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Letter to the Editor

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Initial Background in Size Exclusion Chromatography

I started off my career as a biotechnologist in the early eighties, of the last century, using mainly size exclusion chromatography to identify biologically active substances, from ovine broncho alveolar macrophages co-cultured with lymphocytes, from the lungs of young sheep infected with Jaagsiekte Retrovirus (JSRV). As such, size exclusion chromatography afforded the direct isolation and subsequent characterization of ovine monocyte/Macrophage Chemotactic Factor (MCF), as well as ovine (lung) Tumour Cell Growth Factor (TCGF) from cell culture supernatant, used to perform the broncho alveolar lavage on the excised lungs of sheep suffering from the pulmonary adenomatosis, caused by this retrovirus [1]. The outcome of this study produced a model of lung cancer in sheep, which essentially involved the transformation of surfactant producing type II pneumocytes, which produced a MCF that attracted blood monocytes to the lungs of diseased sheep. When these monocytes then differentiated into alveolar macrophages, a TCGF was produced, being excaberated by the presence of lung lymphocytes [2].

While the methodology of producing this disease might be frowned upon by animal rights groups today, since the only way to grow this virus was by passage through the lungs of newborn lambs, this model was subsequently vindicated by the fact that the condition, known as 'jaagsiekte' or 'chasing disease', when mature infected animals collapsing when chased, with surfactant foam exuding from their nostrils, could not be reproduced in young goats, because the primary lesions became encapsulated with fibroblasts. As such, the encapsulation process effectively prevented the onset of jaagsiekte in goats, because the circulation of MCF in the lungs was being stopped, right at the outset of the disease.

Reverse Phase Chromatography

Subsequent biotechnology based research took me into gradient elution reverse phase chromatography, used to identify the breakdown products of C - Reactive Protein (CRP) produced by livers from patients suffering with chronic inflammatory conditions. As such, characterisation of these products from CRP served to provide a better understanding of how they subsequently down regulated circulating neutrophil activity, thereby providing a kind of chemical breaking effect on the inflammatory process [3]. Other isocratic elution reverse phase chromatography was used to measure alterations in plasma concentrations of vitamin C, vitamin E, and beta carotene, in the blood of smokers exposed to mineral dust in South African gold mines [4].

Phytochemistry on Selected South African Indigenous Plants

More recently, I became involved with the phytochemistry of selected indigenous plants in South Africa, namely *Sutherlandia frutescens* and *Athrixia phylicoides*. In the former case, a series of pre-chromatographic extractions followed by recombination of enriched extracts subsequently analysed on a Liquid Chromatographic Mass Spectroscopy (LC-MS) system, produced some substances of biological interest, which were found to be able to influence the amounts of IL8 produced by cultured HL60 cells [5].

This recurring theme of using chromatographic analysis to identify phytochemical substances of biological interest, then culminated in the combination of LC-MS extracted peaks with comparable peaks produced with Thin Layer Chromatography (TLC) on extracts from *Athrixia phylicoides* [6]. These TLC plates were then exposed to selected cultures of bacteria, to identify biological activity of the observed bands, using standard bioautography techniques.

As such, direct bioautography involves the suspension of microorganisms (MO's) such as bacteria or fungi, growing in a suitable substrate, to be applied to a processed, dried TLC plate. After incubation of this plate with the selected MO's under suitable temperature and humidity, subsequent growth, or lack thereof, in relation to each banded extract, can be visualized when a developing agent, such as tetrazolium salt, is converted to a corresponding brown or orange coloured formazan by dehydrogenases present in living MO's [7].

A General View on the State-of–the-Art of Chromatographic Separation Techniques

Having thus introduced some of my background in biotechnology, as it relates to chromatographic separation techniques, over a period of four and a half decades, I still come back to the usefulness of TLC, as being one of the most versatile and applicable for the identification of biologically active substances. For example, according to Waksmundzka-Hajnos, Sherma and Kowalska [8], each TLC plate is used once, with simpler preparation methods, compared to those of GC and HPLC approaches, which require multiple samples and standards. In addition, orthogonal two-dimensional separations can also be effectively performed using TLC, with two different mobile phases, to achieve good separation of components within complex sample mixtures. They also refer to the use of Dragendorff's reagent (KBiI4) in the TLC system for the identification of heterocyclic bases found in alkaloids, as well as ninhydrin for identification of compounds containing an amino group in their structure, such as found in amines and amino acids, plus 2-(diphenylboryloxy)-ethylamine-polyethylene glycol (PEG), for identification of polyphenols.

During the mid 90s, LC-MS started to be coupled with Nuclear Magnetic Resonance (NMR) instrumentation, as reviewed by Wolfender, Ndjoko & Horstettman [9]. As such, with the development of pulse field gradients and solvent suppression technology, complex mixtures of organic molecules could now be elucidated, particularly where phytochemical extracts were involved. With the advent of shielded magnets, Nuclear Magnetic Resonance equipment (NMR) could now be placed next to LC-MS instrumentation, forming an LC-NMR-MS system, to provide a platform for analyzing the metabolites of novel drugs in development. In addition, the LC-NMR-MS system could also be switched to on-line Solid-Phase Extraction (LC-SPE-NMR), thereby providing even better capacity to identify possible new drugs from complex sample mixtures [10].

Today, the whole process has come full circle with the paradigm involving a Bio Arena system making use of Over Pressured Layer Chromatography (OPLC) coupled with direct bioautography, to characterise bioactive compounds from medicinal plant sources, but also involving bio-guided semi-preparative TLC fractionation protocols which produce LC-MS/MS spectra of compounds of interest. More robust separation of bioactive compounds, under this scenario, reviewed by Choma & Jesionek [11], can be achieved by hyphenated techniques using TLC with-MS, desorption by a spray beam (DESI) or Direct Analysis in Real Time (DART) interfaces.

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