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Development of dipstick-based immuno-chemiluminescence techniques for the rapid detection of dichlorodiphenyltrichloroethane

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ABSTRACT: The occurrence of organochlorine pesticides in the environment has been a major concern, due to their high persistence and the possible impacts of their exposure to humans. Dichlorodiphenyltrichloroethane (DDT) is most hazardous and one of the most widely used organochlorine insecticides. DDT and its main metabolites are highly stable to physical, chemical and biological degradation and are therefore still being detected in many parts of the world. The present study describes dipstick-based immuno-chemiluminescence method for the detection of DDT with high sensitivity. Anti-DDT antibodies raised in chicken (IgY) were used as the biological sensing elements by immobilizing onto nitrocellulose membrane strips in a chemiluminescence (CL)-based dipstick technique. The photons generated during the biochemical interaction were directly proportional to the DDT concentration. A mean recovery of 81.2–95.6% was obtained for DDT-spiked fruit juice samples with 2.8–4.6% relative standard deviation (RSD). Using the proposed dipstick-based immuno-CL method, DDT was detected with linearity in the range 0.05–1 ng/mL, having a limit of detection (LOD) of 0.05 ng/mL. This method can be used for the rapid, reliable detection of DDT pesticide.

Keywords: dipstick; DDT; chemiluminescence; IgY antibodies; immunosensor

Introduction

Organochlorine pesticides constitute the largest group of compounds in the list of priority pollutants for agricultural applications, due to their high persistence in the environment (1). The ability of these pesticides to impart toxicity and bioconcentrate, and consequently their ubiquitous distribution in the biosphere, has caused a severe impact due to their possible effects on the quality of life (2). DDT is one of the most hazardous and widely used organochlorine insecticides. DDT and its main metabolites are highly stable to physical, chemical and biological degradation and have been detected in the adipose tissues of animals (3). Therefore, DDT has been restricted in most countries due to its toxic effects, such as neurotoxicity and endocrine disrupting activity in wildlife (4,5).

The adverse effects of organochlorine pesticides on health have led to the development of newer methods for detection of chlorinated pesticides in environmental samples. Gas chromatography–electron capture detection (GC–ECD), high-pressure liquid chromatography (HPLC) with UV detection and GC–mass spectrometry (GC–MS) are some of the reported analytical methods for the detection of DDT in environmental samples (6–8). However, these conventional methods have limitations, such as being time consuming, requiring expensive instrumentation and needing pretreatment of the samples. Therefore, there is a need for an efficient qualitative/quantitative method to estimate DDT at sensitive levels. Immunosensor and dipstick assays are promising alternatives to overcome the above-mentioned disadvantages. These techniques have gained tremendous ground in the last few years, owing to the advantages of simplicity, reliability and rapidity (9). Chemiluminescence (CL)-based analytical methods are very sensitive for the detection of analytes, even at very low concentration (10). In recent years the CL method has become an attractive tool in pesticide detection, due to numerous advantages, including sensitivity, rapidity and inexpensive instrumentation.

The present study emphasizes the development of simple dipstick techniques based on immuno-chemiluminescence (immuno-CL) for the sensitive detection of DDT in agricultural and other sources. This technique can be performed with no or a less well-equipped laboratory and is suitable for on-site field studies.

Experimental

Reagents

All chemicals were of analytical grade and solutions were prepared with double-distilled water. *p-p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], DDA [2,2-bis(4-chlorophenyl)-acetic acid], horseradish peroxidase (HRP), urea hydrogen peroxide (U–H_2O_2), luminol, Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Nitrocellulose (NC) membrane strips (0.45 μm pore size, 6 × 75 cm length, 130 μm thickness) were obtained from Advanced Microdevices (Ambala, India). All other reagents were obtained from standard sources.

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