Introducti...
1. INTRODUCTION

1.1 ALZHEIMER’S DISEASE

The changes in cognitive function that occur with aging range, in severity, from mild to devastating. Cognition remains virtually intact in some individuals as they grow older, while others become dependent on caregivers. A variety of neurodegenerative diseases, as enumerated in Figure 1, are responsible for the condition (Uttara et al. 2009).

![Figure 1: Various neurodegenerative diseases](image)

Out of all these neurodegenerative diseases, Alzheimer’s disease (AD) is a public health issue affecting one in every ten persons over the age of 65. Particularly, in the group of individuals over 85, one in every three tend to develop Alzheimer’s disease, leading to dementia. Owing to improved life expectancy, as our aging populations expand rapidly, the financial and emotional toll of AD continues to rise (Carter & Lippa 2001; Zhu & Sano 2006). Clinically, AD is characterized by progressive mental deterioration and significant changes in personhood, thought depending upon the speed of the intellectual decay (Reisberg et al. 2011). While the episodic memory is lost quite early, the short-term memory is preserved until very late in the course of AD development. Therefore, impairment of memory is an early feature of AD (Behl 19...
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Radloff et al. 2011).

1.1.1 Pathology of Alzheimer’s

Alois Alzheimer, in 1907, described a “peculiar disease of the cortex”, on the basis of “strange alterations of the neurofibrils” and “foci which are built up by peculiar substance spread over the whole cortex” in silver-stained sections of brain (Behl 1999). A century later, the structures found by Alzheimer became known as neurofibrillary tangles (NFTs) and as senile plaques loaded mainly with amyloid β protein (Figure 2).

![Figure 2: Neurofibrillary tangles and amyloid plaques in Alzheimer’s brain](image)

Figure 2 illustrates the pathological changes characteristic of AD. AD predominantly a disorder of the brain region that controls the human central nervous system (CNS), the cerebral cortex. A continually ongoing sequence of degenerative changes can be found in the affected cortical and subcortical brain areas (Hyman et al. 1990; Braak & Braak 1991; Fjell et al. 2009).

Extracellular deposits in plaques consist of Amyloid β, a 39 ± 43 amino acid long peptide (Ikonomovic et al. 2006; Bolmont et al. 2007; Kubis & Janusz 2007). Intracellular fibrils (NFTs) occur in both dying and already degenerated neurons. The ultrastructural analysis of NFTs reveals paired helical filaments which are comprised of the microtubule-associated tau proteins in the form of an insoluble polymer. These characteristics are quite similar to each other in the different types of AD (e.g., early and late-onset).
1.1.1.1 Oxidative stress leading to Alzheimer’s

1.1.1.1.1 Vulnerability of neurons to free radicals

Free radicals, i.e., the molecules with unpaired electrons, provide high reactivity. In order to acquire a more stable energy level, the free radical can accept another electron or a hydrogen atom from another molecule. With the oxidative phosphorylation reactions that build the center of the respiratory pathway in the mitochondria of cells, molecular oxygen is reduced to water by the successive acceptance of electrons and protons. During this process, three reactive oxygen species (ROS) are formed: superoxide, hydroxyl radicals, and the intermediate hydrogen peroxide. Although $\text{H}_2\text{O}_2$ is chemically not a radical, it is a ROS because it can be readily converted to hydroxyl radicals in the presence of $\text{Fe}^{2+}$ via the so-called Fenton reaction:

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO}^- + \text{OH}^-$$

Since long, iron has been known as a very reactive element which can catalyze oxidation reactions and also the generation of ROS (Aust et al. 1985; Halliwell et al. 1992; Gebril et al. 2011). Compared to all other tissues, which can potentially be damaged by ROS, the brain is particularly vulnerable to oxidative processes for several reasons:

i. Neurons of the CNS are almost completely dependent on the oxidative phosphorylation reactions in order to generate adenosine triphosphate as energy source.

ii. For the normal adult brain, glucose is the major nutrient and, therefore, the brain has a high glucose metabolism and respiratory turnover.

iii. Neuronal membranes of the brain consist of high concentrations of polyunsaturated fatty acids, which are potential substrates for the peroxidation by hydroxyl radicals.

iv. The brain has an overall high concentration of catalytic iron.

v. The brain has only low levels of antioxidant defense enzymes compared to other tissues.

1.1.1.1.2 Signs of oxidation in Alzheimer’s brain

Various products of oxidation reactions and mediators of oxidative stress are
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found in association with the histopathological AD hallmarks, including malondialdehyde, advanced glycation end products (AGEs), carbonyls, nitrotyrosine, SOD and heme oxygenase (Pappolla et al. 1992; Colton et al. 1994; Yan et al. 1994; Good et al. 1996; Smith et al. 1996; Axelsen et al. 2011). Many investigations have been conducted in past years using post-mortem AD brain material to detect such oxidation end products. Basically, all cellular macromolecules (protein, DNA, lipids) could be found in an oxidized form in the AD tissue (Subbarao et al. 1990; Mecocci et al. 1994; Smith et al. 1995b; Smith et al. 1995a).

1.2 TREATMENT STRATEGIES OF ALZHEIMER’S

Because the etiology and pathogenesis of AD have not been clearly defined as yet, the therapeutic target central to the pathological process still needs to be found. Verily, the current strategies to help patients during the course of this devastating disease are directed against various factors and events that are associated with AD (Fan & Chiu 2010; Chopra et al. 2011; Herrmann et al. 2011; Lin & Luo 2011; Palmer 2011; Sureda et al. 2011). The major histopathological hallmarks of this neurodegenerative disorder have already been known for over a century. Although many landmark findings have been made in recent AD research lately, yet the cause(s) of AD are not precisely known. Consequently, current clinical trials are designed on the basis of one particular current hypothesis of AD’s pathogenesis (e.g., use of acetylcholine esterase inhibitors according to the acetylcholine deficiency hypothesis).

1.2.1 Currently Employed Treatments

Slowing down the progression of symptoms in AD, particularly during the early stages when people are fully autonomous, would result in tremendous benefits to the patients along with their caregivers and societal costs. Such a stabilization strategy would logically target primary or secondary pathophysiological events of AD. As a consequence, there are multiple credible hypotheses around which therapeutic agents can be developed. Since the most prominent neuropathological findings in AD are neurofibrillary tangles, neuritic plaques, and amyloid deposition in neural tissues and vessels, agents that stabilise cytoskeletal function by preventing or reversing the formation of these abnormalities (e.g., antiamyloid
drugs) may provide disease-specific neuroprotective effects (VanDenBergh 2000). However, these agents are presently unavailable for clinical use. Neurochemical mechanisms other than those described above may also play a role in cell death and neurodegeneration in AD, including neuroimmune dysfunction, free radical formation, neurotransmitter deficits and alterations in brain homeostasis (Cacabelos et al. 1995). Histopathological studies suggest that inflammatory mediators are active at sites of neurodegeneration in AD and represent targets for therapeutic intervention by agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) (Aisen & Davis 1994).

Compounds such as antioxidants (e.g., selegiline, tocopherol (i.e., vitamin E)) and methyl-D-aspartate (NMDA) antagonists have shown potential as neuroprotective agents in other disease conditions and may also prove beneficial for treating AD. The most studied therapeutic approach to the treatment of AD relies on enhancing neuronal pathways that have been affected by the disease. Figure 3 summarizes the various treatment strategies for AD.

**Figure 3: Treatment strategies for AD**

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<tr>
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<th>Novel Potential Strategies</th>
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<td>• Rivastigmine</td>
<td>• Antioxidants (e.g., flavonoids)</td>
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<td>• Tacrine</td>
<td>• NMDA receptor antagonists (e.g., Memantine)</td>
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1.2.1.1 Cholinergic deficit

The cholinergic hypothesis claims that low levels of acetylcholine lead to cognitive decline (Cummings & Kaufer 1996). Numerous areas in the brain contain neuronal cholinergic innervation. One of the most consistent neurochemical changes in AD is loss of cortical and hippocampal cholinergic innervation, with atrophy and loss of the basal cholinergic neurons that project to the cortex and hippocampus. Each of these specific brain regions plays an important role in memory, learning and behavioural domains. The pharmacological activity of acetylcholine is regulated through neuronal cell surface receptors, specifically muscarinic and nicotinic receptors. Pre-synaptic muscarinic and nicotinic receptors assist in the regulation of acetylcholine neurotransmission. Investigators have reported consistent decreases in the number of muscarinic and nicotinic receptors in patients with AD. Additionally, dramatic loss of cortical enzyme activity of both choline acetyltransferase and acetylcholinesterase (AChE) is found in AD. These are the main enzymes involved with production and degradation of acetylcholine (Jann 1998). AD can therefore be described as a marked disturbance of the cholinergic system involving molecular enzyme activity, specific receptors and degeneration of specific neurons. As a result of these findings, enhancement of the cholinergic system remains a major goal for many of the pharmaceutical agents that are currently in use or under development for the treatment of AD. Potential treatment strategies to counter the cholinergic deficit are summarized in Box 1.

Box 1: Potential treatment strategies to counter the cholinergic deficit

- Supplementation of acetylcholine precursors
- Augmentation of acetylcholine releasers, which facilitate the release of acetylcholine from presynaptic end terminals
- Treatment with muscarinic agonists, which mimic acetylcholine on postsynaptic receptors
- Modulating the glutamatergic system by blocking the NMDA (N-methyl D-aspartate) type of glutamate receptor (e.g., memantine).
- Treatment with cholinesterase inhibitors (ChEIs), which increase levels of acetylcholine by preventing enzymatic breakdown of the neurotransmitter.
### 1.2.1.2 Cholinesterase inhibitors

At the present time, ChEIs are the only agents that have been shown to produce significant improvements on cognitive, behavioural and global performance indices in large controlled trials of patients with AD. ChEIs inhibit the activity of AChE, an enzyme that breaks down acetylcholine at the synapse. This inhibition results in the subsequent increased availability of the neurotransmitter for binding to muscarinic and nicotinic receptors. Increases in acetylcholine concentration can enhance the muscarinic effect and may improve memory and cognition in a person with AD (Cummings & Kaufer 1996; Jann 1998). This strategy is theoretically analogous to replenishing dopamine in the treatment of patients with Parkinson’s disease. Similarly, increasing AChE concentration does not reverse the neuronal destruction that has already occurred; therefore, the overall therapeutic goal with ChEIs is tentatively defined as a delay or decrease in the progression of cognitive or behavioural disease symptoms. Treatment success can be enhanced when initiated early in the disease process and is probably less successful after large amounts of neuronal damage have occurred during later stages of the disease.

Tacrine is the first centrally acting anti-ChE to be introduced for AD. In clinical trial, tacrine produced significant improvement in memory attention praxis, reason and language. However, it does not alter course of the underlying disease process. Frequent side-effects and hepatotoxicity have restricted its use. Donepezil, a cerebroselective and reversible anti-AChE, produces measurable improvement in several cognitive as well as non-cognitive (activities of daily living) scores in AD, which is maintained at least up to two years. The benefit is ascribed to elevation of Ach levels in the cortex, especially in the surviving neurons that project from basal forebrain to cerebral cortex and hippocampus. Therapeutic doses produce only weak peripheral AChE inhibition, thus cholinergic side effects are mild. Because of long $T_{1/2}$ ($\sim 70$ h), donepezil is administered once daily at bed time, a distinct advantage over rivastigmine and galantamine which require twice daily dosing. It is generally well-tolerated and not hepatotoxic. Galantamine is a natural alkaloid, which selectively inhibits cerebral AChE and has some direct agonistic action on nicotinic receptors as well. Galantamine has produced cognitive and behavioral benefits in AD which are
comparable to rivastigmine and donepezil. It is well tolerated but needs twice daily dosing. Rivastigmine is considered a 'pseudoirreversible' cholinesterase inhibitor that forms a carbamoylated complex with the enzymes, and has been reported to inhibit both AChE and BChE with equal potency (Nordberg & Svensson 1998; Jann 2000). After single-dose administration, enzyme inhibition was reported to persist for 10 to 12 hours (Sramek et al. 1996). This longer duration of action is unique among cholinesterase inhibitors.

1.2.2 Novel treatment strategies for AD

Because a substantial dysfunction and loss of cholinergic neurons occur in AD, the major therapeutic approach is to replace this existing neurotransmitter deficiency (Lockhart et al. 2009; Osborn & Saunders 2010; Schneider et al. 2011). The cholinergic system plays a central role in learning and memory. The presence of acetylcholine is not only necessary for the above-mentioned processes, but can also ameliorate learning deficits and restore memory following the damage to the nucleus basalis magnocellularis, the brain area that provides the major cholinergic innervation of the neocortex (Winkler et al. 1995; Nieto-Escamez et al. 2002; Hasselmo & Giocomo 2006). Drugs like tacrine hydrochloride and donepezil are inhibitors of the enzyme cholinesterase that degrades the neurotransmitter acetylcholine in the synaptic cleft. Acetylcholine levels, which get decreased in AD, can be locally increased by blocking its degradation. However, AD is not just a disease of a neurotransmitter deficiency, but is rather characterized by a massive synaptic loss and neuronal degeneration. Thus, novel neuroprotectant strategies which can arrest the cause of this disease need to be explored.

1.2.2.1 Antioxidants as neuroprotectants

Antioxidant therapy has been discussed for AD as well as for a variety of other neurodegenerative disorders, such as Parkinson’s and ischemia, and for age-related disorders in general (Agostinho et al. 2010; Campos-Esparza Mdel & Torres-Ramos 2010; Kelsey et al. 2010; Kim et al. 2010; Lee et al. 2010; Piau et al. 2011). Numerous free radical scavengers, such as Vitamin E (α-tocopherol), the pineal hormone melatonin, the lazaroids (21-aminosteroids) and mifepristone, have been employed with fruition in experimental paradigms of neuronal cell death in vitro and in vivo, (Lucca et al. 1997; Heim et al. 2000; Wang & Wang 2006;
Dhikav & Anand 2007; Grases et al. 2010; Sen et al. 2010; Yang et al. 2010). Some clinical trials employing the antioxidant, Vitamin E, have successfully been completed (Fotuhi et al. 2008; Isaac et al. 2008). This first success raised high hopes with respect to the concept of an antioxidant therapy fueling further intensive research and additional clinical trials on this topic. Of course, novel antioxidants should have improved properties compared to vitamin E, such as an increased lipophilicity, an easy blood brain barrier crossing and, therefore, enhanced activity. Such a molecule could be a female sex hormone estrogen or estrogen-derivatives, or plant based polyphenolic compounds like flavonoids.

1.2.2.2 Flavonoids

The flavonoids constitute a class of natural compounds that has been the subject of considerable scientific and therapeutic interest recently. The flavonoids are ubiquitous to green plant cells and, therefore, could be expected to participate in the photosynthetic process (Havsteen 2002). A detailed evidence of the role of flavonoids is known in gene regulation and growth metabolism. The mutagenic role of flavonoids is of particular interest to botanical taxonomists. It also serves as a reminder to medical practitioners of the potential dangers of the consumption of natural products. Nutritionists estimate the average intake of flavonoids by humans on a normal diet is 1-2 g per day.

The flavonoids appear to have played a major role in the successful medical treatments of ancient times, and their use has persevered up to now. The recent interest in the properties of the flavonoids has several converging explanations:

i. Since flavonoids are pigments ubiquitous to green plant cells, and are highly diversified, as well as easily separable with modern chromatographic equipment, scientists have long used the pattern of occurrence of these compounds for taxonomical studies. This approach is a substitute for full sequencing of the genome and only an indirect reflection of the hereditary traits, but the procedure is quick, easy, and useful.

ii. Another reason for the increasing interest in the flavonoids is that the pharmaceutical industry, true to its tradition, has always been searching for new medical herbs, the functional compounds of which can serve as a starting point for the development of optimal derivatives. During such scanning procedures, flavonoids possessing interesting properties were
iii. A third reason for growing activity in the field of flavonoid biochemistry is the persistent claim by many medical practitioners of the beneficial effects of treatment with natural products, which proved to be rich in flavonoids. Subsequently the existence of many interesting effects of the flavonoids has been proven (Dhawan et al. 2003; Dhawan et al. 2004; Dhawan & Dhawan 2006; Guo et al. 2010; Kumar et al. 2010; Fraga et al. 2011; Pick et al. 2011).

1.2.2.2.1 Basic structure and types of flavonoids

The term “flavonoids” is a collective noun indicative of plant pigments, mostly derived from benzo-γ-pyrole (Figure 4), synonymous with chromone (Croft 1998; Hassig et al. 1999; Havsteen 2002).

![Figure 4: Benzo-γ-pyrole, the basic chemical moiety in all flavonoids](image)

The group comprises anthocyanidines, hydroxyl-4-dihydroflavonoles; glycosides of anthocyanidines; flavonoles, 2-phenyl-3-hydroxychromones; isoflavonoles, 3-phenyl-2-hydroxychromones; flavones, 2-phenylchromones; isoflavones, 3-phenylchromones; flavanes, 2-phenyl-3-dihydrochromones, 2-phenylflavanones; isoflavones, 3-phenyl-2-dihydrochromones; flavanols, 2-phenyl-3-hydro-3-hydroxychromones; isoflavanols, 2-hydro-2-hydroxy-3-phenylchromones; aurones, benzofurones; and coumarins.

1.2.2.2 Antioxidant potential of flavonoids

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage (de Groot 1994; Bhogal et al. 2010; Coaccioli et al. 2010; Miranda-Vilela et al. 2010; Korotkova et al. 2011). The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood. However, one of
the most important mechanistic events seems to be lipid peroxidation, which results in cellular membrane damage. This cellular damage causes a shift in the net charge of the cell changing the osmotic pressure, leading to swelling and eventually, cell death. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. To protect themselves from reactive oxygen species, living organisms have developed several effective mechanisms (Nijveldt et al. 2001; Lipinski et al. 2010). Flavonoids can interfere with three different free radical-producing systems:

1.2.2.2.2.1 Direct radical scavenging

Flavonoids can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals (Chiodo et al. 2010; Kalaivani & Mathew 2010; Limem et al. 2010). Flavonoids are oxidized by radicals, resulting in a more stable and less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following reaction, where R⋅ is a free radical and O⋅ is an oxygen free radical:

\[
\text{Flavonoid (OH)} + R\cdot \rightarrow \text{Flavonoid (O\cdot)} + RH
\]

Selected flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical called peroxynitrite. Quercetin, epicatechin and rutin are powerful radical scavengers (Hanasaki et al. 1994; Kerry & Abbey 1997; Edenharder & Grunhage 2003). The scavenging ability of rutin may be due to its inhibitory activity on the enzyme, xanthine oxidase. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro (Kerry & Abbey 1997).

1.2.2.2.2 Nitric oxide scavenging

Several flavonoids, including quercetin, result in a reduction in ischemia-reperfusion injury by interfering with inducible nitric-oxide synthase activity (Shoskes 1998; Mahat et al. 2010; Dal-Ros et al. 2011). Nitric oxide is produced by several different types of cells, including endothelial cells and macrophages. Although the early release of nitric oxide through the activity of constitutive nitric-
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Oxidase synthase is important in maintaining the dilation of blood vessels (Thomas et al. 1998; Bohlen et al. 2009), the much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage. When flavonoids are used as antioxidants, free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage. Also, nitric oxide can be viewed as a radical itself, and it is was reported that nitric oxide molecules are directly scavenged by flavonoids (van Acker et al. 1995; Messaoudene et al. 2011). Therefore, it has been speculated that nitric oxide scavenging plays a role in the therapeutic effects of flavonoids.

1.2.2.2.3 Xanthine oxidase inhibition

The xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissues, especially after ischemia-reperfusion (Sanhueza et al. 1992; Elahi et al. 2009; Ono et al. 2009). Both xanthine dehydrogenase and xanthine oxidase are involved in the metabolism of xanthine to uric acid. Xanthine dehydrogenase is the form of the enzyme present under physiologic conditions, but its configuration is changed to xanthine oxidase during ischemic conditions. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (i.e., re-oxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals. At least 2 flavonoids, quercetin and silibin, inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury (Chang et al. 1993; Pauff & Hille 2009).

A detailed account of pathology and treatment strategies of AD has recently been published by us (Kapil et al. 2011).

1.3 DRUG DELIVERY TO BRAIN

1.3.1 Barriers to brain drug delivery

Various drug delivery technologies have been developed to boost drug delivery to many parts of body today. But for the scientists designing new medicines and drug delivery systems to treat various central CNS diseases like epilepsy, AD, schizophrenia, brain tumors, etc., the brain is proving to be a formidable challenge (Kapil et al. 2010a). There are three barriers that limit drug transport to the brain parenchyma. These are the blood-brain barrier (BBB), localized in the capillaries in
the brain; the blood cerebrospinal-fluid barrier (BCSFB), which is presented by choroid plexus epithelium in the ventricles; and the ependyma, which is an epithelial layer of cells covering the brain tissue in the ventricles and limits transport of compounds from the cerebral spinal fluid (CSF) to the brain tissue (Boer & Gaillard 2007).

1.3.1.1 Blood brain barrier

The BBB is situated at the interface of blood and brain and its primary function is to maintain the homeostasis of the brain. Furthermore, the BBB is not uniform throughout the brain because the capillaries in the circumventricular organs are fenestrated (Begley 2004). Figure 5 gives a schematic representation of the barriers present in the CNS.

![Figure 5: Various barriers affecting drug delivery to central nervous system](Figure adapted from Kapil et al. 2010a)

The human BBB has a total blood vessel length of approximately 600 Km. In every cubic centimeter of cortex contains the amazing sum of 1 Km of blood vessels. It has an estimated surface area of approximately 20 m², which is similar to the BCSFB.

Micro-vessels make up an estimated 95% of the total surface area of the BBB,
represent the principal route by which chemicals enter the brain. Vessels in brain were found to have somewhat smaller diameter and thinner walls than vessels in other organs.

Also, the mitochondrial density in brain micro-vessels was found to be higher than in other capillaries, not because of more numerous or larger mitochondria, but because of the small dimensions of the brain micro-vessels and consequently smaller cytoplasmic area. In brain capillaries, intercellular clefts, pinocytosis, and fenestrae are virtually nonexistent; exchange must pass transcellularly. Therefore only lipid-soluble solutes, that can freely diffuse through the capillary endothelial membrane, may passively cross the BBB, (Lo et al. 2001) as shown in Figure 6.

At present, many transport systems have been discovered that play an important role in maintaining BBB integrity and brain homeostasis and also influence drug transport to the brain.

Figure 6: Transport of solutes across blood-brain barrier (Figure adapted from Kapil et al. 2010a)

1.3.2 Primordial approaches to cross BBB

Traditionally, the pharmaceutical companies have chosen uncharged, lipophilic compounds as CNS drugs, as they have a greater plausibility of getting across the BBB. Apart from this, early efforts to manipulate BBB in favor of drug delivery have focused on prising apart the tight junctions between the endothelial cell
Hypertonic solutions were introduced into the circulation via carotid artery essentially shrinking the cells so that the junctions open up. This provided a window of about 30 minutes during which a CNS drug was to be administered, again through the carotid artery. But the mechanism was non-specific and during the treatment, brain was open to other potentially toxic substances in the blood too (Bryan 2004).

1.3.3 Novel strategies for enhanced CNS drug delivery

To circumvent a multitude of barriers inhibiting CNS penetration by potential therapeutic agents, myriad drug delivery strategies have been developed. These strategies generally fall into one or more of the following three categories:

(i) Manipulating drugs
(ii) Disrupting the BBB
(iii) Finding alternative routes for drug delivery

1.3.3.1 Lipid-mediated transport

The penetration of a xenobiotic into the CNS tissue is favored by its low molecular weight, lack of ionization at physiological pH, and lipophilicity. Heroin, a diacyl derivative of morphine, is a notorious example that crosses the BBB about 100 times more easily than its parent drug just by being more lipophilic. Thus, a long-standing goal of a drug delivery scientist has been the lipidization of water soluble drugs, whereby chemical medications are used to block existing hydrogen bond-forming groups on the parent drug molecule. Despite extensive applications of medicinal chemistry, till date, there is not even a single FDA-approved drug that exemplifies the conversion of a poor brain penetrating molecule into a high brain-penetrating one.

1.3.3.2 Carrier-mediated transport

An alternative technique to increase brain penetration is to modify drugs, such that there is increased carrier-mediation of the drug. For instance, $\alpha$-carboxylation of the water-soluble catecholamine drug results in the formation of a neutral amino acid. Whereas the BBB penetration of the catecholamine is very low, the $\alpha$-amino acid may then penetrate the BBB at pharmacologically significant rates via carrier-mediated transport.
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1.3.3.3 Intraventricular/Intrathecal Route

The intracerebroventricular (ICV) approach injects drug into the cerebrospinal fluid (CSF) compartment. The entire CSF pool in the human brain is turned over every 4–5 h and four to five times per day. Drugs can be infused intraventricularly using an Ommaya reservoir, a plastic basin implanted subcutaneously in the scalp and connected to the ventricles within the brain via an outlet catheter. The intrathecal route employs lumbar injection of the medicament directly into CSF, illustrated in Figure 7. Following ICV or intrathecal drug administration, the concentration of the drug in brain parenchyma diminishes exponentially with each mm of distance removed from the ependymal surface of brain. Usually, the drug level in brain is only 1–2% of that in CSF at just 1–2 mm from the surface (Boado et al. 2003).

![Image of Ommaya reservoir and CSF flow](Image)

**Figure 7:** Drug delivery through intrathecal administration (Figure adapted from Kapil et al. 2010a)

1.3.3.4 Intranasal drug administration

The neural connections between the nasal mucosa and the brain provide a unique pathway for the delivery of therapeutic agents to the CNS. Intranasally administered therapeutic agents reach the CNS via the olfactory and trigeminal neural pathways (Figure 8). Both the olfactory and trigeminal nerves innervate the nasal cavity, providing a direct connection with the CNS (Jogani et al. 2008). One obvious advantage of this method vis-à-vis other strategies is that it is noninvasive.
Figure 8: Intranasal drug delivery to brain (Figure adapted from Kapil 2010a)

Intranasal delivery has been used to target a wide variety of drugs to the CNS. Drugs which have successfully been delivered intranasally to the CNS include zolmitriptan, lidocaine hydrochloride, ropinirole, beta-amyloid (A-beta) peptidogloid mesylate, buspirone hydrochloride, hypocretin, sodium hyaluronan, olanzapine, progesterone, estradiol, gastrin, tacrine, risperidone, etc. Intranasal delivery works best for potent therapeutic agents that are active in the nanomolar range. Even drugs which are substrates for the P-gp efflux transporter operatin the nasal epithelium, have been reported to reach the CNS in efficacious concentrations (Hanson & Frey 2008).

Nonetheless, there have been certain difficulties that need to be overcome to achieve successful brain drug delivery through nasal route, like an enzymatically active and low pH nasal epithelium, and possibility of mucosal irritation, possibility of large variability caused by nasal pathology, such as common cold.

1.3.3.5 Liposomal targeting

The work that has been most accomplished in the domain of brain targeting liposomes has been carried out with PEGylated immunoliposomes. Liposomal carriers access the brain from blood via receptor mediated transcytosis and deliver their content (small drug molecules, plasmid) into the parenchyma, without damaging the BBB. This requires the presence of receptor specific targeting ligands at the tip of 1-2% of the PEG 2000 strands. Targeting ligands are peptidomimetic monoclonal antibodies, i.e., able to trigger activation of receptors (transferrin or insulin receptors) that are highly expressed on the brain capillary endothelium. These antibodies, directed against ex
receptor epitopes, do not interfere with the natural ligand binding sites, thus avoiding competition. Colloidal carriers should have diameter less than 100 nm to fit the loading capacity of these transport systems. Because immunoliposomes are not able to sustain the release of transported compounds, they require frequent administrations to exhibit an extended pharmacological effect (Olivier 2005).

Owing to the above-mentioned potential of liposomes in brain targeting, a variety of therapeutic molecules like dopamine hydrochloride, adenovirus, temovastatin, tamoxifen, doxorubicin, rivastigmine and 5-flourouracil have been successfully targeted to the CNS employing these carriers.

1.3.3.6 **Biochemical blood brain disruption**

Biochemical disruption of BBB is a potentially safer technique of brain drug delivery (Misra 2004). Selective openings of brain tumor capillaries by intracarotid infusion of leukotriene C4 is achieved without concomitant alteration of the adjacent BBB. The biochemical opening utilizes a novel observation that normal brain capillaries appear to be unaffected when vasoactive leukotriene treatments are used to increase their permeability. However, brain tumor capillaries or injured brain capillaries appear to be sensitive to treatment with leukotrienes, and the permeation is dependent on molecular size.

An extensive account of various drug delivery technologies employed for effective brain targeting has recently been published by us (Kapil et al. 2010a).

1.3.3.7 **Nanoparticulate drug delivery**

Nanoparticles are solid colloidal particles, ranging in size from 1 to 1000 nm, and consisting of various macromolecules in which drugs could be adsorbed, entrapped, or covalently attached (Singh et al. 2008b). Generally administered by the intravenous route like liposomes, they have been developed for the targeted delivery of therapeutic or imaging agents. Their stellar merits over liposomes comprise, low number of excipients used in their formulations, the simple procedures for preparation, a high physical stability, and the possibility of sustained drug release that may be suitable in the treatment of chronic diseases. Strategies for nanoparticles targeting to the brain rely on their interaction with specific receptor-mediated transport systems in the BBB (Lockman et al. 2002).
Nanoparticles, made of polybutylcyanoacrylate (PBCA) have been intensively investigated for delivering drugs to brain. Among other biodegradable polymers employed for production of nanoparticles, polymers derived from glycolic acid and from D,L-lactic acid enantiomers are presently the most attractive compounds owing to their biocompatibility and resorbability through natural pathways. Because polylactic acid (PLA) and polylactide-co-glycolide (PLGA) are hydrophobic polymers, lipophilic drugs are easier to formulate (in dissolved state) in PLA/PLGA nanoparticles, than hydrosoluble ones. Despite the water-in-oil in water solvent evaporation technique, the entrapment of hydrophilic drugs may be a challenge due to the drug diffusion from the inner to the outer aqueous phases promoted by the large surface area developed. Basically, drug entrapment efficiency depends on the solid-state drug solubility in the polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end-functional groups (Olivier 2005).

Initially, however, targeting of nanoparticles to the brain proved unsuccessful when administered intravenously. Failure of nanoparticles to reach the CNS in appreciable quantity was due to the uptake by the RES, also known as the mononuclear phagocytic system. The RES is a collective group of mononuclear cells originating from bone marrow that have phagocytic responsibility in removing small foreign particles from the vascular space. While the cells are found throughout the body, a high number of cells are localized in the liver (Kupfer cells), spleen, and bone marrow. The RES significantly removes a large portion (upto 80-85%) of nanoparticles from the vascular space, limiting the exposure of NPs at the cerebrovasculature and thus resulting in decreased drug concentration in the brain (Lockman et al. 2002).

The solution to this problem of rapid uptake of nanoparticles by the RES is coating with surfactants like polaxamine 908 and polysorbate-80. Reducing the uptake of nanoparticles by liver and other organs of the RES will result in increased residence time of the drug in circulation and increased uptake in non-RES organs. Polaxamine 908 and polysorbate 80 used as a surfactant on hydrophobic NPs was shown to reduce RES uptake in the liver when compared with uncoated drug. As a result, surfactant-coated polymeric nanoparticles have been successfully utilized
for intravenous brain targeting of drug compounds characterized by poor brain diffusion as doxorubicin, nerve growth factor, gemcitabine, tacrine, chloramphenicol, rivastigmine, melatonin, dalargin, and methotrexate.

1.3.3.7.1 Solid lipid nanoparticles

There are various hassles associated with the use of these polymeric nanoparticles like residual contamination from the production process, e.g., by organic solvents, polymerization initiation, large polymer aggregates, toxic monomers and toxic degradation products. Other problems include the expensive production methods, a lack of large scale production method and a suitable sterilization method like autoclaving (Muller et al. 2000b). Considering the success of nanoparticles to pass through the BBB and their limitation(s) especially toxicity and stability, a better option developed for drug delivery into the brain is solid lipid nanoparticles (SLNs). The SLNs constitute an attractive colloidal drug carrier system (Kaur et al. 2008). The SLNs consist of spherical solid lipid particles in the nanometer range, which are dispersed in water or in aqueous surfactant solution. They are generally made up of solid hydrophobic core having a monolayer of phospholipid coating. The solid core may contain the drug dissolved or dispersed in the solid high melting fat matrix with the hydrophobic end of the phospholipid chains embedded in the fat matrix.

The SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes while simultaneously avoiding their disadvantages (Polt et al. 1994). The advantages of SLNs are enlisted in Box 2.

1.3.3.7.2 Formulation of SLNs

Many approaches for SLN preparation exist (Mehnert & Mader 2001; Manjunath et al. 2005). The most common methods consist in high pressure homogenization at elevated or low temperatures, microemulsion, solvent emulsification–evaporation or –diffusion, w/o/w double emulsion and high speed stirring and/or sonication. High pressure homogenizations at elevated temperatures and microemulsification are among the most versatile techniques, and for this reason, have been largely employed for SLN preparation.
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Box 2: Advantages of solid lipid nanoparticles

- SLNs particularly those in the range of 120 – 200 nm are not taken up readily by the cells of the RES (Reticulo Endothelial System) and thus bypass liver and spleen filtration (Chen et al. 2004).
- SLNs can achieve controlled release of the incorporated drug for up to several weeks (Muller et al. 1995; zur Muhlen et al. 1998; Muller et al. 2000a). Further, by coating with ligands or attaching them to SLNs, the scope of drug targeting significantly augments (Lockman et al. 2003).
- SLN formulations stable for even three years have been developed. This is of paramount importance with respect to the other colloidal carrier systems (Diederichs & Muller 1994; Freitas & Müller 1998).
- SLNs offer high drug payload.
- SLNs offer excellent reproducibility with cost effective preparation procedures.
- SLNs offer tremendous scope for incorporating drugs of diverse nature, i.e., hydrophilic as well as lipophilic drugs.
- SLNs are biodegradable and hence safe (Siekmann & Westesan 1992; Yang et al. 1999a; Tabatt et al. 2004).
- SLNs offer feasibility of large scale production and sterilization.

Briefly, the high pressure homogenization method regards the production of a lipid and drug melt, which is added to an aqueous surfactant solution at the same temperature. Then, a pre-emulsion is produced by high shear stirring and subsequently processed by a high pressure homogenizer at controlled temperature for several cycles and finally cooled down. In contrast, the microemulsion method consists in the formation of a warm microemulsion containing about 10% melted lipid, 15% surfactant and up to 10% cosurfactant. The microemulsion is then poured upon stirring in a large volume of cold water and the exceeding water is eliminated by ultra-filtration or lyophilization. Both these techniques, avoiding the use of organic solvents, do not lead to residue contaminations. SLN metallic particle contamination, which may occur by using sonication, is avoided as well. Moreover, the elevated temperatures used do not represent an issue because of the limited time of exposure. Another fundamental advantage of these two methods is the scale up feasibility as high pressure homogenization easily allows a laboratory,
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pilot or large scale production. In fact, 50 to 150 kg of SLN dispersions per hour can be obtained by placing two homogenizers in series. Various lipids, surfactants and co-surfactants can be employed for preparation SLNs employing the microemulsification technique.

1.3.3.7.2.1 Selection of lipidic carrier

Various characteristics of lipid matrices used for the production of SLNs for i.v. administration are summarized as Box 3.

Box 3: Desirable characteristics of lipids employed for formulating SLNs

- Capability of producing small size particles (in the nanometer size range) with low content of micro particles (>5 μm)
- Sufficient loading capacity for lipophilic and possible also hydrophilic drugs
- Suitability for sterilization by autoclaving
- Stability in aqueous dispersions, on long-term storage, on lyophilization or spray drying
- Toxicological acceptance and should not leave any toxic residues from the production process (e.g., solvents)

Various lipids (matrix materials) used for the production of SLNs are tristearin, tripalmitin, trilaurin, hard fat or cetylpalmitate (Westesen & Bunjes 1995). SLNs prepared using lipids of less ordered crystal lattices such as glycerylmonostearate and glycerylbehenate favor successful drug inclusion, compared to those prepared using highly ordered crystal packing lipids such as beeswax, cetylpalmitate, tripalmitate and solid paraffin. However, their long-term stabilities are quite different. Within glycerides, the best physical stability was obtained for tripalmitate followed by tribehenin, and is due to the presence of 15% of monoglycerides in tribehenin that possess the surfactant properties. On the other hand, glycerylmonostearate is extremely unstable and considerable particle growth takes place within a few days of preparation. This is attributed to the presence of 50% of monoglycerides in glycerylmonostearate, which are responsible for their physical destabilization (Jenning & Gohla 2000; Jenning & Gohla 2001).
In another study, SLNs prepared using triglycerides like tripalmitin exhibited limited drug payloads and drug expulsion from the crystal lattice, because of high crystallinity of these particles. Although the crystal order was slightly disturbed by mixing different solid lipids (e.g., trimyristin and tristearin), no improvement in drug loading capacity was observed. Further, high drug loading is accomplished by greatly disturbing the crystal order, using mixture of miscible medium chain glyceride oils with solid long chain glycerides to prepare novel SLNs carriers, termed as nanostructured lipid carriers (NLC). Concept of mixing liquid and solid lipids successfully circumvented limited drug-loading capacity of conventional SLNs; however, a partial loss of controlled release properties was observed and is attributed to the decrease in diffusion length of the matrix due to the adherence of oil droplets between the lipid platelets of NLC (Jenning et al. 2000).

Important point to be considered in the selection of drug carrier system is its loading capacity and also the intended use, for instance complex glycerides like hard fats are not suited for controlled release applications because these particles melt at body temperature. Lipophilicity of the glyceride increases as the hydrocarbon chain length increases. Therefore, lipophilic drugs are better soluble in lipid melts of longer fatty acid chain lengths. However, other factors like degree of crystallinity were to be considered in selection of the lipids for formulation of SLNs. Loading capacity of the drug in the lipids depends upon the factors such as miscibility, solubility of drug in lipid melt, physical and chemical structure of the lipid matrix and polymorphic state of lipid materials. Lipids that form highly crystalline particles with a perfect lattice (e.g., monoacid triglycerides) cause drug expulsion. More complex lipids (mixtures of mono, di, and triglycerides containing fatty acids of different chain length) form less perfect crystals with many imperfections. These imperfections provide space to accommodate the drugs.

Melting point of the lipid has influence on average particle size of SLNs dispersion. SLNs prepared by high-pressure homogenization showed increasing average particle size with higher melting point lipids. Purity of the lipids is important to produce SLNs of good quality. Impurities may alter the zeta potential of formulation and there by stability can be affected.
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1.3.3.7.2.2 Selection of emulsifier

Emulsifier should be nontoxic, compatible with other excipients, capable of producing desired size with minimum amount used, and also provide adequate stability to the SLNs, by covering their surface. Another aspect to be considered in the selection of emulsifier is its in vivo fate; for instance, the poloxamer series provide long-circulating properties to SLNs by preventing reticulo endothelial system (RES) uptake, which allows passive targeting whereas polysorbate 80 coated SLNs improved drug delivery to brain targeting (Muller & Wallis 1993; Olbrich et al. 2002).

From literature, it is evident that the type and amount or emulsifier, method of preparation, and sterilization by autoclaving influence the size of the particles and also their stability. The amount of the emulsifier should be optimum to cover the surface of the nanoparticles. Lesser amounts of emulsifier result in particle aggregation and lead to increase in particle size. However, use of excess amount of emulsifier should be avoided to prevent decrease in entrapment efficiency, burst release as observed in case of release studies of SLNs and also toxic effects associated with surfactants. SLNs stabilized with surfactant mixtures (Lipoid S 75/poloxamer 188 or tyloxapol/lecithin) have lower particle size and higher storage stability.

Particle size of SLNs dispersions stabilized with nonionic surfactants is generally larger than those obtained with ionic surfactants. The combination of nonionic surfactants with lecithin also increased the particle size. The combined use of two or more emulsifying agents appears to produce mixed surfactant films at the interface. These mixed surfactants cover the surface efficiently as well as produce sufficient viscosity to promote the stability (Trotta et al. 2003). Uner et al. (2004) studied the influence of surfactants on the physical stability of SLNs formulation and found that 1.5% Tegocare 450 was the most effective stabilizer for the witepsol E 85 SLNs dispersion compared to Tween 80, tyloxapol and Pluronic F68. All these studies indicate that there is no ideal emulsifier and hence, selection of emulsifier should be made by taking the lipid matrix's nature into consideration.
1.3.3.7.3 Prolongation of brain retention of SLNs

The body distribution of SLNs is strongly dependent on their surface characteristics vis-à-vis size, surface hydrophobicity, surface mobility etc. The SLNs have been proposed as suitable system to deliver hydrophilic drugs like diminazine and also for other BCS class IV drugs like paclitaxel, vinblastine, camptothecin, etoposide, cyclosporine etc (Yang et al. 1999a; Yang et al. 1999b; Cavalli et al. 2000; Chen et al. 2001). These carriers can gain access to the blood compartment easily (because of their small size and lipophilic nature) but the detection of these particles by the cells of the reticuloendothelial system (RES) i.e., the mononuclear phagocytic system; MPS cells of the liver (Kupffer) and that of spleen macrophages is a major limitation for their use. Uptake of nanoparticles by RES could result in therapeutic failure due to insufficient pharmacological drug concentration build up in the plasma and hence at the BBB. To overcome these limitations various researchers have tried to increase the plasma half-life of SLNs by the following methods.

1.3.3.7.3.1 Particle size of SLNs

The size and the deformability of particles play a critical role in their clearance by the sinusoidal spleens of humans and rats. Particles must be either small or deformable enough to avoid the splenic filtration process at the interendothelial cell slits (IES) in the walls of venous sinuses. The IES in sinusoidal spleens provides resistance to flow through the reticular meshwork. The endothelial cells of the sinus wall have two sets of cytoplasmic filaments: a set of loosely associated tonofilaments and a set of filaments tightly organized into dense bands in the basal cytoplasm containing actin and myosin, which can probably vary the tension in the endothelial cells and, hence, the size of IES. However, the slit size rarely exceeds 200 to 500 nm in width, even with an erythrocyte in transit. Hence, retention of blood cells and blood-borne particles at the IES depends on their bulk properties, such as size, sphericity, and deformability. These cell slits are the sites where erythrocytes containing rigid inclusions (e.g., Heinz bodies, malarial plasmodia) are believed to be “pitted” of their inclusions, which are eventually cleared by the red pulp macrophages. Therefore, the size of an engineered long circulatory particle should not exceed 200 nm ideally. If larger, then the particle
must be deformable enough to bypass IES filtration. Alternatively, long-circulating rigid particles of greater than 200 nm may act as splenotropic agents and removed later on, if they are not rigid (Moghimi et al. 1993; Moghimi et al. 2001). Hence SLNs of size below 200 nm have an increased blood circulation and thus an increase in the time for which the drug remains in contact with BBB and for the drug to be taken up by the brain (Gohla & Dingler 2001; Chen et al. 2004; Oyewumi et al. 2004).

1.3.3.7.3.2 Surface coating

The high rates of RES mediated detection and clearance of colloidal carriers by liver, significantly reduce the half-life of the drug. The interaction of the colloidal carriers with blood plasma proteins (opsonins) and thus with the membranes of macrophages (opsonization) is believed to be the major criteria for clearance of these systems from the blood stream. Hence to prevent this clearance and to increase their availability at the target site the RES removal of these particulate systems should be prevented. This RES recognition can be prevented by coating the particles with a hydrophilic or a flexible polymer and/or a surfactant. The RES mediated detection and clearance by the liver is believed to be facilitated by the MPS cells. Opsonins, including complement proteins, apolipoproteins, fibronectin and Igs interact with specific membrane receptors of monocytes and tissue macrophages, resulting in their recognition and thus phagocytosis. It is generally admitted that hydrophobic surfaces promote protein adsorption and that negative surfaces activate the complement system and coagulation factors, any shielding of hydrophobic character of the nanoparticles is thus going to stearically stabilize them by providing a dense conformational cloud and thus reducing opsonization and phagocytosis as well as uptake by neutrophilic granulocytes, thus increasing the blood circulation time and hence the bioavailability (Olivier 2005). Coating with polyethylene glycol (PEG), a polymer of hydrophilic nature showed promising results. PEG has high hydrophilicity, chain flexibility, electrical neutrality and lack of functional groups, preventing it from interacting unnecessarily with the biological components. It has been suggested that the PEG’s with a molecular weight between 2000 to 5000 are necessary to suppress plasma protein adsorption; further it has been observed that the thicker the coat, the slower the clearance, and hence a better protection against liver uptake (Chen...
et al. 2001; Kreuter 2001; Oyewumi et al. 2004). Enlarging the molecule/particle slows down kidney ultrafiltration and, thereby allowing better accumulation into the brain and other permeable tissues by the passive enhanced permeation and retention mechanism. It also provides protein shielding which reduces proteolysis within the serum and tissues, and hinders immune surveillance of surface epitopes. Pegylation improves the pharmacokinetic profile of molecules by reducing opsonization, phagocytosis and clearance by the liver and reticulaendothelial system. Other hydrophilic molecules which have been tried are Brij 78, Poloxamer F68 and Brij 68. Cavalli et al. found that parenteral administration of nanoparticles of paclitaxel was more bioavailable than an i.v. injection of the plain drug (Cavalli et al. 2000).

The chemical nature of the overcoating surfactant is of importance, as only polysorbate-coated particles were found to show results in CNS pharmacological effect while a coating with poloxamers (184, 188, 388, or 407), poloxamine 908, Cremophors (EZ or RH40) or polyoxyethylene(23)-laurylether was not effective (Olivier et al. 1999). The reported mechanism of action was the transport of polysorbate-coated nanoparticles across the BBB via endocytosis by the brain capillary endothelial cells. This endocytosis would be triggered by a serum protein, apolipoprotein E, reported to adsorb on polysorbate 20, 40, 60, or 80-coated nanoparticles after a 5-min incubation in citrate-stabilized plasma at 37 °C, but nanoparticles coated with poloxamers 338, 407, Cremophor EL, or RH 40 (Kreuter 2001) could not cross the BBB. The delivery of the drugs to the brain by nanoparticles made of polybutylcyanoacrylate (PBCA) and coated with the nonionic surfactant polysorbate 80 has been intensely investigated (Harris & Chess 2003; Goppert & Muller 2005). Similarly, polysorbates are investigated to have the highest potential to deliver the solid lipid nanoparticles to the brain (Vasir et al. 2005).

1.3.3.7.3.3 Use of ligands

Ligands or homing devices that specifically bind to surface epitopes or receptors on the target sites, can be coupled to the surface of the long-circulating carriers. Certain cancer cells overexpress certain receptors, like folic acid (over-expressed in cells of cancers with epithelial origin), LDL (B16 melanoma cell line shows higher
expression of LDL receptors) and peptide receptors (such as somatostatin analogs, vasoactive intestinal peptide, gastrin related peptides, cholecystokinin, leutanising hormone releasing hormone). Attaching suitable ligands for these particular receptors on to the nanoparticles would result in their increased selectivity (Pardridge 2002; Tiwari & Amiji 2006). Allen et al. (2003) postulated that the presence of specific ligands on the surface of nanoparticles could lead to their increased retention at the BBB and a consequent increase in nanoparticles concentration at the surface of BBB. Thole et al. (2002) reported better interaction with brain endothelial cells and higher intracellular accumulation of stearically stabilized colloidal particles coupled to cationized albumin as compared to bovine serum albumin. Further the cationized albumin is taken up into the brain endothelia via a caveolae mediated endocytic pathway. Intact antibodies have been used as highly specific targeting agents with a high affinity towards their targets. The antibodies act as Trojan horses for delivery of nanoparticles across the BBB. The use of peptidomimetic antibodies which can bind to BBB transcytosis receptor; brain-targeted pegylated immunonanoparticles are also being proposed, such that, the delivery of entrapped actives into the brain parenchyma can be achieved without inducing BBB permeability alteration. Similarly delivery to the brain using nanoparticulate drug carriers in combination with the novel targeting principles of “differential protein adsorption (Path Finder Technology)” has been reported (Muller & Keck 2004). The path finder technology exploits protein in the blood which adsorb onto the surface of intravenously injected carriers for targeting. Apolipoprotein E is one such targeting moiety as discussed above, for the delivery of particles to the endothelials of the BBB. These technologies can also be explored for their feasible application for improving the brain targeting of SLNs.

1.4 BIOENHANCED DRUG DELIVERY SYSTEMS

Oral delivery of over one-half of the drug compounds through gastrointestinal (GI) tract gets thwarted owing to their high lipophilicity and consequently poor aqueous solubility (Singh et al. 2009b). Various oral drug delivery approaches like solid dispersions, inclusion complexes, fast dissolving tablets, lipidic suspensions, emulsions and self-emulsifying systems and gastroretentive systems have been employed for improvement of oral bioavailability of such drug compounds.
1.4.1 Buccal drug delivery systems

The oral cavity is an attractive site for drug delivery due to ease of administration and avoidance of possible drug degradation in the GI tract and first-pass metabolism. There are four potential regions for drug delivery in the oral cavity: namely buccal, sublingual, palatal, and gingival. Buccal drug delivery specifically refers to the delivery of drugs within/through the buccal mucosa to affect systemic pharmacological actions. Buccal-delivered drugs may be used for the treatment of diseases in the oral cavity or for systemic use (Hao & Heng 201).

1.4.1.1 Anatomy and physiology of buccal mucosa

Oral mucosa is lined with an epithelium supported by a connective tissue lamina propria and separated from the epithelium by a basal membrane. The epithelium of oral mucosa is stratified with regional variation in terms of structure and function. Three types of oral mucosa are referred to as masticatory, lining, and specialized mucosa. The physiological structure of buccal mucosa is illustrated in Figure 9. Small vessels and capillaries that open to the internal jugular vein distribute within the lamina propria, thus avoiding the hepatic first-pass clearance of buccal-delivered drugs. Blood flow in the oral mucosa is generally far richer than that in the skin (Veuillez et al. 2001). The non-keratinized mucosa was reported to have approximately a thickness of 500 - 600 μm and a surface area of 50.2 cm².

![Figure 9: Schematic representation of physiological structure of buccal mucosa](image-url)
The secretion of saliva from salivary glands features regional, individual, and time variations. The buccal region contains minor salivary glands. The mucus layer covers the oral mucosal surface and serves to lubricate and protect as well as to act as a wetting agent. Mucin is a group of glycoproteins composed of oligosaccharide side chains attached to a protein core.

Three-quarters of the protein core are heavily glycosylated and impart a gel-like characteristic to mucus. The remaining nonglycosylated groups are involved in cross-linking via disulfide bonds among mucin molecules. Mucus is negatively charged at physiological saliva pH of 5.8–7.4 because of the presence of sialic acids (pKa 2.6) and ester sulfates at the terminals of some pendant oligosaccharide side chains.

### 1.4.1.2 Drug delivery to buccal mucosa

Absorption of drug via the mucous membranes of the oral cavity can occur in either the sublingual, buccal, or local regions. The local region includes all areas other than the former two regions. However, the main similarities, differences, and applications of each membrane are described in this section. In general, the oral mucosa is classified as a somewhat leaky epithelium with a permeability rank order of sublingual > buccal > palatal, based on the thickness and degree of keratinization of the tissues.

Sustained-release systems, which are able to provide sustained drug concentrations in the systemic circulation due to delayed release of the drug from the formulation, are suitable dosage forms for the buccal region of the oral cavity. The lower permeability of this region compared to the sublingual site is ideal for controlled-release systems. Additionally, drug delivery via this site avoids extensive enzyme degradation and first-pass metabolism seen with oral administration, which are desired outcomes for the delivery of therapeutic proteins and peptides. However, the low permeability of this site is not always an attractive feature and, depending on the choice of drug, can be a major limitation. Use of sub-toxic levels of penetration enhancers and targeted delivery may potentially overcome this problem in the buccal region of the oral cavity. Local delivery in the oral cavity has had particular applications in the treatment of toothache, periodontal diseases, and bacterial infections. However, because of its
specificity, local delivery does not have the broad range of applications sublingual and buccal drug administration provides.

1.4.1.3 Formulation considerations

1.4.1.3.1 Physiological aspects

The buccal mucosa has a very limited area for application of the buccal delivery system, thus limiting device size and drug load. The actual area for absorption depends on the size of the dosage form. Generally, a device with a size of 1 - 4 cm² and a daily dose of 25 mg or less would be preferred for buccal delivery.

The epithelial layer is not uniformly hydrophobic and has two possible penetration routes, the transcellular route and the paracellular route (Figure 10).

![Figure 10: Schematic representation of penetration routes during buccal delivery](image)

The lipophilic drugs penetrate mainly through the more lipophilic transcellular route, while the less lipophilic paracellular route, characterized by loosely packed polar intercellular lipids, is the principle route for hydrophilic drugs. Both routes coexist for all drugs, but the route with the least penetration resistance is usually preferred over the other, depending on the physicochemical properties of drugs.

However, smaller paracellular route area limits the penetration of hydrophilic drugs, whereas lipophilic drugs usually have high penetration rates through transcellular routes. Nonionized nicotine permeates mainly via the transcellular pathway while the mono- and di-protonized molecules permeate via paracellular pathway (Nielsen & Rassing 2002). Variation in saliva pH
influence the serum levels of nicotine after administration of nicotine chewing gum (Henningfield et al. 1990).

The secretion of saliva is affected by disease and various stimuli. An acidic excipient can stimulate the secretion of saliva, which is an important consideration in selecting formulation excipients. Saliva has a weak buffering capacity to maintain pH value within local regions. Saliva contains no proteases but moderate levels of esterases, carbohydrases, and phophatases, which may degrade certain drugs. Although saliva secretion facilitates the dissolution of drug, involuntary swallowing of saliva can result in drug loss from the site of absorption. Also, the non-uniform distribution of drugs within saliva on release from delivery systems implies that some areas of the oral cavity might not receive therapeutic levels of drugs (Weatherell et al. 1994), which is thus an important concern in the development of a locally administered buccal drug delivery system. The mucus layer with the thickness in the range from 1 to 400 mm forms a physical barrier to drug permeation through buccal mucosa but can be advantageous for bioadhesive preparations. Short turnover time of mucus layer is detrimental to achieve long-term bioadhesion and sustained drug release (Khanvilkar et al. 2001). Drugs can interact with mucin through electrostatic attractions (e.g., tetracycline), hydrogen bonding (e.g., urea), or hydrophobic interaction (e.g., testosterone) and prevent their transport through the epithelia.

Pathological aspects

Diseases can make the epithelium thicker (hyperplastic) or thinner (atrophic) than normal or even lost (ulcerated). These alter the barrier property of the mucosa and thus increase the permeability of the mucosa, which will tend to facilitate local delivery of drugs for treatment of mucosal diseases. However, this complicates the application of bioadhesive delivery devices for retention and controlled release of drugs. The marked changes at the mucosal surface may include increased desquamation or sloughing, a factor which is detrimental to maintaining bioadhesion. Diseases are also likely to influence the mucus secretion and properties, thus affecting the drug transport and bioadhesive properties. Cancer patients often develop oral candidosis and a substantial decrease in salivary flow after irradiation treatment. The bioadhesive buccal tablet containing miconazole
has been shown to be effective in treatment of infections, but its efficacy was substantially influenced by low saliva secretion (Bouckaert et al. 1996). It is important to understand the nature of mucus under relevant disease conditions before designing effective buccal delivery systems when the mucus layer affects the drug transport to the mucosal surface.

1.4.1.3 Pharmacological aspects

The intended application and target site of drug affect the selection of dosage form. For treatment of oral disease, the residence time and local concentration of the drug in the mucosa are important considerations. For a systemic effect, the amount of drug transported across the mucosa into the circulatory system is a determinant of dosage forms. A diagnostic agent can be delivered to assist the diagnosis of oral mucosal cancer. 5-Aminolevulinic acid and its esters in the form of rinse have been used in photodynamic diagnosis and photodynamic therapy (Fan et al. 1996).

Despite the possible GI and hepatic degradation, buccal delivery confers many advantages for delivering peptide/protein and other drugs that encounter degradation after oral administration (Jay et al. 2002). Several peptides, including thyrotropin-releasing hormone, insulin, octreotide, leuprolide, and oxytocin have been delivered via the buccal route.

1.4.1.4 Pharmaceutical aspects

Factors influencing drug release and penetration within/through buccal mucosa will affect the therapeutic efficacy and should be considered in the formulation design. As the dosage form is to be resident in a highly developed taste-sensing organ, careful considerations for organoleptic factors are needed. Excipients enhancing palatal properties are often required to improve acceptability of dosage form or masking less desirable properties of the bioactive constituent. Some additives can be incorporated to improve drug release pattern and absorption.

1.4.1.4.1 Penetration enhancers

Buccal penetration enhancers are capable of decreasing penetration barrier of the buccal mucosa by increasing cell membrane fluidity, extracting the structural intercellular and/or intracellular lipids, altering cellular proteins, or altering
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mucus structure and rheology. Penetration enhancement may be only drug 
specific at a certain application site. This is particular to the buccal membrane 
since it is non-keratinized and the intercellular lipids are less structured compared 
to the skin stratum corneum. The buccal mucosa is multilayered without tight 
junctions, and effective penetration enhancers for transdermal and/or intestinal 
drug delivery may not have similar effects on buccal drug delivery. Currently, the 
most commonly investigated penetration enhancers include bile salts, fatty acids, 
and sodium lauryl sulfate. It is well known that bile salts play an important role as 
physiological surfactants in the absorption of lipids and lipid-soluble vitamins. 
Bile salts have been extensively employed to enhance the absorption of drugs 
through various epithelia including buccal membranes.

Purified oleic acid was reported to modify, the barrier property of buccal mucosa, 
and thus led to remarkable and continuous hypoglycemia effect after buccal 
delivery of insulin from Pluronic gel (Morishita et al. 2001). Cod-liver oil extract, 
which is a mixture of 16 types of unsaturated and saturated acids, showed 
enhancement effect on ergotamine tartrate through buccal mucosa of guinea pigs 
(Tsutsumi et al. 2002).

Irritation and toxicity are always concerns with penetration enhancers, although 
the oral mucosa is more resistant to damage than other mucosal membranes. To 
date, the information available on buccal absorption enhancement is much less 
than that for transdermal enhancement. The relationships among structure, 
irritation, and enhancement effect of the enhancer have not been clearly 
elucidated. Very few penetration enhancers are available for buccal delivery 
systems, and penetration enhancers are not used in marketed buccal delivery 
systems owing to the lack of a satisfactory profile with respect to irritation and 
effectiveness.

1.4.1.3.4.2 Enzyme inhibitors

Among mucosal routes, the buccal mucosa has relatively low enzymatic activity, 
and drug inactivation is neither rapid nor extensive. However, enzymes in saliva 
and buccal mucosa can still degrade drugs, particularly peptide/protein drugs. 
Enzyme inhibitors such as aprotinin, bestatin, puromycin, and bile salts stabilize 
protein drugs by affecting activity of proteolytic enzymes, altering the
conformation of the peptide drug or forming micelles, and/or rendering the drug less accessible to enzymatic degradation. Particularly, as peptide/protein drugs are transported through the paracellular route, the cytosolic peptidases may be inaccessible for the drugs during their permeation. Peptidase activity on the surface of the porcine buccal mucosa has also been investigated (Walker et al. 2002).

1.4.1.3.4.3 Bioadhesives

The buccal regions are very suitable for a bioadhesive system because of a smooth, relatively immobile surface and accessibility. The major advantages of bioadhesive systems are the increased residence time of drug device in the oral cavity and localization of drugs in a particular region. The bioadhesion process has been explained by electronic, adsorption, wetting, diffusion, and fracture theories. The interaction between the mucus and bioadhesive polymers is a result of physical entanglement and secondary bonding, mainly hydrogen bonding and van der Waals attractions. These forces are related to the chemical structure of the polymers. The functional groups available on the surface of polymer conformation favoring bioadhesion include hydroxyls, carboxyls, amines, and amides. The bioadhesive polymer must have a critical molecular weight and an adequate length to allow chain interpenetration. Anionic polymers are usually preferred due to negatively charged mucin at physiological pH. The general characteristics, which a buccoadhesive polymer should possess, are summarized as Box 4.

1.4.1.3.4.4 Solubility modifiers

Despite the increased bioavailability of hepatically metabolized drugs by buccal delivery, poor solubility of drug in saliva may impede drug release from its device for uptake by buccal mucosa. Solubilization of a poorly water-soluble drug by complexing with cyclodextrin and delivering via the buccal mucosa is advantageous in increasing drug absorption and bioavailability. Buccal tablets of danazol-sulfobutylether 7β-cyclodextrin complex were prepared using different polymers and were evaluated for bioadhesion, in vitro release, and bioavailability in female beagle dogs (Jain et al. 2002). The buccal-administered danazol-sulfobutylether 7β-cyclodextrin complex and the danazol-sulfobutylether 7β-cyclodextrin polycarbophil tablets had absolute bioavailabilities of 64% and 25%,
respectively, which are significantly greater than 1.8% observed for the commercial formulation, Danocrine. The increased bioavailability was attributed to the enhanced solubility due to complexation and the avoidance of extensive hepatic metabolism upon buccal administration. Imidazole antimycotics (e.g., miconazole, econazole, and clotrimazole) are extensively used in the local treatment of fungal infections in the oral cavity. Due to their low water solubility and high lipophilicity, they were released extremely slowly from the lipophilic chewing gum base. Formulating hydroxypropyl-β-cyclodextrin inclusion complex of these antimycotics into chewing gums was found to increase the drug release from the chewing gums (Jacobsen et al. 1999).

**Box 4: Desirable characteristics of buccoadhesive polymers**

- Non-toxicity, non-irritancy and free from leachable impurities
- Good spreadability, wettability, swellability, solubility and biodegradability
- Biocompatibility and good viscoelasticity
- Quickly adherence to buccal mucosa and sufficient mechanical strength
- Good peel, tensile and shear strengths at the bioadhesive range
- Easy availability and low cost
- Bioadhesive properties in both dry and liquid state
- Local enzyme inhibition and penetration enhancement properties
- Acceptable shelf life
- Optimum molecular weight
- Adhesively active groups
- Spatial conformation
- Sufficiently cross-linked but not to the degree of suppression of bond forming
- Inertness against secondary infections such as dental caries

### 1.4.1.4 Types of buccal DDS

#### 1.4.1.4.1 Buccal tablets

Tablets have been the most commonly investigated dosage form for buccal drug delivery to date (Salamat-Miller et al. 2005). Buccal tablets are small, flat, and oval, with a diameter of approximately 5-8 mm. Unlike conventional tablets, buccal
mucoadhesive tablets allow for drinking and speaking without major discomfort (Rathbone et al. 1994). They soften, adhere to the mucosa, and are retained in position until dissolution and/or release is complete. These tablets can be applied to different sites in the oral cavity, including the palate, the mucosa lining the cheek, as well as between the lip and the gum. Successive tablets can be applied to alternate sides of the mouth. The major drawback of buccal bioadhesive tablets is their lack of physical flexibility, leading to poor patient compliance for long-term and repeated use.

If necessary, the drug may be formulated in certain physical states, such as microspheres, prior to direct compression in order to achieve some desirable properties, e.g., enhanced activity and prolonged drug release (Giunchedi et al. 2002). Some newer approaches use tablets that melt at body temperatures. The matrix of the tablet is solidified while the drug is in solution. After melting, the drug is automatically in solution and available for absorption, thus eliminating dissolution as a rate-limiting step in the absorption of poorly soluble compounds.

1.4.1.4.2 Buccal patches

Patches are laminates consisting of an impermeable backing layer, a drug-containing reservoir layer from which the drug is released in a controlled manner, and a bioadhesive surface for mucosal attachment. Buccal patch systems are similar to those used in transdermal drug delivery. Two methods used to prepare adhesive patches include solvent casting and direct milling. In the solvent casting method, the intermediate sheet from which patches are punched is prepared by casting the solution of the drug and polymer(s) onto a backing layer sheet, and subsequently allowing the solvent(s) to evaporate. In the direct milling method, formulation constituents are homogeneously mixed and compressed to the desired thickness, and patches of predetermined size and shape are then cut or punched out. An impermeable backing layer may also be applied to control the direction of drug release, prevent drug loss, and minimize deformation and disintegration of the device during the application period.

1.4.1.4.3 Buccal films

Films are the most recently developed dosage form for buccal administration.
Buccal films may be preferred over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which are easily washed away and removed by saliva. Moreover, in the case of local delivery for oral diseases, the films also help protect the wound surface, thus helping to reduce pain and treat the disease more effectively. An ideal film should be flexible, elastic, and soft, yet adequately strong to withstand breakage due to stress from mouth movements. It must also possess good bioadhesive strength in order to be retained in the mouth for the desired duration of action. Swelling of film, if it occurs, should not be too extensive in order to prevent discomfort.

Buccadhesive films are similar to laminated patches in terms of their flexibility and manufacturing process. They are usually manufactured by a solvent casting method. The drug and polymer(s) are first dissolved in a casting solvent or solvent mixture. The solution is then cast into films, dried, and finally laminated with a backing layer or a release liner. The backing layer helps retard the diffusion of saliva into the drug layer, thus enhancing the adhesion time and reducing drug loss into the oral cavity.

1.4.1.4.4 Buccal gels and ointments

Semisolid dosage forms, such as gels and ointments, have the advantage of easy dispersion throughout the oral mucosa. However, drug dosing from semisolid dosage forms may not be as accurate as from tablets, patches, or films. Poor retention of the gels at the site of application has been overcome by using bioadhesive formulations. Certain bioadhesive polymers, e.g., poloxamer 407 (Miller & Donovan 1982), sodium carboxymethylcellulose (Wong et al. 1999), Carbopol (Kumar et al. 1994), hyaluronic acid (Gurny et al. 1990), and xanthan gum (Meseguer et al. 1993), undergo a phase change from a liquid to a semisolid. This change enhances the viscosity, which results in sustained and controlled release of drugs. However, these polymers have been investigated for this purpose primarily in ocular drug delivery.

Hydrogels are also a promising dosage form for buccal drug delivery. They are formed from polymers that are hydrated in an aqueous environment and physically entrap drug molecules for subsequent slow release by diffusion or
erosion (Cui & Mumper 2003). It has been suggested that hydrogels with mucoadhesive polymers might be useful for periodontitis therapy when incorporated in antimicrobial-containing formulations that are easily introduced into the periodontal pocket with a syringe (Jones et al. 2000; Vinholis et al. 2001; Ikinci et al. 2002). Mucoadhesion helps ensure formulation retention within the pocket.

1.4.2 Gastroretentive drug delivery systems

The absorption of the drug candidate from the GI tract is dictated by the location of the dosage form in the GI tract and the GI content. Some drugs are more efficiently absorbed from the upper part of GI tract while others are absorbed from the lower parts of GI tract. Therefore, in instances where the drug is not absorbed uniformly over the entire GI tract, the rate of drug absorption may not be constant despite the DDS delivering the drug at a constant rate into the GI fluids (Waterman 2007). Many drugs, categorised as once-a-day delivery, have been demonstrated to have suboptimal absorption due to dependence on the transit time of the dosage form, making traditional extended release development challenging. Therefore, a system designed for longer gastric retention will extend the time within which drug absorption can occur in the small intestine.

Longer residence time in the stomach could be advantageous for local action in the upper part of the small intestine, for example, in the treatment of peptic ulcer disease. The GRDDS extends significantly the period of time over which the drugs may be released. Thus, they not only prolong dosing intervals, but also increase patient compliance beyond the level of existing CR dosage forms. This application is especially effective in delivery of sparingly soluble and insoluble drugs. The GRDDS greatly improves the pharmacotherapy of the stomach through local drug release, leading to high drug concentrations at the gastric mucosa (eradicating Helicobacter pylori from the submucosal tissue of the stomach), making it possible to treat stomach and duodenal ulcers (Bardonnet et al. 2006; Badhan et al. 2009; Jain & Jangdey 2009; Shah et al. 2009), gastritis and oesophagitis, reduce the risk of gastric carcinoma and administer non-systemic, CR antacid formulations (calcium carbonate). In addition, by continually supplying the drug to its most efficient site of absorption, the dosage forms allow for more effective oral use of peptide and protein drugs such as calcitonin, erythropoietin, low-molecular-weight heparin,
protease inhibitors and luteinising hormone-releasing hormone analogues (Luessen et al. 1996; Guggi et al. 2003; Schmitz et al. 2005; Venkatesan et al. 2006).

Many drugs show poor bioavailability (BA) in the presence of intestinal metabolic enzymes like cytochrome P450 (CYP3A), abundantly present in the intestinal epithelium (Nishimura et al. 2007; Thummel 2007; Choi et al. 2010). Their activity decreases longitudinally along the small intestine, with levels rising slightly from the duodenum to the jejunum and declining in the ileum and colon. This nonuniform distribution of CYP3A causes regional variability in the absorption of drugs that are the substrates of those enzymes.

There are certain situations where gastric retention is not desirable. Aspirin and non-steroidal anti-inflammatory drugs are known to cause gastric lesions, and slow release of such drugs in the stomach is unwanted.

1.4.2.1 **Factors affecting efficacy of gastroretentive systems**

Various attempts have been made to retain the dosage form in the stomach or small intestine as a way of increasing the retention time as described above. Among these, the floating dosage forms have been used most commonly. However, most of these approaches are influenced by a number of factors that affect their efficacy as a GI retentive system (Mojaverian et al. 1988; Timmermans & Moes 1994; Singh & Kim 2000):

- **Density:** Gastric retention time (GRT) is a function of dosage form buoyancy that is dependent on the density.
- **Size:** Dosage form units with a diameter of more than 7.5 mm are reported to have an increased GRT compared with those with a diameter of 9.9 mm.
- **Shape of dosage form:** Tetrahedron and ringshaped devices with a flexural modulus of 48 and 22.5 kilopounds per square inch (KSI) are reported to have better GRT ~ 90% to 100% retention at 24 h compared with other shapes.
- **Fed or unfed state:** Under fasting conditions, the GI motility is characterised by periods of strong motor activity or the migrating myoelectric complex (MMC) that occurs every 1.5 to 2 h.
- **Nature of meal:** Feeding of indigestible polymers or fatty acid salts can change the motility pattern of the stomach to a fed state, thus decreasing the gastric
emptying rate and prolonging drug release.

- **Caloric content:** GRT can be increased by 4 to 10 h with a meal that is high in proteins and fats.

- **Frequency of feed:** the GRT can increase by over 400 min when successive meals are given compared with a single meal due to the low frequency of MMC.

- **Gender:** Mean ambulatory GRT in males (3.4 ± 0.6 h) is less compared with their age and race-matched female counterparts (4.6 ± 1.2 h), regardless of the weight, height and body surface.

- **Age:** Elderly people, especially those over 70, have a significantly longer GRT.

- **Posture:** GRT can vary between supine and upright ambulatory states of the patient.

- **Concomitant drug administration:** Anticholinergics like propantheline and atropine, opiates like codeine and prokinetic agents like metoclopramide and cisapride.

### 1.4.2.2 Pharmacokinetic advantages of gastroretentive systems

The various pharmacokinetic advantages of gastroretentive systems are enumerated as under:

1. **Enhanced bioavailability:** The bioavailability of riboflavin and levodopa CR-gastroretentive system is significantly enhanced in comparison to administration of non-CR polymeric formulations (Klausner *et al.* 2002; Hoffman *et al.* 2004; Kagan *et al.* 2006). This can be primarily attributed to localization of the drug in its absorption window and reduced P-glycoprotein (P-gp) activity in the duodenum

2. **Reduced frequency of dosing:** For drugs with a relatively short biological half-life, sustained and slow input from CRGRDF may result in a flip-flop pharmacokinetics and enable reduced dosing frequency. This feature is associated with improved patient compliance, and thereby improves therapy.
3. **Reduced fluctuations of drug concentration**: Continuous input of the drug following CR-GRDF administration produces blood drug concentrations within a narrower range compared to the immediate-release dosage forms.

4. **Targeted therapy for local ailments in the upper GI tract**: The prolonged and sustained administration of the drug from the GRDF to the stomach may be advantageous for local therapy in the stomach and the small intestine. By this mode of administration, therapeutic drug concentrations may be attained locally, while the systemic concentrations, following drug absorption and distribution, are minimal.

### 1.4.2.3 Pharmacodynamic advantages of gastroretentive systems

The various pharmacodynamic advantages of gastroretentive systems are enumerated as under:

1. **Improved selectivity in receptor activation**: Minimization of fluctuations in drug concentration also makes it possible to obtain certain selectivity in the elicited pharmacological effect of drugs that activate different types of receptors at different concentrations.

2. **Reduced counter-activity of the body**: In many cases, the pharmacological response, which intervenes with the natural physiologic processes, provokes a rebound activity of the body that minimizes drug activity. Slow input of the drug into the body was shown to minimize the counter-activity leading to higher drug efficiency.

3. **Minimized adverse activity at the colon**: Retention of the drug in the GRDF at the stomach minimizes the amount of drug that reaches the colon. Thus, undesirable activities of the drug in colon may be prevented.

### 1.4.2.4 Gastroretentive technologies

A number of techniques have been pursued to increase the gastric retention of dosage forms by employing a variety of concepts such as floating, swelling,
inflation and adhesion (Stieubel et al. 2006; Arza et al. 2009; Murphy et al. 2009; Kotrek & Adeyeye 2011). These systems have been classified according to the basic principles of gastric retention, as in Figure 11.

Figure 11: Flow chart depicting classification of GRDDS

1.4.2.4.1 Floating drug delivery systems

The concept of floating drug delivery systems (FDDS) was described in the literature as early as in 1968, when Davis disclosed a method for overcoming the difficulty experienced by some persons of gagging or choking while swallowing medicinal pills (Davis 1968). Since then, several approaches have been used to develop an ideal floating delivery system. The various buoyant preparations include hollow microspheres (microballoons), granules, powders, capsules, tablets (pills), and laminated films. Most of the floating systems reported in literature are
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single-unit systems, such as the hydrodynamically balanced systems (HBS) (Ali et al. 2007) and floating tablets. These systems are unreliable and irreproducible in prolonging the residence time in the stomach when orally administered, owing to their all-or-nothing emptying process.

An FDDS have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time (Ichikawa et al. 1991). While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system (Jain et al. 2008a). After release of drug, the residual system is emptied from the stomach. This results in an increased GRT and a better control of the fluctuations in plasma drug concentration. The increase in the GRT thus achieved could improve BA, reduce drug wastage and result in a better control of fluctuations in the plasma drug concentrations (Hu et al. 2010b; Hu et al. 2011).

1.4.2.4.1 Non-effervescent FDDS

Non-effervescent floating properties of DDS can be based on several principles, including low density due to swelling and inherent low density. In case of low density due to swelling, the system swells unrestrained via imbibition of gastric fluid to an extent that it prevents its exit from the stomach. Another type of FDDS, hydrodynamically balanced system (HBS), is a floating single-unit dosage form with sustained drug release consisting of a capsule, which contains a mixture of drug and hydrocolloids. Following contact with gastric fluid, the systems take up water and swell. As the increase in volume is more than the increase in mass during swelling, the densities of these devices decrease, acquiring a value of less than unity. Thus, after a certain lag time the systems start to float. The influence of several processing and formulation parameters on the floating properties of this type of matrix tablet has been studied by different research groups (Chaturvedi et al. 2010; Chen et al. 2010; Garse et al. 2010; Singh et al. 2010f). Recently a glycerol mono oleate (GMO) matrix was proposed as a gastroretentive carrier system (Kumar et al. 2004).

The HBS must comply with three major criteria (Cargill et al. 1988).

- It must have sufficient structure to form a cohesive gel barrier.
• It must maintain an overall specific gravity lower than that of gastric contents i.e., 1.004-1.010 g/mL.

• It should dissolve slowly enough to serve as a “reservoir” for the delivery system.

1.4.2.4.1.2 **Effervescent FDDS**

1.4.2.4.1.2.1 **Gas generating systems**

These are matrix types of systems prepared with the help of swellable polymers such as methylcellulose and chitosan and various effervescent compounds, e.g., sodium bicarbonate, tartaric acid, and citric acid. They are formulated in such a way that when in contact with the acidic gastric contents, CO₂ is liberated and gets entrapped in swollen hydrocolloids, which provides buoyancy to the dosage forms.

1.4.2.4.1.2.2 **Volatile liquid containing systems**

The gastric retention time (GRT) of a DDS can be sustained by incorporating an inflatable chamber, which contains a liquid, e.g., ether, cyclopentane that gasifies at body temperature to cause the inflation of the chamber in the stomach. These gastro-inflatable DDS are osmotically controlled floating systems containing a hollow deformable unit that can convert from a collapsed to an expanded position, and returns to the collapsed position after an extended period.

1.4.2.4.1.2.3 **Intragastric osmotically controlled DDS**

It consists of an osmotic pressure-controlled drug delivery device and an inflatable floating support in a bioerodible capsule. When the device reaches the stomach, bioerodible capsule quickly disintegrates to release the DDS. The floating support is made up of a deformable hollow polymeric bag containing a liquid that gasifies at body temperature to inflate the bag. The osmotic pressure controlled drug delivery device consists of two compartments:

• A drug reservoir compartment
• An osmotically active compartment

The drug reservoir compartment is enclosed by a pressure-responsive collapsible
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bag, which is impermeable to vapors and liquids and has a drug delivery orifice. The osmotically active compartment contains an osmotically active salt and is enclosed within a semipermeable housing.

In stomach, the water in the gastric fluid is continuously absorbed through the semipermeable membrane into the osmotically active compartment to dissolve osmotically active salt. An osmotic pressure is thus created, which acts as a collapsible bag, and in turn forces the drug reservoir compartment to reduce its volume and activate the release of the drug solution formulation through the delivery orifice. The floating support is also made to contain a bioerodible plug that erodes after a predetermined time to deflate the support. The deflated DDS is then excreted from the stomach.

1.4.2.4.2 Advantages of FDDS

An FDDS offers numerous advantages over conventional DDS:

✓ **Sustained drug delivery**: An FDDS can remain in the stomach for several h and therefore significantly prolong the GRT of numerous drugs. The assumed prolongation in the gastric retention is postulated to cause sustained drug release behavior and offers the advantage in having uniform and consistent blood levels of medication by administrating a single dose of medication, which releases active ingredient over an extended period of time (Fix et al. 1993; Singh et al. 2011c).

✓ **Site-specific drug delivery**: Targeting of drug to stomach appears to be useful for all substances intended to produce a lasting local action on the gastroduodenal wall. For instance, the eradication of *Helicobacter pylori* requires the administration of various medications several times a day resulting in poor patient compliance (Adebisi & Conway 2011).

✓ **Pharmacokinetic advantages**: Any solute released in the stomach will empty together with fluids and have the whole surface of small intestine available for absorption. This should particularly be useful when an absorption window exists in proximal small intestine. In addition, with the total GI transit duration is increased, a greater amount of drug may be delivered and thus the relative BA will consequently be increased (Khan & Dehghan 2011;
Introduction

Singh & Chaudhary 2011).

1.4.2.4.3 Disadvantages of FDDS

In addition to the afore-mentioned advantages, floating drug delivery systems also suffer from some disadvantages described as under:

× The main disadvantage of floating systems is that they require sufficiently high levels of fluid in the stomach for the DDS to float therein and work efficiently. However, this can be overcome by administrating the dosage form with a glass full of water (200-250 mL) with frequent meals or by coating the dosage form with bioadhesive polymers, thereby enabling them to adhere to the mucous lining of the stomach wall.

× Drugs that are unstable and destroyed in the gastric environment are poor candidates for FDDS.

× Drugs that are irritant to the gastric mucosa or induce gastric lesions are not good candidates for FDDS.

× Drugs that are absorbed throughout the GI tract should be discarded for FDDS as prolonging the GRT of such drugs appears to offer no advantage in terms of BA.

× Poorly acid soluble drugs may show dissolution problem in gastric fluid and, consequently may not be released to a sufficient extent. It might, therefore, be advisable not to exploit FDDS with these drugs.

× Finally, for the selective delivery of a few drugs in the colon, e.g., corticosteroids, 5-aminosalicylic acid, prolonging the GRT through FDDS may prove inferior to other specifically designed oral colonic DDS.

1.4.2.4.4 High density systems

The density of a DDS is an important factor influencing the gastric residence time. High-density devices use their weight as a retention mechanism. When the density of the system is larger than that of the gastric juice, the device settles down to the bottom of the stomach, remaining located below the pylorus (Murphy et al. 2009). Commonly used excipients are barium sulphate, zinc oxide, titanium dioxide and
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Iron powder, etc. These materials increase density by up to 1.5-2.4 g/cm³. However, no successful high density system has been marketed.

1.4.2.4.5 Bioadhesive drug delivery systems

Bioadhesive DDS (BDDS) are used to localize a delivery device within the lumen to enhance the drug absorption in a site-specific manner. This approach involves the use of bioadhesive polymers, which can adhere to the epithelial surface in the stomach.

1.4.2.4.5.1 Bioadhesion

Intimate contact between the delivery system and mucosa will improve both the effectiveness and efficiency of the delivery system. Bioadhesion in simple terms can be described as the attachment of a synthetic or biological macromolecule to a biological tissue. An adhesive bond may form either with the epithelial cell layer, the continuous mucus layer or a combination of the two (Chowdary & Srinivas 2000; Dodou et al. 2005). The term mucoadhesion is used specifically when the bond involves mucous coating and an adhesive polymeric device, while cytoadhesion is the cell-specific bioadhesion. Adhesion between mucin and mucoadhesive polymers is usually analyzed based on the molecular attractive and repulsive forces.

Mucoadhesives are synthetic or natural polymers which are capable of interacting with biological materials and being retained on them for prolonged period of time. Bioadhesive or mucoadhesive DDS utilize the bioadhesive property of certain water soluble polymers that become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body (Woodley 2001; Hombach & Bernkop-Schnurch 2010).

1.4.2.4.5.1.1 Mechanism of bioadhesion

The mechanisms of bioadhesion have not fully elucidated. To develop a good adhesive system, it is important to understand the forces responsible for adhesive bond formation. Two steps have been described in adhesive bond formation:

1. Intimate contact of the polymer by wetting
2. Interpenetration and diffusion between the polymeric bioadhesive and
mucosa.

Bioadhesive bonds formed can be either mechanical or chemical in nature.

1.4.2.4.5.1.2 Bioadhesive polymers

Polymers that can adhere to either hard or soft tissues are employed for the purpose of bioadhesion. These are generally the polymers with numerous hydrophilic function groups that can form hydrogen bonds, e.g., carboxyl, hydroxyl, amide and sulfate groups. In the presence of water, hydrogen bonding appears to play a significant role in mucoadhesion. Some mucoadhesive polymers can hydrate in an aqueous media to form a gel.

The mucoadhesives used in oral drug delivery should meet the following requirements:

- Adhesiveness with the mucus layer, to provide adequate contact
- Ability to swell and allow drug release
- Ability to prolong the residence time of the drug at the site of administration
- Lack of interaction with the active drug, to allow the drug to be released and absorbed through the mucosal surface
- Biocompatibility with the mucosal surface, to avoid cytotoxicity or other irreversible alterations of the mucosal surface
- Biodegradability, to allow the physical clearance of the mucosal surface
- Remain unaffected by hydrodynamic conditions, food and pH
- Stability on storage or during the shelf-life of dosage form
- Cost-effectiveness

Bioadhesive polymers are classified on the basis of their charge as per Scheme 1. On the basis of polymer charge, the following important conclusions can be made:

- Cationic and anionic polymer binds more effectively than neutral polymers.
- Polyanions are better than polycations in terms of binding/potential toxicity and further, water insoluble polymers give greater flexibility in dosage form design.
- Anionic polymers with sulfate group bind more than with carboxylic groups.
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Degree of binding is proportional to the charge density of the polymer.

Scheme 1: Classification of bioadhesive polymers on the basis of charge

<table>
<thead>
<tr>
<th>Cationic</th>
<th>Anionic</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Polybrene</td>
<td>• Na CMC</td>
<td>• Bovine serum albumin</td>
</tr>
<tr>
<td>• Poly-L-lysine</td>
<td>• Dextran sodium</td>
<td>• Dextran</td>
</tr>
<tr>
<td>• Polyllysine</td>
<td>• Poly acrylic acid</td>
<td>• Ficoll</td>
</tr>
<tr>
<td></td>
<td>• Poly-L-aspartic acid</td>
<td>• Polyethylene glycol</td>
</tr>
<tr>
<td></td>
<td>• Polystyrene sulfonic</td>
<td>• Polyethylene pyrrolidone</td>
</tr>
<tr>
<td></td>
<td>acid</td>
<td>• Methylcelluloses and their</td>
</tr>
<tr>
<td></td>
<td>• Polyvinyl sulfate</td>
<td>derivatives</td>
</tr>
<tr>
<td></td>
<td>• Heparin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Hyaluronic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Poly glutamic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• γ-Carageenan</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Chondrointin sulfate</td>
<td></td>
</tr>
</tbody>
</table>

These bioadhesive polymers are having different characteristics like:

- **Hydrophilic polymers** are water soluble polymers that swell when in contact with water and eventually undergo complete dissolution. These polymers show high bioadhesiveness to the mucosa in the dry state, but the bioadhesive nature deteriorates as they start dissolving.

- **Hydrogels cross linked polymers** swell when they come in contact with water. The extent of swelling depends on the degree of cross linking. When mobile at the wet mucosal surface they orientate various carboxylic groups present in them towards mucosa and interpenetrate deeper into the mucosa.

- **Thermoplastic polymers** are hydrophobic polymers and include both bioerodible and non-bioerodible polymers. Absorption studies have shown that mucin has strong affinity for hydrophobic surfaces. Additionally, these polymers are non-toxic and non-irritant.

1.4.2.4.5.2 Factors affecting bioadhesion

Many factors can affect the bioadhesive strength of the polymer; some being dependent on the surrounding media while the others on nature of the polymer.
The adhesiveness of a bioadhesive polymer is determined by its intrinsic polymeric properties and the environment in which it is placed, and is influenced by several factors.

### 1.4.2.4.52.1 Bioadhesive polymer related factors

**Molecular weight:** Numerous studies have indicated that there is a certain molecular weight at which bioadhesion is maximum. The interpenetration of polymer molecules is favoured for low molecular weight polymers, whereas physical entanglement is favoured for high molecular weight polymers. The optimum molecular weight for maximum bioadhesion depends on the type of polymer.

**Concentration of the active polymer:** There is an optimum concentration of polymer that corresponds to the best bioadhesion. For solid dosage forms, a direct relationship between polymer concentration and adhesive strength has been reported (Duchene et al., 1988).

**Flexibility of polymer chains:** Flexibility is important for interpenetration and entanglement. As water-soluble polymers become cross-linked, the mobility of individual polymer chain decreases. As the cross-linking density increases, the effective length of the chain which can penetrate into the mucus layer decreases even further thereby reduces the mucoadhesive strength.

**Spatial conformation:** Besides molecular weight or chain length, spatial conformation of a molecule is also an important factor for bioadhesion. Despite high molecular weight of 19.5 megadaltons for dextrans, they have similar bioadhesive strength to that of PEG with molecular weight of 0.2 megadaltons.

### 1.4.2.4.52.2 Environment related factors

**pH:** It has a notable effect on mucoadhesion. It influences the charges on the surface of both the mucus and the polymer. This is due to the differences in the dissociation of functional groups present on the polymer and on the glycoprotein portion of the mucus.

**Initial contact time:** This determines the extent of swelling and interpenetration of the polymer chain. The mucoadhesive strength increases as the initial contact time...
increases.

*Initial pressure:* The pressure initially applied to the mucoadhesive-tissue contact site can affect the depth of interpenetration. If high pressure is applied for a sufficiently long period of time, polymers become mucoadhesive even though they do not have attractive interaction with mucin.

*Swelling:* Swelling depends both on polymer concentration and on the presence of water. Interpenetration of chains is easier as polymer chains are disentangled and free of interaction. However, when swelling is too great, a decrease in bioadhesion occurs due to the formation of slippery surface.

*Selection of model substrate surface:* Some changes (physical or biological) may occur in the mucus gel or tissues under experimental conditions thus making handling and treatment of biological substrates during testing of mucoadhesive as important.

1.4.2.4.5.2.3 *Physiological factors*

Physiological factors like mucin turn over rate and the condition of mucosal layer (normal or diseased) also at times influences mucoadhesion.

1.4.2.4.6 *Advantages of bioadhesive DDS*

Bioadhesive dosage forms have numerous advantages described as under:

- **Stability:** A drug experiences a pH range of 1-8 across the GI tract, a particular drug may be stable at one pH and unstable at another. Bioadhesive dosage forms by localizing the drug to an optimal site of its maximal stability tend to increase stability of certain drugs. Also, these systems by disallowing a proper contact of drug with food components may protect the former from the attack by latter.

- **Improved bioavailability:** Penetration enhancers and enzyme inhibitors could be brought in close proximity to the site of action, resulting in an improved bioavailability. Many drugs are subject to extensive first-pass metabolism, when administered orally. This can possibly be avoided by employing other routes of administration, utilizing the concept of bioadhesion.
• Peptide delivery: The strong interaction between the polymer and mucous lining of the tissue helps increase the contact time and permit localisation which is an essential factor when modification of tissue permeability is important for delivery of macromolecules, such as peptides and proteins.

• Ocular sustained release preparation: Upon administration to the eye, there is an extensive drug loss due to a highly efficient precorneal loss process. Besides the dilution of the drug in the cul-de-sac by the tears, a subsequent drainage results in a comparatively lowered contact time of the drug with the absorbing tissue. To overcome this problem, bioadhesive polymeric substances can be used which adhere to the precorneal surface through non-covalent bonds and increase the duration of interaction between the absorbing tissue and the drug (Kumar & Himmelstein 1995).

• Mucosal protection: Bioadhesive dosage forms could protect the GI mucosa from ulceration caused by the NSAIDs.

• Local action: Bioadhesives could provide effective means of increasing the local therapeutic effects of many drugs thus aiding in drug targeting by the non-invasive routes.

1.4.2.4.7 Disadvantages of bioadhesive DDS

Apart form the above-mentioned advantages, bioadhesive DDS also suffer from some disadvantages mentioned below:

× lon/pH sensitivity: Polyanionics as polyacrylic acid are highly sensitive to the ionic environment. Thus, the use of polyacrylates in an ion rich environment may interfere with the adhesive properties of the polymer. Sufficient adhesiveness may be obtained at a specific pH range only. Rheological properties of Carbopol 934 samples were found to be substantially influenced by the environmental pH.

× High viscosity: Since these polymers give a highly viscous solution, the viscosity of the system could impede the delivery of the drug to the absorbing surface.

× Loss of mucoadhesive activity: An increased wetting of the polymer may lead to the formation of non adhesive slippery mucilage. For a drug with a specific site
of absorption, absorption process may cease once the bioadhesive system has moved past the absorption site. Besides, mucoadhesion during the GI transit of the DDS will be limited by a relatively rapid mucus turnover. Thus renewal of the mucus layer may be a limiting factor in bioadhesion. Rapid loss of the bioadhesive properties of DDS may occur by soluble mucins or mucin degradation products.

\[ \textbf{GI irritation:} \] For orally administered products containing irritant drugs, ingestion with an insufficient amount of water may result in the adherence of the dosage form to the esophageal mucosa, thus aggravating the local toxicity of such drugs.

1.4.2.4.8 Swelling and expanding systems

Another approach to retain a dosage form in the stomach is by increasing its size above the diameter of the pylorus, evening the widest state during the housekeeper wave. Due to significant inter-individual variations, the cutoff size cannot be given exactly. Approximately, the dosage form should be > 13 mm; however, even bigger units have been observed to be emptied from the stomach.

1.4.3 Evaluation of GI retention

\textit{In vivo} visualization is a crucial parameter for evaluating the GI tract retention characteristics of the dosage form. To characterize GRDFs, the following techniques, some of which were recently introduced for pharmaceutical applications, have been proposed:

1.4.3.1 Radiology

This method is the state-of-art in preclinical evaluation of gastroretentivity. Its major advantages as compared to \(\gamma\)-scintigraphy are simplicity and cost. However, use of X-ray in biopharmaceutical studies involving healthy volunteers (Tugcu-Demiroz et al. 2004) has declined as the obvious risk dictates strict limitations, regarding the amount of exposure. A commonly used contrast agent is barium sulfate, often used in high concentrations e.g., 40\% or more, for exemplifying tablets. In order to reduce the formulative change while attaining \textit{in vivo} imaging, contrast aluminium threads obtained from surgical gauze pads have been
compounded into GRDFs during their preparation. Radiopaque markers may be applied to study the effects of GRDFs or experimental models of their evaluation on gastric emptying, including negating obstructions (Klausner et al. 2002).

1.4.3.2 Magnetic marker monitoring

This technique, developed by Weitschies et al. (1994) follows magnetically marked dosage forms by magnetic source imaging and therefore requires very sensitive biomagnetic measurement equipment. The method has no radiation exposure and is completely safe (Weitschies et al. 1994). It yields successive data regarding the location of the dosage form along the GI tract and enables assessment of the intragastric location of the dosage form.

1.4.3.3 Ultrasonography

Ultrasonic waves reflected at substantially different acoustic impedances across an interface enable the imaging of some abdominal organs (Robertson & Baker 2001). Ultrasound is not routinely used in oral biopharmaceutics because it does not delineate the intestine. Additionally, most dosage forms do not have sharp acoustic mismatches across their interface with the physiological milieu.

1.4.3.4 γ-scintigraphy

γ-scintigraphy is a technique whereby the transit of a dosage form through its intended site of delivery can be non-invasively imaged in vivo via the judicious introduction of an appropriate short lived γ-emitting radioisotope (Goole et al. 2008; Perkins et al. 2008; Goodman et al. 2010; Kapil et al. 2010b; Stockis et al. 2010). The observed transit of the dosage form can then be correlated with the rate and extent of drug absorption. Information such as the site of disintegration or dispersion can also be obtained. The stellar advantages of γ-scintigraphy over the earlier methods include the following:

- Gives very little radiation exposure to the participating subjects compared to roentgenography (X-rays)
- Gives both qualitative as well as quantitative results which is not possible with other techniques
- Is totally non-invasive
Introduction

- Allows for the *in vivo* evaluation of dosage forms under normal physiological conditions

γ-scintigraphy requires the presence of short-lived γ-emitting radioactive isotope in the dosage form that can be detected *in vivo* by an external gamma camera. γ-scintigraphy combined with knowledge of physiology and dosage form design can help define these variables. The resulting insight can be used to accelerate the formulation development process and help ensure success in early clinical trials. Products with little chance of attaining their goals can be abandoned earlier. The performance of viable products can be clearly demonstrated to regulatory authorities or potential investors.

1.4.3.4.1 Selection of radioisotopes

The most commonly used radionuclides to correlate GI behavior of the dosage form are Technetium-99m (\(^{99m}\)Tc) and Indium-111 (\(^{111}\)In). Using these two isotopes and the conventional labeling process, simple dosage forms, such as direct compression tablets and capsules, can be easily monitored for their *in vivo* behavior.

1.4.3.4.2 Methods of radiolabeling

The radiolabeling can be achieved either by direct incorporation of a radioactive tracer into the preparation (conventional methods), or neutron activation of a dosage form that contains a non-radioactive tracer. The quantity of tracer to be incorporated into a formulation to render it suitable for γ-scintigraphic study is very small and does not compromise the performance of the delivery system.

**Direct incorporation:** In conventional methods, γ-emitting isotope is incorporated into the dosage form of interest prior to its manufacture. The nuclide (\(^{99m}\)Tc or \(^{111}\)In) is added, in liquid form, to an aliquot of one of the excipients present in the formulation. The resulting mixture is dried and incorporated into the formulation. This radiolabeled dosage form is administered via its appropriate route and followed externally with gamma camera. This method is non-specific and does not involve the direct radiolabeling of the molecule. This method can be used to radiolabel tablets, capsules, suppositories, aerosols, liquids and enemas. However, their application is limited to a simple dosage form made in small-scale
introduction

batches. The conventional method is not suitable for complex dosage form, such as a sustained-release or delayed-release formulations, which require long manufacturing times or unique manufacturing equipment. Production scale batches cannot be radiolabeled by conversation methods because of large amounts of radioactive isotopes needed and the radiation exposure to the manufacturing personnel.

It is possible to label the drug directly by substitution of a radioactive atom for a native atom in the molecule. A related approach is to use radioisotopes whose chemical and physical properties are similar to the rest atom. This approach has been used to radionlabel aluminium containing antacids with a radioisotope of indium since both atoms occur in group IIIb of the periodic table. The second method is to radiolabel an inert marker whose physical behavior mimics that of the drug. Often, the materials used are ion-exchange resins, particles, or solutions, which are not absorbed, e.g., chelates of diethylenetriaminepentaacetic acid (DTPA) with $^{99m}$Tc or $^{111}$In.

Neutron Activation $\gamma$-Scintigraphy: $\gamma$-radiation-emitting isotopes used in scintigraphy can be either intrinsically active (Technetium) or intrinsically stable isotopes that are subsequently activated. The most suitable radioactive isotope for many GI drug delivery systems is $^{153}$Sb, which can be incorporated in dosage forms as samarium oxide. Samarium oxide is virtually insoluble in the GI tract. It can be activated in the thermal neutron flux of a nuclear reactor to the $^{153}$Sb isotope, which emits $\gamma$-radiation. Such radiation is similar to X-rays and can be imaged using a clinical gamma camera.

In the neutron activation method, small amount (µg or mg) of an appropriate stable isotope ($^{153}$Sb, $^{138}$Ba or $^{171}$Er) is incorporated into formulation similar to that of any other excipient prior to the manufacture of the dosage form. Once manufactured, the dosage form is exposed to a neutron source (nuclear reactor), and the stable isotope is converted to a radioactive $\gamma$-emitting isotope ($^{153}$Sb, $^{138}$Ba or $^{171}$Er) that can be easily followed by a gamma camera. Formulations are imaged using a gamma camera 48 hours after neutron activation.

1.4.3.4 Choice of devices for $\gamma$-scintigraphy

Nuclear imaging is mostly conducted with Planar or SPECT (Single Photon
Introduction

Emission Computed Tomography) cameras and by using radionuclides that emit γ-radiation that emit energies between 100 and 250 KeV. The gamma camera is composed of an array of photomultiplier tubes coupled to a sodium iodide crystal. The interaction of a γ-photon from the source with the crystal leads to the production of flash and detected by photomultiplier tubes. Gamma camera imaging can be carried out using two alternative methods: static imaging in which single acquisitions are stored, and dynamic imaging in which a sequence of data of varying frame time can be obtained.

1.4.3.4 Limitations of γ-scintigraphy

A limitation of the technique of γ-scintigraphy is that very little anatomical information is gained, unless the formulation outlines easily recognize organs, such as the stomach and large bowel. When non-disintegrating matrix systems are studied, identification of the position of the object becomes difficult, and it is necessary to administer a second radiopharmaceutical to outline the GI tract. A radionuclide with a different energy is chosen, and it is usually better to use a lower energy than that used to label the preparation, for e.g., a solution of 99mTc-DTPA administered with a tablet labeled with 111In. when the softer isotope is used to mark the tablet together with the indium as a liquid marker, there is scatter-down of energy from the indium into the technetium channel, which has to be corrected. The correction is made by subtracting a fixed proportion of one channel from the other.

Other limitations include, the inability to accurately quantify the activity in the small bowel because of its coiled nature, it is not possible to label all the compounds/drugs of interest, the γ-scintigraphic assembly is expensive, and the method requires qualified personnel for operation. The continuous exposure to natural sources of radiation, cosmic rays and naturally occurring radionuclides, needs a mention (Jain et al., 2005).

1.4.4 Drug release from gastroretentive systems

Mucoadhesive polymers that are generally employed for the purpose are hydrophilic in nature. The mechanism of release of drug from hydrophilic matrices has been reviewed by Korsemeyer et al. (1983b). When swellable
Introduction

Hydrophilic dosage forms come in contact with aqueous medium, various events that take place are:

1. Partial hydration of the polymer followed by the dissolution of the drug at the surface, resulting in an immediate release.
2. Penetration of the solvent molecules into free spaces present on the surface between macromolecular chains.
3. If the thermodynamic compatibility of the dissolution medium with the polymer is favourable, the glass transition temperature of polymer is lowered below the room temperature, leading to chain relaxation of the polymer, resulting in swelling and formation of a gel.
4. Water continuously penetrates the matrix, the gel expands and dissolution of soluble solute inside the matrix and after that dissolution of drug through the gel layer takes place.
5. Simultaneously attrition/erosion of outermost layer and release of insoluble particles occurs.
6. Tailing-off period starts when the water reaches the center of the system and the concentration of drug falls below the solubility value. The final stage is characterized by a reduction in the release rate.

Therefore, the release of an active principle by a matrix system is produced by two simultaneous mechanisms (Vazquez MJP et al. 1992).

a) Erosion or attrition of the outermost, least consistent gel layers.

b) Dissolution of the active principle in the liquid medium and diffusion through the gel barrier when formed.

1.4.4.1 Mathematical analysis of drug release

Korsmeyer et al., (1983a) used a simple empirical equation to describe the general solute release behaviour from the CR polymer matrices.

\[ \frac{M_t}{M_\infty} = k t^n \]  ...(1)

To bring about the computation of the kinetic constant (k) and release exponent (n) values for each dosage form unit pertaining to Eqn. 1, regression analysis is accomplished between the logarithm of fractional drug released versus logarithm
Introduction

of time data, as in the Eqn. 2.

\[ \log \frac{M_t}{M_\infty} = \log k + n \log t \]  \hspace{1cm} \textcolor{red}{(2)}

The slope of this log-log relationship fetched the value of \( n \), while the antilogarithm of its intercept yielded the value of \( k \).

The kinetic constant, \( k \), defines the structural and geometric characteristics of the ER delivery device. The value of \( n \) represents the drug release exponent characterizing different release mechanisms (Korsemeyer et al. 1983a; Pepass 1985; Ritger & Peppas 1987a, b).

This mathematical model, also known as the Power Law, has been used very frequently to describe the drug release from DDS (Ahuja et al. 2007; Garg & Gupta 2009; Nagda et al. 2009; Zugasti et al. 2009). For some swellable matrices, departure from Fickian mechanism is seen and the behavior of drug release is termed as non-Fickian. It arises from coupling of the diffusion (Case I) and molecular relaxation (Case II) phenomena (Peppas & Sahlin 1989). Case II transport is characterized by the zero-order release kinetics and a unity value of “\( n \)”. Accordingly, the non-Fickian release behavior of swellable matrices is further analyzed using an equation wherein the diffusion and relaxation mechanisms of transport are considered simultaneously:

\[ \frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \]  \hspace{1cm} \textcolor{red}{(3)}

where, the first term on the right hand side is the Fickian contribution and the second term is the Case-II relaxation contribution. The constants, \( k_1 \) and \( k_2 \), express the respective contributions of the diffusion and the polymer relaxation mechanisms, allowing quantitative evaluation of their importance on overall release. The coefficient \( m \) is purely Fickian diffusion coefficient for device of any shape and its determination is based on the aspect ratio, \( 2a/l \), where, \( a \) is the radius and \( l \) is the thickness of the device (Peppas & Sahlin 1989). The percentages of Fickian and relaxational drug release can also be determined from the values of \( k_1 \) and \( k_2 \). These semi-empirical equations are applied to phenomenological analysis of release behavior from matrix systems and hence are advantageously used to approach the constant release of drug during development of ER matrix
formulations. Numerous reports cite the use of these equations for evaluation of drug release of systems where drug diffusion occurs through the polymeric network, yet these can successfully be employed for spheres and cylinders too. However, use of these equations for analysis of drug release data has to be done judiciously, since such mathematical relationships are used only for systems where the drug diffusion coefficient is clearly concentration independent (Pepass 1985). Scheme 2 enlists values of “n” for varied device geometries indicating the transport mechanism (Ritger & Peppas 1987b; Peppas et al. 2000; Rinaki et al. 2003).

**Scheme 2:** Release exponent and mechanism of drug release from various swellable oral drug delivery systems (Singh & Kapil 2010)

<table>
<thead>
<tr>
<th>Values of Diffusional Release exponent (n) for Varied Device Shapes</th>
<th>Drug transport Mechanism</th>
<th>Rate as a function of time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin films</td>
<td>Cylinders</td>
<td>Spheres</td>
</tr>
<tr>
<td>0.50</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>$0.50 &lt; n &lt; 1$</td>
<td>$0.45 &lt; n &lt; 0.89$</td>
<td>$0.43 &lt; n &lt; 0.85$</td>
</tr>
<tr>
<td>1.00</td>
<td>0.89</td>
<td>0.85</td>
</tr>
<tr>
<td>$n &gt;1.00$</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In a nutshell, the model can be described in its ‘explicative’ form as Figure 12.

### 1.4.4.2 Factors affecting drug release

It has been observed that the release rate of the drug from the matrix system depends on the type of matrix, characteristics of the polymer and the incorporated drug substance, matrix additives and other technological variables. Some of the factors studied extensively by several workers are described below:

#### 1.4.4.2.1 Polymer characteristics

Polymer is the fundamental component of hydrophilic matrix system, which controls the rate of drug release by formation, through hydration, of diffusion and erosion resistant gel layers. Various properties of polymer that affects the release rate are:
Introduction

Oral Drug Delivery System

\[ \frac{M_t}{M_\infty} = k_d t^n \]

Is the system swellable?

Yes: \[ \frac{M_t}{M_\infty} = k_1 t^n + k_2 t^{2n} \]

No

Kinetic constant (k)

Release exponent (n)

Diffusion coefficient (k_1)

Polymer relaxation coefficient (k_2)

n = 0.5

0.50 < n < 1.00

n = 1

n > 1

Fickian diffusion

Non-Fickian transport

Case II transport

Super case II transport

Figure 12: Explicative representation of Korsemeyer-Peppas model (adapted from Singh & Kapil 2010)

Polymer viscosity: Polymers with different molecular weights and degrees of reticulation, exhibiting a range of viscous efficiencies, which affect the drug release are available. It is well established that as the molecular weight or viscosity of the polymer increases, release rate of the drug from the formulation decreases (Lapidus & Lordi, 1966; Vazquez et al., 1992).

Ratio of polymer to drug in dosage forms: The proportion of polymer is usually employed as a control variable of drug release rate. An inverse relationship was reported between polymer to drug ratio and release rate of drug.

Polymer hydration: It is important to study the hydration/swelling process for polymers and polymeric combinations used because swelling of polymer may influence and control the drug release. An inverse relationship is reported between polymer’s ‘constant rate swelling’ and ‘constant rate dissolution’.

Other important properties of polymers include polymer composition and polymer particle size.
1.4.4.2 Drug characteristics

Drug’s characteristics have also shown to play an active role in the swelling behaviour of HPMC and MC matrices. The rate at which the gel layer was produced varied with the addition of drug to the matrix. Various properties of drugs influencing the release rate are:

**Drug solubility:** Hydrophilic matrices have proved to be useful in the formulation of both water-soluble drugs as well as insoluble drugs. The release of water soluble drugs is controlled by diffusion of drug through the gel, and that of insoluble drugs is controlled by erosion of the gel exposing fresh surfaces containing undissolved drug; and for poorly water soluble drugs by a combination of diffusion and gel erosion.

**Drug loading:** Kim and Fassihi (1997) have shown that release rates of diltiazem HCl increased with higher drug loading. This behaviour is attributed to matrix composition, high drug solubility, and drug diffusion which causes greater channel formulation in the swollen matrix at higher drug loadings.

1.4.4.2.3 Other technological variables

**Compression force:** Tortuosity, porosity and density of the compact depends upon the force used in compression. However, several reports indicate that the tablet hardness might influence only the initial phase of drug release (Perioli et al. 2008; Santos et al. 2010). Once the polymer gets hydrated dissolution profiles remain similar with tablets prepared by different compression forces, since the hydrated matrix is independent of initial porosity.

**Temperature:** An increase in the release rate was observed as the temperature of the medium increased. The phenomenon was attributed to increased diffusivity at a higher temperature.

**pH of the hydrophilic matrix and dissolution medium:** The viscosity of the gel barrier formed on tablet surface and the rate of hydration is relatively independent of the environment pH. Release rate of drugs will not be affected by pH unless drug solubility varies greatly over the normal pH range. This can be explained on the basis of the fact that gums are macromolecular acids and are good buffers and
hence, the liquid penetrating the tablet after formation of gel barrier will attain a fairly constant pH in the gel, regardless of original. The addition of agents to modify the pH of the matrix’s near environment has emerged as a mean of adjusting the release rates of active principles with pH dependent solubilities and therefore, optimize, BA.


tublets: Korsmeyer et al. (1983a) indicated that the initial porosity of the hydrated matrix plays an important role and to explain their findings of an inverse relation between initial porosity and the release kinetics of potassium chloride from HPMC matrices, they postulated that the air entrapped in the pores acts as a barrier to the transport of active principle. Hence, release followed the Higuchian pattern only when air was removed from the tablets before subjecting them to dissolution.

Other important technological factors encompass thickness, shape and size of tablet, hydrodynamic conditions of the matrix and manufacturing process.

1.4.4.2.4 Effect of other additives

Effect of lubricant and diluent: The effect of incorporating diluents, both soluble and insoluble, is well documented. These diluents, if present in higher concentrations bring about a marked increase in the release rate of water soluble drugs. This effect has been attributed to an expansion of the gel layer, if the excipients swell and are insoluble or the impossibility of a continuous gel barrier formation, if the matrix incorporates insoluble materials which don’t swell. An increase in the release rate is usually observed on addition of water-soluble diluents like lactose to the matrix. These diluents tend to diffuse outward into the dissolution medium thereby, decreasing the tortuosity of the matrix.

Other polymers: The use of mixtures of polymers represents a potential way of achieving the required release properties. In addition to the possibilities offered by mixing polymers with different viscous efficiencies, e.g., different non-ionic cellulose ethers, to given gel barriers of varying consistency, mixing non-ionic and ionic varieties in the right proportions can lead to formulations of hydroaluble active principles with zero order release profiles. Reports on the use of HPMC and
Carbopol combination to achieve CR are also available.

1.5 EVALUATION OF PHARMACOKINETICS

Pharmacokinetics plays key role in the development of new drugs and drug delivery systems. Notwithstanding the \textit{in vitro} design considerations of an oral CRDDS, its end objective is always pharmacokinetic, i.e., sustenance of the \textit{in vivo} plasma level time profile of the drug in its therapeutic window for extended periods of time. This is usually accomplished by modulating drug release kinetics such that the desired plasma levels of drug are maintained for the desired period (Liu \textit{et al.} 1995; Homero de Souza Filho \textit{et al.} 2010; Khandave \textit{et al.} 2010). Pharmacokinetics, during the last five decades, has emerged from a purely mathematical description of time course of drug concentration in the body to a discipline that is fully integrated with pharmaceutics, mathematical modeling and clinical therapeutics. Usually for such pharmacokinetic studies, the data on the drug levels in biological fluids, at periodic intervals, is processed by a series of equations involving mathematical and statistical precepts. Consequently, the collection, analysis and presentation of pharmacokinetic data have become an essential part of developing not only the new drugs but diverse DDS too. Use and scope of the pharmacokinetic approaches have expanded enormously by encompassing pharmacodynamic vistas, physiologically based modes, nonlinear kinetics, population methods and system theory methods. Box 5 outlines some vital definitions associated with pharmacokinetics and allied sciences.

\textbf{Box 5: Terminology of important terms related to classical pharmacokinetics}

<table>
<thead>
<tr>
<th>Terms and their Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Biodisposition}: Cumulative process of distribution and elimination of drugs from the body</td>
</tr>
<tr>
<td>\textit{Biotransformation}: Chemical alteration of a drug that occurs by virtue of its sojourn in a biological system. Also \textit{drug metabolism}</td>
</tr>
<tr>
<td>\textit{Disposition}: The study of time course of drug distribution to various body tissues, and elimination from the body primarily through renal excretion and biotransformation</td>
</tr>
<tr>
<td>\textit{Model}: A hypothetical structure that can be used to characterize, with reproducibility, the behavior and fate of a drug in a biological system, when given through a certain route of administration and in a specific dosage form</td>
</tr>
<tr>
<td>\textit{Empirical Model}: Mathematical model describing the pharmacokinetic behavior</td>
</tr>
</tbody>
</table>
### Introduction

#### Terms and their Explanation

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Explicative Model:</strong></td>
<td>Schematic flow chart depicting pharmacokinetic behavior of a drug</td>
</tr>
<tr>
<td><strong>Compartment:</strong></td>
<td>An entity described by certain volume and concentration, with maximal intra-compartmental homogeneity and maximum inter-compartmental heterogeneity. Usually, it is a tissue or a group of tissues with similar blood flow rate and drug affinity. Also called as <em>Pool</em>.</td>
</tr>
<tr>
<td><strong>Multi-Compartment Model:</strong></td>
<td>A hypothesis envisioning finite number of well-connected pools</td>
</tr>
<tr>
<td><strong>Lag Time:</strong></td>
<td>Latent period of time elapsed between drug intake and perceptible drug concentration</td>
</tr>
<tr>
<td><strong>Steady state levels, (C_p^\infty):</strong></td>
<td>Relatively invariant plasma drug levels during the equilibrium plateau phase obtained following multiple administration of drug</td>
</tr>
<tr>
<td><strong>(C_{\text{max}}):</strong></td>
<td>An acronym for “peak” drug concentration in plasma/serum, after its administration prior to administration of subsequent dose during single or multidose kinetics, usually in (\mu g/mL)</td>
</tr>
<tr>
<td><strong>(C_{\text{min}}):</strong></td>
<td>Minimum or “trough” concentration of a drug observed after its administration just prior to the administration of a subsequent dose during multidose pharmacokinetics</td>
</tr>
<tr>
<td><strong>Swing:</strong></td>
<td>Proportional rise in plasma drug conc., ((C_{\text{max}} - C_{\text{min}})/C_{\text{min}}) during multidose kinetics</td>
</tr>
<tr>
<td><strong>Therapeutic Window:</strong></td>
<td>Difference between minimum effective and toxic levels of a drug</td>
</tr>
<tr>
<td><strong>Clearance, (Cl):</strong></td>
<td>The rate at which a drug is cleared from a body fluid, e.g., plasma or blood (usually in (L/h) or (ml/min)). Clearance may be from total body ((Cl_b)) or kidneys ((Cl_k)) or liver ((Cl_l))</td>
</tr>
<tr>
<td><strong>Biological Half-Life, (t_{1/2}):</strong></td>
<td>Time (usually in h) for drug levels in the terminal linear phase of plasma conc.-time curve to be halved. Mathematically obtained as (0.693/t_{1/2})</td>
</tr>
<tr>
<td><strong>Urinary Availability, (f_r):</strong></td>
<td>Fraction of drug excreted (%) through renal pathway</td>
</tr>
<tr>
<td><strong>Volume of Distribution, (V_d):</strong></td>
<td>It is volume where a drug appears to distribute, given as the ratio of dose to drug conc. (L or L/Kg). It may be (V_{d_{\text{app}}}, V_{d_{\text{so}}}, V_{d_{\text{area}}}, V_{d_{p}}, V_d, V_{d_{ip}}), etc.</td>
</tr>
</tbody>
</table>

Today, several approaches and computational techniques have been postulated and put into use for estimating pharmacokinetic parameters and simulating the pharmacokinetic performance of a drug from its delivery device. Besides the traditional compartmental modeling approach, these include, the relatively more recent noncompartmental stochastic and system deconvolution approaches (Almeida et al. 2010; Tassaneeyakul et al. 2010; Ullah et al. 2010; Jackson et al. 2011;
Pharmacokinetic parameters like mean residence time (MRT), mean absorption time (MAT), plateau time (half-value duration, $C_{\text{max}}$), %PTF (peak-to-peak fluctuations), % fluctuations in area under curve (AUC) etc. are popularly employed for pharmacokinetic characterization of CR delivery systems. Particularly, the utility of multi-dose pharmacokinetic simulations of CR delivery systems using superposition principle is unparalleled.

### 1.5.1 One-compartment body model

Most drugs make the body "behave" as if it consists of a single pharmacokinetic compartment. Tissue and plasma levels of drug rapidly and simultaneously reach equilibrium in all the tissues to which it is distributed. A plot of plasma level versus time after intravenous administration can be rectified into only a straight line of negative slope, which can intersect the ordinate at only one point indicating only one volume of distribution can be calculated.

Empirically, a one compartment open body model (1-CBM) is represented in Figure 13.

![Drug flow diagram](image)

**Figure 13:** Diagrammatic representation of one compartment body model

### 1.5.2 Noncompartmental pharmacokinetics

Despite the global popularity of compartmental modeling, controversies and confusion have invariably existed for quite sometime regarding its validity and appropriateness for pharmacokinetic data treatment. There have been a number of questions and misgivings about the use of such models. Several disadvantages of compartmental modeling, given below in Box 6, call for the alternative approaches to noncompartmental modeling.

Noncompartmental approaches have developed and evolved as both alternatives and adjuncts to compartmental models. Albeit these methods are hardly novel, their usage for pharmacokinetic data analysis has been novel and significant. Many relationships used in the former practice have been derived originally...
compartmental context for supplemental use in these models.

**Box 6: Potential shortcomings of classical compartmental analysis**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The basic assumption of compartmental modeling, i.e., the body is consisting of relatively few, kinetically homogeneous compartments, seems to be quite unrealistic and difficult to justify from physiological reality.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Unambiguous identification of correct model is often not possible because more than one model of comparable complexity can be consistent with available data.</td>
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<td>Model identification and parameter estimation are confounded by vanishing exponentials.</td>
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<td>Microscopic rate constants do not conform to reality.</td>
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<td>Likelihood of flip-flop situation particularly for drugs with short biological half-life following non-instantaneous administration.</td>
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<td>Differing number of exponential terms are frequently employed in the empirical mathematical expression of pharmacokinetic models.</td>
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<td>Higher models are intricate in expression and comprehension.</td>
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<td>Differing model selection for the same drug in the same or different animals.</td>
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<td>9</td>
<td>Quantitative magnitude of classical pharmacokinetic parameters is a function of the selected model.</td>
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<td>10</td>
<td>There is no parameter to depict the performance of controlled release drug products.</td>
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<td>11</td>
<td>Pharmacokinetic comparison amongst congeneric or non-congeneric drug series can not be pragmatically plausible.</td>
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<td>12</td>
<td>Computation for drugs exhibiting nonlinear pharmacokinetic behavior is tedious.</td>
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<td>13</td>
<td>Incongruity exists for in silico prediction of ADME parameters using Quantitative Structure Pharmacokinetic Relationship (QSPkR) Studies.</td>
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<td>14</td>
<td>Elaborate computer software is almost an imperative requirement.</td>
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<td>15</td>
<td>Only use of effective experimental designs shows optimal usage of data.</td>
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Motivating factors for the development of noncompartmental methods include criticisms of the compartmental approach as discussed above. Another impetus for noncompartmental method has been the desire to develop methods of estimating pharmacokinetic parameters that do not require tedious and somewhat subjective method of nonlinear regression. The approach has also some times been known as model-independent or model-free approach. Box 7 outlines various advantageous features.
Box 7: Meritorious visages of noncompartmental pharmacokinetic modeling

- Simplistic modeling as based usually on integration of data
- Applicability to virtually any linear body model
- Easy detection of nonlinearity using independence of pharmacokinetic parameters
- Fewer, less restrictive, easily verifiable and realistic assumptions
- Similar and naïve general equations for drugs facilitating contrast

Amongst the popular noncompartmental methods propounded for pharmacokinetic data analysis are the statistical moment approach, linear system analysis and the recirculatory models. The information on the myriad perspectives of the subject lie scattered as over a scores of sparse reviews and book chapters. Linear system analysis tends to describe the entire time course of plasma drug concentration in body in a relatively more comprehensive manner. This approach may be exploited for prediction of systemic drug concentration by convolution, and estimation of time course of drug absorption by deconvolution, and has even been used for establishing Level A in vitro-in vivo correlations. The recirculatory models tend to describe drug disposition in terms of repeated cycles through the body circulatory system (Moriwaki et al. 2004; Avram et al. 2007; Naidoo et al. 2009; Masui et al. 2010). It can be argued that such models reflect better than even the so called physiologically based compartmental models.

1.5.2.1 Statistical moment approach

The technique is doubtlessly the most accepted and indisputable noncompartmental approach for pharmacokinetic data analysis. It has gained a significant degree of use and acceptance in pharmacokinetic literature in the last two decades. Although the statistical moments have been used extensively as a practical technique in various sciences since the fifties, the first report on their use in describing cholesterol kinetics appeared in 1969 (Perl & Samuel 1969). The application of statistical moment approach to pharmacokinetics was virtually simultaneously reviewed by Yamaoka et al. (1978) and Cutler (1978). Chanter (1985) questioned the validity of using statistical moments for estimating MRT. In the same year, Landaw & Katz (1985) indicated errors in the Chanter postulate and Benet (1985) suggested some alternative measures to compute MRT for 2-
CBM kinetics. Gillespie and Veng-Pedersen (1985) ultimately ended the controversy in the same year by re-establishing the application of moment theory for all the linear pharmacokinetic systems. Assumptions made while hypothesizing such a non-compartmental model are fewer, less restrictive, easily verifiable and realistic ones. Another impetus for these “model-independent” methods has been the desire to develop methods of estimating pharmacokinetic parameters that do not require tedious and somewhat subjective method of nonlinear regression.

1.5.2.1.1 Mean time concept

Based on integration of data, the statistical moment technique banks upon parameters like mean residence time (MRT), applicable to virtually any linear body model. After the drug is administered, the drug molecules distribute themselves throughout the body and stay in the body for differing time periods. Some molecules may stay longer in the body while other will leave immediately after absorption. Thus, the term, MRT describes the average time for which the drug molecules reside in the body. The residence time for the drug molecules in the dose may be sorted according to their residence time into groups. The total residence time is the summation of the number of molecules in each group multiplied by their residence times. Thus, MRT is the ratio of sum of the residence times of all the drug molecules divided by total number of such molecules. To accomplish this, the drug dose may be converted to number of moles by dividing the dose in grams by molecular weight. Multiplying the number of moles by the Avogadro’s number (6.023 \times 10^{23}) yields the number of molecules which turns out to tune of 10^{20}. Hence for estimation of pharmacokinetic parameters, one needs to determine residence times of each of such 10^{20} molecules and eventually calculate their mean and variance as MRT and variance of residence time (VRT) respectively.

Residence times of such large number of molecules can be rationally considered to follow a normal distribution curve, as per the Gaussian equation.

\[
P(X) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(x - \mu)^2}{2\sigma^2}}
\]

Accordingly, as per the Gaussian relationship, the residence times of drug
molecules in body following a single dose, when drawn versus their number, can be regarded as a statistical normal frequency distribution curve.

For such Gaussian normal distribution, the curve tends to be symmetric about the axis with the area lying under particular range as ascertainable. Hence the value corresponding to the zenith of this frequency curve or peak of this unimodal distribution is MRT. As it is known that nearly 68.23% of area under normal distribution curve is encompassed between mean (μ) ± 1 standard deviation (σ), the value of σ and eventually, its square, variance (i.e., σ²) can easily be monitored. Verily, using conventional means of averaging the residence times of zillions of the drug molecules is a daunting rather impossible task. Statistical moments, on the other hand, make the task enormously simplified.

Pharmacokinetically, the movement of individual drug molecules through a body compartment is governed by probability. Some drug molecules may hit the target (stocha) as all may not get absorbed (or metabolized or distributed or excreted) and rest may form a random scatter around a general cluster. The analysis of statistical moments is, in fact, the analysis of the probability distribution resulting as a consequence of this stochastic process.

1.6 IN VITRO/ IN VIVO CORRELATIONS (IVIVC)

The term IVIVC first appeared in pharmaceutical literature as a result of awareness of concept of in vivo bioavailability and of in vitro dissolution rate determination. An endeavor to relate in vitro dissolution and in vivo pharmacokinetic results is referred to as IVIVC analysis (Amidon et al. 1995; Uppoor 2001; Emami 2006). Thus, IVIVC can verily be defined as “a quantitative rational relationship between a biological property (e.g., Cmax, AUC or tmax), or a parameter desired from a biological property produced by a dosage form, with a physicochemical property or characteristic of the same dosage form”.

The United States Pharmacopeia (USP) defines IVIVC as “the establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form and a physicochemical property or characteristic of same dosage form”.

71
However, the US Food and Drug Administration (FDA) describes IVIVC as “a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response.” Generally, the in vitro property is the rate or extent of drug dissolution or release, while the in vivo response parameter is the plasma drug concentration or amount of drug absorbed.

In the last a few years, the concept of IVIVC has extensively been discussed by pharmaceutical scientists, particularly for extended release (ER) drug products. The ability to predict the expected bioavailability characteristics accurately and precisely through dissolution profile characteristics is a long sought-after goal of the pharmaceutical scientists.

A report from a 1987 ASCPT/DIA/APS/FDA-sponsored workshop entitled Report of the Workshop on CR Dosage Forms: Issues and Controversies indicated that the state of science and technology at that time did not permit consistently meaningful IVIVC for ER dosage forms and encouraged IVIVC as a future objective. Dissolution testing was considered useful only for process control, stability, minor formulation changes, and manufacturing site changes.

1.6.1 Significance of IVIVC

The concept of IVIVC has gained tremendous attention in pharmaceutical industry, academia, and regulatory sector. It acts as a tool to reliably correlate in vitro drug dissolution data and in vivo drug absorbed. Such a tool shortens the drug development period, economizes the resources and leads to improved product quality (Byron et al. 2010; Yang 2010).

The main objective of establishing IVIVC is to facilitate in vitro dissolution studies to serve as surrogate for in vivo bioequivalence testing, thus supporting biowaiver, especially during scale-up and post approval changes (SUPAC). Understanding and controlling the relationship between in vitro release and in vivo response in a compound plays a critical role in development of modified release formulations, generics, fixed dose combination products, and drug delivery systems. During the manufacturing and marketing of any therapeutic agent, development and optimization of formulation are integral parts which are indeed time consuming.
and costly procedures. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in healthy human volunteers may be required to prove that the new formulation is bioequivalent with the old one. The implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. A validated IVIVC, here, can be used to predict in vivo behavior of the formulation to assess likelihood of success before entering it in a biostudy, thus, eliminating many biostudies that are unnecessary.

With increasing introduction of modified release and novel drug delivery systems, it is obligatory to understand the concept of IVIVC in greater depth. Conducting dissolution analysis with IVIVC is a fast and inexpensive method for obtaining optimal formulations as opposed to slow and expensive bioavailability or bioequivalence studies that provide "hit or miss" results. Earlier, the IVIVC is implemented in the drug development process, easier and more cost-effective is its implementation for all the future changes in the formulation. Box 8, in a nutshell, summarizes the key advantages of IVIVC in a typical pharmaceutical R & D set-up.

**Box 8: Key advantages of IVIVC**

- Bypasses the hassles of biostudy by serving as a surrogate for human bioequivalence studies
- Helps to reduce costs by obviating the need to perform expensive bioavailability/bioequivalence human trials
- Speeds up the product development process
- Demonstrates bioequivalence when certain pre-approval changes are made in formulation, equipment, manufacturing process or in manufacturing site.
- Improves product quality using more meaningful dissolution specifications
- Reduces "regulatory burden" due to biostudies

**1.6.2 IVIVC model development**

In the process of IVIVC development, the in vitro drug release parameters which resemble in vivo drug performance are identified. The appropriate design of in vitro dissolution tests, capable of discriminating between the formulations with
Introduction

different bioavailabilities, plays a major role in the ability of the IVIVC predictability. Therefore, it is essential that in vitro dissolution tests closely reflect in vivo situations, when they are used to establish an IVIVC. Thus for all categories, it is anticipated that well-designed dissolution test is a key prognostic tool in the assessment of both, the drug’s potential for oral absorption and the bioequivalence of its formulations.

1.6.2.1 Dissolution studies in development of IVIVC

The purpose of in vitro dissolution studies in drug development process is to assess the lot-to-lot quality of a drug product, guide development of new formulations; and ensure continuing product quality and performance after certain changes, such as changes in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process (de Campos et al.; Gray et al. 2009; Bajerski et al. 2010). However, from the IVIVC standpoint, dissolution serves as a surrogate for drug bioavailability. Thus more rigorous dissolution standards may be necessary for the biowaiver. Generally, a dissolution methodology, which is able to discriminate between the study formulations with different release patterns and reflects the in vivo behavior, should be used to establish an IVIVC. The in vitro dissolution release of a formulation can be modified to facilitate the correlation development. Changing dissolution testing conditions such as the stirring speed, choice of apparatus, pH of the medium, and temperature may alter the dissolution profile. Once a discriminating system is developed, dissolution conditions should be the same for all formulations tested in the biostudy for development of the correlation and should be fixed before further steps towards correlation evaluation are undertaken.

Dissolution profiles of the given formulations can be compared using different techniques. These techniques can be divided as model independent, model dependent and ANOVA-based approaches. Different results can be discerned depending on the method used for the comparison. Important model-independent approaches encompass the ratio-test procedures and pair-wise procedures.

These approaches compare dissolution profiles of drug formulations furnishing
tangible basis to formulate dissolution specifications. Of the various types of test procedures performed, 90% confidence interval for mean ratio percent dissolved, percent dissolution efficiency (%DE), and mean dissolution time (MDT, Eqn. 5) are the popular ones. Use of percent dissolved data is mostly preferable as it is the simplest and most natural calculation of three approaches and allows the establishment of criteria at any time point, unlike mean time for complete dissolution, which could be calculated only after nearly the entire drug is dissolved.

\[
MDT = \frac{\int_{0}^{\infty} W_d(t) dt}{\int_{0}^{\infty} W(t) dt} = \frac{ABC}{W_0}
\]  

... (5)

where, ABC (area between curves) is the shaded area as shown in Figure 14, \( W_d(t) \) is cumulative drug amount dissolved at any time, and \( W_0 \) is the actual (not labeled) quantity of drug available for dissolution. ABC can be estimated arithmetically using trapezoidal rule.

\[ 
\text{ABC} = \int_{0}^{\infty} W_d(t) dt - \int_{0}^{\infty} W(t) dt 
\]

\[ 
W(t) = W_0 - W_d(t) 
\]

Figure 14: Area between the curves

1.6.3 Levels of IVIVC

Five correlation levels have been defined in the FDA guidance namely level A, level B, level C, level D, and multiple level C (Emami 2006; Byron et al. 2010). The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of given dosage form.
1.6.3.1 Level A IVIVC

This is the most common type of correlation observed in new drug applications (NDAs) submitted to the FDA. From regulatory perspectives, it is considered to be the most useful. It is the highest category of correlation, where a point to point correlation exists between the entire in vitro dissolution (or drug release) time course and the entire in vivo response time course, e.g., the time course of plasma drug concentration or amount of drug absorbed, or in vivo dissolution of the drug from the dosage form (Donato et al. 2008; Ghosh et al. 2008; Ochoa et al. 2010; Scheubel et al. 2010). This estimation of in vivo absorption profile from the concentration-time data can be achieved through the two-stage deconvolution methods. Wagner-Nelson (WN, Eqn. 6) is specifically suited for cases obeying one-compartmental (1-CBM) open body model.

\[
\frac{(X_A)_T}{(X_A)_\infty} = \frac{C_T + K \int_0^T Ct}{K \int_0^T Ct} \quad \text{... (6)}
\]

Analogously, for cases obeying two-compartmental (2-CBM) open body model, Loo-Riegelman (LR, Eqn. 7) method is more suited.

\[
\frac{(X_A)_T}{(X_A)_\infty} = \frac{C_T + K_{10} \int_0^T Ct + (V_c/X_c)(X_p)_T}{K_{10} \int_0^T Ct} \quad \text{... (7)}
\]

where, \((X_A)_T\) = amount of drug absorbed into systemic circulation at time ‘t’; \((X_A)_\infty\) = amount of drug absorbed into systemic circulation at infinite time; \(C_T\) = plasma concentration of drug at time ‘t’; \(K\) = first-order elimination rate constant; \(K_{10}\) = apparent first order elimination rate constant of drug from the central compartment; \(X_p\) = amount of drug in the central compartment; \(V_c\) = apparent volume of the central compartment

Subsequent to the estimation of the in vivo absorption profile, the relationship with in vitro dissolution is evaluated. An example of establishing “level A” IVIVC in
In "Level A" IVIVC, generally a linear correlation model is observed which expressed as a simple equation (Eq. 17) between the \textit{in vivo} drug absorptive and drug dissolved (released).

\[ Y_{\text{in vivo absorbed}} = mX_{\text{in vitro drug dissolved}} + C \]

In this equation, \( m \) is the slope of the relationship, and \( C \) is the intercept. \( m = 1 \) and \( C = 0 \), indicating a linear relationship. However, depending on the nature of the modified-release system, some data are better fitted using other models, such as Sigmoid, Weibull, Higuchi, or Hixson-Crowell.

Eqn. 14 may be applied to most formulations with comparable \textit{in vitro} duration of release. However, for dosage forms with complicated mechanisms of release, which are of longer duration, \textit{in vitro} release may not be in the same scale as the \textit{in vivo} release. Thus, in order to model such data, it is necessary to incorporate time-scaling and time-shifting parameters within the model. The kind of data that is routinely encountered in the development of \textit{in vivo} release dosage forms.

\textit{In vivo} release rate (\( X'_{\text{vivo}} \)) can also be expressed as a function of \textit{in vitro} release rate (\( X'_{\text{rel,vivo}} \)) with parameters (\( a, b \)), which may be empirically selected and used using appropriate mathematical processes as shown in Eqn. 8. An iterative method may be used to compute the time-scaling and time-shifting parameters.
Nonlinear IVIVC are observed when *in vitro* dissolution profile runs ahead or behind the *in vivo* input profile as seen in Figure 16a. The resulting non linear IVIVC can be made linear by log transformation (Figure 16b) using an exponential equation for correlation.

![Figure 16: (a) Nonlinear IVIVC (b) Log transformation of nonlinear IVIVC](image)

### 1.6.3.2 Level B IVIVC

If the entire profile can not be used for correlation, data reduction that leads to comparable and preferably analogous parameters on the *in vivo* and the *in vitro* side is required. The statistical moment theory, a model-independent method, uses empirical distribution functions for the purpose. Level B correlation, as depicted in Figure 17, is established by comparing the mean *in vitro* dissolution time (MDTvivo) of the product to either mean *in vivo* residence time (MRT) or the mean *in vivo* dissolution time (MDTvivo). Level B correlation, like Level A, utilizes all of the *in vitro* and *in vivo* data but is not considered to be a point-to-point correlation because it does not uniquely reflect the actual *in vivo* plasma level curve, since there are a number of different *in vivo* curves that will produce similar mean residence time values.

Besides MRT, Mean Transit Time (MTT) and Mean Absorption Time (MAT) are other parameters used in oral pharmacokinetics. While MTT, quite similar to MRT, is mean time a molecule needs to leave a kinetic space, MAT can be estimated as per Eqn. 9.

\[
MAT = MRT_{oral} \frac{MRT_{iv}}{MRT_{iv}} \quad \ldots \text{(9)}
\]
In shear contrast to the traditional compartmental pharmacokinetics, one of the remarkable features of the stochastic approach is that even drug formulation performance can be determined using moment parameters. Some instances of such moment parameters include Mean Dissolution Time (MDT, Eq. 10) and Mean Disintegration Time (MDIT).

$$MDT = \frac{MAT_{solution}}{MAT_{solid}}$$ ...\(\text{(10)}\)

The most vital advantage of mean time parameters is their rational additivity across the whole gamut of processes involved in the drug fate of solid oral dosage forms, both \textit{in vitro} and \textit{in vivo}, encompassing disintegration, dissolution, absorption and disposition. The continuum of parameters is pictorially represented in Figure 18.

### 1.6.3.3 Level C IVIVC

It establishes a single point relationship between \textit{in vitro} dissolution parameter (e.g., \(t_{90}\), or percent dissolved in 4 h) and a pharmacokinetic parameter (AUC, \(t_{\text{max}}\) or \(C_{\text{max}}\)). One dissolution time point (like \(t_{90}\), \(t_{50}\), etc.) is usually compared to one pharmacokinetic parameter (like AUC, \(t_{\text{max}}\), \(C_{\text{max}}\), etc.). Therefore, it represents a single point correlation and does not reflect the complete shape of the plasma profile, which is a crucial factor that indicates the performance of modified-release products. Level C is the weakest level of correlation, as the partial relationship between absorption and dissolution is only established.
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Because of its obvious limitations, a level C correlation has limited usefulness in predicting \textit{in vivo} drug performance and is subject to the same caveats as a Level B correlation in its ability to support product and site changes as well as justification of quality control standard extremes. Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected. While the information may be useful in formulation development, waiver of an \textit{in vivo} bioequivalence study (biowaiver) is generally not possible.

1.6.3.4 Multiple Level C IVIVC

A multiple point level C relationship is almost equivalent to level A. It relates one or more pharmacokinetic parameters of interest (like \(C_{\text{max}}\), AUC, or any other suitable metrics) to the amount of drug dissolved at various time points. It can be employed to justify biowaiver(s) provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. A relationship should be demonstrated at each time point at the same parameter such that the effect on the \textit{in vivo} performance of any change in dissolution can be assessed, at least three dissolution time points covering the early, middle, and late stages of the dissolution profile are required. If
such a multiple level C correlation is achievable, then the development of a “level A” correlation is also likely.

### 1.6.3.5 Level D IVIVC

It is a nonparametric rank order correlation between the *in vitro* dissolution parameter and an *in vivo* pharmacokinetic parameter. It is usually based on ordinal (but not quantitative) data, thus considered to be a weaker correlation.

### 1.6.4 IVIVC in the light of BCS

Class I compounds like metoprolol propranolol, labetolol, diltiazem, verapamil, enalapril, phenylalanine and caffeine posses high permeability and solubility. They are expected to be well absorbed owing to their high $A_n$ and high $D_n$, unless they are unstable, form insoluble complexes, are secreted directly from gut wall, or undergo first pass metabolism. When a class I drug is formulated as an ER product in which the release profile controls the rate of absorption, and the solubility and permeability of the drug is site independent, a level A correlation is most likely. However, once the permeability is site dependent a level C correlation is expected. The major challenge in development of drug delivery system for class I drugs is to achieve a target release profile associated with a particular pharmacokinetic and/or pharmacodynamic profile. Formulation approaches include both control of release rate and certain physicochemical properties of drugs like pH-solubility profile of drug.

Class II compounds like phenytoin, danazol, ketoconazole, mefenamic acid, nifedipine, flurbiprofen, diclofenac, naproxen, piroxicam and ketoprofen, possess high permeability and low solubility. They have a high $A_n$ but low $D_n$, which means absorption is faster than dissolution rate i.e., drug is absorbed quickly after dissolution, thus dissolution is the rate limiting step. For class II drugs, therefore, a strong correlation between dissolution rate and the *in vivo* performance can be established. When a class II drug is formulated as an ER product, and the solubility and permeability of the drug is site independent, a level A correlation is expected. However, once the permeability is site dependent little or no IVIVC is expected. The systems that are developed for class II drugs are based on their solubility enhancement. The key techniques include, micronization, lyophilization,
addition of surfactants, emulsion formulations, microemulsion systems, and use of
complexing agents like cyclodextrins.

Class III drugs, like cimetidine, acyclovir, neomycin, famotidine, nadolol, atenolol
and ranitidine, possess low permeability and high solubility. They are rapidly
dissolving and permeability is the rate-controlling step in drug absorption.
Furthermore, Class III drugs exhibit a high variability in rate and extent of
absorption, but if dissolution is fast such that 85% of drug dissolves in 15 minutes,
the variation could be attributed to GI transit, luminal contents, and membrane
permeation rather than dosage form factors. As drug permeation is rate
controlling, limited or no IVIVC is expected. Class III drugs require the
technologies that address to fundamental limitations of absolute or regional
permeability. Peptides and proteins constitute the part of class III and the
technologies handling such materials are on rise now days.

Class IV like taxol, furosemide, cyclosporine and terfenedine possess low
permeability and solubility. This class of drugs exhibit significant problems for
effective oral delivery, no IVIVC is expected. Class IV drugs present a major
challenge for development of drug delivery system and the route of choice for
administering such drugs is parenteral with the formulation containing solubility
enhancers.

1.6.5 Applications of IVIVC

1.6.5.1 Biowaivers for changes during drug product manufacturing

A biowaiver, using an IVIVC developed with two formulations/release rates, for a
non-narrow therapeutic index drug will likely be granted for an ER drug product
for Level 3 manufacturing and non-release controlling excipient changes and as
defined in SUPAC-MR.

1.6.5.1.1 Biowaivers for lower strengths

If an IVIVC is developed with the highest strength, waivers for changes made on
the highest strength and any lower strength may be granted if these strengths are
compositionally proportional or qualitatively the same, the in vitro dissolution
profiles of all the strengths are similar, and all strengths have the same release mechanism.

1.6.5.2 Approval of new strengths

This biowaiver is applicable to strengths lower than the highest strength, within the dosing range that has been established to be safe and effective, if the new strengths are compositionally proportional or qualitatively the same; have the same release mechanism; have similar in vitro dissolution profiles; and are manufactured using the same type of equipment and the same process at the same site as other strengths that have bioavailability data available.

For generic products to qualify for this biowaiver, one of the situations listed in Box 9 should exist.

1.6.5.2 Product development

The pharmaceutical applications of IVIVC are not limited merely to obtaining biowaivers for new formulations. The concepts of IVIVC can be well-utilized at various stages of product development resulting in the formulation of more valuable and robust formulations. The long standing dream of product development scientists of predicting in vivo behavior of formulations by examining their in vitro release profiles can only be realized by IVIVC.

Box 9: Conditions for generic products to qualify for biowaiver

- Bioequivalence has been established for all strengths of the reference product
- Dose proportionality has been established for the reference product, and all reference product strengths are compositionally proportional or qualitatively the same
- Bioequivalence is established between the generic product and the reference product at the highest and lowest strengths and, for the reference product, all strengths are compositionally proportional or qualitatively the same
- Obtaining “category 2d” biowaivers: The difference in predicted means of $C_{\text{max}}$ and AUC should be no more than 10%, based on dissolution profiles of the highest strength and the lower strength product

A newer approach of in vitro/ in vivo matching (IVIVM) comes to the rescue of the scientists in this regard. The technique principally involves simulation of in vivo
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profiles from in vitro data using model-independent deconvolution technique shown in Figure 19.

Observed in vitro drug release profiles Simulated in vivo serum drug prc

Figure 19: In vitro/ in vivo matching (IVIVM) using rational con simulations

1.6.5.2.1 Setting-up of dissolution media

In vitro dissolution specifications should generally be based on the performa the clinical/bioavailability lots. These specifications may sometimes be wi so that scale-up lots, as well as stability lots, meet the specifications asso with the clinical/bioavailability lots. This approach is based on the use of vitro dissolution test as a quality control test without any in vivo significance though in certain cases (e.g., ER formulations), the rate limiting step absorption of the drug is the dissolution of the drug from the formulatic IVIVC adds in vivo relevance to in vitro dissolution specifications, beyond ba batch quality control. In this approach, the in vitro dissolution test beco meaningful predictor of in vivo performance of the formulation, and dissc specifications may be used to minimize the possibility of releasing lots that be different in in vivo performance.

It is relatively easier to establish a multipoint dissolution specificatic modified-release dosage forms. The FDA guidance describes the proced setting dissolution specifications in cases of level A, multiple level C and 1 correlation and where there is no IVIVC. Once an IVIVC developed, it sho used to set specifications in such a way that the fastest and lowest release allowed by the upper and lower dissolution specifications result in a ma difference of 20% in the predicted Cmax and AUC. Predicted plasma concentr
and the consequent AUC and C\textsubscript{max} can be calculated using convolution or any other appropriate modeling techniques, described earlier.

1.7 FORMULATION BY DESIGN (FbD)

Development of an effective oral drug delivery system (DDS), however, invariably involves rational blending of diverse functional and non-functional polymers and excipients. Optimizing the formulation composition and the manufacturing process of such a drug delivery product to furnish the desired quality traits is, therefore, a Herculean task. The traditional approach of optimizing a formulation or process essentially involves studying the influence of one variable at time (OVAT), while keeping others as constant. Using this OVAT approach, the solution of a specific challenging property can be achieved somehow, but attainment of the true optimum composition or process is never guaranteed (Singh et al. 2005a). This may ostensibly be ascribed to the presence of interactions, i.e., the influence of one or more variables on others. The final product may be satisfactory, but mostly sub-optimal, as a better formulation might still prevail for the studied conditions.

Design of experiments (DoE), on the other hand, is an optimization technique meant for products and/or processes, developed to evaluate all the potential factors simultaneously, systematically and rapidly. Its implementation invariably encompasses the use of statistical experimental designs, generation of mathematical equations and graphic outcomes, portraying a complete picture of variation of the response(s) as a function of the factor(s), which can never be obtained employing the traditional OVAT approach.

Lately, a holistic DoE-based philosophy of Quality by Design (QbD) has been slowly permeating into the mindset and practice in the industrial environs (Skrđla et al. 2009; Huang et al. 2011). This popularity of QbD in pharma circles is largely attributable to the recent impetus provided by the ICH, FDA and EMEA through their respective federal guidance’s. Since DoE has much wider domain of application, recently we have proposed, on the heels of QbD, a terser jargon, viz. “Formulation by Design (FbD)”, applicable specifically to the use of DoE in drug formulation development (Singh et al. 2011a). Table 1 succinctly enumerates the merits of FbD over the OVAT methodology.
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Table 1: Comparison of OVAT and FbD methodology

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<thead>
<tr>
<th>Attribute</th>
<th>OVAT</th>
<th>FbD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choice of optimum formulation</td>
<td>May result only in sub-optimal solutions</td>
<td>Yields the best possible formulation</td>
</tr>
<tr>
<td>Interaction among the ingredients</td>
<td>Inept to reveal possible interactions</td>
<td>Estimates any synergistic or antagonistic interactions among constituents</td>
</tr>
<tr>
<td>Scale-up and post approval changes</td>
<td>Very difficult to design a formulation slightly differing from the desired formulation</td>
<td>Changes in the optimal formulation can easily be incorporated, as response variables quantitatively govern a set of input variables</td>
</tr>
<tr>
<td>Resource-economics</td>
<td>Highly resource-intensive, as it leads to unnecessary runs and batches</td>
<td>Economical, as it furnishes information on process performance after minimal trials</td>
</tr>
<tr>
<td>Time-economics</td>
<td>Highly time-consuming, as each product is individually evaluated for its performance</td>
<td>Can simulate the process behavior using model equations</td>
</tr>
</tbody>
</table>

Owing to such numerous benefits of FbD, the recent years have witnessed an increase in the development of various DDS, both oral and non-oral, optimized using FbD (Ivic et al. 2010; Singh et al. 2010). Figure 20 pictographically depicts the number of FbD studies reported in literature in the past five decades.

![Figure 20: Various drug delivery formulations optimized using FbD until (Singh et al. 2011a)](image)
Usually, specific terminology, both technical and otherwise, is employed during FbD practice. To facilitate better clarity of precepts of FbD of DDS, important terms have been compiled in Box 10.

**Box 10: Vital terminology employed during FbD of drug delivery**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optimize</strong></td>
<td>Make as perfect, effective, or functional as possible</td>
</tr>
<tr>
<td><strong>Optimized</strong></td>
<td>Improved product to accomplish the objectives of a development scientist using DoE and computers</td>
</tr>
<tr>
<td><strong>Optimization</strong></td>
<td>Implementation of systematic approaches to achieve “the best” combination of product and/or process characteristics under a given set of conditions</td>
</tr>
<tr>
<td><strong>Independent Variables</strong></td>
<td>Input variables, which are directly under the control of the product development scientist</td>
</tr>
<tr>
<td><strong>Quantitative Variables</strong></td>
<td>Variables that can take numeric values</td>
</tr>
<tr>
<td><strong>Categorical Variables</strong></td>
<td>Qualitative variables which can not be quantified</td>
</tr>
<tr>
<td><strong>Runs or Trials</strong></td>
<td>Experiments conducted according to the selected experimental design</td>
</tr>
<tr>
<td><strong>Factors</strong></td>
<td>Independent variables, which influence the product/process characteristics or output of the process</td>
</tr>
<tr>
<td><strong>Design Matrix</strong></td>
<td>Layout of experimental runs in matrix form, as per experimental design</td>
</tr>
<tr>
<td><strong>Knowledge Space</strong></td>
<td>Scientific elements to be considered and explored on the basis of previous knowledge as product attributes and process parameters</td>
</tr>
<tr>
<td><strong>Design Space</strong></td>
<td>Multidimensional combination and interaction of input variables and process parameters, demonstrated to provide quality assurance</td>
</tr>
<tr>
<td><strong>Control Space</strong></td>
<td>Domain of design space selected for the detailed study</td>
</tr>
<tr>
<td><strong>Levels</strong></td>
<td>Values assigned to the factor</td>
</tr>
<tr>
<td><strong>Constraints</strong></td>
<td>Restrictions imposed on the factor levels</td>
</tr>
<tr>
<td><strong>Response Variables</strong></td>
<td>Characteristics of the finished drug product or the in-process material</td>
</tr>
<tr>
<td><strong>Critical Quality Attributes</strong></td>
<td>Parameters ranging within appropriate limits, which ensure the desired product quality</td>
</tr>
<tr>
<td><strong>Critical Process Parameters</strong></td>
<td>Independent process parameters most likely to affect the quality attributes of a product or intermediates</td>
</tr>
<tr>
<td><strong>Critical Formulation Attributes</strong></td>
<td>Formulation parameters affecting critical quality attributes</td>
</tr>
<tr>
<td><strong>Interaction</strong></td>
<td>Lack of additivity of factor effects</td>
</tr>
</tbody>
</table>
**Introduction**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antagonism</td>
<td>Undesired negative change due to interaction among factors</td>
</tr>
<tr>
<td>Synergism</td>
<td>Desired positive change due to interaction between factors</td>
</tr>
<tr>
<td>Effects Plot</td>
<td>Plot between the magnitude of various coefficients for the effects and/or interactions against the response variable</td>
</tr>
<tr>
<td>Main effect</td>
<td>The effect of a factor averaged over all the levels of other factors</td>
</tr>
<tr>
<td>Nuisance Factor</td>
<td>Uncontrollable factors which complicate the estimation of main effect or interactions</td>
</tr>
<tr>
<td>Orthogonality</td>
<td>If the estimated effects are due to the main factor of interest and independent of interactions</td>
</tr>
<tr>
<td>Confounding</td>
<td>Lack of orthogonality</td>
</tr>
<tr>
<td>Resolution</td>
<td>The measure of the degree of confounding</td>
</tr>
<tr>
<td>Coding (or normalization)</td>
<td>Process of transforming a natural variable into a non-dimensional coded variable</td>
</tr>
<tr>
<td>Factor Space</td>
<td>Dimensional space defined by the coded variables</td>
</tr>
<tr>
<td>Experimental Domain</td>
<td>Part of the factor space, investigated experimentally for optimization</td>
</tr>
<tr>
<td>Blocks</td>
<td>A set of relatively homogenous experimental conditions, wherein every level of the primary factor occurs the same number of times with each level of nuisance factor.</td>
</tr>
<tr>
<td>Response Surface</td>
<td>Graphical depiction of the mathematical relationship</td>
</tr>
<tr>
<td>Empirical Model</td>
<td>Mathematical model describing factor-response relation using polynomial equations</td>
</tr>
<tr>
<td>Response Surface Plot</td>
<td>3-D graphical representation of a response plotted between two independent variables and one response variable</td>
</tr>
<tr>
<td>Contour Plot</td>
<td>Geometric illustration of a response obtained by plotting one independent variable against another, while holding the magnitude of response and other variables as constant</td>
</tr>
</tbody>
</table>

Based on prior multi-disciplinary knowledge impacting various product attributes and/or process parameters, the foremost task ahead for a researcher is to identify the “knowledge space” i.e., entire space worth exploring from the possible vast ocean of scientific information. A “knowledge space”, thereby, encompasses all those product and process variables that may even minutely affect the overall product quality. A “design space” has to be demarcated involving “selected few” most influential variables from the “knowledge space”. “Control space”, a further subset construct of this “design space”, is the experimental domain earmarked for detailed studies within the refined ranges of input variables.
"Design space" uses systematic approach coupled with archival data to connect the "knowledge space" to "control space". Figure 21 portrays the hierarchy of knowledge, design and control space. Verily, FbD hits the bull’s eye using five strengths viz. apt choice of experimental designs, accurate computer-aided optimization, meticulous drug product development, precise definition of design and control space, and identification of critical quality attributes (CQAs), critical formulation attributes (CFAs) and critical process parameters (CPPs). Figure 21 pictorially illustrates the concept.

Figure 21: Inter-relationship between knowledge, design and control space (Singh et al. 2011a)

Figure 22: Involving five cardinal elements, FbD aims to hit the bull’s eye (Singh et al. 2011a)
Introduction

The theme of DoE optimization methodology provides thought-through and thorough information on diverse DoE aspects organized in a five-step sequence illustrated in Figure 23.

- The PhD study begins with Step I, where an endeavor is made to explicitly ascertain the drug delivery objective(s). Various CQAs or response variables, which pragmatically epitomize the objective(s), are earmarked for the purpose. All independent product/process variables are also listed.

- In Step II, the response variables which directly represent the product quality (e.g., particle size for nanoparticles, emulsification time for self-emulsifying systems) are selected. Also, selection of "prominent few" influential factors among the "possible many" input variables is conducted using experimental designs, the process popularly termed as "screening" (Murphy 2003). The formulator, at times, can even bypass the rigor of screening process to choose these factors, viz. CQAs and/or CPPs by virtue of his experience, wisdom and previous knowledge. Factor influence studies are usually conducted later to quantify the effect of factors and to determine the interactions, if any. Experimental studies are also undertaken to define the broad range of factor levels.

- During Step III, a suitable experimental design is worked out, accordingly, to map the responses on the basis of the study objective(s), responses being explored, number and the type of factors, and factor levels, viz. high, medium or low. The niceties of using important experimental designs along with their pros and cons are discussed in subsequent sections. A design matrix is subsequently generated to guide the drug delivery scientist. The drug delivery formulations are experimentally prepared according to the chosen experimental design, and the chosen response variables are evaluated meticulously.

- In Step IV, a suitable numeric model is proposed on the basis of experimental data thus generated, and its statistical significance discerned. Response surface methodology (RSM) is employed to relate a response variable to the levels of input variables. Optimum formulation compositions are searched within the experimental domain, employing graphical or numerical techniques.
Figure 23: Five step strategy of formulating DDS using FbD (Singh et al. 2011a)

- **Step V** is the ultimate phase of the FbD exercise, involving validation (response predictive ability of the proposed design model). Drug delivery performance of some studies, taken as the confirmatory runs, is assessed vis-à-vis that predicted using RSM, and the results are critically compared. The optimum formulation is scaled-up and set forth ultimately for the production cycle.

### 1.7.1 FbD experimental designs

An experimental design constitutes the pith of the entire DoE exercise (Singh et al. 2008a). Before the selection of an experimental design, it is essential to demarcate the experimental domain (i.e., the area to be investigated) within the factor space (i.e., the broad range of factor studies). To accomplish this task, first a pragmatic range of experimental domain is embarked upon and the levels and their numbers are selected so that the optimum lies within its realm. While selecting the level one must see that the increments between them should be realistic. Too wide increments may miss finding the useful information between the levels, while too narrow range may not yield accurate results.

There are numerous types of experimental designs to choose from. Various commonly employed experimental designs for RSM, screening and factorial influence studies during pharmaceutical product/process development include:
Introduction

A. Factorial designs
B. Fractional factorial designs
C. Plackett-Burman designs
D. Star designs
E. Central composite designs
F. Box-Behnken designs
G. Equiradial designs
H. Mixture designs
I. Taguchi designs
J. Optimal design

1.7.1.1 Factorial designs

The most frequently employed experimental design, factorial design (FD), is the one in which all levels of a given factor are combined with all levels of every other factor in the experiment (Li 2003). Full FDs involve studying the effect of all the factors \( k \) at various levels \( x \), including the interactions among them, with the total number of experiments being \( x^k \).

An FD can be termed as ‘symmetric’, if the number of levels is the same for each factor, and ‘asymmetric’ in cases of a different number of levels for different factors (Lewis et al. 1999). Figure 24 portrays a \( 2^3 \) FD, in which each point represents an individual experiment.

![Diagrammatic representation of a 2^3 factorial design](image)

**Figure 24: Diagrammatic representation of a 2^3 factorial design**

1.7.1.2 Fractional factorial designs

A fractional factorial design (FFD) is a finite fraction \( (l/x^r) \) of a complete or full FD, where \( r \) is the degree of fractionation and \( x^r \) is the total number of experiments required (Doornbos & Haan 1995). This design is particularly preferred over FD when the number of required experiments exceeds the manageable levels due to an increase in the number of factors or factor levels. Figure 25 graphically
represents an FFD as a hypercube, with its corners represented by spheres, depicting the experiments studied.

![Diagram](image1.png)

**Figure 25:** Diagrammatic representation of a $2^{3-1}$ fractional factorial design

### 1.7.1.3 Central composite design

Also known as Box-Wilson design, the central composite design (CCD) is the most often used design for quadratic models. The design comprises of a combination of a two-level factorial points ($2^n$), axial or star points ($2^n$) and a central point (Box & Wilson 1951). Thus the total number of factor combinations in a CCD is given by $2^n + 2n + 1$. The axial points for a two-factor problem include, $(\pm \alpha, 0)$ and $(0, \pm \alpha)$, where ‘$\alpha$’ is the distance of the axial points from the center. A two-factor CCD is identical to a $3^2$ FD with spherical experimental domain with $\alpha$ as $\sqrt{2} = 1.414$, as shown in Figure 26.

![Diagram](image2.png)

**Figure 26:** Diagrammatic representation of central composite design (spherical domain) with $\alpha = 1.414$

### 1.7.1.4 Plackett-Burman designs

The Plackett-Burman designs (PBDs) are specialized two-level FFDs used
Introduction
generally for screening of $K$, i.e., $N - 1$ factors, where $N$ is a multiple of 4 (Plackett & Burman 1946). Also known as Hadamard designs or symmetrically reduced $2^k$ FDs, the designs can easily be constructed employing a minimum number of trials (Loukas 2001). Because these designs cannot be represented as cubes, they are sometimes called non-geometric designs. PBDs are quite favorably employed during the screening processes.

1.7.1.5 Taguchi design
Genichi Taguchi, a Japanese engineer, proposed several approaches to experimental designs that are sometimes called “Taguchi Methods” (Taguchi 1986). These methods utilize two-, three-, and mixed-level fractional factorial designs. Large screening designs seem to be particularly favored by Taguchi adherents. Taguchi referred an experimental design as “off-line quality control”, as it is a method to ensure good performance in the design stage of products or processes. The aim here is to make a product or process less variable (i.e., more robust) in the face of variation over which we have little or no control.

Table 2 furnishes a comparative account of important experimental designs employed for RSM, listing their advantages and disadvantages.

1.7.2 Factor studies
Systematic screening and factor influence studies are usually carried out as a prelude to DoE optimization. These are often sequential stages in the development process. Screening methods are used to identify important and critical effects. Factor studies aim at quantitative determination of the effects as a result of a change in the potentially critical formulation or process parameter(s). Such factor studies usually involve statistical experimental designs, and the results so obtained provide useful leads for further response optimization studies. Factor studies include screening of influential factors and factor influence studies.

As the term suggests, “screening” is analogous to separating “rice” from “rice husk”, where “rice” is a group of factors with significant influence as response, and “husk” is a group of the rest of the non-influential factors (Singh et al. 2006a). Thus, the entire exercise aims at selecting the active factors and excluding the
unnecessary variables, but not at obtaining complete and exact numerical data on
the system properties.

Table 2: Merits and demerits of various experimental designs

<table>
<thead>
<tr>
<th>Design</th>
<th>Merits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factorial design</td>
<td>• Efficient in estimating main effects and interactions</td>
<td>• Reflection of curvature not possible in a 2 level design</td>
</tr>
<tr>
<td></td>
<td>• Maximum usage of data</td>
<td>• More experiments are required</td>
</tr>
<tr>
<td></td>
<td>• Used for screening of factors, factor influence studies</td>
<td>• Prediction outside the region is not advisable</td>
</tr>
<tr>
<td>Fractional factorial</td>
<td>Suitable for large number of factors or factor levels</td>
<td>Effects cannot be uniquely estimated, as are confounded with interaction</td>
</tr>
<tr>
<td>design</td>
<td></td>
<td>terms. Difficult to construct</td>
</tr>
<tr>
<td>Plackett-Burman design</td>
<td>Suitable for very large number of factors, where even FFDs require a</td>
<td>• Fixed designs in which runs are predetermined and are limited to &lt; 16</td>
</tr>
<tr>
<td></td>
<td>large number of experiments</td>
<td>experiments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Effects confounded as suitable for two levels only</td>
</tr>
<tr>
<td>Central composite</td>
<td>• Allows the work to proceed in stages, i.e., if linear design does</td>
<td>Difficult to practice with fractional values of α</td>
</tr>
<tr>
<td>design</td>
<td>not adequately fit the data, suitable number of experiments can be</td>
<td></td>
</tr>
<tr>
<td></td>
<td>be added to run a CCD and determine the quadratic effects.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Combines the advantages of FDs and star designs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Requires fewer experiments</td>
<td></td>
</tr>
</tbody>
</table>

Having screened the influential variables, a more comprehensive study, i.e., factor influence study, is subsequently undertaken to quantify the effect of factors, and to determine the interactions, if any (Singh et al. 2006a). Herein, the studied experimental domain is less extensive, as quite fewer active factors are studied. The experiments conducted at this step may often be reused during optimization or response modeling phase by augmenting with additional design points. Central
points (i.e., at the intermediate level), if added at this stage, may prove to be useful in identifying the curvature in the response, in allowing the reuse of the experiments at various stages; and if replicated, in validating the reproducibility of the experimental study.

1.7.3 Selection of experimental designs

Choice of a design amongst the various types of available options depends upon the amount of resources available and the degree of control over making wrong decisions (i.e., Type I and Type II errors for testing hypotheses) that the experimenter desires. It is a good idea to choose a design that requires somewhat fewer runs than the budget permits, so that center point runs can be added to check for curvature in a 2-level screening design and backup resources are available to redo runs that have processing mishaps. By and large, low-resolution designs like FDs (full or fractional), PBDs, or Taguchi designs suffice the purpose of simpler screening of a large number of experimental parameters. Screening designs support only the linear responses. Thus, if a nonlinear response is detected, or a more accurate picture of the response surface is required, a more complex design type is necessary. Hence, when the investigator is interested in estimating interaction and even quadratic effects, or intends to have an idea of the local shape of the response surface, the response surface designs, capable of detecting curvatures, are used.

1.7.4 Modelization

Following choice and implementation of an apt experimental design, apt models need to be generated. Generally, the polynomial mathematical equations are obtained, their statistical significance determined and the choice of the apt model made taking the help of model diagnostic plots.

1.7.4.1 Calculation of coefficients of polynomial equations

Regression is the most widely used method for quantitative factors. It cannot be used for qualitative factors, because interpolation between discrete (i.e., categorical) factor values is meaningless. In ordinary least-squares regression (OLS), a linear model, expressed as Eqn. 5, is fitted to the experimental data such that the sum of squared differences between predicted and observed responses is
minimized.

\[ Y = \beta_0 + \beta_1 X_1 + \beta_n X_n \]  

... (5)

Multiple linear regression analysis (MLRA) can be performed for more factors, interactions, and higher order terms. In certain situations, where the factor-response relationship is nonlinear, multiple nonlinear regression analysis (MNLRA) may also be performed. In situations, where there are large numbers of variables, such as in multivariate studies, the methods of partial least squares (PLS) or principal component analysis (PCA) can also be employed for regression (Westerhuis & Coenegracht 1997). PLS is an extension of MLRA and is used when there are fewer observations than the number of predictor variables.

1.7.4.2 Model diagnostic plots

The goodness of fit of a model can be investigated using one or more of the plots explained as under:

- Actual vs. predicted: A graph is plotted between the actual and the predicted response values. This helps in detecting a value or a group of values that are not easily predicted by the model.

- Residuals vs. predicted: Residuals (or error) is the quantitative difference between the observed and the predicted response(s). Studentized residuals are the residuals converted to their standard deviation units.

- Residuals vs. run: This is a plot of the residuals versus the order of the experimental runs. It checks for the “lurking variables” that may have influenced the response during the experiment.

- Residuals vs. factor: This is a plot of the residuals versus any selected factor. It checks whether the variance, not accounted for by the model, is different for different levels of a factor.

- Normal probability plot: It investigates the normal probability distribution of residuals, as judged from the linear trend of the points, when plotted on a probit scale.

- Outlier T: This is a measure of by how many standard deviations the actual value deviates from the value predicted after deleting the point in question.
• Cook’s distance: This provides measures of the influence, potential or actual, of the individual runs (Cook 1977).

• Leverage: This is a measure of degree of influence of each point on the model fit.

• Box-Cox plot for power transforms: The Box-Cox plot is a tool to help in determining the most appropriate power transformation for application to response data (Box & Cox 1964).

1.7.4.3 Optimum search

From the models thus selected, optimization of one response or the simultaneous optimization of multiple responses needs to be accomplished graphically and/or numerically.

1.7.4.3.1 Graphical optimization

Known popularly as response surface analysis, graphical optimization displays the area of feasible response values in the factor space (Schwartz & Connor 1996; Myers 2003; Singh & Ahuja 2004). The experimenter has to make a choice, “trading off” one objective for other(s), according to the relative importance of the objectives considered. The success in locating an optimum lies in the sagacious interpretation and/or comparison of the resulting plots, leading to attainment of the best compromise. One or more of the following techniques may be employed for this purpose.

1.7.4.3.1.1 Location of the stationary point

After completing the experimental work, often the goal of the formulation scientist is to locate the optimum. The nature of the response surface is interpreted graphically, and a stationary point is located, which may be maximum, minimum, or a target value. At this point, the partial derivatives of the response with respect to the design variable are all zeroes. The case in which the stationary point is neither a maximum nor a minimum is known as the saddle point.

1.7.4.3.2 Search methods

Search methods are employed for choosing the upper and lower limits of the
responses of interest. The response surfaces in these search methods, as defined by the appropriate equations, are searched to find the combination of independent variables yielding the optimum. Two major steps, viz. feasibility search and exhaustive grid search, are used. Together, these techniques are also referred to as the brute force method. The feasibility search method is used to locate a set of constraints that are just at the limit of possibility. One selects several values of responses of interest, and a search of the response surface is made to determine whether a solution is feasible. Subsequently, the exhaustive grid search is conducted wherein the feasible experimental range is divided into a grid of smaller sizes and searched methodically (Singh et al. 2006b).

1.7.4.3.1.3 Overlay plots

The contour plots are superimposed over each other to search for a compromise visually. Minimum and maximum boundaries are set for acceptable objective values. The region is highlighted wherein all the responses are acceptable. This is termed as an overlay plot or a combined contour plot. This area, an optimum is located, trading off different responses. Figure 27 illustrates the overlay plot generated during formulation optimization of gastroretentive formulation of trimetazidine (Singh et al. 2008d).

![Overlay plots showing the location of the optimized formulation.](image)

1.7.4.3.2 Mathematical optimization

Graphical analysis is usually preferred in the case of single response. However, in cases of multiple responses, it is usually advisable to conduct numerical analysis.
mathematical optimization first to uncover a feasible region.

1.7.4.3.2.1 Desirability function

Desirability function is a way of overcoming the difficulty of multiple, sometimes opposing responses. In this method, each response is associated with its own partial desirability function. The optimum is the point with the highest value for desirability. The experimenter should study the contour plot of desirability surface around the optimum and combine this with contour plots of the most important responses. A large area or volume of high desirability will indicate a robust formulation or set of processing conditions. Although the method requires appropriate computer software, yet it is a highly useful and pragmatic method of optimization.

Besides the techniques of “objective function” and “sequential unconstrained minimization technique (SUMT)” have also been utilized to optimize drug delivery systems numerically.

1.7.4.3.3 Sequential search methods

Despite the numerous merits of simultaneous approaches, there are situations where there is hardly any a priori knowledge about the effects of variables (Doornbos & Haan 1995; Schwartz & Connor 1996). Such situations arise when choosing a very extensive experimental domain is difficult or the possible experimental domain is not known at the beginning of the study, thus calling for the application of the sequential optimization methods. In sequential approach, optimization is attempted in a step-wise fashion. Experimentation is started at an arbitrary point in the experimental domain and responses are evaluated.

The inherent advantages of these methods are:

- No need to plan all the experiments simultaneously
- A priori knowledge of the response surface is not essential
- Interactive

However, various disadvantages encompass:
Introduction

• Number of experiments to reach an optimum can not be predicted
• Optimum found may not be the global optimum
• Robustness is not known
• Unsuitable for multiple objective problems
• Attainment of optimum is judged only by the expert developmental scientist(s)
• Mathematical model and complete response surface is not generated
• Yields unreliable results when multiple optima exist
• Applicable only when response surface is continuous

Steepest ascent (or descent) methods are direct optimization methods for first-order designs (Lewis et al. 1999), esp. when the optimum is outside the domain and is to be arrived at rapidly.

1.7.4.3.4 Artificial neural networks

In the last decade, the application of artificial neural networks (ANNs) in the field of pharmaceutical development and optimization of dosage forms has become a blown out topic of discussion in the pharmaceutical literature (Takayama et al. 1999; Rizkalla & Hildgen 2005; Amani et al. 2008; Djekic et al. 2008). The ANNs are model-independent computational paradigms that can simulate the neurological processing ability of the human brain. The neural networks, consisting of interconnected adaptive processing units, so-called neurons, are able to discern complex and latent patterns in the information presented to them. ANN is a computer-based learning system that can be applied to quantify a nonlinear relationship between causal factors and pharmaceutical responses by means of iterative training of data obtained from a designed experiment. The results obtained from implementation of an experimental design are used as input information for learning. Once trained, the neurons of an ANN may be used to forecast outputs from new sets of input conditions.

1.7.4.4 Selection of optimum search methodology

In case of single response, graphical analysis is often opted for (Lewis et al. 1999).
However in case of multiple response variables, certain responses can oppose one another. Accordingly, changes in a factor that improve one response may have a negative effect on another. Since it is not usually possible to obtain the best values for all the responses, optimization principally embarks upon finding experimental conditions where different responses are most satisfactory, over all. Nevertheless, there is a certain degree of subjectivity in weighing up their relative importance. Table 3 provides a ready reference for suitability of various optimization methods for different experimental conditions.

Table 3: Suitability of various optimization methods under variegated situations

<table>
<thead>
<tr>
<th>Optimization method</th>
<th>Model situations for use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphical analysis</td>
<td>Mathematical model of any order, normally no more than 4 factors, preferably in single response</td>
</tr>
<tr>
<td>Desirability function</td>
<td>Mathematical model of any order, number of factors between 2 and 6, multiple responses</td>
</tr>
<tr>
<td>Steepest accent</td>
<td>First-order model, optimum outside the domain, single response</td>
</tr>
<tr>
<td>Optimum path</td>
<td>Second-order model, optimum outside the domain, single response</td>
</tr>
<tr>
<td>Sequential simplex</td>
<td>No mathematical model, direct optimization, single or multiple responses,</td>
</tr>
<tr>
<td>Evolutionary operations</td>
<td>Industrial situation, little variation possible</td>
</tr>
<tr>
<td>Artificial Neural Networks</td>
<td>High levels of predictability obtained, model becomes complicated beyond 3 - 4 factors</td>
</tr>
</tbody>
</table>

1.7.5 FbD optimization of drug delivery formulations

FbD optimization employing various experimental designs has been used for a long time like, the FDs since 1926, the screening designs since 1946, the CCDs since 1951 and mixture designs since 1958 (Fisher 1935; Plackett & Burman 1946; Box & Wilson 1951; Scheffe 1958). The use of FbD optimization techniques, however, permeated into the field of pharmaceutical product development around four decades ago. The first literature report on the rational use of optimization appeared in 1967, when a tablet of sodium salicylate was optimized using an FD
Since 1990, there has been a sudden spurt in the number of published work on the use of rational optimization in drug product development. This may largely be attributed to the realization of significant benefits of systematic FbD techniques, coupled with the ready accessibility of effective and cost-effective computational tools in the armamentarium of a formulator. Amongst the products optimized, the major proportion constitutes the relatively intricate novel DDS, obviously where the benefits of FbD optimization can be better realized. Hardly any study has been reported on the FbD optimization of conventional drug formulations in the last few years.

Extensive literature search carried out in pharmaceutical journals and texts till date, reveals that the FbD optimization techniques have been employed for almost all of these dosage forms, ranging from the simple conventional ones to that of the most intricate novel drug delivery systems. Figure 28 presents a pictorial depiction of the proportion of various kinds of dosage forms formulated using FbD approach.

Diverse experimental designs have been employed for optimization of DDS. Overall Figure 29 shows that FD and its modifications (FFD, PBD, and Taguchi) occupy significant slice of the pie chart, almost one half of the entire chart, indicating their distinct popularity while optimizing the varied kind of DDS. The FDs are verily the orthogonal designs allowing independent estimation of main effects and interactions among various factors. Higher-level FDs have been found to be quite efficient in determining the quadratic effects too. The experimental designs, next in popularity, are the composite designs (CCD, BBD, and CGD) and mixture designs. Composite designs have particularly been quite preferred for investigating second-order effects in drug delivery development. A brief account of optimization of diverse type of drug formulations (i.e., controlled release matrices, floating systems, bioadhesive and buccoadhesive systems, and nanoparticles) is being presented as under with select literature instances of each type.
**Introduction**

- Macroparticulates
- Capsules
- Fast release dosage form:
- TDDS
- Vescicular systems
- Topicals
- Liquids
- Microparticulates
- Tablets
- Controlled release matrix

**Figure 28:** Pie chart showing the proportion of various pharmaceutical dosage forms optimized using FbD (Singh et al. 2011a)

**Figure 29:** Pie chart showing proportion of the individual experimental designs used in pharmaceutical formulations and processes (Singh et al. 2011a)
1.7.5.1 **FbD Optimization of oral CR matrices**

Despite tremendous advancements in diverse drug delivery approaches, oral route remains the most “natural” route of drug administration. And owing to the low cost of oral therapy, ease of administration, and improved patient compliance associated with oral route, more than 50% of DDS available commercially are oral ones. In this context, CR DDS are quite popular as they offer a number of advantages over the conventional dosage forms.

Generally, the CR DDS for oral use are solid dosage forms, based upon the mechanism of diffusion, dissolution or a blend of both to control the release rate of drug. These include, the reservoir devices wherein a polymeric membrane surrounds a drug core, and the matrix devices wherein the dissolved or dispersed drug is distributed uniformly in an inert polymeric matrix. Most DoE literature reports in this category are focused on optimizing the levels of these release rate-controlling polymers.

DoE optimization on oral CR matrix delivery devices started since early eighties (Harris *et al.* 1985; Franz *et al.* 1987). Such devices encompass, the inert matrices like hydrophilic, hydrocolloid, silicone elastomer and the lipid matrices. The common independent variables for all of these have been the quantities of the polymers or other ingredients, while the optimized responses invariably have been the parameters characterizing *in vitro* dissolution profile. The other response variables that have been optimized include, disintegration time, bioavailability, bioequivalence, etc. The literature reports on oral CR dosage forms have been compiled in various tables, categorized on the basis of various types of polymers (natural, semisynthetic, synthetic) and the type of CR dosage form (matrices, dispersions, coated tablets). Table 4 depicts the use of statistical experimental designs in optimization of oral CR matrices along with the selected drug candidate and various input variables (factors) studied.

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Factor(s)/Polymer(s) investigated</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline</td>
<td>Eudragit, ethyl cellulose</td>
<td>FD</td>
<td>(Patel &amp; Amin 2011)</td>
</tr>
</tbody>
</table>
### Introduction

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Factor(s)/Polymer(s) investigated</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venlaflaxine HCl</td>
<td>PEO, Compritol</td>
<td>FD</td>
<td>(Aboelwafa &amp; Basalious 2010)</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>PEG-4000, PVP K30, HPMC K100 and HPMC E50LV</td>
<td>ANN</td>
<td>(Barmpalexis et al. 2010)</td>
</tr>
<tr>
<td>Venlaflaxine HCl</td>
<td>HPMC, ethyl cellulose</td>
<td>CCD</td>
<td>(Madgulkar et al. 2009b)</td>
</tr>
<tr>
<td>Model drug</td>
<td>Carbopol, HPMC</td>
<td>SMD</td>
<td>(Petrovic et al. 2009)</td>
</tr>
<tr>
<td>Venlaflaxine HCl</td>
<td>Carnauba wax, bees wax</td>
<td>FD</td>
<td>(Bhalekar et al. 2008)</td>
</tr>
<tr>
<td>Isosorbide mononitrate</td>
<td>coating level of tablets, sodium carboxymethyl starch</td>
<td>BBD</td>
<td>(Xie et al. 2008)</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>HPMC, PVP</td>
<td>CCD</td>
<td>(Mandal et al. 2007)</td>
</tr>
<tr>
<td>Losartan potassium</td>
<td>HPMC, Na CMC</td>
<td>BBD</td>
<td>(Chopra et al. 2007)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Polyox, Carbopol, lactose</td>
<td>D-OD</td>
<td>(El-Malah et al. 2006)</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>HPMC, ethyl cellulose</td>
<td>CCD</td>
<td>(Chandran et al. 2006)</td>
</tr>
<tr>
<td>Propanolol HCl</td>
<td>Dextran, HPMC, cetyl alcohol</td>
<td>CCD</td>
<td>(Gil et al. 2006)</td>
</tr>
<tr>
<td>Fluoride</td>
<td>HPMC K4M, HPMC K100LV, Eudragit RL PO</td>
<td>SLD</td>
<td>(Tillotson &amp; Sakr 2004)</td>
</tr>
<tr>
<td>Salt of a weakly alkaline drug</td>
<td>HPMC, amount of water, tablet hardness</td>
<td>FD</td>
<td>(Huang et al. 2003)</td>
</tr>
<tr>
<td>Diltiazem HCl</td>
<td>Guar gum, ispaghula husk</td>
<td>FD</td>
<td>(Gohel et al. 2003)</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>HPMC of various viscosity grades, adhesive type, lubricant, preparation method</td>
<td>RSM</td>
<td>(Li et al. 2003a)</td>
</tr>
<tr>
<td>Drug(s)</td>
<td>Factor(s) /Polymer(s) investigated</td>
<td>Design</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>--------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Eudragit L 100, compression force</td>
<td>CCD</td>
<td>(Ibric et al. 2003)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>PEG 6000, lactose, stearic acid</td>
<td>RSM</td>
<td>(Grassi et al. 2003)</td>
</tr>
<tr>
<td>Verapamil HCl</td>
<td>HPMC, Na CMC</td>
<td>CCD</td>
<td>(Singh et al. 2002)</td>
</tr>
<tr>
<td>Weakly soluble acidic drug</td>
<td>HPMC of various grades, hardness</td>
<td>FD</td>
<td>(Khanvilkar et al. 2002)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Eudragit RS PO, compression force</td>
<td>CCD</td>
<td>(Ibric et al. 2002)</td>
</tr>
<tr>
<td>Didanosine</td>
<td>Eudragit RS-PM: Ethocel 100 ratio, drug content</td>
<td>Doehlert</td>
<td>(Sanchez-Lafuente et al. 2002)</td>
</tr>
<tr>
<td>Ketorolac tromethamine</td>
<td>HPMC:Na CMC ratio, EC, drug content</td>
<td>FD</td>
<td>(Vatsaraj et al. 2002)</td>
</tr>
<tr>
<td>Captopril</td>
<td>Eudragit RS of various grades, HPMC</td>
<td>FD</td>
<td>(Khattab et al. 2001)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Deaggregating agent, compression pressure, precipitating water</td>
<td>BBD</td>
<td>(Zaghloul et al. 2001)</td>
</tr>
<tr>
<td>Metoprolol tartrate</td>
<td>HPMC, HEC, DCP</td>
<td>FD</td>
<td>(Minarro et al. 2001)</td>
</tr>
<tr>
<td>Diltiazem HCl</td>
<td>Various grades of carrageenan and cellulose acetate propionate, ionic strength, buffer conc.</td>
<td>FD</td>
<td>(Bonferoni et al. 2000)</td>
</tr>
<tr>
<td>Lobenzarit disodium</td>
<td>Eudragit RS-PO, MCC, volume of granulation solvent</td>
<td>CCD</td>
<td>(Boza et al. 2000)</td>
</tr>
<tr>
<td>Diltiazem HCl</td>
<td>Succinic acid-treated ispaghula husk, DCP</td>
<td>FD</td>
<td>(Gohel et al. 2000)</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>Pectin, EC, granulating fluid</td>
<td>D-OD</td>
<td>(Ahrabi et al. 2000)</td>
</tr>
</tbody>
</table>

Various natural or semisynthetic polymers have been taken as factors, invariably
Introduction
to control or modify the release rate of the drug. The natural polymers used comprise ispaghula husk, guar gum, xanthan gum, pectin, carrageenan, algic acid, etc. Semisynthetic polymers that have been frequently employed include, mainly the cellulose derivatives, i.e., hydroxypropylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, sodium carboxymethylcellulose, ethylcellulose, etc. Some studies on the gums involve the treatment with acid or alkali to modify the swelling properties of the naturally existing gum and subsequent optimization of their proportion, to be used in SR matrices. Experimental designs have also successfully been employed in case of core-in-cup type of compressed SR matrices, studying role of non-swellable polymers and the other process variables in retarding the release of soluble (caffeine) and insoluble (ibuprofen) drugs employing FDs.

The experimental designs of choice for optimizing oral SR tablets have largely been FD, FFD and CCD, with some reports on the use of SMD, Box-Behnken design (BBD), D-optimal design (D-OD).

1.7.5.2 FbD Optimization of floating systems

Floating systems constitute an important share of gastroretentive formulations due to their ability of releasing the drug in its absorption window in a sustained manner. Such floating systems have been optimized mainly using factorial and composite designs for the buoyant properties of the dosage form and drug release profile, as indicated in Table 5.

Table 5: Literature reports on FbD optimization of floating systems

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Polymer(s)/Factor(s)</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nimodipine</td>
<td>HPMC, PEG, PVP</td>
<td>D-OD</td>
<td>(Barmpalexis et al. 2011)</td>
</tr>
<tr>
<td>Ranitidine HCl</td>
<td>HPMC, xanthan gum</td>
<td>FD</td>
<td>(Jain et al. 2010)</td>
</tr>
<tr>
<td>Tramadol HCl</td>
<td>Carbopol, HPMC</td>
<td>CCD</td>
<td>(Singh et al. 2010f)</td>
</tr>
<tr>
<td>Metoprolol succinate</td>
<td>sodium alginate, Na CMC, magnesium alumino</td>
<td>BBD</td>
<td>(Boldhane &amp; Kuchekar 2010)</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>HPMC, NaHCO₃, stearic</td>
<td>SLD</td>
<td>(Rajab et al. 2010)</td>
</tr>
<tr>
<td>Drug(s)</td>
<td>Polymer(s)/Factor(s)</td>
<td>Design</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------------</td>
<td>--------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>Na alginate, Na CMC</td>
<td>FD</td>
<td>(Boldhane &amp; Kuchekar 2009)</td>
</tr>
<tr>
<td>Ranitidine HCl</td>
<td>Ethyl cellulose, HPMC</td>
<td>FD</td>
<td>(Roy &amp; Shahiwala 2009)</td>
</tr>
<tr>
<td>Domeperidone</td>
<td>Carbopol, HPMC, Na alginate</td>
<td>BBD</td>
<td>(Prajapati et al. 2008)</td>
</tr>
<tr>
<td>Calrithromycin</td>
<td>HPMC K4M, HPMC K15M</td>
<td>D-OD</td>
<td>(Patel et al. 2006)</td>
</tr>
<tr>
<td>Metoprolol tartarate</td>
<td>Polymer:drug, polymer:polymer</td>
<td>FD</td>
<td>(Narendra et al. 2006)</td>
</tr>
<tr>
<td>Ranitidine HCl</td>
<td>Citric acid anhydrous, stearic acid</td>
<td>FD</td>
<td>(Dave et al. 2004)</td>
</tr>
<tr>
<td>Calcium</td>
<td>HPMC K4M: HPMC K100LV, CP</td>
<td>FD</td>
<td>(Li et al. 2003b)</td>
</tr>
<tr>
<td>Calcium</td>
<td>HPMC of various grades, HPMC:CP ratio, MS</td>
<td>Taguchi</td>
<td>(Li et al. 2002)</td>
</tr>
<tr>
<td>Calcium</td>
<td>HPMC, citric acid, MS</td>
<td>CCD</td>
<td>(Li et al. 2001)</td>
</tr>
<tr>
<td>Captopril</td>
<td>HPMC, Eudragit RS of various grades</td>
<td>FD</td>
<td>(Khattab et al. 2001)</td>
</tr>
<tr>
<td>Sotalol HCl</td>
<td>Na CMC:HPMC, EC:polyplasdone</td>
<td>CCD</td>
<td>(Chueh et al. 1995)</td>
</tr>
<tr>
<td>Furosemide</td>
<td>HPMC viscosity, HPMC:drug ratio</td>
<td>FD</td>
<td>(Menon et al. 1994)</td>
</tr>
</tbody>
</table>

### 1.7.5.3 FbD optimization of bioadhesive systems

Bioadhesive systems find wider applicability in drug delivery. These systems can be applied at various mucosal sites like GI mucosa, buccal, nasal, vaginal, rectal, etc., thus offering site-specificity. Such systems employ the principal of bioadhesion and thus provide prolonged residence time at the site of application. Oral mucoadhesives, besides enhanced residence time in GI tract, also offer advantages over other type of CR dosage forms like, bioavailability enhancement, targeted drug delivery, etc. The bioadhesive formulations have been optimized for bioadhesion strength and drug release profile. The independent variables have
been the type or amount of bioadhesive polymers like cellulose derivatives, polyacrylates, polymethacrylates, etc. Many types of bioadhesive formulations have been optimized like gels, tablets, semisolids, with input variables varying from 2 to 4 and the response variables from 1 to 5. Mainly, the FDs have been employed for optimization with some isolated studies using CCD and mixture design. The process optimization studies involve optimization of various bioadhesive characteristics of the formulations. Table 6 provides the list of bioadhesive DDS optimized using DoE techniques.

<table>
<thead>
<tr>
<th>Type</th>
<th>Drug</th>
<th>Polymer(s)/Factor(s)</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccoadhesive</td>
<td>Miconazole nitrate</td>
<td>HPMC, PVP</td>
<td>Simplex</td>
<td>(Madgulkar et al. 2009a)</td>
</tr>
<tr>
<td>Oral mucoadhesive</td>
<td>Hydralazine HCl</td>
<td>Carbopol, HPMC</td>
<td>CCD</td>
<td>(Singh et al. 2009a)</td>
</tr>
<tr>
<td>Buccoadhesive</td>
<td>5-FU</td>
<td>Gantrez, HPMC</td>
<td>FD</td>
<td>(Dhiman et al. 2008)</td>
</tr>
<tr>
<td>Buccoadhesive</td>
<td>Propanolol</td>
<td>Prehydration time, contact force</td>
<td>BBD</td>
<td>(Munusur et al. 2007)</td>
</tr>
<tr>
<td>Oral mucoadhesive</td>
<td>Atenolol</td>
<td>CP, Na CMC</td>
<td>FD</td>
<td>(Singh et al. 2006b)</td>
</tr>
<tr>
<td>Buccoadhesive</td>
<td>Metoprolol tartrate</td>
<td>Carbopol: HPMC, HPMC: Na alginate</td>
<td>CCD</td>
<td>(Narendra et al. 2005)</td>
</tr>
<tr>
<td>Buccoadhesive</td>
<td>Progesterone</td>
<td>HPMC, CP 934P, mannitol</td>
<td>SLD</td>
<td>(Bain et al. 2002)</td>
</tr>
<tr>
<td>Buccoadhesive</td>
<td>Diltiazem HCl</td>
<td>HPMC, CP</td>
<td>FD</td>
<td>Singh &amp; Ahuja 2002</td>
</tr>
<tr>
<td>Oral mucoadhesive</td>
<td>Insulin</td>
<td>Eudragit (L100 &amp; S100)</td>
<td>CCD</td>
<td>(Uraizee et al. 2000)</td>
</tr>
<tr>
<td>Oral mucoadhesive</td>
<td>Verapamil HCl</td>
<td>HPMC, Na CMC, MCC</td>
<td>SLD</td>
<td>(Elkheshen &amp; Hosny 1999)</td>
</tr>
</tbody>
</table>


### Introduction

<table>
<thead>
<tr>
<th>Type</th>
<th>Drug</th>
<th>Polymer(s)/Factor(s)</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucoadhesive dental semisolid</td>
<td>Tetracycline</td>
<td>HEC, PVP</td>
<td>FD</td>
<td>(Jones &amp; Pearce 1996)</td>
</tr>
<tr>
<td>Mucoadhesive ocular drug delivery system</td>
<td>Timolol maleate</td>
<td>Dextran stabilizers, surfactants, pH of the aqueous phase</td>
<td>FD</td>
<td>(Das et al. 1995b)</td>
</tr>
<tr>
<td>Oral mucoadhesive tablet</td>
<td>Sotalol HCl</td>
<td>Na CMC:HPMC ratio, EC: polyplasdone ratio</td>
<td>CCD</td>
<td>(Chueh et al. 1995)</td>
</tr>
<tr>
<td>Mucoadhesive nasal gel</td>
<td>Propranolol HCl</td>
<td>Viscosity of CP, solvent composition</td>
<td>SC-LD</td>
<td>(Chu et al. 1991)</td>
</tr>
</tbody>
</table>

1.7.5.4 **FbD Optimization of nanoparticulate systems**

Now-a-days, nanoparticles employing various biodegradable and synthetic polymers, are becoming popular for providing various benefits like site specificity, enhancement in bioavailability, controlled release of various drugs, etc, with fruition. Such systems can either be injected i.v. applied with site-specificity (e.g., opthalamic, nasal, pulmonary, etc.). Table 7 gives an account on the optimization literature on nanoparticulates.

### Table 7: Literature reports on DoE optimization of nanoparticulate systems

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Polymer(s)/Factor(s)</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>Compritol, Tween 80</td>
<td>CCD</td>
<td>(Dhawan et al. 2011)</td>
</tr>
<tr>
<td>Insulin</td>
<td>pH of polymer solution, conc. ratio of polymer/insulin</td>
<td>D-OD</td>
<td>(Mahjub et al. 2011)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Lipid, surfactant</td>
<td>CCD</td>
<td>(Gonzalez-Mira et al. 2011)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>Type and concentration of cryoprotectant</td>
<td>D-OD</td>
<td>(Varshosaz et al. 2010)</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>Volume of co-solvent, volume of non-solvent</td>
<td>FD</td>
<td>(Yadav &amp; Sawant 2010)</td>
</tr>
<tr>
<td>Rivastigmine</td>
<td>PLGA, PBCA</td>
<td>FD</td>
<td>(Joshi et al. 2010)</td>
</tr>
<tr>
<td>Drug(s)</td>
<td>Polymer(s)/Factor(s)</td>
<td>Design</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>--------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Amount of miglyol, PLGA</td>
<td>CCD</td>
<td>(Kollipara et al. 2010)</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Glycerol monostearate, poloxamer, isopropyl alcohol</td>
<td>FD</td>
<td>(Shah &amp; Pathak 2010)</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>BSA, ethanol</td>
<td>CCD</td>
<td>(Zu et al. 2009)</td>
</tr>
<tr>
<td>Insulin</td>
<td>Calcium chloride, chitosan, albumin</td>
<td>BBD</td>
<td>(Woitiski et al. 2009)</td>
</tr>
<tr>
<td>Insulin lauryl sulphate</td>
<td>PLGA/INS complex weight ratio, PVA/acetone volume ratio</td>
<td>CCD</td>
<td>(Shi et al. 2008)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Boric acid addition to inner aqueous phase, drug conc. in inner aqueous phase, oil/outer aqueous phase ratio, homogenization cycles number</td>
<td>FD</td>
<td>(Dillen et al. 2004)</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>Emulsifier, homogenization pressure</td>
<td>Taguchi</td>
<td>(Yang &amp; Zhu 2002)</td>
</tr>
<tr>
<td>Aceclofenac</td>
<td>PCL, Miglyol 812, drug content</td>
<td>CCD</td>
<td>(Alonso et al. 2000)</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>Polymer, surfactant, temperature of aqueous phase, needle gauge, volume of organic phase</td>
<td>RCCD</td>
<td>(Molpeceres el al. 1996; Molpeceres et al. 1997)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>PCL, Migliol 840, acetone</td>
<td>FD</td>
<td>(Calvo et al. 1996)</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>PLGA, needle gauge, injection rate</td>
<td>RCCD</td>
<td>(Chacón et al. 1996)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>PLA, acetone, methylene chloride</td>
<td>FD</td>
<td>(Wehrlé et al. 1995)</td>
</tr>
<tr>
<td>Timolol maleate</td>
<td>Dextran of various grades, Pluronic F68, Tween 20, pH of the aqueous phase</td>
<td>FD</td>
<td>(Das et al. 1995a)</td>
</tr>
<tr>
<td>Metipranolol</td>
<td>Polysobutylcyanoacrylate, PCL, type and volume of the oil encapsulated</td>
<td>FD</td>
<td>(Losa et al. 1993)</td>
</tr>
</tbody>
</table>

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### Introduction

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Polymer(s)/Factor(s)</th>
<th>Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>Butyl cyanoacrylate monomer amount, temperature, pH of aqueous phase, drug content</td>
<td>CCD</td>
<td>(Carpignano et al. 1991)</td>
</tr>
</tbody>
</table>

### 1.8 DRUG AND EXCIPIENT MONOGRAPHS

#### 1.8.1 Rivastigmine: Drug profile

##### 1.8.1.1 Physicochemical properties

Rivastigmine has an empirical formula of $C_{14}H_{22}N_2O_2.C_4H_6O_6$ (hydrogen tartrate salt, Figure 30) and a molecular weight of 400.43. Rivastigmine tartrate is a white to off-white, fine crystalline powder that is very soluble in water, soluble in ethanol and acetonitrile, slightly soluble in n-octanol and very slightly soluble in ethyl acetate. The distribution coefficient at 37°C in n-octanol/phosphate buffer solution pH 7 is 3.0.

![Figure 30: Chemical structure of rivastigmine tartarate](image)

##### 1.8.1.2 Absorption and distribution

Rivastigmine exhibits rapid and nearly complete oral absorption, with $T_{max}$ of 0.8 (0.5 in some reports) to 1.7 hours. Food slows absorption and results in a decrease of $C_{max}$ of about 30% with an increase in the extent of absorption, also by 30% (Spencer & Noble 1998). Rivastigmine should be taken with meals (breakfast and dinner), which decreases GI problems. NAP-226-90, its decarbamylated metabolite, is also rapidly detected within 2 hours of administration (Jann 2000). About 96% of a radiolabelled dose is absorbed, but the presystemic biotransformation is high, indicating a significant first-pass effect with an AUC
ratio of oral to intravenous drug of 0.355 (35% bioavailability) (Spencer & Noble 1998; Jann 2000). Protein binding of rivastigmine is quite low at 40%, with about 40 to 50% of rivastigmine bound with red blood cells. The volume of distribution of rivastigmine and its metabolite are 1.8 to 2.7 and 4.3 to 5.9 L/kg, respectively.

**1.8.1.3 Metabolism and Elimination**

Rivastigmine is not significantly metabolized by hepatic oxidative CYP isoenzymes. Rivastigmine is rapidly and extensively metabolised, primarily by cholinesterases, to the NAP-226-90 metabolite (Spencer & Noble 1998; Jann 2000). NAP-226-90 may then undergo N-demethylation and/or sulphate conjugation. After metabolism, it undergoes rapid renal elimination. Accumulation of rivastigmine and NAP-226-90 does not occur after repeated administration of 1 to 6 mg/day. The $t_{1/2\beta}$ of rivastigmine from the CSF was reported to range from 0.31 to 2.95 hours with multiple dose administration.

**1.8.1.4 Effects of age, hepatic and renal impairment**

The $t_{1/2\beta}$ of rivastigmine is slightly prolonged in the elderly compared with adult volunteers (ranges 0.88 to 1.25 vs 0.80 to 0.99 hours), which does not appear to be clinically significant (Cutler et al. 1998). In patients with renal and moderate hepatic impairment, the AUC of rivastigmine was 1.4-fold and 2.3-fold higher, respectively, than in adult healthy controls. The AUC of NAP-226-90 was also 0.8-fold lower in renally impaired patients, but 1.5-fold greater in hepatically impaired patients. Significant differences in the $t_{1/2\beta}$ of parent drug were not found in patients with moderate or severe renal impairment. The $t_{1/2\beta}$ of NAP-226-90 was longer only in patients with severe renal impairment (6.0 vs 3.4 hours). Although the AUC of rivastigmine was higher in patients with hepatic impairment, specific dosage recommendations may not be needed in these special populations. Rivastigmine has not been studied in patients with severe hepatic impairment (Jann et al. 2002; Williams et al. 2003).

**1.8.1.5 Mechanism of action**

Rivastigmine’s precise mechanism of action is unknown. It binds to both the esteratic and ionic sites of AChE, preventing the enzyme from metabolizing ACh. Several isoforms of AChE have been identified, with identical amino acid
sequences, but with different posttranslational modifications, anatomic and microanatomic locations, and functions.

Rivastigmine also inhibits the activity of BuChE, which is more active in patients with AD (McGleenon et al. 1999; Simon et al. 2000). Rivastigmine selectively inhibits cholinesterases in the CNS, using cerebrospinal fluid (CSF) activity as a marker. The inhibition of AChE and BuChE has been demonstrated for up to 12 months in patients with mild dementia (McGleenon et al. 1999; Williams et al. 2003). Although AChE selectively hydrolyzes ACh, BuChE also degrades other substrates such as various neuroactive peptides. Therefore, inhibiting both AChE and BuChE results in higher levels of ACh in the brain. Over the course of AD, AChE activity decreases progressively, whereas BuChE activity increases progressively. Therefore, in early stages of AD, inhibition of AChE is more important, but as the disease progresses, BuChE inhibition contributes more to reducing the cholinergic deficit. Both AChE and BuChE are associated with formation of amyloid plaque and NFTs, the two pathologic criteria for the diagnosis of AD. Advanced plaque formation shows as much as 87% BuChE activity and 80% AChE activity, which has been demonstrated to accelerate the formation of Aβ.

1.8.2 Quercetin: A potential anti-Alzheimer’s flavonoid

Quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (Figure 31), is a yellow, crystalline solid with a bitter taste, which is insoluble in water, slightly soluble in alcohol, and soluble in glacial acetic acid and aqueous alkaline solutions.

Animals are unable to synthesize the flavone nucleus, thus quercetin is exclusively found in the plant kingdom. Quercetin is found in various food products and plants, including fruits, seeds, vegetables, tea, coffee, bracken fern, and natural dyes. Foods rich in quercetin include black and green tea (Camellia sinensis; 2000–2500 mg/kg), capers (1800 mg/kg), lovage (1700 mg/kg), apples (44 mg/kg), onion, especially red onion (1910 mg/kg), red grapes, citrus fruit, tomato, broccoli and other leafy green vegetables, and a number of berries, including raspberry, bog whortleberry (158 mg/kg, fresh weight), lingonberry (cultivated 74 mg/kg, wild 146 mg/kg), cranberry (cultivated 83 mg/kg, wild 121 mg/kg), chokeberry.
Introduction

(89 mg/kg), sweet rowan (85 mg/kg), rowanberry (63 mg/kg), sea buckthorn berry (62 mg/kg), crowberry (cultivated 53 mg/kg, wild 56 mg/kg), and the fruit of the prickly pear cactus.

Figure 31: Chemical structure of quercetin

1.8.2.1 Antioxidant potential of quercetin

Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation (Hollman & Katan 1997; Sakanashi et al. 2008). Lipid peroxidation is the process by which unsaturated fatty acids are converted to free radicals via the abstraction of hydrogen (Young & McEneny 2001). The subsequent free radicals are oxidized by molecular oxygen to create lipid peroxy radicals. This process is propagated by the resulting lipid peroxy radicals extracting hydrogen from other unsaturated fatty acid molecules to create more free radicals. It is catalyzed, in part, by the presence of trace amounts of transition metal ions. Lipid peroxidation can create deleterious effects throughout the body, such as cardiovascular and neurodegenerative diseases; however, it can be terminated by antioxidants, like quercetin, which interfere by reacting with the radicals formed (Adedapo et al. 2008; Singh et al. 2009c; Kim et al. 2011).

The oxidation of low-density lipoproteins (LDL) can result in the formation of atherosclerotic plaques, leading to cardiovascular disease (Tabuchi et al. 2007; Pedersen et al. 2010; Tsimikas & Miller 2011). However, several studies have illustrated quercetin’s ability to inhibit LDL oxidation. A 21% reduction in cardiovascular disease mortality has been observed when the intake of quercetin was greater than 4mg/d (Graf et al. 2005). Chopra et al. (2000) gave one group of males 30 mg/d of quercetin and another group 1 g of red wine powdered extract for two weeks, prior to which there had been a placebo period for all participants so that each could be their own control. The red wine extract was in the form of a
powder and contained several flavonoids, among which quercetin constituted 3.5 mg per gram of powder. Every participant was required to keep a journal of their intake of specific foods, including: fruits, vegetables, chocolates, fruit juices, milk, and alcohol. Vitamins C and E plasma concentrations were also measured along with flavonoids. They reported that the red wine extract and quercetin inhibited LDL and there was no effect on plasma concentrations of vitamin C and E. However, plasma concentrations of LDL remained constant. Chopra and co-workers suggested that LDL-cholesterol is only lowered by quercetin in hyperlipidemic patients; otherwise, quercetin inhibits LDL oxidation (Chopra et al. 2000).

The vulnerability of brain lipid membranes to lipid peroxidation is thought to lead to neurodegenerative disease, such as Alzheimer’s and Parkinson’s disease (Sanyal et al. 2009; Khuwaja et al. 2010; Skoumalova et al. 2011). In early nineties, Balazs and Leon (1994) found that oxidative stress occurring in the brain membrane lipids is associated with the extracellular accumulation of amyloid beta-peptide, which precedes neural losses in Alzheimer’s patients. Yet, formation of amyloid plaques can be prevented by taking antioxidants (Roth et al. 1999; Ansari et al. 2009). In this situation, quercetin does not only stop the propagation of lipid peroxidation, but also increases glutathione (GSH) levels. GSH is part of the neuron’s defense against oxidative damage. When the superoxide radical is formed, the radical can be converted to the hydrogen peroxide radical by superoxide dismutase; however, GSH can convert hydrogen peroxide to oxygen and water, preventing the formation of free radicals.

Quercetin can also reduce inflammation by scavenging free radicals. Free radicals can activate transcription factors that generate pro-inflammatory cytokines, which are often found to be elevated in patients that suffer from chronic inflammatory diseases (Boots et al. 2008). Chronic prostatitis is not well understood, but it is thought that the disease inflames the genital tract. Alexander et al. (1998), examined semen samples taken from normal men and men with chronic prostatitis, measuring the levels of the cytokines tumor necrosis factor-alpha and interleukin-1 beta. The results showed that men with the disease had higher levels of both pro-inflammatory cytokines in seminal plasma. In another study, Shoskes and co-workers (Shoskes et al. 1999) administered men with chronic prostatitis 500
mg of quercetin twice a day for one month. As a result, 67% of the men had a 25% improvement in symptoms.

Oxidative stress can cause cell death by means of prolonged elevations of intracellular Ca^{2+} concentrations (Sakanashi et al. 2008). Elevated levels of Ca^{2+} concentrations lead to which can lead to strokes and acute neuronal losses (Nicotera & Orrenius 1998). However, quercetin can protect cells suffering oxidative stress and thus prevent Ca^{2+}-dependent cell death (Sakanashi et al. 2008).

In a 15 year study following 550 middle-aged men, those with a flavonol intake greater than 30 mg/d had a 60% reduction in their risk for strokes (Hollman & Katan 1997).

Quercetin can also protect against the more obvious environmental causes of free radicals, such as smoking. Cigarette tar is a source of free radicals, which has been found to damage erythrocyte membranes. Begum and Terao (2002) found that the quercetin aglycone and its conjugate metabolites (quercetin-3-O-β-glucuronide and quercetin-3-O-β-glucoside) could protect erythrocytes from the membranous damage that is caused by smoking. The control used in the study was flavone, which has the basic structure of quercetin but no hydroxyl groups, and it had no effect on the erythrocytes. This indicated that the hydroxyl groups are important to the antioxidant properties of quercetin.

1.8.2.2 Pharmacokinetics of quercetin

Quercetin follows a two-compartment open-body model after its i.v. administration (60 mg/m²) over a period of 30 s (Gugler et al. 1975). The distribution half-life is found to widely range between 0.7 - 7.8 min, with a median value of 6 min. The elimination half-life, analogously, varies grossly between 3.8 - 86 min, with a median value of 43 min. The clearance is 0.23 - 0.84 L/min/m², with a median value of 0.28 L/min/m². The median volume of distribution is observed to be 3.7 L/m². An excellent correlation coefficient of 0.89 is construed between the dose in mg/m² and AUC indicating linear pharmacokinetics in the studied dose range. No obvious relationship, however, is noticeable between toxicity and pharmacokinetic parameters (Ferry et al. 1996).

Following i.v. administration, very low amounts of quercetin are able to enter the CNS due to the presence of the natural defense, blood brain barrier. Thus,
quercetin has to be formulated in such a way so as to facilitate its entry into the brain in order to reap the benefits of its anti-oxidant potential to the fullest. This is particularly of interest when the objective of investigation is reduction of oxidative stress to prevent neurodegeneration in disorders like AD.

1.8.2.3 Quercetin: Physicochemical characteristics

The physicochemical properties of quercetin are compiled as under:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS No.</td>
<td>117-39-5; 73123-10-1; 74893-81-5 (Base)</td>
</tr>
<tr>
<td></td>
<td>6151-25-3 (Dihydrate)</td>
</tr>
<tr>
<td>EINECS No.</td>
<td>204-187-1</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{15}H_{10}O_{7}</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>302.24</td>
</tr>
<tr>
<td>Density</td>
<td>1.799 \text{g} \cdot \text{cc}^{-1}</td>
</tr>
<tr>
<td>Physical state</td>
<td>Yellow crystalline powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>310 - 317 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>Sublimes</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Vapour density</td>
<td>8.1</td>
</tr>
<tr>
<td>Flash point</td>
<td>112 °C</td>
</tr>
<tr>
<td>pKa</td>
<td>7.2</td>
</tr>
</tbody>
</table>

1.8.3 Compritol: Excipient profile

Compritol® 888 ATO is a solid lipid based on glycerol esters of behenic acid (C_{22}). It consists of glycerol tribehenate (28 - 32%), glycerol dibehenate (52 - 54%) and glycerol monobehenate (12 - 18%). The main fatty acid is behenic acid (> 85%) but other fatty acids (C16 - C20) are also present. Due to the presence of partial acylglycerols, Compritol® 888 ATO has an amphiphilic character. Its hydrophilic-lipophilic balance (HLB) is ~2. In addition, this lipid has a peroxide value lower than 6 meq O_{2} kg^{-1}, indicating a relatively high chemical stability, which is an important aspect in formulation of SLNs (Souto et al. 2006).

**Solubility:** Insoluble in water, ethanol, n-hexane and mineral oils; soluble in chloroform

**Appearance:** Fine powder
Introduction

**Odour:** Faint

**Melting point:** 75 - 85 °C

**Acid value:** < 4.00 mg KOH·g⁻¹

**Saponification value:** 145 to 165 mg KOH·g⁻¹

**Iodine value:** < 3.0 gI²/100 g

**Peroxide value:** < 6.0 meqO₂/Kg

**Water content:** < 0.1%

**Free glycerol content:** < 1.0%

**Total monoglycerides content:** 15.0 to 23.0%

**Total diesters content:** 40.0 - 60.0%

**Total trimesters content:** 21.0 - 35.0%

**Palmitic acid (C₁₆):** < 3.0%

**Stearic acid (C₁₈):** < 5.0%

**Arachidic acid (C₂₀):** < 10.0%

**Behenic acid (C₂₂):** > 83.0%

**Erucic acid:** < 1.0%

1.8.4 Polysorbate 80: Excipient profile

Polysorbate 80 (commercially also known as Tween® 80, a registered trademark of ICI Americas, Inc. and other trade names) is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. Polysorbate 80 is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups which are polymers of ethylene oxide. In the nomenclature of polysorbates, the numeric designation following polysorbate refers to the lipophilic group, in this case the oleic acid. Polysorbate 80 is often used in food and pharmaceutical products as an emulsifier.

**IUPAC name:** Polyoxylethylene (20) sorbitan monooleate

**Synonyms:** Polyoxylethylene 20 sorbitan monooleate; polyethylene oxide sorbitan mono-oleate; polyoxylethylene sorbitan monooleate; polyoxylethylene sorbitan oleate; Sorethytan (20) monooleate

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**Introduction**

**Chemical structure:**

![Chemical structure diagram]

**Molecular formula:** $C_{64}H_{124}O_{26}$

**Physical state:** Oily liquid

**Odour:** Fatty (slight)

**Colour:** Clear amber, yellow

**pH (1% solution):** 7.0

**Molar mass:** 1310 g.mol$^{-1}$

**Density:** 1.06 - 1.09 g.mL$^{-1}$

**Boiling point:** $>100\degree C$

**Viscosity:** 300 - 500 centistokes

**Vapor pressure:** $<0.1$ KPa

**Solubility:** Very soluble in water, soluble in methanol, toluene, alcohol, cottonseed oil, corn oil, ethyl acetate; insoluble in mineral oil

**1.8.5 Carbopol: Excipient profile**

It is a synthetic high molecular weight polymer of acrylic acid crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56-68% of carboxylic acid (COOH) groups, calculated on the dry basis. The carboxyl group provided by the acrylic acid backbone of the polymer are responsible for many of the product characteristics. Carbopols designated with the suffix P, e.g., Carbomer 934P, are the only pharmaceutical grades of polymers accepted for oral or mucosal contact products.

**Non-proprietary names:** Carbomer (BP)

**Chemical name:** Carboxy polymethylene

**Empirical formula:** $(C_3H_4O_2)_x (-C_3H_5 sucrose)$

**Grades:** 907, 910, 934, 934P, 940, 941, 971P, 974P, 980 & 981
Introduction

Structural formula:

\[
\begin{array}{c}
\text{H} \\
\text{C} \\
\text{H}
\end{array}
\]

Molecular weight: Between $1 \times 10^6$ and $4 \times 10^6$

Description: occurs as white, fluffy, acidic, hygroscopic powder with a slight characteristic odour.

Density (bulk): 1.76 g/cm³

Density (tapped): 1.4 g/cm³

pH: 2.5 - 3.0 (1% aqueous solution)

Melting point: Decomposition occurs at 260°C

Solubility: Practically soluble in water, and after neutralization in ethanol (95%) and glycerin.

Incompatibilities: Carbopol is discoloured by resorcinol and is incompatible with phenol, cationic polymers, strong acids and high concentration of electrolytes. Trace levels of iron and the transition metals can catalytically degrade carbomer dispersion.

Safety: Used in non-parenteral medicines and is non-toxic and non-irritant and there is no evidence of allergic reactions or hypersensitivity in humans.

Applications: It is used as suspending, thickening, emulsifying and gelling agent. In tablet formulations, carbopol is used as a binder during either direct compression or wet granulation processes. It is also employed in cosmetics. It is frequently used as the bioadhesive component in mucoadhesive ointments, gels and tablets. Since the drug release rate from the solid formulation of CP is slow, it is difficult to take advantage of the
Introduction

polymers mucoadhesive property in oral administration of fast acting drugs. Freeze dried sodium salt of CP (FNaCP), may be used to improve the release rate of drug from the formulation of CP.

1.8.6 HPMC: Excipient profile

Non-proprietary name: Hydroxypropyl methylcellulose

Chemical name: Cellulose, 2-Hydroxypropylmethylether

Structural formula:

\[
\begin{array}{c}
\text{CH}_2\text{OH} \\
\text{H} \\
\text{OR} \\
\text{H} \\
\text{OR} \\
\text{H} \\
\text{CH}_2\text{OR} \\
\end{array}
\]

Molecular Weight: Approx. 86,000

Description: Odourless, tasteless, white, yellowish white or grayish white, hygroscopic fibrous powder or granules.

Apparent density: 0.25 - 0.70 g/cm³

Browning temperature: 190 - 200 °C

Charring temperature: 225 - 230 °C

Percent moisture: 3% maximum

Gel Point: 50° C - 90° C depending on the grade

Ash: 1.5% - 3.0% depending on the grade

Specific gravity: Approximately 1.3

Surface activity: Provides some surfactancy in solutions. Surface tensions for such solutions range from 42-56 dynes/cm

Ionic charge: Being nonionic, it will not complex with metallic salts and ionic organics to form insoluble precipitates, thus presenting less compatibility problems.

Gel formation: Undergoes a reversible transformation from sol to gel upon heating and cooling.

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**Introduction**

**Solubility:** Soluble in cold water, forming a viscous colloidal solution, practically insoluble in hot water, acetone and toluene, insoluble in alcohol, ether and chloroform, but soluble in mixtures of methyl alcohol and methylene chloride. Certain grades are soluble in aqueous acetone and other organic solvents.

**Enzyme resistance:** Comparatively enzyme resistant, providing excellent viscosity stability during long-term storage.

**Stability:** Very stable in dry conditions. Solutions are stable at pH 3.0-11.0. Aqueous solutions are liable to be affected by microorganisms. When used as a viscosity-increasing agent in ophthalmic solutions, an antimicrobial agent, such as benzalkonium chloride, should be incorporated.

**Incompatibilities:** Extreme pH conditions and oxidizing materials.

**Types and grades:** Various grades of HPMC, official in USP, BPC, IP, are frequently employed as polymers for controlling drug release. For BPC/IP grades the name is followed by a number indicating the apparent viscosity of a 2% w/v solution in millipascal seconds at 20°C. For USP grade HPMC, the name is followed by a four-digit number. The first two digits refer to the approximate content of the methoxy (-OCH$_3$) groups and the second two digits refer to the approximate content of the hydroxypropoxy group (-OCH$_2$CHOHCH$_3$) in percent, calculated on dry basis (105°C for two h). The HPMC grades are being marketed by various manufacturers, the prominent being Methocels by the Dow Chemical Company, USA and Metolose by the Shin Etsu Chemical products Ltd., UK. Table 8 enlists the compendial limits for the contents of methoxy/hydroxypropoxy groups.

**Applications:** HPMC is perhaps the most commonly used film-forming agent in tablet film coating. Lower viscosity grades are used in aqueous film coating and higher viscosity grades are used in solvent film coating. The concentration varies from 2 to 10% depending upon the viscosity grade of the polymer. It is used as a binder in tablet granulations in concentration of 2 - 5%. High-viscosity grades are used to retard the release of water soluble drugs. It has also been used as a thickening agent added to vehicles for eye drops and artificial tear solutions at
Introduction

0.45 - 1.0% concentrations.

Table 8: Substitution type, trade names and limits of methoxy and hydroxypropoxy groups in hydroxypropyl methylcellulose

<table>
<thead>
<tr>
<th>Substitution Type (USP)</th>
<th>Trade Name</th>
<th>Methoxy (%)</th>
<th>Hydroxypropoxy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dow</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>1828</td>
<td>Methocel J</td>
<td>16.5</td>
<td>20.0</td>
</tr>
<tr>
<td>2208</td>
<td>Methocel K</td>
<td>19.0</td>
<td>24.0</td>
</tr>
<tr>
<td>2906</td>
<td>Methocel F</td>
<td>27.0</td>
<td>30.0</td>
</tr>
<tr>
<td>2910</td>
<td>Methocel E</td>
<td>28.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Table 9 enlists the various pharmacopoeial specifications for HPMC.

Table 9: Pharmacopoeial specifications for HPMC

<table>
<thead>
<tr>
<th>Test</th>
<th>USP</th>
<th>BPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>---</td>
<td>6.0 - 8.0</td>
</tr>
<tr>
<td>Apparent Viscosity</td>
<td>Viscosity Grade</td>
<td>Viscosity</td>
</tr>
<tr>
<td></td>
<td>Viscosity (20°C)</td>
<td>Viscosity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20°C)</td>
</tr>
<tr>
<td>≤ 100 cps</td>
<td>80 -120%</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15-25</td>
</tr>
<tr>
<td>&gt; 100 cps</td>
<td>75 - 140%</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110-140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>350-550</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1200-1800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3750-5250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12000-18000</td>
</tr>
<tr>
<td>LOD*</td>
<td>≤ 5% by weight,</td>
<td>≤ 10 %</td>
</tr>
<tr>
<td>Residue on ignition</td>
<td>≤ 1.5 % (&gt; 50 cps)</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>≤ 3.0 % (≤ 50 cps)</td>
<td>------</td>
</tr>
<tr>
<td>Sulfated ash</td>
<td>----</td>
<td>≤ 1.0 %</td>
</tr>
<tr>
<td>Arsenic</td>
<td>≤ 3 ppm</td>
<td>≤ 2 ppm</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>≤ 0.001 %</td>
<td>------</td>
</tr>
<tr>
<td>Lead</td>
<td>----</td>
<td>≤ 5 ppm</td>
</tr>
</tbody>
</table>

It is a protective colloid which prevents droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments and hence is used as an emulsifier, suspending agent and stabilizer in gels and ointments.
Introduction

1.8.7 Polyethylene oxide: Excipient profile

Polyethylene oxide is a nonionic homopolymer of ethylene oxide, where n represents the average number of oxyethylene groups (200-100,000).

**Non-proprietary names**: Polyethylene oxide (USP)

**Synonyms**: Polyox; polyoxirane; polyoxyethylene

**Chemical name**: Polyethylene oxide [25322-68-3]

**Grades**: WSR N-10, WSR N-80, WSR N-750, WSR N-3000, WSR 205, WSR 1105, WSR N-12K, WSR N-60K, WSR 301, WSR 303

WSR stands for water soluble resins. Grade units are classified on the basis of number of repeat units and molecular weight.

**Structural Formula**: \((\text{CH}_2\text{CH}_2\text{O})_n\)

**Molecular weight**: Between \(1 \times 10^5\) and \(7 \times 10^6\)

**Description**: White to off-white, free-flowing powder.

**Physiochemical properties**

- **Density (true)**: 1.3 g/cm³
- **Melting point**: 65 – 70 °C
- **Solubility**: Polyethylene oxide is soluble in water and a number of common organic solvents such as acetonitrile. It is insoluble in aliphatic hydrocarbons, and most alcohols.
- **Storage**: Store in tightly sealed containers in a cool, dry place. Avoid exposure to high temperatures since this can result in viscosity reduction.
- **Incompatibilities**: Polyethylene oxide is incompatible with strong oxidizing agents.
- **Safety**: Animal studies suggest that polyethylene oxide has a low level of toxicity regardless of the route of administration. It is poorly absorbed from the GI tract but appears to be completely and rapidly eliminated. The resins are neither skin irritants, nor sensitizers, nor do they cause eye irritation.
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Applications: It can be used as a tablet binder at concentrations from 5-85%. The higher molecular weight grades provide delayed release via the hydrophilic matrix approach. Polyethylene oxide is an excellent mucoadhesive polymer. Low levels are effective thickeners, although alcohol is usually added to water-based formulations to provide improved viscosity stability. PEO films demonstrate good lubricity when wet. It can be radiation crosslinked in solution to produce a hydrogel. The hydrogels so produced have been used in wound care applications.

1.8.8 Sodium CMC: Excipient profile

**Non-proprietary names**: BP: Carmellose sodium  
USP: Carboxymethylcellulose sodium

**Chemical name**: Cellulose, carboxymethyl ether, sodium salt

**Structural formula**: ![Structural formula](image)

**Molecular weight**: 90,000-700,000

**Description**: It occurs as white or buff colored, odorless, hygroscopic powder or granules. The B.P. specifies a sodium content of 6.5-10.8% and U.S.P. specifies it as 6.5-9.5%.

**Physiochemical properties**

**Apparent density**: 0.75 g/cm³

**Moisture content**: ≤10 %

**Specific gravity**: 1.0068

**Gel formation**: Undergoes a reversible transformation from solution to gel upon heating and cooling, respectively.

**Sulfated ash**: 20.0-33.3%

**Ionic charge**: Anionic nature

**Solubility**: Practically soluble in acetone, ethanol, ether and toluene. Easily dispersed in water at all temperatures, forming clear, colloidal solutions. The aqueous solubility varies with the degree of substitution.

**Storage**: It should be stored in airtight containers in a cool
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and dry place.

Incompatibility: It is incompatible with strongly acidic solutions and with soluble salts of iron and some other metals, such as aluminium, mercury and zinc. It is also incompatible with xanthan gum. Precipitation can occur at pH < 2 and when mixed with ethanol (95%). It also forms complex coacervates with gelatin and pectin. It additionally forms a complex with collagen and is capable of precipitating certain positively charged proteins.

Stability: CMC sodium is a stable, though hygroscopic material. Under high humidity conditions, it can absorb a large quantity (> 50%) of water. Aqueous solutions are stable between pH 2-10. Generally, solutions exhibit maximum viscosity and stability at pH 7-9. Sterilization of solutions by heating and gamma radiations results in a reduction in viscosity. Aqueous solution stored for prolonged periods should contain an antimicrobial preservative.

Safety: It is generally regarded as non-toxic and non-irritant material.

Labeling: Label to indicate the apparent viscosity in millipascal seconds of a 2% w/v solution or, where the viscosity is low, the concentration of the solution to be used and the apparent viscosity in millipascal seconds.

Viscosity: Various grades of sodium CMC are commercially available which have differing aqueous viscosities: aqueous 1% w/v solutions with viscosities of 5-4000 mpa (5-4000 cps) may be obtained. It is available as a low viscosity, medium viscosity and high viscosity grades.

Applications

It is widely used in oral and topical pharmaceutical formulations primarily for its viscosity increasing properties. It is used as tablet binder and disintegrant and to stabilize emulsions.