Stereo-separations of Peptides by Capillary Electrophoresis and Chromatography

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Method Article

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Abstract

Small peptides \(di-, tri-, tetra- penta- hexa etc. and peptides) control many chemical and biological processes. The biological importance of stereomers of peptides is of great value. The stereo-separations of peptides are gaining importance in biological and medicinal sciences and pharmaceutical industries. There is a great need of experimental protocols of stereo-separations of peptides. The various chiral selector used were polysaccharides, cyclodextrins, Pirkle's types, macrocyclic antibiotics, crown ethers, ligand exchange, etc. The attempts have been made to develop stereo-separations protocols for peptides using capillary electrophoretic and chromatographic techniques. In addition to these, the optimization strategies of stereo-separations were also discussed in the details. The efforts are also made to discuss the future perspectives of peptides stereo-separations.

Introduction

Its 21st century scientists are attempting to provide the best lives to the society. Medication is one of the most important aspects in our lives. Chirality in the drugs is a complex phenomenon creating confusion in medications. The demand of chiral drugs is increasing constantly due to different pahramaceutical activities of drugs enantiomers. One enantiomers may be active while the other inactive, toxic or ballast; leading to various side effects and problems \[1]. It is because of chiral nature of our biological systems. Mostly biological reactions are stereo-selective because of different enantioselective distribution rates, metabolisms, excretion and clearances of enantiomers. Due to these facts, scientists, clinicians, industrialists, academicians and government authorities are asking data for optically active drugs and other biological important molecules. US FDA, Health Canada, European Committee for Proprietary Medicinal Products and Pharmaceutical and Medical Devices Agencies of Japan, have banned the marketing of all racemic drugs \[2-4]. Small peptides \(monomers n < 6) are of great importance because of contribution in various biological processes. The biological activities of small peptides include protein synthesis, fertility, neurotransmission, inflammation process, pathogenic microorganisms activities and other functions of human beings. These functions made peptide vital molecules in drug development and health care \[5-7]. Besides, these peptides are also being used as biological markers in the biological systems \[8]. The small peptides are also considered as important molecules in food and nutrition industries. For examples, aspartame, carnosine, etc. are being prepared at industrial scale \[7]. It is important to mention here that biological functions of peptides are stereoselective; especially related to enzymatic reactions. In view of these facts, stereo-separation of small peptides is very important in drugs development and health care. Stereo-separation of peptides may be achieved by capillary electrophoresis and chromatography. Literature has many papers on stereo-separation of peptides \[9-12]. Recently, Ali et al. \[13] reviewed stereo-separations of small peptides by capillary electrophoresis and chromatography techniques. It was observed that all these papers contain sufficient information on stereo-separations of peptides but no one describes the experimental procedures, methods development and optimization strategies in details, which are urgently required at laboratory level globally. Therefore, the attempts have been made to describe a protocol for stereo-separations of peptides by capillary electrophoresis and

chromatography. The present article describes the state-of-the-art of stereo-separations of peptides using capillary electrophoresis, chromatography. The efforts have been made to discuss optimization strategies and future perspectives of stereo-separations of peptides.

Reagents

All solvents and reagents should be of HPLC and AR grades Optically active pure and racemic peptides standards Deionised water Acetonitrile Methanol Reagents for the preparation of phosphate, acetate and borate buffers. Required chiral selectors Acids and bases for pH adjustment

Equipment

Capillary Electrophoresis Instrument . Personal computer \(PC) for data acquisition . Fused silica capillaries \(~ 50 cm effective length with 50 or 75 µm inner diameter) . Special capillary cutting blade . pH meter . UV–Vis. Spectrometer . Degasification unit . Filtration unit . Micro balance

Procedure

1. Prepare stock solutions of peptides \(optically active pure and racemic) in water \(0.1 mg/mL). 2. Prepare required BGE and dissolve suitable and appropriate amount of chiral selector in it. 3. Protocols given in Figure 2 should be used before selecting and preparing BGE. 4. Filter through 0.45 µm membrane and degas by sonication. 5. Rinse the capillary for 5 min. with 0.5 M NaOH followed by 10 min with deionize water. 6. Fill BGE in CE reservoirs and dip the ends of capillary into them. 7. Rinse capillary for 5 min with BGE. 8. Inject racemic peptides sample solutions. 9. Apply appropriate potential and run CE instrument. 10.. Among the measurements, rinse capillary for 2 min with BGE from time to time. 11. Optimize the stereo-separations. 12. Identify the resolved enantiomers by running standard pure optically active stereomers. 13. Wash capillary by deionised water before stopping HPLC instrument. 14. Calculate capillary electrophoretic parameters using standard equations \[14]. 15. Determine the qualitative and quantitative stereo-separations.

Timing

Rinse the capillary for 5 min. with 0.5 M NaOH followed by 10 min with deionize water. Rinse capillary for 5 min with BGE Among the measurements, rinse capillary for 2 min with BGE from time to time.

Troubleshooting

1. CE is gaining importance in stereo-separations of peptides. 2. CE instrument has two injection modes i.e. electrokinetic and pressure injection modes. 2. pH meter should be calibrated using pH 4.0 and pH 10.0 standards. 4. First filter about 10 mL of deionized water in order to remove any impurities from filtration unit. 5. pH of electrolyte is a crucial parameter and should be adjusted as per the requirements.

6. The addition of organic modifiers improves the stereo-separations. 7. Generally, organic modifiers are toxic to health. 8. Care should be taken to avoid skin contact, inhalation and swallowing. 9. Organic modifiers should be handled with cautions using gloves, glasses, etc. 10. These organic modifiers should be stored in cool, dry and well ventilated places. 11. Both ends of capillary should be sealed by heating; if instrument is kept for long time.

Anticipated Results

Stereo-separation of small peptides is a growing research area since early 1990s. The metabolism of D-amino acid containing peptides stimulated stereo-separations of peptides \[55,56]. The stereo-separations methods are gaining advancement with respect to time. For example, switching from indirect methods to direct stereo-separations and a gradual replacement of protocols requiring derivatization of samples to the analysis of underivatized peptides are the advancements. The cyclodextrines and macrocyclic antibiotics are the most commonly used chiral selectors. However, in recent time polysaccharides and other chiral selectors have been used. During last few years, hyphenation of CE and HPLC with MS detectors, 2D-LC \[57] and the development of miniaturized analytical devices are other developments \[58]. Reducing analysis time and complication of analyzed samples have economic impact \[59]. Fast speed UPLC instrument has not been used in chiral chromatograph1 of peptides. But UPLC is used for stereo-separations of derivatized amino acids \[60]. Therefore, it is expected that UPLC may acquire a good position in stereoseparations of peptide. The stereo-separation of peptides diastereomers is important in physiological researches and industries. This is due to the fact that multi-components, multistereoisomer mixtures are found in the food and pharmaceutical industries. Such situation will expand industries with respect to enantiopure peptides. Keeping these facts into consideration, the stereo-separations of peptides at preparative scale is the requirement of today. Literature survey indicates only one report at preparative scale \[61]. Therefore, there is a great need for stereo-separation of peptides at preparative scale. Really, optically active pure di- and tri-peptides can be obtained by chiral synthetic methods but not larger peptides. This is a niche for preparative chromatographic methods. It is assumed that more attention will be drawn to the area in pharmaceutical industries for peptide stereo-separations

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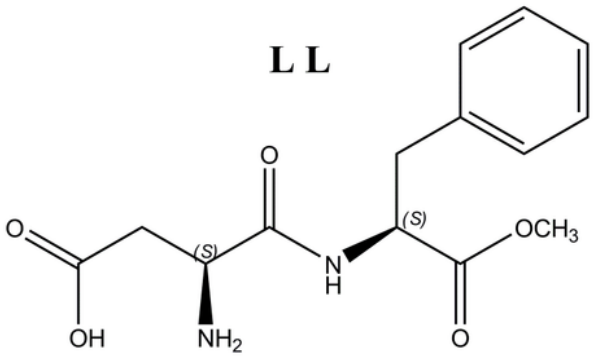
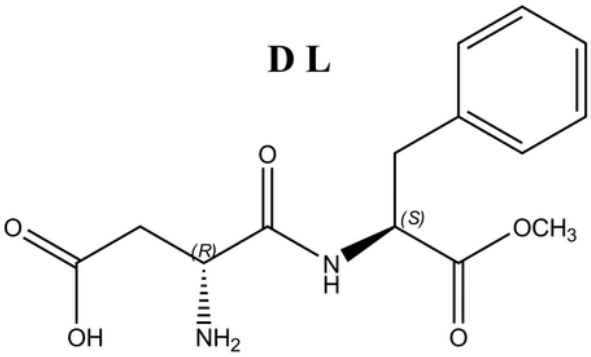
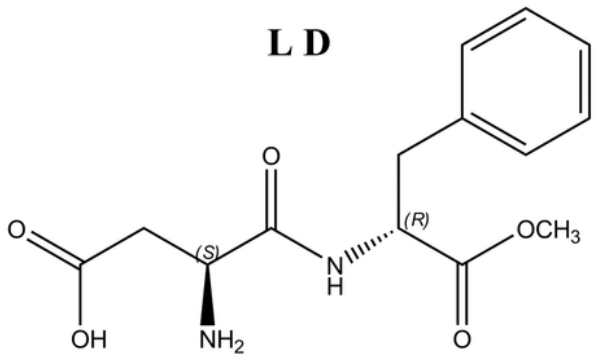
Figures

Figure 1

Figures Word Document containing all 7 figures







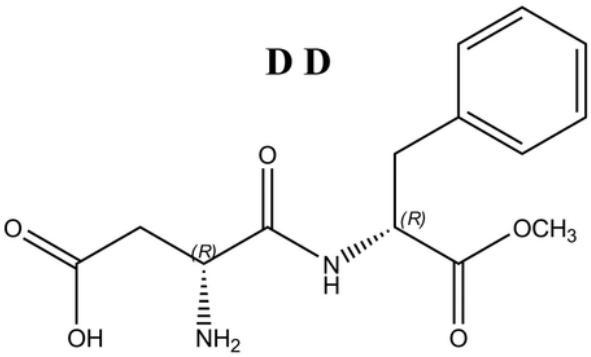
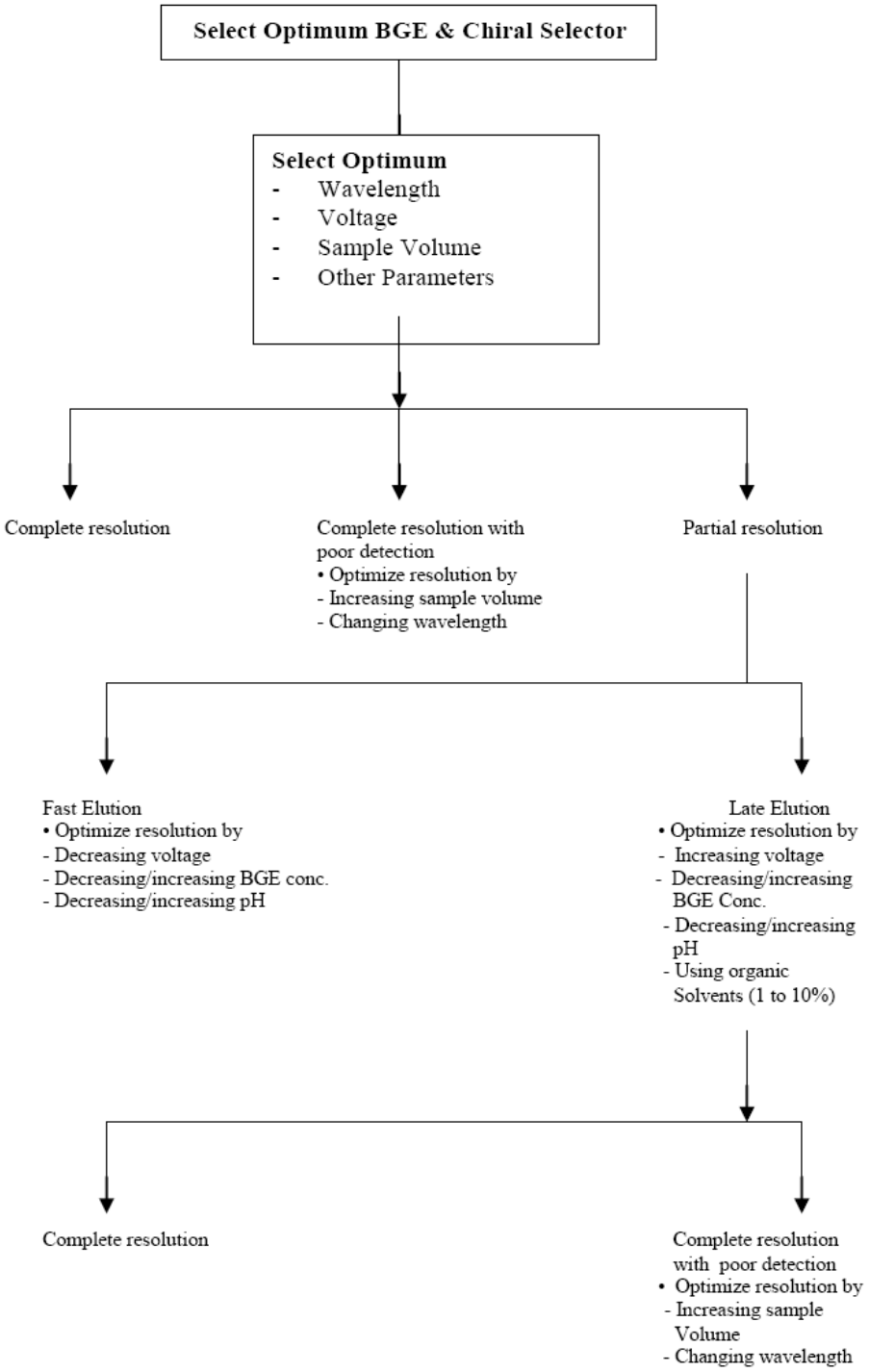


Figure 2

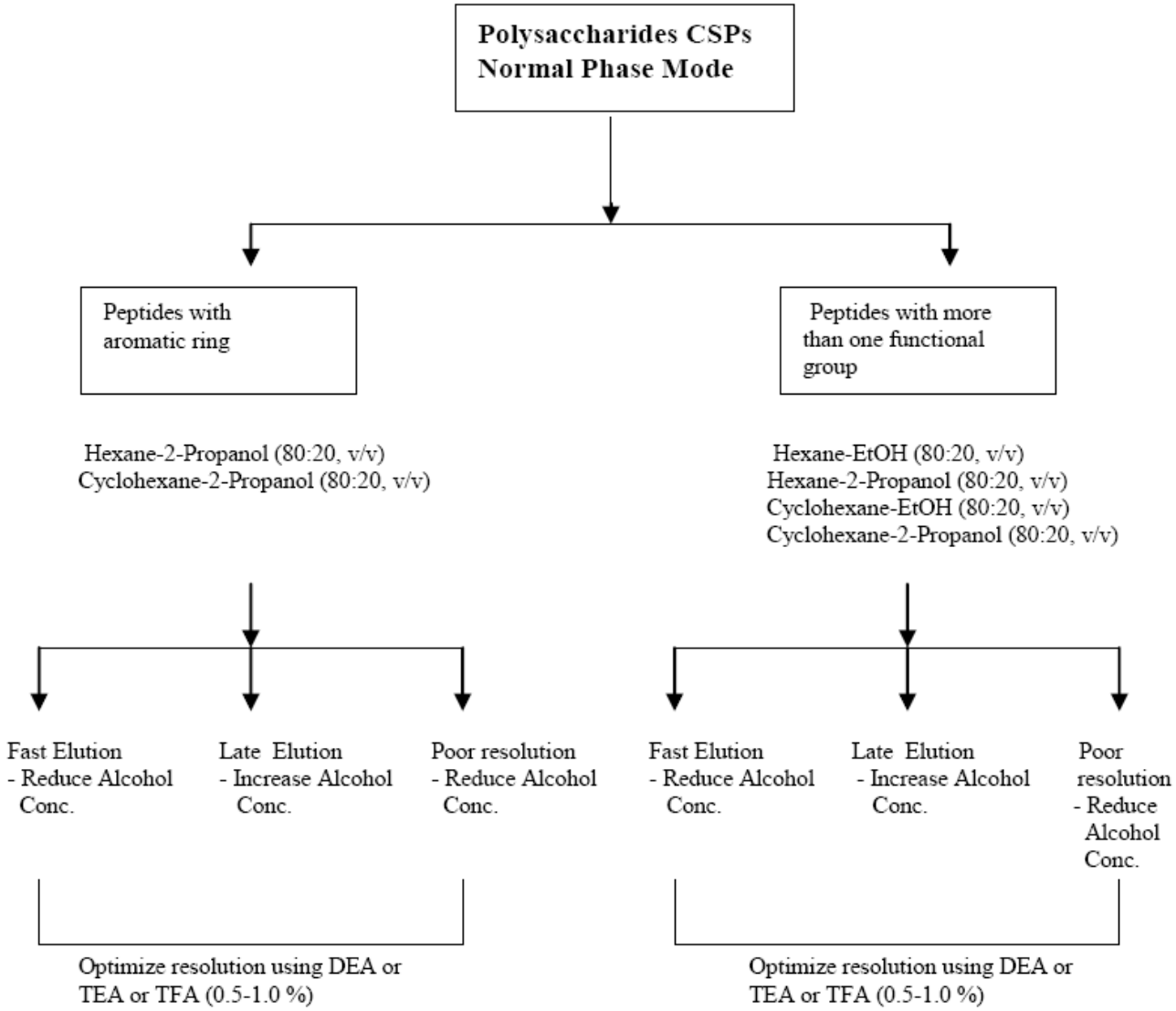
Figure 1 Diastereomers of N-(α-Aspartyl)-phenylalanine. LL-aspartame is sweet.



Note: This is a brief outline of the procedure to follow for stereo-separations by CE. However, other variations may be carried out.

Figure 3

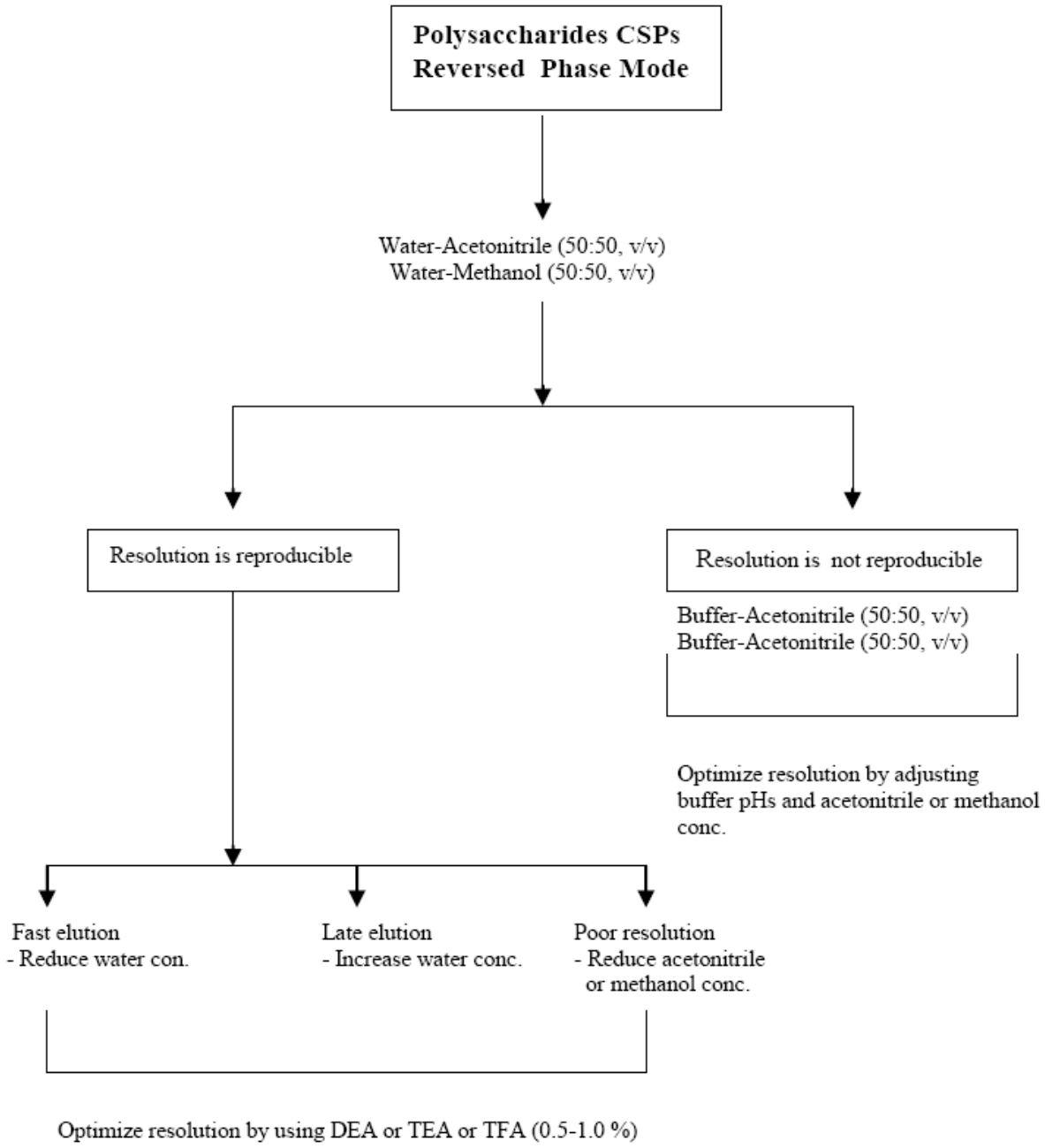
Figure 2 Protocol for development and optimization of CE conditions for chiral resolution.



Note: This is a brief outline of the procedure to follow in developing stereo-separations on polysaccharides CSPs under normal phase mode. However, other mobile phases may be used.

Figure 4

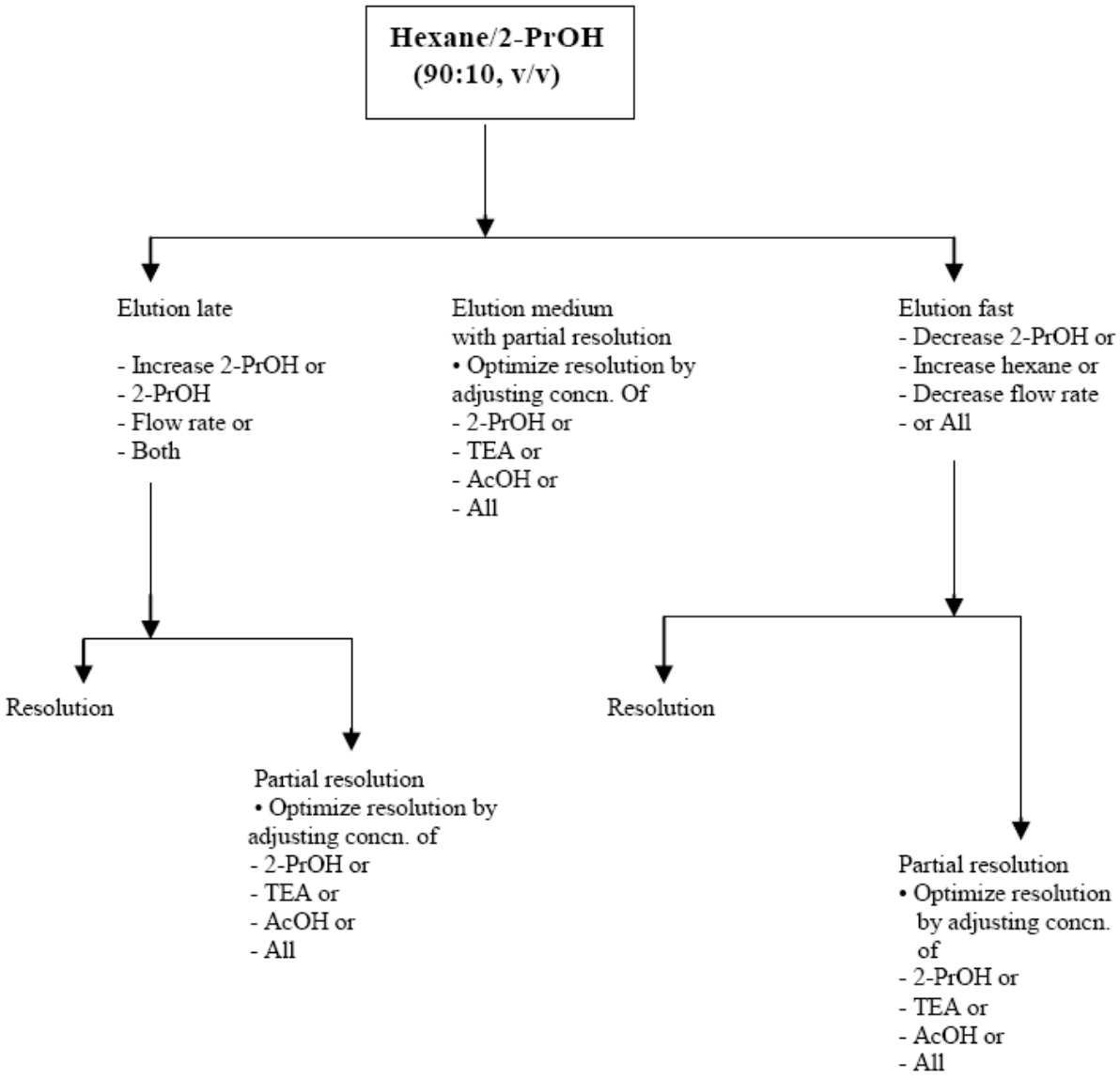
Figure 3 Protocol for development and optimization of mobile phases on polysaccharides CSPs under normal phase mode.



Note: This is a brief outline of the procedure to follow in developing stereo-separations on polysaccharides CSPs under reversed phase mode. However, other mobile phases may be used.

Figure 5

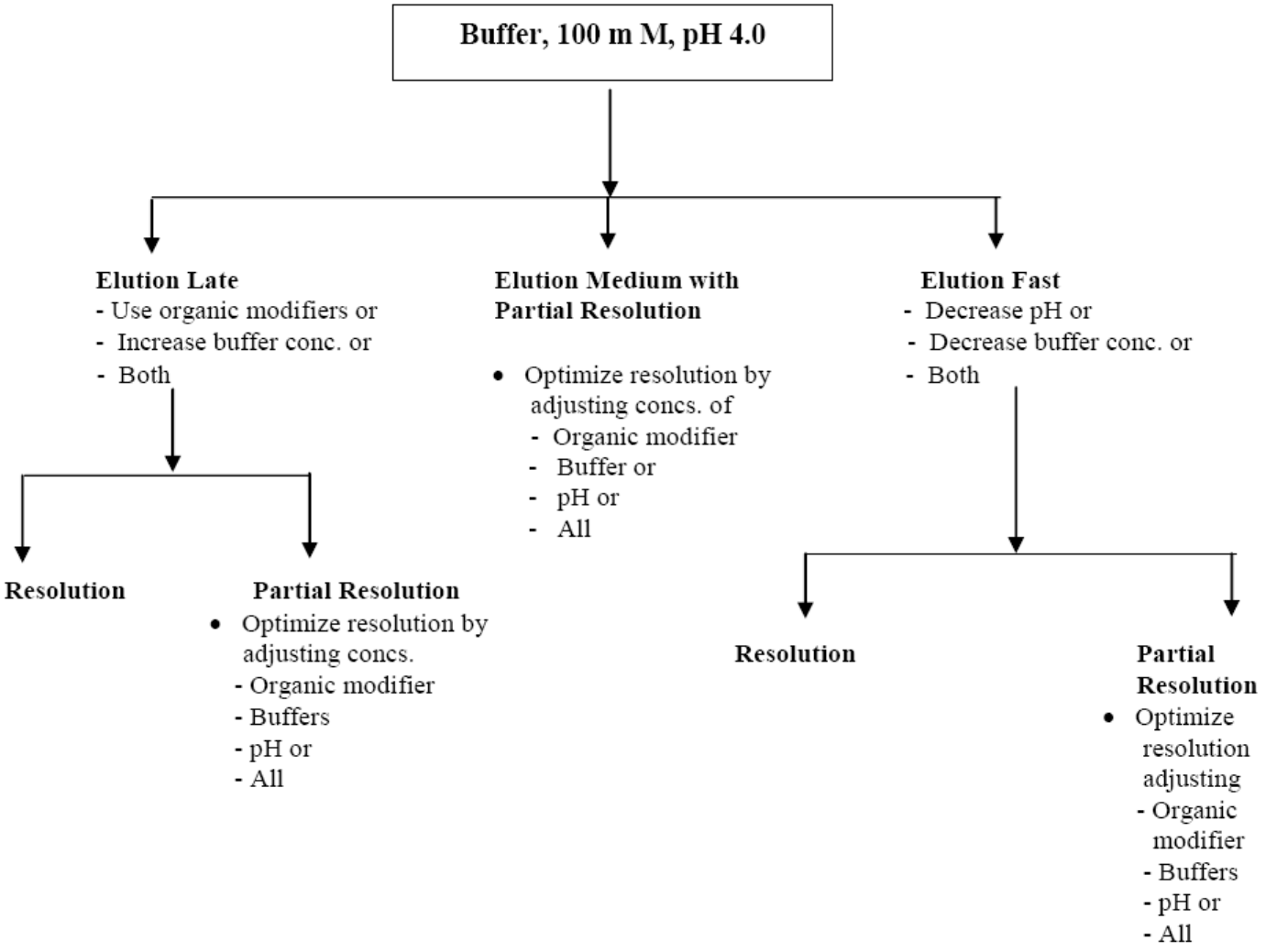
Figure 4 Protocol for development and optimization of mobile phases on polysaccharides CSPs under reversed phase mode.



Note: This is a brief outline of the procedure to follow in developing stereo-separations on CDs based CSPs.

Figure 6

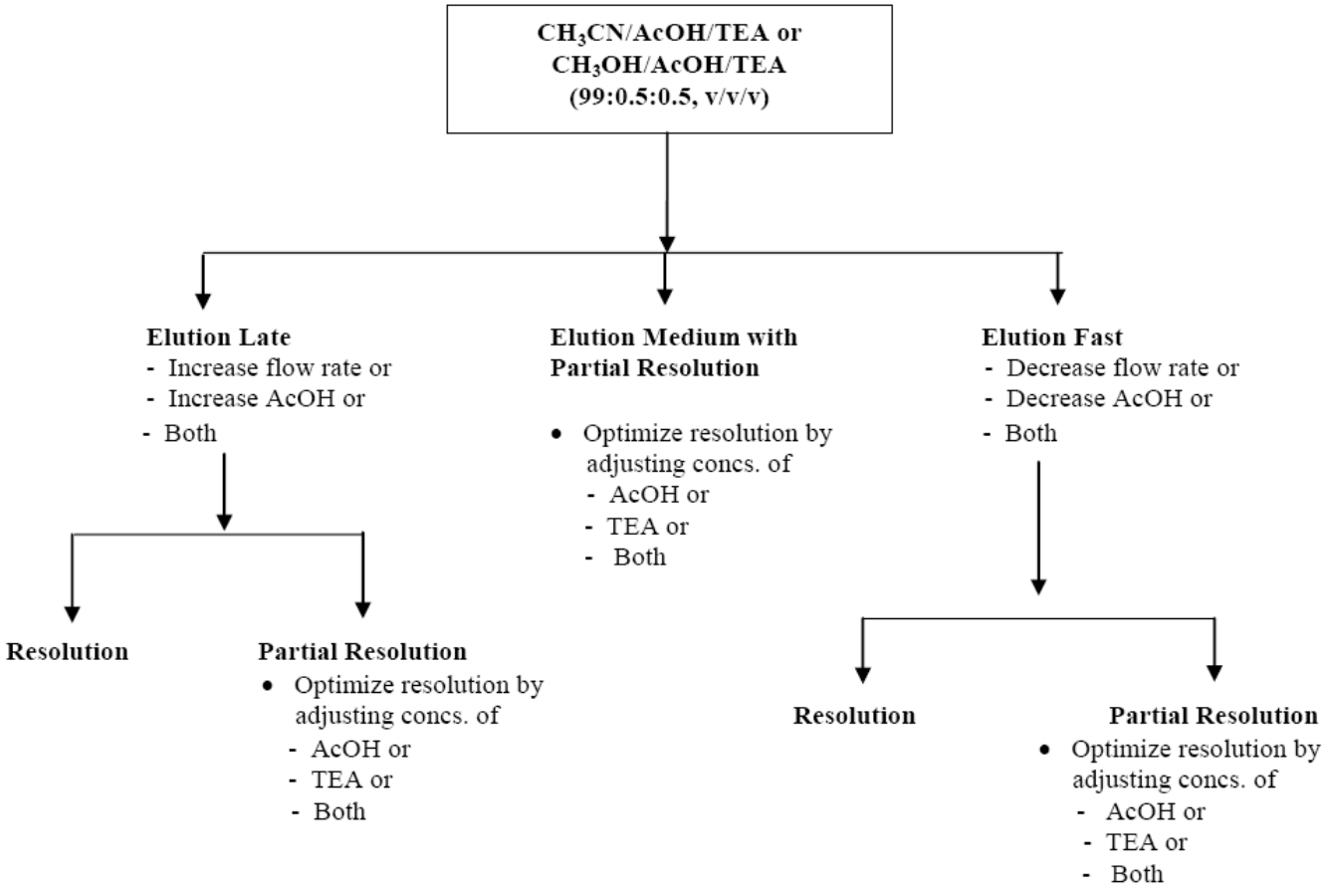
Figure 5 Protocol for development and optimization of normal mobile phases on CDs based CSPs under normal phase mode.



Note: This is a brief outline of the procedure to follow in developing stereo-separations on CDs based CSPs.

Figure 7

Figure 6 The Protocol for development and optimization of normal mobile phases on CDs based CSPs under reversed phase mode.



Note: This is a brief outline of the procedure to follow in developing stereo-separations on CDs based CSPs.

Figure 8

Figure 7 The protocol for the development and optimization of mobile phases on CDs based CSPs under polar organic phase mode.