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ARTICLE

Isatin *N*-phenylsemicarbazone: Effect of substituent and concentration on anion sensing selectivity and sensitivity

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The effect of substituent and concentration on anion sensing selectivity and sensitivity of nine new easily synthesized isatin *N*-phenylsemicarbazone *E*-isomer sensors ***IIIa***-***XIa*** was investigated. The substantial difference between the association constants for ***IIIa***-***XIa*** interaction with strongly and weakly basic anions allows detection of F^- or CH_3COO^- anions even at high weakly basic anion excess. Substitution in position 5- of the isatin ring and *para*- substitution on the phenyl ring in the phenylsemicarbazide chain influence sensor:anion complex stoichiometry and thus also sensor sensitivity. Detection limits of $3\text{-}4\times10^{-7}$ mol dm^{-3} for F^- and CH_3COO^- anions by sensors ***IVa*** and ***Va*** bearing electron-withdrawing substituents are among the lowest published detection limits for these anions in organic solvents. The high selectivity and sensitivity of sensor ***VIa*** allows confident F^- detection at tolerated fluoride drinking-water level. The solution dilution leads to dramatic change in sensor selectivity. Consequently, one *E*-isomer can be used to sense both strongly and weakly basic anions. On the other side, higher sensor solution concentrations increase F^- and CH_3COO^- anion detection range, similar to the additional *Z*-isomer utilization.

Introduction

Ion and neutral molecule recognition and signalling are currently amongst the most intense emerging areas of supramolecular chemistry. While anions play a very important role in many chemical and biological processes, their identification and quantification are quite complex; especially in biological systems. One way to overcome this problem is to utilize new optical receptors specifically designed for individual anions. Supramolecular chemistry has focused on the development of selective colorimetric or fluorescent anion sensors for the last twenty years [1-4], and researches including Wenzel, Chudzinski, Bergamaschi, Jiménez, Martínez-Máñez and Gale continue this work.[5-10]

Anions and receptors interact together particularly through hydrogen bonding interactions.[4,11-14] Due to the facile synthesis and easily-tunable NH acidity, amides, ureas and thioureas persist as the most widely employed hydrogen bond donor groups in anion receptor systems.[10]

Anion induced tautomerism and the light and thermally initiated mutual *E*- and *Z*-isomer transformations of two efficient and easily synthesized isatin *N*-phenylsemicarbazone colorimetric sensors were investigated in our recent three studies (ESI Scheme S1†).[15-17] Therein, the interaction of F^-, AcO^-, $H_2PO_4^-$, Br^- and HSO_4^- anions with *E*- and *Z*-isomers of isatin-3-4-phenyl(semicarbazone) ***I*** and *N*-methylisatin-3-4-phenyl(semicarbazone) ***II*** as sensors influenced the equilibrium ratio of the individual sensor tautomeric forms in the liquid phase.

The *E*- and *Z*- sensor isomers differed in sensitivity, selectivity and sensing mechanism. UV-Vis spectroscopy readily determined the equilibrium ratio of the individual tautomeric forms affected by; (1) the inter- and intra-molecular interaction modulation of isatinphenylsemicarbazone molecules by anion induced changes in receptor molecule solvation shells and (2) the sensor-anion interaction with urea hydrogens. Appropriate selection of experimental conditions resulted in a high degree of sensor selectivity for some investigated anions. Sensors ***Ia***-***IIb*** provided both excellent signal to noise ratio and wide detection range. Detection of F^- or CH_3COO^- anions at high weakly-basic anion excess was also possible. Furthermore, due to excellent *E* isomer sensitivity in organic media, these isomers can be used for F^- or CH_3COO^- sensing in semi-aqueous media. The photodegradation of *E*-isomer anion sensors ***Ia*** and ***IIa*** due to light initiated *E-Z* isomerization may complicate the anion detection, therefore care should be taken when interpreting data for quantitative determination of anions using ***Ia*** and ***IIa*** *E*-isomers. However, photochemical *E-Z*

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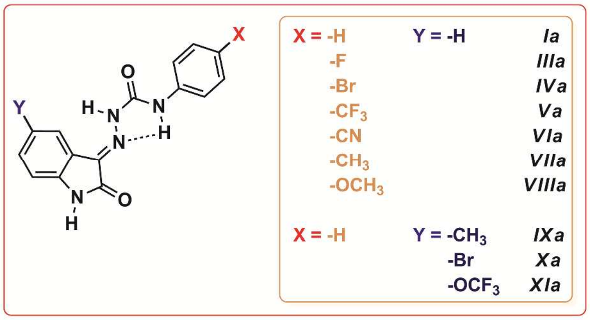
isomerization efficiency is relatively low at $\Phi_{E\text{-}Z} < 0.01$, and this allows reliable detection of strongly basic anions using ***Ia*** or ***IIa***. In addition, the easy *E*-isomer transformation to the corresponding *Z*-isomer and the utilization of both isomers significantly increases the detection range for F^- and CH_3COO^- anions valid for ***Ia*** or ***IIa*** in organic media from 10^{-6}-10^{-4} mol dm^{-3} to 10^{-6}-10^{-2} mol dm^{-3} of F^- or CH_3COO^-. Although zero efficiency of back photochemical *Z-E* isomerization excludes the use of isatinphenylsemicarbazones ***I*** and ***II*** as molecular switches, the absence of thermally initiated *E-Z* isomerization and both photochemically and thermally initiated back *Z-E* isomerizations in strongly interacting polar solvents are beneficial for ***Ia*** and ***IIa*** *E*-isomer application in chemical actinometry.

Strongly basic anions influence the solvation shell of the *E*-isomer self-associate, and this interaction leads to formation of the non-associated hydrazide. Concurrently, strongly basic anions interact mostly with the highly acidic NH hydrogen of hydrazide resulting in hydrazonol formation. This is sharply contrasted with most urea and thiourea based sensors which interact with CH_3COO^- in double hydrogen bonding to the Y-shaped urea/thiourea moiety.[9,18,19,...] The reason for this anomalous isatin *N*-phenylsemicarbazones behaviour is the lower energy of its *s-cis*, *s-trans* –NHCONH– structural fragment conformation compared to the Y-shaped *s-trans*, *s-trans* –NHCONH– arrangement.[17]

Herein, the substituent and dilution effect on anion sensing of nine new isatin *N*-phenylsemicarbazone *E*-isomers ***IIIa***-***XIa*** is reported (Scheme 1); including the determination of light initiated *E-Z* isomerization quantum yield ($\Phi_{E\text{-}Z}$). The properties of these nine sensors with various substituents in position 5- of the isatin or in the phenyl ring *para*- position are compared with those for the unsubstituted ***Ia*** *E*-isomer. This research is necessary for practical application of these compounds as anion sensors and also for development and design of new effective anion sensors. In addition, concepts of sensing selectivity, sensitivity, detection range and detection limit for these colorimetric sensors, together with mathematical analysis of the well-known relation for association constants K_{ass} determination, are discussed in this paper.



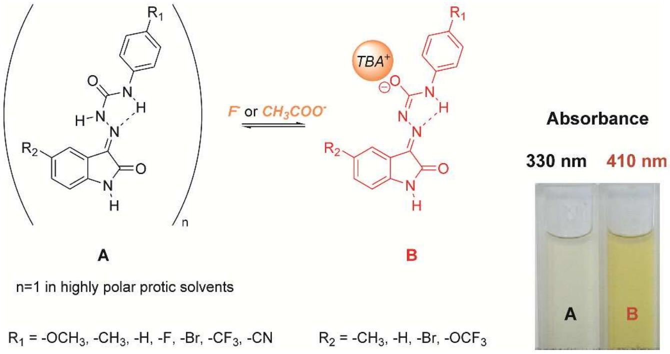
Scheme 1. Molecular structure of studied isatin *N*-phenylsemicarbazones ***IIIa***-***XIa***.

Results and Discussion

Sensor Selectivity

Anion presence affects the degree of ***IIIa***-***XIa*** self-association in the DMF solution through intermolecular interactions.[16] In the presence of strongly basic anions (F^- and CH_3COO^-), the ***IIIa***-***XIa*** *E*-isomers transform from their self-associate hydrazide A (keto) form to hydrazonolate (enolate) form B (Scheme 2).

This transformation is accompanied by a decrease in absorption intensity at approximately 330 nm and increased absorption at approximately 410 nm (Fig. 1 and ESI Figs. S1†, S2†).



Scheme 2. Presumed interaction of derivatives ***Ia*** and ***IIIa***-***XIa*** with F^- (or CH_3COO^-) anions during ***Ia*** solution titration with TBA^+F^- (or $TBA^+CH_3COO^-$).

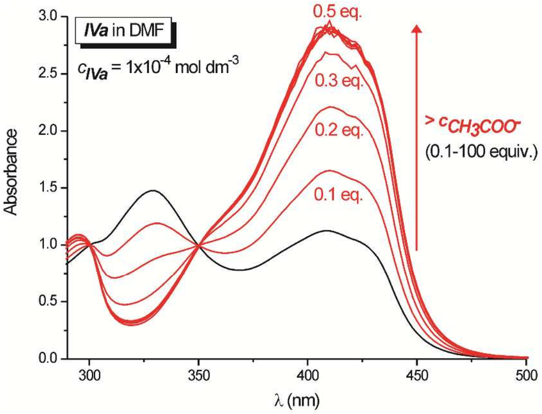


Fig. 1. Evolution of the UV-VIS spectrum of isatin *N*-phenylsemicarbazone *E*-isomer ***IVa*** in DMF during ***IVa*** solution titration with $TBA^+CH_3COO^-$ (c_{IVa} = 1×10^{-4} mol dm^{-3}; $c_{CH3COO-}$ = 0 mol dm^{-3}, 1×10^{-5} mol dm^{-3}, 2×10^{-5} mol dm^{-3}, 3×10^{-5} mol dm^{-3}, 4×10^{-5} mol dm^{-3}, 5×10^{-5} mol dm^{-3}, 1×10^{-4} mol dm-3, 5×10^{-4} mol dm^{-3}, 1×10^{-3} mol dm^{-3} and 1×10^{-2} mol dm^{-3}).

Similar to the result observed for ***Ia***, the addition of weakly basic anions such as Br^- and HSO_4^- shifts the tautomeric hydrazide/hydrazonol equilibrium for all substituted ***IIIa***-***XIa*** derivatives to the hydrazide form A. Absorption band intensity at approximately 410 nm decreases in the presence of these anions in contrast to the F^- and CH_3COO^- effect (ESI Fig. S3†, S4†).

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Because the anion-receptor 1:1 K_{ass} constants higher than 10^5 cannot be determined precisely by UV-VIS spectroscopy (see Appendix A), sensor selectivity was investigated in DMF:H_2O (9:1; v/v) solvent mixture (Table 1; ESI Fig. S5†, S6†). However, the large K_{ass} sensitivity to additional anion-receptor interactions in the high anion concentration region does not allow correct selectivity comparison for ***IIIa-XIa*** sensors from their K_{ass} values alone (see Appendix B).

Interestingly, the –CN, –Br and –OCF_3 electron-withdrawing substituents in ***VIa***, ***IXa*** and ***Xa*** change the sensor:anion binding stoichiometry to 2:1 and thus further complicates ***IIIa-XIa*** K_{ass} comparison. In these cases, one anion binds two sensor molecules (Table 1; ESI Fig. S7†).

Table 1. Association constants K_{ass} (mol^{-1} dm^3) of the studied *E*-isomers of isatin *N*-phenylsemicarbazones ***Ia*** and ***IIIa-XIa*** with anions in DMF at 298.16 K.

Anion Compd	Association constants –K_{ass}			
	F$^-$	R^2	*CH_3COO^-*	R^2
Ia	$(6.7 \pm 0.2) \times 10^3$	0.9963	$(1.6 \pm 0.5) \times 10^4$	0.9673
IIIa	$(5.2 \pm 0.2) \times 10^3$	0.9906	$(7.7 \pm 0.2) \times 10^3$	0.9934
IVa	$(1.2 \pm 0.1) \times 10^4$	0.9810	$(1.2 \pm 0.1) \times 10^4$	0.9864
Va	$(2.9 \pm 0.7) \times 10^4$	0.9421	$(2.2 \pm 0.3) \times 10^4$	0.9710
VIa	$(5.6 \pm 1.8) \times 10^4$	0.9869	$(8.6 \pm 4.1) \times 10^{6a}$	0.9581
VIIa	$(5.3 \pm 0.1) \times 10^3$	0.9784	$(9.0 \pm 0.6) \times 10^3$	0.9898
VIIIa	$(5.4 \pm 0.8) \times 10^3$	0.9853	$(1.2 \pm 0.0) \times 10^4$	0.9874
IXa	$(5.1 \pm 0.6) \times 10^4$	0.9942	$(4.8 \pm 2.3) \times 10^{7a}$	0.9728
Xa	$(5.7 \pm 3.2) \times 10^{7a}$	0.9663	$(1.7 \pm 0.9) \times 10^{8a}$	0.9574
XIa	$(2.4 \pm 0.1) \times 10^3$	0.9967	$(1.3 \pm 0.1) \times 10^4$	0.9697

[a] – sensor:anion 2:1 complex – K_{ass} in mol^{-2} dm^6; $c_{E\text{-isomer}}$ = 1×10^{-4} mol^{-1} dm^3; R^2 – coefficient of determination (average value)

Despite the above mentioned complexities encountered in K_{ass} determination for 1:1 ***IIIa-XIa*** complexes with strongly basic anions, the K_{ass} values higher than 1×10^4 for ***IIIa-XIa***:*F*$^-$ and ***IIIa-XIa***:*CH_3COO^-* 1:1 complexes and the K_{ass} above 1×10^7 for sensor:anion 2:1 complexes indicate exceptionally strong *F*$^-$ and *CH_3COO^-* anion binding. Therefore, strongly basic anion addition leads rather to the NH group deprotonation than to the hydrazide/hydrazonol tautomeric equilibrium shift resulting in sensor:hydrazonol 1:1 complex formation bounded by one hydrogen bond (as we assumed in our previous article).[16] Absorbance increase at 410 nm during titration process in non-polar $CHCl_3$, where the hydrazonol is not formed, supports this conclusion.

In the case of sensor:anion 2:1 complexes, one aggregate (dimer) molecule is transformed to hydrazonolate B and the second hydrazide molecule is stabilized by the solvent as hydrazonol form. The tendency to form 2:1 complexes therefore correlates fairly well with the hydrazonol form stabilization by solvation that increases in isatin *N*-phenylsemicarbazones with electron-withdrawing substituents (ESI Figs. S8†, S9†).

Substantial difference between the K_{ass} values for ***IIIa-XIa*** interaction with strongly and weakly basic anions allows detection of *F*$^-$ and *CH_3COO^-* anions even at high weakly basic anion excess (K_{ass}=10^1-10^2 for *Br*$^-$ or *HSO_4^-* anions).

Sensor Sensitivity

Sensitivity denotes sensor ability to detect an analyte at a specified concentration. Sensor sensitivity depends not only on sensor-anion interaction strength (K_{ass}) but also on sensor signal changes following anion-binding.

The sensitivity of isatin *N*-phenylsemicarbazone ***IIIa-XIa*** *E*-isomers was compared by introducing the Γ sensitivity parameter which describes detection-wavelength absorbance changes in the presence of 1 equivalent of anion.

Substitution in position 5- of the isatin ring and *para*-substitution in the phenylsemicarbazide chain phenyl ring influence Γ value (Fig. 2 and ESI Fig. S10†). Although mainly electron-withdrawing substituents increase the Γ sensor sensitivity value, Γ increase correlates rather with the sensor ability to form 2:1 complexes than with the increasing strength of electron-withdrawing group. However, the absence of sensitivity icrease in ***Xa*** forming the most stable 2:1 complex (Table 1) and the sensitivity order change in DMF compared to DMF:H_2O solvent mixture point out the complexity of sensor/sensor, sensor/anion and sensor/solvent interactions (Fig. 2 and ESI Fig. S10†). Combination of these processes creates the complexity of resultant substituent influence on sensor sensitivity.

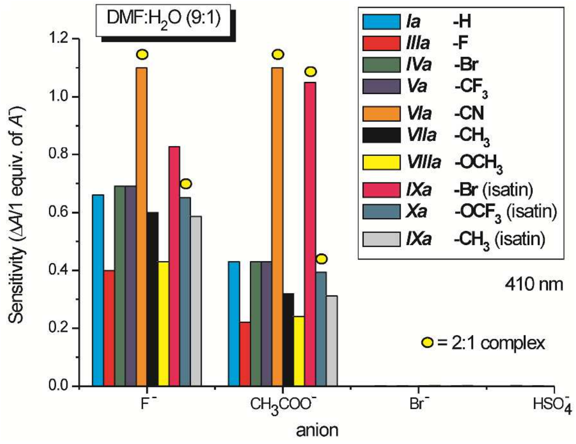

Fig. 2. The sensitivity Γ (to 1 equiv. of A^-) of the studied *E*-isomers of isatin *N*-phenylsemicarbazones ***Ia*** and ***IIIa-XIa*** at 410 nm for anions in DMF:H2O (9:1; v/v) solvent mixture at 298.16 K ($c_{E\text{-isomer}}$ = 1×10^{-4} mol^{-1} dm^3 for *F*$^-$ and *CH_3COO^-* sensing and $c_{E\text{-isomer}}$ = 1×10^{-5} mol^{-1} dm^3 for *Br*$^-$ and *HSO_4^-* sensing).

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The ***IIIa***-***XIa*** *E*-isomer sensitivity for weakly basic Br^- and HSO_4^- anions at 10^{-5} mol dm^{-3} sensor concentration in solution is almost 1000-times lower than for F^- and CH_3COO^- anions.

Detection limit (3σ/S) is the lowest concentration level determined statistically different from a blank with 99% confidence and quantification limit (10σ/S) is the level above which quantitative results are obtained with a specified degree of confidence. The calculated detection and quantification limits for F^-, CH_3COO^-, Br^- and HSO_4^- anions using the most important isatin *N*-phenylsemicarbazone *E*-isomer sensors from the ***Ia***-***XIa*** set are summarized in Table 2. The determined 3σ/S for strongly basic F^- and CH_3COO^- anions by sensors ***IVa*** and ***Va*** are amongst the lowest published detection limits for these anions in organic solvent.[20-23] At 10^{-5} mol dm^{-3} sensor concentration, the tautomeric hydrazide-hydrazonol equilibrium is completely shifted to the hydrazonol side, and isatin *N*-phenylsemicarbazones can be used as sensors for weakly basic anions. Sensor solution dilution thus leads to dramatic change in sensor selectivity. Therefore, the one *E*-isomer can be used for both strongly and weakly basic anion detection. The determined 3σ/S for Br^- and HSO_4^- anions in DMF for some isatin *N*-phenylsemicarbazone sensors is highlighted in Table 2. Their values are in the 0.2-0.4 mmol dm^{-3} range.

Table 2. Detection (3σ/S) and quantification (10σ/S) limits in μmol dm^{-3} for selected anions using the most important isatin *N*-phenylsemicarbazone *E*-isomer sensors from the ***Ia***-***XIa*** set in DMF at 298.16 K (refer to absorbance at 410 nm).

	3σ/S [μM]				**10σ/S** [μM]			
Anion Compd	F^- (a)	CH_3COO^- (a)	Br^- (b)	HSO_4^- (b)	F^- (a)	CH_3COO^- (a)	Br^- (b)	HSO_4^- (b)
Ia	0.7	-	-	-	2.3	-	-	-
IVa	0.4	0.3	280	200	1.3	1.0	930	680
Va	0.3	1.0	290	-	1.1	3.5	960	-
VIa	1.0	0.6	-	-	3.2	2.0	-	-
VIIa	0.5	0.9	-	-	1.6	3.0	-	-
IXa	-	1.3	-	-	-	4.5	-	-
Xa	-	-	320	280	-	-	1100	930
XIa	-	-	220	-	-	-	720	-

μM = μmol dm^{-3} = 10^{-6} mol dm^{-3}; [a] – $c_{E\text{-isomer}} = 1 \times 10^{-4}$ mol^{-1} dm^3; [b] –$c_{E\text{-isomer}} = 1 \times 10^{-5}$ mol^{-1} dm^3

Detection Range

Detection range is an important factor in anion sensing. The utilization of *Z*-isomers with lower sensitivity can significantly increase the F^- or CH_3COO^- anion detection range valid for ***IIIa***-***XIa*** in organic media from approximately 0.005-1 equivalent to 0.005-100 equivalent of isatin *N*-phenylsemicarbazone (from 5×10^{-7}- 10^{-4} mol dm^{-3} to 5×10^{-7}- 10^{-2} mol dm^{-3} of F^- and CH_3COO^-)(Figs. 3 and S11†). The photochemical *E*-*Z* isomerization efficiency for ***IIIa***-***XIa*** is relatively low at $\Phi_{E\text{-}Z} < 0.01$. This is similar to the situation in the unsubstituted ***Ia*** *E*-isomer and it allows ***IIIa***-***XIa*** reliable detection of strong basic anions (ESI Table S1†).

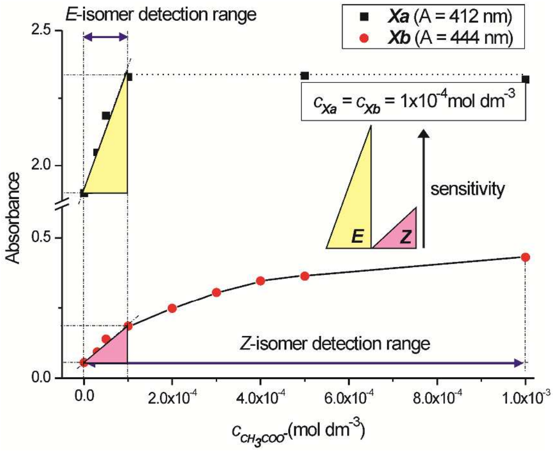

Fig. 3. CH_3COO^- anion detection range and the sensitivity of ***Xa***-*E*- and ***Xb***-*Z*-isomer sensors.

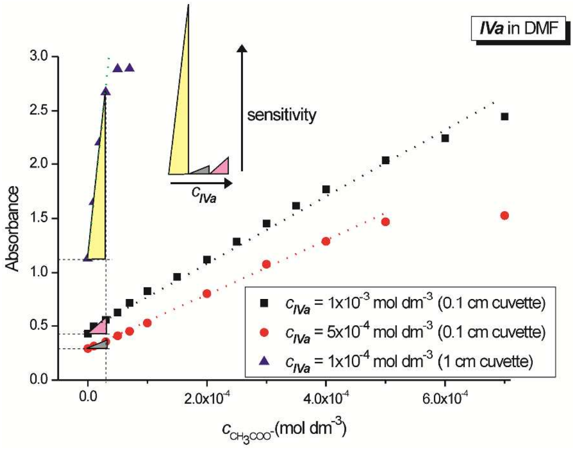

Fig. 4. CH_3COO^- anion detection range increase using *E*-isomer sensor ***IVa*** accomplished by sensor concentration increase and simultaneous decrease in optical path length.

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Furthermore, the $\Phi_{E\text{-}Z}$ values for substituted ***IIIa-XIa*** *E*-isomers at 10^{-4} mol dm^{-3} concentration are approximately 2-fold lower than for the unsubstituted ***Ia*** *E*-isomer. $\Phi_{E\text{-}Z}$ decrease following electron-withdrawing substitution in position 5- of the isatin ring and also in *para*- position on the phenyl ring is connected with the enhanced tendency towards hydrazonol formation (ESI Figs. S8†, S9†).[15] Unexpected $\Phi_{E\text{-}Z}$ decrease following also electron-donating substitution is probably linked to the enhanced aggregate variability. Neither excitation nor solution heating *Z*-isomers led to back conversion to the corresponding *E*-isomers; similar to the situation in the unsubstituted ***Ia*** *Z*-isomer.

Sensor concentration increase and simultaneous optical path length decrease also increase *E*- isomer sensors ***IIIa-XIa*** anion detection range (Fig. 4). Although increased ***IVa*** concentration decreases sensor sensitivity, it allows acetate anion detection range increase from 3×10^{-7}-5×10^{-5} mol dm^{-3} to 3×10^{-7}-1×10^{-3} mol dm^{-3} CH_3COO^-.

Anion detection in aqueous media

Detection of anions in aqueous environments is currently one of the most interesting target in the chemosensor field.[19] However, this is unachievable for most designs relying on hydrogen bonding, since even minute amounts of water disrupt these interactions. Moreover, sensor molecules are often such complex constructs that several synthetic steps are required for their preparation.[10,20,...]

E-isomers ***IIIa-XIa*** are synthesized in a one step facile condensation of isatine with the corresponding phenylsemicarbazide in high reaction yield. Due to excellent ***IIIa-XIa*** *E*-isomer sensitivity in organic media, these isomers can also be used for F^- or CH_3COO^- sensing in semi-aqueous media (Fig. 5).

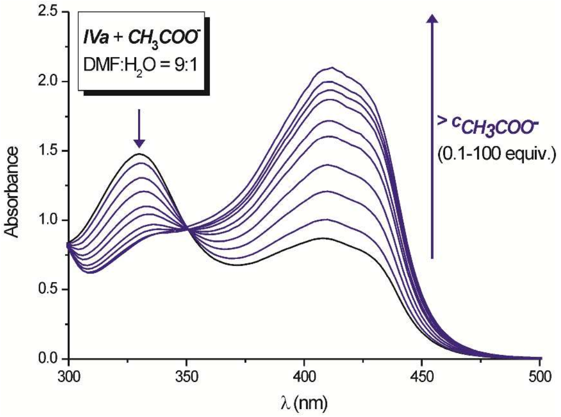

Fig. 5. Evolution of the UV-VIS spectrum of isatin *N*-phenylsemicarbazone *E*-isomer ***IVa*** in DMF:H2O 9:1 solvent mixture during IVa solution titration with TBA^+ CH_3COO^- ($c_{Ia} = 1\times10^{-4}$ mol dm^{-3}; $c_{F-} = 0$ mol dm^{-3}, 1×10^{-5} mol dm^{-3}, 5×10^{-5} mol dm^{-3}, 1×10^{-4} mol dm^{-3}, 2×10^{-4} mol dm^{-3}, 3×10^{-4} mol dm^{-3}, 5×10^{-4} mol dm^{-3}, 7×10^{-4} mol dm^{-3}, 1×10^{-3} mol dm^{-3} and 1×10^{-2} mol dm^{-3}).

The determined low detection limit $3\sigma/S = 1.3\times10^{-6}$ mol dm^{-3} ($10\sigma/S = 4.3\times10^{-6}$ mol dm^{-3}) for F^- anions in 9:1 DMF:H_2O mixture by sensor ***VIa*** allows the confident F^- detection at the tolerated fluoride level of 1.5 mg L^{-1} (0.17 mmol dm^{-3}) in drinking water. On the other side, *E*-isomer ***IXa*** with low $3\sigma/S = 1.4\times10^{-6}$ mol dm^{-3} for CH_3COO^- in 9:1 DMF:H_2O mixture can be a useful sensor for CH_3COO^- determination in biological samples with low F^- concentration. However, because the addition of strongly basic OH^- anions has the same effect as F^- or CH_3COO^- anion addition, care must be taken when interpreting data in semi-aqueous media from aqueous samples at pH above 10.[16] In addition, the $CO_2(g)/HCO_3^-/CO_3^{2-}$ equilibrium may also play an important role.

Conclusion

This paper investigated the effect of substituent and concentration on anion sensing selectivity and sensitivity of nine new easily synthesized isatin *N*-phenylsemicarbazone *E*-isomer sensors ***IIIa-XIa***.

Although the large K_{ass} sensitivity to additional anion-receptor interactions in the high anion concentration region does not allow correct selectivity comparison for ***IIIa-XIa*** sensors from their K_{ass} values, it can be concluded that the K_{ass} values higher than 1×10^4 in DMF:H_2O (9:1; v/v) solvent mixture and K_{ass} higher than 1×10^7 for 2:1 anion-sensor interactions indicate exceptionally strong F^- and CH_3COO^- anion binding. Addition of strongly basic anion leads to the NH group deprotonation and not to the sensor:hydrazonol 1:1 complex formation that we assumed in our previous article. In the case of sensor:anion 2:1 complexes, one aggregate (dimer) molecule is deprotonated to hydrazonolate B and the second hydrazide molecule is stabilized by solvation as the hydrazonol form. The tendency to form 2:1 complexes correlates fairly well with the hydrazonol form stabilization that increases in isatin *N*-phenylsemicarbazones with electron-withdrawing substituents. Substantial difference between the K_{ass} values for ***IIIa-XIa*** interaction with strongly and weakly basic anions allows the detection of F^- or CH_3COO^- anions even at high weakly basic anion excess. Although mainly electron-withdrawing substituents increase the sensor sensitivity (Γ parameter), Γ increase correlates rather with the sensor ability to form 2:1 complexes than with the increasing strength of electron-withdrawing group.

Although photodegradation of ***IIIa-XIa*** *E*-isomer anion sensors due to light initiated *E-Z* isomerization can complicate anion detection, the photochemical efficiency for substituted ***IIIa-XIa*** *E*-isomers at 10^{-4} mol dm^{-3} concentration is even approximately 2-fold lower than for the unsubstituted ***Ia*** *E*-isomer, and this allows reliable detection of strong basic anions. Moreover, utilization of both *E*- and *Z*-isomers increases the F^- and CH_3COO^- anion detection range for ***IIIa-XIa*** in organic media from approximately 5×10^{-7}- 1×10^{-4} mol dm^{-3} to 5×10^{-7}-10^{-2} mol dm^{-3}. A further efficient method of increasing anion detection range up to 10^{-3} mol dm^{-3} for ***IIIa-***

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XIa *E*-isomer sensors is simultaneous sensor concentration increase and optical pathway length decrease.

Sensor ***VIa***'s low detection limit for F^- anions in 9:1 DMF:H_2O mixture and the simultaneous absence of detectable CH_3COO^- anion levels allow confident F^- detection at tolerated drinking-water fluoride level.

Appendix A. Behaviour of the $A = f(c_{A-})$ function.

$A = f(c_{A-})$ curve behaviour at boundary conditions (A_{lim} = const or K_{ass} = const, respectively) and sensor concentration of 1×10^{-4} mol dm^{-3} is depicted in Figs. A1 and A2. Fig. A2 shows that steepness of the $A = f(c_{A-})$ curve and thus sensor sensitivity are directly linked to the K_{ass} value for the sensor:anion 1:1 complex describing sensor selectivity; particularly in the low K_{ass} region. However, $A = f(c_{A-})$ curve steepness does not depend on further K_{ass} increase in the high log K_{ass} >5 region. Therefore, K_{ass} higher than 10^5 cannot be determined precisely by UV-VIS spectroscopy.

The $A = f(c_{A-})$ function, described by Eq. (A1),

$$A = A_0 + 1/2c_0(A_{lim} - A_0)\left[c_0 + c_{A^-} + 1/K_{ass} - \sqrt{(c_0 + c_{A^-} + 1/K_{ass})^2 - 4c_0c_{A^-}}\right]$$

, (A1)

can be easily modified in a simple manoeuvre: The radicand in this equation is positive for arbitrary K_{ass} >0 (and K_{ass} is always higher than 0) because:

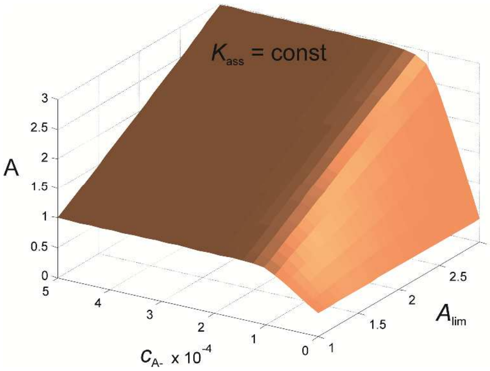

Fig. A1. $A = f(c_{A-})$ curve behaviour at boundary conditions (K_{ass} = const = 1×10^6 mol^{-1} dm^3; c_{sensor} = 1×10^{-4} mol dm^{-3}).

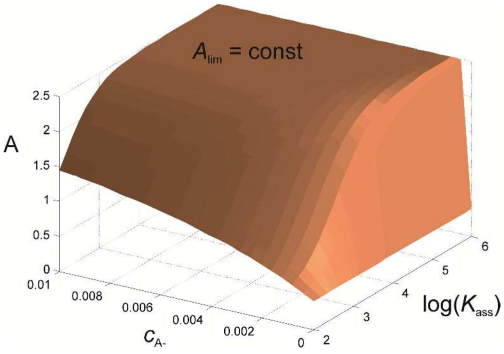

Fig. A2. $A = f(c_{A-})$ curve behaviour at boundary conditions (A_{lim} = const = 2.5; c_{sensor} = 1×10^{-4} mol dm^{-3}).

$$\left(c_0 + 1/K_{ass} + c_{A^-}\right)^2 - 4c_0c_{A^-} = \left(1/K_{ass} - c_0 + c_{A^-}\right)^2 + 4c_0/K_{ass} \; .$$

(A2)

Further, the bracketed term in equation (A2) can be multiplied by unity (× [... + √]/[... + √]), which gives:

$$A = A_0 + (A_{lim} - A_0)\frac{2c_{A^-}}{c_0 + 1/K_{ass} + c_{A^-} + \sqrt{\left(1/K_{ass} - c_0 + c_{A^-}\right)^2 + 4c_0/K_{ass}}} \; .$$

(A3)

Because the complex fraction on the right side of Eq.(A3) is always positive for arbitrary K_{ass} >0, it is clear that the A_{lim}-A_0 term determines the A vs. c_{A-} behaviour. When A_{lim}-A_0 > 0, then A is the ascending function of c_{A-}; and vice-versa.

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For small c_{A-} values ($c_{A-}\rightarrow0$), Eq. (A3) can be rewritten as (the complex fraction behave as x/(c_0 + 1/K_{ass})):

$$A = A_0 + \frac{\left(A_{lim} - A_0\right)}{c_0 + 1/K_{ass}}c_{A^-} \; .$$

(A4)

Therefore, for small c_{A-} values, the steepness of the $A = f(c_{A-})$ curve directly indicating the sensor sensitivity is expressed as:

$$\frac{\left(A_{lim} - A_0\right)}{c_0 + 1/K_{ass}} \; .$$

(A5)

This expression clearly indicates that if the sensor concentration c_0 is sufficiently higher than the inverted K_{ass} value (c_0 >> 1/ K_{ass}), sensor sensitivity is directly described by the (A_{lim}-A_0)/c_0 ratio and does not depend on K_{ass} value; as previously mentioned.

Appendix B. Complexities in K_{ass} determination.

The K_{ass} value strongly depends on sensor absorbance increase at high anion concentration above 1 equivalent, despite similar slope sensitivity in the initial low concentration portion of the A = $f(c_{A-})$ curve (Fig. A3).

However, absorbance at anion concentration above 1 equivalent can be influenced by additional anion-sensor interactions, and these influences distort K_{ass} value determination. For example, F^- concentration above 1

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equivalent leads to loss of the intramolecular hydrogen bond in the *E*-isomer ***Ia*** structure and simultaneous hydrazonolate C formation (ESI Scheme S2†).[16] This structural change results in hydrazonolate B absorption band hypsochromic shift in *E*-isomer ***Ia*** UV-VIS absorption spectra at 410 nm. However, absorbance at 410 nm further increases up to the 10 sensor equivalent and significantly affects the sensor K_{ass} value (ESI Fig. S12†).

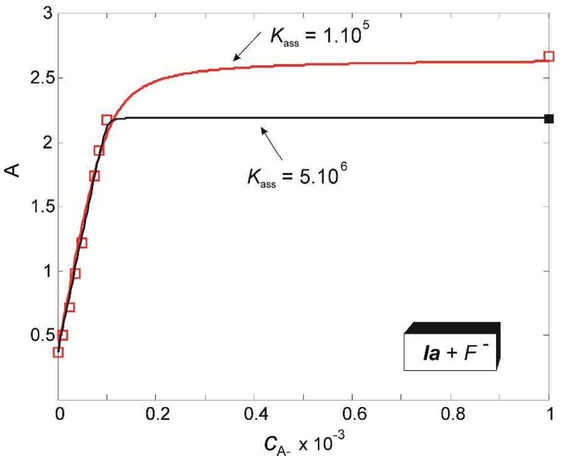

Fig. A3. K_{ass} value dependence on sensor absorbance increase at anion concentration over 1 equivalent (c_{A-} > 1×10^{-4} mol dm^{-3}) for the 1:1 ***IXa***:*F*$^-$ anion-receptor complex. The red squares depict experimental values, the black square is the model point, and red (black) line shows non-linear fitting.

This large K_{ass} sensitivity to additional anion-receptor interactions in the high anion concentration region does not allow correct selectivity comparison for ***IIIa***-***XIa*** sensors from their K_{ass} values alone. Moreover, both the lower correlation coefficient for 1:1 complex and Job's plot indicate sensor:anion 2:1 complex stoichiometry in some cases, and this further complicates ***IIIa***-***XIa*** K_{ass} comparison (Table 1; ESI Figs. S7†).

Experimental Section

Synthesis

Semicarbazides were prepared using a modified procedure from the literature [24]. Phenyl chloroformate (20 mmol) was added dropwise to a mixture of aniline (20 mmol), pyridine (20 mmol) and dry CH_2Cl_2 (40 mL) in an ice-water bath and stirred at room temperature for 18 hours. The mixture was then evaporated under reduced pressure and the residue was poured into saturated NaCl for salting out. The precipitate was filtered, dried, and stirred in hydrazine hydrate (80%, 10 ml) at room temperature for 4hours and then filtered off and recrystallized from ethanol.

General procedure: A solution of phenylsemicarbazide (6 mmol) in hot absolute EtOH (25 mL) was added to a solution of R-substituted isatine (6 mmol) in hot absolute ethanol (50 mL); R = –H (Sigma-Aldrich), –Br (Acros Organics), $-OCF_3$ (Sigma-Aldrich) and $-CH_3$ (Sigma-Aldrich). The reaction mixture was refluxed for 2 hours and the desired product precipitated. In the case of ***Xb*** *Z*-isomer, the filtrate was evaporated and the residue was purified by column chromatography using silica gel as the stacionary phase and hexol (a mixture of methylpentanes and hexane; φ_r ~ 2:1)/ethyl acetate (φ_r = 2:3) as the mobile phase.

For further data on the synthesis and characterization of semicarbazones ***Ia*** and ***IIIa***-***XIa***, see ESI Synthesis†.

Spectroscopic measurements

Electronic absorption spectra were obtained on a HP 8452A diode array spectrophotometer (Hewlett Packard, USA). The *N*,*N*-dimethylformamide (DMF) solvent was UV-spectroscopy grade (Uvasol®, Merck, Germany), and DMF was dried with CaH_2 and distilled under reduced pressure. All photochemical measurements were performed at 25°C in the dark, with only 405 nm LED diodes Thorlabs as light sources with optical power of *P* = 6 mW.

Titration experiments

MATERIALS

All anions in the titration experimentswere added in the form of tetrabutylammonium (*TBA*$^+$) salts purchased from Sigma-Aldrich (USA), and used without further purification.

GENERAL METHOD

All titration experiments were carried out in DMF or DMF:distilled H_2O solvent mixture (φ_r = 9:1) at 298.16 K. The ***IIIa***-***XIa*** sensor solutions were titrated with the corresponding anion solutions of *F*$^-$, *AcO*$^-$, *Br*$^-$ or HSO_4^- to obtain 1×10^{-4} mol.dm^{-3} or 1×10^{-5} mol.dm^{-3} sensor concentrations of the resultant solution. The titration process in the anion concentration range of 1×10^{-5} mol.dm^{-3} to 1×10^{-2} mol.dm^{-3} was monitored by UV-VIS spectroscopy (in a 1 cm cuvette; 0.1 cm cuvette was used to increase the detection range).

ASSOCIATION CONSTANT DETERMINATIONS

The anion-receptor association constants K_{ass} of the studied isatinphenylsemicarbazones with the particular anions in 1:1 complex stoichiometry were determined using the well-known relation describing the complex anion concentration:[25]

$$A = A_0 + \frac{A_{\mathrm{lim}} - A_0}{2c_0}\left[c_0 + c_{\mathrm{A^-}} + 1/K_{\mathrm{ass}} - \left[\left(c_0 + c_{\mathrm{A^-}} + 1/K_{\mathrm{ass}}\right)^2 - 4c_0 c_{\mathrm{A^-}}\right]^{1/2}\right], \tag{1}$$

where: A_0 is the absorbance of free isatinphenylsemicarbazone, *A* is the isatin *N*-phenylsemicarbazone absorbance measured after anion addition, A_{lim} is the isatinphenylsemicarbazone absorbance measured with excess of the particular anion, c_0 is the overall concentration of isatinphenylsemicarbazone and c_{A-} is the overall concentration of the added anion A$^-$.

For further data on the association constant for sensor:anion 1:1 and 2:1 complexes, and determination of the

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detection and quantification limits for semicarbazones ***IIIa-XIa***, see ESI Titration experiments†.

Light initiated *E-Z* isomerization

GENERAL PROCEDURE

Photochemical measurements were performed using the apparatus described elsewhere (Fig.7 in Refrence 26 without ultrasonic horn H and lens L_1 and using Ocean Optics SD 2000 diode array spectrophotometer). The light sources were four 405 nm LED diodes Thorlabs with overall incident photon flux $I_0=5.6\pm0.1\times10^{-4}$ mol s^{-1} dm^{-3}. Molar extinction coefficients of *E*-isomers are higher than for *Z*-isomers at this wavelength.[15] The actual compound concentrations in air-saturated solutions during irradiation in a 1 cm quartz fluorescence cuvette were measured spectrophotometrically in right-angle arrangement (HP 8452A), and LED light sources were turned off during concentration measurements. The incident concentration of the studied *E*-isomers was c_0 =1.10^{-4} mol.dm^{-3}.

For further data on *E-Z* isomerization quantum yield ($\Phi_{E\text{-}Z}$) determination for isatinphenylsemicarbazone *E*-isomers ***IIIa-XIa*** in DMF solution, see ESI Light initiated *E-Z* isomerization†.

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Notes and references

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Electronic Supplementary Information (ESI) available: Including additional data for Experimental section, Schemes S1, S2, Tables S1, Figures S1-S13 and additional References. See DOI: 10.1039/b000000x/

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Isatin *N*-phenylsemicarbazone: Effect of substituent and concentration on anion sensing selectivity and sensitivity

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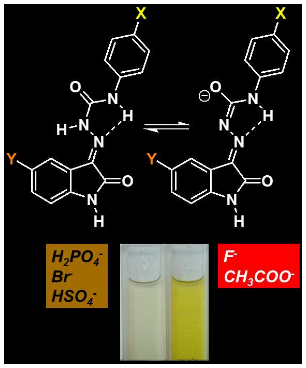

Efficient and easily synthesized sensors for F^- or CH_3COO^- anion sensing in semi-aqueous media based on keto-enol/enolate equilibria