International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 958-963 www.ijprajournal.com ISSN: 2249-7781

Nano Technologies for Chiral Seperations

[1]Sanikommu Kalpana, [2]Kondepudi Lalitha, [3]Saripalli Sri Lakshmi*

[1]Student, [2]Student, [3]Assistant professor

[1]Department of Pharmaceutical Analysis

[1]Jawaharlal Nehru Technological University,JNTUK

[1]School of Pharmaceutical Sciences and Technologies Kakinada,Andra Pradesh,India-533003

Submitted: 05-10-2023 Accepted: 15-10-2023

ABSTRACT
Nano High performance liquid chromatography (Nano HPLC) and Nano Capillary Elecrophoresis(NCE) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. Enantiomeric ratios of chiral medicines' activity, bioavailabilities, and biodegradation are crucially relevant to medical procedures. Only homochiral medication is safe for humans. The first and most crucial phase is the chiral examination of biological material. This article was prepared with an aim to review different aspects of Nano HPLC and NCE, such as principle, types, instrumentation and application.

Key words : nano liquid chromatography, nano capillary electrophoresis,enatiomer,chirality.

I. INTRODUCTION

The word "Chirality," which Lord Kelvin first used in 1883, is derived from the Greek word kheir or chiros signifying hardness. A chiral object is one that has two non-superposable mirror images of each other and lacks the three components of symmetry (plane of symmetry, centre of symmetry, and axis of symmetry).

Although the optional isomers of a racemic medication, which make up the majority of chiral medicines, can exhibit various pharmacological actions in biological systems, they are administered as racemates. Obviously, the issue is more complicated the more chiral centres are found in a pharmacological molecule. It would be considered practical to administer eutomer in order to secure the best therapeutic outcome. However, since racemization can happen in vivo, administering a single enantiomer to humans does not guarantee preclude adverse effects or tissue or organ damage. Ibuprofen serves as an illustration of chiral inversion with no adverse effects. An enzymatic process transforms the inactive (R) - (-) isomer into the active (S)- (+) form. It is possible to think of the R isomer as a prodrug of the (S)-enantiomer.

Thalidomide, which was marketed as a sedative in its racemic form in the late 1960s, is a bad example. even when used in the racemic form, a safe and beneficial sedative. When thalidomide was prescribed to pregnant women, it was shown that in vivo inter conversion into the hazardous (-) - isomer caused catastrophic abnormalities of embryos, even when the medication was applied in the therapeutic and safe (+)-form.

For several other medications, chirality is crucial in the design and development of the drug. Chiral molecules can be separated and identified using a variety of techniques. The numerous chromatography and electrophoresis modes are the most significant.

II. SEPERATION/ RESOLUTION OF ENANTIOMERS:

Resolution is the process of separating an enantiomer from a racemic material. The different approaches can be categorised into two groups: favoured crystallisation and the conventional method, which involves the enzymatic degradation of one of the enantiomers. Modern technologies include spectroscopic, electrophoretic, and chromatographic methods.

2.1 CLASSICALMETHODS:

In the enzymatic method, resolution is accomplished through a biochemical mechanism that destroys one enantiomeric form. Several microorganisms, including bacteria, moulds, and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 958-963 www.ijprajournal.com ISSN: 2249-7781

yeast, selectively assimilate one form while discarding the other when given the chance to grow in a solution containing racemic mixtures.
For instance, the dextrorotatory form of a racemic solution of ammonium tartrate is destroyed when the common mould Penicillium glaccum is added, resulting in the solution becoming levorotatory.

2.2 MODERN METHODS

1.Chromatography Methods

- ❖ Gas Chromatograpy
- ❖ Liquid Chromatography
- High performance liquid chromatography
- Super critical Fluid chromatography
- Capillary electrochromatography
- Thin layer chromatography
- Stimulated moving bed chromatography
- Nano liquid chromatography

2. Capillary Electrophoresis
3.Biosensors
4. Membranes
5. Crystallization
6.Biotransformation

Recently, it has been realized that drugs remain in our body for long time at tracelevels of nano and picogram levels. Besides, chiral pollutant are also present at these trace concentrations. The presence of unwanted enantiomers in the body and environment is not desirable. During the course of time, the chiral identities at trace levels accumulate into the body and environment leading to various side effects and diseases. In view of these fact, it is important to explore analytical methods capable to distinguish stereo isomers at nano level detection limits. Recently 2 techniques i.e., NLC and NCE have emerged.

INSTRUMENTATION

NANO LIQUID CHROMATOGRAPHY

An LC system can be made smaller by downscaling each component, including the injection, column , pumps, connecting tubing, and the interface with the detector.

PUMP

A pump system is necessary for Nano-LC because it allows for gradient elution at nanoscale levels, consistent nano flow rates, and stability during the separation. 500nL/min or Smaller flow rates are necessary. Split and splitless pumps are the two main systems that can be utilized in Nano-LC. The passive split system and active split systems are two categories of split systems. Splitter distributes the pump's high flow rate between the column and restrictor in the passive split system. The flow stability and accuracy of passive split system are compromised despite being straight forward and reasonably priced. Compared to passive split systems, active split systems have better flow stability and display better repeatability, although the majority of the mobile phase is still lost. The "Solvent replenishment" systems and the "Continuous flow" systems can be subdivided. Split less systems are currently utilised frequently in Nano-LC. These systems have greater reproducibility and prevent solvent losses. Compared to the split systems, Nano flow rates.

NANO COLUMNS

Although columns with a diameter of 10mm can be used, Nano-LC columns with a diameter of 75mm are the most common in nano-LC Separations, These column offers a good balance between defectability , loadability, and robustness.

PACKED COLUMNS

Polyimide coated fused silica capillaries are utilized to make the Packed columns used in nano LC columns that offers flexibility greater mechanical resistance, and a range of interior dimensions, although nano columns also made of stainless steel and titanium tubes. They may be filled by a monolithic bed of silica -based particles. Alternatively , much less frequently, walls that have been approximately covered in organic or inorganic materials. The most widespread particle sizes ranges from 3 to 5 mm for packed columns particle filled small i.d. Columns are challenging to prepare.

MONOLITHIC COLUMNS

Organic or inorganic based synthesis methods can be used to process monoliths, and biocompatible materials are intriguing alternatives in biospecific analysis this type of column does not require frits because the stationary phase is fixed to a porous (silica or polymer) structure that forms throughout the column that wall's column. Single rods of organic or inorganic material ,known as monolithic stationary phases, Can be the capillary column produces. Due to their great porosity, monolithic columns do not require frets materials allow for higher mobile phase flow rates, which shorten separation times.

INJECTION

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 958-963 www.ijprajournal.com ISSN: 2249-7781

The maximum injections volumes for columns are typically a few microlitres and can be expressed as a function of the column length , Plate number ,Retention factor, or other characteristics , and are generally a few nanolitres. In nano-LC, small injected quantities are a significant issue since they result in loss of detectability, but higher injected volumes causes a band broadening effect that reduces the effectiveness of separation, particularly for poorly preserved compounds.

Commercial auto samplers, which typically operate at microliter levels, need to be adjusted in order to be used in the nano liter range. A split valve between the injector and the column may be used to get around this.

DETECTION

The detection methods used for nano – LC seperations are the same as those used for HPLC seperations. Due to its low cost, broad variety of applications, and usage of online detection, Diode array detection(DAD) is frequently utilized in nano-LC. However, when on-column detection is used, detectability is constrained due to the nano coloumn's short route lenth. Longer light routes provided by carefully tuned detector cells help to overcome this. Inductively coupled plasma and Laser induced fluorescence are also used in nano-LC detection.

Good detectability and a universal detection technique, like those offered by MS detection, are typically requirements for biomedical and pharmaceutical applications.The nano from the column is sufficient for MS coupling through various nanospray interfaces, especially elecrospray ionization(ESI) which only needs a small amount of eluent from the LC column is frequently 100-500nL\min.

NANO CAPILLARY ELECROPHORESIS

Nano-capillary electrophoresis has a comparable set up to traditional capillary electrophoresis.The only difference is how small all the parts have been made. The instrumentation of nano capillary electrophoresis briefly covered in the following subsections.

SEPERATION CHIP

The need for studies at the nano- and pico-scales has forced researchers to create nano-capillary electrophoresis based on microchips. High speed, a smaller sample size, and a high level of separation effectiveness are just a few of its many benefits. Microchannel networks are used for sample pre- and post-handling, chemical reactions, separation, and detection as part of the integration of all nano-capillary electrophoresis components onto a chip.

Planar chips technique for downsizing and integration in nano-capillary electrophoresis was published by Manz et al. in 1992b. Typically, the cross-channel chips used in nano-capillary electrophoresis are constructed of glass, silica, or another material such poly(dimethylsiloxane), with a separation channel length of 5 cm and a cross-sectional size of 50 20 lm. Due to its excellent heat conductivity and electrical insulation, glass is regarded as the greatest material. Additionally, electroosmotic flow can be used for electro kinetic pumping in place of mechanical pumping. Due to its high transparency in the ultraviolet area, quartz is the optimum glass for this application.

Several techniques, including photolithography, wet chemical processes, and dry etching, are used to produce the microchips. Effectively employed in nano-capillary electrophoresis is the standard background electrolyte of conventional capillary electrophoresis. Nano-capillary electrophoresis doesn't require a specific background electrolyte. However, high-performance liquid chromatography and analytical grade solvents and reagents are required. In nano-capillary electrophoresis, phosphate buffers with various pH values and ionic strengths are typically employed. However, other buffers have also been utilised, including acetate, borate, and ammonium citrate. To enhance separation, several organic modifiers such acetonitrile and methanol may be utilised. Additionally, nano-capillary electrophoresis may make use of the standard surfactants employed in conventional capillary electrophoresis.

SAMPLE INJECTION

Low mass detection limits and sample injection are crucial for repeatable results in nano-capillary electrophoresis (in absolute units). Of fact, given the small volume needed, injecting samples into nano-capillary electrophoresis is a difficult task (Futterer et al. 2004). For nano-capillary electrophoresis analyses to be successful, carefully injected small amounts of samples are required. In nano-capillary electrophoresis, electrokinetic (Alarie et al. 2001), sequential (Fang et al. 1999), pressure-pinched (Bai et al. 2002), hydrodynamically (Solignac and Gijs 2003), hydrostatic pressure (Gai et al. 2004), push/pull

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 958-963 www.ijprajournal.com ISSN: 2249-7781

(Wu et al. 2004), and bias-free pneumatic are the primary sample injection modes (Cho et al. 2005).

A straightforward sample loading technique for nano-capillary electrophoresis was published by Ito et al. in 2005. Sample loading was done using a polymer microchip (dimethylsiloxane). At the sample polymer interface, the discontinuous electrophoretic mobility may be advantageous. Additionally, the sample compaction impact was only mildly size dependent. Pneumatic sample injection in nano-capillary electrophoresis was described by Cho et al. in 2005. The approach was demonstrated to be able to inject a 10.0 nL sample without sampling bias. Fluorescein, dichlorofluorescein, and red ink were utilised as test substances by the authors. Zhang et al. (2006) described an injection technique that takes advantage of flows produced by electrokinetic, hydrostatic, and negative pressure forces. A single syringe pump and a power source with consistent voltage were used to complete the work. In the headspace of a sealed sample waste reservoir, a partial vacuum was created during loading. Rhodamine 123 and fluorescein sodium had migration time precisions of 3.3% and 1.5%, respectively.

DETECTION

The ability to detect in nano-capillary electrophoresis is also essential for completing nanoanalysis tasks. The most widely used detectors include nuclear magnetic resonance, time-of-flight mass spectrometry, inductively coupled plasma, fluorescent, conductivity, atomic fluorescence spectrometry, atomic emission spectrometry, atomic absorption spectroscopy, and atomic fluorescence spectrometry. Despite the applications for very tiny detectors, it is still challenging to achieve detection sensitivity at the nanoscale. Various detection advancements have been developed periodically to address this issue. The accurate and appropriate hyphenation of the detectors using nano-capillary electrophoresis is one of the advances. It was found that hyphenation caused many detectors to become more sensitive. It is discovered that the sensitivity of ultraviolet-visible detectors increases with detector cell volume. Currently, nano-capillary electrophoresis can be used in combination with mass spectrometer chip-based detectors to produce pretty strong sensitivity.

Capacitively coupled contactless conductivity detection in nano-capillary electrophoresis was described by Alves Brito-Neto et al. in 2005. The theoretical and practical complexity of detection was presented by the writers. The authors conducted modelling studies and hypothesised that the electrolyte co-ion and counter-ion, cell geometry, its placement, and operation frequency all affect sensitivity. The experimental setup can benefit from this publication. Using a contactless conductivity detector, Chen et al. (2007) described the detection of potassium ions with a detection limit of 10 lmolL-1. The benefits come from the independence of detecting electrodes in nano-capillary electrophoresis. Additionally, it was simple to substitute detection in the various positions of the available channel for chip and other activities. A straightforward glass/PDMS microfluidic chip for on-line sample preparation and contactless conductivity detection was described by Zhai et al. (2014). The authors automatically carried out sample extraction, injection, separation, and detection in that order. The stated detection limit for contactless conductivity detection was 2.5 lg mL-1.

A capacitively connected contactless conductivity detector for NH4+, K+, Ca2+, Na, Mg2+, Li+, Br, Cl, NO2-, NO3-, SO42-, and F ions was optimised by Mahabadi et al. (2010). The 1.5 to 0.3 lM detection limits were used. The quantity of ions examined and the detection limit suggested a decent level of detection. Numerous studies have been published on the measurement of contactless conductivity in nano-capillary electrophoresis.

REFERENCES

[1]. Kelvin L in Kallenborn r, Huhnefuss H, Chiral Environmental Pollutants: Trace Analysis and Ecotoxicology , Springer-Verlag, Berlin (2000).
[2]. Hegstrom R, Kondepudi DK, Sci Am 262: 108 (1900).
[3]. Stinson SC, Chem Eng News 72: 38 (1994).
[4]. Testa B, Trends Pharmacol Sci 60 (1986).
[5]. Aitken RA, Parker D, Taylor RJ, Gopal J, Kilenyi RN, in Asymmetric Synthesis, Blackie Academic & Professional, New York (1992).
[6]. Blaschke G, Kraft HP, Fickentscher K, Kohler F, Drug Res 29: 1640 (1749).
[7]. Knoche B, Blaschke G, J Chromatogr A 666: 235 (1994).
[8]. Faller J, Huhnerfuss H, Krebber R, Ludwig P, E nviron Sci Technol 25: 676 (1991).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 958-963 www.ijprajournal.com ISSN: 2249-7781

[9]. Pastuer L, C R Acad Sci 26: 535 (1848).
[10]. Yandamuri N, Dinakaran SK. Advanced Study of Nano Liquid Chromatography and Its Application-A Review. World J. Pharm. Res. 2015 Jan 28;4:1355-67.
[11]. Gama MR, Collins CH, Bottoli CB. Nano-liquid chromatography in pharmaceutical and biomedical research. Journal of chromatographic science. 2013 Aug 1;51(7):694-703.
[12]. Ali I, Alam SD, Al-Othman ZA, Farooqi JA. Recent advances in SPE–chiral-HPLC methods for enantiomeric separation of chiral drugs in biological samples. Journal of Chromatographic Science. 2013 Aug 1;51(7):645-54.
[13]. Rieux, L., Sneekes, E.-J., Swart, R.; Nano LC: Principles, evolution, and state-of-the-art of the technique; LC-GC North America, (2011); 29: 926–934.
[14]. Zotou, A.; An overview of recent advances in HPLC instrumentation; Central European Journal of Chemistry; (2012); 10: 554–569.
[15]. COLUMNS
[16]. Mariana Roberto Gama, Carol H. Collins and Carla B.G. Bottoli, Nano-Liquid Chromatography in Pharmaceutical and Biomedical Research, Journal of Chromatographic Science, 2013; 1–10.
[17]. 5. Laurent Rieux, Evert-Jan Sneekes, Remco Swart, Nano LC: Principles, Evolution, and State-of-the-Art of the Technique, LCGC North America, 29(1): 926-35.
[18]. Chevert, J.P., Ursem, M., Salzman, J.P.; Instrumental requirements for nanoscale liquid chromatography; Analytical Chemistry, (1996); 68: 1507–1512
[19]. He´ron, S., Tchapla, A., Chervet, J.P.; Influence of injection parameters on column performance in nanoscale high-performance liquid chromatography; Chromatographia, (2000); 51: 495–499
[20]. Zotou, A.; An overview of recent advances in HPLC instrumentation; Central European Journal of Chemistry; (2012); 10: 554–569.
[21]. Heus, F., Giera, M., de Kloe, G.E., van Iperen, D., Buijs, J., Nahar, T.T., et al.; Development of a microfluidic confocal fluorescence detection system for the hyphenation of nano-LC to on-line biochemical assays; Analytical and Bioanalytical Chemistry, (2010); 398: 3023–3032.
[22]. Pro¨frock, D.; Progress and possible applications of miniaturized separation techniques and elemental mass spectrometry for quantitative, heteroatom-tagged proteomics; Analytical and Bioanalytical Chemistry, (2010); 398: 2383–2401.
[23]. She, Y.F., Smith, R.D.; Advanced nanoscale separations and mass spectrometry for sensitive high-throughput proteomics; Expert Review of Proteomics, (2005); 2: 431–447.
[24]. Ali I, Aboul-Enein HY, Gupta VK (2009) Nano Chromatography and Capillary Electrophoresis Pharmaceutical and Environmental Analyses. Wiley, Hoboken. ISBN 978-0-470-17851-5
[25]. Bai X, Lee HJ, Rossier JS, Reymond F, Schafer H, Wossner M, Girault HH (2002) Pressure pinched injection of nanolitre volumes in planar micro-analytical devices. Lab Chip 2:45–49. doi:10.1039/B109247B
[26]. Brivio M, Oosterbroek RE, Verboom W, van den Berg A, Reinhoudt DN (2005) Simple chip-based interfaces for on-line monitoring of supramolecular interactions by nano-ESI MS. Lab Chip 5:1111–1122. doi:10.1039/B510534J
[27]. Buettgenbach S, Wilke R (2005) A Capillary electrophoresis chip with hydrodynamic sample injection for measurements from a continuous sample flow. Anal Bioanal Chem 383:733–737. doi:10.1007/s00216-005-3346-6
[28]. Chen Z, Li Q, Li O, Zhou X, Lan Y, Wei Y, Mo J (2007) A thin cover glass chip for contactless conductivity detection in microchip capillary electrophoresis. Talanta 71:1944–1950
[29]. Chin CD, Linder V, Sia SK (2007) Lab-on-a-chip devices for global health: past studies and future opportunities. Lab Chip 7:41–57. doi:10.1039/B611455E
[30]. Ito T, Inoue A, Sato K, Hosokawa K, Maeda M (2005) Autonomous polymer loading and sample injection for microchip electrophoresis. Anal Chem 77:4759–4764. doi:10.1021/ac050122
[31]. Futterer C, Minc N, Bormuth V, Codarbox JH, Laval P, Rossier Viovy JL (2004) Injection and flow control system for

microchannels. Lab Chip 4:351–365. doi:10.1039/B316729A

[32]. Kenyon SM, Meighan MM, Hayes MA (2011) Recent developments in electrophoretic separations on microfluidic devices. Electrophoresis 32:482–493. doi:10.1002/elps.201000469

[33]. Fredrickson CK, Fan ZH (2004) Macro to micri interfaces for micro fluidic devices. Lab Chip 6:526–533. doi:10.1039/B410720A

[34]. Fang Q, Wang FR, Wang SL, Liu SS, Xu SK, Fang ZL (1999) Sequential injection sample introduction microfluidic-chip based capillary electrophoresis system. Anal Chim Acta 390:27–37. doi:10.1016/S0003-2670(99)00183-X

[35]. Wang J, Chen G, Muck A (2003) Movable contactless-conductivity detector for microchip capillary electrophoresis. J Anal Chem 75:4475–4479. doi:10.1021/ac030122k

[36]. Zamfir AD (2007) Recent advances in sheathless interfacing of capillary electrophoresis and electrospray ionization mass spectrometry. J Chromatogr A 1159:2–13. doi:10.1016/j.chroma. 2007.03.115

[37]. Zhao L, Bao J, Li Y (2013) Recent advances in microchip electrophoresis Appl. Chem Ind 42:2263–2266. doi:10.1002/ elps.200800615