Synthesis of water-soluble carboxylic and acetic acid-substituted poly(thiophenes) and the application of their photochemical properties in homogeneous competitive immunoassays

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Novel water-soluble poly(thiophene) derivatives are synthesized and used as labels in homogeneous competitive immunoassays for antigens and haptens.

The label still remains the main focus in immunoassay technology[1] and for some time we have been considering water-soluble conductive polymers to match many of the characteristics of ideal label candidates, *i.e.* applicability in homogeneous assay formats on common automated analysers.[2] On the one hand, conductive polymers, and polymers of thiophene derivatives in particular, are sensitive to oxido-reduction and reflect the changes in their electronic structure by displaying important chromatic transitions in the UV–VIS range.[3,4] Some polymers even reflect these changes by emitting luminescence[5] or phosphoresence.[6] On the other hand, antigen–antibody interactions induce a local pH change around the complex,[7] which can be reported by a conductive polymer label. Such an approach has already been successful with poly(acetylene) in a solid-phase photometric sensor intended to detect ligand–receptor interactions.[8]

Up to now, [9,10] water-soluble conductive polymers have been obtained from monomers substituted with a limited alkyl chain terminated by a sulfonate group. Because such a substituent is not particularly suited for coupling to either antigens or haptens, we synthesized poly(thiophene-3-carboxylic acid) (PT-C) and poly(thiophene-3-acetic acid hydrazide) (PT-AAH) by chemical oxidation. The monomers were respectively obtained from Sigma–Aldrich (Bornem, Belgium) and Maybridge Chemical Co (Tintagel, UK).

The syntheses were performed by reacting, over 5 h at room temperature with magnetic stirring, 72 mmol of ferric chloride and 14.4 mmol of ammonium persulfate with 14.4 mmol of the respective monomers in HCl (0.1 dm^3; 1.2 mol dm^{-3}). The polymer solution was recovered by filtration after the pH of the reaction mixture had been adjusted to 12 with NaOH.

Gel permeation HPLC of the solutions showed that the mixtures contained approximately 20% of polymers of 15–20 kDa (approximately 120 repeat units), the remaining being made of oligomers. The UV–VIS spectra of the polymer solutions were recorded at different pH's. As shown in Fig. 1, the optical density changes recorded at 380 nm (λ max) for both polymers correlated linearly with pH 6–9, *i.e.* the pH range

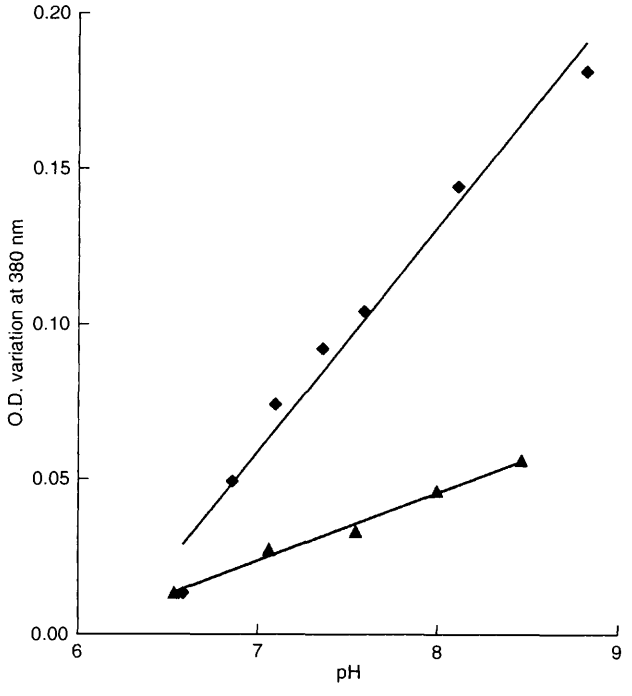

Fig. 1 Optical density changes at 380 nm as a function of pH for PT-C (diamonds) and PT-AAH (triangles). The polymers were diluted 1 : 100 (*v* : *v*) in 0.1 mol dm^{-3} phosphate buffer of which the pH was adjusted with either HCl or NaOH (0.1 mol dm^{-3}). A UV–VIS spectrum was recorded at each pH after subtraction of the buffer blank.

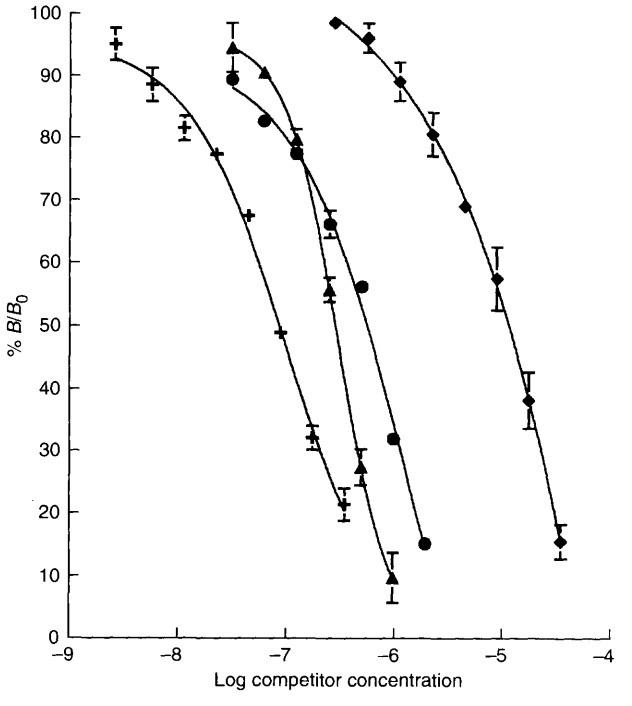

Fig. 2 Immunoassay dose–response curves for human albumin (triangles) and theophylline (diamonds) obtained with PT-C as the label and for human C-reactive protein (crosses) and human albumin (circles) with PT-AAH as the label. The antibodies were respectively diluted 1 : 200 for albumin, 1 : 100 for theophylline and 1 : 1 200 (*v* : *v*) for C-reactive protein in 0.2 mol dm^{-3} phosphate-buffered saline pH 7.4, containing 40 g dm^{-3} poly(ethylene glycol) 6000. The fraction of tracer bound was between 0.3 and 0.5.

where most antibody–antigen interactions occur (r = 0.98 for PT-C and r = 0.99 for PT-AAH).

We then labelled two protein antigens (human albumin and human C-reactive protein) and a hapten-protein conjugate (theophylline-8-bovine serum albumin) with the polymers by a standard two step carbodiimide method. The tracers obtained were purified by gel filtration chromatography and applied in competitive immunoassays which were performed in a Cobas Mira automated analyser (Hofmann-La Roche). After simultaneous programmed addition of the antibody, tracer and sample to the cuvette, the incubation lasted 20 min at 37 °C and the O.D. variation was recorded at 340 nm. The theophylline antigen used for labelling and the rabbit anti-theophylline antibody were purchased from OEM Concepts (Toms River, N.J. 08755). The source of the other reagents and the procedures used are detailed elsewhere.[11] Normalized dose–response curves obtained in the three analytical systems are shown in Fig. 2. The results displayed are representative of the wide analytical range (nanomolar up to higher micromolar) to which such homogeneous assay techniques can be applied. This compares favourably to the usually non-homogeneous radio- and chemi-luminescent-immunoassay technologies.[12,13] Among the homogeneous assay formats, the enzyme multiplied immunoassay technique (EMIT) is probably that which best matches the one described here in terms of simplicity of use. Whilst best known for its use in therapeutic drug monitoring, assays for protein antigens have been reported with least detectable doses of 30–100 nmol dm^{-3}.[14] In the present case, the least detectable dose observed for C-reactive protein is ten times lower. Some other homogeneous systems, such as luminescence, scintillation proximity or flow-cytometric fluorescence immunoassays can quantitate analytes in the picomolar and even femtomolar range, but they require many manipulations, use complex and costly reagents, and depend on a specific instrument.[1]

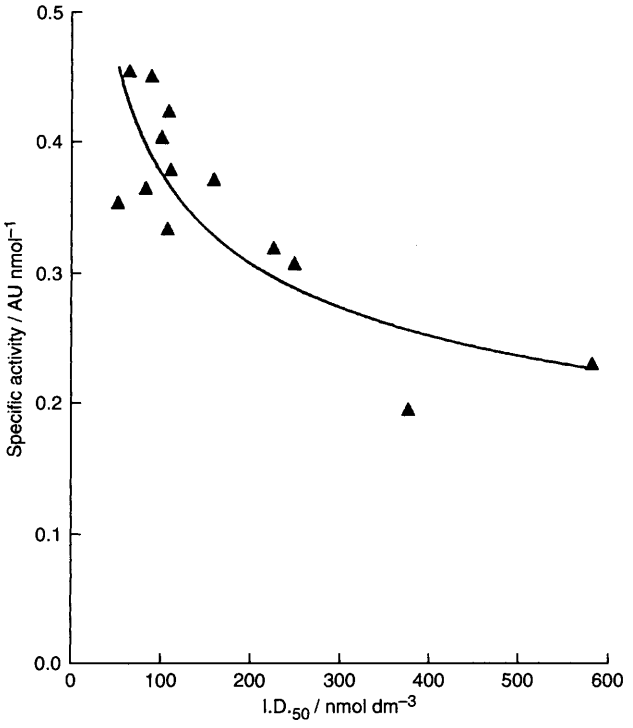

Fig. 3 Relationship between the specific activity of human albumin tracers and the assay sensitivity of the corresponding immunoassay dose–response curves as expressed by the interpolated dose at 50% B/B_0 (ID_{50})

Finally, we prepared several batches of human albumin tracers by labellling the protein with increasing polymer concentrations and examined the relationship between specific activity (ratio of photometric signal intensity over mass of labelled protein) and assay sensitivity (dose of unlabelled human albumin inhibiting tracer-binding by 50%). As shown in Fig. 3, the higher the specific activity, the better the sensitivity, like in standard radioimmunoassay.[12,15] This further relates the photometric signal measured to the conductive polymer label and consequently allows this signal to be distinguished from possible immunoturbidity.

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