1. Introduction

1.1 Alzheimer's disease and its pathophysiology

Alzheimer's disease (AD), is a progressive and devasting neurological disorder, affecting approximately about 50 million people across the globe, which is supposed to affect around 75 million people by 2030, and 131.5 million by 2050 [1–3]. The American Alzheimer's Association states that about 5.8 million people are found to have Alzheimer's, with almost one person per 65 seconds generating AD [4]. The disease has become the 5th leading cause of death in America [5,6]. It origins to a progressive decline in cognitive activities and memory (declarative memory) along with behavioral and personality changes [5–8]. The main reason for neurocognition is the impaired activity of acetylcholine in the forebrain [2,9]. Aβ plaque and neurofibrillary tangles formed due to hyperphosphorylation of τ-protein are the hallmarks of AD [10,11]. Histopathological findings of the AD after autopsy were found to have neuronal losses, amyloid aggregates, fibrillary tangles alongside amyloid angiopathy [12–15].

The AD is of two types namely Early-onset AD (EOAD) and Late-onset AD (LOAD). EOAD arises mostly earlier than 65 years of age and LOAD or sporadic AD, which is age-related and happens after 65 years of age [16,17]. Only 1 to 6% of the total AD is considered to be early onset. EOAD is generally familial AD, occurring mainly due to the aggregation of Aβ plaques, hyperphosphorylation of tau proteins and mutations in presenilin genes (autosomal dominant) [18–20]. On the other hand, LOAD is primarily due to disturbances in the neurotransmission and ApoE [10,21]. Several hypotheses have been proposed, most common of which are β-Amyloid hypothesis and Tau (τ) hypothesis. Others include the cholinergic hypothesis and the ApoE pathway [15,22,23].

(a) Aβ hypothesis

It is also known as the amyloid cascade hypothesis; it is the most common hypotheses for Alzheimer's, but recently researches have shown that this hypothesis is not much accountable for the disease. According to this hypothesis, abnormal metabolism of the beta-amyloid (Aβ) causes the development of harmful plaques in the brain [24,25]. Aβ is generated from the Amyloid Precursor Protein (APP) which is decoded from the APP gene situated on chromosome 21. During cellular metabolic processes, the APP might be cleaved by three different types of enzymes named (i) α-secretase, (ii) β-secretase and (iii) γ-secretase. APP has glycosylated N-terminal that projects out of the cell and C-terminal projecting inside the cell [24,26]. When cleaved by α secretase, the N-terminal leaves by forming APPα and C-terminal is left behind with 99 amino acids [27,28].

When cleaved by β-secretase, it gives APPβ and 99 AA C-terminal, this C-terminal is further cleaved by γ secretase to give a soluble form of Aβ peptide ($A\beta_{40}$ oligomer), an insoluble form of Aβ ($A\beta_{42}$ oligomer) and a C-terminal (Fig. 1.1). In normal physiological conditions, the breakdown of APP takes place by α-secretase enzyme which forms soluble APPα, can be easily cleared from the brain during regular metabolic processes [23,29]. On the other hand, when abnormally cleaved by β and γ-secretase, there is an imbalance in the formation and clearance of Aβ proteins, which gets aggravated into soluble oligomers. This later coalesces into insoluble fibrils and forms large, toxic aggregate termed as senile plaques [26]. The $A\beta_{42}$ oligomers aggregate and form plaque which causes oxidative damage due to reactive oxygen species (ROS), imbalance in calcium homeostasis, promotion of tau hyperphosphorylation and mitochondrial damage [30]. This also causes microglial activation which causes simulate astrocyte-neuron to generate even additional $A\beta_{42}$. Such aggregates of $A\beta_{42}$ are accountable for neuronal and vascular deterioration in AD patient's brain [26,31].

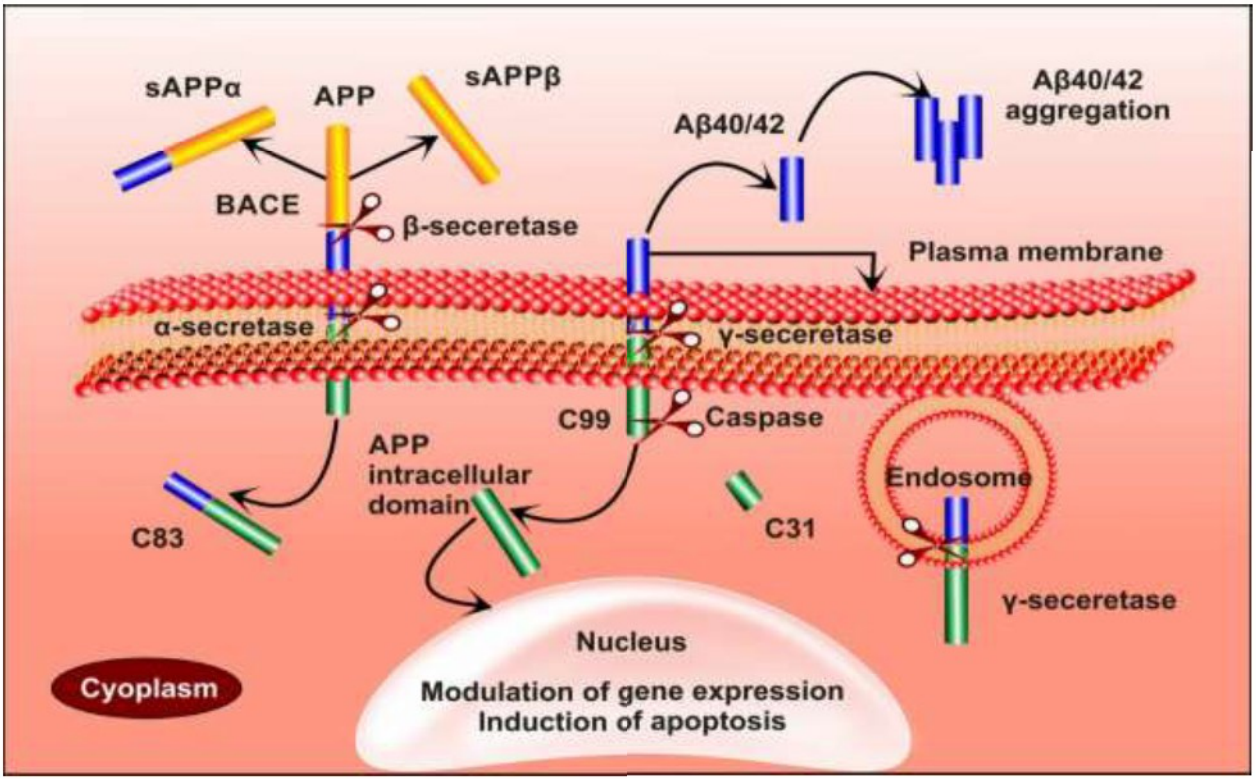

Fig. 1.1: Represents the formation of Aβ- cleavage by α-secretase on N-terminal side results in the formation of secreted APPα (sAPPα), leaving the C-terminal with 88-amino acids (non-amyloidogenic pathway). Cleavage of the APP by β-secretase on N-terminal results in the formation of sAPPβ and going the C-terminal with 99 amino acids. Subsequent cleavage of the C-99 terminal by γ- secretase results in the accumulation of $A\beta_{40/42}$ ($A\beta_{40}$ soluble form and Aβ-42 insoluble form), leaving an internal C-terminal domain (amyloid pathway) (Adapted with permission from [20])

(b) Tau (τ) hypothesis

τ protein is a microtubule-associated protein (MAP) responsible for maintaining the structure of neurons by providing stability to microtubules [32]. The structure of a neuron is disturbed because of detachment of τ protein from microtubule as a result of an imbalance of phosphatase and kinase enzyme in AD [33]. In AD, hyperphosphorylation of the τ-protein takes place that leads to the growth of paired helical filaments (PHF) which aggregates to form neurofibrillary tangles (NFT) (Fig. 1.2) [34].

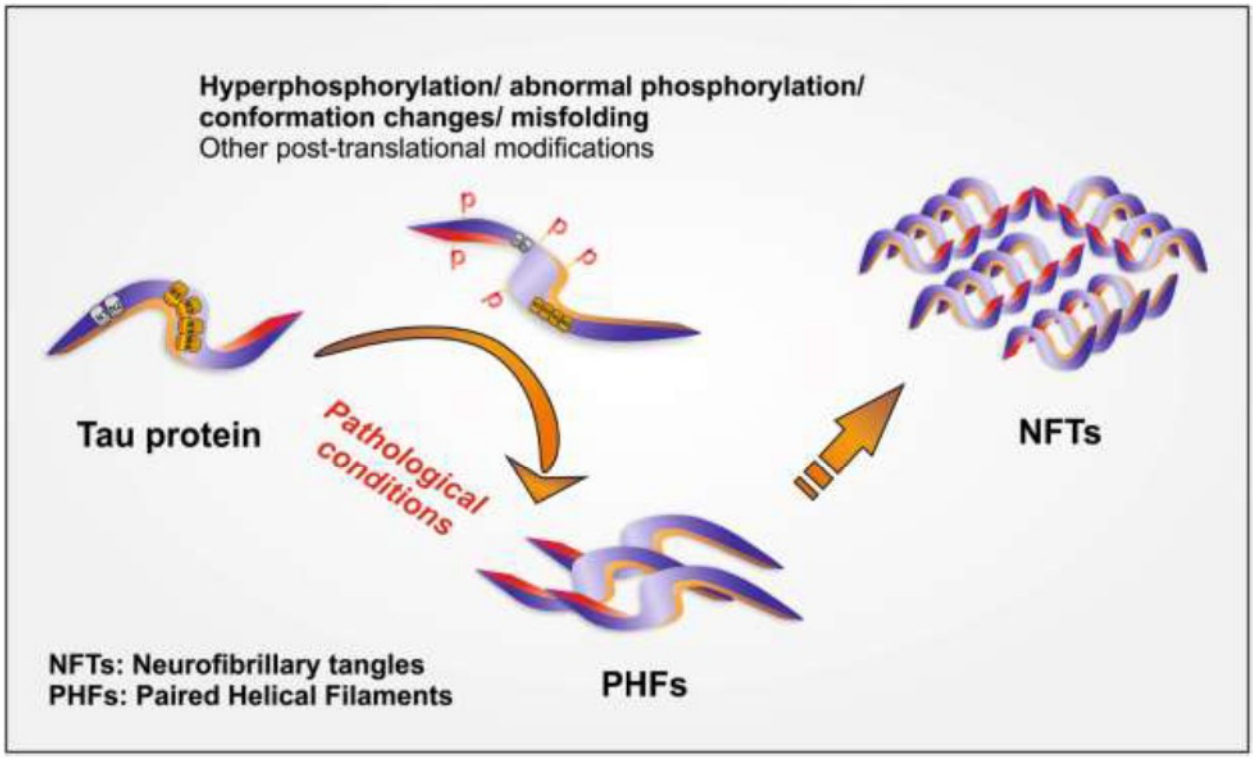

Fig. 1.2: Formation of neurofibrillary tangles of τ–protein (Adapted with permission from [20])

It causes activation of astrocytes and microglial cells which causes neuroinflammation and immune response [35]. Besides, hyperphosphorylated tau proteins get aggregated into the NFT as they cannot be transported across the axons [36,37]. Therefore, they worsen the pathological condition by promoting oxidative stress, which further exacerbates the disease by leading to necrosis, mitochondrial dysfunction, and apoptosis [38]. The pre-fibrillar tau can also form aggregates (soluble oligomers or insoluble granules) that contribute to neurodegeneration by producing disconnections in the synapse and neuronal death [39]. Tau mutation alone is found to lead to dementia rather than AD, unlike the mutated APP [37]. Two models are used in proposing this hypothesis (Fig. 1.3) and the series model states that rise in the Aβ levels leads to the

enhancement in tau phosphorylation which further increases the severities of AD. The dual pathway says that both Aβ and hyperphosphorylated tau lead to a neuronal loss in the AD [40,41].

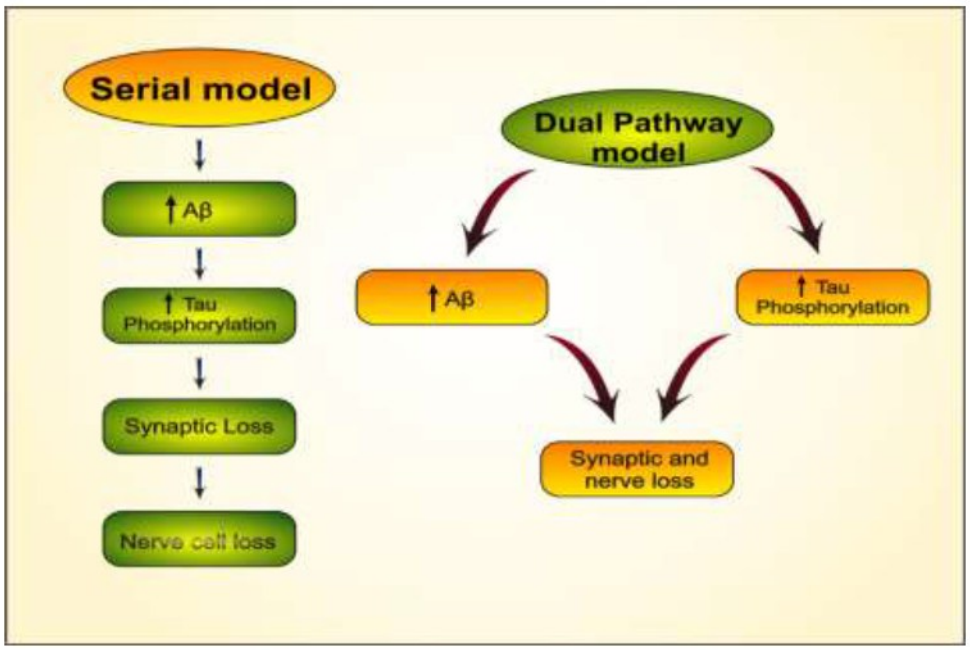

Fig. 1.3: Represents the neuronal loss pathway because of Aβ and tau pathway in the AD. The serial model states that the rise in Aβ levels increases the tau phosphorylation that leads to the AD. The dual path says that both Aβ and hyperphosphorylated tau lead to a neuronal loss in the AD (Adapted with permission from [23])

(c) Cholinergic hypothesis

During LOAD, there is nearly 75% loss of cholinergic neurons, which hence leads to memory loss and cognitive impairment [18]. It is because of the degeneration of basal forebrain cells that help in the formation of the hippocampus [9,42]. Also, because of the reduction in acetyl CoA due to the diminishing activity of pyruvate dehydrogenase, Ach synthesis is said to drop down [38]. Therefore, treatment for an AD with Ach agonist and AChEI would of a significant impact in reversing memory impairment in such patients by increasing regional glucose uptake of the brain and insulin which will thereby increase the synthesis and release of ACh in the brain [43]. But the long-term cure is not effective with AChEI's like rivastigmine, donepezil, and galantamine [19,44].

(d) ApoE pathway

ApoE is a key cholesterol transporter in the brain that is formed by astrocytes, present along with ApoJ and ApoA1 [18,45]. ApoE acts as a lipid carrier and repairs injuries in the brain apart from their support during synaptogenesis. They mediate lipoprotein transport in the brain via transporters like low-density lipoprotein receptor-related protein (LDLR)-1, that are actively

engaged in Aβ clearance from the brain [46–48]. Thus, plaque buildup can also be because of decreased LRP-1 in the AD condition [49]. ApoE has 3-alleles-E_2, E_3, and E_4. In which, E_4 carriers have increased the risk of developing AD with higher levels of plasma inflammatory markers which increases the risk of neuroinflammation either by increasing the formation of $A\beta_{1-42}$, aggregation of formed Aβ or decreased Aβ clearance [49,50]. E2 and E3 are protective and optimistically related to cognition on aging, along with enhancing the glucose uptake and glycolysis in the brain [50–53]. Currently, the use of ApoE is attractive for the target specific delivery of drugs to the brain [50,54,55].

1.2 Overview of BBB molecular architecture and transport strategies to the brain

Brain is a highly sensitive organ of our body compared to the other organs. Although several advancements in the pharmaceutical field, the brain target drug delivery is still a challenging task [56]. This is mostly due to the existence of barriers such as the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The presence of BBB is the major obstacle for any substance that wants to travel from blood to brain [57,58]. BBB is important in defending the brain cells from the toxic substances by impeding the passage of these materials from the blood to the brain tissue [57,59]. But at the same time, it obstructs various drugs to enter into CNS thereby limiting their therapeutic benefits [60]. Maintaining the environment and homeostasis is another important function of BBB [58,61,62].

1.2.1 BBB molecular structure

BBB is mainly designed by endothelial cells of the brain capillaries with tight junctions, these are also known as BMECs (Brain microvessel endothelial cells). The luminal membrane of the endothelial cells is towards the blood while the abluminal membrane is towards the extracellular matter of the brain [63]. Other components of BBB include immune cells, astrocytes, pericytes and basement membrane [64]. Neurovascular unit (NVU) is the collection of the cells that regulate the flow of cerebral blood flow (CBF) and the functions of BBB. NVU comprises of endothelial cells, neurons, vascular smooth muscle cells (VSMCs), microglia, pericytes, astrocytes, mast cells and oligodendrocytes [65–67].

Endothelium shares the basal membrane with astrocytes and pericytes. Pericytes are the mural cells that cover the endothelial cells in capillaries (in arterioles and arteries the mural cells present are VSMCs) [68,69]. These cells interact with other cells to maintain the CNS homeostasis. Its various functions include maintaining BBB integrity, macrophage activity and most importantly

maintaining the barrier property of BBB. Its deficiency has been associated with the downregulation of proteins that form the tight junctions thus affecting the integrity of BBB [69–72].

Astrocytes are star-shaped glial cells that have processes called 'perivascular endfeet' that is in contact with the microvessel wall [73]. Astrocytes perform mainly the function of maintaining the microenvironment within the BBB. This is done by balancing parenchymal water and metabolites, regulation of innate immunity and monitoring of electrochemical activity [74,75].

Basement Membrane is an extracellular matrix that surrounds the vessels and pericytes and is in contact with astrocyte end. It is comprised of two layers: vascular and parenchymal. The vascular layer is secreted by endothelial cells and pericytes whereas the parenchyma layer is secreted by astrocytes. The role of the basement membrane is to provide a physical barrier and network for chemical signaling [76,77].

1.2.2 Strategies for delivery to the brain

Despite of the fact that the BBB causes hindrance in achieving therapeutic concentration in the brain, several alternative strategies have been reported to enhance the transport of therapeutic agents to the brain [78–82].

The following are the approaches:

a) Modification of the molecule
b) Direct delivery to the brain
c) BBB disruption/modification
d) Utilizing the endogenous transport system
e) Intranasal administration

(a) Modification of the molecule

The transport mechanism of the molecules through BBB by passive diffusion is reliant on lipophilicity and molecular weight of the molecule. Molecules with more lipophilicity and log P values ranging in between 1.5 - 2.5 can able to cross the BBB [83]. Modifications in the structure of the molecules can enhance their membrane permeation and passive diffusion [84]. Thus, to boost the BBB permeability of molecules, it can be designed as prodrug analogs or lipophilic scaffolds.

The drug molecules can be converted into a lipophilic form by attaching lipophilic groups for lipidization. Morphine and heroin are classic examples of improved BBB penetration by addition

of lipophilic groups. Chemical conversion of morphine to heroin results in more than 100-fold increase in lipophilicity that leads in 100-fold more uptake in the brain [85].

Prodrugs are inactive analogs of the molecules which undergoes enzymatic or chemical alterations in the biological conditions to form pharmacologically active drug moiety. Based on the design of prodrug, the conversion may happen prior or after absorption into a selective organ [86,87].

(b) Direct delivery to the brain

The complications related to BBB permeation can be resolved by direct administration of designed formulations to the brain. The higher amount of the molecules in the brain can be achieved by direct administration into the brain via implants, injections, and infusions [88,89]. However, this delivery method has several limitations such as limited drug permeation due to high elimination rate and slow diffusion of the molecule and also a highly invasive process which increases the risk of infections, inflammation, edema and damage to the brain parenchyma [90].

This includes various routes such as intracerebroventricular (ICV), intracerebral, convection-enhanced delivery (CED), intrathecal and intratympanic.

(i) Intracerebroventricular (ICV): Drug is directly injected into the cerebrospinal fluid (CSF) filled lateral ventricle of the drug. Drug is either introduced by the implantable reservoir or by a pump (more desirable because of continuous and stable delivery) [91]. The disadvantage of this technique is that it is invasive requiring skull penetration and 90-98% of the drug is lost because the drug can penetrate the parenchyma only by diffusion (very slow) and CSF is continuously replaced at a higher rate, leading to loss of drug before reaching the brain tissue [92,93]. Thus, drug concentration cannot be maintained in the brain [90,94].

(ii) Intracerebral: It is also called as intraparenchymal because the drug is directly delivered to parenchyma using implant or injection [82,91,95]. Less penetration through brain parenchyma is limiting the use of this route.

(iii) Convection enhanced delivery (CED): In this technique drug solution is infused via small diameter catheters that are seated in the parenchymal interstitial space using minor surgical procedures [96,97]. Here positive pressure is applied by using pumps, therefore fluid conviction is also present along with diffusion which increases the bioavailability when compared to the above mentioned routes [98]. Size of particles plays a key role in the drug distribution profile. Smaller particles have a better distribution profile than larger particles [99]. Precautions must be

taken in applying pressure and in the placement of the catheter as it may cause tissue injury or formation of air bubbles [91,96,100].

(iv) Intrathecal: It is the least invasive in the category. Drug is injected via lumbar puncture in the subarachnoid space of the spinal cord. The drug reaches the brain parenchyma by the flow of CSF [101,102]. Leptomeningeal transport is responsible for the delivery of the drug to the parenchyma. This type of transport depends upon the location and volume of injection [70,102]. This is the most viable option for the delivery of nanoparticles as well as macromolecules in substantial quantity, therefore targeting is possible using this route [102,103].

(v) Intratympanic: This involves an injection of drug in the middle ear cavity that reaches to perilymph (extracellular fluid present in the cochlea of the inner ear) through the round window membrane (RWM). Perilymph has an ionic composition similar to that of CSF and communicates to CSF via the perilymphatic duct, causing the drug to reach the brain by bypassing the blood-labyrinthin barrier. Researchers have suggested that macromolecules up to size 1 μm can easily cross RWM [104]. Studies concluded that nanoparticles administered via intratympanic route are more helpful in treating neurodegenerative disorders [105–107].

(c) BBB disruption/modification

The existence of tight junctions hinders the paracellular movement of the active substances in the BBB. Modulators from biological or chemical substances leading to physical stimuli like electromagnetic field and focused ultrasound causes temporary alterations of these tight junctions promotes the improvement in the brain concentrations of drugs [108]. However, it is associated with several changes in the normal biological environment of the CNS like epilepsy, ischemic stroke, brain tumors and several neurodegenerative disorders as well [109,110].

Hyperosmotic disruption of the BBB follows the process of drawing out of the water from the endothelial cells into the blood vessel lumen, leading to the shrinkage of the endothelial cells. The resulting loss of water from the brain leads to vasodilatation and stretching of the endothelial cells. The most commonly used osmotic agents are mannitol, urea and arabinose. Intracarotid infusion of mannitol maintains the permeable state of the BBB for 15 min. to 4h depending on the size of the molecule [111].

Intraarterial vasoactive agents are the chemical BBB disruptors that cause temporary inflammation in the endothelial cells. Destabilization of the endothelial cell membrane by alkylglycerols causes BBB disruption of about 3-15 min. Its disruption leads to the entry of plasma albumin and other

protein components of blood into the brain, that are highly toxic to brain endothelial cells [112]. Cytokine, interleukin, histamine, leukotriene c4, bradykinin, cereport (RMP-7), nitric oxide, 5-hydroxytryptamine (5-HT) and surfactants like polysorbate 80 or sodium dodecyl sulfate have also been explored for enhancing BBB permeation [113].

Focused ultrasound (FUS) mediated delivery is a non-invasive and targeted process for the BBB opening to deliver the therapeutic agents into the brain with negligible side effects. FUS is dependent on acoustic energy and is stored at a target region of the brain [114]. When the electrical energy is supplied, the transducer converts it into mechanical motion, thereby creating an ultrasound that proliferates through the skull. This causes acoustic fluid streaming, pressure variation, local hyperthermia and cavitation in the brain resulting in enhancement of BBB permeability. The magnetic resonance imaging (MRI) assisted with FUS helps in better BBB opening thereby leading to improvement in therapeutic and diagnostic capabilities [114–118].

(d) Utilizing the endogenous transport system

The BBB is composed of various influx and efflux transporters which can control the permeability of drug molecules into the brain. Efflux transporters like P-glycoproteins (P-gp) and multidrug resistance-associated protein (MRP) family members are actively involved in driving substrates back into the blood stream before they cross the brain parenchyma. The uptake of molecules can be attained by P-gp modulators at various stages by usage of P-gp transport inhibitors. Tariquidar a promising third generation P-gp inhibitor, which inhibits by binding to P-gp [119]. These transport mechanisms can be broadly divided into five groups which are (i) carrier-mediated transport (CMT) (ii) ion transport (iii) receptor-mediated transport (RMT) (iv) adsorption-mediated transport (v) cell-mediated transport and (vi) active efflux transport.

CMT is accountable for the transport of molecules such as amino acid, glucose, hexoses, neuropeptides, and vitamins into the brain [120]. For transportation of these molecules into the brain, various carriers are present at the BBB such as amino acid transporters, glucose transporters, monocarboxylic acid transporters, choline transporters, nucleobase transporters etc.[121,122]. The fabrication of certain vector molecules leads to an increase in the proficiency of CNS drug delivery through BBB. This occurs via specific ligands for these receptors that are involved in RMT. These include insulin receptors, LDLR and transferrin receptor (TfR). Despite of these, the antibody molecules that directly bind to the insulin receptor or transferrin receptor (TfR) developed as a vector showed effective transport through BBB. Another type of receptor i.e. LDLR and other

groups of natural apolipoprotein ligands such as ApoB and ApoE have been considered for the efficient transfer of nanoparticles loaded with therapeutic protein to the brain. Apart from these, there are various types of new RMT targets such as single domain llama antibodies (FC5 and FC44) and rabies virus glycoprotein [123].

The delivery through the transport system has the following characteristics:

i. Surface modifications of nanoparticles with some specific ligands for transporting therapeutic agents to the BBB and a hydrophilic nature to extend the biological circulation time of the molecule
ii. The drug molecule is altered by conjugation with a polymer or ligand that acts as a homing device and alters the intrinsic properties
iii. The delivery systems must be non-toxic, non-immunogenic and able to interact with receptors at the BBB
iv. The carriers should be target-selective and increase the efficiency
v. All these delivery systems must be in a controlled and uniform manner to govern the *in vivo* fate of the molecule

All the listed criteria need to full filled for an effective target delivery system [124–126].

(e) Intranasal administration

The human nose comprises of the olfactory region having sub-mucous space which directly continues with the CSF flow tract situated on the olfactory lobe. Intranasal administration leads to direct delivery of a drug into the CSF, whereas the drug is transferred through the nasal epithelium and it travels through the arachnoid membrane which differentiates the sub-mucous space of the nose and reaches the region of olfactory CSF compartment [127]. Lipophilic molecules delivered into the nose possess some distribution efficiency into the olfactory CSF [128]. However, large and hydrophilic molecules do not enter into the olfactory CSF from the nose. The intranasal route for delivery of active agents to the brain is an emerging alternative route to oral and parenteral delivery since it overcomes the limitation of these systems [129]. It is a non-invasive and effective approach for bypassing the major hurdle to brain drug delivery i.e., BBB. This route has various advantages over others including rapid onset of action, reduced systemic exposure, avoidance of pre-systemic degradation, increased bioavailability [130]. For efficient transport of therapeutic actives into the brain through the intranasal route, a detailed knowledge in the physiology and anatomy of the nasal system, pathophysiology, drug transport strategies, formulation, and practical

consideration is an essential requirement. Two major approaches in the intranasal delivery include the olfactory nerve in the olfactory region and trigeminal nerve in the respiratory epithelium which is in contact with both CNS and environment. These serve as a gateway for direct entry of drugs into brain parenchyma via this route [3,7].

1.3 Nanocarriers for drug delivery

The application of nanocarriers has demonstrated promising outcomes in the delivery of drug molecules to the brain. It has been well reported that most of the potent CNS molecules that are unable to cross BBB, could be improved by novel drug delivery systems to attain better therapeutic efficacy. Nanoparticles are colloidal drug delivery systems ranging in the particle size diameter from 1 – 1000 nm containing the active drug molecule encapsulated in macromolecular materials [131–133]. Nanocarrier drug delivery systems not only enhance the drug's solubility but also have several other advantages such as dosage reduction, controlled release of drugs, reduced side effects, target specific drug distribution, improved half-life of the drug, enhanced bioavailability, and thereby can resolve various drawbacks associated with CNS active drugs. Most interestingly, nano-formulations often act at the molecular level to facilitate cellular drug uptake or block efflux pathways like P-gp or by targeting specific receptors, thus further improving the pharmacokinetic and pharmacodynamic drug profile. Depending on the method of preparation technique nanoparticles, polymeric micelles, polymersomes, nanospheres or nanocapsules can be fabricated which shows different properties of release kinetics and *in vivo* behavior also [132–135].

1.3.1 Nanoparticles for brain delivery

The various possibilities for the mechanism of delivery of nanoparticles to the brain were explained below:

a) Inhibition of efflux transporters particularly P-gp
b) Opening of tight junctions at the BBB
c) Nanoparticles will be endocytosed by endothelial cells of the brain capillaries that result in the release of drug with in the brain cells
d) Transcytosis through the endothelial cell layer
e) Surface modifications with surfactants like polysorbate 80, poloxamers like Pluronic F68, low-density lipoprotein receptors like apolipoprotein B or E, insulin receptors, transferrin receptor etc.

Moreover, enhanced brain uptake of the drug molecules via nanocarriers delivery system can be attained with several other approaches like vasoactive agents, cationization, olfactory delivery, hyperosmotic approach, focused ultrasound mediated delivery [135,136]. A wide range of polymers are available both synthetic and natural for the development of nanoformulations [137,138]. Biocompatibility and biodegradability are key parameters which need to be considered while selection of the polymers. They have significant impact on the characterization of formulation, entrapment efficiency, drug loading and also in release kinetics [139,140].

1.3.2 Polymeric nanoparticles for targeted drug delivery

Polymeric nanoparticulate drug delivery systems have been well explored for the delivery of active molecules to the brain [141–143]. They possess significant advantages such as enhanced bioavailability, circulation time, controlled/sustained release and selective or target distribution to the tissues. Moreover, these delivery systems have been extensively used to enhance the brain uptake of therapeutic agents [141,142,144].

Polymers like polybutyl cyanoacrylates (PBCA), polycaprolactone (PCL), poly (D, L-lactide) (PLA), poly (D, L-lactide-co-glycolide) (PLGA), di-block polymers like mPEG-PLA or mPEG-PCL are extensively utilized for the delivery of molecules to the brain. Many investigations have been determined to deliver drug molecules to the brain by utilizing polymeric nanoparticulate drug delivery systems. Previously several reports have been published from our group for improving brain uptake by using polymeric nanoparticles. PLGA and PCL loaded etoposide nanoparticles were developed and investigated for pharmacokinetic and biodistribution studies with radiolabeled etoposide. The results exhibited an increased residence of nanoparticles as related to free drug and also improved the bioavailability by selective distribution with improved brain uptake and reduced etoposide associated toxicity. Imatinib mesylate loaded PLGA nanoparticles were prepared and assessed its pharmacokinetic and biodistribution pattern in another study. The results exhibited that these nanoparticles enhanced the extent of drug permeation with increased mean residence time in the brain than free drug alone [145]. In another study, olanzapine loaded PCL nanoparticles were fabricated and further surface modified with polysorbate 80 for the optimized formulation to boost the brain targeting efficacy. Further, these nanoparticles improved the brain efficacy as compared with the free drug alone [146]. Moreover, surface modifications of polymeric nanoparticles with surfactants or brain lipids are a useful approach to increase the brain uptake of therapeutics. Various studies have been reported in this direction and it has been proved that

surface modifications resulted in improved brain concentration level of the drug molecules as compared with free drug alone [147–149].

1.3.3 Methods of preparation

Different preparation techniques have been reported for the fabrication of polymeric nanoparticles. Based on the preparation techniques the following methods have been discussed [150–154].

(a) Emulsification / solvent evaporation

In this method, polymer and drug are solubilized in an organic solvent. The solution is added dropwise into an aqueous phase and the resulting emulsion is evaporated. During emulsification, homogenization methods are used to disperse organic solvent having polymer-drug complex into the aqueous phase. Further, the organic solvents are evaporated under vacuum using a rota-evaporator. Polymers such as PLA, PCL, PLGA, mPEG-PLA, mPEG-PCL, poly hydroxybutyrate etc. are utilized for the fabrication of nanoparticles [155,156].

(b) Film hydration technique

In this method, polymer and drug are solubilized in an organic solvent, the solution is transferred into a round bottom flask and is evaporated at 40-50 °C until a thin dry film is formed on the inner surface of the round bottom flask using a rota-evaporator. The flask is then dried over a vacuum line for about 12 h. The dried film is then rehydrated with the aqueous phase and then vortexed till the thin dry film is no longer visible. Different polymers like PLGA, mPEG-PLA, mPEG-PCL, D-α-Tocopherol polyethylene glycol 1000 succinate etc. are widely used for the preparation of nanoparticles [157,158].

(c) Nanoprecipitation technique

In this method, selected polymer and drug are dissolved in an organic solvent and are precipitated by diffusing into aqueous solution in the presence or absence of stabilizers. The polymer is deposited in between organic and aqueous phase and is precipitated into nanoform immediately due to fast diffusion from the organic solvent. For this, aqueous miscible organic solvents like acetone, acetonitrile, methanol etc. are utilized for the fabrication of polymeric nanoparticles. Several polymers such as PLA, PCL, PLGA, mPEG-PLA, mPEG-PCL, poly hydroxybutyrate etc. are extensively utilized for the fabrication of nanoparticles [153,159,160].

(d) Salting out with synthetic polymers

In this, to separate aqueous miscible organic phase from the aqueous solution, salting out method is utilized. It is also known as a modified process of emulsification or solvent diffusion method.

Polymer and drug are solubilized in miscible organic solvents like dimethyl sulfoxide, acetone, acetonitrile etc. Further, it is added to the aqueous solution containing electrolytes or non-electrolytes are used as salting out agents. [161,162].

(e) Desolvation of macromolecules

It is the other technique for the preparation of nanoparticles, whereas desolvation is performed by charge or pH changes or by the addition of desolvating agents. This technique is mostly preferred for thermolabile drugs due to no heat process is utilized during the preparation process. Moreover, macromolecular materials such as gelatin, casein, serum albumin etc. are utilized in this method [163,164].

(f) Supercritical fluid technology

This is one of the recent methods used for the fabrication of nanoparticles. In this, polymer and drug are solubilized in compressed or supercritical fluid and then passed through a nozzle where the solution is expanded. The solute particles get precipitated into nanosize after the evaporation of supercritical fluid. Further this technique is having an advantage in free of solvent residuals due to the complete removal of supercritical fluid during the process. Several formulations have been reported by using this method including protein delivery systems also resulted in promising therapeutic effects. The high investment is necessary for the initial set up and solubility of polar substances in the supercritical fluid is limited, hence this technique is less suitable for hydrophilic drugs [165–167].

1.3.4 Characterization of polymeric nanoparticles

Characterization of polymeric nanoparticles is important and is required for the assessment of stability, quality, release kinetics and the *in-vivo* fate of the delivery system. The important in-vitro evaluation methods include assessment of particle size and its distribution, surface charge, morphology and thermal behavior [153,168,169].

(a) Particle size, distribution and zeta potential

Particle size and distribution pattern plays a critical role in the overall performance of nanoparticulate drug delivery system. It can impact on the stability, release kinetics and biological behavior of designed polymeric nanoparticles. The normal particle size of polymeric nanoparticles ranges between 10-1000 nm. Although, for target specific delivery to CNS the particle size in between 50-300 nm is preferred. This range provides enhanced cellular uptake, easier permeability and site specificity. Several factors influence the size and distribution pattern of nanoparticles like

composition of formulation, preparation technique and process variables. Non-destructive techniques such as dynamic light scattering (DLS), quasi elastic light scattering (QELS), quasi light scattering (QLS), photon correlation spectroscopy (PCS) and multi-angles light scattering (MALS) are utilized for the assessment of hydrodynamic particle size and distribution in the liquid dispersion. Ideally, polydispersity index below 0.2 describes a narrow size distribution range where, more than 0.5 represents broad size distribution [170–172].

Zeta potential is another important parameter that gives information about the stability of polymeric nanoparticles. Nano-dispersion stabilized electrostatistically requires minimum ± 30 mv for good stability of nanoparticles. Higher charge over the surface of nanoparticles results in the repulsion of nanoparticles, thereby reducing the tendency to agglomerate. Zeta potential measurement is integrated with the DLS instrument, to estimate the particle size, polydispersity index and zeta potential [172,173].

(b) Particle morphology

In particle morphology studies, the shape and surface structure of the nanoparticles are estimated. As compared to spherical particles, anisotropic particles have more surface area and rapid diffusion pathways [172,174]. The surfactant amount required is more in the case of non-spherical particles for maintaining their stability. Particle morphology plays an important role in entrapment efficiency, drug loading, drug release pattern, pharmacokinetics, biodistribution and site-specific delivery of polymeric nanoparticles. Moreover, it also significantly influences on cellular uptake, interaction with cells and receptor binding [175–177]. Microscopy techniques like transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM) are utilized for estimating the particle size, shape, aggregation state, surface morphology and also the core structure of polymeric nanoparticles [172,178].

(c) Entrapment efficiency and Drug loading

Entrapment efficiency is one of the key factors need to be optimized during formulation development to reduce the cost of the designed formulation. It represents the percent quantity of drug which gets encapsulated in the polymeric nanoparticle. The developed formulations are optimized to achieve a maximum percent entrapment efficiency to reduce the cost and enhance the therapeutic efficacy [168,172,177].

Drug loading is the ratio of the amount of drug present in nanoformulation and the weight of the polymer utilized for preparation. The drug loading and entrapment efficiency are estimated directly

by digesting the polymeric nanoparticles in suitable organic solvents or indirectly by determining free drug content present in the supernatant collected after separating the nanoparticles from the aqueous dispersion by ultra-centrifugation [168].

(d) *In-vitro* drug release

The release pattern of polymeric nanoparticles is significantly dependent on the nature and properties of the polymer utilized for the fabrication of nanoparticles. The controlled release of drugs from the polymeric nanoparticles is directly proportional to the hydrophobic moiety of the selected polymer which results in prolonged circulation time and shielded from enzymatic degradation in biological conditions. Drug release pattern from the polymeric nanoparticles might be either by erosion of the matrix or diffusion of drug. Moreover, the drug release pattern is bi-phasic in nature with an initial burst release might be due to the existence of drug over the surface of the nanoparticles thereby sustained or controlled release from the core of the formulation. Besides, the release pattern of polymeric nanoparticles is dependent on the lipophilicity of the polymers utilized for preparation. Stabilizers also have a critical role in the drug release profile, as they regulate the binding of enzymes like lipase or co-lipase to the polymeric nanoparticles [172,174]. The drug release pattern is estimated by using different methods such as dialysis membrane, continuous flow etc. Although, the dialysis membrane is an extensively used method for the assessment of release pattern in polymeric nanoparticles [179].

1.3.5 Stability of polymeric nanoparticles

Stability assessment of any delivery system is a significant and essential parameter that needs to be considered. During long-term storage conditions, aggregation of nanoparticles may arise due to collision resulting in perikinetic flocculation. It has been observed that, a pearl like effect can be noticed in a highly concentrated polymeric nanoparticular dispersions leading to network formation which hampers perikinetic and collision flocculation. Further, diluting with suitable quantity leads to network disturbance and releasing discrete nanoparticles.

Agglomeration of polymeric nanoparticles during storage in dispersion form leads to the physical instability of the formulation. Thus, to maintain their nanoparticular properties like particle size, polydispersity index, entrapment efficiency and to prevent any bacterial contamination in the aqueous phase, a suitable approach needs to be used to ensure the stability of polymeric nanoparticles. The addition of a preservative to the nanoparticle dispersion and removal of water by lyophilization are widely applied methods to maintain their physical stability.

The destabilizing effect of preservatives is influenced by various parameters like lipophilicity of nanoparticles, ability to decrease the zeta potential, surface attachment of preservative, the interaction of preservative with the nanoparticles etc. Further, preservatives should have some properties such as non-ionic, hydrophilicity, minimal interaction with nanoparticle surface and non-significant effect on zeta-potential to have a stable and preserved polymeric nanoparticles [172].

Freeze-dried formulations must be resuspended in aqueous phase with a quick reconstitution time and have an adequate appearance and lower redispersibility index value. Additionally, it should not alter significant variations in particle size, polydispersity index and entrapment efficacy of the polymeric nanoparticles. Several carbohydrates and polymers such as sucrose, mannitol, dextrose, lactose, sorbitol, trehalose, Avicel RC-591 etc. are well explored as cryoprotectants [180–182].

1.3.6 *In-vivo* fate of polymeric nanoparticles

The *in-vivo* fate of the polymeric nanoparticles is dependent on several parameters like properties of the polymer used, route of administration, the particle size of nanoparticles, surface modifications, interaction with biological proteins or enzymes and so on. Thus, it is essential to explore the pharmacokinetics, bio-distribution pattern and therapeutic efficacy of the designed polymeric nanoparticles [160].

1.4 Objectives of the current research work

Donepezil is an anti-Alzheimer's drug approved for the symptomatic treatment of cognitive disorders. It is a piperidine derivative and an acetylcholinesterase inhibitor with neuroprotective activity [183]. However, it causes increased gastric acid secretion which promotes the risk for developing ulcers, gastric irritation, gastrointestinal bleeding, nausea, diarrhea etc. Earlier studies have been reported that, it follows a linear pharmacokinetic profile on oral administration with complete absorption and relative bioavailability of about 100%. However, only 15-20% of the administered dose reaches the brain [184,185].

Moreover, repeated administration of donepezil at a dose of 5 mg every day leads to several side effects and patient incompliance particularly in Alzheimer patients. Further, donepezil is highly distributed to all tissues and is metabolized rapidly [186]. Thus, it is necessary to design a formulation with a targeted approach to selective distribution. In the previous studies, PLGA loaded donepezil microparticles were prepared and characterized to achieve a sustained release formulation [187,188]. Similarly, in another study donepezil loaded PLGA-PEG nanoparticles

were fabricated and evaluated for its controlled release behavior and their ability to enhance the destabilizing effect of amyloid-beta fibrils [189].

Designing a novel polymeric nanoparticulate delivery system will provide an effective system for improving the delivery of donepezil to the brain, enhanced biological circulation time and changes in the distribution pattern leading to achieve higher concentrations of donepezil in the brain. In addition, surface modifications with specific ligands to enhance the brain uptake efficiency, thereby dose reduction and reduced side effects. Polymeric nanoparticles are biocompatible, biodegradable and less toxic in nature with higher drug loading capacity, site-specific delivery and provide controlled or sustained release of the drug.

By considering the above facts, the present research purpose is to design, develop and optimize a delivery system that will enhance the brain uptake with reduced side effects.

Therefore, present research work is aimed to:

- To design and develop the novel polymeric nanoparticulate drug delivery system with enhanced brain uptake
- Characterization of the developed polymeric nanoparticles and optimization of various process parameters
- Evaluation of optimized polymeric nanoparticles in cell culture studies
- To conduct pharmacokinetics, biodistribution and efficacy evaluation studies in intracerebroventricular-amyloid beta$_{1\text{-}42}$ induced rat model

References:

[1] J. Meldolesi, Alzheimer's Disease: key developments support promising perspectives for therapy, Pharmacol. Res. (2019) 104316.

[2] R. Khoury, K. Patel, J. Gold, S. Hinds, G.T. Grossberg, Recent progress in the pharmacotherapy of Alzheimer's disease, Drugs Aging. 34 (2017) 811–820.

[3] M. Agrawal, S. Saraf, S. Saraf, S.G. Antimisiaris, M.B. Chougule, S.A. Shoyele, A. Alexander, Nose-to-brain drug delivery: An update on clinical challenges and progress towards approval of anti-Alzheimer drugs, J. Control. Release. 281 (2018) 139–177.

[4] M.J. Prince, World Alzheimer Report 2015: the global impact of dementia: an analysis of prevalence, incidence, cost and trends, Alzheimer's Disease International, 2015.

[5] M. Wadman, US government sets out Alzheimer's plan, Nature. 485 (2012) 426.

[6] A. Association, 2019 Alzheimer's disease facts and figures, Alzheimer's Dement. 15 (2019) 321–387.

[7] A. Alexander, S. Saraf, Nose-to-brain drug delivery approach: A key to easily accessing the brain for the treatment of Alzheimer's disease, Neural Regen. Res. 13 (2018) 2102.

[8] H. Hampel, A. Vergallo, L.F. Aguilar, N. Benda, K. Broich, A.C. Cuello, J. Cummings, B. Dubois, H.J. Federoff, M. Fiandaca, others, Precision pharmacology for Alzheimer's disease, Pharmacol. Res. 130 (2018) 331–365.

[9] G.S. Watson, S. Craft, Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease, Eur. J. Pharmacol. 490 (2004) 97–113.

[10] S. Tiwari, V. Atluri, A. Kaushik, A. Yndart, M. Nair, Alzheimer's disease: pathogenesis, diagnostics, and therapeutics, Int. J. Nanomedicine. 14 (2019) 5541.

[11] G.D. Stanciu, A. Luca, R.N. Rusu, V. Bild, S.I. Beschea Chiriac, C. Solcan, W. Bild, D.C. Ababei, Alzheimer's Disease Pharmacotherapy in Relation to Cholinergic System Involvement, Biomolecules. 10 (2020) 40.

[12] M. Shinohara, N. Sato, Bidirectional interactions between diabetes and Alzheimer's disease, Neurochem. Int. 108 (2017) 296–302.

[13] S.O. Bachurin, S.I. Gavrilova, A. Samsonova, G.E. Barreto, G. Aliev, Mild cognitive impairment due to Alzheimer disease: Contemporary approaches to diagnostics and pharmacological intervention, Pharmacol. Res. 129 (2018) 216–226.

[14] M. Agrawal, S. Saraf, S. Saraf, S.G. Antimisiaris, N. Hamano, S.-D. Li, M. Chougule, S.A.

Shoyele, U. Gupta, Ajazuddin, others, Recent advancements in the field of nanotechnology for the delivery of anti-Alzheimer drug in the brain region, Expert Opin. Drug Deliv. 15 (2018) 589–617.

[15] M. Agrawal, D.K. Tripathi, S. Saraf, S. Saraf, S.G. Antimisiaris, S. Mourtas, M. Hammarlund-Udenaes, A. Alexander, others, Recent advancements in liposomes targeting strategies to cross blood-brain barrier (BBB) for the treatment of Alzheimer's disease, J. Control. Release. 260 (2017) 61–77.

[16] P. Tellechea, N. Pujol, P. Esteve-Belloch, B. Echeveste, M.R. Garc\'\ia-Eulate, J. Arbizu, M. Riverol, Early-and late-onset Alzheimer disease: Are they the same entity?, Neurol. (English Ed. 33 (2018) 244–253.

[17] G.P. Morris, I.A. Clark, B. Vissel, Questions concerning the role of amyloid-β in the definition, aetiology and diagnosis of Alzheimer's disease, Acta Neuropathol. 136 (2018) 663–689.

[18] P. dos Santos, C. Leide, P.F. Ozela, M. de Fatima de Brito Brito, A.A. Pinheiro, E.C. Padilha, F.S. Braga, H.T. Carlos, C.B.R. dos Santos, J. Rosa, others, Alzheimer's disease: a review from the pathophysiology to diagnosis, new perspectives for pharmacological treatment, Curr. Med. Chem. 25 (2018) 3141–3159.

[19] L.A. Craig, N.S. Hong, R.J. McDonald, Revisiting the cholinergic hypothesis in the development of Alzheimer's disease, Neurosci. Biobehav. Rev. 35 (2011) 1397–1409.

[20] S.K. Dubey, M.S. Ram, K.V. Krishna, R.N. Saha, G. Singhvi, M. Agrawal, S. Saraf, S. Saraf, A. Alexander, others, Recent Expansions on Cellular Models to Uncover the Scientific Barriers Towards Drug Development for Alzheimer's Disease, Cell. Mol. Neurobiol. 39 (2019) 181–209.

[21] M. Nizzari, S. Thellung, A. Corsaro, V. Villa, A. Pagano, C. Porcile, C. Russo, T. Florio, Neurodegeneration in Alzheimer disease: role of amyloid precursor protein and presenilin 1 intracellular signaling, J. Toxicol. 2012 (2012).

[22] M.E. Conway, Alzheimer's disease: targeting the glutamatergic system, Biogerontology. (2020) 1–18.

[23] S.K. Dubey, K.K. Lakshmi, K.V. Krishna, M. Agrawal, G. Singhvi, R.N. Saha, S. Saraf, S. Saraf, R. Shukla, A. Alexander, Insulin mediated novel therapies for the treatment of Alzheimer's disease, Life Sci. (2020) 117540.

[24] J. Wang, B.J. Gu, C.L. Masters, Y.-J. Wang, A systemic view of Alzheimer disease—insights from amyloid-β metabolism beyond the brain, Nat. Rev. Neurol. 13 (2017) 612.

[25] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science (80-.). 297 (2002) 353–356.

[26] A. Kumar, A. Singh, Ekavali, A review on Alzheimer's disease pathophysiology and its management: An update, Pharmacol. Reports. 67 (2015) 195–203.

[27] S. Musardo, E. Marcello, Synaptic dysfunction in Alzheimer's disease: From the role of amyloid β-peptide to the α-secretase ADAM10, Eur. J. Pharmacol. 817 (2017) 30–37.

[28] X. Du, X. Wang, M. Geng, Alzheimer's disease hypothesis and related therapies, Transl. Neurodegener. 7 (2018) 2.

[29] G.S. Bloom, Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis, JAMA Neurol. 71 (2014) 505–508.

[30] A. Kumar, S. Dogra, Neuropathology and therapeutic management of Alzheimer's disease--An update, Drugs Future. 33 (2008) 433–446.

[31] M.P. Mattson, Pathways towards and away from Alzheimer's disease, Nature. 430 (2004) 631–639.

[32] E.-M. Mandelkow, E. Mandelkow, Biochemistry and cell biology of tau protein in neurofibrillary degeneration, Cold Spring Harb. Perspect. Med. 2 (2012) a006247.

[33] J. Kalra, A. Khan, Reducing Aβ load and tau phosphorylation: Emerging perspective for treating Alzheimer's disease, Eur. J. Pharmacol. 764 (2015) 571–581.

[34] K. Iqbal, F. Liu, C.-X. Gong, I. Grundke-Iqbal, Tau in Alzheimer disease and related tauopathies, Curr. Alzheimer Res. 7 (2010) 656–664.

[35] C. Laurent, L. Buée, D. Blum, Tau and neuroinflammation: What impact for Alzheimer's Disease and Tauopathies?, Biomed. J. 41 (2018) 21–33.

[36] F.P. Chong, K.Y. Ng, R.Y. Koh, S.M. Chye, Tau proteins and tauopathies in Alzheimer's disease, Cell. Mol. Neurobiol. 38 (2018) 965–980.

[37] E. Giacobini, G. Gold, Alzheimer disease therapy—moving from amyloid-β to tau, Nat. Rev. Neurol. 9 (2013) 677.

[38] S.C. Correia, R.X. Santos, G. Perry, X. Zhu, P.I. Moreira, M.A. Smith, Insulin-resistant brain state: the culprit in sporadic Alzheimer's disease?, Ageing Res. Rev. 10 (2011) 264–273.

[39] S. de la Monte, Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease, Curr. Alzheimer Res. 9 (2012) 35–66.

[40] L.M. Ittner, J. Götz, Amyloid-β and tau—a toxic pas de deux in Alzheimer's disease, Nat. Rev. Neurosci. 12 (2011) 67–72.

[41] S.A. Small, K. Duff, Linking Aβ and tau in late-onset Alzheimer's disease: a dual pathway hypothesis, Neuron. 60 (2008) 534–542.

[42] M. Sarter, V. Parikh, Choline transporters, cholinergic transmission and cognition, Nat. Rev. Neurosci. 6 (2005) 48–56.

[43] P.J. Whitehouse, Cholinergic therapy in dementia, Acta Neurol. Scand. 88 (1993) 42–45.

[44] P.-P. Liu, Y. Xie, X.-Y. Meng, J.-S. Kang, History and progress of hypotheses and clinical trials for Alzheimer's disease, Signal Transduct. Target. Ther. 4 (2019) 1–22.

[45] J. Kim, J.M. Basak, D.M. Holtzman, The role of apolipoprotein E in Alzheimer's disease, Neuron. 63 (2009) 287–303.

[46] R.P. Koldamova, I.M. Lefterov, M.I. Lefterova, J.S. Lazo, Apolipoprotein AI directly interacts with amyloid precursor protein and inhibits Aβ aggregation and toxicity, Biochemistry. 40 (2001) 3553–3560.

[47] A.C. Paula-Lima, M.A. Tricerri, J. Brito-Moreira, T.R. Bomfim, F.F. Oliveira, M.H. Magdesian, L.T. Grinberg, R. Panizzutti, S.T. Ferreira, Human apolipoprotein A--I binds amyloid-β and prevents Aβ-induced neurotoxicity, Int. J. Biochem. Cell Biol. 41 (2009) 1361–1370.

[48] J. Ghiso, E. Matsubara, A. Koudinov, N.H. Choi-Miura, M. Tomita, T. Wisniewski, B. Frangione, The cerebrospinal-fluid soluble form of Alzheimer's amyloid β is complexed to SP-40, 40 (apolipoprotein J), an inhibitor of the complement membrane-attack complex, Biochem. J. 293 (1993) 27–30.

[49] T.S. Salameh, E.M. Rhea, W.A. Banks, A.J. Hanson, Insulin resistance, dyslipidemia, and apolipoprotein E interactions as mechanisms in cognitive impairment and Alzheimer's disease, Exp. Biol. Med. 241 (2016) 1676–1683.

[50] C.-C. Liu, T. Kanekiyo, H. Xu, G. Bu, Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy, Nat. Rev. Neurol. 9 (2013) 106.

[51] L. Wu, X. Zhang, L. Zhao, Human ApoE isoforms differentially modulate brain glucose and ketone body metabolism: implications for Alzheimer's disease risk reduction and early

intervention, J. Neurosci. 38 (2018) 6665–6681.

[52] D.J. Berlau, M.M. Corrada, E. Head, C.H. Kawas, APOE ε2 is associated with intact cognition but increased Alzheimer pathology in the oldest old, Neurology. 72 (2009) 829–834.

[53] A. Sen, D.L. Alkon, T.J. Nelson, Apolipoprotein E3 (apoE3) but not apoE4 protects against synaptic loss through increased expression of protein kinase Cϵ, J. Biol. Chem. 287 (2012) 15947–15958.

[54] C.R.A. Mondadori, D.J.-F. de Quervain, A. Buchmann, H. Mustovic, M.A. Wollmer, C.F. Schmidt, P. Boesiger, C. Hock, R.M. Nitsch, A. Papassotiropoulos, others, Better memory and neural efficiency in young apolipoprotein E ε4 carriers, Cereb. Cortex. 17 (2007) 1934–1947.

[55] P.B. Verghese, J.M. Castellano, D.M. Holtzman, Apolipoprotein E in Alzheimer's disease and other neurological disorders, Lancet Neurol. 10 (2011) 241–252.

[56] D. Furtado, M. Björnmalm, S. Ayton, A.I. Bush, K. Kempe, F. Caruso, Overcoming the blood--brain barrier: the role of nanomaterials in treating neurological diseases, Adv. Mater. 30 (2018) 1801362.

[57] M.D. Sweeney, A.P. Sagare, B. V Zlokovic, Blood--brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders, Nat. Rev. Neurol. 14 (2018) 133.

[58] B. V Zlokovic, The blood-brain barrier in health and chronic neurodegenerative disorders, Neuron. 57 (2008) 178–201.

[59] A. Montagne, S.R. Barnes, M.D. Sweeney, M.R. Halliday, A.P. Sagare, Z. Zhao, A.W. Toga, R.E. Jacobs, C.Y. Liu, L. Amezcua, others, Blood-brain barrier breakdown in the aging human hippocampus, Neuron. 85 (2015) 296–302.

[60] N.J. Abbott, A.A.K. Patabendige, D.E.M. Dolman, S.R. Yusof, D.J. Begley, Structure and function of the blood--brain barrier, Neurobiol. Dis. 37 (2010) 13–25.

[61] J. Interlandi, Breaking the brain barrier, Sci. Am. 308 (2013) 52–57.

[62] B. V Zlokovic, Neurodegeneration and the neurovascular unit, Nat. Med. 16 (2010) 1370–1371.

[63] J. Temsamani, J.-M. Scherrmann, A.R. Rees, M. Kaczorek, Brain drug delivery technologies: novel approaches for transporting therapeutics, Pharm. Sci. Technolo. Today. 3 (2000) 155–162.

[64] Y. Sharif, F. Jumah, L. Coplan, A. Krosser, K. Sharif, R.S. Tubbs, Blood brain barrier: A review of its anatomy and physiology in health and disease, Clin. Anat. 31 (2018) 812–823.

[65] B. V Zlokovic, Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders, Nat. Rev. Neurosci. 12 (2011) 723–738.

[66] N.J. Abbott, Blood--brain barrier structure and function and the challenges for CNS drug delivery, J. Inherit. Metab. Dis. 36 (2013) 437–449.

[67] W.A. Banks, From blood--brain barrier to blood--brain interface: new opportunities for CNS drug delivery, Nat. Rev. Drug Discov. 15 (2016) 275.

[68] R. Daneman, L. Zhou, A.A. Kebede, B.A. Barres, Pericytes are required for blood--brain barrier integrity during embryogenesis, Nature. 468 (2010) 562–566.

[69] B.T. Hawkins, T.P. Davis, The blood-brain barrier/neurovascular unit in health and disease, Pharmacol. Rev. 57 (2005) 173–185.

[70] X. Yi, D.S. Manickam, A. Brynskikh, A. V Kabanov, Agile delivery of protein therapeutics to CNS, J. Control. Release. 190 (2014) 637–663.

[71] R.D. Bell, E.A. Winkler, A.P. Sagare, I. Singh, B. LaRue, R. Deane, B. V Zlokovic, Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging, Neuron. 68 (2010) 409–427.

[72] M.D. Sweeney, S. Ayyadurai, B. V Zlokovic, Pericytes of the neurovascular unit: key functions and signaling pathways, Nat. Neurosci. 19 (2016) 771–783.

[73] R.M. Ransohoff, P. Kivisäkk, G. Kidd, Three or more routes for leukocyte migration into the central nervous system, Nat. Rev. Immunol. 3 (2003) 569–581.

[74] A. Wong, M. Ye, A. Levy, J. Rothstein, D. Bergles, P.C. Searson, The blood-brain barrier: an engineering perspective, Front. Neuroeng. 6 (2013) 7.

[75] N.J. Abbott, L. Rönnbäck, E. Hansson, Astrocyte--endothelial interactions at the blood--brain barrier, Nat. Rev. Neurosci. 7 (2006) 41.

[76] L. Sorokin, The impact of the extracellular matrix on inflammation, Nat. Rev. Immunol. 10 (2010) 712–723.

[77] P.D. Yurchenco, B.L. Patton, Developmental and pathogenic mechanisms of basement membrane assembly, Curr. Pharm. Des. 15 (2009) 1277–1294.

[78] A.R. Jones, E. V Shusta, Blood--brain barrier transport of therapeutics via receptor-mediation, Pharm. Res. 24 (2007) 1759–1771.

[79] Y. Chen, L. Liu, Modern methods for delivery of drugs across the blood--brain barrier, Adv. Drug Deliv. Rev. 64 (2012) 640–665.

[80] D. Gopalan, A. Pandey, N. Udupa, S. Mutalik, Receptor specific, stimuli responsive and subcellular targeted approaches for effective therapy of Alzheimer: Role of surface engineered nanocarriers, J. Control. Release. 319 (2020) 183–200.

[81] E. Neuwelt, N.J. Abbott, L. Abrey, W.A. Banks, B. Blakley, T. Davis, B. Engelhardt, P. Grammas, M. Nedergaard, J. Nutt, others, Strategies to advance translational research into brain barriers, Lancet Neurol. 7 (2008) 84–96.

[82] M.I. Alam, S. Beg, A. Samad, S. Baboota, K. Kohli, J. Ali, A. Ahuja, M. Akbar, Strategy for effective brain drug delivery, Eur. J. Pharm. Sci. 40 (2010) 385–403.

[83] D.J. Begley, Delivery of therapeutic agents to the central nervous system: the problems and the possibilities, Pharmacol. Ther. 104 (2004) 29–45.

[84] W.M. Scheld, Drug delivery to the central nervous system: general principles and relevance to therapy for infections of the central nervous system, Rev. Infect. Dis. 11 (1989) S1669--S1690.

[85] L. Juillerat-Jeanneret, The targeted delivery of cancer drugs across the blood--brain barrier: chemical modifications of drugs or drug-nanoparticles?, Drug Discov. Today. 13 (2008) 1099–1106.

[86] A.J. Wiemer, D.F. Wiemer, Prodrugs of phosphonates and phosphates: crossing the membrane barrier, in: Phosphorus Chem. I, Springer, 2014: pp. 115–160.

[87] K. M Huttunen, J. Rautio, Prodrugs-an efficient way to breach delivery and targeting barriers, Curr. Top. Med. Chem. 11 (2011) 2265–2287.

[88] L.K. Fung, W.M. Saltzman, Polymeric implants for cancer chemotherapy, Adv. Drug Deliv. Rev. 26 (1997) 209–230.

[89] L.S. Hwang, D. Krivosheya, M.A. Vogelbaum, Clinical Methods of Nervous System Drug Delivery for Tumors, in: Nerv. Syst. Drug Deliv., Elsevier, 2019: pp. 429–446.

[90] A.G. De Boer, P.J. Gaillard, Drug targeting to the brain, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 323–355.

[91] M.F. Bennewitz, W.M. Saltzman, Nanotechnology for delivery of drugs to the brain for epilepsy, Neurotherapeutics. 6 (2009) 323–336.

[92] A.M. Cook, K.D. Mieure, R.D. Owen, A.B. Pesaturo, J. Hatton, Intracerebroventricular

administration of drugs, Pharmacother. J. Hum. Pharmacol. Drug Ther. 29 (2009) 832–845.

[93] A.J. Atkinson, Intracerebroventricular drug administration, Transl. Clin. Pharmacol. 25 (2017) 117–124.

[94] W.M. Pardridge, Drug targeting to the brain, Pharm. Res. 24 (2007) 1733–1744.

[95] A. Misra, S. Ganesh, A. Shahiwala, S.P. Shah, others, Drug delivery to the central nervous system: a review, J Pharm Pharm Sci. 6 (2003) 252–273.

[96] E. Allard, C. Passirani, J.-P. Benoit, Convection-enhanced delivery of nanocarriers for the treatment of brain tumors, Biomaterials. 30 (2009) 2302–2318.

[97] W. Debinski, S.B. Tatter, Convection-enhanced delivery for the treatment of brain tumors, Expert Rev. Neurother. 9 (2009) 1519–1527.

[98] R.H. Bobo, D.W. Laske, A. Akbasak, P.F. Morrison, R.L. Dedrick, E.H. Oldfield, Convection-enhanced delivery of macromolecules in the brain, Proc. Natl. Acad. Sci. 91 (1994) 2076–2080.

[99] M.Y. Chen, A. Hoffer, P.F. Morrison, J.F. Hamilton, J. Hughes, K.S. Schlageter, J. Lee, B.R. Kelly, E.H. Oldfield, Surface properties, more than size, limiting convective distribution of virus-sized particles and viruses in the central nervous system, J. Neurosurg. 103 (2005) 311–319.

[100] C. Mamot, J.B. Nguyen, M. Pourdehnad, P. Hadaczek, R. Saito, J.R. Bringas, D.C. Drummond, K. Hong, D.B. Kirpotin, T. McKnight, others, Extensive distribution of liposomes in rodent brains and brain tumors following convection-enhanced delivery, J. Neurooncol. 68 (2004) 1–9.

[101] J.D. McAdoo, D.S. Warner, R.N. Goldberg, M.P. Vitek, R. Pearlstein, D.T. Laskowitz, Intrathecal administration of a novel apoE-derived therapeutic peptide improves outcome following perinatal hypoxic--ischemic injury, Neurosci. Lett. 381 (2005) 305–308.

[102] M.I. Papisov, V. V Belov, K.S. Gannon, Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS, Mol. Pharm. 10 (2013) 1522–1532.

[103] P. Calias, W.A. Banks, D. Begley, M. Scarpa, P. Dickson, Intrathecal delivery of protein therapeutics to the brain: a critical reassessment, Pharmacol. Ther. 144 (2014) 114–122.

[104] G. Chen, X. Zhang, F. Yang, L. Mu, Disposition of nanoparticle-based delivery system via inner ear administration, Curr. Drug Metab. 11 (2010) 886–897.

[105] M. Zhang, J. He, W. Zhang, J. Liu, Fabrication of TPGS-Stabilized Liposome-PLGA hybrid nanoparticle via a new modified Nanoprecipitation approach: in vitro and in vivo evaluation, Pharm. Res. 35 (2018) 199.

[106] X. Zhang, G. Chen, L. Wen, F. Yang, A. Shao, X. Li, W. Long, L. Mu, Novel multiple agents loaded PLGA nanoparticles for brain delivery via inner ear administration: in vitro and in vivo evaluation, Eur. J. Pharm. Sci. 48 (2013) 595–603.

[107] T. Feng, X. Huang, R. Ni, W.L.L. Suen, Y. Chau, Nanoparticles for drug delivery targeting neurodegeneration in brain and eye, in: Nanomater. Drug Deliv. Ther., Elsevier, 2019: pp. 149–183.

[108] A. Bystritsky, A.S. Korb, P.K. Douglas, M.S. Cohen, W.P. Melega, A.P. Mulgaonkar, A. DeSalles, B.-K. Min, S.-S. Yoo, A review of low-intensity focused ultrasound pulsation, Brain Stimul. 4 (2011) 125–136.

[109] B. Obermeier, R. Daneman, R.M. Ransohoff, Development, maintenance and disruption of the blood-brain barrier, Nat. Med. 19 (2013) 1584.

[110] J.M. Herndon, M.E. Tome, T.P. Davis, Development and maintenance of the blood--brain barrier, in: Prim. Cerebrovasc. Dis., Elsevier, 2017: pp. 51–56.

[111] R. Gabathuler, Approaches to transport therapeutic drugs across the blood--brain barrier to treat brain diseases, Neurobiol. Dis. 37 (2010) 48–57.

[112] N. Vykhodtseva, N. McDannold, K. Hynynen, Progress and problems in the application of focused ultrasound for blood--brain barrier disruption, Ultrasonics. 48 (2008) 279–296.

[113] M. Chaturvedi, Y. Molino, B. Sreedhar, M. Khrestchatisky, L. Kaczmarek, Tissue inhibitor of matrix metalloproteinases-1 loaded poly (lactic-co-glycolic acid) nanoparticles for delivery across the blood--brain barrier, Int. J. Nanomedicine. 9 (2014) 575.

[114] A. Burgess, K. Hynynen, Noninvasive and targeted drug delivery to the brain using focused ultrasound, ACS Chem. Neurosci. 4 (2013) 519–526.

[115] H.-L. Liu, M.-Y. Hua, P.-Y. Chen, P.-C. Chu, C.-H. Pan, H.-W. Yang, C.-Y. Huang, J.-J. Wang, T.-C. Yen, K.-C. Wei, Blood-brain barrier disruption with focused ultrasound enhances delivery of chemotherapeutic drugs for glioblastoma treatment, Radiology. 255 (2010) 415–425.

[116] S.R. Sirsi, M.A. Borden, State-of-the-art materials for ultrasound-triggered drug delivery, Adv. Drug Deliv. Rev. 72 (2014) 3–14.

[117] H. Chen, E.E. Konofagou, The size of blood--brain barrier opening induced by focused ultrasound is dictated by the acoustic pressure, J. Cereb. Blood Flow Metab. 34 (2014) 1197–1204.

[118] T.D. Azad, J. Pan, I.D. Connolly, A. Remington, C.M. Wilson, G.A. Grant, Therapeutic strategies to improve drug delivery across the blood-brain barrier, Neurosurg. Focus. 38 (2015) E9.

[119] Y. Su, P.J. Sinko, Drug delivery across the blood--brain barrier: why is it difficult? How to measure and improve it?, Expert Opin. Drug Deliv. 3 (2006) 419–435.

[120] A. Tsuji, Small molecular drug transfer across the blood-brain barrier via carrier-mediated transport systems, NeuroRx. 2 (2005) 54–62.

[121] Q.R. Smith, Carrier-mediated transport to enhance drug delivery to brain, in: Int. Congr. Ser., 2005: pp. 63–74.

[122] M.A. Rauf, F.U. Rehman, M. Zheng, B. Shi, The Strategies of Nanomaterials for Traversing Blood-Brain Barrier, in: Nanomedicine Brain Dis., Springer, 2019: pp. 29–57.

[123] V.M. Pulgar, Transcytosis to cross the blood brain barrier, new advancements and challenges, Front. Neurosci. 12 (2019) 1019.

[124] E. Joseph, R.N. Saha, Advances in brain targeted drug delivery: nanoparticulate systems, J. PharmaSciTech. 3 (2013) 1–8.

[125] A. Khosa, R.N. Saha, G. Singhvi, Drug delivery to the brain, in: Nanomater. Drug Deliv. Ther., Elsevier, 2019: pp. 461–514.

[126] A. Khosa, G. Singhvi, R.N. Saha, G. Gupta, Drug delivery to the CNS., Panminerva Med. 60 (2018) 226.

[127] W.M. Pardridge, Drug transport across the blood--brain barrier, J. Cereb. Blood Flow Metab. 32 (2012) 1959–1972.

[128] C.L. Graff, G.M. Pollack, Nasal drug administration: potential for targeted central nervous system delivery, J. Pharm. Sci. 94 (2005) 1187–1195.

[129] P. Merkus, H.-J. Guchelaar, D.A. Bosch, F.W.H.M. Merkus, Direct access of drugs to the human brain after intranasal drug administration?, Neurology. 60 (2003) 1669–1671.

[130] S. Sood, K. Jain, K. Gowthamarajan, Intranasal therapeutic strategies for management of Alzheimer's disease, J. Drug Target. 22 (2014) 279–294.

[131] G. Karthivashan, P. Ganesan, S.-Y. Park, J.-S. Kim, D.-K. Choi, Therapeutic strategies and

nano-drug delivery applications in management of ageing Alzheimer's disease, Drug Deliv. 25 (2018) 307–320.

[132] H. Derakhshankhah, S. Sajadimajd, S. Jafari, Z. Izadi, S. Sarvari, M. Sharifi, M. Falahati, F. Moakedi, W.C.A. Muganda, M. Müller, others, Novel therapeutic strategies for Alzheimer's disease: Implications from cell-based therapy and Nanotherapy, Nanomedicine Nanotechnology, Biol. Med. (2020) 102149.

[133] J.S. D'Arrigo, Nanotherapy for Alzheimer's disease and vascular dementia: Targeting senile endothelium, Adv. Colloid Interface Sci. 251 (2018) 44–54.

[134] Y. Luo, H. Yang, Y.-F. Zhou, B. Hu, Dual and multi-targeted nanoparticles for site-specific brain drug delivery, J. Control. Release. 317 (2020) 195–215.

[135] M. Huang, X. Gu, X. Gao, Nanotherapeutic strategies for the treatment of neurodegenerative diseases, in: Brain Target. Drug Deliv. Syst., Elsevier, 2019: pp. 321–356.

[136] H.L. Wong, X.Y. Wu, R. Bendayan, Nanotechnological advances for the delivery of CNS therapeutics, Adv. Drug Deliv. Rev. 64 (2012) 686–700.

[137] M.L. Hans, A.M. Lowman, Biodegradable nanoparticles for drug delivery and targeting, Curr. Opin. Solid State Mater. Sci. 6 (2002) 319–327.

[138] M. Dash, F. Chiellini, R.M. Ottenbrite, E. Chiellini, Chitosan—A versatile semi-synthetic polymer in biomedical applications, Prog. Polym. Sci. 36 (2011) 981–1014.

[139] J. Kreuter, Drug delivery to the central nervous system by polymeric nanoparticles: what do we know?, Adv. Drug Deliv. Rev. 71 (2014) 2–14.

[140] S. Wohlfart, S. Gelperina, J. Kreuter, Transport of drugs across the blood--brain barrier by nanoparticles, J. Control. Release. 161 (2012) 264–273.

[141] D. Bennet, S. Kim, Polymer nanoparticles for smart drug delivery, chapter, 2014.

[142] C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, L. Bernardino, Nanoparticle-mediated brain drug delivery: overcoming blood--brain barrier to treat neurodegenerative diseases, J. Control. Release. 235 (2016) 34–47.

[143] E. Joseph, G. Singhvi, S. Banerjee, Nanoparticulate Drug-Delivery Systems for Brain Targeting, Nanocarriers Brain Target. Princ. Appl. (2019) 207.

[144] Y. Zhu, C. Liu, Z. Pang, Dendrimer-Based Drug Delivery Systems for Brain Targeting, Biomolecules. 9 (2019) 790.

[145] M. Snehalatha, K. Venugopal, R.N. Saha, A.K. Babbar, R.K. Sharma, Etoposide loaded PLGA and PCL nanoparticles II: biodistribution and pharmacokinetics after radiolabeling with Tc-99m, Drug Deliv. 15 (2008) 277–287.

[146] E. Joseph, S. Reddi, V. Rinwa, G. Balwani, R. Saha, DoE based Olanzapine loaded poly-caprolactone nanoparticles decreases extrapyramidal effects in rodent model, Int. J. Pharm. 541 (2018) 198–205.

[147] A. Ambruosi, A.S. Khalansky, H. Yamamoto, S.E. Gelperina, D.J. Begley, J. Kreuter, Biodistribution of polysorbate 80-coated doxorubicin-loaded [14C]-poly (butyl cyanoacrylate) nanoparticles after intravenous administration to glioblastoma-bearing rats, J. Drug Target. 14 (2006) 97–105.

[148] J. Chang, Y. Jallouli, M. Kroubi, X. Yuan, W. Feng, C. Kang, P. Pu, D. Betbeder, Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood--brain barrier, Int. J. Pharm. 379 (2009) 285–292.

[149] B. Petri, A. Bootz, A. Khalansky, T. Hekmatara, R. Müller, R. Uhl, J. Kreuter, S. Gelperina, Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly (butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants, J. Control. Release. 117 (2007) 51–58.

[150] M. Nahar, T. Dutta, S. Murugesan, A. Asthana, D. Mishra, V. Rajkumar, M. Tare, S. Saraf, N.K. Jain, Functional polymeric nanoparticles: an efficient and promising tool for active delivery of bioactives, Crit. Rev. Ther. Drug Carr. Syst. 23 (2006) 259–318.

[151] S. Bhatia, Nanoparticles types, classification, characterization, fabrication methods and drug delivery applications, in: Nat. Polym. Drug Deliv. Syst., Springer, 2016: pp. 33–93.

[152] R.A. Petros, J.M. DeSimone, Strategies in the design of nanoparticles for therapeutic applications, Nat. Rev. Drug Discov. 9 (2010) 615–627.

[153] M. Munjal, Nanoparticles-Preparation, technology, evaluation and used in targeted drug delivery system, Technology. 14 (2018) 15.

[154] V. Wagner, A. Dullaart, A.-K. Bock, A. Zweck, The emerging nanomedicine landscape, Nat. Biotechnol. 24 (2006) 1211–1217.

[155] J. Park, P.M. Fong, J. Lu, K.S. Russell, C.J. Booth, W.M. Saltzman, T.M. Fahmy, PEGylated PLGA nanoparticles for the improved delivery of doxorubicin, Nanomedicine Nanotechnology, Biol. Med. 5 (2009) 410–418.

[156] G. Morral-Ruíz, C. Solans, M.L. García, M.J. García-Celma, Formation of pegylated polyurethane and lysine-coated polyurea nanoparticles obtained from O/W nano-emulsions, Langmuir. 28 (2012) 6256–6264.

[157] H. Li, L. Yan, E.K.Y. Tang, Z. Zhang, W. Chen, G. Liu, J. Mo, Synthesis of TPGS/curcumin nanoparticles by thin-film hydration and evaluation of their anti-colon cancer efficacy in vitro and in vivo, Front. Pharmacol. 10 (2019).

[158] K. Hadinoto, A. Sundaresan, W.S. Cheow, Lipid--polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review, Eur. J. Pharm. Biopharm. 85 (2013) 427–443.

[159] F. Danhier, N. Lecouturier, B. Vroman, C. Jérôme, J. Marchand-Brynaert, O. Feron, V. Préat, Paclitaxel-loaded PEGylated PLGA-based nanoparticles: in vitro and in vivo evaluation, J. Control. Release. 133 (2009) 11–17.

[160] N. Bertrand, P. Grenier, M. Mahmoudi, E.M. Lima, E.A. Appel, F. Dormont, J.-M. Lim, R. Karnik, R. Langer, O.C. Farokhzad, Mechanistic understanding of in vivo protein corona formation on polymeric nanoparticles and impact on pharmacokinetics, Nat. Commun. 8 (2017) 1–8.

[161] M.N.V.R. Kumar, U. Bakowsky, C.M. Lehr, Preparation and characterization of cationic PLGA nanospheres as DNA carriers, Biomaterials. 25 (2004) 1771–1777.

[162] E. Allémann, R. Gurny, E. Doelker, Preparation of aqueous polymeric nanodispersions by a reversible salting-out process: influence of process parameters on particle size, Int. J. Pharm. 87 (1992) 247–253.

[163] C.J. Coester, K. Langer, H. Von Briesen, J. Kreuter, Gelatin nanoparticles by two step desolvation a new preparation method, surface modifications and cell uptake, J. Microencapsul. 17 (2000) 187–193.

[164] C. Weber, C. Coester, J. Kreuter, K. Langer, Desolvation process and surface characterisation of protein nanoparticles, Int. J. Pharm. 194 (2000) 91–102.

[165] P. Chattopadhyay, B.Y. Shekunov, D. Yim, D. Cipolla, B. Boyd, S. Farr, Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system, Adv. Drug Deliv. Rev. 59 (2007) 444–453.

[166] Y.J. Chen, R.X. Jin, Y.Q. Zhou, J. Zeng, H. Zhang, Q.R. Feng, Preparation of solid lipid nanoparticles loaded with Xionggui powder-supercritical carbon dioxide fluid extraction

and their evaluation in vitro release, Zhongguo Zhong Yao Za Zhi= Zhongguo Zhongyao Zazhi= China J. Chinese Mater. Medica. 31 (2006) 376–379.

[167] A.J. Thote, R.B. Gupta, Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release, Nanomedicine Nanotechnology, Biol. Med. 1 (2005) 85–90.

[168] E. Locatelli, M.C. Franchini, Biodegradable PLGA-b-PEG polymeric nanoparticles: synthesis, properties, and nanomedical applications as drug delivery system, J. Nanoparticle Res. 14 (2012) 1316.

[169] A. Kumari, S.K. Yadav, S.C. Yadav, Biodegradable polymeric nanoparticles based drug delivery systems, Colloids Surfaces B Biointerfaces. 75 (2010) 1–18.

[170] A.A. Galyean, W.N. Vreeland, J.J. Filliben, R.D. Holbrook, D.C. Ripple, H.S. Weinberg, Using light scattering to evaluate the separation of polydisperse nanoparticles, Anal. Chim. Acta. 886 (2015) 207–213.

[171] T. Yamaguchi, T. Mori, K. Aoki, R. Oda, M. Yasutake, A. Nakamura, K. Takahashi, T. Shigehuzi, H. Kato, Characterization of the volume-based or number-based size distribution for silica nanoparticles using a unique combination of online dynamic light scattering having a uni-tau multi-bit correlator and high resolution centrifugal field-flow fractionation se, Anal. Sci. (2020) 19P351.

[172] C.I.C. Crucho, M.T. Barros, Polymeric nanoparticles: A study on the preparation variables and characterization methods, Mater. Sci. Eng. C. 80 (2017) 771–784.

[173] S. Honary, F. Zahir, Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 1), Trop. J. Pharm. Res. 12 (2013) 255–264.

[174] B.L. Banik, P. Fattahi, J.L. Brown, Polymeric nanoparticles: the future of nanomedicine, Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology. 8 (2016) 271–299.

[175] S.M. Moghimi, A.C. Hunter, T.L. Andresen, Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective, Annu. Rev. Pharmacol. Toxicol. 52 (2012) 481–503.

[176] N.P. Truong, M.R. Whittaker, C.W. Mak, T.P. Davis, The importance of nanoparticle shape in cancer drug delivery, Expert Opin. Drug Deliv. 12 (2015) 129–142.

[177] M. Ballauff, Y. Lu, "Smart" nanoparticles: preparation, characterization and applications, Polymer (Guildf). 48 (2007) 1815–1823.

[178] J.B. Hall, M.A. Dobrovolskaia, A.K. Patri, S.E. McNeil, Characterization of nanoparticles for therapeutics, (2007).

[179] S. D'Souza, A review of in vitro drug release test methods for nano-sized dosage forms, Adv. Pharm. 2014 (2014).

[180] P. Fonte, S. Reis, B. Sarmento, Facts and evidences on the lyophilization of polymeric nanoparticles for drug delivery, J. Control. Release. 225 (2016) 75–86.

[181] L. Niu, J. Panyam, Freeze concentration-induced PLGA and polystyrene nanoparticle aggregation: Imaging and rational design of lyoprotection, J. Control. Release. 248 (2017) 125–132.

[182] M.E. Ali, A. Lamprecht, Spray freeze drying as an alternative technique for lyophilization of polymeric and lipid-based nanoparticles, Int. J. Pharm. 516 (2017) 170–177.

[183] P. Zambrano, M. Suwalsky, M. Jemiola-Rzeminska, K. Strzalka, B. Sepúlveda, M.J. Gallardo, L.F. Aguilare, The acetylcholinesterase (AChE) inhibitor and anti-Alzheimer drug donepezil interacts with human erythrocytes, Biochim. Biophys. Acta (BBA)-Biomembranes. 1861 (2019) 1078–1085.

[184] M. Dooley, H.M. Lamb, Donepezil, Drugs Aging. 16 (2000) 199–226.

[185] E.Y. Shintani, K.M. Uchida, Donepezil: an anticholinesterase inhibitor for Alzheimer's disease, Am. J. Heal. Pharm. 54 (1997) 2805–2810.

[186] C. Christodoulou, P. Melville, W.F. Scherl, W.S. MacAllister, L.E. Elkins, L.B. Krupp, Effects of donepezil on memory and cognition in multiple sclerosis, J. Neurol. Sci. 245 (2006) 127–136.

[187] W. Guo, P. Quan, L. Fang, D. Cun, M. Yang, Sustained release donepezil loaded PLGA microspheres for injection: Preparation, in vitro and in vivo study, Asian J. Pharm. Sci. 10 (2015) 405–414.

[188] P. Zhang, L. Chen, W. Gu, Z. Xu, Y. Gao, Y. Li, In vitro and in vivo evaluation of donepezil-sustained release microparticles for the treatment of Alzheimer's disease, Biomaterials. 28 (2007) 1882–1888.

[189] I. Baysal, G. Ucar, M. Gultekinoglu, K. Ulubayram, S. Yabanoglu-Ciftci, Donepezil loaded PLGA-b-PEG nanoparticles: their ability to induce destabilization of amyloid fibrils and to cross blood brain barrier in vitro, J. Neural Transm. 124 (2017) 33–45.