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INDUSTRIAL AUTOFOCUSING —A NEW TECHNOLOGY FOR LARGE-SCALE ISOELECTRIC FOCUSING

O. SOVA
Institute of Animal Physiology of Slovak Academy of Sciences, Palackého 12, 040 01 Košice (Czechoslovakia)

SUMMARY

A new system for autofocusing, *i.e.*, for isoelectric focusing without carrier ampholytes has been developed which offers for the utilization of large-scale isoelectric focusing in industry. A number of possible applications of this method are discussed, *e.g.*, the isolation and purification of proteins, drugs, antibiotics, whole cells (yeasts, bacteria, viruses) as well as the purification of nonionized substances. The production capacity of the system may be as high as 1000 kg.

INTRODUCTION

I have previously[1] drawn attention to autofocusing as a new method for isoelectric focusing without carrier ampholytes. This method was developed for industrial large scale isoelectric separation of various compounds. The production capacity of this method was limited by the conductivity (concentration) of the material to be separated and by the dimensions of the apparatus.

In order to demonstrate of the possibilities of this method, at first the 440-ml LKB electrofocusing columns were used. Later larger columns up to 50 l in volume were developed[2]. But this method of construction of suitable apparatus for industrial large scale autofocusing has many shortcomings[3].

Columns need a gradient of sucrose or another non-ionized viscous material to prevent jumbling of the fractions of separated material, caused by diffusion. In a large column there are, however, some kilograms of low-molecular-weight compounds which it is necessary to eliminate after focusing. On the other hand, Joulean heat can cause convection problems and thus prevent the use of annular column or laminar flow electrophoresis. Therefore there seems to be little interest at the moment in industrial applications of electrofocusing[4].

In this paper a new apparatus is presented for large scale industrial autofocusing which has almost unlimited capacity, and some industrial applications are described.

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MATERIALS AND METHODS

The apparatus is a simple box (8 × 8 × 26 cm) of 1 l in volume, made from electrically non-conducting material, such as acrylate, plastic mass, ceramics, etc. The open top of the box is provided with a cover made from the same material. At the opposite sides of the cover there are two holes of 0.3 cm in diameter, through which the electrodes are introduced.

The inside of the box comprises a totally convection-free space, where autofocusing is realized in the horizontal direction (Fig. 1). The solution is separated without sucrose or other stabilizing material, because in the convection-free space it is thoroughly stabilized[5].

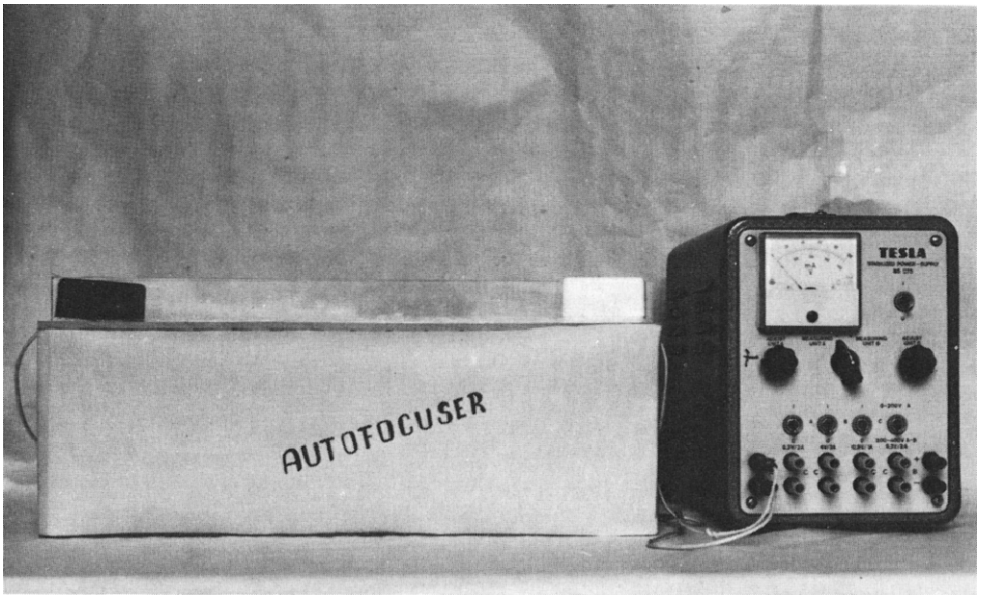


Fig. 1. Photograph of a typical large-scale set-up of volume, 10 l.

The apparatus is assembled as follows. The material to be separated is dissolved in pure distilled water, so that the conductivity of the solution is less than 800 μS, and then poured into the apparatus. The cover is applied and the electric power is connected to the electrodes. No anolyte or catholyte is used. The platinum electrodes are immersed immediately into the solution to be separated. The autofocusing is always carried out at a power of 3 W at a variable field strength from 200 to 1000 V, until the current decreases to zero. Then power is switched off and the vessel opened. The focused solution may be divided into equal fractions and measured in the usual way for pH, etc.

Different apparatus of various volumes from 75 ml up to 1000 l was examined and various compounds were separated. Other details have been reported elsewhere[1,6].

RESULTS

Fig. 2. shows the separation of the virus strain La Sota by autofocusing in a 2-l apparatus. Along the step-like pH gradient formed by dissolution of virions in distilled water, four peaks are formed at p*I* 4.18, 4.33, 11.49 and 12.74. Only the peak at p*I* 4.33 was contained the pure and active viruses. Similar results were obtained for separations of whole cells of bacteria or yeasts.

The antigens K 88, K 99 and enterotoxin LT were autofocused in a 5-l apparatus for preparing a vaccine against piglet diarrhoea. Fig. 3 shows the purification of K 99 antigen. This simple electrofocusing yielded a very pure antigen which could be employed for as many as 1000 doses of vaccine[6].

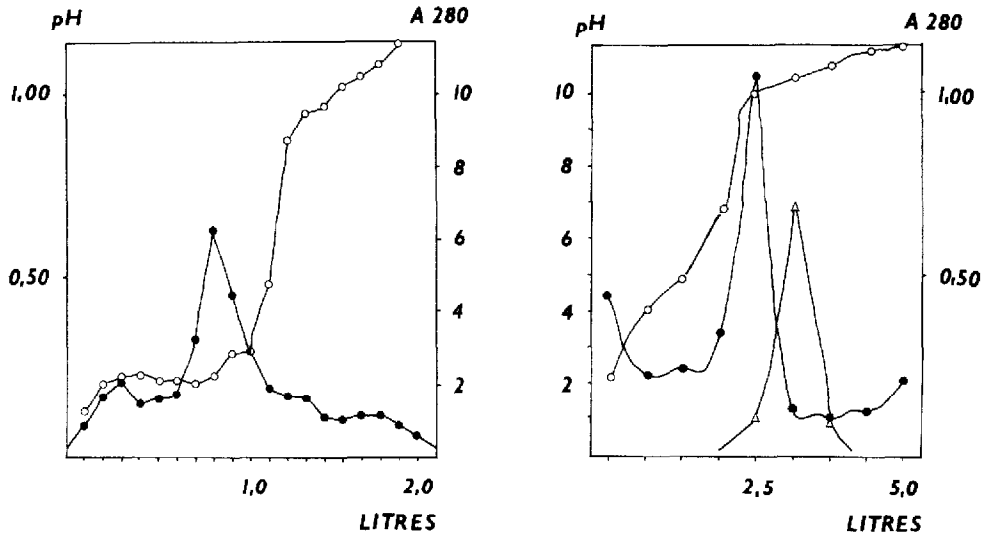


Fig. 2. Industrial autofocusing of the virus strain La Sota. ○—○, pH gradient; ●—●, UV absorption at 280 nm.

Fig. 3. Industrial autofocusing of K 99 antigen. ○—○, pH gradient; ●—●, absorbance at 280 nm; △—△, titre of the activity.

Fig. 4 shows the isolation of lectin from peas. After simple electrofocusing of dialysed pea proteins in a 2-l apparatus a very pure lectin was isolated (16 g/l).

As an example of autofocusing of antibiotics 500 g of a tetracycline were purified in a 10-l apparatus. Fig. 5 shows that the tetracycline was divided into four fractions with p*I* 2.15, 2.43, 6.50 and 10.17, only the third and fourth being active (see Table I). Similar results were obtained for other antibiotics[6].

Fig. 6 shows the autofocusing of xylose, where the impurities were focused to both sides of the apparatus and the superpure sugar was remained in the middle. The capacity of such a 1000-l apparatus is as high as 600 kg of sugar (while the viscosity is not influencing the focusing effect).

Fig. 7 shows the purification of beer. By autofocusing it is possible to separate

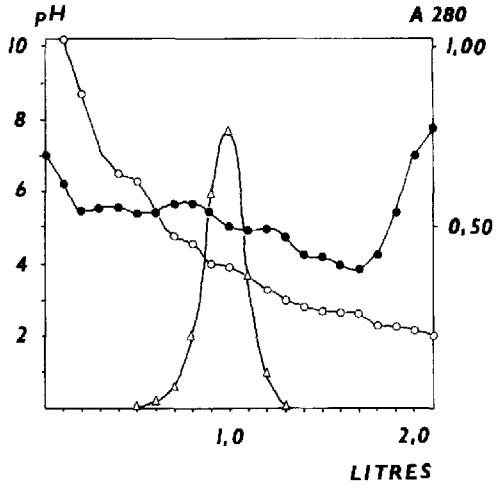


Fig. 4. Isolation of lectin from peas by industrial autofocusing. O—O, pH gradient; ●—●, absorbance at 280 nm; △—△, agglutination effect.

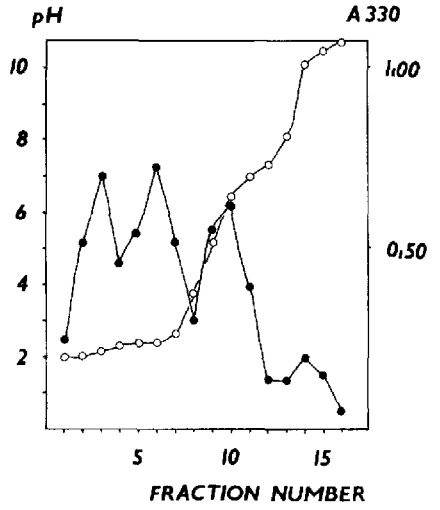


Fig. 5. Purification of tetracycline by industrial autofocusing. O—O, pH gradient; ●—●, absorbance at 330 nm.

TABLE I

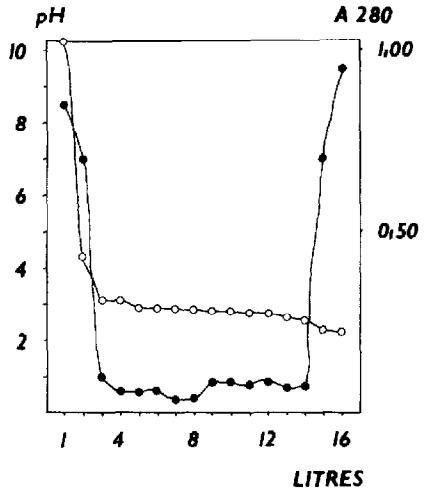
SENSITIVITY OF THE BACTERIA *ESCHERICHIA COLI* (E.c.) AND *STREPTOCOCCUS FAECIS* (Str.) TO THE INDIVIDUAL FRACTIONS OF TETRACYCLINE (TC) AFTER SEPARATION BY INDUSTRIAL AUTOFOCUSING

	E.c. 186	*E.c. 290*	*E.c. 292*	*E.c. 277*	*E.c. 278*	*Str. 315*	*Str. 316*
Total TC	–	+ +	–	+	+	+ + +	+ +
Fraction I	–	–	–	–	–	+	–
Fraction II	–	–	–	–	–	+	–
Frasction III	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Fraction IV	–	+ + +	+ +	+ +	+ +	+ + +	+ + +

beer into many fractions, differing in colour, flavour and other merits. On the other hand, it is very interesting that the nitrosoamines were focused at basic pH. The purification of other "nonionized" substances, such as brandies, was estimated.

DISCUSSION

To date the largest apparatus examined has a volume of 1000 l, but since the operation of the smallest and the largest one is very similar it seems that the construction of still larger apparatus will have no shortcomings. The apparatus is usually operated at room temperature, or in a cooled room when a lower temperature is required. At room temperature the cooling of the apparatus is sufficient provided that the focusing at 3 Watts is carried out.



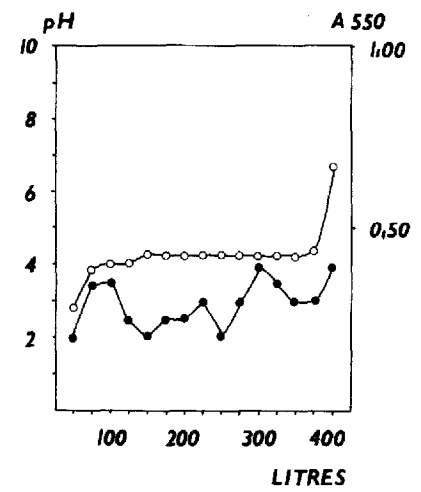


Fig. 6. Purification of xylose by industrial autofocusing. ○—○, pH gradient; ●—●, absorbance at 280 nm.

Fig. 7. Industrial autofocusing of beer. ○—○, pH gradient; ●—●, absorbance at 550 nm.

The use of autofocusing, however, means a different approach to the isolation and purification of various materials. For example, to date, the isolation of K 88 and similar antigens was carried out through cultivation, centrifugation, isolation of pili, saline precipitation, gel filtration, etc., as many as twelve steps could be required[8]. In the new method, after isolation of pili by stirring in distilled water, direct autofocusing yields the pure antigen for use as a vaccine[7].

The same situation is similar in the isolation of enzymes and other compounds[9]. Separation of antibiotics and drugs offers the possibility to isolate only the effective fractions, thus diminishing the total doses needed for treatment and perhaps yielding of non-allergic drugs[6]. Autofocusing offers the standardization of liquids in food technology by adjustment of pH, colour and flavour without addition of various chemicals, as is usually done at present, but by simple elimination of undesirable fractions.

It seems that industrial autofocusing is a most important and most suitable method for biotechnology. Continuous work is also possible.

However, only widespread application of autofocusing in convection-free apparatus on an industrial scale will demonstrate all of its advantages and shortcomings. At present only seven factories in Czechoslovakia are using this method for production of superpure products.

REFERENCES

1 O. Sova, *J. Chromatogr.*, 320 (1985) 15–22.
2 O. Sova, *Czech. Pat.*, 211,857 (1981).
3 N. B. Egen, *Electrophoresis*, 83 (1984) 547.
4 J. C. Janson, Pharmacia, personal communication, 1983.
5 O. Sova and K. Boďa, *Czech. Pat. pending*, 1643-83 (1983).

6 O. Sova, *New Trends in Antimicrobial and Anticancer Chemotherapy, Prague, April 1984*, Abstracts, p. 186.
7 O. Sova and M. Viest, *Czech. Pat. pendings*, 01376, 01377 and 01378 (1983).
8 F. R. Mooi and F. K. De Graaf, *FEMS Microbiol. Lett.*, 5 (1979) 17.
9 V. Žúber, O. Jr. Križanová, J. Zachar and O. Sova, *Ionic Channels and Membrane Receptors (International Symposium), Smolenice, May, 1984, Abstracts*, p. 38.