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INFLUENCE OF CATIONS AND ANIONS ON THE SEPARATION PROPERTIES OF MACROPOROUS POLYSTYRENE-TYPE RESINS FOR CEPHALOSPORINS

W. VOSER* and K. WEISS
Biotechnology K-693, Ciba-Geigy Limited, CH-4002 Basle (Switzerland)

SUMMARY

Cephalosporin C can be separated from deacetyl- and deacetoxycephalosporin C, cephalosporin N (penicillin N) and other impurities when an aqueous solution is percolated at neutral pH through a macroporous polystyrene-type adsorbent, followed by elution with deionized water. For a quantitative separation, alkaline-earth metal ions should be the only cations present or the molar ratio of alkali metal to alkaline-earth metal cations should be $\geqslant 1:2$. Surprisingly, an unfavourable cation ratio can be corrected by adding α-hydroxy-γ-methylmercaptobutyrate anion. This is the first instance in which the separation properties of macroporous polystyrene-type adsorbents depend on the nature of the inorganic cations present in the feed solution. Differences exist in the degree of separation obtained between commercial resins.

INTRODUCTION

During the last two decades, numerous macroporous adsorbents of the polystyrene type without any functional groups were marketed by most of the leading manufacturers of ion-exchange resins (see Table I). These resins are characterized by pore volume (0.5–4.5 ml/g), specific surface area (200–800 m^2/g), pore diameter (40–1300 Å), pore size distribution and bead size distribution. They are recommended for the adsorption of water-soluble organic compounds with hydrophobic and hydro-

TABLE I

POLYSTYRENE-TYPE MACROPOROUS ADSORBENTS AND THEIR MANUFACTURERS

Resins	*Manufacturer*
Amberlite* XAD-1, -2, -4	Rohm and Haas
Diaion HP-10, -20, -21, -30, -40, -50	Mitsubishi
Duolite ES-861, -862, -866	Dia-Prosim
Imac Syn-46, -72	Akzo Chemie
Kastel S-111, -112, -114	Montedison
Lewatit OC-1031	Bayer
Relite ADS	Resindion

* First commercially available resins.

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philic molecular properties (ref. 1 and the technical notes on these resins of the manufacturers). Inorganic salts and highly hydrophilic organic substances, such as carbohydrates, some amino acids and peptides, are only slightly or not adsorbed when percolated through the resins in a column. Carboxylic acids and organic bases are more strongly adsorbed as free acids or bases (undissociated form) than as the corresponding inorganic salts (dissociated form).

In the course of our experiments on the isolation and determination of cephalosporin C (CC), which is a secondary metabolite of *Cephalosporium acremonium*, and its by-products (see Table III), it was observed that neutral culture filtrates contain one or more factors that influence the separation properties of polystyrene-type macroporous adsorbents. The relevant factors were evaluated using Diaion HP-20, which has been mentioned in a Japanese patent by Meiji Seika[2] for the separation of cephalosporin N (CN) from CC at neutral pH. The separation properties of some other commercially available resins were also evaluated.

EXPERIMENTAL

Feed solutions

Culture filtrates were prepared by filtration of the culture broth at harvest pH using Dicalite (diatomaceous earth) as a filter aid. In order to work under uniform conditions in all experiments, the filtrate was concentrated to about 20% and freeze-dried. A solution containing about 30 g/l of CC was then prepared.

For the experiments with aqueous solutions of varying composition, technical-grade sodium, ammonium or magnesium salts of CC [composition *ca.* 90% CC, 5% deacetyl-CC (DA-CC), 1.5% deacetoxy-CC (DAO-CC)] were dissolved in deionized water to a concentration of about 30 g/l of CC. Various amounts of alkaline-earth metal chlorides or sulphates, sodium acetate or the sodium salt of α-hydroxy-γ-methylmercaptobutyric acid (HMBA), methionine (MET) and α-hydroxybutyric acid (HBA) were added as required.

Resin

Diaion HP-20, commercial grade, was used.

Column

The diameter was 50 mm, the bed height *ca.* 500 mm and the bed volume (BV) 1 l. The bottom had a low dead volume. The column was closed and provided with a pressure indicator. The separation was performed under a slight overpressure, which was adjusted with a needle valve in the exit line. The solutions were fed with a piston pump. The supernatant liquid on the resin was kept to a minimum.

Operating conditions

The feed volume was 200 ml, containing *ca.* 6 g of CC, with a pH of 6.5. The eluent was deionized water at a flow-rate of 1 BV/h. The fraction volume was 0.2 BV and the overpressure *ca.* 0.5 bar.

Analytical evaluation

The absorbance was measured at 260 nm. CC, DA-CC and DAO-CC were determined by high-performance liquid chromatography (HPLC) and CN microbiologically.

RESULTS

Separation properties of Diaion HP-20 for cephalosporin mixtures of different origins

When an aqueous alkali metal salt solution of technical-grade CC (ammonium behaves like an alkali metal), a culture filtrate from a methionine-free or a methionine-containing medium was percolated through Diaion HP-20 followed by deionized water, the following results were observed (see Table II, Nos. 1–3). No. 1: CC was separated from DA-CC, CN and DAO-CC. On concentration and crystallization by addition of isopropanol, CC was isolated as the mixed calcium–magnesium salt containing 0.5 mole of alkaline-earth metal cation per mole of CC. No. 2: the CC peak was split into two maxima, the first having only a marginal separation. No. 3: the separation of CC was only marginal.

TABLE II

RESULTS OF SEPARATION EXPERIMENTS USING DIAION HP-20

No.	*Composition of feed solution*	*Elution curve maxima (BV)*			
		DA-CC	*DAO-CC**	*Cephalosporin C*	
				Peak 1	*Peak 2*
1	Culture filtrate from methionine-containing media**	1.2	1.4	–	2.8
2	Culture filtrate from methionine-free media***	1.5	1.8	2.2	3.2
3	Aqueous solutions of alkali metal salts (including ammonium) of CC	1.2	1.4	1.6	–
4	No. 3 + insufficient alkaline-earth metal ions§	1.2	1.4	1.8	2.8/3.2
5	No. 3 + sufficient alkaline-earth metal ions§§; inorganic anion Cl^-	1.4	1.6	–	3.2
6	No. 5, but inorganic anion SO_4^{2-}	1.2	1.6	–	2.8
7	Aqueous solution of alkaline-earth metal salts of CC	1.6	2.2	–	2.8
8	No. 4 + acetate, MET or HBA ions**	1.2	1.4	1.8	3.2
9	No. 4 + HMBA ions**,§§§	1.2	1.4	–	2.8
10	No. 3 + HMBA ions**	1.2	1.4	1.8	–

* Also position of cephalosporin N.

** Molar ratio CC: alkali metal: alkaline-earth metal $\approx$ 1:8:2.5 (cation ratio 1:0.31).

*** Molar ratio CC: alkali metal: alkaline-earth metal $\approx$ 1:7:1.5 (cation ratio 1:0.21).

§ Molar ratio alkali metal: alkaline-earth metal $<$ 1:2.

§§ Molar ratio alkali metal: alkaline-earth metal $\geqslant$ 1:2.

§§§ $\geqslant$ 0.5 mole per mole of CC.

Here there are one or more factors in the culture filtrate that determine the separation properties of Diaion HP-20. The main constituents of the culture filtrates are shown in Table III.

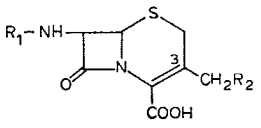
Influence of inorganic cations (see Table II, Nos. 4–7)

When increasing molar ratios of a water-soluble alkaline-earth metal salt were added to an aqueous alkali metal salt solution of technical-grade CC (CC crystallizes as the monosodium salt), an increasing second peak of CC at 2.7–3.2 BV appeared

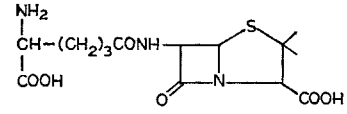
TABLE III

MAIN KNOWN CONSTITUENTS OF CULTURE FILTRATES

Formulae and abbreviations:



R_1	R_2	*Product*
$= NH_2$	$-OCOCH_3$	Cephalosporin C (CC)
$CH-(CH_2)_3CO-$ | COOH	$-OH$	Deacetylcephalosporin C (DA-CC)
	$-H$	Deacetoxycephalosporin C (DAO-CC)
$H-$	$-OCOCH_3$	7-Aminocephalosporanic acid



= cephalosporin N (CN ≡ penicillin N)

$CH_3-S-CH_2-CH_2-CH(OH)-COOH$ = α-hydroxy-γ-methylmercaptobutyric acid (HMBA)

Charge	*Inorganic*	*Organic*
Neutral	Unknown	Carbohydrates, peptides
Cationic	Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}	Unknown (peptides?)
Anionic	Cl^-, SO_4^{2-}	CC^-, $DA\text{-}CC^-$, $DAO\text{-}CC^-$, CN^-, $HMBA^-$ (in the case of methionine-containing media), other organic acids, peptides

and the first peak disappeared. At a molar ratio of alkali metal to alkaline-earth metal cations equal to or greater than 1:2 the first peak of CC was absent and a complete separation of CC was achieved. In this instance only the molar ratios of the two cations are important and not their ratio with respect to CC. A complete separation was also achieved when an aqueous solution of an alkaline-earth metal salt of technical-grade CC was used. Thus 0.5 mole with respect to CC is sufficient if alkali metal ions are absent. In this instance the resin must be washed with acid to prevent interference by small amounts of alkali metal ions, which are difficult to remove after alkaline regeneration of the resin.

The results indicate that alkaline-earth metal cations influence the separation properties of Diaion HP-20, and that Mg, Ca, Sr and Ba added as water-soluble salts, *e.g.*, chlorides, have identical properties. The alkali metal to alkaline-earth metal molar ratio is decisive, and not the CC to cation ratio. The minimum cation ratio for a complete separation is 1:2. In the absence of alkali metal ions 0.5 mole of alkaline-earth metal cations with respect to CC is sufficient for a good separation of CC.

The factor or factors that are responsible for the elimination of the negative effect of the unfavourable cation ratio (see the second footnote to Table II) in culture filtrates of methionine-containing media remain to be identified.

Influence of organic anions (see Table II, Nos. 8–10)

From the results in Table II, Nos. 1 and 2, it is obvious that the separation properties of Diaion HP-20 are also correlated with the presence of methionine in the nutrient medium initially, which is virtually completely metabolized by the end of the fermentation. One of its main metabolites is HMBA. Surprisingly, it was found that the addition of 0.5 mole or more of HMBA as the sodium salt to an aqueous solution of technical-grade CC with an unfavourable cation ratio resulted in a complete separation of CC from its by-products. However, in the absence of alkaline-earth metal cations no improvement in the separation properties occurred. Acetate, MET or HBA had no effect.

Hence the HMBA anion, which is a metabolite of methionine, can eliminate the effect of an unfavourable cation ratio, but has no effect in the absence of alkaline-earth metal cations.

Influence of inorganic anions (see Table II, Nos. 5 and 6)

During the evaluation of the different alkaline-earth metal cations it was observed that chloride and sulphate anions also had a slight influence on the separation properties of Diaion HP-20 in the presence of sufficient alkaline-earth metal cations, but the difference in the maxima was only 0.4 BV.

Separation behaviour and physical properties of some commercial resins (see Table IV)

The results of preliminary experiments with commercial resins and their relevant physical properties are summarized in Table IV. Three groups can be distinguished. Variations in efficiency (tailing, capacity, etc.) that are at least partly due to differences in particle size exist within the three groups. The differences in separation behaviour cannot be correlated with any physical property of the resins, including the pore size distribution (not shown in Table IV).

TABLE IV

SEPARATION BEHAVIOUR AND PHYSICAL PROPERTIES OF SOME COMMERCIAL RESINS

Resin	*Separation properties*	*Surface area (m^2/g)*	*Pore volume (ml/g)*	*Maximum of CC (BV)*
Amberlite XAD-1	Marginal	100	—	1–1.4
Diaion HP-30		570	0.87	
Amberlite XAD-2	Medium	300	0.684	1.8–2.5
Diaion HP-10		501	0.64	
Imac Syn-72		470	—	
Amberlite XAD-4	Good	784	0.976	2.8–4
Diaion HP-20		718	1.16	
Diaion HP-40		704	0.63	
Diaion HP-50		590	0.81	
Duolite ES-861		~500	~0.900	
Imac Syn-46		750	—	
Kastel S-112		450–600	1–1.8	
Lewatit OC-1031		350–400	—	

DISCUSSION

This is the first example to be described of the separation properties of polystyrene-type adsorbents for carboxylic acids depending on the nature of inorganic cations and/or their ratio in the feed solution. As yet there is no explanation for the complex effects of cations and anions on the separation properties of macroporous adsorbents or of the differences amongst the commercial resins. These complex effects seem to exist for all cephalosporins with an acetoxymethyl group in position 3, because they were also observed for 7-aminocephalosporanic acid and derivatives thereof. Such phenomena reduce the value of the use of the resins in analytical determinations or the industrial recovery of cephalosporin C, but they do allow the preparation of pure cephalosporin C. Cephalosporin C is isolated as the mixed calcium–magnesium salt, which is not very suitable for transformation to 7-aminocephalosporanic acid.

The separation properties of these resins at acidic pH, which is the basis for the recovery procedure of Ciba-Geigy[3], are not cation or anion dependent.

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