Spectroscopic characteristics of the cationic dye basic orange 21

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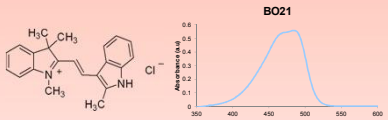
Leukocyte staining with the cationic dye, Basic Orange #21 (BO21), can improve leukocyte differential counting, routinely used to assess general health, help diagnose the cause of abnormal white blood cell count and monitor other diseases and conditions affecting specific leukocytes. The spectroscopic aspects of BO21 and their changes, upon interaction with biological molecules within the live cell in general, and with various leukocytes (white blood cells) in particular, were examined. Spectroscopic characteristics were studied in bulk solutions under several conditions (pH, viscosity) and with various solvents (salts, proteins). The focus of this study is the influence of Heparin, an organic anion common to a specific type of leukocyte, on the BO21 spectra, and the mechanism of the interaction.

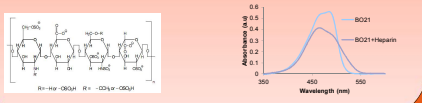
Basic Orange # 21 (BO21)

Basic Orange # 21 is also known as Astrazon Orange G and Albright Orange. Its absorption spectrum has a peak at 484nm. This spectrum shows no sensitivity to changes in solute pH, viscosity, salts or proteins.



Heparin

Heparin, an organic anion common to a specific type of leukocyte, causes a blue shift of the spectra to 465 nm.

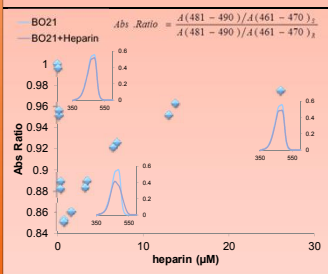


BO21-Heparin Interaction

electrostatic bond: cation - polyanion

		Absorbance at 484 nm	
Replacement	Concentration	BO21+Rep	BO21+Rep.+Hep
H_2O		**0.59**	**0.49**
PBS	0.15M	0.57	0.57
$(NH_4)SO_4$	0.2M	0.58	0.59
NaAC (CH_3COONa)	0.2M	0.58	0.59
NaCl	0.2M	0.58	0.58

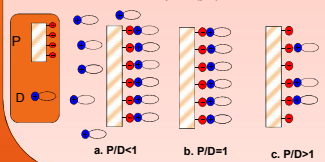
Electrostatic interaction was examined by replacing Heparin ion with inorganic salts. No significant change in BO21 absorption was observed.



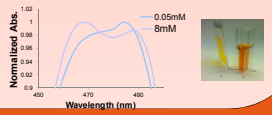
BO21 aggregation

BO21(D) tends to bind Heparin in its bonding sites (P):

a. P/D <1; Fully, in the presence of spares D.
b. P/D ~1; Fully, with no spares D.
c. P/D >1; Partially, with no spares D.



High concentration BO21 absorption spectrum measurements in thin layer result in the same blue shift



Fluorescence Polarization (FP) and Life Time (FLT)

$$FP = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Results: FP of BO21
In water > 0.450
In water, in the presence of Heparin < 0.200

Perrin equation:

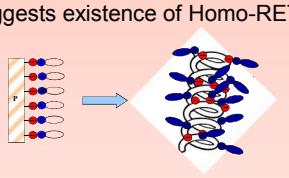
$$\left(\frac{1}{p} - \frac{1}{3}\right) = \left(\frac{1}{p_0} - \frac{1}{3}\right)\left(1 + \frac{\tau_f}{\tau_c}\right)$$

Presence of Heparin does not alter FLT of BO21:

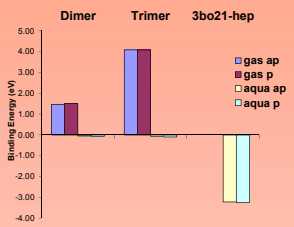
$$\tau^f_{BO\ 21} = 0.267 \pm 0.123\,[ns]$$

$$\tau^f_{BO\ 21+Heparin} = 0.35 \pm 0.205\,[ns]$$

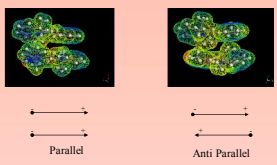
This suggests existence of Homo-RET

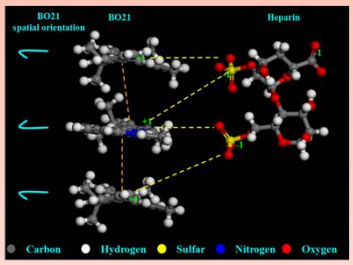


BO21-Heparin Bonding Energy



We propose a mechanism based on computational quantum chemistry. According to this model the molecular electrical dipoles of BO21 dimers and trimers are oriented in a parallel fashion. Surprisingly, the related binding energies are even lower than those calculated for anti-parallel arrangements.

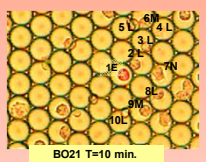


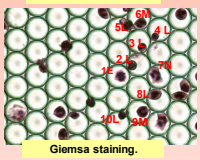


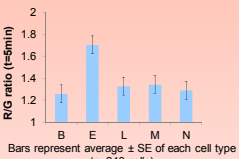
The BO21-Heparin complexes formed are governed by electrostatic interaction wherein the positive charge of BO21 interacts with the negative charge of Heparin. Additionally, π-cation interactions between stacked BO21 molecules stabilize the complexes.

Quantum calculations were performed with the DMol³ module in Material Studio (Accelrys, USA)

BO21 stained leukocytes inspection in live cell array (LCA)







Following image acquisition of live BO21-stained leukocytes, same sample in LCA was fixed in-situ and giemsa stained in order to correlate between types of leukocytes and BO21 red/green (R/G) ratio.
Initial results indicated that R/G ratio of BO21 stained eosinophil is significantly higher than that of other sub types of leukocytes.

Conclusions

1. Of the intracellular molecules, Heparin seems to be the cause for the blue shift in absorption spectrum of BO21.
2. The mechanism that stands behind this shift is the induction of high orders of BO21 structures, rather than coulombic interaction between heparin and BO21.
3. Low FP values of Heparin-bound BO21 are due to Homo-RET.

Reference

Kasdan, Harvey L., et al. "The white IRIS leukocyte differential analyzer for rapid high-precision differentials based on images of cytoprobe-reacted cells." *Clinical chemistry* 40.9 (1994): 1850-1861.