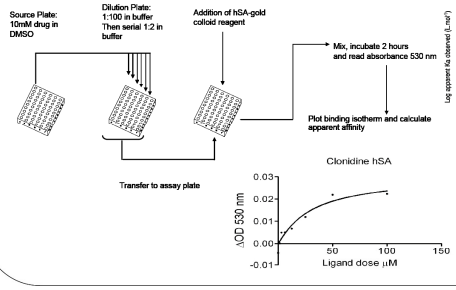
Gold Nanoparticles Solution

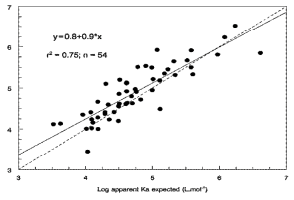
2. TECHNOLOGY ADVANTAGES

- Homogeneous technique: mix and read
- Applicable to various systems:
  - Ligand-receptor/binding proteins
  - Charge interactions
  - Enzymatic reactions
  - Redox reactions
- Sensitivity (down to pM with proteins)
- Measurement of signal in the visible spectrum
- Reproducibility
- Ease of reagent preparation



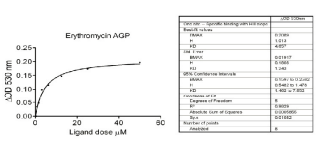
3. ALBUMIN BINDING

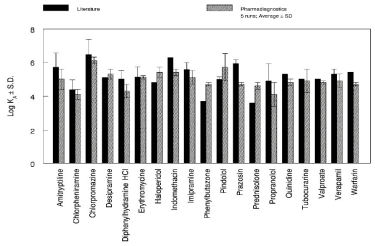




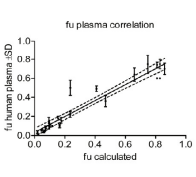
4. AGP BINDING

Protocol identical to that of albumin





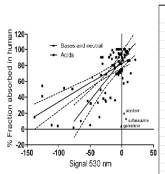
5. FRACTION UNBOUND IN PLASMA



Best-fit values	fu human plasma
Slope	0.8347 ± 0.04661
Y-intercept when X=0.0	0.03154 ± 0.02154
X-intercept when Y=0.0	-0.03778
1/slope	1.198
95% Confidence Intervals	
Slope	0.7387 to 0.9307
Y-intercept when X=0.0	-0.01283 to 0.07591
X-intercept when Y=0.0	-0.09877 to 0.01419
Goodness of Fit	
r²	0.9277
Sy.x	0.07599
Is slope significantly non-zero?	
F	320.8
DFn, DFd	1.000, 25.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	27
Maximum number of Y replicates	1
Total number of values	27
Number of missing values	0

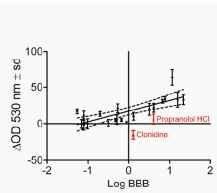
Calculated from experimentally obtained affinity values for albumin and AGP

6. FRACTION ABSORBED GI TRACT



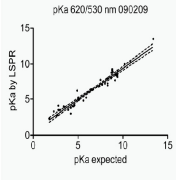
Best-fit values	Bases and neutrals	Acids
Slope	0.4806 ± 0.0371	1.024 ± 3.7612
Y-intercept when X=0.0	82.10 ± 2.669	98.45 ± 4.865
X-intercept when Y=0.0	-170.7	96.43
1/slope	2.079	0.9881
95% Confidence Intervals		
Slope	0.3325 to 0.6283	-3.6320 to 5.342
Y-intercept when X=0.0	76.27 to 87.92	76.53 to 96.42
X-intercept when Y=0.0	-228.4 to -137.9	-119.1 to -57.68
Goodness of Fit		
r²	0.5587	0.5948
Sy.x	17.40	20.92
Is slope significantly non-zero?		
F	56.98	39.43
DFn, DFd	1.000, 45.00	1.000, 28.00
P value	< 0.0001	< 0.0001
Deviation from zero?	Significant	Significant
Data		
Number of X values	47	30
Maximum number of Y replicates	1	1
Total number of values	47	30
Number of missing values	33	50

7. Log BBB



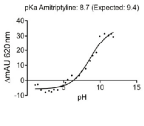
Best-fit values	ΔOD 530nm
Slope	14.64 ± 2.920
Y-intercept when X=0.0	17.32 ± 2.432
X-intercept when Y=0.0	-1.183
1/slope	0.06830
95% Confidence Intervals	
Slope	8.508 to 20.78
Y-intercept when X=0.0	12.21 to 22.43
X-intercept when Y=0.0	-2.133 to -0.7366
Goodness of Fit	
r²	0.5828
Sy.x	10.64
Is slope significantly non-zero?	
F	25.15
DFn, DFd	1.000, 10.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	20
Maximum number of Y replicates	1
Total number of values	20
Number of missing values	2

8. EXPERIMENTAL pKa

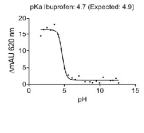


Best-fit values	
Slope	0.3892 ± 0.03113
Y-intercept when X=0.0	0.5445 ± 0.2231
X-intercept when Y=0.0	-0.5769
1/slope	1.125
95% Confidence Intervals	
Slope	0.3264 to 0.4520
Y-intercept when X=0.0	0.06438 to 0.9646
X-intercept when Y=0.0	-1.151 to -0.06766
Goodness of Fit	
r²	0.9499
Sy.x	0.5832
Is slope significantly non-zero?	
F	815.8
DFn, DFd	1.000, 43.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	45
Maximum number of Y replicates	1
Total number of values	45
Number of missing values	0

Base



Acid

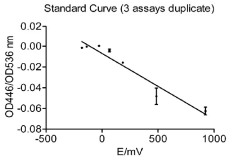


9. REDOX POTENTIAL

Use of a composite nanomaterial made of gold nanoparticles covered by a poly(aniline) derivative (Englebienne & Van Hoonacker, J. Coll. Interface Sci. 292[2005] 445-454). The nanocomposite is more sensitive to redox reactions than the polyaniline derivative.

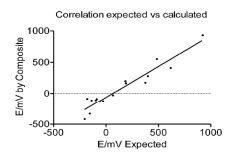
GNP | PANI pH4 | PANI pH9 | Composite pH4 | Composite pH9

Standard Curve (3 assays duplicate)



Best-fit values	
Slope	-0.00060349 ± 0.000002928
Y-intercept when X=0.0	-0.008004 ± 0.0010192
X-intercept when Y=0.0	-168.8
1/slope	-1656.0
95% Confidence Intervals	
Slope	-0.00060940 to -0.00059757
Y-intercept when X=0.0	-0.008035 to -0.004476
X-intercept when Y=0.0	-168.8 to -23.17
Goodness of Fit	
r²	0.9216
Sy.x	0.0006924
Is slope significantly non-zero?	
F	476.1
DFn, DFd	1.000, 40.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	7
Maximum number of Y replicates	6
Total number of values	42
Number of missing values	0

Correlation expected vs calculated



Best-fit values	
Slope	0.9920 ± 0.08027
Y-intercept when X=0.0	-62.59 ± 28.26
X-intercept when Y=0.0	63.10
1/slope	1.008
95% Confidence Intervals	
Slope	0.8198 to 1.164
Y-intercept when X=0.0	-123.2 to -1.970
X-intercept when Y=0.0	2.136 to 119.1
Goodness of Fit	
r²	0.9160
Sy.x	103.2
Is slope significantly non-zero?	
F	152.7
DFn, DFd	1.000, 14.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	16
Maximum number of Y replicates	1
Total number of values	16
Number of missing values	0

An oxidant has more chances to be cytotoxic than a reductant

10. TURBIDIMETRY BASED SOLUBILITY ASSAY

Compound	Water solubility expected µM	Water solubility observed µM	Fed µM Average ± sem (# assays)	Fasted µM Average ± sem (# assays)
Paracetamol	92,610	>500	31.4±9.5 (3)	>500 (4)
Aspirin	25,549	>500	>500 (2)	>500 (1)
Pentobarbital Na	2,734	>500	56.7±2.3 (3)	485 (1)
Acetobutolol	770	>500	55.4±4.1 (2)	139.5_31 (2)
Diazepam	176	200	113.4±22.7 (3)	105.8±33.4 (5)
Ibuprofen	237	220	31.9±18.4 (3)	964±489 (5)
Triamterene	190	120	83.5 (1)	12.9 (1)
Oxacilline Na	33	45	168.7±1.7 (3)	25.2±8.1 (5)
Furosemide	18	20.5	13.2±2.6 (3)	16.4±5.4 (4)
Chlorpromazine	8	5	25_8 (2)	37.2±9.1 (5)
Erythromycin	1.96	2.7	8.5±5.6 (3)	45±11.6 (5)
Fluconazole	3.3	19.8	448.8±99 (2)	150.3±81.4 (3)