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CONCENTRATION AND PURIFICATION OF NEWCASTLE DISEASE VIRUS BY MICROFILTRATION AND EXCLUSION LIQUID CHROMATOGRAPHY

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Summary. — Newcastle disease virus (NDV) was concentrated and purified by microfiltration and exclusion liquid chromatography (ELC) on macroporous glass (MPG). The purified NDV eluted as one peak; its yield was strain-dependent, the degree of purification was 95 % to 99 %.

Key words: concentration and purification of NDV; macroporous glass; hydrophobicity of viruses

Chromatographic techniques of virus purification have been intensively developing in recent years (Bresler *et al.*, 1969; Haller, 1965; Bresler *et al.*, 1975). The development of these techniques have been promoted by the appearance of a new promising adsorbent — macroporous glass (MPG) which proved suitable for purification of a variety of viruses (Ross and Castro, 1968; Balayan *et al.*, 1971; Mchedlishvili *et al.*, 1975; Krashenyuk *et al.*, 1982).

Rosenbergová and coworkers (1981) have demonstrated that Newcastle disease virus (NDV, strain Kansas) can be purified using exclusive liquid chromatography (ELC) on glass beads with pore diameter of 150 nm. Using the sorbent Bio-Glass 1500 (Bio Rad) these authors have shown that ELC method allowed to separate proteins that were impossible to remove by sedimentation, although this procedure failed to discriminate between "virus" and "contamination" peaks in the mixture.

The purpose of the present paper has been to find out whether or not it is possible to concentrate different NDV strains by microfiltration and to purify virus suspensions by ELC on MPG with pore diameter of 200 nm.

Infectious allantoic fluid (AF) of 10-day-old chick embryos was used as virus source. Three strains of NDV were tested: La-Sota, B_1 and Bor-74. VAF were concentrated 17 to 20-fold by microfiltration in MF-02 cells (Special Design Office of the U.S.S.R. Academy of Sciences, Leningrad) on polysulphonamide membranes with pore diameter 60 nm (All-Union Research Institute of Synthetic Resins, Vladimir, U.S.S.R.) at a pressure of 4 atmospheres.

Exclusion chromatography was made on MPS-2000V-GCh (specific surface 25—55 m^2/g and specific pore vol 1.7 to 2.5 cm^3/g). MPS was modified by polyvinylpyrrolidon (PVP) in order to decrease the adsorption activity of MPG (Molodkin and Mchedlishvili, 1976). On columns H/D-40/I

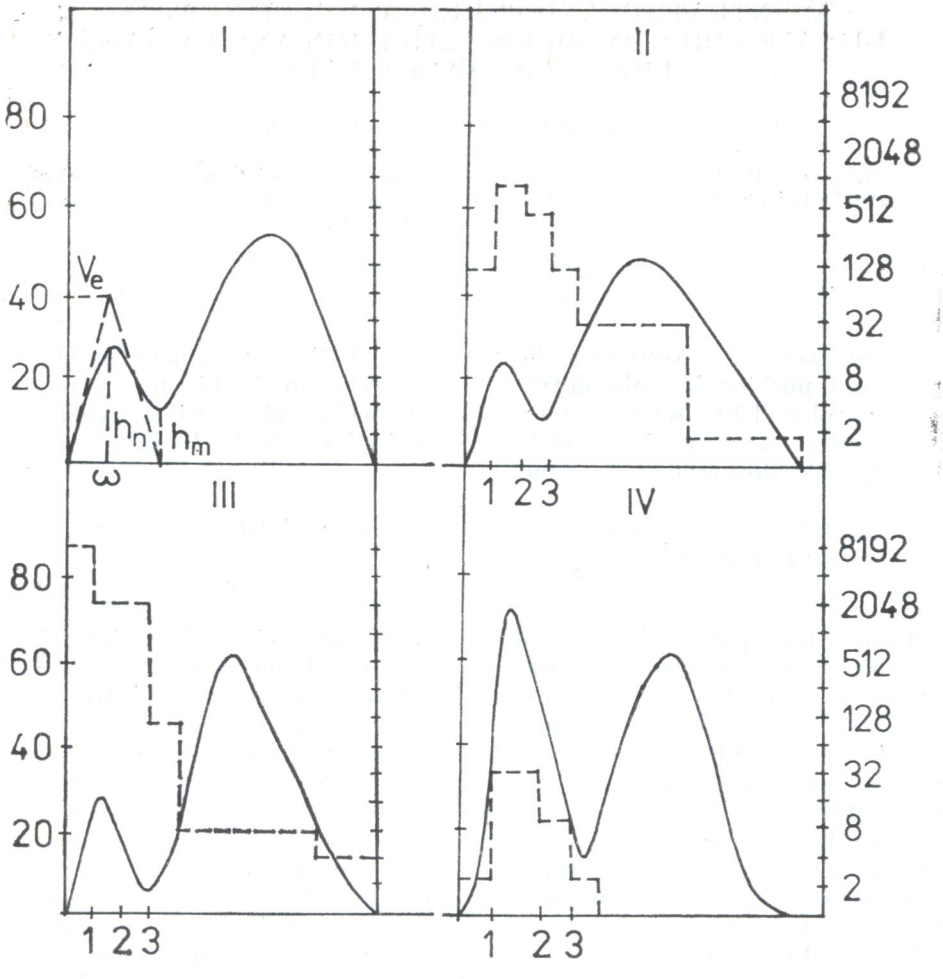

Fig. 1.

Chromatograms of the separation of infectious AF for the 3 NDV strains

Abscissa — vol of the eluate, fraction No. (ml); ordinates: left ordinate — absorption (%) at λ = 260 nm, 100—T %(solid line), right ordinate — haemagglutination titre reciprocals (dashed line).

I — chromatographic scheme and basic chromatographic characteristics: V_e — yielded volume: ω — peak width; $K_B = (h_n - h_m)$: h_n — criterion of peak separation;

II — strain La-Sota;

III — strain B_1;

IV — strain Bor-74.

(column vol 31.4 cm³) the sample was applied in a vol of 1.0 ml (3.2 % of the colum vol). Samples were eluted with 0.01 mol/l Na-phosphate buffer solution with NaCl concentration 0.15 mol/l at a rate of 100 ml/hr cm². The chromatograms were recorded with the help of REPPS-1M

Table 1. Concentration of the three NDV strains by microfiltration method

Strain	Sample	Protein concentration (mg/ml)	HA titre	Vol (ml)	Multiplicity of concentration vol	Multiplicity of concentration protein	Virus yield as measured by HA activity* (percentage)
La-Sota	Original material	7.20	1 : 256	350			100
	Concentrate	71.40	1 : 65 536	20	17.7	9.9	1463
B_1	Original material	4.40	1 : 512	400			100
	Concentrate	61.40	1 : 524 288	20	20.0	13.9	5120
Bor-74	Original material	n. m.	1 : 16	400			100
	Concentrate	91.26	1 : 256	20	20.0	n. m.	80

* Virus yield (percentage) was calculated from the equation:

$$\frac{\text{Volume of concentrate} \times 1/\text{titre} \times 100\ \%}{\text{Volume of original material} \times 1/\text{titre of original material}} = x\ \%$$

n.m. = not measured

system (Central Design Office of the U.S.S.R. Academy of Sciences). Protein concentration was measured using a biuret micromethod (Bailey, 1965) and haemagglutinating activity (HA) of the virus was determined in haemagglutination test with 1 % chick erythrocyte suspension performed in Takachi titrator.

By 17- to 20-fold concentration of virus suspension, the protein concentration increased 9.9 to 13.9-fold, which indicates certain degree of virus purification from contaminating allantoic fluid proteins. Low-titre HA (1 : 2 to 1 : 8) detected in the filtrate can be accounted for free HA which did not become incorporated into virions. It is noteworthy that during microfiltration of strains La-Sota and B_1 the HA titer had significantly increased; the virus yield (according to HA) was as high as 1463 % for strain La-Sota and as high as 5120 % for strain B_1 (Table 1). This seemed to be related to the removal of HA inhibitors of the NDV strains tested in the course of microfiltration. It was previously reported (Syurin, 1963) that nonspecific inhibitors of NDV haemagglutination are probably present in the AF. However, no reports have been published on this problem ever since. Our results suggest that the NDV strains tested differ in sensitivity to HA inhibitors, but this suggestion needs further verification.

The MPG chromatograms of various NDV strains (chromatography after preliminary concentration) shown in Fig. 1 revealed patterns resembling to those obtained under analogous conditions during purification from infections AF of human influenza virus (Krashenyuk *et al.*, 1982). The virus yield during chromatography, as measured by HA titre, was 5.1 % for strain La-Sota, 4.7 % for strain B_1 and 57.8 % for Bor-74. Criterion of efficiency

Table 2. Purification of the three NDV strains by elution chromatography on MPG

Strain	Fraction No., titre in HA	Virus yield as measured by HA activity (%)	Peak concentration of protein (μg/ml)	**Degree of purification in terms of protein (%)	***Specific activity	K_B
La-Sota	1. 1 : 128 2. 1 : 1024 3. 1 : 512	5.1	54.3	99.54	18 86	0 41
B_1	1. 1 : 8192 2. 1 : 2048 3. 1 : 2048	4.7	99.47	99.47	150.31	0.80
Bor-74	1. 1 : 2 2. 1 : 32 3. 1 : 16	57.8	215.0	98.16	0.15	0.81

* Volume of the eluated fraction 2.0 ml.

** Original protein concentrations in the samples applied to the column for each NDV strain are presented in Table 1.
Degree of purification in terms of protein was calculated as follows:

$$\frac{\text{(quantity of protein applied to the column} - \text{peak quantity of protein)}}{\text{quantity of protein applied to the column}} \times 100\ \% = x\ \%$$

*** Specific activity: ratio of HA titre reciprocal (1/titre in HA) to protein concentration in the sample.

of peak separation (K_B, see legend to the Figure) was 0.41, 0.80 and 0.81, respectively. Variations in separation criterion for the three NDV strains were independent of protein concentration in the sample applied to the column. They seemed determined rather by the presence of other infectious AF components which had been comparable in their hydrodynamic size to that of the virions. We believe that lipopolysaccharide complexes might have such effect. Parameters of chromatographic purification of virus suspensions on MPG are presented in Table 2. Since chromatographic conditions in the virus yield during ELC seemed to be related either to the quantity of the protein applied to the column, or to dissimilarities of the surface properties of these viruses.

The investigations carried out with different strains of human influenza virus have shown the unequal hydrophobicity of their virions (Krashenyuk and Sukhanova, 1984; Stepanov *et al.*, 1984). Using ELC on MPG it has been demonstrated that different clones prepared by immunoselection from the same influenza virus strains had unequal hydrophobicity (Krashenyuk *et al.*, 1984). Additional studies are needed, however, to test whether this is also true for NDV.

Chromatographic pattern of HA revealed only one elution peak of the virus, which disagrees with the data reported by Rosenbergová *et al.*, (1981). These discrepancies may be due to the fact that components of the second

HA peak (free haemagglutinin) observed in the paper cited were removed by microfiltration in our experiments.

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