

Gel Permeation Chromatography. I. A New Method for Molecular Weight Distribution of High Polymers

J. C. MOORE, *Texas Basic Research Department, The Dow Chemical Company, Freeport, Texas*

Synopsis

Polystyrene gels crosslinked in the presence of diluents have been made in fine-mesh bead form suitable for packing into chromatographic columns. A series of narrow molecular weight range polymer fractions was eluted through such columns with aromatic and chlorinated solvents. Effluent concentrations were detected and recorded by a continuous differential refractometer. The fractions were shown to be efficiently separated. Columns capable of separating adjacent polymeric samples of high molecular weight were prepared from gels crosslinked in the presence of large amounts of diluents having little or no solvent action on polystyrene. Smaller proportions of diluents and those with more solvent action yielded columns with lower molecular weight permeability limits. Such studies provided a unique quantitative view of the topology of the gels. They also demonstrated that rapid repetitive molecular weight distribution data can be obtained in this way on polymers for which solvents compatible with the gels are available.

Introduction

Early in their work on ion-exclusion, Wheaton and Bauman¹ found that many nonionic substances of low molecular weight were separated by elution with water through a column packed with ion-exchange resin particles. Since that time the techniques of column chromatography on crosslinked gels have become broadly applicable to separations of large from small molecules in aqueous solutions. Lathe and Ruthven² showed that the separating range could be greatly extended by using swollen starch grains as column packing, differentiating between a globulin and hemoglobin, for example, of molecular weights 150,000 and 67,000, respectively. Porath and Flodin^{3,4} have made available a series of hydrophilic gel column packings and have introduced the term "gel filtration" for the process, which has achieved considerable usefulness. Lea and Sehon⁵ have recently described other hydrophilic gels of this nature. However, being swellable only in aqueous media, their use is limited to the separation of water-soluble substances.

Hydrophobic gels of high permeability have not been available. Several interesting separations were reported by Cortis-Jones⁶ on columns packed with crosslinked polystyrene. His investigation did not, however, extend to large molecules. Vaughan⁷ showed that some separation of low molec-

ular weight polystyrenes did occur when they were eluted with benzene through polystyrene beads so lightly crosslinked that they had swollen in benzene to 51 times the volume of the dry polymer. However, all these gels were crosslinked in the absence of diluents, while the hydrophilic gels referred to were crosslinked in aqueous solution. This is a significant difference. Lloyd and Alfrey^{8,9} have pointed out that gel networks of altered structure are produced by crosslinking in the presence of a diluent which is a solvent for the monomer. If the diluent is a nonsolvent for the resulting polymer, the gel may be still further altered by precipitation to give a rugged internal structure, and outstanding properties of stability and permeability are claimed for ion exchange resins based on such polymers.¹⁰⁻¹² The properties of gels prepared in the presence of various diluents have been investigated by Lloyd and Alfrey,¹³ and diluents of intermediate solvent power were shown to give gels of intermediate properties.

Samples and Experimental Method

From the foregoing considerations, it appeared likely that we could prepare polystyrene beads with sufficient crosslinking to confer a desirable amount of rigidity, and still regulate the permeability of the network over a wide range by varying the amount and nature of the diluent present at the time of crosslinking. The permeabilities of the resulting gel networks were revealed by eluting a series of very similar compounds covering a wide range of molecular weights through a column packed with the gel. The properties of these marker materials are shown in Table I. In the low to medium molecular weight range the commercially available polypropylene glycols served as standards. A series of anionically polymerized polystyrenes covered the range from medium to high molecular weights.¹⁴ In both cases the samples, while not monodisperse, gave well defined peaks. Aromatic or chlorinated eluting solvents chemically similar to the polystyrene gel were used to suppress adsorption and partition effects as much as pos-

TABLE I
Sample Materials

Polymer	Mol. Wt., \bar{M}_w	\bar{M}_w/\bar{M}_n
Anionic polystyrenes:		
S1	13,850	1.50
S102	82,000	1.05
S105	154,000	1.04
S108	267,000	1.08
S1159	570,000	1.05
S12	1,197,000	1.19
S114	3,500,000	1.24
Polypropylene glycols: The Dow Chemical Co.*		

* Average molecular weight by endgroup analysis as indicated in the identifying number, as: P-2000.

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sible, and tailing was not evident with these samples. Eluent composition was followed by a Waters continuous differential refractometer, with a Rinco fraction collector adapted to mark the recorder chart at each volume increment.

Experimental Results and Discussion

A series of polystyrene gels with toluene as diluent in increasing proportions showed several significant changes. To avoid a soft compressible gel with resulting poor flow properties in the packed column, an increase in the proportion of diluent required a corresponding increase in the degree of crosslinking. With diluent and crosslinker increasing together, the permeability limit rose gradually at first, then rapidly at high dilution. With a rigidly crosslinked gel, the packed column became easier to pack and to use, less affected in volume by changes of eluting solvent. The compositions of these gels are shown in Table II. The permeability of each gel was then visualized by plotting the elution volume of each sample peak against the logarithm of the average molecular weight of the sample. These plots showed slanting lines, often quite straight for a considerable range, from an upper limit of permeability sharply defined at the interstitial volume, down to a nebulous lower limit around the total liquid

TABLE II
Permeabilities of Styrene Gels with Varying Proportions of Toluene

Gel	Gel composition			M.W. permeability limit	Notes
	Styrene, wt.-%	Divinyl- benzene, wt.-%	Toluene, wt.-%		
PSX8	92	8	0	1,000	
PSX4	96	4	0	1,700	
PSX1	99	1	0	3,500	Rubbery
PSX0.1	99.9	0.1	0		Too soft to pack
A	79.1	4.2	16.7	2,500	
B	65.7	5.7	28.6	c.a. 7,000	
C	30	10	60	7,000	
D	24.8	2.5	72.7		Too soft to pack
E	9	11	80	250,000	

volume of the column, in which region the elution volume was determined more by polarity factors than by molecular size. Examples of the elution tracings obtained and the resulting permeability curves are shown in Figures 1 and 2.

Great increases in permeability were found when the nature of the diluent was changed. In this series the gels were made by polymerizing a mixture of 21.8% styrene, 18.2% commercial (55%) divinylbenzene, and 60% diluent by weight. Varying the diluent by progressively replacing toluene with *n*-dodecane caused regular increases in permeability, but a limit was

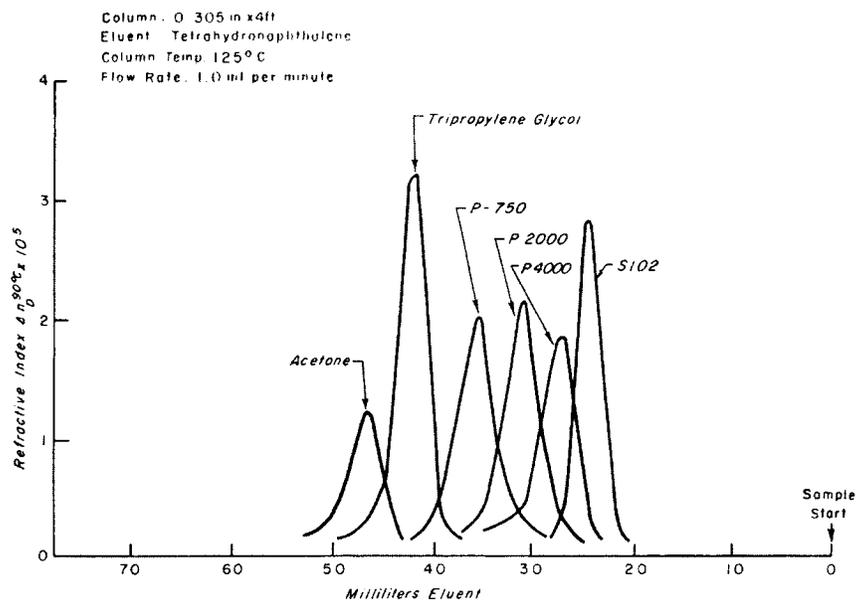


Fig. 1. Superimposed traces of elution peaks obtained with gel C.

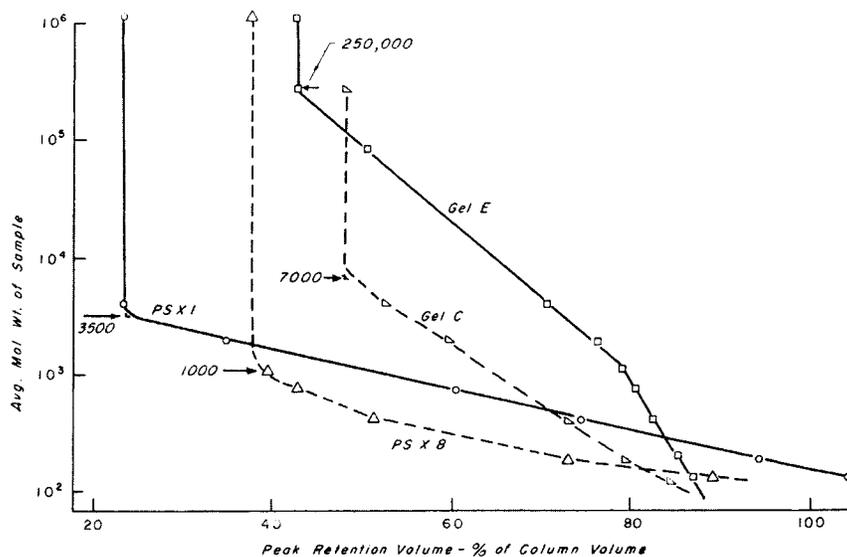


Fig. 2. Representative permeability curves for gels in Table II and determination of permeability limits.

reached when the diluent was 75% dodecane. Above that point the porous-structured beads were coated with a thin but impermeable skin. A non-solvent on the more polar side, isoamyl alcohol, progressively replacing diethylbenzene as diluent, showed very little increase in permeability at

TABLE III
Permeabilities of Gels with Various Diluents, All Made from 30% Styrene, 10% Divinylbenzene, 60% Diluent*

Gel	Diluents, parts/100 parts of gel	M.W. permeability limit
C	60 Toluene	7×10^3
F	30 Toluene, 30 diethylbenzene	1.5×10^4
G	60 Diethylbenzene	1.2×10^4
H	45 Toluene, 15 <i>n</i> -dodecane	1×10^5
I	30 Toluene, 30 <i>n</i> -dodecane	3×10^5
J	15 Toluene, 45 <i>n</i> -dodecane	2×10^6
K	10 Toluene, 50 <i>n</i> -dodecane	$<2 \times 10^3$
L	40 Diethylbenzene, 20 isoamyl alcohol	ca. 3.6×10^3
M	20 Diethylbenzene, 40 isoamyl alcohol	ca. 8×10^6
N	13.3 Diethylbenzene, 46.7 isoamyl alcohol	ca. 10^{10}
O	60 Isoamyl alcohol	Extremely high

* "Styrene" is a mixture of styrene and ethylvinylbenzene.

first, probably because of loss of some of the isoamyl alcohol to the aqueous continuous phase in which the beads were polymerized. With larger proportions of isoamyl alcohol in the diluent during polymerization, the permeability increased rapidly, far beyond the molecular sizes of the test materials used. This series of tests is summarized in Table III and Figures 3 and 4. The changes of gel structure indicated by the permeability curves were further shown in electron micrographs prepared by Dr. E. B. Bradford (Fig. 5). To preserve the swollen gel structure for electron microscopy, the porous polymer beads were soaked in a mixture of acrylate monomers, potted by polymerizing in a small gelatin capsule, and sectioned.

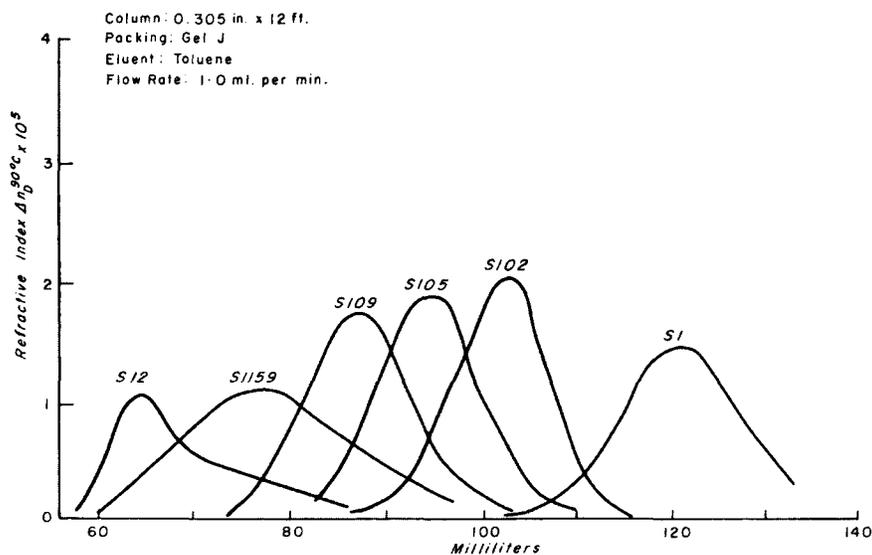


Fig. 3. Superimposed traces of elution peaks for polystyrene samples (Table I) obtained with gel J.

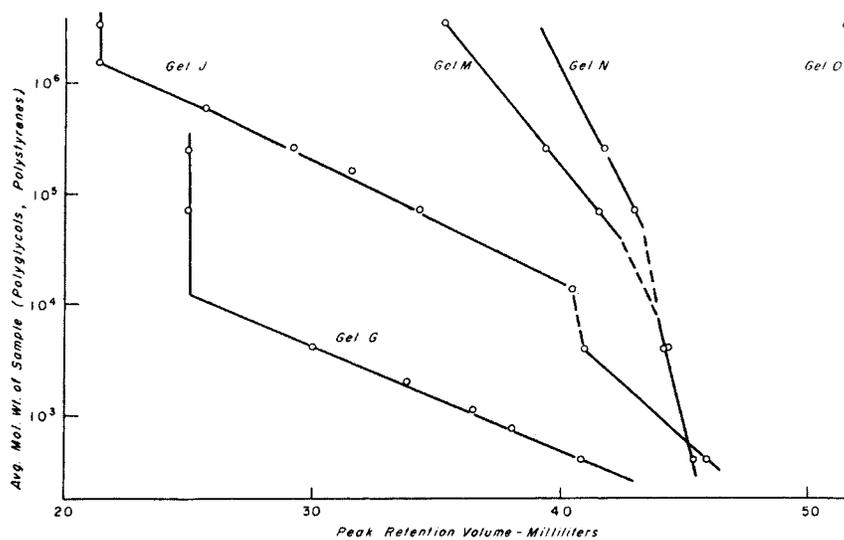


Fig. 4. Representative permeability curves for gels from Table III.

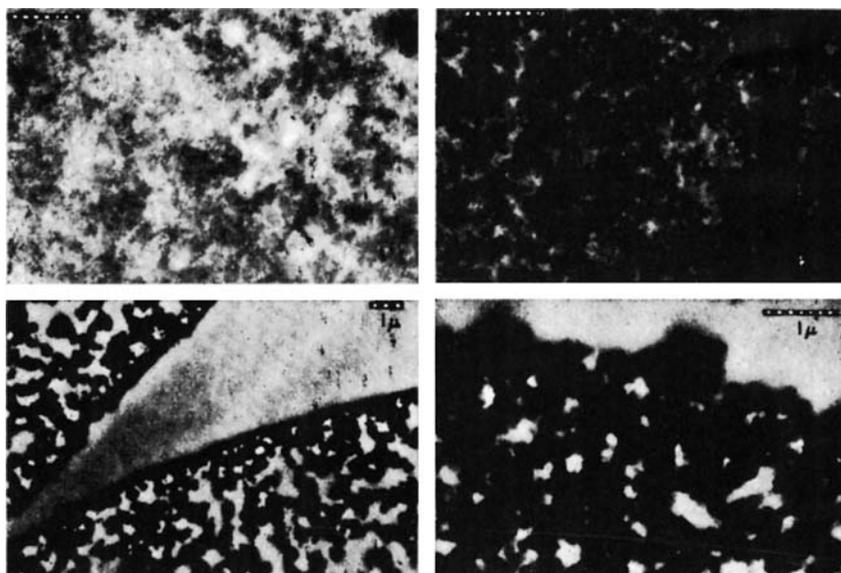


Fig. 5. Electron micrographs of thin sections of gels from Table III: (a) gel C; (b) gel G; (c) gel K; (d) gel M.

The chromatographic separation appears to be close to an equilibrium process, in which the solute molecules very rapidly diffuse into all parts of the gel network not mechanically barred to them. With small samples at slow flow rates, very sharp peaks can be obtained. With larger samples or with faster flow rates, the peaks are broader, but not earlier as would be expected if diffusion rates were the basis of separation. Even large mole-

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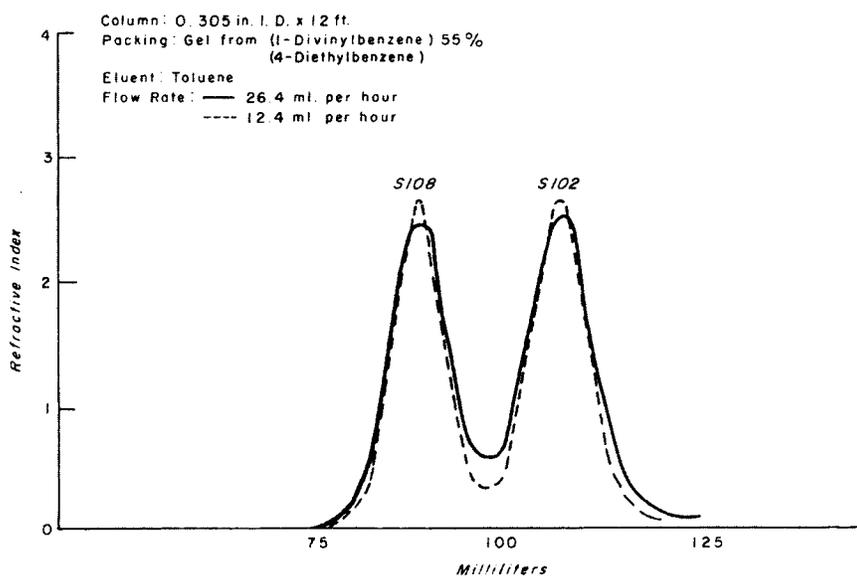


Fig. 6. Separation of two narrow-distribution styrenes at two flow rates.

cules do not appear to affect the permeability of the gel to small molecules, so that the elution pattern of a mixture can be expected to match the sum of the elution patterns of the components, as long as overloads are avoided. These effects were shown in the separation of two polystyrenes by elution with toluene through a column of 0.305 in. i.d. and 12 ft. long. The column was packed with a 200–325 mesh fraction of a bead polymer made from a mixture of one part commercial 55% divinylbenzene and four parts diethylbenzene. The 1.0 ml. sample contained 6.7 mg. each of styrene polymers S108 and S102 (Table I) in toluene. Figure 6 shows the tracings of effluent refractive index at two flow rates, 26.4 ml./hr. and again at 12.4 ml./hr. At the slower rate the resolution was improved, but the peaks were not shifted. Samples of the effluent were evaporated and examined by ultracentrifugation, and the separation was shown to be complete.

While the separation of the different polymeric species from the same monomer can be correlated quite well with molecular weight, nonhomologous compounds do not always correlate so simply. With larger molecules, the molecular size seems to be the prime factor with polarity becoming more important at lower molecular weights. These factors were demonstrated with a gel column of lower permeability, 1.3 cm. diameter and 109 cm. long. The packing was 100–200 mesh PSX4 polystyrene (Table II) swollen in methylene chloride, packed and eluted with the same solvent. Four compounds were sampled, using 0.25 ml. of 1% solution. The elution volumes of the resulting peaks show the expected inverse relationship to molecular model dimensions, with some deviation probably due to polarity, and no correlation with molecular weight, as shown in Table IV.

TABLE IV
 Inverse Correlation of Peak Elution Volume with Molecular Size

Substance	Elution volume, ml.	Molecular size, A.	M.W.
Bis (glycidyl ether) of Bisphenol A $(\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_4\text{OCH}_2\text{CH}(\text{CH}_2\text{O}))_2$	75	18.5	340
Dibromotetrachloroxylylene <i>m</i> -(CH_2Br) $_2\text{C}_6\text{Cl}_4$	108	13.5	402
Mixed xylenes, $(\text{CH}_3)_2\text{C}_6\text{H}_4$	109	8.9	106
Perchloroethylene, C_2Cl_4	122	7.7	166

It is apparent that polystyrene gels, crosslinked in the presence of appropriate diluents, can be used in column chromatography to make molecular size fractionations over an extremely wide range. The term "gel permeation chromatography" is proposed for this technique. It suggests and describes a mechanism of fractionation in which solute molecules are separated by their permeation into a gel which offers different internal volumes to molecules of different sizes over an extended range. This mechanism is prominent in the separation of small nonionic hydrophilic molecules by water elution through beds of ion-exchange resins, as noted much earlier by Wheaton and Bauman,¹ and in the "gel filtration" fractionation of hydrophilic molecules on Sephadex.^{3,4} However, to us the term "gel filtration" has not seemed apt for a fractionation of extended range. It did seem apt for the short-column, sievelike separations with which the Sephadex gels were first reported.

This work has shown that structurally modified polystyrene gels, eluted with compatible solvents, have utility in making molecular size fractionations of hydrophobic macromolecules. It seems quite possible that suitable copolymer-diluent systems may be found to yield gels of adequate strength and rigidity with any desired combination of solvent compatibility and permeability. This would indeed open a broad field of usefulness to gel permeation chromatography.

Important contributions of many other persons to this study are gratefully acknowledged. Thanks are particularly due to W. G. Lloyd and T. Alfrey for consultations on the control of permeability in crosslinked gels, to D. R. Asher and D. B. Parrish for some of the fine-mesh bead preparations, to H. W. McCormick for the anionically polymerized styrenes and the ultracentrifuge work, to E. B. Bradford for the electron microscopy, and to M. C. Arrington for technical assistance.

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Résumé

On a préparé des gels de polystyrène pontés en présence de diluants sous forme de fines perles convenant à l'entassement dans les colonnes chromatographiques. Une série de fractions polymériques de poids moléculaires voisins est élue à travers de telles colonnes avec des solvants aromatiques et chlorés. On détermine et enrégistre les concentrations sortante à l'aide d'un réfractomètre continu différentiel. Les fractions sont ainsi efficacement séparées. On prépare des colonnes capables de séparer des échantillons de polymères de poids moléculaires voisins élevés à partir de gels pontés en présence de grandes quantités de diluants possédant une action solvatante faible ou nulle sur le polystyrène. Des proportions plus faibles de diluants et ceux possédant une plus forte action solvatante fournissent des colonnes présentant des limites de perméabilité des moléculaires plus faibles. De telles études donnent une vue quantitative unique de la topologie des gels. Elles démontrent également que des résultats rapides et reproductibles de distributions de poids moléculaires peuvent être obtenus de cette façon sur des polymères pour lesquels des solvants compatibles avec les gels sont existants.

Zusammenfassung

In Gegenwart von Verdünnungsmitteln vernetzte Polystyrolgele wurden in Feinsieb-Kugelform zur Verwendung in chromatographischen Säulen erzeugt. Eine Reihe engverteilter Polymerfraktionen wurde durch solche Säulen mit aromatischen und chlorierten Lösungsmitteln eluiert. Die Effluentkonzentrationen wurden mit einem kontinuierlich arbeitenden Differentialrefraktometer bestimmt und registriert. Es trat eine wirksame Trennung der Fraktionen ein. Zur Trennung von hochmolekularen Polymerproben mit benachbartem Molekulargewicht wurden Säulen mit Gelen gebaut, die in Gegenwart grosser Mengen von Verdünnungsmitteln mit geringer oder fehlender Lösungswirkung für Polystyrol vernetzt worden waren. Kleinerer Gehalt an Verdünnungsmitteln und Verdünnungsmitteln mit grösserer Lösungswirkung für Polystyrol lieferten Säulen mit niedrigeren Molekulargewichtspermeabilitätsgrenzen. Diese Untersuchungen ergaben einzigartige Aufschlüsse über die Topologie der Gele. Sie zeigt ferner, dass auf diese Weise für Polymere, für welche mit den Gelen verträgliche Lösungsmittel vorhanden sind, rasche und reproduzierbare Ergebnisse für die Molekulargewichtsverteilung zugänglich sind.

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