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ADSORPTION AND CHROMATOGRAPHIC PROPERTIES OF MODIFIED SILICA SORBENTS FOR THE PRODUCTION OF VIRAL PREPARATIONS

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SUMMARY

The adsorption and chromatographic properties of modified silica sorbents for application to different viruses have been investigated. Minimal adsorption of viruses was observed on macroporous silicas with hydrophilic properties of the sorbent surface. The universal sorbent for the gel-permeation chromatography of viruses was macroporous silica, chemically modified with polyvinylpyrrolidone (PVP). The viruses were purified on this sorbent without of loss of biological activity. Preparative chromatography on PVP–silica was used for virus vaccine production.

INTRODUCTION

Chromatography on porous silicas is one of the few methods that have been successfully applied to the concentration and purification of virus particles[1–4]. However, as a result of specific features of viruses undergoing chromatography (relatively large dimensions, the presence of complex proteins and lipids on their surface and their low resistance to many physico-chemical effects), the choice of the optimal system for the isolation of viruses is a complex problem.

Virus particles, like proteins, are strongly adsorbed on porous silica sorbents. For most viruses, the isoelectric point is in the pH range 4–5. In this range, the charge on their surface changes, but this change has virtually no effect on adsorption. The adsorption of viruses decreases markedly under alkaline conditions, these conditions being strictly individual for each virus[5]. However, for many viruses alkaline conditions are unfavourable as their biological activity decreases and some viruses, *e.g.*, the rabies virus, are completely inactivated. Hence, it is advisable to use modified porous silicas for the chromatography of viruses.

In order to prevent strong adsorption of viruses on the surface of silicas, sorbents modified with various molecules have been prepared. For example, modification of silica by treatment with serum blood components decreased the adsorption of influenza virus[6]. However, such types of modification are unacceptable because of the low stability of the modified surface under the conditions applied.

Silica beads pre-treated with poly(ethylene oxide) have been used for the purification of viruses by gel-permeation chromatography (GPC), but the coverage was not very stable[2]. Because many viruses are used as vaccine preparations, it is essential

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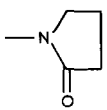
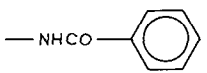
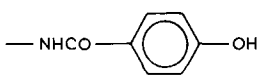
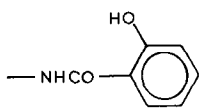
TABLE I

ADSORPTION OF DIFFERENT VIRUSES ON CHEMICALLY MODIFIED SILICA

Buffer, 0.05 *M* Tris–HCl containing 0.13 *M* NaCl (pH 7.8).

Sample No.	*Type of silica*	*Functional groups*	*Virus yield (%)*				
			Toga-	*Mixo-*	*Rabdo-*	*Entero-*	*Hepatitis B*
1	Amino-silica	$-(CH_2)_3NH_2$	0.01	0.03	0	0	0.05
2	Tris-silica	$-NHC(CH_2OH)_3$	80.0	90.0	20.0	20.0	–
3	C_3-Carboxyl-silica	$-(CH_2)_3COOH$	90.0	70.0	30.0	50.0	50.0
4	C_{10}-Carboxyl-silica	$-(CH_2)_{10}COOH$	10.0	1.0	0.2	2.0	0.1
5	Hydroxypropyl-silica	$-(CH_2)_3OH$	50.0	10.0	2.0	10.0	–
6	PVP–Silica		95.0	95.0	90.0	95.0	50.0
7	Benzoyl-silica		0.5	2.0	0.02	0.1	1.0
8	4-Hydroxybenzoyl-silica		30.0	60.0	7.0	0.5	1.0
9	Salicyloyl-silica		5.0	10.0	1.0	0.5	–
10	Glycero-silica	$-OCH(OH)CH_2OH$	90.0	90.0	80.0	70.0	–
11	C_8-Silica	$-(CH_2)_7CH_3$	0.05	0.1	0	0	0
12	Albumin-modified silica		90.0	95.0	90.0	90.0	20.0

that the sorbents are sufficiently stable against dissolution during the chromatographic process in order to preclude penetration of the modifier into the eluate as an impurity. For this purpose, silicas modified by chemical bounding are used. In previous papers[7–10] we described some sorbents based on macroporous silica, chemically modified by different reagents. These sorbents were used for the chromatographic purification of viruses such as tick-borne encephalitis (TBE) virus and rabies virus.

In this paper we summarize the results of our experience on the chromatography of viruses of different classes and characterize the effect of the chemical structure of the surface layer on the selection of the chromatographic conditions.

EXPERIMENTAL

Sorbents

The silicas used were macroporous glasses, MPS-1000-VGKh and MPS-2000-VGKh (Gorky, U.S.S.R.), with particle diameters of 100–200 μm, pore diameters of 110 and 220 nm and porosities of 1.6–1.8 cm^3/g. These macroporous glasses were modified with various reagents and are characterized in Table I.

Sorbents 1, 3, 4, 10 and 11 (Table II) were prepared by silanization of silica with the corresponding silane by a described method[11]. Sorbent 5 was prepared by treatment of silica with γ-aminopropyltriethoxysilane with subsequent conversion of the amino group to a hydroxy group as described previously[6,9]. Chemically bonded PVP–silica (sorbent 6) was prepared in accordance with a previous method[12]. Sorbents 2, 7–9 and 12 were prepared as described previously[8,10].

The coating stability was checked by measuring the amount of modifier via elemental analysis after several washings with pure water and the eluents used in the chromatographic process. No desorption could be detected. The same results were obtained on checking the coating after eluting in more than 100 chromatographic cycles.

Viruses

Investigations were performed with viruses (Table II) that were typical representatives of their parent families. The viruses were propagated in cell cultures or in allantoic medium as described[10,13–15]. Virus-containing fluid was pooled and clarified by centrifugation at 10000 *g* for 40 min at 4°C.

For adsorption studies the viruses were purified by ultracentrifugation[5,10,14,15]

TABLE II

CHARACTERISTICS OF THE VIRAL PREPARATIONS

Family	*Virus*	*Strain*	*Middle virion size (nm)*	*pI*	*Cultivation*
Flaviviridae	TBE	Sophin	50	5.0	Cell line
	West Nile	Egypt (O)	46	5.2	Cell line
Rhabdoviridae	Rabies	Vnukovo-32	75 × 180	6.3	Cell line
Entheroviridae	Polio, type 1	Lsc 2ab	28	4.9	Cell line
Ortomyxoviridae	Influenza	X-79	95	5.3	Allantois
Hepadnoviridae	Hepatitis B	ayw	42	8.0	Serum

with subsequent dialysis against 0.05 *M* Tris–HCl containing 0.13 *M* sodium chloride solution (pH 7.8).

The contents of TBE, rabies, polio and West Nile viruses in the initial samples and the eluate were determined by intracerebral titration in mice[10] and plaque assay, using 10-fold dilutions of the samples[5,13]. The contents of influenza and hepatitis B viruses were measured by enzyme immunoassay using a described modification[16].

Electron microscopy of the viral preparations was carried out by the method of negative contrasting[5,8].

Chromatography

Chromatography was carried out with the standard system for liquid chromatography (LKB, Bromma, Sweden), consisting of a Multiperpex pump (Model 2115), a Uvicord S11 (Model 2838) and Superrac fraction collector (Model 2211), analytical glass columns (5.0–8.0 × 1.0 cm I.D.) and a preparative glass column (60 cm × 10 cm I.D.) of our own construction.

For detection the absorbance was measured at 280 nm with an LKB detector. In some instances the protein concentration in the samples and eluate fractions was determined by Lowry's method.

The adsorption of the viruses was studied on analytical columns (8 cm × 1 cm I.D.) filled with the modified silica and equilibrated with 0.05 *M* Tris–HCl buffer containing 0.13 *M* sodium chloride (pH 7.8). Thereafter 0.5 ml of virus sample was injected on to the column and eluted with the same buffer at a rate of 0.5 cm/min. Fractions of 0.5 ml were collected and assayed for virus content.

Adsorption chromatography

A 5 cm × 1.0 cm I.D. column was filled with an aqueous suspension of chemically modified MPS (sorbent 5) and 80 ml of virus suspension were passed through the column at a rate of 0.5 cm/min. Virions were adsorbed on the carrier, the column being "saturated" with virus. After passage of the virus suspension, non-adsorbed virus and proteins were washed out of the column with 20 ml of 0.05 *M* Tris–HCl buffer (pH 7.5) at the same rate. Virus adsorbed on the carrier was eluted with 0.1 *M* Tris–HCl buffer (pH 8.1). Fractions of 2 ml were collected and assayed for virus and protein contents.

Preparative GPC was carried out in a 60 cm × 10 cm I.D. column filled with PVP-modified porous glass (MPS-1000-VGKh) equilibrated with 0.05 *M* Tris–HCl buffer containing 0.13 *M* sodium chloride (pH 7.8).

A sample of virus of volume 0.8 l was placed on the column and eluted at a rate 1 cm/min. The eluate was collected in the fractions tested for the content of virus activity and protein.

RESULTS AND DISCUSSION

The adsorption of viruses on modified silicas was studied under conditions of maximum stability of their biological properties (the pH of the eluents was 7.7–7.8). The results of studies on the adsorption of viruses on modified silicas are listed in Table I.

Virtually complete adsorption of myxo-, toga-, entero- and rabdoviruses and

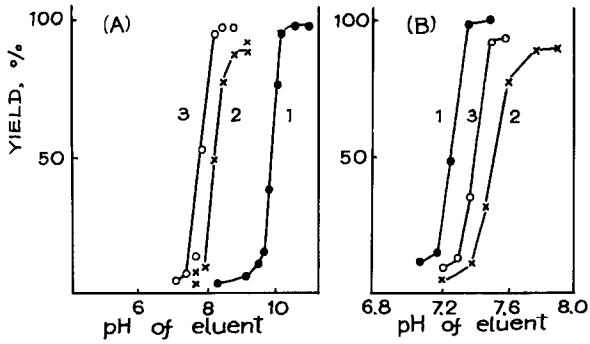


Fig. 1. Virus yield plotted against pH of the eluent in GPC on (A) unmodified and (B) PVP-modified silica. Sorbent, macroporous glass (MPS-2000-VGKh). Viruses; 1 = polio virus; 2 = TBE virus; 3 = influenza virus.

a hepatitis B virus was observed on silicas with hydrophobic surface properties (sorbents 7, 9, 11) and on amino-silicas (sorbent 1). The shielding of the hydrophobic surface by the hydroxyl or carboxyl groups noticeably decreased the adsorption of toga- and myxoviruses (sorbents 4 and 8). Slight adsorption of viruses was observed when carboxyl- and Tris-modified silicas were used. Virtually complete virus elution was achieved from silicas modified with PVP. The hepatitis B virus was an exceptions; its elution from this sorbent under the experimental conditions used was *ca.* 50%.

The results of our experiments agreed with previously published applications of PVP to prevent the adsorption of proteins[17,18] on the silica surface. We used sorbents with covalently bonded PVP instead of sorbents with adsorbed PVP as described elsewhere[17,18].

The results obtained for modified silicas make it possible to draw conclusions concerning the forces of interaction between the virus particles and the silica surface. The existence of hydrophobic interactions is the basic condition of virus adsorption (sorbents 4, 7, 9 and 11). Adsorption can be decreased by inducing a negative charge on the silica surface (sorbents 3 and 4). It can be completely eliminated if the surface is also modified with hydrophilic groups (sorbents 2, 10 and 12).

The dependence of virus elution on the pH of the eluent was investigated for a number of modified silicas. Fig. 1 shows as an example the dependence of virus elution on the pH of the eluent for unmodified silicas and silicas modified with PVP.

TABLE III

GEL-PERMEATION CHROMATOGRAPHY OF VIRUSES ON PVP-MODIFIED SILICA

Chromatography was carried out on an 8.0 × 1 cm I.D. column; volume of virus sample, 0.5 ml.

Virus sample	*Medium*	*Pore size of silica (nm)*	*Virus yield (%)*	*Degree of virus purification (%)*
Influenza	Allantoic fluid	200	90	99.1
Rabies	Cultural fluid	200	85	99.3
TBE	Cultural fluid	100	95	99.6
West Nile	Cultural fluid	100	90	99.3

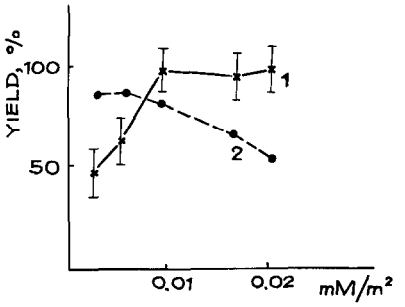


Fig. 2. Yields of TBE virus and ballast proteins in GPC on Tris-modified porous glass (MPS-1000-VGKh), plotted agains surface modifier concentration (mM/m^2). 1, Purified virus yield (%); 2, ballast protein yield (%).

Complete elution of poliomyelitis, tick-borne encephalitis and influenza viruses from the PVP–silica is observed when eluents of pH 7.4–7.8 are used. The viruses are eluted almost completely from the unmodified silicas at pH 10.3 (for poliomyelitis), 8.7 (TBE) and 8.3 (influenza virus). Hence, the use of modified silicas makes it possible to reduce considerably the pH of the eluents and to standardize the chromatographic processes for many viruses (Table III).

Virus elution from silicas also depends on the concentration of the modifier on the sorbent surface. For the Tris-modified silica, the dependence of the elution of the tick-borne encephalitis virus on the concentration of the grafted modifier was determined under the conditions of GPC (Fig. 2). It was found that the minimum concentration of the modifier ensuring effective virus elution is 0.01 mM per m^2 of surface. This result was subsequently taken into account in the modification of the surface with other substances.

The results obtained in the study of virus adsorption on chemically modified silicas makes it possible to select a sorbent suitable for either GPC or adsorption

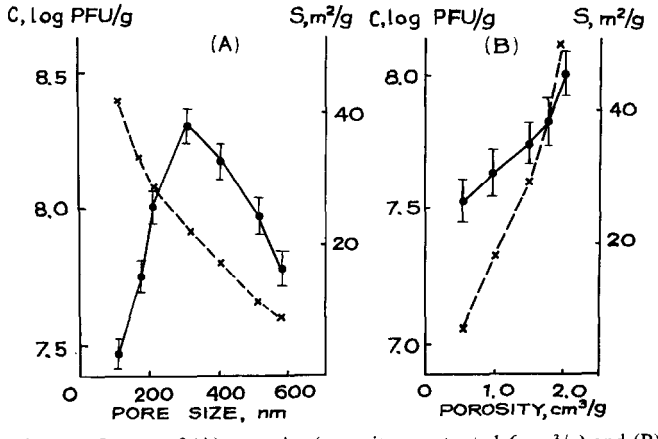


Fig. 3. Influence of (A) pore size (porosity constant, 1.6 cm^3/g) and (B) porosity (pore size constant, 200 nm) on capacity (●) of MPS in adsorption chromatography of TBE virus. ×, Specific surface area of MPS, m^2/g. PFU = titre of infectious virus in plaque-forming units.

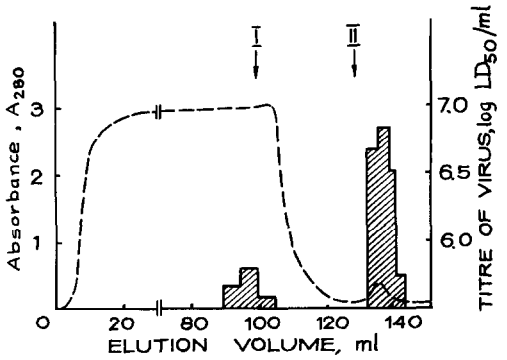


Fig. 4. Adsorption chromatography of rabies virus. (I) Elution buffer, 0.05 *M* Tris–HCl (pH 7.5); (II) desorption buffer, 0.1 *M* Tris–HCl (pH 8.1). Broken line, absorbance (A_{280}); solid line, virus titre as log LD_{50}/ml.

chromatography. However, apart from the physico-chemical properties of the sorbent surface, the separation process is also affected by the geometric parameters of the silica. In particular, it was established for tick-borne encephalitis virus that its diffusion into the pores of silica becomes measurable only if the pore size exceeds 100 nm. The optimum pore size was also determined for adsorption chromatography. Silicas with a pore diameter of 300–400 nm exhibited the maximum capacity for the tick-borne encephalitis virus. In this instance, the virus was effectively adsorbed on the inner pore surface. When silicas of larger pore size were used, the capacity decreased as a result of the decrease in the specific surface area of the sorbent (Fig. 3).

We used these relationships in the preparation of concentrated and purified viral preparations. In particular, it was established for the rabies virus that when the virus is adsorbed on unmodified porous silicas and on sorbents modified with benzoic acid, it is virtually completely inactivated. For concentration of the virus by adsorption chromatography, oxypropyl-silica was used. Its surface exhibited less pronounced hydrophobic properties[10]. With this sorbent, a concentrated rabies virus was obtained that completely retained its biological properties (Fig. 4).

At present, chromatography on modified silicas is used for obtaining viral preparations (vaccines). Adsorption chromatography is used for the concentration and purification of tick-borne encephalitis, influenza and hepatitis B viruses. If the sorbent and the conditions of the adsorption and elution processes are chosen correctly, 50–100-fold concentration and purification to 99% and above of viral preparations can be achieved for preparative applications.

Adsorption chromatography has been developed not only for infectious but also for inactivated viruses. It is particularly important for the concentration and purification of such pathogenic viruses as tick-borne encephalitis, rabies and hepatitis B viruses. The immunological activity of inactivated preparations was determined after chromatography by various tests, in particular by immuno-electron microscopy.

The use of modified PVP–silicas for GPC made it possible to purify preparative amounts of viruses of tick-borne encephalitis, rabies, poliomyelitis, influenza and hepatitis B with minimal losses. This silica was found to be effective in the purification of inactivated viral preparations pre-concentrated by different methods (Fig. 5).

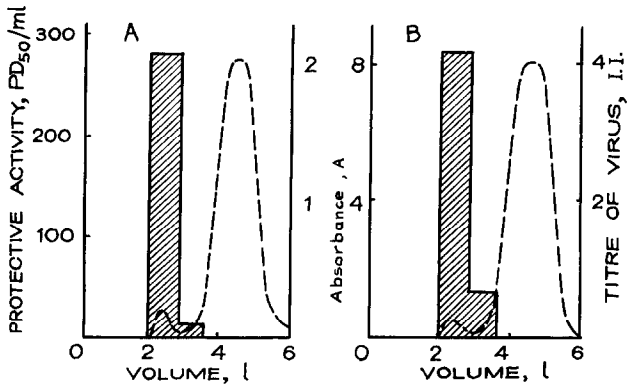


Fig. 5. Preparative GPC of formalin-inactivated TBE virus (A) and inactivated rabies virus (B). Broken lines, absorbance (A_{280}); solid lines, protective dose (PD) activity or immunogenicity index (I.I.).

The purification of preparations of rabies virus concentrated by ultrafiltration and of tick-borne encephalitis virus concentrated by adsorption chromatography is very effective. Both preparations were prepared in more than 99% purity with maintenance of immunological activity and were used as experimental vaccines.

In recent years, combinations of several chromatographic methods for the isolation of individual substances have been widely used. It is usual to obtain viral preparations by a combination of adsorption chromatography and GPC. This combination makes it possible to obtain standardized preparations ready for use. The use of GPC in the last stage not only provides additional removal of protein impurities but also leads to the replacement of the alkaline by the neutral buffer. Moreover, the substances used for the inactivation or conservation of the virus are also removed.

Hence, the use of modified porous silicas makes it possible to obtain highly purified preparations with characteristics such that they can be used directly as vaccines or preparations for sensitive diagnostics.

REFERENCES

1 R. J. Barton, *J. Gen. Virol.*, 35 (1977) 77.
2 G. L. Hawk, J. A. Cameron and L. B. Dufailt, *Prep. Biochem.*, 2 (1972) 193.
3 S. E. Bresler, D. P. Dobychin, A. G. Popov and A. T. Sukhodolova, *Mol. Biol.*, 3 (1969) 29.
4 W. Haller, *Virology*, 33 (1967) 740.
5 I. V. Krasilnikov, L.B. Elbert, M. Khanina and S. Sobolev, *Arch. Virol.*, 79 (1984) 241.
6 B. V. Mchedlishvili, S. P. Zhdanov, N. V. Katushkina, V. M. Kolikov, Kh. Ya. Kipper, E. V. Koromaldi, V. M. Molodkin, S. E. Bresler, L. M. Molodkina, O. A. Vostryukhina and I. L. Potokin, in T. V. Pradze (Editor), *Inactivated Influenza Vaccine*, Medicine, Leningrad, 1976, p. 43.
7 I. V. Krasilnikov, L. B. Elbert, L. L. Mamonenko, V. V. Pogodina, G. L. Krutyanskaya, S. E. Bresler, V. M. Kolikov, B. V. Mchedlishvili, V. N. Borisova, N. S. Golovina and L. A. Nachapetyan, *Vopr. Virusol.*, 22 (1977) 685.
8 I. V. Krasilnikov, L. B. Elbert, V. N. Borisova and L. A. Nakhapetyan, *Acta Virol.*, 29 (1985) 273.
9 V. N. Borisova, I. V. Krasilnikov, B. V. Mchedlishvili, L. A. Nakhapetyan and L. B. Elbert, *Author's Certificate (U.S.S.R.)*, No. 1071306, 1981; *Byull. Izobret.*, No. 5 (1984) 17.
10 I. V. Krasilnikov, T. A. Aksenova and B. V. Mchedlishvili, *Acta Virol.*, 25 (1981) 205.
11 P. J. Robinson, P. Dunnil and M. D. Lilly, *Biochim. Biophys. Acta*, 242 (1971) 659.

12 L. B. Zubakova, V. N. Borisova, S. K. Koroleva, V. Ya. Davidov and G. N. Philatova, *Zh. Prikl. Khim.*, 40 (1987) 1491.
13 F. X. Heinz, Ch. Kunze and H. Tauma, *J. Med. Virol.*, 6 (1980) 213.
14 D. Trent, *J. Virol.*, 22 (1977) 608.
15 G. N. Trushinskaya, V. T. Ivanova and L. Ya. Zakstelskaya, *Acta Virol.*, 28 (1984) 487.
16 A. P. Ivanov, *Arch. Virol.*, 67 (1981) 71.
17 L. Letot, J. Lesec and C. Quivoron, *J. Liq. Chromatogr.*, 4 (1981) 1311.
18 J. Köhler, *Chromatographia*, 21 (1986) 573.