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SELECTIVE SEPARATION OF AMINO ACIDS BY REACTIVE EXTRACTION

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ABSTRACT. The method of reactive extraction with di-(2-ethylhexyl)phosphoric acid (D2EHPA) for the separation of a range of amino acids is studied. Amino acids are extracted by means of an interfacial reaction of ionic exchange type, if the amino acid exists as a cation in aqueous solution. The separation yield is controlled by the pH value of the aqueous phase, which is due to the acidic or basic character of each amino acid.

The results obtained on the individual reactive extraction indicated the possibility of the amino acids selective separation as a function of the pH value of aqueous solution and the acidic or basic character of each amino acid. Thus, an operation flow for selective separation has been elaborated and demonstrated experimentally. Using multistage extraction, the total separation of the following amino acids groups has been performed: neutral amino acids (l-glycine, l-alanine, l-tryptophan) at pH = 5 - 5.5 (nine extraction stages), basic amino acids (l-lysine, l-arginine) and l-cysteine at pH = 4 - 4.5 (ten extraction stages), l-histidine at pH = 3 - 3.5 (five extraction stages), and acidic amino acids (l-aspartic acid, l-glutamic acid) at pH = 2 - 2.5 (three extraction stages).

The proposed extraction method can be developed and used for the selective separation of amino acids from fermentation broths or protein hydrolysates.

INTRODUCTION

The amino acids can be obtained by biosynthesis or from protein hydrolysis, but their separation from fermentation broths or protein hydrolysates is rather difficult. Amino acids dissociate in aqueous solutions, forming characteristic ionic species as a function of the solution pH value. For this reason, their solubility in nonpolar solvents is very low. Generally, for the amino acids separation from fermentation broths or protein hydrolysates ionic exchange, crystallization at the isoelectric point or chromatography are used.

The liquid - liquid extraction of amino acids is only possible by adding into the organic phase extractants such as phosphoric acid derivatives [1,2], high molecular weight quaternary aliphatic amines [3-7] or crown-ethers [8-10]. In this article, the separation of some amino acids of acidic character (l-aspartic acid, l-glutamic acid), basic character (l-histidine, l-lysine, l-arginine) or neutral character (l-glycine, l-tryptophan, l-cysteine, l-alanine) by reactive extraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) is explored experimentally and the results discussed. Using the experimental data of the study on the individual reactive extraction, the selective separation of the considered amino acids from a mixture has been analyzed.

The results indicated the possibility of the selective separation by reactive extraction of individual amino acids or groups of amino acids as a function of the

solution pH value. This method is considered an efficient alternative for the fractionation of amino acids mixtures compared to existing techniques.

EXPERIMENTAL PART

The experiments have been carried out in two steps. In the first step, the individual reactive extraction of each amino acid has been analyzed. For this purpose, an extraction column with vibratory mixing has been used, this laboratory equipment being described in detail in previous papers [11,12]. The phase mixing was made by mean of a perforated disk with 45 mm diameter and 20% free section. The vibrations had a frequency of 50 s^{-1} and 4 mm amplitude. The mixer position was maintained at the initial contact interface between the aqueous and organic phases. The extraction time was of 1 minute. The resulted emulsion was evacuated at the base of the column and broken in a centrifugal separator at 5000 rpm.

The initial concentration in aqueous solutions and the pK values of the studied amino acids are given in Table 1 (α-NH_3^+ - the NH_3^+ group in (position from the COOH group, R - the amino acid radical, pH_i - the isoelectric point of the amino acid [13]).

Table 1.

The initial concentrations and the pK values of the extracted amino acids.

Amino acid	Initial concentration		pK_1	pK_2	pK_3	pH_i
	g/l	M	COOH	α NH_3^+	R	
l-aspartic acid	4.65	0.035	2.09	9.82	3.87	3.0
l-glutamic acid	5.14	0.035	2.19	9.66	4.28	3.2
l-cysteine	4.23	0.035	1.96	8.18	10.3	5.1
l-tryptophan	7.14	0.035	2.38	9.39	-	5.9
l-glycine	2.62	0.035	2.35	9.78	-	6.1
l-alanine	3.11	0.035	2.34	9.87	-	6.1
l-histidine	5.42	0.035	1.77	9.2	6.20	7.6
l-lysine	5.11	0.035	2.18	8.95	10.5	9.7
l-arginine	6.09	0.035	2.02	9.04	12.5	10.8

For extraction experiments a solution of 48.3 g/l (0,15 M) D2EHPA in butyl acetate has been used. The volume ratio between the aqueous solution and organic solvent was of 1, each phase volume being 50 ml.

The initial solution pH adjustement has been made with a solution of 4% w/w sulfuric acid, or 5% w/w sodium hydroxide, depending on the desired pH value (pH values were determined using a digital pH meter). The pH values have been recorded throughout each experiment and any pH change was noted.

In the second step of the experiments, the selective separation of amino acids from a mixture by reactive extraction has been studied. Each amino acid had an initial concentration in the mixture of 0,015 M. The extractions were carried out in the same laboratory equipment, similar experimental conditions being used.

The extraction degree has been calculated by means of the amino acid concentrations in the initial solution and in the raffinate. The amino acids

concentrations have been measured by high performance liquid chromatography (HPLC) (HP 1090 liquid chromatograph).

RESULTS AND DISCUSSION

The reactive extraction of individual amino acids

The reactive extraction of amino acids with D2EHPA occurs by means of an interfacial chemical reaction of the ion exchange type:

$$R\text{-}CH(NH_3^+)\text{-}COOH_{(aq)} + HP_{(o)} \rightleftharpoons R\text{-}CH(NH_3^+)\text{-}COOH.P^-_{(o)} + H^+_{(aq)}$$

where HP is the extractant. As it can be observed, the separation is possible if amino acids exist as cation in aqueous solution, as found at a low pH values. At the same time, if the solution pH is too low, then the extractant will become protonated and thus unable to extract the cations [1].

The effect of the pH value on the extraction degree is depicted in Figs. 1-3 for each class of amino acid.

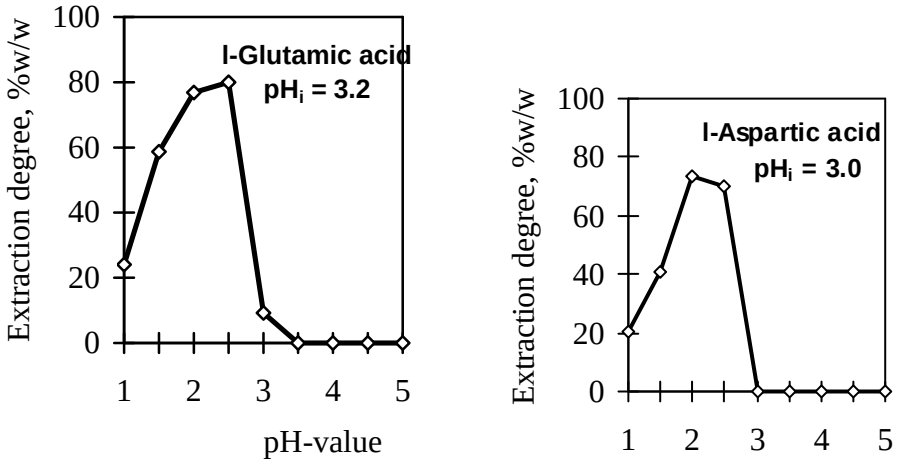


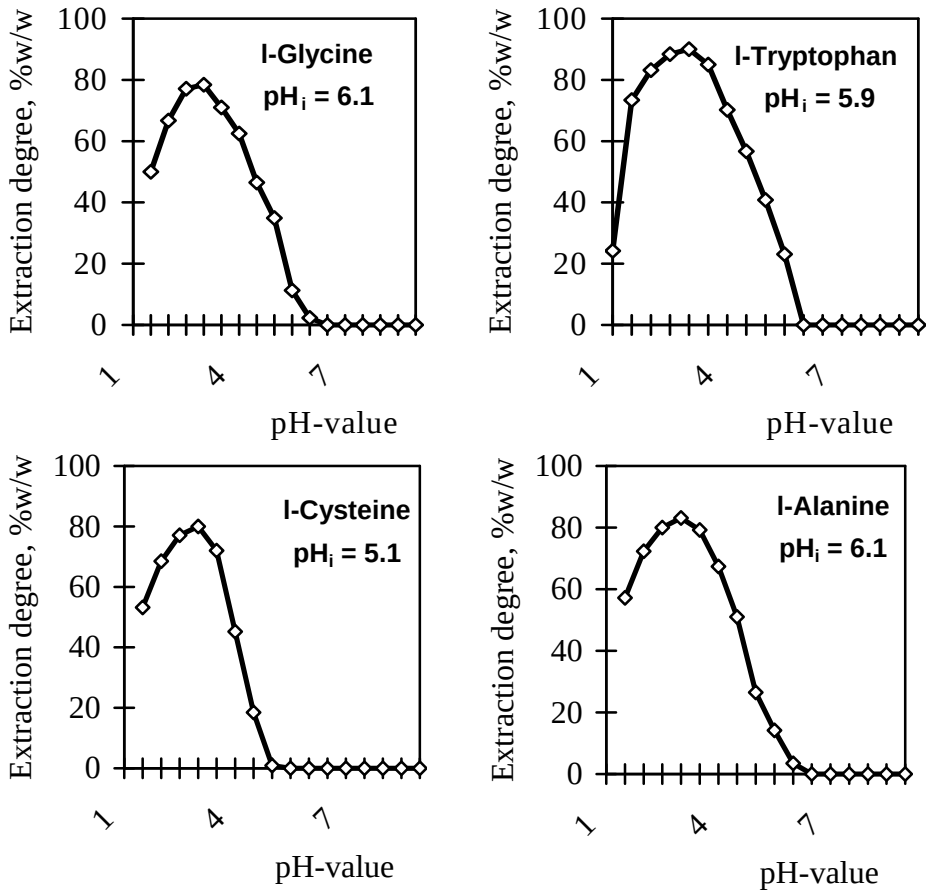
Figure 1. Effect of aqueous solution pH value on the reactive extraction degrees of acidic amino acids with D2EHPA dissolved in butyl acetate.

For all cases, the extraction degree reaches a maximum value as pH increases, corresponding to a maximum pH extraction domain of 2 - 3, followed by a strong decrease. The maximum is the result of the two opposite phenomena which occur as pH value increase: the increase of the concentration of extractant active form, which is able to react with the amino acid, and the decrease of the total amount of amino acid presenting a cationic form.

The reactive extraction of the amino acids with acidic character (l-aspartic acid, l-glutamic acid) occurs with maximum efficiency at pH 2.5, the extraction degree values being comparable (77% w/w for l-aspartic acid, 80% w/w for l-glutamic acid). Because of the increase of the zwitterion concentration in aqueous solution for both amino acids, any further increase of the pH value leads to the strong decrease of the extraction yield. At the isoelectric point of each amino acid the reactive extraction become impossible (i.e. yields $\cong$ 0).

In the case of neutral amino acids (l-glycine, l-alanine, l-tryptophan, l-cysteine), the maximum extraction yields are obtained at pH 3, these values varying between 78.4% w/w for l-glycine and 90% w/w for l-tryptophan as a function of the amino acid hydrophobicity. For higher pH values, the separation efficiency decreases, as a result of the amino acids COOH dissociation, and reaches zero at isoelectric point.

Figure 2. Effect of aqueous solution pH value on the reactive extraction degrees of neutral



amino acids with D2EHPA dissolved in butyl acetate.

The amino acids with basic character exist as dianions at low pH values. For this reason, the mechanism of the reactive extraction with D2EHPA can be described using the following interfacial chemical reaction:

R_1^+-CH(NH_3^+)-$COOH_{(aq)}$ + $2HP_{(o)}$ ⟷ R_1^+-CH(NH_3^+)-$COOH.P^-_{2\,(o)}$ + $2H^+_{(aq)}$
(R_1^+ being the dissociated radical of amino acid).

The maximum separation yield for the basic amino acids studied (l-histidine, l-lysine, l-arginine) varies between 57.8% w/w for l-histidine and 68% w/w for l-lysine and l-arginine, being reached at pH value of 2.5 - 3. The difference is due to the more pronounced dissociation of l-histidine at this pH value and, thus, to the formation of dication - anionic species in aqueous solution. The increase of pH value leads to a significant decrease of the extraction degrees, which become zero at pH 4 for l-histidine, and pH 5 for the other two amino acids.

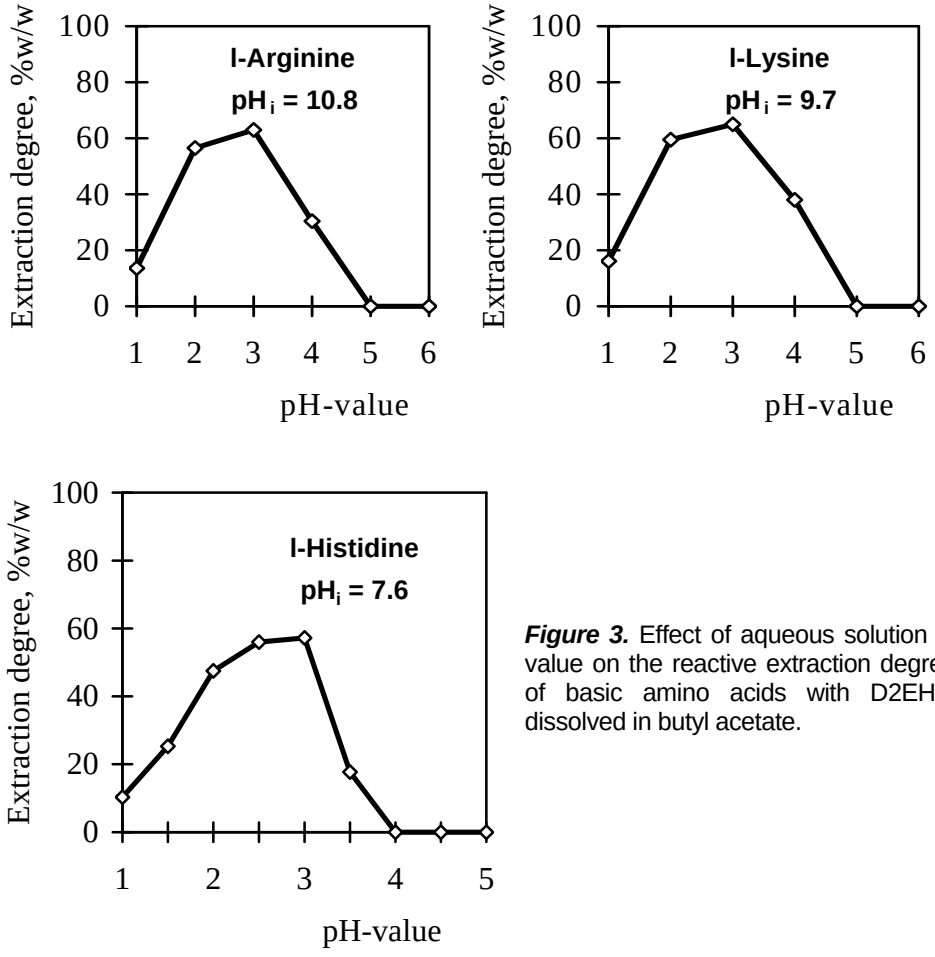


Figure 3. Effect of aqueous solution pH value on the reactive extraction degrees of basic amino acids with D2EHPA dissolved in butyl acetate.

Compared to the acidic and neutral amino acids, the reactive extraction of basic amino acids become impossible at pH values lower than the isoelectric point, as a result of the total dissociation of the -COOH group with formation of dication - anionic forms and a small amount of zwitterions.

The selective extraction of amino acids

The preliminary studies on the reactive extraction of individual amino acids indicated the possibility of the selective separation of different groups of amino acids with similar acidic properties as a function of aqueous solution pH value. In order to checking this hypotheses, the amino acids were extracted from a mixture with D2EHPA, at different pH values, the results being shown in Fig. 4.

In can be observed that all amino acids are extracted, with different yields, for a pH domain of 1.5 - 3. Over this interval, the extract contains only the amino acids with neutral and basic character. For pH 5 - 6, only the neutral amino acids are extracted, and for pH > 6 the extraction becomes impossible.

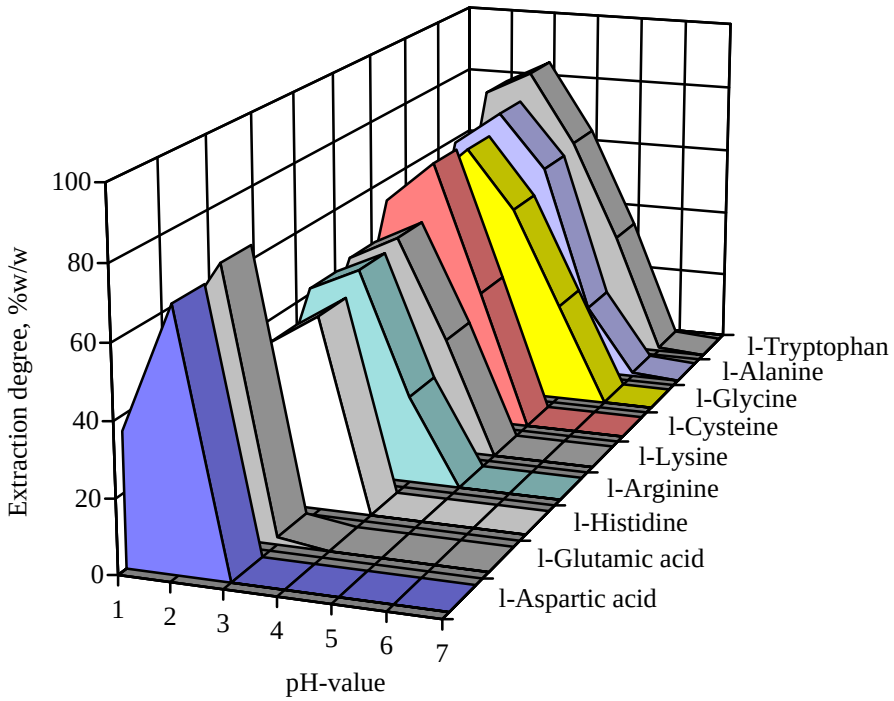


Figure 4. Effect of aqueous solution pH value on the reactive extraction degrees of amino acids from a mixture with D2EHPA dissolved in butyl acetate.

Using these data, a process flow sheet for the selective separation of the amino acids by reactive extraction with D2EHPA has been elaborated and applied (Fig. 5). Because the extraction degrees of each group of amino acids are rather low at the required pH values for selective separation, for total recovery of the considered amino a multistage extraction has been used. The extraction conditions are given in Table 2.

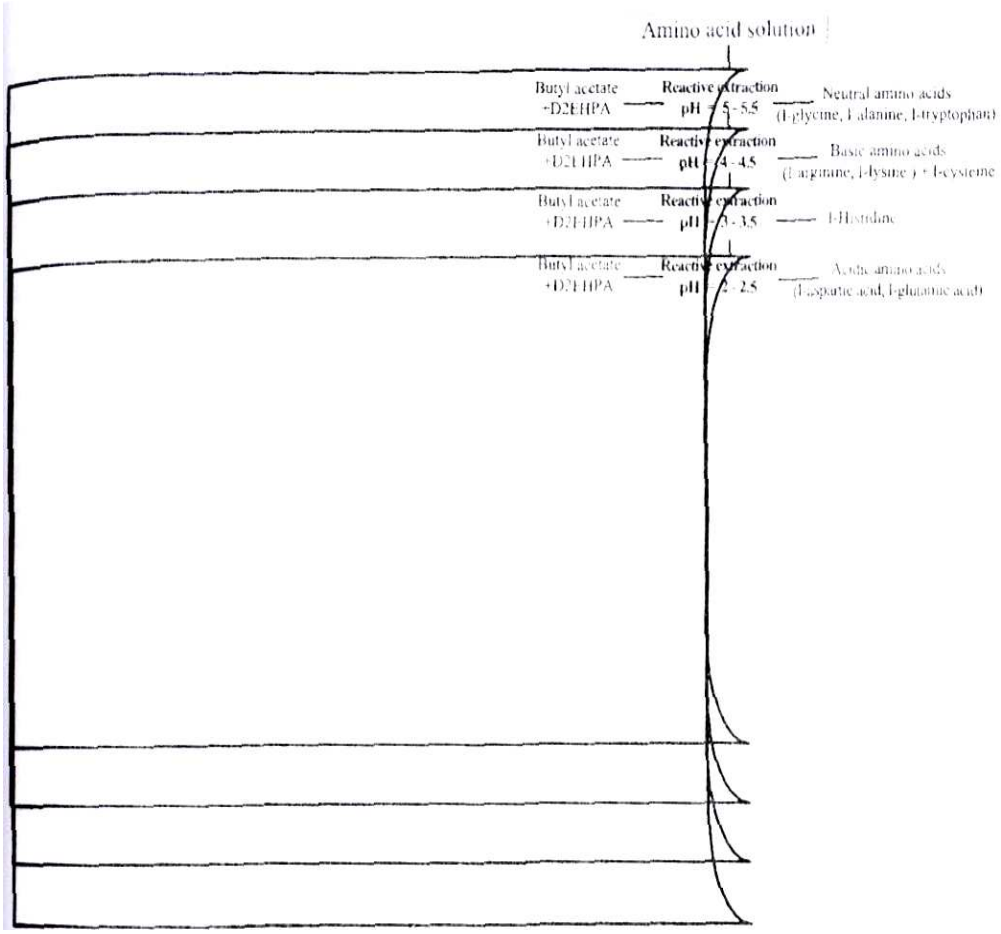


Figure 5. Operation chart for the selective extraction of amino acids with D2EHPA dissolved in butyl acetate.

Table 2.

The experimental conditions for the reactive extraction and separation of amino acids from a mixture.

Extracted amino acids	**pH domain**	**One stage extraction degree**, % w/w	**Number of extraction stages**
Neutral amino acids (- l-cysteine)	5 - 5.5	20 - 25	9
Basic amino acids (- l-histidine) + l-cysteine	4 - 4.5	18 - 25	10
l-Histidine	3 - 3.5	40	5
Acidic amino acids	2 - 2.5	60 - 63	3

CONCLUSIONS

Amino acids are extracted with D2EHPA by means of an interfacial reaction of ionic exchange type, if the amino acid exists as a cation in aqueous solution. The separation yield is controlled by the pH value of the aqueous phase, which is due to the acidic or basic character of each amino acid.

The individual extraction of amino acids indicated that the maximum yields are reached for a pH domain of 2 - 3, then strongly decreasing with the pH increase. Thus, for acidic and neutral amino acids, the extraction becomes impossible at the isolelectric point, and for basic amino acids at a pH value lower than pH_i, as a result of the carboxylic group dissociation.

By means of the pH influence on reactive extraction of the considered amino acids, an operation flow for selective separation has been elaborated and demonstrated experimentally. Using multistage extraction, the total separation of the following amino acids groups has been performed: neutral amino acids (l-glycine, l-alanine, l-tryptophan) at pH 5 - 5.5, basic amino acids (l-lysine, l-arginine) and l-cysteine at pH 4 - 4.5, l-histidine at pH 3 - 3.5, and acidic amino acids (l-aspartic acid, l-glutamic acid) at pH 2 - 2.5.

The proposed extraction method can be developed and used for the selective separation of amino acids from fermentation broths or protein hydrolysates.

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