VANCOMYCIN AND ITS BETA-CYCLODEXTRIN COMPLEX: PHYSICOCHEMICAL CHARACTERIZATION AND ITS CYTOTOXICITY EVALUATION ON HUMAN GLIAL CELL LINE

by

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ZARIF MOHAMED SOFIAN
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LIST OF ABBREVIATIONS

µl	Microliter
µg	Microgram
g	Gram
mg	Milligram
m	Meter
min	Minute
ml	Milliliter
cm	Centimeter
mm	Millimeter
µm	Micrometer
S.D	Standard deviation
M	Molar
mM	Millimolar
UV/Vis	Ultraviolet/visible
nm	Nanometer
kV	Kilovolt
mA	Milliampere
i.v	Intravenous

i.p	Intraparetional
IC_{50}	50 % inhibitory culture medium concentration
LD_{50}	50% lethal dose
s.c	Subcutaneous
rpm	Revolutions per minute
FTIR-ATR	Fourier Transform Infrared Spectrascopy-Attenuated Total Reflactance
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
SEM	Scanning Elelctron Microscopy
TGA	Thermogravimetric Analysis
XRD	X-ray diffractrometry
HPLC	High Performance Liquid Chromatography

LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
θ	Theta
KCl	Potassium chloride
v/v	Volume/volume
w/w	Weight/weight
$MgSO_4$	Magnesium sulphate
NaCl	Sodium chloride
NaH_2PO_4	Monosodium phosphate
$NaHCO_3$	Sodium bicarbonate
O_2	Oxygen

VANCOMYCIN DAN VANCOMYCIN/BETA -SIKLODEKSTRIN KOMPLEKS: PENCIRIAN FISIKOKIMIKAL DAN PENILAIAN SITOTOKSISITI TERHADAP SEL GLIAL MANUSIA

ABSTRAK

Dalam kajian ini, kompleks vancomycin, sejenis antibiotik glikopeptida spektrum luas bersama β-CD pada nisbah molar yang sama dihasilkan dan dinilai bagi mengkaji tahap kesesuaiannya dalam pembentukan jenis antibiotik baru yang bersifat *prolonge-released*. Kompleks β-CD/vancomycin disediakan melalui dua kaedah iaitu penyejuk-keringan dan pengulian. Pembentukan kompleks disahkan melalui Analisis Difraktometri X-ray (XRD), Analisis Termal (TGA), Imbasan Mikroskop Elektron (SEM) dan juga Spektroskopi Transformasi Inframerah (FT-IR). Kesemua data yang direkodkan menyokong antara satu dengan yang lain membuktikan pembentukan kompleks. Difraktogram XRD yang diperoleh mempamerkan penurunan ketara sifat kristaliniti bagi setiap kompleks menunjukan kehadiran vancomycin di dalam molekul kompleks. Perubahan ketara morfologi permukaan dapat dilihat antara kompleks dengan β-CD dan vancomycin pada imej-imej SEM yang diambil. Perbezaan profil termal yang diperoleh antara kompleks dan molekul pemula pula menandakan kewujudan interaksi antara β-CD dan vancomycin. Selain itu, variasi pada spektra IR termasuk pengembangan, anjakan dan pengurangan keamatan relatif jalur penyerapan dapat dilihat pada spektra kedua-dua kompleks. Kehilangan jalur penyerapan molekul tetamu dapat dijadikan sebagai pengesahan pembetukan kompleks β-CD/vancomycin. Walaubagaimanapun, daripada data yang diperoleh, kompleks pengulian terbukti lebih efisien dalam

memanjangkan tempoh penghantaran vancomycin berbanding kompleks penyejuk-kering. Kesan sitotoksik vancomycin dan kompleksnya turut dikaji ke atas sel glial manusia dengan menggunakan kaedah MTS. Kesan kebergantungan terhadap dos secara linear diikuti dengan kesan secara *hermetic-like biphasic* dapat dilihat selepas tempoh inkubasi selama 72 jam. Peningkatan proliferasi sel secara signifikan (p<0.001) dapat dikesan pada kepekatan yang rendah : $\leq$ 18.75 μg/ml untuk sel yang didedahkan kepada β-CD dan juga kompleksnya sementara $\leq$ 9.38 μg/ml untuk sel yang terdedah kepada vancomycin. Namun, penurunan proliferasi sel secara signifikan (p<0.001) pula direkodkan pada kepekatan yang tinggi $\geq$ 150 μg/ml pada semua kumpulan sel yang dikaji. 50 % perencatan (IC_{50}) *in vitro* dicapai pada kepekatan 115.95 μg/ml (untuk β-CD), 116.48 μg/ml (untuk vancomycin) and 115.44 μg/ml (untuk kompleks β-CD/vancomycin). Secara keseluruhannya, berdasarkan keupayaan β-CD untuk secara signifikan (p<0.001) memanjangkan tempoh penghantaran vancomycin selain nilai IC_{50} yang tinggi secara relatif yang diperoleh di dalam kajian ini menjadikan hasil kompleks yang dikaji sebagai produk yang berpotensi untuk digunakan sebagai *site-specific administration* bagi rawatan jangkitan terpencil pada sistem saraf pusat (CNS) oleh bakteria gram-positif pada masa hadapan.

VANCOMYCIN AND ITS BETA-CYCLODEXTRIN COMPLEX: PHYSICOCHEMICAL CHARACTERIZATION AND ITS CYTOTOXICITY EVALUATION ON HUMAN GLIAL CELL LINE

ABSTRACT

In this present study, the equimolar complexes of vancomycin, a broad-spectrum glycopeptide antibiotic with β-CD were prepared to evaluate their suitability for the development of a prolonged-release form of the antibiotic. β-CD/vancomycin complexes were prepared accordingly via freeze-drying and kneading techniques. The formation of the complexes was confirmed through X-ray Diffractometry (XRD), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and Fourier Transfrom Infrared Spectroscopy (FT-IR). The results obtained were all in good agreement suggesting the successive formation of the aforementioned binary systems. The XRD diffractograms of the complexes showed marked reduction in crystalinity which indicates the presence of vancomycin in the complexes. In the SEM microimages captured, significant difference in surface morphology was observed between the complexes and the starting materials. Distinct thermal profiles between the complexes and the pure molecules recorded signify the existence of possible reciprocal interactions between the two molecules. Spectral variations included broadening, shifting and reduction in relative intensities of the absorption bands were also observed in the IR spectra of both complexes. Apart from that, the disappearance of guest signals in the complexes spectra can be considered as a confirmation for the formation of β-CD /vancomycin complexes. However, from the experiments it was found that the kneaded complex was more efficient in prolonging the

delivery of vancomycin compared to the freeze-dried complex. The possible cytotoxic effects of vancomycin and its complex with β-CD on human glial cell were assayed by means of MTS assay. A linear dose-dependency cytotoxicity followed by hermetic-like biphasic dose-dependence was observed after incubation period of 72 hours. Significant (p<0.001) increase of cell proliferation was observed at lower concentrations: $\leq$ 18.75 μg/ml for cells treated with β-CD and their complex while $\leq$ 9.38 μg/ml for cells treated with vancomycin. In contrary, regardless of the treatments given, significant (p<0.001) reduce in cell survival was found at higher concentrations $\geq$ 150 μg/ml. In particular, 50 % inhibitory (IC_{50}) *in vitro* were achieved at the concentrations of 115.95 μg/ml (for β-CD), 116.48 μg/ml (for vancomycin) and 115.44 μg/ml (for β-CD/vancomycin complex). All in all, due to the ability of β-CD to significantly (p<0.001) control the delivery of vancomycin over a prolonged period of time provided with relatively high IC_{50} value calculated from this study renders this complex a promising product to be used in the future for site-specific administration of vancomycin for the treatment of localized gram-positive bacterial infections in CNS.

CHAPTER 1
INTRODUCTION

Medicinal chemistry is a branch of scientific disciplines which provides a common ground between chemistry, medical and pharmaceutical fields. It is an interdisciplinary field which involves designing, formulating, synthesizing as well as isolating of pharmaceutical drugs neither from plants nor synthetically produced for potential therapeutic use. Over the past few decades, technology advancement in the related areas has brought researches closer to meeting the goals of maximum efficacy with minimal toxicity and aggravation.

For years, the development of a controlled and targeted drug delivery system has become of great interest to researches as the system will enable the delivery of the drugs at the desirable therapeutic level directly to the targeted areas for prolonged periods of time while avoiding high systemic doses. The used of polymeric materials including polymer micelles and polymeric microspheres likewise hydrogel-like materials has been reported to be effective in developing the system (Blanchemain *et al*., 2008; Figueiras *et al*., 2007; Silva-Jùnior *et al*., 2008; Sohajda *et al*., 2009; Veyries *et al*., 1999).

1.1 Cyclodextrins (CDs)

Generally, degradation of starch via enzymatic process will result in the formation of dextrins; a long series of linear or branched chain of malto-oligomers. Dextrins are heterogenous, amorphous and hygroscopic substances. They are produced in large quantities for numerous industry purposes. However, when the starch is degraded by the cyclodextrin-transglycosylase enzyme (CGT-enzyme), the primary product of the chain splitting undergoes an intramolecular reaction to form the α-1,4-linked cyclic dextrins or also known as cyclodextrins (CDs) in the absence of water molecule (Szetjli, 1998).

In 1891, the first publication on CDs was reported by Villiers, a French author following his observation on some unidentified crystalline substances during the fermentation process of starch (Szetjli, 1998). According to Szetjli (2004), the long history of the revolutionary process of CDs can be divided into three major stages which started with the "*discovery*" period from 1891 to the middle of 1930s, followed with the "*exploratory*" period from 1936 until 1970 and ended up with the "*utilization*" period from 1970 onward.

1.1.1 Properties and classification of CDs

CDs are characterized as crystalline, homogenous, non-hygroscopic substances. The structure of CD is often described as a truncated cone. The exterior surface is hydrophilic while the interior cavity is relatively hydrophobic (Wang *et al*., 2007a). They are alpha-cyclodextrin (α-CD, also known as Schardinger's α-dextrin, cyclomaltohexaose, cyclohexaglucan, cyclohexaamylose, ACD, C6A), beta-cyclodextrin (β-CD, also known as Schardinger's β-dextrin, cyclomaltoheptose, cycloheptaglucan, cycloheptaamylose, BCD, C7A) and gamma-cyclodextrin (γ-CD, also known as Schardinger's β-dextrin, cyclomaltooctatose, cyclooctaglucan, cyclooctaamylose, GCD, C8A) which are formed by six, seven and eight glucopyranose units, respectively (Szetjli, 2004).

Theoretically, larger CDs with ten or more ring members could be prepared. Nonetheless, due to their expected high solubility and weak complex forming ability, the challenge seems to be unrewarding. On the other hand, CDs with fewer than six glucopyranose units cannot be formed for steric reasons. All hydroxyl groups are located on the edges of the cavity where the secondary hydroxyl groups are placed on the edge with wider diameter while the primary hydroxyl groups accommodated the other edge with narrower diameter. The free rotation of the primary hydroxyl groups reduces the effective diameter of the cavity (Szetjli, 2004). Figure 1.1 exhibits the chemical structure of the three common types of native or parent CDs.

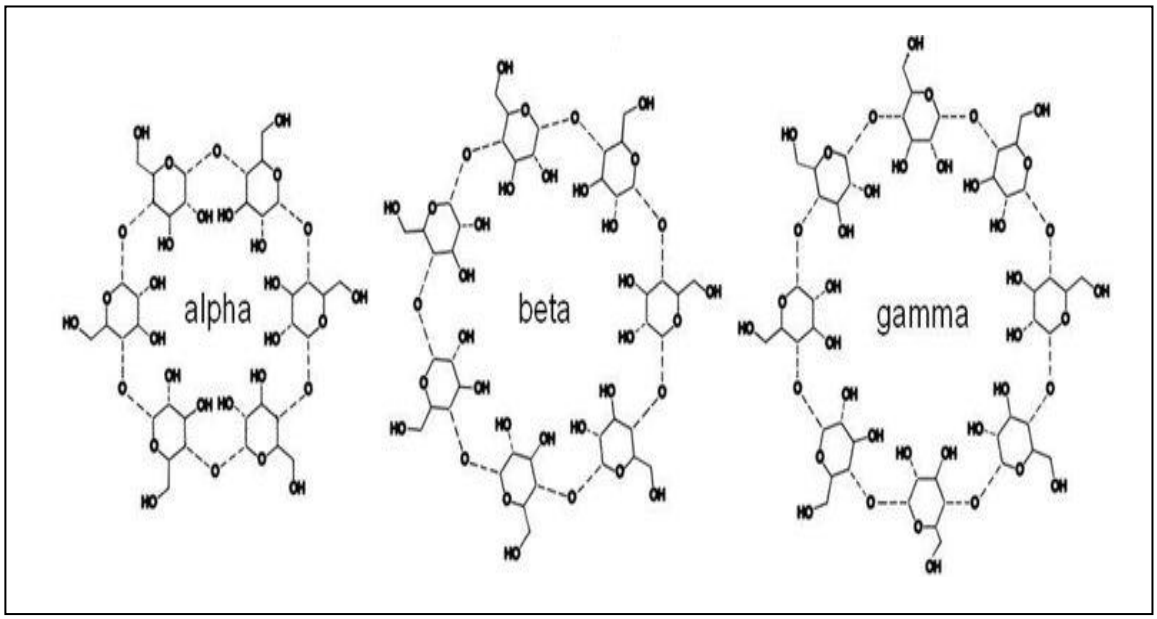

Figure 1.1 Chemical structure of native cyclodextrins; alpha-cyclodextrin (α-CD), beta-cyclodextrin (β-CD) and gamma-cyclodextrin γ-CD (Szetjli, 2004)

The hydrogen bonds and also the glucosidic-oxygen bridges (Figure 1.2) linked the glucopyranose units together and help to keep the cavity in shape.

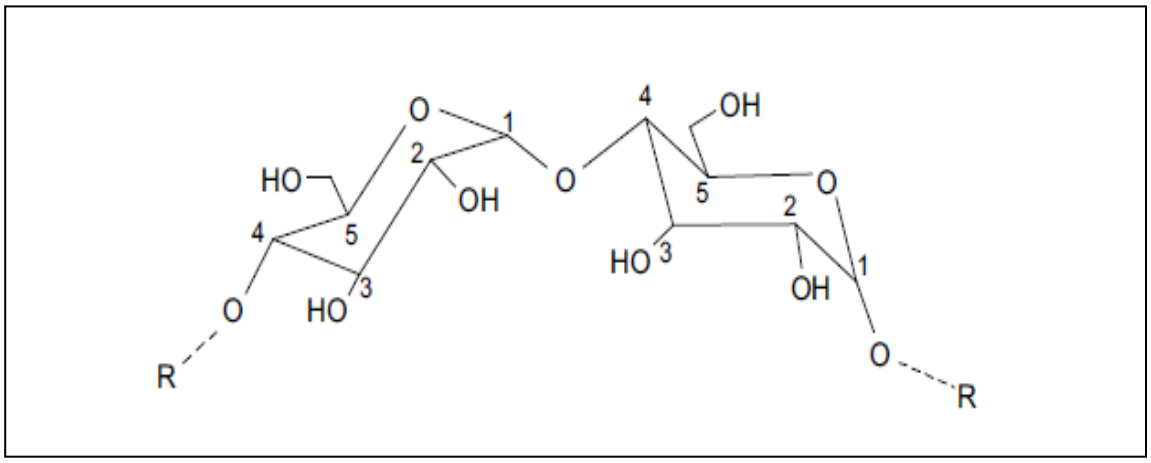

Figure 1.2 The glucosidic oxygen bridge α-(1, 4) between two molecules of glucopyranose (Astray *et al*., 2009)

The nonbonding electron pairs of the glucosidic-oxygen bridges are directed towards the inner part of the cavity, producing a high electron density environment and lending some Lewis-base characters to it. The C-2-OH group of one glucopyranose unit can be linked with the C-3-OH group of the adjacent glucopyranose unit via hydrogen bond (Szetjli, 2004). Figure 1.3 shows the approximate geometry of α-CD, β-CD and γ-CD.

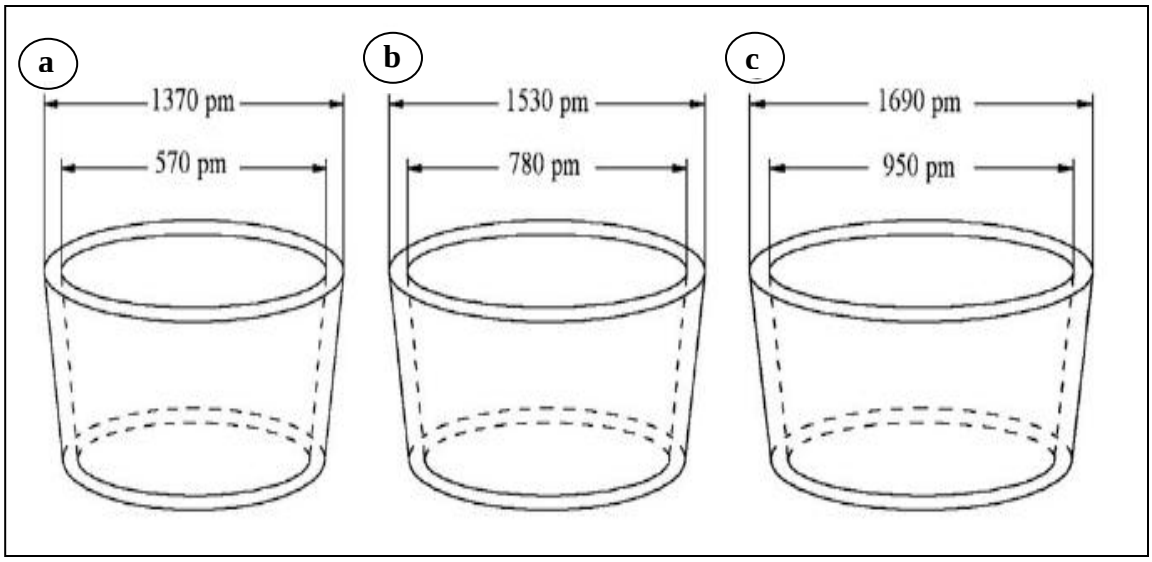

Figure 1.3 Approximate geometry of a) α-CD, b) β-CD and c) γ-CD (Astray *et al*., 2009)

A complete secondary belt is formed by these hydrogen bonds in the β-CD molecule contributing to a rigid structure of the β-CD. Thus, the formation of these intramolecular hydrogen bondings in β-CD explained its lowest solubility of all CDs. In contrast, because of one glucopyranose unit is in a distorted position in the α-CD molecule, a complete secondary belt cannot be formed. As a consequence, only four out of six possible hydrogen bonds can be established. The γ-CD is a non-coplanar, more flexible structure and therefore it is the most soluble CD (Astray *et al*., 2009). Amongst

the common types of CDs, β-CDs appeared as the most accessible, the lowest-priced and generally the most useful of all (Martin Del Valle, 2004). Apart from that, reports by other authors have shown the ability of β-CDs to improve the physicochemical properties of the entrapped guest molecule including to sustained-release of water-soluble drugs (Hirayama and Uekama,1999; Keipert *et al*., 1996). Table 1.1 summarizes the characteristics of α-CD, β-CD and also γ-CD.

Table 1.1 Characteristics of α-CD, β-CD and also γ-CD (Frömming and Szetjli, 1994)

	α	**β**	**γ**
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Solubility in water g/100 ml	14.5	1.85	23.2
Cavity diameter, Å	4.7 - 5.3	6.0 - 6.5	7.5 - 8.3
Height of torus, Å	7.9 $\pm$ 0.1	7.9 $\pm$ 0.1	7.9 $\pm$ 0.1
Approx. volume of cavity, $Å^3$	174	262	427
Approx. cavity volume in 1 mol CD (ml) in 1 g CD (ml)	104	1257	427
Crystal forms (form water)	Hexagonal plates	Monoclinic parallelelograms	Quadratic prisms
Crystal water weight, %	10.2	13.2-14.5	8.13-17.7
Diffusion constant at 40 ^oC	3.443	3.223	3.000
p*K* (by potentiometry) at 25 ^oC	12.332	12.202	12.081
Partial molar volumes in solution (ml mol^{-1})	611.48	703.8	801.2

1.1.2 CD inclusion complexes and host-guest concepts

The most unique characteristic of CDs is their capability to form inclusion complex with a broad range of molecules ranging from solid, liquid and gases. Inclusion in CDs exerts profound effects on the physicochemical properties of the entrapped guest molecules as they are temporarily caged within the host cavity. The potential guest molecules including straight or branched aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gaseous and polar compounds such as halogens, oxyacids and amines (Schmid, 1989). This inclusion phenomena results in solubility enhancement of highly insoluble guests, stabilization of labile guests against degradative effects of oxidation, visible or UV light and heat, control of volatility and sublimation, physical isolation of incompatible compounds, chromatographic separations, taste modification by masking off the flavour, unpleasant odours and controlled release of drugs and flavours (Szetjli, 1998; Hollands *et al*., 1999; Bharwaj *et al*., 2000).

The term *Einschlussverbindung* (or inclusion compound) was introduced by Sclenk in 1950 as cited by Frömming and Szetjli (1994). There are some other names used in the literature, such as adduct, clathrate, molecular compound, cryptate and complex. Inclusion complexes are entities built up from two or more molecules, in which the guest molecules are entrapped (totally or partially) only by physical forces without covalent bonding (Astray *et al*., 2009). The main driving force of the complex formation is the release of enthalpy-rich water molecules from the CD cavity.

Water molecules are displaced with the more hydrophobic guest molecules present in the solution to attain an apolar-apolar interaction and reduce of CD ring strain resulting in a more stable energy state (Szetjli, 1998). The interactions between host (H) and guest (G) normally are based on simultaneous non-covalent interactions between single binding sites, A (acceptor) and D (donor), etc. No covalent bonds are formed nor broken during the formation of the inclusion complex. Figure 1.4 illustrates an example of the CD structure and inclusion complex formation through host-guest interactions (Frömming and Szetjli, 1994).

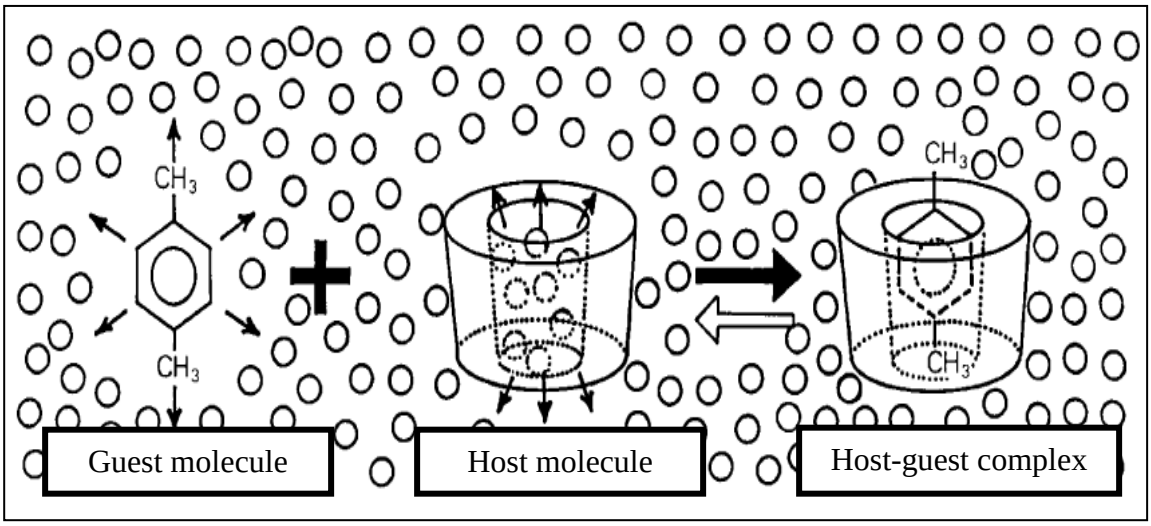

Figure 1.4 Cyclodextrin structure and host-guest complex formation (Frömming and Szetjli, 1994)

1.1.3 Toxicological aspects of beta-cyclodextrin (β-CD)

The novel attempt to evaluate the toxic effects of β-CD was initiated by French and Thomas back in 1957. The experiments were carried out by feeding the rats with highly purified β-CD in which β-CD was partly included in the carbohydrate diet of the animals. The results were rather discouraging as it was reported in the monograph that the animals refused to eat the test diet except in very small quantities and were found dead in the following week. However, cause of death was not revealed in the postmortem reports (Frömming and Szetjli, 1994). Since then, further studies have been carried out and none of them came out with similar results as reported by French and Thomas.

When administrated intravenously (i.v) to rats, the median lethal dose (LD_{50}) of β-CD was 788 mg/kg. Sign of nephrotoxicity including severe nephrosis but no deaths was observed in rats given with 1, 2, 4 or daily injection of β-CD at 450 mg/kg. Daily subcutenous (s.c) injection of 200 mg β-CD (equivalent to 840 mg/kg) over a period of seven days of treatment resulted in a strong swelling of the kidneys as well necrosis in the subcutenous connective tissues. The LD_{50} via intraparetonial (i.p) route of β-CD were 373 and 356 mg/kg in male and female rats, respectively. As in mice, the values showed small deviation as the values calculated were 372 mg/kg for male and 331 mg/kg in female. In addition, when administrated orally, the LD_{50} were as follows: 12.5 g/kg in mice, 18.8 g/kg in rats and > 5g/kg in dogs (Bellringer *et al.*, 1994).

Moreover, the cytotoxicity studies of β-CD have been reported before and the results were found to be varied depending on the types of cell studied (Hipler *et al*., 2007; Piel *et al*., 2004; Tilloy *et al*., 2006)

1.2 Vancomycin

1.2.1 Structure

Vancomycin was first isolated from *Amycolatopsis orientalis* which was found in Borneo Island in 1956. However due to its impurities, only 30 years later vancomycin starting to gain increasingly popularity in medical application and serves as antibiotic of last resort in the treatment of gram-positive bacterial infections (Veyries *et al*., 1999; Loll and Axelsen, 2000). According to commonly accepted classification which is based on type of residues at position I and III, glycopeptide antiobiotics can be classified into five different classes in which class I-IV are proven to have antimicrobial activities (Loll and Axelsen, 2000).

Generally, all glycopeptide antibiotics share a common heptapeptide core of seven amino acid residues with three characteristic rings system in the aromatic side chains of residues 2 and 4, 4 and 6, and 5 and 7 which are covalently joined in addition to diverse composition of sugar moieties and attachment sites of chlorine. Vancomycin together with balhimycin, eremomycin, chloroeremomycin and LY 33328 are in class I glycopeptide antibiotics. In comparison to the other subclasses of glycopeptide antibiotics, class I glycopeptides bearing aliphatic amino acids at position I and III. Class II glycopeptides (e.g. actinoidin) possess aromatic residues while class III (e.g. ristocetin) and IV (e.g. teicoplanin) share similar general architecture, but however, class IV in addition have a fatty acid attached to the sugar residue. On the other hand, class V glycopeptides (e.g. complestatin) only have two instead of three ring systems (Loll and

Axelsen, 2000). Figure 1.5 shows the chemical structure of class I glycopeptide antibiotics.

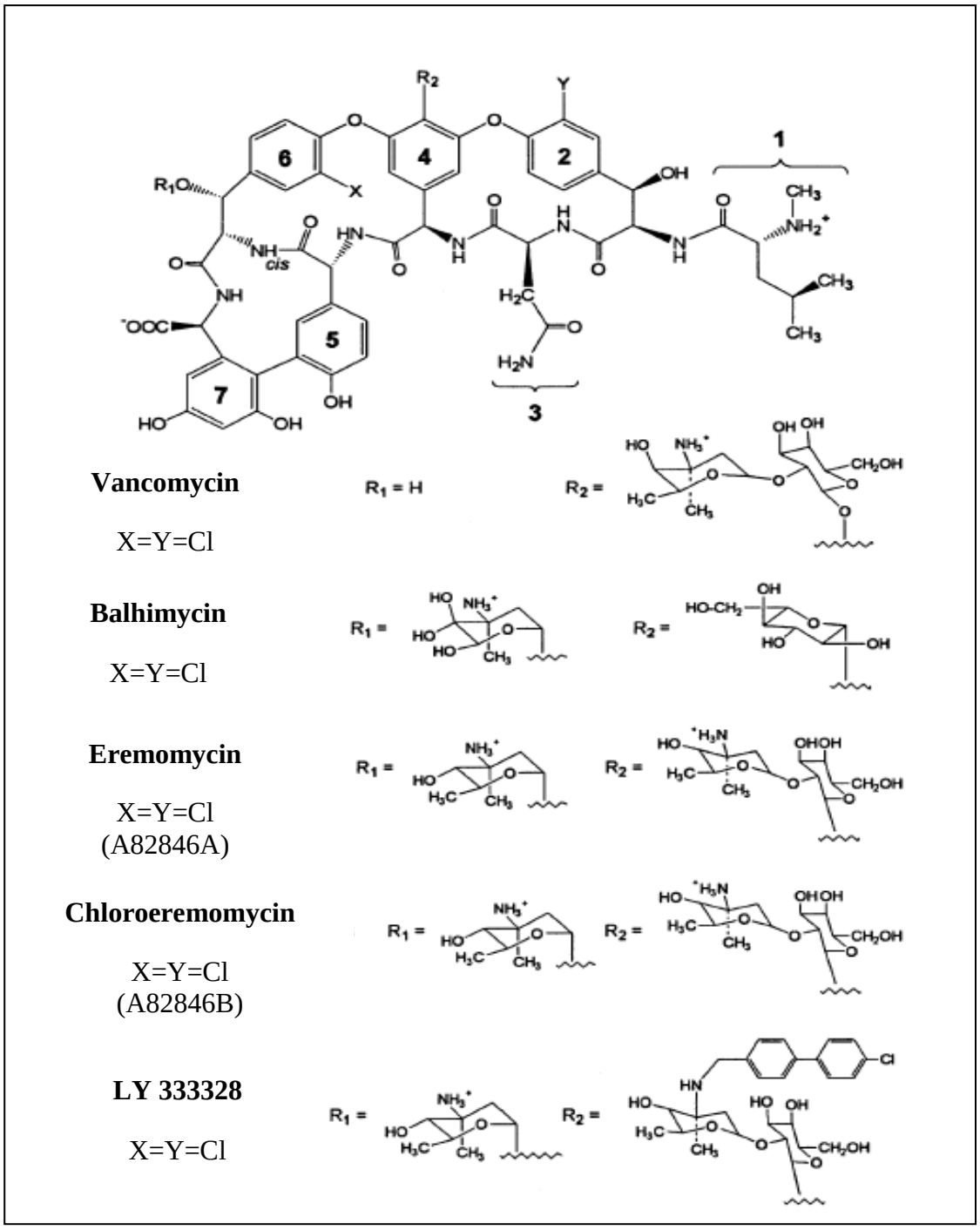

Figure 1.5 Chemical structure of class I glycopeptide antibiotics (Loll and Axelsen, 2000)

1.2.2 Mechanism of action

Vancomycin has a relatively high molecular mass (~1400 Da) and this makes vancomycin and its analogues unable to penetrate the inner membrane of bacterial cell wall. In order to deal with such deficiency, vancomycin will bind to the bacterial cell wall and interfere during the late phases of peptidoglycon biosynthesis. The inhibition of peptidoglycon biosynthesis is due to binding of vancomycin to the –D-Ala-D-Ala terminus of peptidoglycon in bacterial cell wall. By doing such, vancomycin blocks the transglycosilation and transpeptidation steps and cause the bacterial cells become susceptible to lysis (Loll and Axelsen, 2000).

Vancomycin is known for its ability to form dimer in aqueous solution (Backes *et al*., 1998). The first report on aggregation of vancomycin in an aqueous solution was first studied and documented by Neito and Perkins in 1971. It was shown that in the dimer, hydrogen bond interactions were formed between the back sides of the two antibiotic molecules (Figure 1.6). The resulting formation of homodimer increases the affinity towards cell wall fragment up to a factor of ten. In turn, cell wall fragments were reported to enhance dimerization by factors of two to 100 (Loll and Axelsen, 2000). Based on this information one can assume that the process of dimerization plays potent role in the physiological mode of action of a number of vancomycin-type antibiotics.

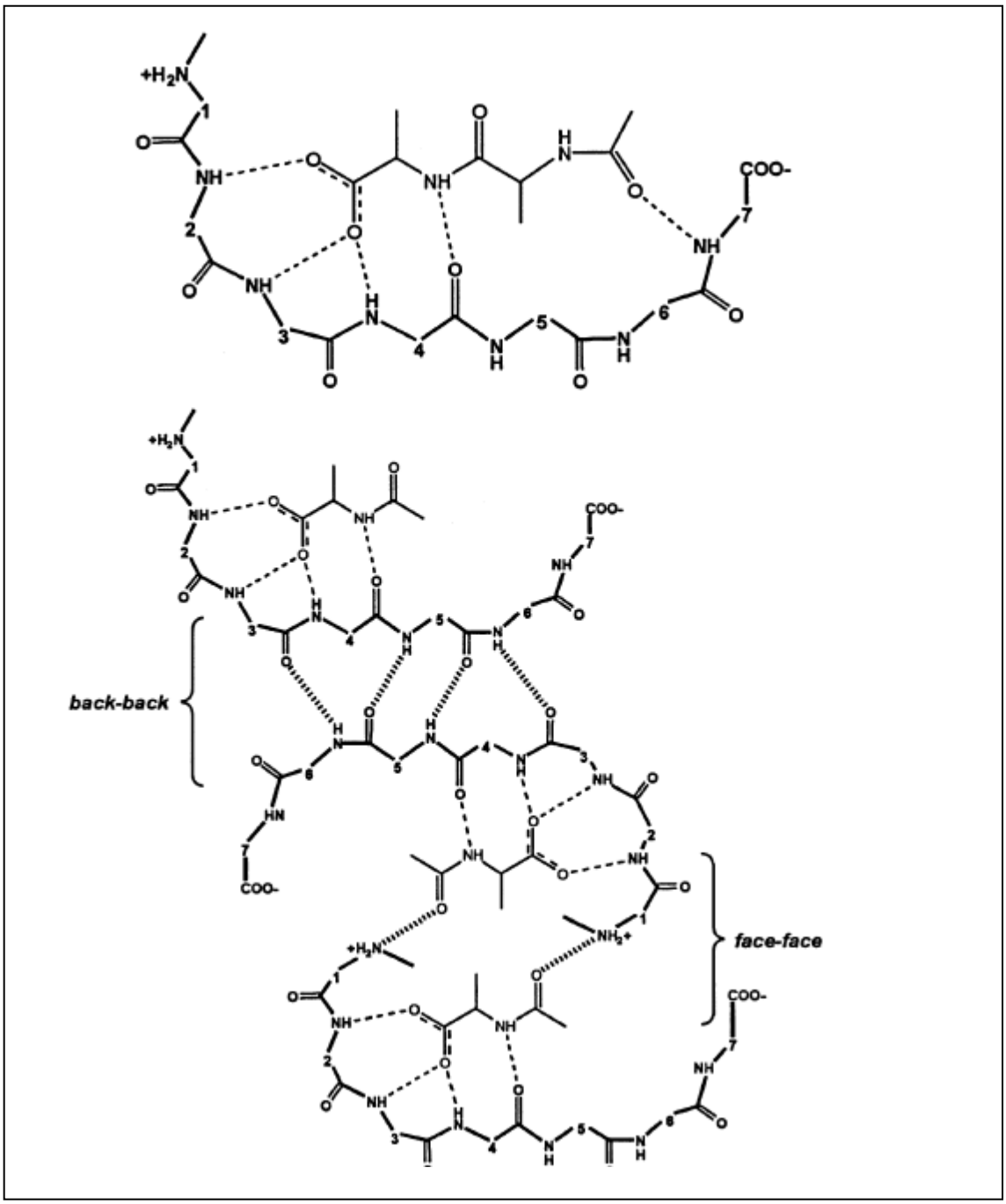

Figure 1.6 Schematic intermolecular relationships. (*Top*) The antiparallel orientation of Ac-D-Ala-D-Ala relative to the heptapeptide backbone of a glycopeptide antibiotic, and its intermolecular hydrogen bond configuration. (*Bottom*) The dimeric relationships seen in crystals of vancomycin/Ac-D-Ala complex. The back-to-back interface is stabilized by four hydrogen bonds as well as numerous hydrophobic contacts (Loll and Axelsen, 2000).

1.2.3 Toxicological aspects of vancomycin

During 1960s, the leading role of vancomycin was replaced by the less toxic agents such as methicillin, semisynthetic penicillins and cephalosporins due to its impurities issues (Loll and Exelsen, 2000). After more than 20 years of studies done on the toxicological aspects of this drug, the results showed that the administration of this drug may cause few adverse affects in animal models including local irritation following parenteral administration, nephrotoxicity and a significant drop of blood pressure after rapid intravenous (i.v) administration in dogs (Wold and Turnipseed, 1981). Similar observations were also reported in clinical use of vancomycin where local irritation at the site of injection, nephrotoxicity and histamine-like responses were reported occasionally. In addition, vancomycin administration showed no signs of ototoxicity unless given in combination with aminoglycosides in laboratory animals (Farber and Moellering, 1983).

Wold and Turnipseed (1981) summarized their findings on the acute toxicity study of vancomycin in laboratory animals as follows: i.v median lethal dose (LD_{50}) of vancomycin for rats, mice and dogs were 319, 489 and 292 mg/kg, respectively. Rats and mice were found death in clonic convulsion after immediate dosing which signifed direct effects of the drug on the central nervous system (CNS). Moreover, from the results of their experiments, they then hypothesized that renal failure may associate with the mortality in dogs following several days of vancomycin administration. In contrary, administrations of vancomycin via subcutaneous (s.c) and oral (even at high concentration of 5g/kg) resulted in no deaths in mice. However, for s.c route, extensive

necrosis and sloughing of the subcutaneous tissue were observed. The intraparetional (i.p) LD_{50} of vancomycin for rats and mice were 2218 mg/kg and 1734 mg/kg. Similarly to β-CD, previous reports on the cytotoxic effects of vancomycin also revealed mixture of results as the cytotoxic effects were shown to be depending on the nature of the cells (King and Smith, 2004; Yoeruek *et al*., 2008). Nonetheless, to the best of our knowledge, none of them were ever performed on human glial cells.

1.3 Glial cells

1.3.1 Types of glial cells in human CNS

Glial was first named in 1856 by Rudolf Virchow (Somjen, 1988). Generally, in human central nervous system (CNS), glial cells or also known as neuroglials refer to the major constituent of human brain cells including astrocytes, oligodendrocytes and microglials. Figure 1.7 illustrates the types of cells found in human CNS. Relatively to neurons, glial cells present at the ratio of ~ 1.65:1 in human cortex (Sherwood *et al*., 2006; Verkhratsky and Toescu, 2006). Astrocytes or astroglials are the main class and probably the most studied of neuroglials followed by oligodendrocytes and microglial. Astrocytes can be further sub-classified in two different types which are protoplasmic and fibrous astrocytes mostly based on the cell morphology (Miller and Raff, 1984). The size of human protoplasmic astrocyte was reported at about 2.5-3 times larger than fibrous astrocyte (Oberheim *et al*., 2009).

According to Goldman (2001), astrocytes are originated from two sources. During early development, astrocytes are believed to form from elongated precursors that have their cell body in or near the ventricular zone and a process that stretches radically to terminate at the surface of the neural tube or developing brain. Later in development, astrocytes are generated from a distinct set of germinal areas, the subventricular zone. As for oligodendrocytes, the origin of these cells is still uncertain, however in analogy with the cord, ventral structures are most likely to be the major source for the production of oligodendrocytes (Richardson *et al*., 2006). In addition to

that, study by Kaur *et al*. (2001) suggested that some of microglial may arise from the pial mesenchymal macrophages that appear to originate from the yolk sac precursors.

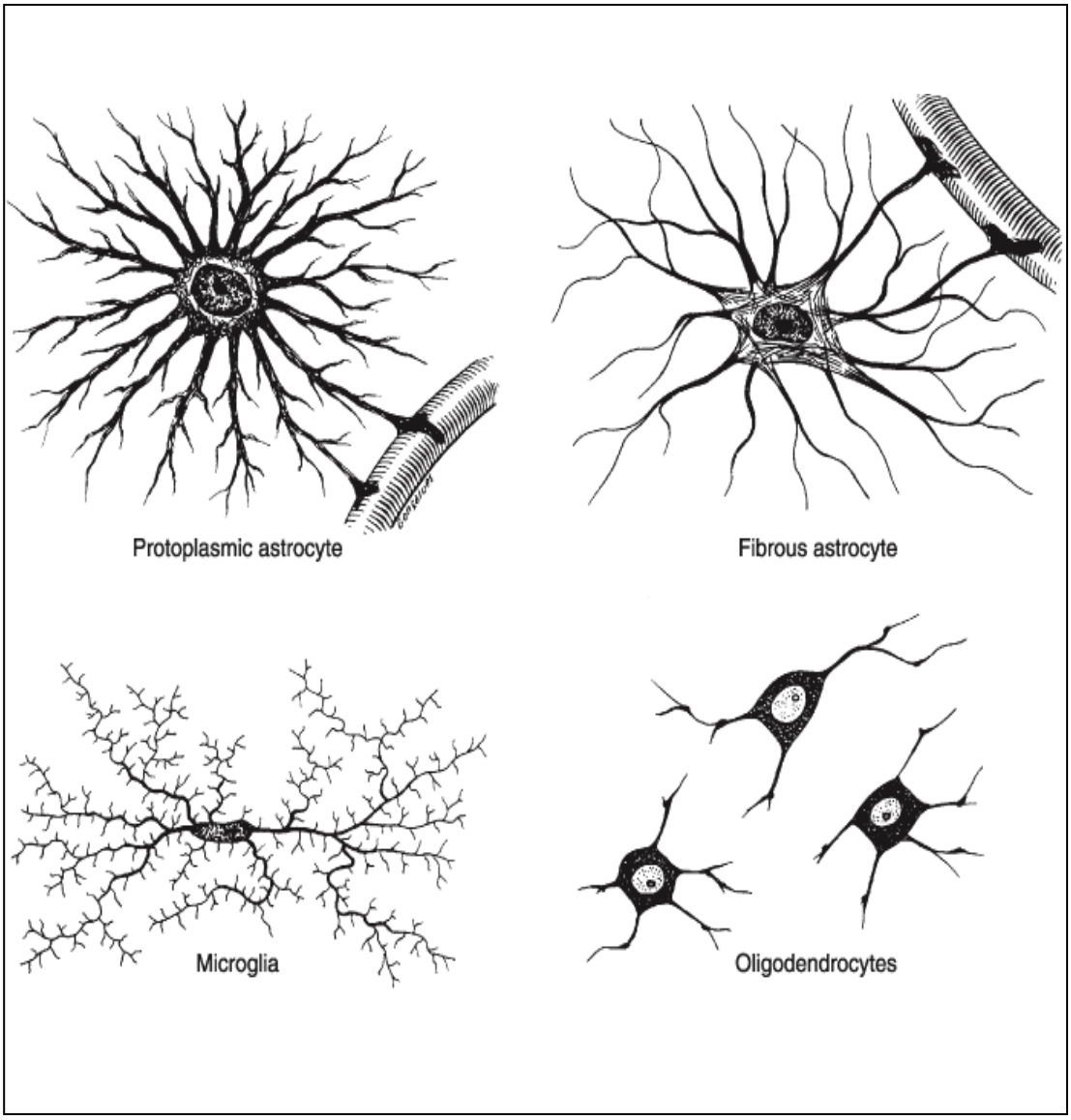

Figure 1.7 Types of glial cells in human CNS (Ganong WF. Review of medical physiology 22nd edition (http://www.accessmedicine.com)

1.3.2 Importance of glial cells in human CNS

In mammalian brain, astrocytes divide the grey matter into relatively independent structural units. The protoplasmic astrocytes occupy their own territory and create the microanatomical domains within the limits of their processes (Bushong *et al*., 2004). Within the confines of these anatomical domains, the membrane of astrocytes covers synapses and neuronal membranes as well as sends the process to plaster the wall of the neighbouring blood vessel with the end foot (Figure 1.8).

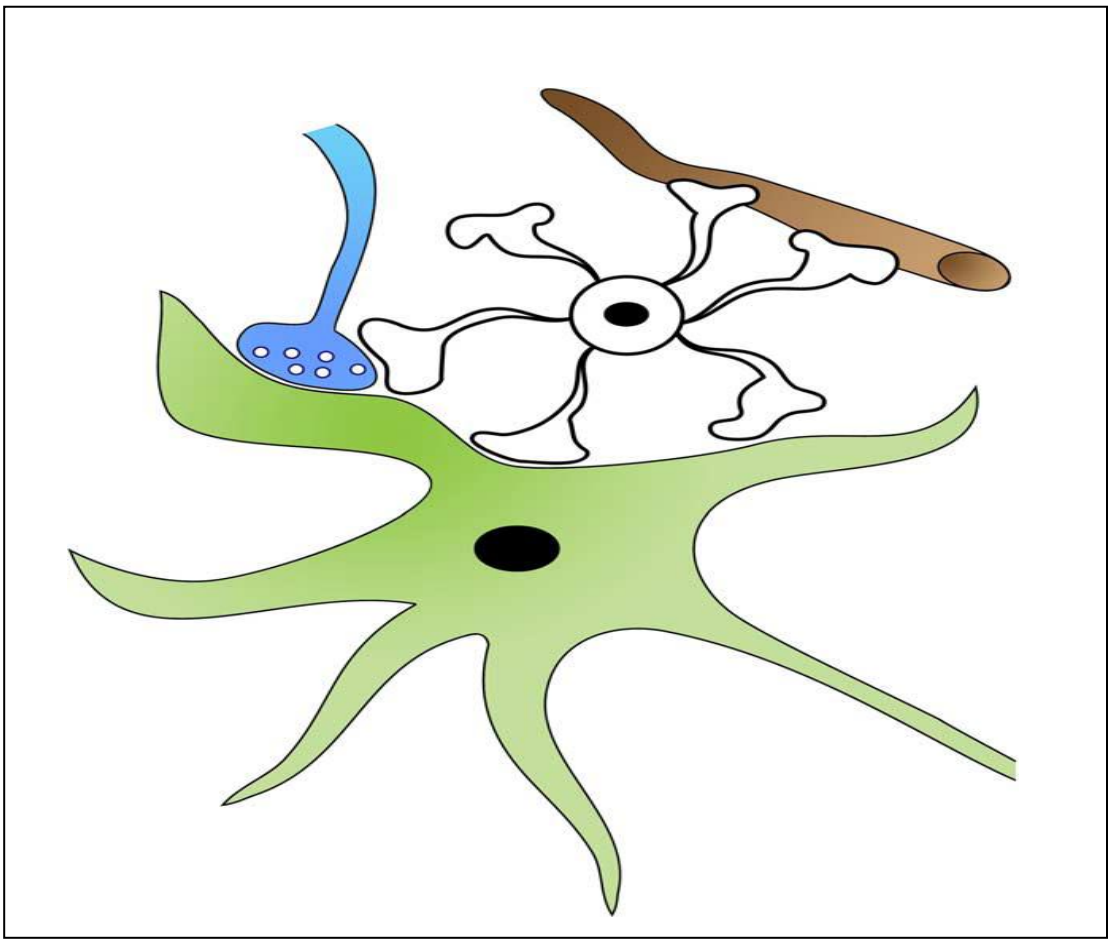

Figure 1.8 The general arrangement between astrocytes, capplilaries and neurons. Astrocytes (*white*), capillaries (*brown*), neurons (*green*) and synaptic junctions (*blue*) (Prienger, 2002).

Meanwhile, oligodendroytes which are the second major class of neuroglials are actively communicating with neurons. This reciprocal signaling profoundly contributes to overall brain plasticity (Fields, 2008). From the homeostatic aspect, astrocytes appeared as the core cellular element of the extracellular homestasis in the brain. The control over the concentrations of ions, neurotransmitters, neuromodulators, metabolites and other active substances is of a paramount importance for normal operation of neural networks (Simard and Nedergaard, 2004). Neuroglials are non-excitable and therefore are unable to use plasmalemmal ion conductances to rapidly convey the information. Nonetheless, neuroglials on the other hand are able to develop of propagating signals either through the gap junctions or via the release of gliotransmitters. Conceptually, glial cells can perform all physiological processes as neurons do although the neuroglials execute these physiological processes in a specific and distinct way (Condorelli *et al*., 1999; Gallo and Ghiani, 2000; Fraser *et al*., 1995; Verkhratsky and Toescu, 2006).

Besides that, glial cells are believed to play the leading role in initiation, progression and outcome of neurological diseases. Indeed in higher mammals, neuroglials are fully responsible for brain homeostasis, brain metabolic support and brain defense, as well as in every type of lesion to the CNS. In a way, this shows that neuroglials are important to withstand and repair the damage (Matute, 2010; Rossi and Volterra, 2009). The pathological of neuroglials are diverse including various programmes of activation, which are essential for limiting the areas of damage, producing neuro-immune responses and for the post-insult remodeling and recovery of neural function (Hasnich and Kettenmann, 2007; Rolls *et al*., 2009).

1.4 Recent studies

Study by Tilloy *et al*. (2006) was conducted in order to evaluate the potential use of methylated-β-cyclodextrins (M-β-CD), a type of β-CD derivative in enhancing the delivery of doxorubicin (DOX), an anti-tumoral agent across an *in-vitro* model of blood-brain barrier (BBB). β-CD and two methylated derivatives were used at a concentration preserving the BBB intergrity (1, 1 and 2.5 mM for β-CD, Rame-β-CD and Crysme-β-CD, respectively). The concentration of DOX was fixed at 1 μM. In the study, it was found that M-β-CD was able to improve the delivery of DOX across the brain capillary endothelial cells (BCEC). The results showed that M-β-CD was able to reduce the P-gp activity by extracting the cholesterol which led to an enhancement of delivery of P-gp substrates. Release of cholesterol from BCEC was found to be dependent on the nature as well as the concentration of CDs used. The cholesterol efflux was equal to 16 %, 21 % and 31 % for 1mM β-CD, 1mM Rame- β-CD and 2.5 mM Crysme- β-CD.

Cannavá *et al*. (2009) reported the formation of an amphiphilic cyclodextrin/genistein complex via emulsification-difussion method at 1:1 molar ratio in water medium. Genistein (Gen) is an isoflavone belonging to the class of phytoestrogens has been considered as a potential remedy for many kinds of diseases. Gen molecules were shown to form a partially included complex with cyclodextrin. The lack of inclusion was confirmed by monitoring the significant differences in the spectral features of the Fourier Transform Infrared Spectrascopy-Attenuated Total Reflactance (FTIR-ATR) spectrum of complex with respect to the physical mixture, revealed in the

O-H, C=O, C=C, C-O-C and C-O stretching region. The differences evidenced the functional groups involved in the activation of the host-guest interaction during complexation process.

Juelin *et al*. (2009) reported the role played by CD in enhancing the uptake of ribavirin (RBV) into the brain. RBV is a broad-spectrum antiviral agent and a water-soluble synthetic nucleoside. It is ineffective against major viral encephalitis because of its failure to cross the blood-brain barrier. RBV improved the survival of subacute sclerosing panencephalitis (SSPE) virus-infected hamsters when only administrated intracranially and did not improve their survival when given intraperitoneally suggesting its failure to cross the BBB. In this study, RBV specific extraction from brain tissue was developed based on a solid phase extraction and quantified by high performance liquid chromatography (HPLC) at different interval points after intraperitoneal injection of single or multiple doses of free RBV or the complex RBV/alpha-cyclodextrin. The amount of RBV was significantly higher ($p<0.001$) for the tested doses (40 or 100 mg/kg) when the drug was injected as a complex with alpha-cyclodextrin in healthy of measles virus-infected mice. The results showed that RBV reached its effective concentration when injection in a complex formulation and proved that cyclodextrins are of a great interest for the treatment of human infectious diseases of central nervous system.

Figueiras *et al*. (2007) performed a study to evaluate the formation of solid-state inclusion complexes of omeprazole (OME) with the native and the chemically modified β-CD and their dissolution profiles. The complexes were prepared via kneading, freeze-

drying and spray-drying techniques in a 1:1 molar ratio. The formation and physicochemical characterization of the complexes were investigated by Differential Scanning Calorimetry (DSC), Fourier Transform-Infrared (FTIR), X-ray Diffractometry (XRD) and Scanning Electron Microscopy (SEM). Significant differences were observed between the dissolved drug amounts in pure, physically mixed and complexed forms ($p < 0.001$) as well as in the dissolution rate in artificial saliva media. The results proved that the complexation effectively enhanced solubility of OME, which consequently can increase its bioavailability and might improve its pharmaceutical potential.

1.5 Research objectives

1.5.1 General objective

In this study, the equimolar complexes of vancomycin, a broad-spectrum glycopeptides antibiotic with beta-cyclodextrin (β-CD) were prepared to evaluate their suitability for the development of a prolonged-release form of the antibiotic. Three specific objectives were studied accordingly.

1.5.2 Specific objectives

(i) To prepare and to characterize β-CD/vancomycin complexes formed by freeze-drying and kneading techniques.

(ii) To study the dissolution patterns of vancomycin upon complexation with β-CD produced via freeze-drying and kneading processes by using High Performance Liquid Chromatography (HPLC).

(iii) To evaluate the cytotoxicity effects of vancomycin and its complex with β-CD in human glial cell line.