

Changes in emission induced by non-covalent analyte-fluorophore interactions in silica gel as a general detection procedure for TLC

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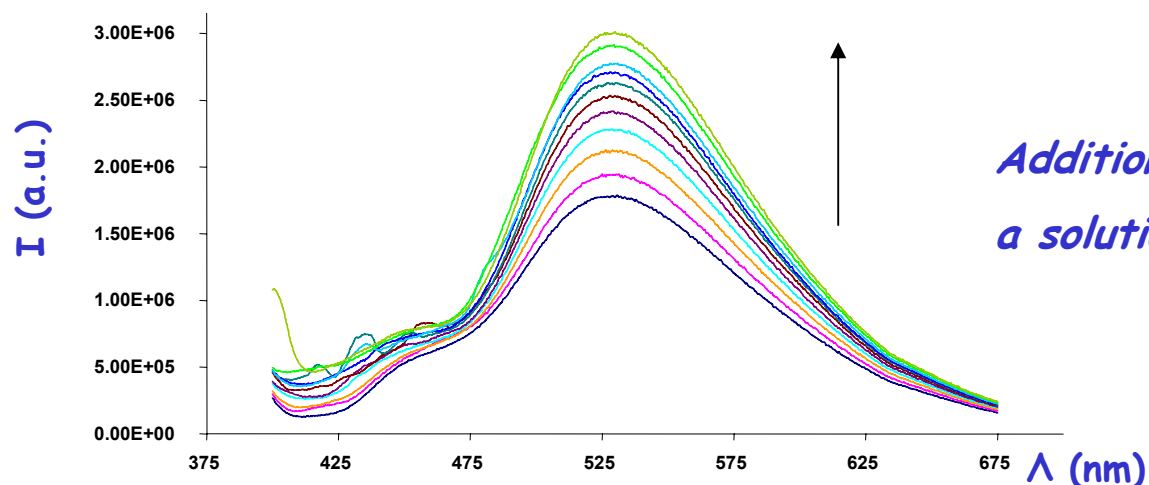
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HPTLC- Helsinki, 2008

Introduction

FDIC: A general detection procedure

A wide number of compounds, including *non-fluorescent ones*, induce changes in the fluorescence spectrum (either increase or quenching) of berberine cation that exclusively affect emission intensity



Additions of non-fluorescent n-dodecanol to a solution of berberine in methanol

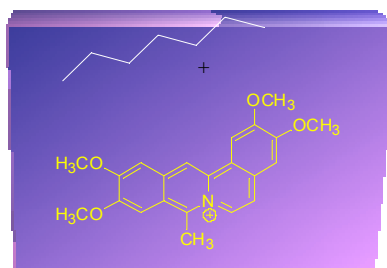
Saturated hydrocarbons (alkanes) using HPTLC

(Anal.Chem. 2000, 72, 1759)

Increase of fluorescent emission

$h\nu$

$\lambda_{exc} = 365 \text{ nm}$

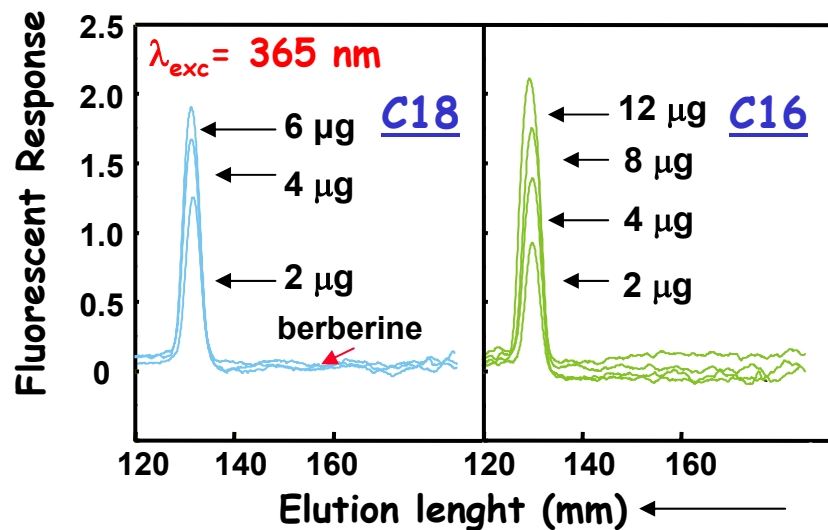


$\lambda_{em} > 450 \text{ nm}$

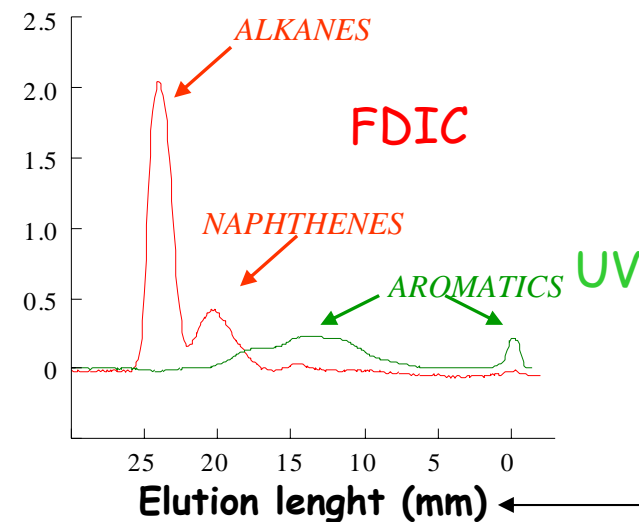
Examples of TLC-FDIC (berberine cation, 60 ppm)



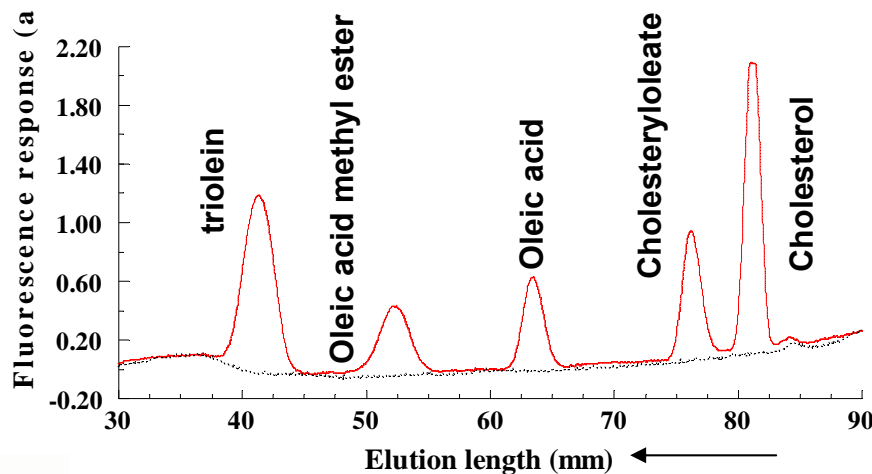
Increases in emission: ALKANES
(Org.Lett. 2000, 2, 2311)



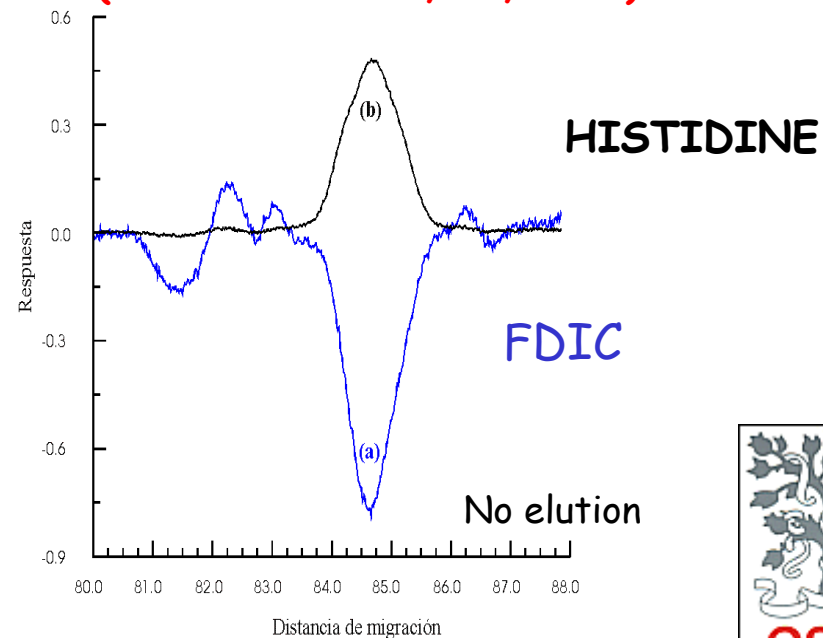
HYDROCARBON TYPES IN DIESEL
(J.Sep. Sci. 2003, 26, 1665)



Increases in emission: all kind of LIPIDS
(J.Chromatogr. A 2007, 1146, 251)



Quenching: polar molecules
(Anal.Chem. 2006, 78, 3699)



Aims

Which fluorophores give FDIC?

Is FDIC a general phenomenon from the point of view of fluorophores?

-  Photophysics
-  Theoretical model
-  Experimental validation with other fluorophores in TLC

Is it possible to control radiative and non-radiative decay processes through non-covalent interactions to design TLC-based procedures ?

To assist analysts in the design of effective FDIC-based detection systems for TLC

Theoretical model to explain changes in emission

1.- Contribution to emission increases: refractive index

 *A modern revision of Quantum Electrodynamics Theory*

2.- Contribution to emission increases: nonspecific, weak electrostatic interactions

 *Evidence of interaction*

 *Model based on the energy of electrostatic interaction*

 *Ion-induced dipole interaction*

 *Dipole-induced dipole interaction*

3.- Contribution to quenching: specific interactions

1.- Contribution to emission increases: refractive index (n)

$$\phi = \frac{k_r}{k_r + k_{nr}}$$

Variation with n usually considered as negligible
Emission considered in vacuum

Quantum-Electrodynamics: k_r is not an intrinsic property of fluorophores
 (D. Topygin. J. Fluorescence 2003, 13, 201)

$$k_r = f^2 n k_{r0}$$

FLUOROPHORE OPTICAL PROPERTIES
 k_{r0} intrinsic spontaneous emission

MEDIUM PROPERTIES $f = \frac{3n^2}{2n^2 + 1}$ A local field correction

★ And n is related to polarizability (α) of probe environment $\frac{4}{3} \pi \alpha = \frac{n^2 - 1}{n^2 + 2} \frac{M}{d}$
 For silica gel medium, n is related to α_{analyte}




A GENERAL PROPERTY OF FLUOROPHORES

$$\uparrow \phi = \frac{k_r \uparrow}{k_r + k_{nr}}$$

2.- Contribution to emission increases: nonspecific, weak electrostatic interactions

Evidence of interaction

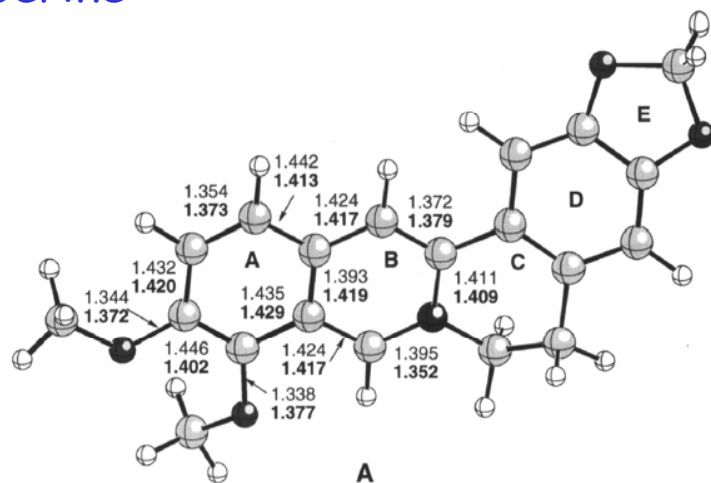
Using methods of Quantum Mechanics and Computational Chemistry

-  Berberine is a nearly planar cation with a remarkable conformational rigidity
-  Frontier orbitals (HOMO and LUMO) are not affected
-  Fluorescent emission is produced by a nonspecific interaction between the alkane and the π -system of berberine, and there are intermolecular distances close to Van der Waals contacts

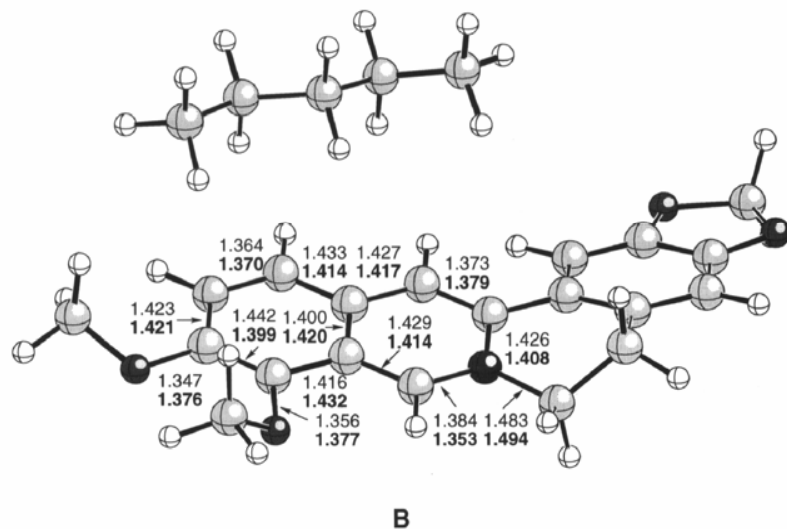
Methods: **Ground state:** semiempirical Hamiltonian PM3 at Hartree-Fock (HF) level; **1st excited state:** 3X3CI-HE/PM3 level; **Molecular Mechanics:** AMBER force field, Montecarlo

Berberine-pentane geometry and frontier orbitals

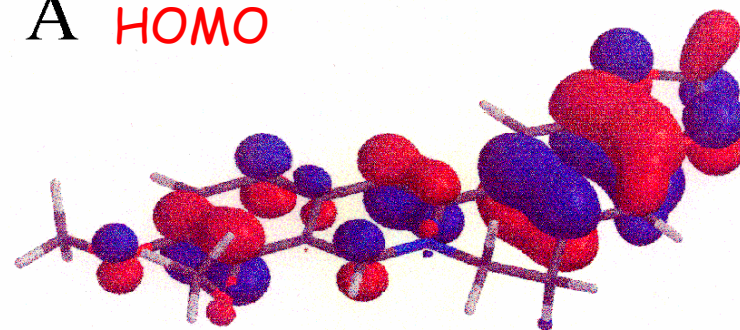
Berberine



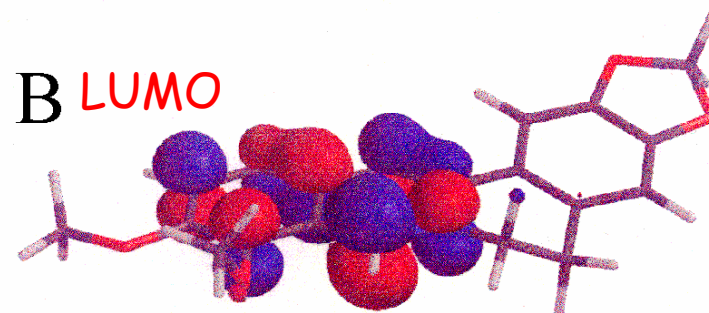
Berberine-pentane complex



A HOMO



B LUMO

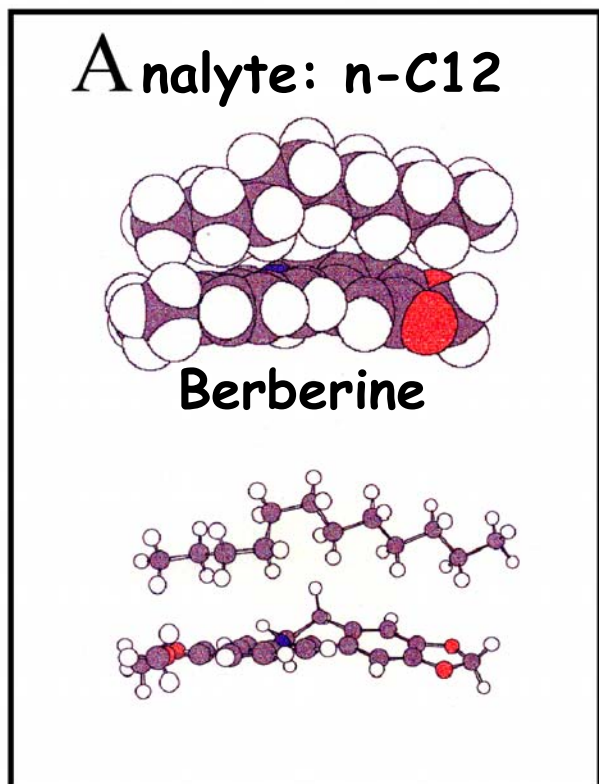


Bond distances in Å

Bold numbers: ground state

Light numbers: excited state

 A MODEL based on the energy of electrostatic interaction



AMBER force field, ΔE (kcal mol⁻¹)

- Berberine-C₁₂: -7.04

- Berberine-C₁₄: -7.50

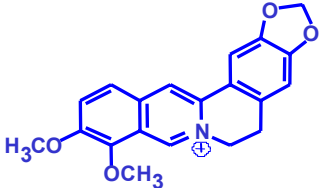
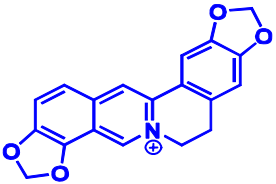
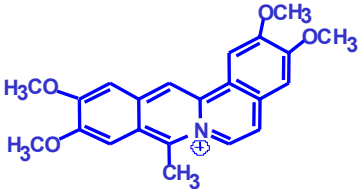
ΔI proportional to energy of interaction

Through alkane polarizability

The higher the chain length (the higher α), the higher the interaction.
The higher the protection

Ion-induced dipole interaction (TLC data):

$$\Phi_{i,p} = U_k \frac{e^2 \cdot Z_p^2}{8 \cdot \pi \cdot \epsilon_0 \cdot r^4} \alpha_i \quad \longrightarrow \quad \Delta I_{i,p} = A_p + B_p \alpha_i$$

	<u>Analytes series</u>	<u>Sample load</u> (μg)	<u>λ_{exc}</u> (nm)	<i>B_p</i>	<i>r</i> ²
 Berberine cation *	Alkanes (n= 12-24)	0.8	365	52	0.99
	Alcohols (n= 10-22)	0.8	365	70	0.97
 Coptisine cation *	Alkanes (n= 10-22)	1	367	132	0.98
 Coralyne cation *	Alkanes (n= 12-24)	0.8	410	217	0.99
	Alcohols (n= 10-22)	0.8	410	169	0.97

*Berberine: 60 ppm; coptisine: 15 ppm; coralyne: 6 ppm

Dipole-induced dipole interaction (TLC data):

$$\Phi_{DP,i} = \frac{U_k}{(4\pi\epsilon_0)^2} \frac{\alpha_i \cdot \mu_{DP}^2 + \alpha_{DP} \cdot \mu_i^2}{r^6} \longrightarrow \begin{cases} \Delta I_{DP,i} = A_{DP} + B_{DP} C_{DP,i} \\ C_{DP,i} = \alpha_i \mu_{DP}^2 + \alpha_{DP} \mu_i^2 \end{cases}$$

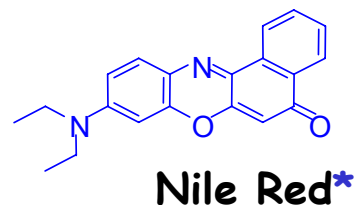
Analytes series

Sample load (μg)

λ_{exc} (nm)

B_p

r^2



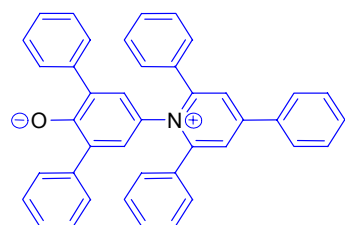
Alkanes (n= 16-24)

1

370

163

0.98



Alkanes (n= 16-24)

5

300

45

0.99

Alcohols (n= 10-18)

8

300

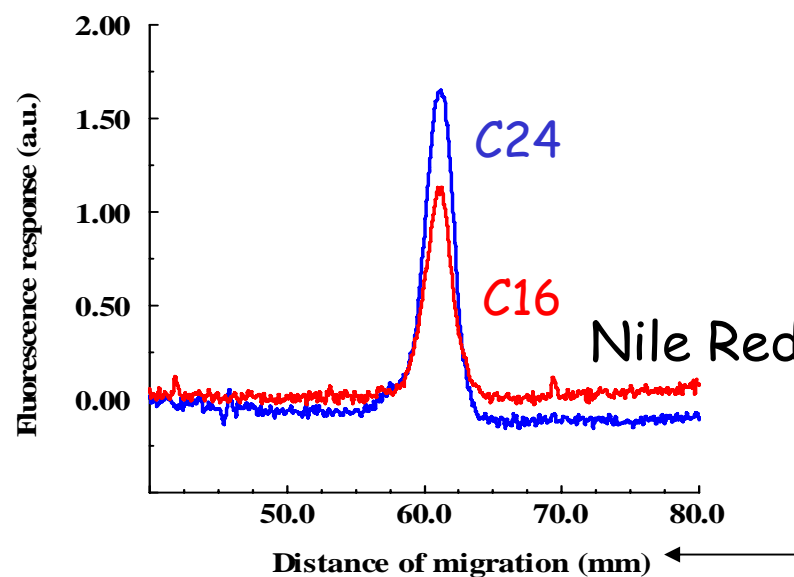
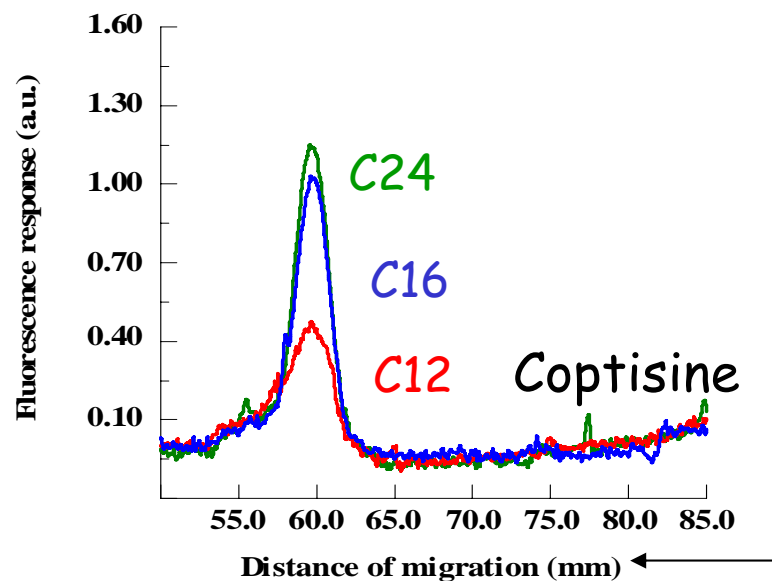
0.24

0.99

Reichardt's Dye*

Non-specific, dipolar-induced interactions between probe and the corresponding analyte contribute to the efficiency of the fluorescence emission, creating a microenvironment that isolates the fluorescent probe and prevents non-fluorescent decay mechanisms.

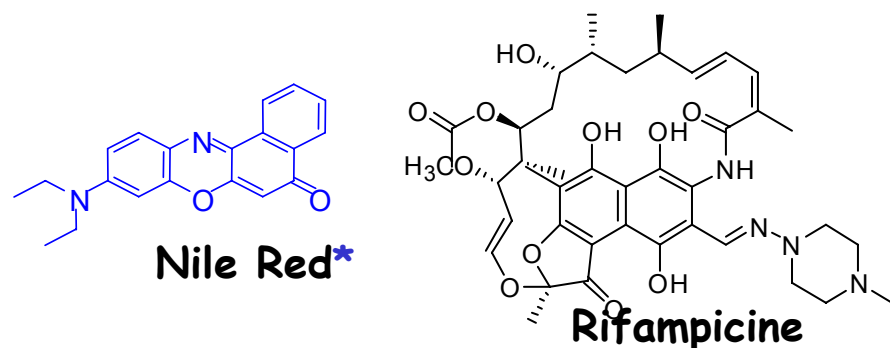
$$\uparrow \phi = \frac{k_r}{k_r + k_{nr}} \downarrow$$



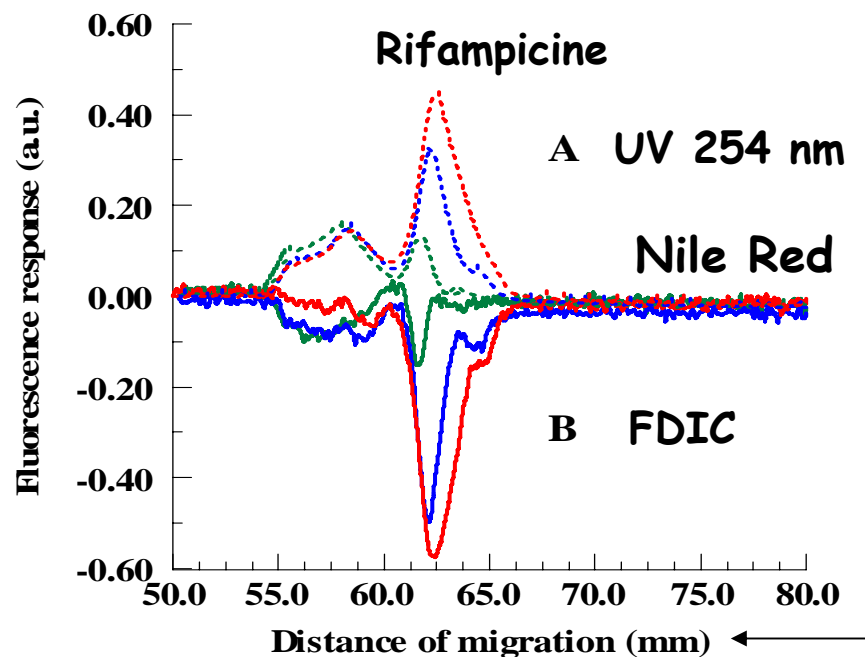
Planar TLC system favours analyte-fluorophore interaction

3.- Contribution to quenching: specific interactions

Negative peaks can be attributed to quenching produced by net specific interactions between polar compounds and probe



$$\downarrow \phi = \frac{k_r}{k_r + k_{nr}} \uparrow$$



The final emission for a given system depends on the balance between radiative and non-radiative decay processes which is determined by the balance between non-specific and specific, non-covalent interactions

Conclusions

- * FDIC is a general property of fluorophores in the presence of analytes. This has been deduced from models based on QED that considers k_r not in vacuum but in a medium of a certain refractive index, and on energy of electrostatic interaction theory.
- * Although the interactions involved are weak, it is possible to tailor sensitive TLC detection procedures based on FDIC through emission increases or quenchings between a probe and analytes in different media, through non-covalent interactions
- * Useful as a general detection method; for detecting non-fluorescent analytes; for selective detection; for obtaining information on interactions involved in a system

Acknowledgements

