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Smartphone analyzers for on-site testing of food quality and safety

Development of a smartphone-based assay using cholinesterase strips for the on-site determination of organophosphates

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Introduction

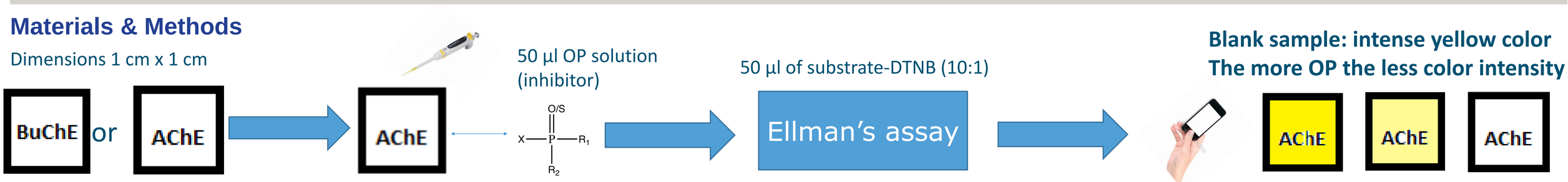
- Smartphones, as colorimetric detectors, is a concept of increasing popularity and great potential
- While accurate determination of organophosphates pesticides (OPs) demands laborious and time-consuming methodologies like LC/MS & GC/MS, rapid screening can be achieved based on the principle that OPs inhibit the action of the enzymes called cholinesterases (ChE)
- The inhibition is correlated to color development which is measured using a smartphone

Aim of the study

- Introduce the possibility of unqualified citizens to act like scientist thanks to simplicity and rapidity of the proposed smartphone-based assay
- The use of cotton strips with immobilized ChE combined with smartphone as an analytical platform for the on-site, sensitive and fast detection of OPs
- Investigation and optimization of the following parameters: substrates for the enzymatic reaction, concentration of the substrates and chromogenic agents, reaction time between enzyme-inhibitor and color development time

Materials & Methods

Dimensions 1 cm x 1 cm



Immobilized enzyme on cotton

- Purchased by Oritest company
- Enzymatic activity of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) was investigated
  - -AChE: 0.05, 0.2, 2 nkat/cm^2
  - -BuChE: 0.2 & 1 nkat/cm^2

Enzyme-OP reaction

- The duration of the reaction was checked and optimized for each OP
- OPs tested: dichlorvos, paraoxon, methamidophos

Color development reaction

- Ellman's and indoxyl acetate (IDA) assay were used
- Ellman's assay was further investigated, because IDA assay was not capable for rapid determination
- Four different substrates and one chromogenic reagent (5,5'-dithiobis(2nitrobenzoic) acid, DTNB) were tested in various concentrations in order to find which one achieves the best color intensity

Colorimetric detection using smartphone

- Huawei P8 lite was used as colorometric detector
- RGB color space values were measured using Color Grab free application
- OP concentration was correlated to saturation value (S%) from HSV color space
- HSV values are calculated from RGB values

Results & Discussion

1. Effect of substrate concentration on color intensity

- An increase in substrate concentration results to a higher color intensity as the enzyme catalyzes greater amount of the substrate
- The use of AcThI as the substrate resulted to the highest color intensity

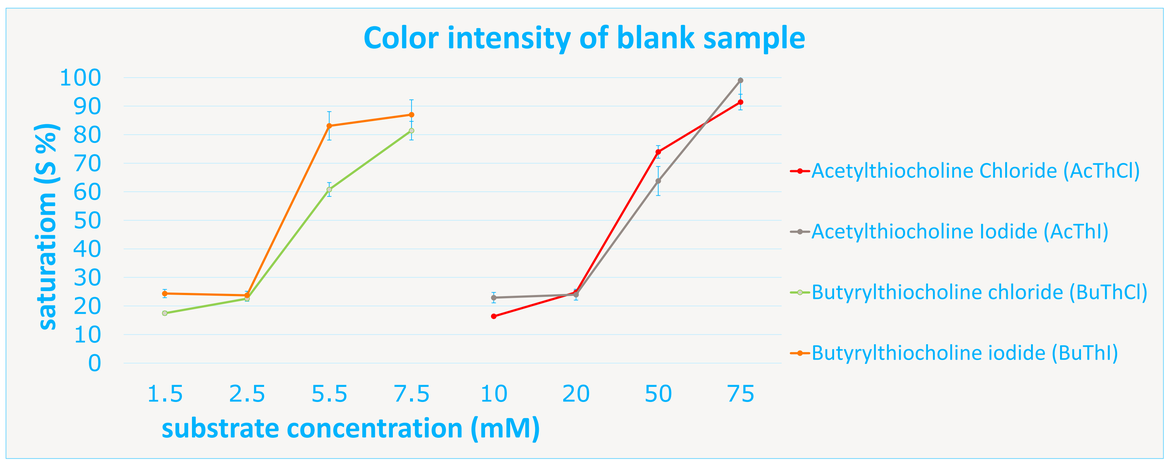


Figure 1. Effect of substrate concentration on BuChE 1 nkat/cm^2 using 5,5'-dithiobis(2nitrobenzoic) acid (DTNB) as chromogenic reagent

2. Effect of enzyme concentration on color intensity

- Greater color intensity obtained using 1 nkat/cm^2 BuChE compared to 0.2 nkat/cm^2
- However, lower limits of detection can be achieved with 0.2 nkat/cm^2
- Noticeable is that 0.2 nkat/cm^2 did not provide robust results 5 days after the purchase

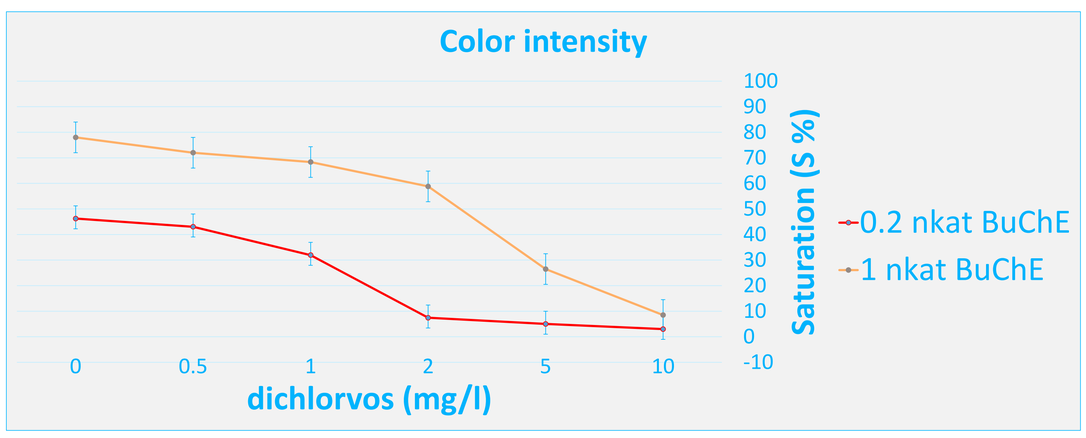


Figure 2. Color intensity of the assay at five different levels of inhibition using AcThI (50 mM) as the substrate and DTNB (5mM) as the chromogenic reagent

3. Effect of OP on assay duration

- 2 nkat/cm^2 AChE and 1 nkat/cm^2 BuChE had similar assay duration
- Metamidophos needed longer assay time, compared to dichlorvos and paraoxon, proving that is a weaker inhibitor of the enzymes
- Concentration of the substrates do not affect the duration of the assay

Table 1. Assay duration using 50 mM AcThI as substrate and 5 mM DTNB as chromogenic reagent.

OP	Enzyme-OP reaction		Color development reaction	
Dichlorvos	AChE: 8 min	BuChE: 5 min	AChE: 2 min	BuChE: 5 min
Paraoxon	AChE: 8 min	BuChE: 5 min	AChE: 2 min	BuChE: 5 min
Metamidophos	AChE: 15 min	BuChE: 15 min	AChE: 5 min	BuChE: 5 min

4. Detection of OP using the smartphone-based assay

- Calibration curves for dichlorvos were made using 2 nkat/cm^2 AChE and 1 & 0.2 nkat/cm^2 BuChE
- A logarithmic trendline was observed, which is usual for enzyme inhibition
- Inhibition% (I%)= dIntensity= $S\%_{blank}$-$S\%_{sample}$

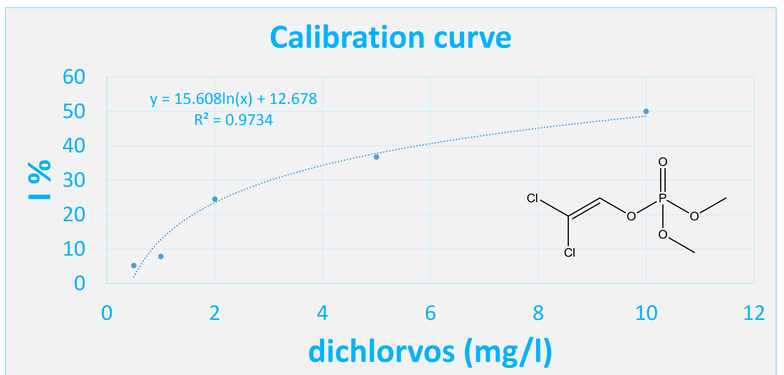


Figure 3. Calibration curve for dichlorvos dissolved in water using AChE (2 nkat/cm^2) based assay with AcThI (50 mM) as the substrate and DTNB (5mM) as the chromogenic reagent

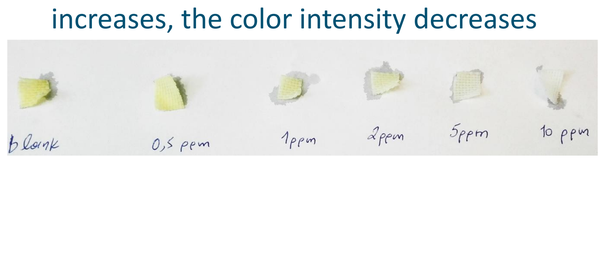


Figure 4. The view of the AChE based assay. As the concentration (mg/l) of dichlorvos increases, the color intensity decreases

Conclusions

1. The inhibitory efficiency of OPs differs largely, therefore for each analyte the detection conditions should be modified
2. This preliminary data set shows the prospective of the colorimetric smartphone assay to be used and validated in real food matrices

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