Exploring Drug Interactions: A Sneak Peek with FIDA

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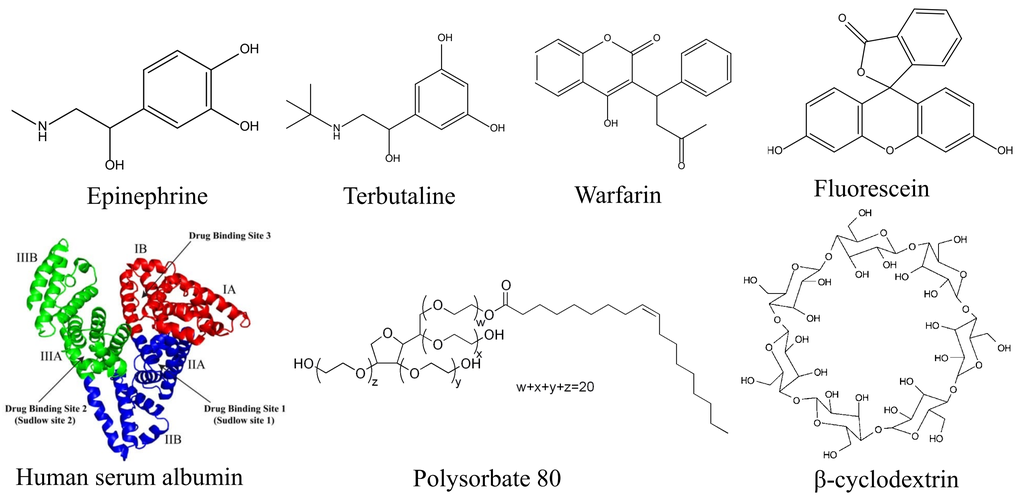
OBJECTIVES

Characterization of drug binding interactions is essential in formulation development. The broad applicability of FIDA for interaction studies was assessed towards:

- β-cyclodextrin binding with model drugs epinephrine, terbutaline, and warfarin.
- Model drug – surfactant interactions involving polysorbate-20 and polysorbate-80.
- Temperature dependence of fluorescein - human serum albumin binding (15 - 25℃).



METHODOLOGY

- Indicator (drug) solutions were prepared by mixing the indicator with buffer or analyte (β-CD, surfactant, HSA) in 67 mM phosphate buffer, pH 7.40.
- FIDA analysis: indicator plug was injected (50 mbar for 10 s) into the analyte-filled standard capillary, followed by mobilization with the analyte solution at 400/800 mbar. Flush between samples with 1 M NaOH and buffer.
- In-capillary mixing was used for β-CD study, and pre-mixing for surfactant and HSA studies.
- Epinephrine, terbutaline, and warfarin were detected at 280 nm, and fluorescein at 480 nm.

1. DRUG - β-CD INTERACTIONS

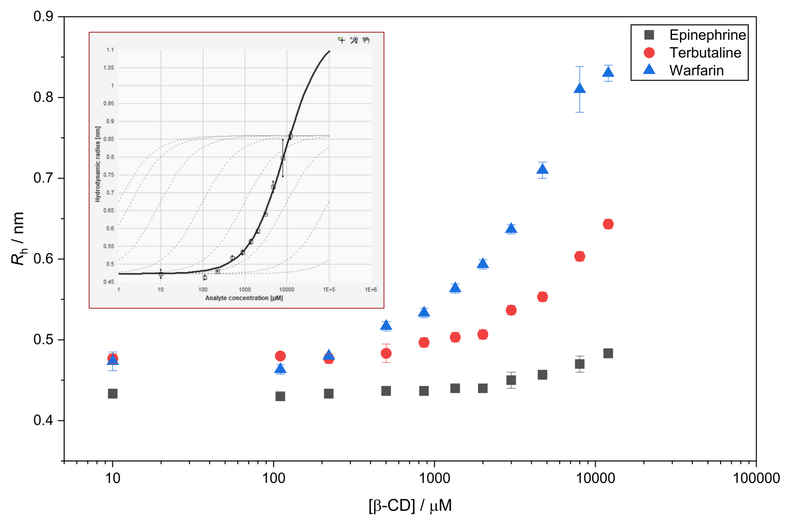


Fig 1.1: Binding of epinephrine, terbutaline, and warfarin to β-CD at 25℃ by in-capillary mixing. Strength of interaction is in the order warfarin > terbutaline > epinephrine. The insert shows a sample binding curve fitted to obtain the K_D.

Drug	K_D 25℃ (mM)	K_D 37℃ (mM)	K_D 45℃ (mM)	K_D 55℃# (mM)	K_D Reported value[1, 2]
Warfarin	$3.4 \pm 3.5 \times 10^{-1}$	$4.0 \pm 3.1 \times 10^{-1}$	$5.5 \pm 4.7 \times 10^{-1}$	$1.2 \times 10^{1} \pm 3.1$	$1.8 \pm 6.5 \times 10^{-2}$
Terbutaline	$1.1 \times 10^{1} \pm 1.3$	$1.7 \times 10^{1} \pm 6.2$	$6.5 \times 10^{1} \pm 3.1$	-	1.7
Epinephrine*	$2.9 \times 10^{1} \pm 1.1 \times 10^{1}$	-	-	-	2.7

Table 1.1: Drug - β-CD dissociation constants at different temperatures.
*Estimated K_D values are less reliable for epinephrine since the data points do not cover the complete binding isotherm.
#K_D estimation at high temperature was unreliable due to background fluorescence and noise.

FIDA detects weak interactions between model drugs and β-CD. K_D values are estimated assuming 1:1 binding. Limited solubility of β-CD (16.5 mM) prevents generation of full binding isotherms.

2. FLUORESCEIN - HSA BINDING

Temp ℃	15	25	37	45	55
K_D (μM)	$1.8 \times 10^{1} \pm 2.0$	$1.8 \times 10^{1} \pm 1.5$	$2.6 \times 10^{1} \pm 1.5$	$3.3 \times 10^{1} \pm 1.6$	4.4×10^{1}

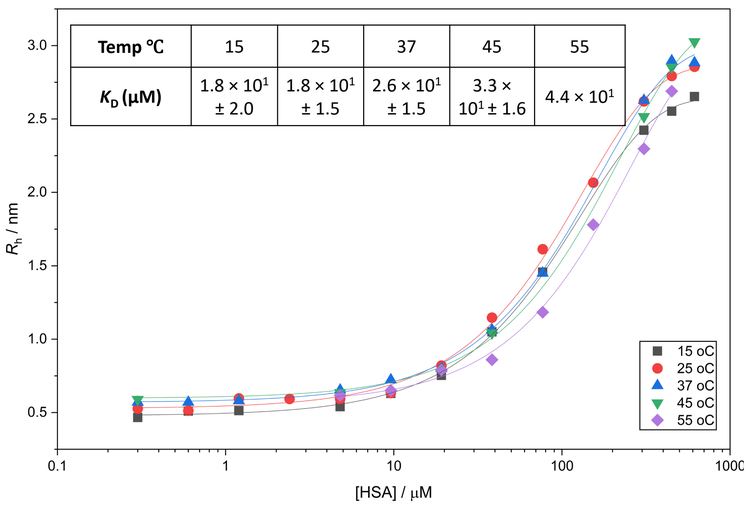


Fig 2.1: Fluorescein - HSA binding curves at 15-55℃.

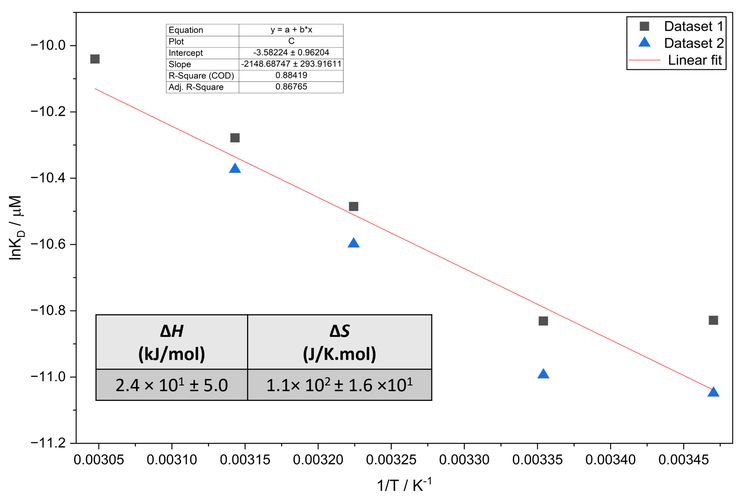


Fig 2.2: van't Hoff plot and thermodynamic parameters for fluorescein - HSA binding.

Fluorescein – HSA interactions can be described by 1:1 model when indicator concentration is kept low, e.g., [Fluorescein] << [HSA]. van´t Hoff plot shows variability between two independent series of experiments ([HSA] concentration measurement considered major source of variability).

3. DRUG - SURFACTANT INTERACTIONS

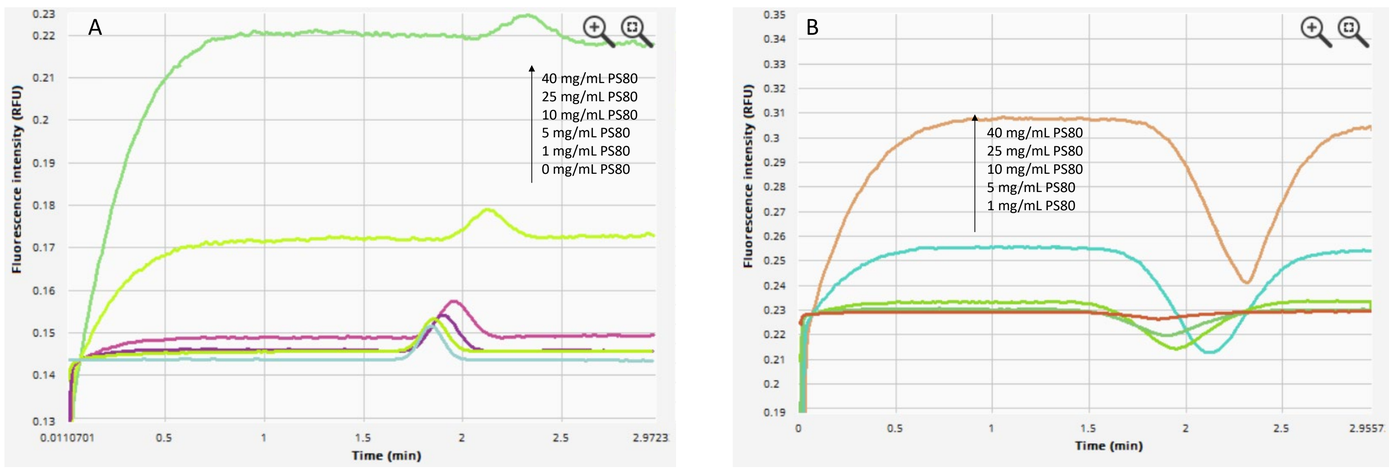


Fig 3.1: Taylorgrams: (A) 25 μM warfarin (premix) and (B) "buffer/blank" (in-cap) in presence of polysorbate 80 at 25℃ (λ_{em} = 280 nm).

FIDA detects increased (macro) viscosity with PS80 concentration. Auto-fluorescence of PS80 is used for measurement of PS80 micelle diffusivity (and sizing). Micelle R_h in good agreement with values from diffusion NMR [3] (R_h 4.2 -5.0 nm using FIDA vs 4.5 nm using NMR).

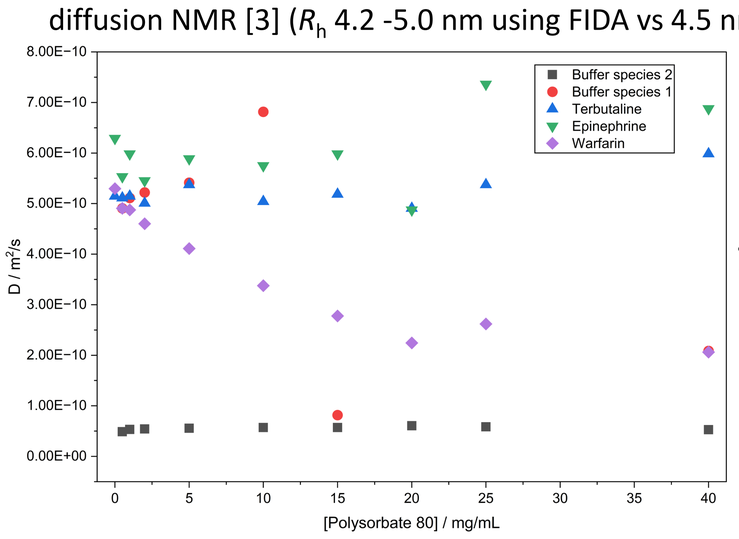


Fig 3.2: Diffusivity of PS80 micelles (species 2), low-MW species (species 1), terbutaline, epinephrine, and warfarin as a function of polysorbate 80 concentration at 25℃ using FIDA.

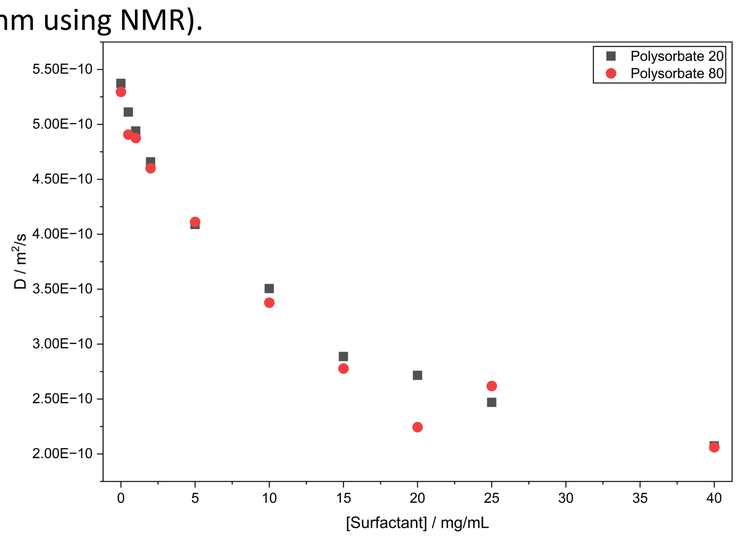


Fig 3.3: Change in the diffusion coefficient of warfarin with increasing surfactant concentration is plotted for polysorbate 80 and polysorbate 20. The effect on diffusivity is similar for both surfactants.

Diffusion coefficients obtained indicate that terbutaline and epinephrine do not interact with PS80 micelles in contrast to warfarin (Fig. 3.2). Warfarin interacts roughly to the same extent with PS20 and PS80. Note, viscosity compensation leads to over correction when determining Rh.
Next step is to estimate degree of drug interaction with polysorbates.

CONCLUSIONS

- Weak drug - β-CD interactions were detected by FIDA. K_D values estimated from incomplete binding isotherms. Additional support for binding obtained from changes in fluorescence intensity and fluorescence ratio.
- Temperature dependent binding (including van´t Hoff plot for assessing thermodynamic parameters) is determined using FIDA without additional material consumption.
- FIDA facilitates insight into surfactant/micelle properties (based on auto-fluorescence) and drug - surfactant interactions relevant to drug design.
- FIDA offers a versatile setup for assessing various types of drug interactions (requiring minimum of method development) with multiple readouts and low material consumption.

References:

1. Siva et al. (2012). *Physics and Chemistry of Liquids*, 50(4), 434–452.
2. Karadağ, et al. (1994). *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 20*, 23–32.
3. Lafitte et al. (2007). *Langmuir*, *23,* 10933-10939

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