A novel approach for discriminating of lipophilic bisphosphonate-based pharmaceuticals using a potentiometric array

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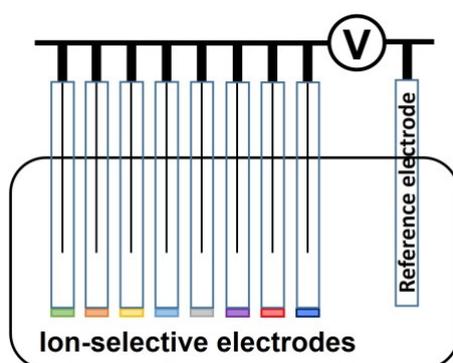2. Materials and Methods

2.2. Instrumentation

Fig. S1: Schema of experimental array used in the present work.

3. Results and Discussion
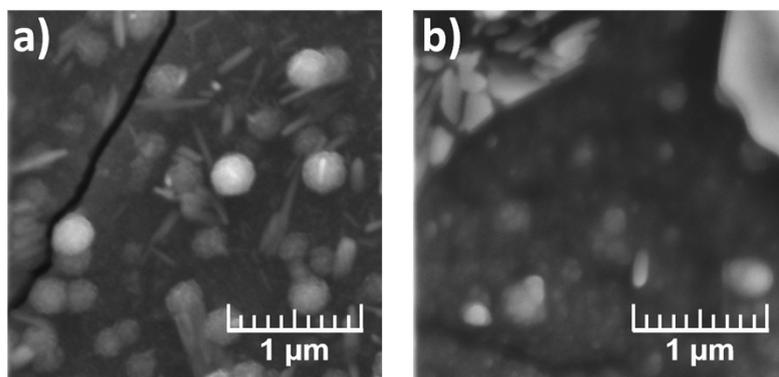
3.2. SEM and EDS analysis

Fig. S2. SEM images at 100,000x magnification of PANI-coated membranes **a)** blank (1A) and **b)** TDDMACl-based (2A).
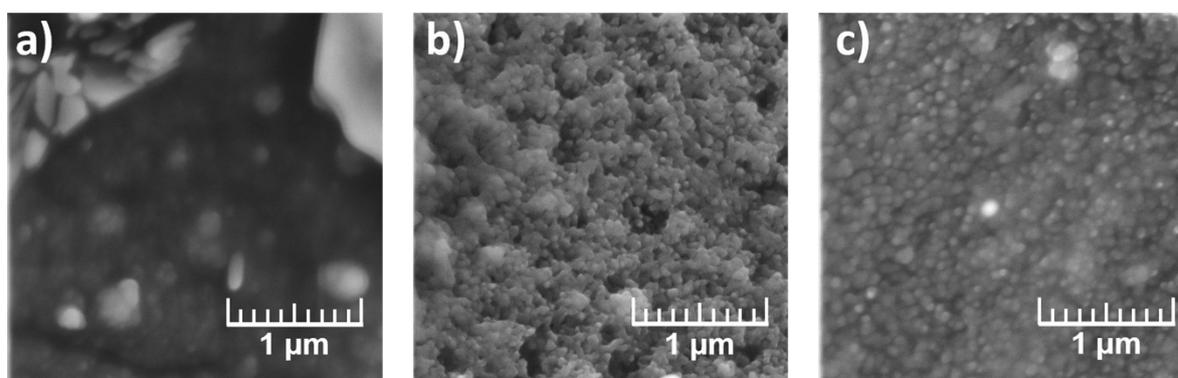

Fig. S3. SEM micrographs at 100,000× magnification of PANI-coated membranes based on an anion exchanger (TDDMACl), prepared with different salts in the polymerization mixture: **a)** Sample 2A (no added salt) shows a more compact and uniform structure. **b)** Sample 2B (with NaCl) exhibits a morphology with high surface roughness. **c)** Sample 2C (with Na$_2$SO$_4$) displays a heterogeneous surface with finer dispersed features, indicating altered nucleation behavior due to sulfate doping.
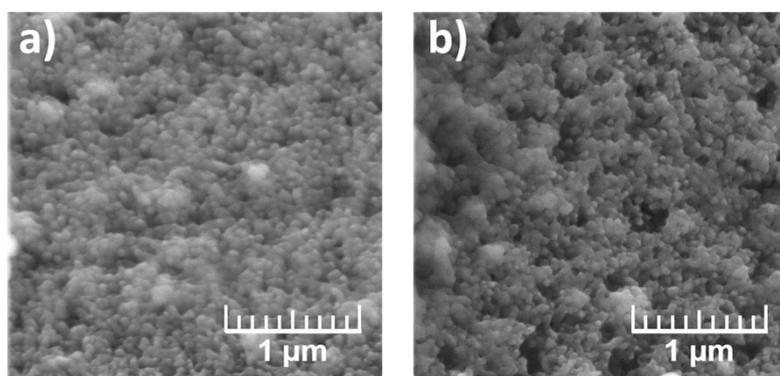

Fig. S4. SEM images at 100,000× magnification showing the effect of monomer form on the morphology of the PANI layer on the membrane surface. **a)** Sample 2B* (aniline base + NaCl) and **b)** Sample 2B (aniline hydrochloride + NaCl) both exhibit granular polymer structures with high surface roughness. Only subtle morphological differences are visible.
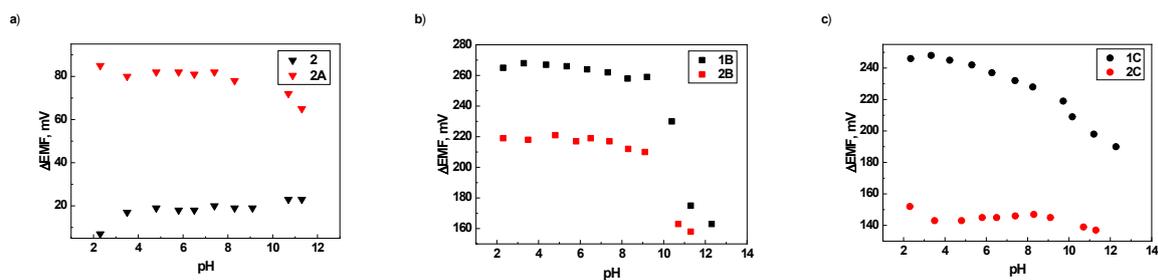
3.3. Potentiometric measurements

Fig. S5. pH dependence of experimental membranes prepared in this study and used in the sensor array.
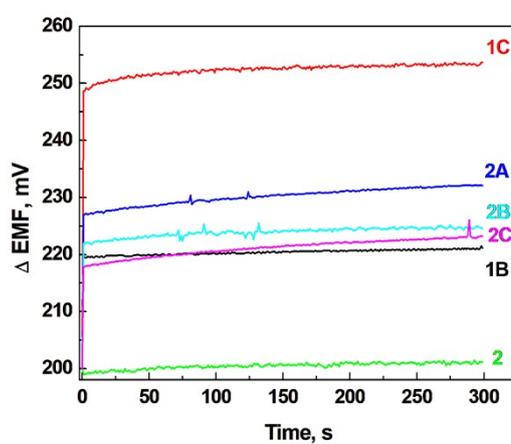

Fig. S6. Potentiometric signal of experimental membranes towards ibandronate recorded in time with the sensor array used in the present study.

Table S1. Potentiometric parameters of experimental ion-selective membranes prepared without (membrane 1) and with (membrane 2) the anion-exchanger modified by the PANI layer towards tested bisphosphonates (n=3).

ISM	Alendronate			
	Linear regression equation	Correlation coefficient	Linear concentration range, mol L^{-1}	C_{min}, mol L^{-1}
1A	$-19.2 \log C +193.7$	0.9778	1.0×10^{-5} - 5.9×10^{-3}	9.7×10^{-6}
1B	$-27.8 \log C +211.43$	0.9375	5.0×10^{-5} - 5.9×10^{-3}	3.1×10^{-5}
1C	$-14.6 \log C +171.8$	0.9668	5.0×10^{-5} - 5.9×10^{-3}	7.2×10^{-6}
2	$-29.1 \log C +193.7$	0.9348	5.0×10^{-5} - 5.9×10^{-3}	1.1×10^{-5}
2A	$-25.6 \log C +193.7$	0.9705	1.0×10^{-5} - 5.9×10^{-3}	6.0×10^{-6}
2B	$-23.4 \log C +229.5$	0.9934	1.0×10^{-5} - 5.9×10^{-3}	9.8×10^{-6}

ISM	Clodronate			
	Linear regression equation	Correlation coefficient	Linear concentration range, mol L^{-1}	C_{min}, mol L^{-1}
1A	$-35.1 \log C +191.0$	0.9999	4.3×10^{-4} - 5.5×10^{-3}	1.2×10^{-4}
1B	$-11.7 \log C +185.80$	0.9751	5.0×10^{-5} - 5.5×10^{-3}	7.3×10^{-6}
1C	$-18.7 \log C +179.3$	0.9567	5.0×10^{-5} - 5.5×10^{-3}	2.6×10^{-5}
2	$-18.9 \log C +105.8$	0.9965	4.3×10^{-4} - 5.5×10^{-3}	1.5×10^{-4}
2A	$-39.3 \log C +185.9$	0.9999	1.0×10^{-5} - 5.5×10^{-3}	6.1×10^{-6}
2B	$-36.7 \log C +183.0$	0.9999	1.0×10^{-5} - 5.5×10^{-3}	6.0×10^{-6}
2C	$-35.9 \log C +190.1$	0.9999	1.0×10^{-5} - 5.5×10^{-3}	7.3×10^{-6}

ISM	Risedronate			
	Linear regression equation	Correlation coefficient	Linear concentration range, mol L^{-1}	C_{min}, mol L^{-1}
1A	$-15.1 \log C + 101.5$	0.9795	5.0×10^{-5} - 1.8×10^{-3}	1.7×10^{-6}
1B	$-22.8 \log C + 109.0$	0.9215	5.0×10^{-5} - 1.8×10^{-3}	5.0×10^{-6}
1C	$-14.6 \log C + 170.2$	0.9215	1.0×10^{-5} - 1.8×10^{-3}	2.6×10^{-6}
2	$-28.1 \log C +122.61$	0.9348	1.0×10^{-5} - 1.8×10^{-3}	1.7×10^{-6}
2A	$-29.8 \log C + 59.6$	0.9845	1.0×10^{-5} - 1.8×10^{-3}	5.3×10^{-6}
2B	$-25.7 \log C + 78.1$	0.9777	1.0×10^{-5} - 1.8×10^{-3}	4.0×10^{-6}
2C	$-27.6 \log C + 82.0$	0.9955	1.0×10^{-5} - 1.8×10^{-3}	7.1×10^{-6}

| ISM	Ibandronate			
	Linear regression equation	Correlation coefficient	Linear concentration range, mol L^{-1}	C_{min}, mol L^{-1}
1A	$-18.9 \log C + 85.0$	0.9766	4.3×10^{-4} - 2.9×10^{-3}	6.8×10^{-6}
1B	$-18.1 \log C + 162.5$	0.9838	5.0×10^{-5} - 2.9×10^{-3}	9.5×10^{-6}
1C	$-34.0 \log C + 71.2$	0.9918	4.3×10^{-4} - 2.9×10^{-3}	2.4×10^{-6}
2	$-17.6 \log C + 241.2$	0.9939	1.0×10^{-5} - 2.9×10^{-3}	4.6×10^{-6}
2A	$-17.3 \log C + 226.4$	0.9895	1.0×10^{-5} - 2.9×10^{-3}	6.8×10^{-6}
2B	$-20.3 \log C + 234.6$	0.9783	5.0×10^{-5} - 2.9×10^{-3}	1.4×10^{-5}
2C	$-20.0 \log C + 102.0$	0.9846	5.0×10^{-5} - 2.9×10^{-3}	5.6×10^{-6}
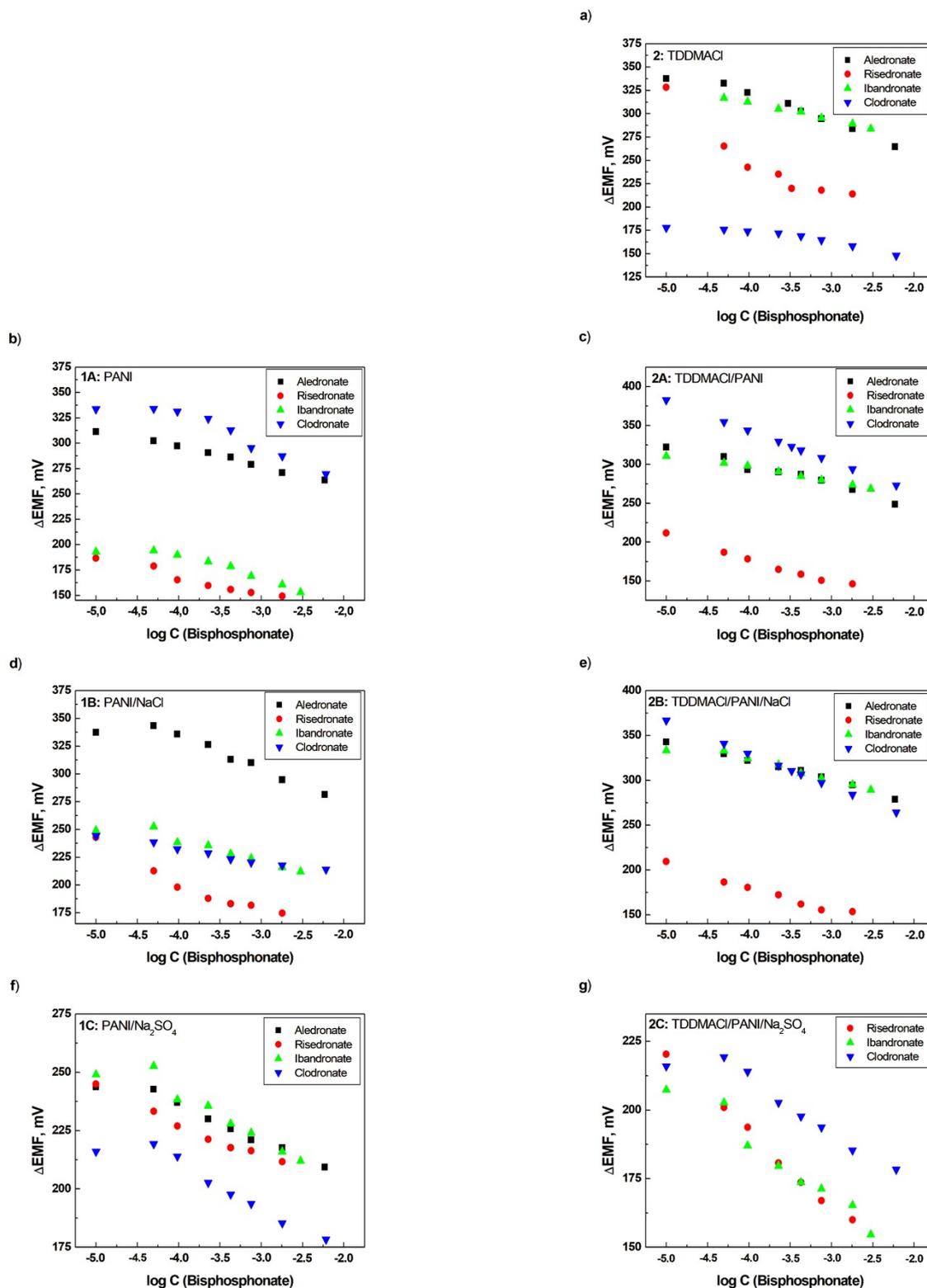
Fig. S7. Potentiometric response of PANI-coated experimental ion-selective membranes prepared without (membrane1) and with (membrane 2) the anion-exchanger to tested bisphosphonates*

*If no Nernstian portion of the response to the primary/interferents ions is obtained, no selectivity coefficient should be determined. In such a case, it is best to report the calibration curve to represent the selectivity. We follow a recipe for experimentalists [**S1**: E. Bakker, E. Pretsch and P, Buhlmann, Selectivity of Potentiometric ion sensors, *Anal. Chem.* 2000, **72**, 1127–1133. https://doi.org/10.1021/ac991146n.].

Table S2. Potentiometric signals of experimental PANI-modified membranes towards tested pharmaceuticals based on bisphosphonates in time (n=5).

| Measurement	Bisphosphonate	Experimental membranes					
		1B	1C	2	2A	2B	2C
1 day	A	210 ± 4	262 ±8	189 ± 3	214 ±2	209 ± 2	222 ± 1
	C	182 ±6	241 ± 4	168 ± 1	191 ± 1	191 ± 1	209 ± 7
	I	222 ± 1	264 ± 13	198 ± 2	227 ± 4	223 ± 1	222 ± 1
	R	198 ± 2	260 ± 2	181 ± 2	204 ± 1	202 ± 2	218 ± 1
2 day	A	215 ± 2	234 ± 5	185 ± 1	211 ± 1	208 ± 1	209 ± 2
	C	205 ± 4	229 ± 3	172 ± 2	190 ± 2	194 ± 2	189 ± 2
	I	218 ± 1	237 ± 16	193 ± 4	216 ± 2	210 ± 1	220 ± 2
	R	208 ± 2	229 ± 7	180 ± 2	201 ± 1	201 ± 1	200 ± 1
3 day	A	215 ± 1	243 ± 7	179 ± 1	207 ± 1	198 ± 1	198 ± 1
	C	205 ± 1	225 ± 6	161 ± 1	185 ± 3	189 ± 1	175 ± 4
	I	213 ± 1	248 ± 17	187 ± 3	209 ± 1	197 ± 3	202 ± 1
	R	208 ± 1	230 ± 2	168 ± 4	197 ± 1	191 ± 5	188 ± 1

3.4. Bisphosphonate discrimination
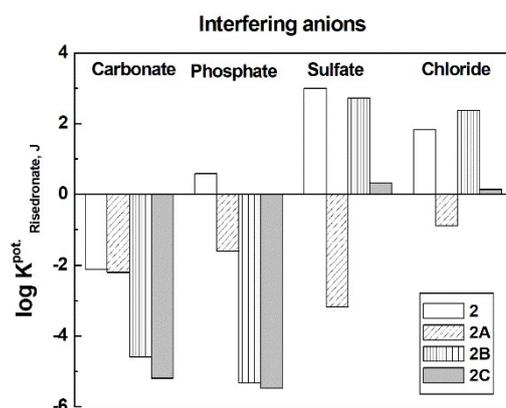
Fig. S8. Influence of possible inorganic interferents on the potentiometric selectivity of unmodified and PANI-modified ion-selective membrane based on anion-exchanger.
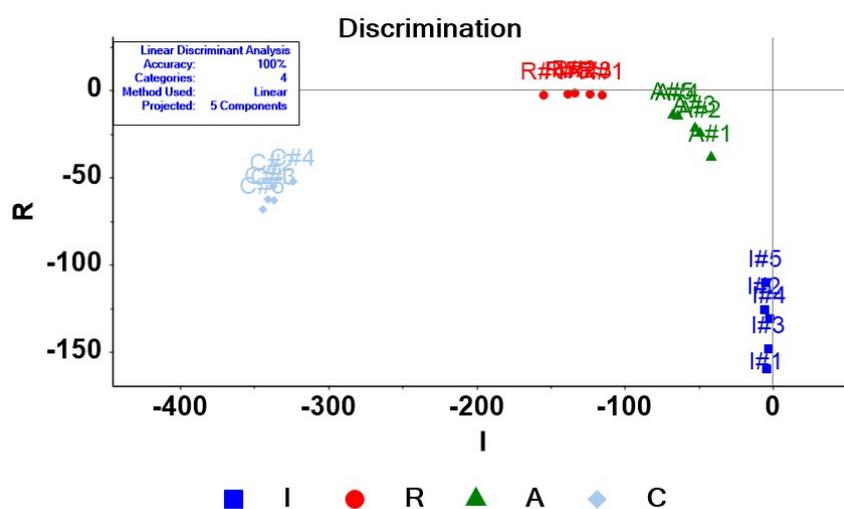

Fig. S9. LDA discrimination chart (sample distance to individual models) for model samples.

Table S3. Confusion matrix obtained by PCA-LDA method for model samples.

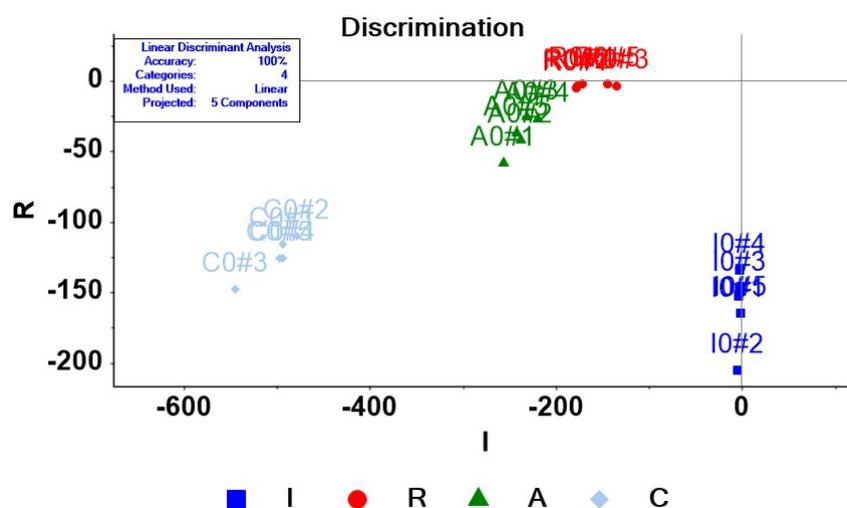| Confusion		Actual			
		I	**R**	**A**	**C**
Predicted	**I**	5	0	0	0
	R	0	5	0	0
	A	0	0	5	0
	C	0	0	0	5

Fig. S10. LDA discrimination chart (sample distance to individual models) for real samples.

Table S4. Confusion matrix obtained by PCA-LDA method for real samples.

Confusion		Actual			
		I	**R**	**A**	**C**
Predicted	**I**	5	0	0	0
	R	0	5	0	0
	A	0	0	5	0
	C	0	0	0	5