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## Letter to the Editor

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### Isotachopheresis and isoelectric focusing of soil humic substances in polyacrylamide gel

Sir,

The use of the term "isoelectric focusing" by Curvetto *et al.*<sup>1</sup> in describing the separation of soil humic substances would appear questionable, and it is doubtful that the observed  $pI$  values of separated fractions are truly meaningful. I have also observed fractionation of humic substances on acrylamide gels with carrier ampholytes, but the migration is continually toward the anode, regardless of the placement of the sample. This would suggest that true isoelectric focusing, in which fractions will migrate to their  $pI$  and stop, is not the prevailing phenomenon.

What is taking place is instead a form of electrophoresis involving isoelectric buffers of very low conductivity, as described by Fullarton and Kenny<sup>2</sup>. With Ampholine carrier ampholytes, the conductivity is enormously decreased after the ampholytes have reached their respective  $pI$ ; the buffering capacity, however, remains high. The use of Ampholines in this manner will decrease the conductance by at least tenfold over conventional buffer systems, permitting the application of a higher-voltage gradient and resulting in what often proves to be superior separations.

The final position of the humus fraction in the acrylamide gel is, therefore, a function of time rather than of position in the pH gradient. The position does not yield a true  $pI$ , and any value determined from the pH gradient curve does not represent a reliable or reproducible physical property of the humus fraction.

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1 N. R. Curvetto, N. A. Balmaceda and G. A. Orioli, *J. Chromatogr.*, 93 (1974) 248.

2 J. R. Fullarton and A. J. Kenny, *Biochem. J.*, 116 (1970) 147.

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