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Comparative Study of separation of Amino Acid in between solvent extraction & solvent Sublation method

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ABSTRACT

Biomolecules are organic molecules which include a large polymeric molecule such as protein, polysaccharides, lipids& nucleic acid as well as small molecules such as primary metabolites, secondary metabolites and natural product. Biomolecules are produced naturally in living organism. Separation of biomolecules by traditional techniques represents serious problems owing to high dilution in fermentation broth and other factors. Therefore new separation techniques (i.e, solvent sublation) have been developed. In this editorial we will focus on the separation of L-phenyl alanine (amino acid) by solvent extraction & solvent sublation method. The solvent sublation technique is one of adsorption bubble separation methods (ABSM) in which the material (i.e, aqueous solution of L-phenyl alanin) adsorbed on the surface of ascending bubbles and then collected by a solvent in the column upper zone. On the other hand, Solvent extraction is very common process in which an acidic extractant di phosphoric acid (D2EHPA) is selected as an active carrier. Though Solvent extraction is very common process used in many fields but it has certain disadvantages. In this article we will compare these two techniques in the separation of l-phenyl alanin.

KEYWORDS: Adsorptive Bubble Separation Method (ABSM), Biomolecule, L-phenyl alanin, Solvent sublation, Solvent extraction

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INTRODUCTION

Phenyl alanine is an essential amino acid found in breast milk in mammals & a number offoods including meat, poultry, fish, cottage, cheese, lentils, peanuts and sesame seed. The L-isomers are used to biochemically form proteins, coded for by DNA. It is encoded by the codons UUU & UUC. Separation of amino acids has become an important goal in analytical chemistry as they are present in a variety of biological, industrial and environmental samples. In the extraction of α-amino acids it was demonstrated that proton transfer reactions occur, by Yang-Sheng Liu et al[1]. They also explained the complicated phenomenon present in the extraction of l-tryptophan.One of the process showed that 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.D.Cascaval carried out separation of some amino acids of acidic character (l-aspartic acid, l-glutamis acid), basic character (l-histidine, l-lysine, l-arginine) and neutral character (l-glycine, l-tryptophan, l-cysteine) by reactive extraction with D2EHPA[2]. 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 increasing of pH.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, as a result of the carboxylic group dissociation.The separation and recovery of amino acids using stream processing are demonstrated byM. Teramoto et al[3]. The liquid-liquid extraction of L-phenyl alanin is performed by adding extractants such as phosphoric acid. The liquid–liquid extraction of amino acids is only possible by adding into the organic phase extractants such as phosphoric acid derivatives and, high molecular weight quaternary aliphatic amines and crown-ethers. 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) was explored experimentally by other investigators[4]. Using the experimental data of the study on the individual reactive extraction, the selective separation of the considered amino acids from a mixture is analyzed. 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 had been explored earlier by A.K. Neil (1998). This method is considered an efficient alternative for the fractionation of amino acids mixtures compared with existing techniques. The reactive extraction of amino acids with di(2-ethylhexyl) phosphoric acid (D2EHPA) occurs by means

of an interfacial chemical reaction of the ion exchange type.It is observed that 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.

1.1. Characteristics of l-phenylalanine

L-phenylalanine is chemically known as (S)-alpha-Amino-beta-phenylpropionic acid**,** 3-phenyl-L-alanine**,**Phe**,** β-phenyl-L-alanine. It is an essential aromatic amino acid that is a precursor of melanin; dopamine; noradrenalin (norepinephrine), and thyroxine. It appears as odorless white crystalline powder and slightly bitter in taste.Its Molecular Weight is 165.19 and Chemical formula is $C_9H_{11}NO_2$. It is soluble in water and Solubility is 2.965 g/100ml at 25°C. Its Boiling point is 563°F at 760 mm Hg (sublimes) and Melting Point is 541°F (decomposes).

SYNTHESIS OF L-PHENYLALANINE

L-phenylalanine can be synthesized by following ways

2.1. Chemical synthesis

From benzalhydantoin, which is obtained by condensation of benzaldehyde with hydantoin. This hydantoin is then converted into the hydantoin of phenylalanine, by reduction, and the amino-acid then obtained by hydrolysis with barium hydroxide. It requires only three operations (two if hydriodic acid is used as a reducing agent), and the yield of amino-acid is excellent. The only drawback of this synthesis is the price of the hydantoin.

2.2. Biosynthesis

It is a method for the direct, one-step enzymatic conversion of trans-cinnamyl methyl ester to L-phenylalanine methyl ester. The reverse reaction of phenylalanine ammonia lyase from *Rhodotorulaglutinis* was utilized for this conversion. Insolubility of substrate trans-cinnamyl methyl ester in aqueous buffer solution was overcome by employing an organic-aqueous biphasic system, heptane: 0.1 m Tris-sulfate buffer, pH 9.0 (2:1). Different conditions were optimized for the maximal conversion such as time (16–18 h), temperature (30°C), pH (9.0), concentration of substrates, 0.1 m trans-cinnamyl methyl ester and 1 m $(NH_4)_2$ SO_4, and nature of the organic solvent (heptanes); about 70% conversion of substrate to product was obtained under these conditions. Formation of the product, l-phenylalanine methyl ester, was identified by paper chromatography and was further confirmed by autoradiography and NMR spectral analyses.

METHODS OF SEPERATION

3.1. Extraction

Extraction is an important technique in chemistry and biotechnology. It is usually applied in biotechnology as the first step in the recovery of primary and secondary metabolites. The main problems with the recovery of primary and secondary metabolites by extraction are the complexity of industrial cultivation media, meaning that several by-products are extracted as well as the desired main product. Various alternative extraction techniques, like reactive extraction and aqueous two-phase extraction, have been investigated in order to improve the selectivity of the product recovery. The liquid–liquid extraction of amino acids is only possible by adding into the organic phase extract ants such as phosphoric acid derivatives and, high molecular weight quaternary aliphatic amines and crown-ethers.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) was explored experimentally by other investigators. Using the experimental data of the study on the individual reactive extraction, the selective separation of the considered amino acids from a mixture is analyzed. 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 had been explored earlier by A.K. Neil (1998). This method is considered an efficient alternative for the fractionation of aminoacids mixtures compared with existing techniques. The concentration of amino acids using supported liquid membranes and D2EHPA as the carrier were carried out by PiotrWieczoreka et al[5].The extraction of l-phenylalanine with D2EHPA dissolve in n-octane was carried out by Yang-Sheng Liu.The reactive extraction of amino acids with di(2-ethylhexyl) phosphoric acid (D2EHPA) occurs by means of an interfacial chemical reaction of the ion exchange type.Where HP is the extractant.It is observed that 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. The recovery of amino acids from aqueous solution was demonstrated by Travis Cameron and Scott M. Hussonusingmixed organic extractants[6].

3.2. Solvent Sublation

The name of this method arises from the fact that an ionic species called the colligend is removed by the addition of surface active collector of opposite charge to that of the colligend. The complex formed by coulombic attraction is called “sublate” and the process of lifting the sublate by

gas bubbles is called “sublation”. This technique was first introduced by Sebba& it has been widely applied to remove surface-active compounds and hydrophobic compounds, such as surfactants, dyes, complex compounds, and active components in natural products. This technique is an adsorptive bubble separation process in which surface-active and hydrophobic compounds in water are adsorbed on the bubble surfaces of an ascending gas stream and then collected in an organic layer placed on top of the water column. It has significant potential for separation of the low concentration product. The solvent solution process follows first-order kinetics. A characteristic parameter, apparent activation energy of attachment of the sublate to bubbles, was estimated at a value of 9.48 kJ/mol[7].The solvent sublation technique consists of the generation of small gas bubbles, surfactant and organic solvent. The size of bubbles is of major importance with preference in very small bubbles. This method consist a several advantages like- Significant potential for separation of the low concentration product, Simultaneous separation and purification, Ability to handle feed composition and flow-rate changes without loss of efficiency, recycle capability, and low energy requirements. Moreover, because of the simple design requirements, sublation may be carried out in many types of existing equipment, such as horizontal or vertical tanks, accumulators, phase separators, columns, or retention ponds, with minimal modifications. The added flexibility and low cost of solvent sublation makes it an economically attractive solution to wastewater problems – particularly the treatment of highly diluted aqueous solutions. Another advantage of solvent sublation is that in addition to the soluble organic fraction, the suspended fraction, such as fine oil droplets, also may be removed by means of flotation. Because of soft process of mass transfer and non-reversible bubble mass transfer, solventsublationcan effectively avoid the emulsification and increase the distribution ratio between aqueous and organic phase. This process is used is used for the separation and determination of trace metals. Naphthalene and phenanthrene are readily removed from aqueous systems by solvent sublation into mineral oil. Several amino acids like L-lysine; L-arginine has been separated using solvent sublation. The solvent sublation technique was applied for the separation and enrichment of l-Arg using dodecylbenzene sulfonic (DBSA)as the surfactant, di(2-ethylhexyl)phosphoric acid(P_{204})as the extractant and n-heptane as the organic solution. The solvent sublation was compared with the solvent extraction. The experimental results showed that enrichment ratio of 16.2 and removal rate of 97.2% to l-Arg were obtained by the solvent sublation under the conditions of room temperature, 0.09 g/L l-Arg aqueous solution 250 mL, DBSA concentration 0.15 g/L,the initial pH 7.0,volume of n-heptane 10 mL, gas flow rate of 200 mL/min . Separation and purification of Penicillin G from fermentation broth has been done with this technique and the floation product which was separated &purified from the fermentation broth under optimal condition are quantitatively analyzed by HPLC and it is compared with traditional solvent

extraction .the result shows that solvent sublation not only increases the separation efficiency but also efficiently reduce the BA emulsification in separation process[8]. Inspite of having a lot of advantages it possesses some limitation. As sublation is mainly selective for sparingly soluble or hydrophobic compounds, almost any oil-based solvent such as mineral oil, a heat-transfer fluid, or lubricating oil is preferred. For ionic solutes like pentachlorophenol, which have lower distribution coefficients in oil-based solvents, high-molecular-weight alcohols or ethers may be preferred. Also, the addition of surfactants or adjustments of pH are other means of affecting the relative distribution of ionic solutes.

4. CONCLUSION

From the above comparison it is observed that solvent sublation is more adventitious than solvent extraction. Solvent sublation method is found more effective in the separation of the low concentration product, simultaneous separation and purification and in the ability to handle feed composition and flow-rate changes without loss of efficiency. Hence this newer separation technique is proved to be an optional way of solvent extraction.

5. ACKNOWLEDGEMENT:

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