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BIOAVAILABILITY AND DRUG ABSORPTION

Oral bioavailability is the rate and extent of absorption, which is a process of movement of the unchanged drug from its site of administration to systemic circulation. Latter is important for designing and developing the drug dosage regimen. Most of the drugs produce their effects only after they have entered into the bloodstream. The rapidity of absorption and site of absorption depend on chemical and physical properties of the drug. The rate of absorption effects the duration and intensity of the drug action, thus providing a correlation between the plasma concentration and the therapeutic response.

With oral route being regarded as the most compliant of all the existing modes of administration, it is of paramount importance to design a drug in a form possessing enhanced oral bioavailability (Varma et al., 2010). However, a multiple processes, which occur in sequence or simultaneously and are driven by multiple molecular determinants, are involved upon oral administration of drug molecules. These processes determine the extent of exposure to a drug after oral administration. For example, the landmark analysis by Lipinski and colleagues showed that particular physicochemical attributes of a molecule are associated with high or low oral bioavailability (Lipinski et al., 1997). Molecular weight >500, lipophilicity >5 (calculated LogP), total hydrogen bond acceptors >10, and total hydrogen bond donors>5 are all properties identified as those that would decrease the likelihood of good oral absorption (Lipinski et al., 1997). Similar observations were made by others in an effort to define descriptors that can provide a rationale for establishing qualitative, semiquantitative, and quantitative structure-absorption relationship (QSAR) models (Bergstrom et al., 2003; Linnankoski et al., 2006; Veber et al., 2002). The dependence of human intestinal absorption on the readily accessible physicochemical properties like lipophilicity, molecular size, hydrogen bonding capacity, polar surface area, and number of free rotatable bonds has been demonstrated (Bergstrom et al., 2003; van de Waterbeemd, 1998; Veber et al., 2002). Identification of these basic physicochemical properties as determinants is consistent with notions regarding the ability of small organic molecules to pass
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through lipid bilayer membranes. However, oral exposure is determined not only by absorption through membranes of the gastrointestinal tract but also by the extent to which organs just after absorption are able to extract these orally administered drugs. The most important organs causing first-pass extraction include the liver (which extracts by metabolism, uptake transport, and biliary secretion) and the intestine (which extracts mainly by metabolism) (Galetin et al., 2008; Lin et al., 1999; van Herwaarden et al., 2009). Thus, both the drug absorption and hepatic and intestinal extraction must also be taken into account to better understand the relationship between physicochemical properties and oral exposure. A drug that is completely but slowly absorbed may fail to show therapeutic response as the plasma concentration for desired effect is never achieved. On the contrary, a rapidly absorbed drug easily attains the therapeutic level to elicit pharmacological effect, thus both the rate and the extent of drug absorption are important.

After oral administration, absorption of drugs may occur at various body sites between the mouth and the rectum. In general, the higher is the absorption of drug along the length of the alimentary tract, the more rapid will be its action. However, because the drug substances differ in their chemical and physical properties and in the forms in which they are presented to the body, a given drug may be better absorbed from a particular site of the gastrointestinal tract from one dosage form than the other. To achieve a desired therapeutic objective, a drug product must deliver the active drug at an optimal rate and in sufficient amount (s). The process involves disintegration, deaggregation and subsequent release of the drug and finally dissolution of the drug in the aqueous fluids at the absorption site and movement of the dissolved drug through the GI membrane into the systemic circulation.

In general, drugs are modified into convenient dosage form to ensure acceptability, physicochemical stability during the shelf life, uniformity of composition and dosage, and optimum bioavailability using suitable administration route. Thus, the expedition to transform the new chemical entities into clinically useful drugs is posed to be a major challenge of drug delivery and design. In consequence, considerable research efforts have been made in the application of absorption, distribution, metabolism, excretion (ADME) sciences in drug design (Mager, 2006; Rostami-Hodjegan and Tucker, 2007; Varma et al., 2004).
BLOOD BRAIN BARRIER (BBB)

Blood–brain barrier (BBB) presents a dynamic and complex interface between blood and the central nervous system (CNS) that controls the exchange between the blood and brain compartments, consequently playing a key role in brain homeostasis and providing protection against many toxic compounds and pathogens.

The term ‘bluthirnschranke’ or the 'blood–brain barrier' was first used by Lewandowsky (1900) (Lewandowsky, 1900) while studying the limited permeation of potassium ferrocyanate into the brain. The staining experiment performed by Paul Ehrlich (a bacteriologist), gave a direct evidence showing the presence of a hypothetical barrier, when all the organs except the brain were stained after an injection of aniline dyes into the body (Ehrlich, 1906).

However, experiment performed by Edwin Goldmann (one of Ehrlich's students) in 1913, confirmed the existence of some sort of compartmentalization (Goldmann, 1913). He demonstrated that an injection of a dye directly into the spinal fluid of the brain stained only the brain. At that time, it was thought that the blood vessels themselves were responsible for the barrier, as no obvious membrane could be found. The concept of the BBB (then termed hematoencephalic barrier) was finally proposed by Lina Stern in 1921. It was not until the introduction of the scanning electron microscope to the medical research fields in the 1960s that the actual membrane could be demonstrated.

Further studies have showed that endothelial tight junctional complexes physically limit solute exchanges between the blood and the brain. Latter being illustrated by injecting horseradish peroxidase intravascularly, showing diffusion between endothelial cells (EC) lining skeletal and cardiac vessels, thought it did not pass through the EC in cerebral microvasculature (Reese and Karnovsky, 1967).

Structural make-up of BBB

BBB is a unique selective barrier formed by a complex system of endothelial cells, pericytes, astrocytes as well as basal lamina. Astrocytic end-feet processes form around 99% of the CNS capillaries, yet they do not contribute to the physiological activity of the BBB.
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Although BBB permeability itself is controlled by the biochemical properties of brain microvascular endothelial cells (BMVEC) (Pardridge, 1999), microvascular biology of the brain is a result of interactions between these cells with the basement membrane and neighboring glial cells (Kaur and Ling, 2008), such as microglia and astrocytes, as well as neurons and perivascular pericytes (Zlokovic, 2008). Altogether these constitute the neurovascular unit (NVU) (Choi and Kim, 2008; Persidsky et al., 2006), essential for both health and function of the CNS (Hawkins and Davis, 2005).

Pericytes

Pericytes (also known as vascular smooth muscle cells, mural cells, or myofibroblasts) are important cellular constituents of the capillaries and post-capillary venules (Dore-Duffy, 2008), having a close physical association with the endothelium (Figure 1).

They share the same basement membrane with the EC (Bagley et al., 2005) and cover 22 to 32% of the capillaries (Kim et al., 2005b). The location of pericytes on the microvessel and the degree of coverage varies considerably between different microvessel types (Allt and Lawrenson, 2001), seeming to correlate with the degree of tightness of the interendothelial junctions (Lai and Kuo, 2005). The vascular pericyte synthesizes most elements of the basement membrane including a number of proteoglycans. In addition, pericyte synthesis and release of laminal proteins is thought to be a critical step in the differentiation of the BBB (Dore-Duffy, 2008).

Figure 1. Pericytes have a close physical association with the endothelium covering up to 32% of the capillaries (Cardoso et al., 2010)
Astrocytes

These are the glial cells whose endfeet form a lacework of fine lamellae closely opposed to the outer surface of the BBB endothelium and resp basement membrane (Abbott, 2002) (Figure 2). Their major role lies in promoting proteoglycan synthesis thus increasing selectivity of charge on BMVEC consequently playing a vital role in the induction of BBB functions. Further, the proximity of neuronal cell bodies to brain capillaries which suggests the usefulness of these interactions for a functional NVU (Persidsky et al., 2006). In some areas, CNS, the microvessels lack astrocytic ensheathment but still exhibit some features, which most likely are due to the soluble factors acting from the limitans’ or the subarachnoid cerebro-spinal fluid (Abbott, 2002). However, some show a loss and restoration of barrier integrity, in vivo, following a temporal focal loss of astrocytes (Persidsky et al., 2006; Willis et al., 2004). Attempts to recover BBB properties in BMVEC cultures have included co-culturing BMVEC and astrocytes and/or astrocyte-conditioned medium (Colgan et al., 2008). Astrocytes may therefore modulate the BBB phenotype without being directly involved in physical BBB properties (Figure 2).

Figure 2. Astrocytes’s endfeet form a fine lamellae closely opposed to the outer surface of the capillary endothelium (Cardoso et al., 2010)
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**Endothelial cells (EC)**

EC are known to play a key role in BBB properties. BMVEC interact intimately with other brain cells of the NVU, and hence can act as mediators between blood and brain (Calabria and Shusta, 2008). Their main function involves regulation of selective transport and metabolism of substances from blood to brain and vice-versa (Zheng et al., 2003). It is this communication between EC and other surrounding cells which enhances the barrier functions consequently resulting in maintenance of proper brain homeostasis (Choi and Kim, 2008).

Brain capillary endothelium is 50–100 times tighter than peripheral microvessels as a result of special properties that cause severe restriction of the paracellular pathway for diffusion of hydrophilic solutes (Abbott, 2002). Further, EC cytoplasm has a uniform thickness lacking fenestrae, with a low pinocytotic activity and a continuous basement membrane (de Boer and Gaillard, 2006). Additionally, the BMVEC have a negative surface charge that repulses negatively charged compounds (de Boer and Gaillard, 2006). They have a greater number and volume of mitochondria compared with endothelium in other organs. These characteristics enhance the energy potential (Persidsky et al., 2006), providing energy for enzymes to break down compounds and allowing various selective transport systems to actively transport nutrients and other compounds into and out of the brain (de Boer and Gaillard, 2006). BMVEC lining the vascular wall have narrow junctional complexes that eliminate gaps or spaces between cells and prevent any free diffusion of blood-borne substances into the brain parenchymal space (Zlokovic, 2008). In fact, the cerebral microvasculature lining is characterized by the presence of an elaborated junctional complex that includes mainly tight junction (TJ) and adherens junction (AJ) proteins (Hawkins and Davis, 2005). Gap junctions have also been identified at the BBB, but their role in the barrier function is not clear (Zlokovic, 2008).

Endothelial cells are tethered to the basement membrane through focal adhesions, which consist mainly of transmembrane proteins (Kumar et al., 2009b) that also participate in intercellular adhesion (Wolburg and Lippoldt, 2002; Wolburg et al., 2009). The transmembrane proteins have been classified into three families of cell adhesion molecule (CAM) according to their structure: selectins, immunoglobulin...
superfamily, and integrins (Lee and Benveniste, 1999). In EC, integrins play an important role during angiogenesis and in the maintenance of vascular integrity (Wolburg et al., 2009). They function as adhesion receptors, in addition to transmitting chemical signals and mechanical forces between the matrix and the cytoskeleton (Yuan, 2003). The focal adhesion complex also contains a host of signaling molecules, such as focal adhesion kinase, Src tyrosine kinases and Rho GTPases that participate in integrin engagement and focal adhesion assemblage; this way, the contractile and adhesive components interact with each other, playing a determinant role in the assembly and disassembly of focal adhesions, which allows a dynamic control of cell–matrix interactions, endothelial contractility and permeability properties (Yuan, 2003).

The endothelial cytoskeleton of brain plays a critical role in establishing interendothelial junctional integrity and endothelial–extracellular matrix adherence. As in other cell types, the three primary elements of the cytoskeleton are actin filaments, intermediate filaments and microtubules. Actin filaments are composed of globular monomers of G-actin, which polymerize to form helical and asymmetrical filaments of F-actin that form contractile bundles and filamentous networks, essential for the maintenance of cell shape and integrity (Kierszenbaum, 2007). At the BMVEC, bands of F-actin are anchored to proteins involved in the adhesion to extracellular matrix (Kierszenbaum, 2007) and linked to membrane and cytosolic proteins involved in TJ and AJ to form a structure denoted as the actin-rich adhesion belt (Stamatovic et al., 2008), sometimes also referred to as perijunctional actin (Terry et al., 2010). EC at the BBB have a unique pattern of receptors and specific transport systems that facilitate the uptake of important nutrients and hormones, in addition to active pumps that help to regulate the concentrations of ions, metabolites and xenobiotics in the brain (Zheng et al., 2003; Zlokovic, 2008).

**BMVEC perform various functions which involve:**

1. Efficient supply of metabolites to the brain, thus contributing to the maintenance of the brain's ionic homeostasis by protecting the CNS from a large variety of potentially harmful hydrophobic compounds (Betz, 1992; Choi and Kim, 2008).
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2. A fundamental role in neurogenic niches, the micro-anatomical units where neurogenesis occurs in the adult mammalian brain. In these niches, mainly local to the subventricular zone, progenitor cells are in close proximity to the capillaries (Goldberg and Hirschi, 2009; Tavazoie et al., 2008), which lack astrocyte endfeet pericyte coverage, giving them direct access to vascular and blood-derived signals.

FUNCTIONS OF BBB

The BBB performs various functions as elaborated in Figure 3 (Persidsky et al., 2006; Petty and Lo, 2002). Maintenance of homeostasis in the CNS is achieved through the regulation of ion balance (Hawkins and Davis, 2005; Persidsky et al., 2006; Wolburg and Lippoldt, 2002) and influx/efflux of compounds (Chaudhuri, Khan, 2005). This is essential in protection against harmful substances, variation in blood composition and breakdown of concentration gradients (Petty and Lo, Wolburg and Lippoldt, 2002).

The BBB is present in all brain regions, except in those regulating the autonomic nervous system and endocrine glands of the body, where vessels permit diffusion of blood-borne molecules across the vessel (Ballabh et al., 2004).

Figure 3. Various functions of the BBB
DELIVERING THERAPEUTICS TO BRAIN

In today’s scenario, a major challenge in treating most brain disorders is overcoming the difficulty of delivering therapeutic agents to specific regions of the brain by crossing the BBB. The barrier, a physiological checkpoint selectively allows the entry of certain molecules from blood circulation into the brain. The dilemma for scientists is to overcome the limited transport across the BBB which strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers.

Factors affecting permeation across the BBB

The ability of a particular substance to cross the BBB and enter the brain is dependent upon several factors:

1. Drug related factors at the BBB: Concentration at the BBB and the size, flexibility, conformation, ionization (nonionized form penetrates better) (Newton, 2006) and lipophilicity of the drug molecule, its cellular enzyme stability and cellular sequestration, affinity for efflux mechanisms (i.e. P-glycoprotein), hydrogen bonding potential (i.e. charge), affinity for carrier mechanisms, and effect on all of the above by the existing pathological conditions (Levin, 1980; Pardridge, 1999) are important factors monitoring the permeability of drug agent into the brain.

2. The physicochemical characteristics e.g. octanol-water partition coefficient (log Po/w) of the therapeutic agent is one of the most informative parameter. In this regard the rule of 2 is generally accepted i.e. the value of log Po/w nearing 2 is considered optimal (Gupta, 1989; Levin, 1980). However, increasing the lipophilicity with an intent to increase the permeability would increase the volume of distribution (Bergstrom et al., 2003) and also the rate of oxidative metabolism by cytochrome P450 (Levin, 1980; van deWaterbeemd et al., 2001). Peripheral factors including systemic enzymatic stability, plasma protein binding affinity, uptake of the drug into other tissues, clearance rate, and effects of existing pathological conditions are also important.

The lipophilicity of a given drug or compound is inversely related to the degree of hydrogen bond formation that occurs with surrounding water. The presence of certain chemical moieties in the drug like terminal amide, primary amines or amides...
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and hydroxyl group favors hydrogen bond formation resulting in a decreased lipophilicity. Thus, for a compound to be transported through the BBB, the cumulative number of hydrogen bonds should not go beyond 8–10. Therefore for small drugs, increasing lipophilicity i.e. decreasing hydrogen bonds, has a positive impact on capillary permeability and drug transfer to the brain while for large drug molecules with molecular weight above 400 Da or for those with strong polarity, the capillary permeability will remain low regardless of the lipophilicity (Newton, 2006).

Even though the BBB effectively seals off the brain to the access of polar blood-borne solutes, some polar solutes such as glucose and amino acids are essential nutrients and metabolites for the brain. The endothelial cells of the cerebral capillaries must maintain a high level of expression of transporters for these essential solutes in order to facilitate their entry into the brain (Begley, 2003). The tight junctions act as a fence in the cell membrane, in that they prevent the free movement of transmembrane proteins and lipid rafts between the luminal and the abluminal surfaces of the endothelial cells. This fence property of the TJ enables the maintenance of a polar expression of many transporters present in the membranes of the cerebral endothelial cells and allows some transporters to be expressed solely in the luminal membrane and some in the abluminal membrane. As a result of this polarized transporter expression, for a range of solutes, transport can be primarily directed from blood to brain and for other solutes in the opposite direction, removing them from the CNS. This differentiation of the cerebral ECs to form a tight polarized BBB, rather than an open endothelium seen in other tissues, is thought to be the result of a close cellular association with both astrocytes and pericytes, both of which are closely applied to the basement membrane surrounding the abluminal surface of the cerebral capillaries. The astrocytes, the cell bodies of which are situated in the brain parenchyma, have end feet, which project down to the cerebral capillaries and spread in a network over the abluminal surface. Although there is still considerable debate about the factor(s), which induce the very specific differentiation of the BBB endothelium, there are probably both cell surface molecules (cell–cell contact) and soluble factors involved.

Several specialized transport mechanisms of solute transfer across endothelial cells and into the brain interstitium are also present within the BBB e.g.
carrier system for monosaccharides, monocarboxylic acid, neutral amino acids, basic amino acid, acidic amino acids, amines, purine bases, nucleosides, vitamins and hormones. The more lipophilic substances that are present in the blood can diffuse passively directly through the lipid of the cell membrane and enter the endothelial cells and brain by this means. In general, there is a relationship between lipophilicity and brain penetration for these solutes and the more lipid soluble a molecule, the more readily it will enter the CNS. Some of these lipophilic substances do not, however, enter the brain as readily as one might expect or predict from their lipid solubility. These solutes, and in many cases their metabolites, are actively removed from the CNS by efflux transporters. Various efflux transport pathways like P-glycoprotein and active organic acid present in choroid plexus may also be active in brain EC and thus help to remove unwanted substances (Newton, 2006). On the other hand presence of tight junctions and the lack of aqueous pathways between cells greatly restrict the movement of polar solutes across the cerebral endothelium (Kreuter, 2001).

**STRATEGIES TO CROSS BBB**

Numerous attempts have been made to overcome the BBB to transport drugs across it. The most frequent and successful attempts necessitate chemical modification of drugs or opening of BBB by osmotic methods. The biology-based strategies for drug delivery to the brain are majorly based on the utilization of endogenous transport systems within the BBB. The transport systems may be broadly classified as carrier-mediated transport (CMT), active efflux transports (Orecchioni et al., 2003), and receptor-mediated transport (RMT) (de Boer et al., 2003; Jong and Huang, 2005). These transporters play important roles in the influxes and/or effluxes of drugs including antimicrobial agents in brain capillary EC that form the BBB (Figure 4).

**NANOTECHNOLOGY**

Nanotechnology is defined as the design, characterization, production and application of structures devices, and systems by controlling shape and size at nanometer scale.
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- **a** Paracellular aqueous pathway
- **b** Transcellular lipoplastic pathway
- **c** Transport proteins
- **d** Receptor-mediated transcytosis
- **e** Adsorptive transcytosis

**Figure 4.** A schematic diagram of the endothelial cells that form the BBB and their associations with the perivascular endfeet of astrocytes. The main routes for molecular traffic across the BBB are shown (Abbott et al., 2006)

- **a** Normally, the tight junctions severely restrict penetration of water-soluble compounds, including polar drugs. 
- **b** However, the large surface area of the lipid membranes of the endothelium offers an effective diffusive route for lipid-soluble agents. 
- **c** The endothelium contains transport proteins (carriers) for glucose, amino acids, purine bases, nucleosides, choline and other substances. Some transporters are energy-dependent (for example, P-glycoprotein) and act as efflux transporters. AZT, azidothymidine. 
- **d** Certain proteins, such as insulin and transferrin, are taken up by specific receptor-mediated endocytosis and transcytosis. 
- **e** Native plasma proteins such as albumin are poorly transported, but cationization can increase their uptake by adsorptive-mediated endocytosis and transcytosis. Drug delivery across the brain endothelium depends on making use of pathways **b**–**e**; most CNS drugs enter via route **b**

Nanotechnology has greatly attracted the interest of the researchers in the field of medicine because of the superior properties achieved with nanoparticles as compared to the same materials of a larger size. This is mainly attributed to the increased surface area, consequently, enhancing properties such as reactivity,
strength, electrical characteristics and in vivo behavior. Additionally, drug nanoparticles, with their small size can penetrate through small capillaries a taken up by cells allowing the drug release at an optimum rate and dose. This help achieving targeted drug delivery as a result of enhanced therapeutic effe reduced toxicity and side effects.

Nanoparticles prepared using biodegradable materials allows sus release within the target site over a period of days or even weeks. Various ty nanoparticles are being proposed as carriers for drug and diagnostic agents. include, polymeric nanoparticles; nanosuspensions and nanocrystals; pol micelles; ceramic nanoparticles; liposomes; fullerenes and dendrimers; m nanoparticles; nanoshells coated with gold; nanomers and carbon nanotube solid lipid nanoparticles.

Macromolecules, extracellular matrix, and cell density are important fac the partition of nanoparticulate therapeutic agents into target tissues and c The structure of the polymer and the method of trapping drugs in the nanop will define the drug release kinetics and characteristics. Various methods hav used for the preparation of nanoparticles (Jeanneret, 2006). However, it be necessary to develop tools to entrap drugs into carrier systems, capable of rel the drug at a right place and at defined time. Moreover, the delivery systems a therapeutic agents must resist hydrostatic, hydrophilic/hydrophobic biophysical/biochemical barriers, resistance to biotransformation, degradatio clearance mechanisms. It must be noted that the use of biodegradable pol matrices solves the issue of removal of the device after delivery of the drug. nanoparticle based therapeutics in various phases of clinical trials and approved by FDA are presented in Table 1 and 2.

Various types of surface modifications of nanospheres made of p (synthetic or natural) aggregates or nanoliposomes in which the drug is dissolved, entrapped, encapsulated, or covalently attached, is also p (Panyam and Labhasetwar, 2004; Savic et al., 2003). The design of the ch bonds linking the drugs to their carriers is also of potential interest for the se release of the therapeutic agents (Reents et al., 2002; Schoenmakers et al.,
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However, the drug-loaded targeting and transport-enhancing nanoparticles match the mechanical properties and degradation rates that are needed for their applications (Grazia Cascone et al., 2002). The most commonly used polymer, polyethylene glycol (PEG), is a flexible water-soluble molecule that can be easily functionalized for chemical modification as well as for copolymerization with other polymers (Lee et al., 2003). These polymers have features such as controllable mechanical properties and degradation rates, minimal toxicity, and immune responses (Lu and Chen, 2004).

**Table 1. Nanoparticles based therapeutics in clinical trials***

<table>
<thead>
<tr>
<th>Product/ Brand Name</th>
<th>Nanoparticulate System/Active Ingredient(s)</th>
<th>Delivery Route</th>
<th>Company</th>
<th>Indication</th>
<th>Approval Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurimmune (CYT-6091)</td>
<td>Colloidal gold nanoparticles coupled to TNF and PEG-thiol</td>
<td>Intravenous</td>
<td>Cytimmune Sciences</td>
<td>Solid tumors</td>
<td>Phase II</td>
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<tr>
<td>AuroShell</td>
<td>Fleximer-camptothecin prodrug conjugate</td>
<td>Intravenous</td>
<td>Mersana Therapeutics</td>
<td>Various cancers</td>
<td>Phase I</td>
</tr>
<tr>
<td>CALAA-01</td>
<td>Cyclodextrin containing si RNA incorporated into nanoparticles</td>
<td>Intravenous</td>
<td>Calando Pharmaceuticals</td>
<td>Various cancers</td>
<td>Phase I</td>
</tr>
<tr>
<td>INGN-401</td>
<td>Liposome FUS-1</td>
<td>Intravenous</td>
<td>Introgen Therapeutics</td>
<td>Metastatic, non small cell lung cancer</td>
<td>Phase I</td>
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<tr>
<td>NB-00X</td>
<td>Nanoemulsion droplets (~200 nm)</td>
<td>Topical</td>
<td>Nanobio Corporation</td>
<td><em>Herpes labialis</em> caused by herpes simplex I virus</td>
<td>Phase I</td>
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<tr>
<td>SGT-53</td>
<td>P-53 Liposomes</td>
<td>Intravenous</td>
<td>Synergene Therapeutics</td>
<td>Solid tumors</td>
<td>Phase I</td>
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<tr>
<td>Viva Gel</td>
<td>Dendrimer gel</td>
<td>Topical</td>
<td>Star Pharma Holdings</td>
<td>Vaginal microbicide for the prevention of HIV and genital herpes</td>
<td>Phase II Fast Track</td>
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*www.ClinicalTrials.gov.com*
Table 2. Nanoparticles based therapeutics approved by FDA

<table>
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<th>Company/Alliance</th>
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<tr>
<td>Abelcet</td>
<td>Amphotericin B phospholipid complex</td>
<td>Intravenous</td>
<td>Enzon</td>
<td>Invasive fungal infections in patients who are refractory to or intolerant to conventional Amphotericin B therapy</td>
<td>Nov 199</td>
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<tr>
<td>Abraxane</td>
<td>Paclitaxel (Taxol) bound albumin nanoparticles (~130 nm)</td>
<td>Intravenous</td>
<td>Abraxis AstraZeneca</td>
<td>Metastatic breast cancer patients who have failed combination therapy</td>
<td>Jan 200</td>
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<td>Adagen</td>
<td>Pegylated adenosine deaminase</td>
<td>Intravenous</td>
<td>Enzon</td>
<td>Enzyme replacement therapy for patients with severe combined immune deficiency disease</td>
<td>Mar 199</td>
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<td>Ambisome</td>
<td>Amphotericin B liposomes (~45-80 nm)</td>
<td>Intravenous</td>
<td>Gilead Sciences</td>
<td>Fungal infections</td>
<td>Aug 199</td>
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<td>Amphotec</td>
<td>Colloidal suspension of lipid-based amphotericin B (~115 nm)</td>
<td>Subcutaneous</td>
<td>Sequus</td>
<td>Invasive aspergillosis patients who are refractory to or intolerant to conventional Amphotericin B</td>
<td>Nov 199</td>
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<tr>
<td>Copaxone</td>
<td>Glatiramer acetate (copolymer of L-glutamic Acid, L-alanine, L-tyrosine, and L-lysine)</td>
<td>Subcutaneous</td>
<td>TEVA</td>
<td>Relapsing-remitting multiple sclerosis</td>
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<td>Daunoxome</td>
<td>Encapsulated-daunorubicin citrate liposomes</td>
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<td>Gilead Sciences</td>
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<td>Depocyt</td>
<td>Sustained release cytarabine liposomes</td>
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<td>Skye Pharma Enzon</td>
<td>Lymphomatous Meningitis</td>
<td>Apr 199</td>
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<td>Diprivan</td>
<td>Propofol liposomes</td>
<td>Intravenous</td>
<td>Zeneca Pharma</td>
<td>Anesthetic</td>
<td>Oct 198</td>
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<td>Doxil Caelyx</td>
<td>Pegylated doxorubicin (Adriamycin) HCl liposomes</td>
<td>Intravenous</td>
<td>Orthobiotech Schering-Plough</td>
<td>Metastatic ovarian cancer and AIDS-related Kaposi's sarcoma</td>
<td>Nov 199</td>
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<td>Elestrin</td>
<td>Estradiol gel (0.06%) incorporating calcium phosphate nanoparticles</td>
<td>Transdermal</td>
<td>Biosanté</td>
<td>Treatment of moderate to severe hot flashes in menopausal women</td>
<td>Dec 200</td>
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<td>Epaxal</td>
<td>Hepatitis A vaccine adjuvanted with immune potentiating reconstituted influenza virosomes (IRIV)</td>
<td>Intramuscular</td>
<td>Berna Biotech</td>
<td>Active immunization against hepatitis A for adult and children &gt;12 months (age may vary and depend upon the country)</td>
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<tr>
<td>Estrasorb</td>
<td>Estradiol hemihydrates micellar nanoparticles (emulsion)</td>
<td>Transdermal</td>
<td>Novavax</td>
<td>Reduction of vasomotor symptoms, such as hot flushes and night sweats, in menopausal women</td>
<td>Oct 200</td>
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<table>
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<tr>
<th>Product/Brand Name</th>
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<td>Macugen</td>
<td>Pegylated anti-VEGF aptamer</td>
<td>Intravitreal</td>
<td>OSI Pharmaceuticals Pfizer</td>
<td>Neovascular age-related macular degeneration</td>
<td>December 2004</td>
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<td>Myocet</td>
<td>Liposome-encapsulated doxorubicin-citrate complex</td>
<td>Intravenous</td>
<td>Zeneus Pharma, Sopherion Therapeutics</td>
<td>Cardio-protective formulation of doxorubicin used in late stage metastatic breast cancer</td>
<td>Approved in Europe and Canada</td>
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<tr>
<td>Neulasta</td>
<td>PEG-G-CSF or Pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxy polyethylene glycol)</td>
<td>Subcutaneous</td>
<td>Amgen</td>
<td>Febrile neutropenia</td>
<td>January 2002</td>
</tr>
<tr>
<td>Oncaspar</td>
<td>Pegaspargase</td>
<td>Subcutaneous</td>
<td>Enzon</td>
<td>Leukemia</td>
<td>February 1994</td>
</tr>
<tr>
<td>Pegasys</td>
<td>Peginterferon alfa-2a</td>
<td>Subcutaneous</td>
<td>Nektar, Hoffmann-la Roche</td>
<td>Chronic hepatitis C virus infection</td>
<td>October 2002</td>
</tr>
<tr>
<td>Pegintertron</td>
<td>Peginterferon alfa-2b</td>
<td>Subcutaneous</td>
<td>Enzon, Schering-Plough</td>
<td>Chronic hepatitis C virus infection in patients with compensated liver disease</td>
<td>January 2001</td>
</tr>
<tr>
<td>Renagel</td>
<td>Cross-linked poly(allylamine) resin (Sevelamer Hydrochloride)</td>
<td>Oral Tablets</td>
<td>Genzyme</td>
<td>Control of serum phosphorus in patients with chronic kidney disease on dialysis</td>
<td>October 1998</td>
</tr>
<tr>
<td>Somavert</td>
<td>Pegvisomant (PEG-hgh)</td>
<td>Subcutaneous</td>
<td>Nektar, Pfizer</td>
<td>Acronegaly</td>
<td>March 20</td>
</tr>
<tr>
<td>Triglide</td>
<td>Nanocrystalline fenofibrate</td>
<td>Oral Tablets</td>
<td>Skye Pharma, First Horizon Pharmacuetical Corp.</td>
<td>Lipid disorders; markedly reduces elevated plasma concentrations of triglycerides, LDL and total cholesterol and raises abnormally low levels of HDL</td>
<td>May 2005</td>
</tr>
</tbody>
</table>

# www.fda.gov/ohrms/dockets

Release of drug by the carrier must follow extravasation and transvascular transport and drug therapeutic dosages must be attained (Béduneau et al., 2000). Derivatization or encapsulation into polymeric particles (Duncan, 2003; Feng et al., 2004; Ferrari, 2005) has been reported for enhancing the drug selectivity. Mechanistically, the nanoparticles are generally internalized into cells via fluid phase endocytosis, receptor-mediated endocytosis, or phagocytosis. Various attempts using carrier-mediated systems to transport nanoparticles include the GLUT1, which showed that α-mannose, but not β-mannose derivatives incorporated on the surface of liposomes (Umezawa and Eto, 1988) induced transport across the BBB. Cholesterol derivative coated nanoparticles were transported across brain-derived endothelial...
cells faster by the cation transporter than uncoated nanoparticles (Fenart et al., 1999), possibly also dependent on the lipophilic nature of choline derivatives, whilst thiamine derivatives were not transported (Lockman et al., 2003).

Another remarkable system is the folic acid receptor specifically expressed at the BBB and able to transport doxorubicin-loaded folic acid-decorated nanoparticles (Wu and Pardridge, 1999). Some hydrophilic surfactants, in particular polysorbates, interact with the surface of the BBB (Ambruosi et al., 2006; Brigger, 2004; Kreuter, 2001; Petri et al., 2007), showing effective delivery to the brain. Polysorbate-coated doxorubicin nanoparticles, but not PEG-coated nanoparticles, may be promising for nanoparticulate drug delivery to the brain. Adsorption of apolipoprotein E onto the surfactant-coated nanoparticles favours receptor-mediated processes (Steiniger et al., 2004).

There may be no end to the list of literature, that cites and elaborates on the hefty advantages achieved using the empirically different types of nanoparticulate systems. Nevertheless, various tribulations associated with the use of these polymeric nanoparticles like residual contamination from the production process, for example by organic solvents, polymerization initiation, large polymer aggregates, toxic monomeric degradation products, expensive production methods and absence of a suitable sterilization method (Gohla and Dingier, 2001; Kante et al., 1982; Muller et al., 2000) prompt us to exploit other nanodelivery devices.

**Considering the success of nanoparticles to pass through the BBB and their limitation(s) especially toxicity and stability, another suitable option for drug delivery into the brain would be the first generation nanoparticles i.e solid lipid nanoparticulate systems.**

**SOLID LIPID NANOPARTICLES**

Solid lipid nanoparticles (Figure 5) are one of the novel potential colloidal carrier (Cavalli et al., 2000) systems in the range of 10-1000 nm as alternative materials to polymers which have the capacity to incorporate both the hydrophilic and the hydrophobic drug agents. They are supposed to be identical to oil /water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by a high melting solid lipid (Jenning et al., 2002).
SLNs are generally made up of a solid hydrophobic core with a monolayer of phospholipid coating and are dispersed in water or in aqueous surfactant solution. The drug in solid core may be dissolved or dispersed in the solid high melting fat matrix with the hydrophobic end of the phospholipid chains embedded in the fat matrix (Kaur et al., 2008).

**Advantages of SLNs over polymeric nanoparticles (and other delivery systems like liposomes)**

SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes while simultaneously avoiding their disadvantages (Kaur et al., 2008). The advantages of SLNs include the following:

1. The nanoparticles and the 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).

2. Controlled release of the incorporated drug can be achieved for up to several weeks (Muhlen et al., 1998; Muller et al., 2000). Further, by coating with or attaching ligands to SLNs, there is an increased scope of drug targeting (Allen et al., 2003).

3. SLN formulations stable for even three years have been developed. This is of paramount importance with respect to the other colloidal carrier systems (Freitas and Müller, 1998).
4. High drug payload.

5. Excellent reproducibility with a cost effective high pressure homogenization method as the preparation procedure (Gohla and Dingler, 2001).

6. The feasibility of incorporating both hydrophilic and hydrophobic drugs (et al., 2001; Fundaro et al., 2000).

7. The carrier lipids are biodegradable and hence safe (Siekman Westesen, 1994; Tabatt et al., 2004).

8. Avoidance of organic solvents (Kaur et al., 2008).

9. Feasible large scale production and sterilization (Gasco, 1993; Muller 2000)

METHOD OF SLN PREPARATION

There are two basic production techniques for SLN (Gohla and Dingle Schwarz and Mehnert, 1999).

1) Hot homogenization

2) Cold homogenization

**Hot homogenization**: Lipid is melted to approximately 5°C a melting point, the drug is dissolved or solubilized in the melted lipid, drug containing lipid melt is dispersed in an aqueous surfactant solution at the same temperature. The obtained pre-emulsion is then passed through a high pressure homogenizer (HPH). The product of this process is an emulsion and the cooling of this emulsion leads to crystallization of the solid lipid nanoparticles (Wissing et al., 2004a).

**Demerits**: Method cannot be employed successfully for hydrophilic ingredients/drugs because chances of partitioning of the drug from the lipid to the water phase are high. This may result in a loss of more than half of the drug to the aqueous phase rather than its incorporation into the nanoparticles.

**Cold homogenization**: Drug is incorporated into melted lipid and the melt is cooled up to solidification. Solid material is ground by a mill. Obtained lipidic microparticles are dispersed in a cold surfactant solution.
Review of Literature

room temperature or even at temperature distinctly below room temperature and passed through the HPH to form nanoparticles. The solid state matrix mimics partitioning of the drug to the water phase. It has merits of hot homogenization since even during storage of the aqueous solid dispersion, the entrapment efficiency remains unchanged. A comparison of hot vs cold homogenization is given below (Figure 6).

![Image of a diagram showing the comparison between hot and cold homogenization techniques]

**Common Step:** Melting the lipid at temperature(s) >10°C above the m.p. of the lipid and dissolving/dispersing the drug in the lipidic phase

**Hot homogenization technique:**
- Dispensing the drug-loaded lipidic phase in a hot aqueous solution of surfactant mixture
- Pre-eruision is formed using a high speed slitter
- High pressure homogenization at a temperature above the lipid melting point
- Hot core nanoemulsion
- Separation by cooling to room temperature
- Advantages:
  - Small and uniformly sized nanoparticles are obtained
  - Large scale production is easy
  - Amphiphilic drugs are better entrapped
- Disadvantage:
  - Not suitable for incorporation of hydrophilic and thermolabile drugs

**Cold homogenization technique:**
- Solidification of the drug loaded lipid phase in liquid nitrogen or using dry ice
- Powder mill is used to micronize the solidified phase
- Dispensing the powder in aqueous dispersion medium (pre-mix)
- High pressure homogenization at room temperature
- Advantages:
  - Minimizes the thermal exposure of the sample
  - Can incorporate both hydrophilic and lipophilic drugs
- Disadvantages:
  - Larger particle size is obtained
  - Broader size distribution

**Figure 6. Comparison of hot and cold homogenization technique**

Apart from the two above-explained basic technologies used for production, other methods are also proposed, which include:

- **Ultrasonication or high speed homogenization**

Production of SLNs by sonication are also reported (Boltri et al., 1992; Labouret et al., 1995). The main advantage of the method lies in the use of basic lab equipments which are easily accessible. However, the
particle size distribution obtained minimizes its expected Furthermore, physical instabilities (particle growth upon storage potential metal contamination due to ultrasonication may add shortcoming of the method. Thus, the technique is generally combined with high speed stirring for making a stable formulation.

Solvent emulsification/evaporation

Production of SLNs by emulsification involves the principle of pre (Sjostrom and Bergenstahl, 1992). The first step involves dissolving drug in water-immiscible organic solvent (cyclohexane) which is emulsified in an aqueous phase. As a result of solvent evaporation, the SLNs are formed by precipitation of the lipid in the aqueous medium. Siekmann and Westesen, reported the formation of cholesterol acetate nanoparticles with a mean particle size of 29 nm (Siekmann and Westesen, 1996) by using cholesterol acetate as model drug and lecithin/sodium glycocholate as emulsifier.

Microemulsion based SLN preparation

Gasco and co-workers developed SLN preparation techniques which are based on the dilution of microemulsion (Gasco, 1993). Microemulsions are produced by stirring an optically transparent mixture at 65-70°C characteristically composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine, sodium taurodeoxycholate), co-emulsifiers (sodium monooctylphosphate), and water. The hot microemulsion thus formed is dispersed in cold water (8°C) under stirring. Typical volume ratios of the hot microemulsion to water are in the range of 1:25 to 1:50. According to the literature, the molecular structure is already contained in the microemulsion and therefore, no further dilution is required to achieve submicron particle sizes (Boltri et al., 1993; 1997). In addition to the composition, the temperature gradient and the value of the temperature gradient at the interface are key parameters for the quality of the final lipid nanosuspension. High-temperature gradients facilitate rapid lipid crystallization and aggregation (Cortesi et al., 2002). Because of the dilution step, ac
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Lipid contents are considerably lower compared with the HPH-based formulations.

**SLN preparation by using supercritical fluid**

This technique for SLN production has the advantage of producing SLNs without the use of organic solvents (Chen et al., 2006b; Kaiser et al., 2000). There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS).

**Spray drying method**

It is an alternative procedure to lyophilization in order to transform aqueous SLN dispersion into a drug product. It is an economical method as compared to the process of lyophilization. The method suffers from a major drawback of causing aggregation of particles due to high temperature, shear forces and partial melting of the particle. Freitas and Muller (Freitas and Muller, 1998) recommended the use of high melting-point lipid with >70°C for spray drying. The best result was obtained with SLN concentration of 1% in a solution of trehalose in water or 2% trehalose in ethanol-water mixtures (10:90 v/v).

**Double emulsion method**

Solvent emulsification-evaporation method is used for the preparation of SLN loaded with hydrophilic drugs (Cortesi et al., 2002). Drug partitioning into the external water phase during solvent evaporation is prevented by use of appropriate stabilizer.

**METHODS FOR EFFECTIVE DELIVERY OF DRUG TO THE BRAIN**

The body distribution of SLNs is strongly dependent on their surfactant characteristics like size, surface hydrophobicity and surface mobility. The SLNs have been proposed as suitable systems to deliver hydrophilic drugs like diminazine aceturate also for other BCS class IV drugs like paclitaxel, vinblastine, camptothecin, etoposide and cyclosporine (Cavalli et al., 2000; Chen et al., 2001; Chen et al., 1999). These carriers can gain access to the blood compartment ea
Review of Literature

(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.

Wang et al. have reported the synthesis of 3', 5,-dioctanoyl-5-flouro-2,-deoxyuridine (DO-FUdR) to overcome the limited access of the drug 5-flouro-2,-deoxyuridine (FUdR) and its incorporation into solid lipid nanoparticles. The brain area under the concentration/time curve of DO–FUdR–SLN and DO–FUdR were 10.97- and 5.32-fold higher than that of FUdR, respectively. These results indicated that DO–FUdR–SLN had a good (2 times the free drug derivative) brain targeting efficiency in vivo. These authors report that SLN can improve the ability of the drug to penetrate through the BBB and is a promising drug targeting system for the treatment of CNS disorders (Wang et al., 2002). SLNs consisting of microemulsions of solidified oil nanodroplets loaded with iron oxide and injected systemically into rats have been shown to cross the BBB and accumulate in the brain with long-lasting kinetics (Dupas et al., 1999; Silva, 2008). Iron oxides are classic superparamagnetic magnetic resonance imaging (MRI) contrast agents. Because iron oxides are insoluble in water, they must be delivered as modified colloids for clinical applications, which is usually achieved by coating them with hydrophilic molecules, such as dextrans (Peira et al., 2003). Therefore, the delivery vehicle used is critical in determining the functional properties of the contrast agent. By taking advantage of the ability of SLNs to cross the BBB, nanoparticles complexed with iron oxides may provide new ways to image the CNS using MRI (Silva, 2008).

Further, various researchers have tried to increase the plasma half-life of SLNs by the following methods:

1. **Particle size:** 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 (Moghimi et al., 1991). The IES in sinusoidal spleens provides
resistance to flow through the reticular meshwork. The EC 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 EC and, hence, the size of IES (Drenckhahn and Wagner, 1986). However, the slit size rarely exceeds 200 to 500 nm in width, even with an erythrocyte in transit (Chen and Weiss, 1973). 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 may be removed later on, if they are not rigid (Moghimi et al., 1991). Hence SLNs of size below 200 nm have an increased blood circulation resulting in an improved contact of the drug molecule at BBB and for the drug to be taken up by the brain (Chen et al., 2004; Oyewumi et al., 2004).

2. **Surface coating with hydrophilic polymers/surfactants**: 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. To prevent this clearance and to increase their availability at the target site, the RES removal of these particulate systems should be prevented (Utreja and Jain, 2001). This is achieved 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 immunoglobulins (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 (Moghimi et al., 2001); any shielding of hydrophobic character of the nanoparticles is thus going to stearically stabilize them by providing a dense conformational cloud. This in turn will reduce opsonization and phagocytosis as well as uptake by neutrophilic granulocytes, thus increasing the blood circulation time and hence 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 can 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 (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 RES. Other hydrophilic molecules which have been tried are Brij 78, Poloxamer F68 and Brij68. Cavalli et al. found that parenteral administration of tripalmitin based SLNs of paclitaxel, stabilized by soy phosphatidylcholine were 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, Cremphors (EZ or RH40) or polyoxyethylene(23)-lauryl ether was not effective (Alyautdin et al., 1997). 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.

Delivery of drugs to the brain by nanoparticles made of polybutylcyanoacrylate (PBCA) and coated with the nonionic surfactant polysorbate 80 has been intensely investigated (Goppert and Muller, 2005; Kreuter, 2001). Similarly, polysorbates showed the highest potential to deliver the solid lipid nanoparticles to the brain (Vasir et al., 2005).

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, luteinizing hormone releasing hormone). Attaching suitable ligands for these particular receptors on to the nanoparticles would result in their increased selectivity (Pardridge, 2002). Allen et al. 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 nanoparticle concentration at the surface of BBB. While attempting to prove their assumption, they prepared coated nanoparticles from Brij 78, and emulsifying wax, with thiamine ligand (linked to DSPE via a PEG spacer). The authors however could not achieve prolonged nanoparticle concentration which was attributed to a number of factors including insufficient thiamine ligand coating and a preferred binding of the thiamine ligand to the blood thiamine transporters (Allen et al., 2003). Thole et al. 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 (Thole et al., 2002). 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 (Thole et al., 2002). 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 and 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.

APPLICATION OF SLNs

Solid lipid nanoparticles, with their superior advantages as compared to other drug delivery systems possess a wider range of application in the pharmaceutical arena. Enhancement in bioavailability (BA) by SLNs has been enormously evidenced through the available literature. Further, the achieved enhancement in BA after formulating the drug into SLNs is not only restricted to oral route of administration, but also extends to parenteral, topical, nasal and pulmonary routes. SLNs possess a better stability and ease of large scale production as compared to other colloidal carriers in the category. Several potential applications of SLNs are described below briefly. A list of numerous drugs which have been successfully incorporated into SLNs for a pharmaceutically improved product is presented in Table 3.

SLNs as gene vector carrier

Use of SLNs in the gene vector formulations is well cited in literature. A very recent study (del Pozo-Rodriguez et al., 2010), confirmed the capacity of SLN–DNA
vectors to induce the expression of a foreign protein after intravenous administration, in mice, resulting in transfection of hepatic tissue and spleen. Another study (Choi et al., 2008) demonstrated a new formulation of cationic SLNs produced by the melt homogenization method for gene delivery. The authors found that the SLN-mediated transfection of the p53 gene resulted in efficient high levels of wild-type p53 mRNA and protein expression levels in H1299 cells, which help restoring the apoptotic pathway. Results of the study revealed that cationic SLN-mediated p53 gene delivery may have potential for clinical application as a nonviral vector-mediated lung cancer therapy. Suitability of novel cationic SLN as a nonviral transfection agent for gene delivery has been investigated in several studies (Bondi et al., 2007; Rudolph et al., 2004). The authors described that incorporation of dimeric HIV-1 TAT peptide (TAT2) into SLN gene vectors induced up to 100-fold sequence-dependent increase of gene expression as compared with the mutant TAT2-M1 and was 4- to 8-times higher as compared with DNA complexed with polyethylenimine (PEI) in vitro. Further, there are several reports of SLN carrying genetic/peptide materials such as DNA, plasmid DNA and nucleic acids (Pedersen et al., 2006). A study detailed the formation of genospheres which are stable and homogeneously sized lipid-nucleic acid nanoparticles (70-100 nm), formed as a result of removal of organic solvent into which both lipid and DNA were separately dissolved. In addition, specific targeting can be achieved by insertion of an antibody-lipopolymer conjugated in the particle (Hayes et al., 2006).

SLNs for topical use

Application of SLNs in the area of topical drug delivery is well evidenced from the abundant literature available. The researchers have explored not only the better permeability obtained with developed SLNs but also their potential to sustain the drug release after incorporation into polymeric gel systems.

A recent report by Passerini et al. (Passerini et al., 2009) demonstrated that econazole nitrate loaded SLNs showed better permeation across the skin. A very similar study with miconazole nitrate (MN) loaded SLN for topical application has also been reported recently. Authors demonstrated that MN-loaded SLN-bearing hydrogel were more efficient in the treatment of candidiasis as a result of sustained topical effect and in addition provide quicker relief from fungal infection (Jain et al., 2010).
Another study reports on a desirable sustained release achieved with gel enriched with diclofenac sodium SLN (DSSLN; prepared by hot homogenization method). Topical administration of DSSLN gel demonstrated sustained systemic delivery of the drug and a good anti-inflammatory activity up to 24 h, in comparison to oral and conventional gel administration of diclofenac sodium (Gaddam and Aukunuru, 2010).

Previously SLN loaded with curcuminoids for topical application were developed and characterized by Tiyaboonchai and co-workers, however they do not report any in vitro improvement in comparison to free curcumin (70% release in 12 h by SLN vs. 90% release in 8 h by pure curcumin) (Tiyaboonchai et al., 2007). The light and oxygen sensitivity of curcuminoids was however, strongly reduced by incorporating curcuminoids into this unique type of formulation. An in vivo study with healthy volunteers revealed improved efficiency of a topical application cream containing curcuminoid loaded SLN over that containing free curcuminoids (Tiyaboonchai et al., 2007).

SLNs have been used for topical application of various drugs such as tropolide, imidazole, antifungals, anticancers, vitamin A, isotretinoin, ketoconazole, DNA, flurbiprofen and glucocorticoids (Jain et al., 2010). A local depot for the sustained release of active compound in dermis was achieved by preparation of podophyllotoxin-loaded SLNs (Chen et al., 2006a). Vitamin A-loaded SLNs prepared using glyceryl behenate as a lipid by hot homogenization technique are also reported to result in an improved permeation and sustained release effect of the drug (Jenning et al., 2000). The isotretinoin-loaded lipid nanoparticles were formulated using soyaean lecithin and Tween 80, by hot homogenization method for topical delivery of drug. Latter resulted in an increase of cumulative uptake of isotretinoin in skin (Jenning et al., 2000). Production of the flurbiprofen-loaded SLN gel using polyacrylamide, glycerol and water for topical application confirmed the potential advantage of SLNs for delivering the drug directly to the site of action to result in higher tissue concentrations (Mei and Wu, 2005; Santos et al., 2002).

**SLNs as cosmeceuticals**

SLNs offer a number of advantages for cosmetic products. They can protect the encapsulated ingredients from degradation. Compounds, including coenzyme
Q₁₀ and retinol (Jenning and Gohla, 2001) can remain stable in SLNs over a long time period. They can be used for the controlled delivery of cosmetic agents over a prolonged period of time and have been found to improve the penetration of active compounds into the stratum corneum.

SLNs have occlusive properties making them ideal for potential use in day creams. In-vivo studies have shown that SLN-containing formulation is more efficient in skin hydration than a placebo (Wissing and Müller, 2003). SLNs have also been found to show UV resistant properties which were enhanced when a molecular sunscreen was incorporated and tested (Wissing and Müller, 2001). Enhanced UV blocking by 3,4,5- trimethoxybenzoylchitin (a good UV absorber) was observed when incorporated into SLNs (Song and Liu, 2005). An in vivo study illustrated an increased (31%) skin hydration by addition of 4% SLN to a conventional cream after 4 weeks of topical application (Wissing and Müller, 2001).

SLNs have also been tested in perfume formulations. Chanel's Allure perfume was incorporated into SLNs and nanoemulsions (Wissing et al., 2000). SLN formulations delayed the release of perfume over a longer period of time. This slow release profile is also desirable for the formulation of insect repellent creams.

Although SLNs are promising for cosmetic purposes they suffer some drawbacks. The process of production needs improvement in order to increase the drug loading capacity and prevent the expulsion of the drug during storage. Further, the need to concentrate the SLN dispersion due to their high water content also prevents their extensive exploration for use in cosmeceuticals.

SLNs for potential agricultural applications

Essential oil extracted from Artemisia arboresens L when incorporated in SLN prepared using Compritol® 888 ATO as lipid and poloxamer 188 or Miranol Ultra C32 as surfactant, were able to reduce the rapid evaporation when compared with emulsions. The study showed that SLNs are potential carriers for ecological pesticides in agriculture (Lai et al., 2006).

SLNs as a targeted carrier for anticancer drugs

SLNs have been reported to be useful as drug carriers to treat neoplasms. Tamoxifen, an anticancer drug was incorporated into SLNs to achieve a prolonged
drug release after i.v. administration, for the treatment of breast cancer. The study suggested the effectiveness of tamoxifen-loaded SLN for the treatment of breast cancers as a result of enhanced permeability and retention achieved after incorporating the drug into SLNs. Tumour targeting has also been achieved with SLNs loaded with drugs like methotrexate and camptothecin (Huang et al., 2008; Ruckmani et al., 2006). In a study by Yassin et al. SLNs prepared by double emulsification method, were utilized for investigating the release of 5-fluorouracil (5-FU) inside the colonic medium for local treatment of colon cancer. They concluded that developed SLN system has a high potential to improve the uptake of anticancer drugs inside colon tumors (Yassin et al., 2010). Another study reported an enhanced efficacy of doxorubicin to cause a reduction in breast cancer cells after incorporation into SLNs (Subedi et al., 2009).

Xu et al. (Xu et al., 2009) showed better tolerance and antitumor efficacy in murine model bearing hepatoma after treatment with docetaxel-loaded hepatoma-targeted SLNs (tSLN). The studies on cellular uptake and biodistribution indicated that the better antitumor efficacy of tSLNs was attributed to both the increased accumulation of drug in tumor and higher cellular uptake by hepatoma cells. Further, formulations of mitoxantrone-loaded SLN local injections is also reported to reduce the toxicity and improve its safety and bioavailability (Lu et al., 2006).

**SLNs for improved oral bioavailability**

Apart from the above evidenced role of SLNs in drug delivery, their wider perspective lies in oral BA enhancement. In a recent study, a poorly water-soluble drug cryptotanshinone (CTS) was incorporated into SLNs which were prepared by an ultrasonic and high-pressure homogenization method. The authors reported that the relative BA of CTS in the SLNs was significantly increased when compared with that of a CTS-suspension in the pharmacokinetic study in rats. They attributed the enhanced BA to the improved absorption by employing SLN formulations (Hu et al., 2010). Similar BA enhancement is also reported for Pentoxifylline (PTX) a highly water-soluble drug, post incorporation into SLNs using homogenization-sonication technique (Varshosaz et al., 2010).

Another study by Gota and co-workers, in healthy volunteers demonstrated that plasma levels of curcumin after dosing of a solid lipid curcumin particle (SLCP)
formulation at 650 mg of SLCP was 22.43 ng/ml while dosing an equal quantity of unformulated 95% curcuminoids extract did not produce detectable levels (Gota et al., 2010).

Another study reported an enhancement in relative BA of quercitin loaded SLNs (QT-SLNs) by 5 folds in comparison to quercetin suspension. Further, their pharmacokinetic data revealed a prolonged $T_{\text{max}}$ and mean residence time (MRT) for quercetin in rat plasma after oral administration. The authors attributed the achieved enhanced bioavailability by the SLNs to direct uptake of nanoparticles through the GI tract, increased permeability by surfactants, and decreased degradation and clearance (Li et al., 2009a). Earlier, Luo et al. reported an improvement in relative BA of vinpocetine (VIN) in SLNs (prepared by ultrasonic-solvent emulsification technique) as compared with that of the VIN solution. The improved BA was accredited to the effect of surfactant, on the oral absorption of VIN, with SLN formulations (Luo et al., 2006). Superiority of SLNs to avoid the toxic effects of cyclosporine was established by a pharmacokinetic study, in pigs. The authors compared the pharmacokinetic profile of cyclosporine SLNs in comparison to the developed nanocrystals and a reference conventional formulation, i.e Sandimmun Neoral/Oporal®. They stated that the SLN™ formulation avoids side effects by maintaining the blood concentrations below 1000 ng/ml. Further, they quoted that SLN™ as a drug carrier for oral administration of cyclosporine A shows a low variation in bioavailability of the drug and also simultaneously avoids the plasma peak typical of the conventional Sandimmun® formulation (Müller et al., 2006).

Antitubercular drugs such as rifampicin, isoniazid, pyrazinamide-loaded SLN system prepared by emulsion solvent diffusion technique, were able to decrease the dosing frequency and improve the patient compliance (Pandey et al., 2005). The potential of nasal route for such drugs is also being explored by the same group of scientists.

Thus, SLNs can be used extensively as an alternative to the existing drug carrier systems, providing more flexibility with respect to the area of application and also aspects for commercialization.
<table>
<thead>
<tr>
<th>Drug incorporated</th>
<th>Method of preparation</th>
<th>Lipid phase</th>
<th>Surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>All trans retinoic acid (Lim and Kim, 2002)</td>
<td>Melt homogenization</td>
<td>Tricaprin</td>
<td>Polysorbate 80</td>
</tr>
<tr>
<td>Alpha-lipoic acid (Ruktanonchai et al., 2009)</td>
<td>Hot high pressure homogenization</td>
<td>PEG-8, Beeswax</td>
<td>Poloxamer 188</td>
</tr>
<tr>
<td>Amphoterin B (Lemke et al., 2010)</td>
<td>Hot high pressure homogenization</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Polysorbate 80 and so cholate</td>
</tr>
<tr>
<td>Ascorbyl palmitate (Kristl et al., 2003)</td>
<td>Hot high pressure homogenization</td>
<td>Witepsol E 85</td>
<td>Tegocare 450</td>
</tr>
<tr>
<td>Benzyl nicotinate (Krac et al., 2001)</td>
<td>Melt-emulsification</td>
<td>Dynasan 116 (glyceryl tripalmitate)</td>
<td>Phospholipon 80 (soybean lecithin) and poloxamer 188</td>
</tr>
<tr>
<td>Betamethasone valerate (Sivaramakrishnan et al., 2004)</td>
<td>Hot high pressure homogenization</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Poloxamer 188</td>
</tr>
<tr>
<td>Bupivacaine (Masters and Domb, 1998)</td>
<td>Hot high pressure homogenization</td>
<td>Tristearin</td>
<td>Egg phosphatidylichol</td>
</tr>
<tr>
<td>Calcitonin (Garcia-Fuentes et al., 2005)</td>
<td>Double emulsion-solvent emulsification</td>
<td>Dynasan 116 (glyceryl tripalmitate)</td>
<td>L-a-Lecithin and chito</td>
</tr>
<tr>
<td>Camptothecin (Yang et al., 1999b)</td>
<td>Hot high pressure homogenization</td>
<td>Stearic acid</td>
<td>Poloxamer 188 and soylecithin</td>
</tr>
<tr>
<td>Carmustine (Kuo and Liang, 2011)</td>
<td>Microemulsification solidification</td>
<td>Cocoa butter and stearic acid</td>
<td>Hexadecyltrimethyl ammonium bromide a sodium dodecylsulfate</td>
</tr>
<tr>
<td>Carvedilol phosphate (Chakraborty et al., 2010)</td>
<td>Solvent emulsification evaporation</td>
<td>Stearic acid</td>
<td>Polyvinyl alcohol and sodium taurocholate</td>
</tr>
<tr>
<td>Cholesteryl acetate (Sjostrom and Bergenstahl, 1992)</td>
<td>Solvent emulsification evaporation</td>
<td>Organic solvent</td>
<td>Phosphatidylycholine a sodium glycocholate</td>
</tr>
<tr>
<td>Cholesteryl butyrate (Ugazio et al., 2001)</td>
<td>Microemulsification solidification</td>
<td>Soy phosphatidylichol</td>
<td>Taurodeoxycholate an butanol</td>
</tr>
<tr>
<td>Clobetasol propionate (Hu et al., 2002)</td>
<td>Solvent diffusion</td>
<td>Monostearin</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>Clotrimazole (Souto et al., 2004)</td>
<td>Hot high pressure homogenization</td>
<td>Dynasan 116 (glyceryl tripalmitate)</td>
<td>Tyloxapol</td>
</tr>
</tbody>
</table>

Table 3 continued........
## Review of Literature

<table>
<thead>
<tr>
<th>Drug incorporated</th>
<th>Method of preparation</th>
<th>Lipid phase</th>
<th>Surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine (Venkateswarlu and Manjunath, 2004)</td>
<td>Hot high pressure homogenization</td>
<td>Tristearin, Dynasan 114 (trimyristin)</td>
<td>Epikuron 200 and poloxamer</td>
</tr>
<tr>
<td>Coenzyme Q10 (Wissing et al., 2004)</td>
<td>Hot high pressure homogenization</td>
<td>Cetyl palmitate</td>
<td>Tego Care 450 (polyglyceryl-3 methylglucoside distearate)</td>
</tr>
<tr>
<td>Cryptotanshinone (Hu et al., 2010)</td>
<td>Ultrasonic and high pressure homogenisation</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Sodium dehydrocholate/soy lecithin</td>
</tr>
<tr>
<td>Curcuminoids (Tiyaboonchai et al., 2007)</td>
<td>Microemulsification</td>
<td>Glyceryl monostearate</td>
<td>-</td>
</tr>
<tr>
<td>Cyclosporine A (Varia et al., 2009)</td>
<td>Hot high pressure homogenisation</td>
<td>Imwitor 900 (glycerol monostearate 40–50%)</td>
<td>Tagat S and sodium cholate</td>
</tr>
<tr>
<td>Dexamethasone (Xiang et al., 2007)</td>
<td>Solvent evaporation</td>
<td>Glycerol tristearate</td>
<td>Pluronic F 68 (poloxamer 188) and Lipoid S 75 (soy lecithin)</td>
</tr>
<tr>
<td>Diazepam (Cavalli et al., 1997)</td>
<td>Microemulsification solidification</td>
<td>Stearic acid, Behenic acid, Acidan N12 (monostearate mononitrate glycerol)</td>
<td>Epikuron 200 (phosphatidylcholine 95%) and taurodeoxycholate</td>
</tr>
<tr>
<td>Diazepam Retinol Ubidecarenone (Westesen et al., 1997)</td>
<td>Hot high pressure homogenization</td>
<td>Dynasan 114 (trimyristin)</td>
<td>Soy bean phospholipid (Lipoid S100) and sodium glycocholate</td>
</tr>
<tr>
<td>Diclofenac sodium (Gaddam and Aukunuru, 2010)</td>
<td>Emulsion/solvent evaporation method</td>
<td>Glycerol monostearate</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>Diminazine (Olbrich et al., 2004)</td>
<td>Hot high pressure homogenization</td>
<td>Stearic acid</td>
<td>Polysorbate 80</td>
</tr>
<tr>
<td>Doxorubicin (Fundaro et al., 2000b)</td>
<td>Microemulsification solidification</td>
<td>Stearic acid</td>
<td>Epikuron 200 (phosphatidylcholine 95%) and taurodeoxycholate</td>
</tr>
<tr>
<td>D-penicillamine (Cui et al., 2005)</td>
<td>Microemulsification solidification</td>
<td>Emulsifying wax</td>
<td>Brij 78</td>
</tr>
<tr>
<td>Econazole nitrate (Passerini et al., 2009)</td>
<td>High-shear homogenization</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Tween 80</td>
</tr>
<tr>
<td>Etoposide (Harivardhan Reddy et al., 2005)</td>
<td>Hot high pressure homogenization</td>
<td>Tripalmitin</td>
<td>Hydrogenated soya phosphatidylcholine and sodium tauroglycolcholate</td>
</tr>
<tr>
<td>Ibuprofen (Casadei et al., 2006)</td>
<td>Hot high pressure homogenization</td>
<td>Precirol ATO 5 (glyceryl palmitostearate)</td>
<td>Pluronic F68 and sodium cholate</td>
</tr>
<tr>
<td>Indomethacin (Castelli et al., 2005)</td>
<td>Ultrasonication</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Poloxamer 188</td>
</tr>
</tbody>
</table>

Table 3 continued........................

36
<table>
<thead>
<tr>
<th>Drug incorporated</th>
<th>Method of preparation</th>
<th>Lipid phase</th>
<th>Surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon-alpha (Li et al., 2010)</td>
<td>Double emulsion solvent evaporation</td>
<td>Castor oil</td>
<td>Poly (lactic–glycolic acid)</td>
</tr>
<tr>
<td>Menadione (Westesen et al., 1997)</td>
<td>Hot high pressure homogenization</td>
<td>Dynasan 114 (trimyrstin)</td>
<td>S 100 and tyloxapol</td>
</tr>
<tr>
<td>Miconazole nitrate (Jain et al., 2010)</td>
<td>Hot homogenization method</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Tween 80/ glyceryl monoestearate</td>
</tr>
<tr>
<td>Mifepristone (Hou et al., 2003)</td>
<td>Modified high shear homogenization and ultrasonication</td>
<td>Glyceryl monostearate</td>
<td>Polysorbate 80 and glycerol monostearate</td>
</tr>
<tr>
<td>Mitoxantrone (Lu et al., 2006)</td>
<td>Film dispersion–ultrasonication method</td>
<td>Compritol® 888 ATO (glyceryl behenate)</td>
<td>Pluronic F68, Tween 80 and dimethyldioctadecyl ammonium bromide</td>
</tr>
<tr>
<td>Nevirapine (Kuo and Chung, 2011)</td>
<td>Microemulsification solidification</td>
<td>Compritol® 888 ATO and stearic acid</td>
<td>Polysorbate 80 and dimethyldioctadecyl ammonium bromide</td>
</tr>
<tr>
<td>Nimodipine (Hu et al., 2008)</td>
<td>Solvent diffusion</td>
<td>Monostearin</td>
<td>Sodium dodecyl sulfate</td>
</tr>
</tbody>
</table>
| Paclitaxel (Cavalli et al., 2000) | Microemulsification solidification | Tripalmitin | Epikuron 200 (phosphatidylcholine 95%)
| Pentoxifylline (Varshosaz et al., 2010) | Homogenization followed by sonication | Cetyl alcohol | Lecithin |
| Prednicarbate (Westesen et al., 1997) | Hot high pressure homogenization | Compritol® 888 ATO (glyceryl behenate) | Poloxamer 188 |
| Puerarin (Li et al., 2010) | Double emulsion-solvent emulsification | Monopropionate | Sorbitan monooleate and polyoxyethylene sorbitan monolaurate |
| Quercitin (Li et al., 2009a) | Emulsification and low-temperature solidification method | Glyceryl monostearate | Tween 80 |
| Retinyl acetate Progesterone (Cortesi et al., 2002) | Melt dispersion technique | Glyceryl tristearate (tristearin) Glyceryl tribehenate (tribehenin) Glyceryl tripalmitate (tripalmitin) | Gelatin, pectin, carrageenan polyvinyl alcohol, polyoxyethylene 20, sorbitan trioleate, lauryl sarcosine |
| Tamoxifen (Reddy et al., 2006) | High pressure homogenization | Compritol® 888 ATO (glyceryl behenate) Tristearin | Sodium tauroglycocholate |
| Tetracaine (zur Muhlen et al., 1998) | High pressure homogenization | Dynasan 112 (glyceryl trilaurate) | Pluronic F 68 (poloxamer and lipoid S 75 (sodium lecithin)) |
| Etomidate Prednisolone (zur Muhlen et al., 1998) | Compritol® 888 ATO (glyceryl behenate) | | |

Table 3 continued.....
SCOPE OF COMMERCIALISATION AND SCALE-UP OF SOLID NANOPARTICLES

During the last 10 years the field of nanotechnology has rapidly evolve mostly being limited to a research and scientific venture to a largely commercial undertaking. Hundreds of products that incorporate, or indeed are composed of nanomaterials, have left the laboratory and entered the market common and commercially successful applications of nanomaterials has been delivery of active molecules in pharmaceutical and related fields, such as for agriculture. In evaluating technologies, markets and developers, it is observed the drug delivery ideas are transforming from conceptual state to promising practice sometimes successfully and sometimes not.

The most important driver behind interest in nanomaterials for drug delivery is the increasing intensity of research and competition in delivery technologies overall market for new delivery technologies is growing very rapidly as companies seek to reduce the side effects of drugs, lower the amount of active drug needed to provide a therapeutic effect for cost and safety reasons and differentiate products.
that face commoditization and increasing competition. The interest in these type of products is very high, considering high cost of about $800 million for developing a new chemical entity (NCE). This cost not only involves money but also needs to be translated in terms of time (10-12 years) for any NCE to enter the market and the success rate is 21.5% for the drugs to enter the phase I trials, which eventually get a marketing approval (Bunger et al., 2009).

Today’s US$ 10 billion market for targeted delivery technologies in drugs, medical devices, food, personal care and agricultural chemicals will grow to US$ 24.6 billion by 2013, with approximately 89% of this market being currently, and predicted to remain, in drug delivery (Bunger et al., 2009). Nanomedical products for cancer are one of the projected market segments, which are reported to be worth nearly $20 billion in 2009. Latter is expected to increase at a compound annual growth rate (CAGR) of 11% to reach $33 billion in 2014. Further, nanomedicine for CNS indications is another major market sector, valued at nearly $11 billion in 2009 and expected to reach $18 billion by 2014 (www.bccresearch.com)

Nanomaterials, including nano-milled or sprayed drug crystals, micelles and liposomes, nanoporous implants and many other systems, constitute an important new toolkit in the drug delivery space. Against the backdrop of the growing delivery market, nanomaterials provide new features and functions that other delivery technologies cannot match. For example, the cancer drug paclitaxel is a small molecule that interferes with cell division, killing all fast-multiplying cells including healthy hair and intestinal cells; it is also highly insoluble on its own, and the solvents such as Cremophor and ethanol, in which it is soluble have their own side effects, some of which are significantly harsh. Companies including NanoCarrier, Abraxis and Kereos are developing nanoparticulate reformulation and encapsulation technologies to deliver paclitaxel without the debilitating effects of the drug and the solvents on healthy tissue. With the active ingredient, paclitaxel, now an off-patent commodity that still sells for about US$1 billion annually, the delivery systems are becoming the protectable therapeutic feature of the system.
Review of Literature

With a potential usefulness of SLNs over the polymeric nanoparticles various companies are endeavoring to gain expertise to develop SLNs on large scale so as to capture the commercial market. SLNs and microemulsions are constituted of biomolecules, such as fatty acids, triglycerides and phospholipids. SLNs are solidified droplets of warm microemulsion and so they have a spherical shape with average diameter preferably between 100 and 200 nanometers (may be upto 1000 nm), depending on molecule to be loaded in; size distribution is very narrow. Stealth SLNs can be prepared for i.v. administration, in order to avoid RES recognition, to prolong MRT and to better reach specific organs of the body including the brain.

SLN are solid nanoparticles made from lipids being solid at body temperature (Hou et al., 2003; Manjunath et al., 2005) and (Wissing et al., 2004a) are an alternative nanocarrier system to polymeric nanoparticles, liposomes and emulsions (Deshpande et al., 2009; Kaur et al., 2008). In view of the wide range of routes (dermal, oral, i.v.) by which SLN can be administered, various production techniques for different application routes are reported. Latter use lipids and surfactants (GRAS), or lipids being made from physiological compounds (e.g. glycerides of fatty acids available in the body, and fatty acids present in oils in parenteral nutrition). There exists a huge database on the various techniques and applications of SLNs. (Almeida and Souto, 2007; Bondi et al., 2010). Ability of production of lipidic nanoparticles at lab scale, pilot and large scale is essential, and the latter should provide not only the quantity but also lipid nanoparticles with long-term stability comparable to the lab scale (Weyhers et al., 2006). Some reports are available in the literature about the long-term stability of lipid nanoparticles from lab scale, e.g. till 12 months (Freitas and Müller, 1999) and 24 months (Schwarz, and Mehnert, 1999). However, there is limited long-term stability data available when produced on large scale.

One of the research and development consultancy based company, NANOVECTOR, claims specific skills and expertise in SLNs and microemulsions, which have been studied as colloidal carriers for drug delivery. SLNs are obtained by warm microemulsions by a proprietary method. The company is equipped with all the equipment(s) required to develop on bench scale the SLN and microemulsion
preparations, to characterise them for size, poly-dispersity index, zeta potential, and for chemical analyses.

Thus, prerequisite for the introduction of particulate or nanoparticulate drug carriers into the pharmaceutical market is the availability of a large scale production method. The method itself needs to be able to be qualified and validated, to be accepted by the regulatory authorities apart from being cost-effective. In addition, it should yield a product of a quality, acceptable by the regulatory authorities in regard to the accepted status of excipients and toxicology evaluation of the developed nanoproduct. Although various methods for large scale production of microparticles are established, but developing large scale production methods for nanoparticles are still in their infancy (Muchow et al., 2008). The most common of the existing methods of large scale nanoproduction in pharmaceutical industry is high pressure homogenization (HPH), e.g. for intravenous nanoemulsions (e.g. Intralipid, Lipofundin). The same production method has been extended to produce solid lipid nanoparticles. The HPH technique is used since 1950s for the production of parenteral emulsions. However, a large amount of energy and very high cost of the equipment is involved for manufacturing of a product using HPH.

Still another efficient, easy, cost effective and rugged method of producing SLN is the microemulsification method. In this method SLNs are prepared by the dispersion of warm oil-in-water (o/w) microemulsion in cold water; solid lipids with high melting points are used as the internal phase of the microemulsions. Although scaling up of this method for parenterals, is reported by Marengo et al. (2000), they proposed production from 1 to 100 ml of microemulsion by use of a specially designed apparatus. In the apparatus the warm liquid microemulsion was sterilized through a 0.22 μm membrane filter as the nanodroplets sizes of warm o/w microemulsion are lower than 100 nm. After passing through the filter, the warm microemulsion flows through the interchangeable needle and drops directly into a stirred cold aqueous medium (Marengo et al., 2000).
DRUG AGENTS SELECTED

CURCUMIN

Curcumin is a hydrophobic polyphenol derived from turmeric, the rhizome of the herb *Curcuma longa* L. (Figure 7). Commercial curcumin is a mixture of curcuminoids, containing approximately 77% diferuloylmethane, demethoxycurcumin and 5% bisdemethoxycurcumin. Curcumin is a highly pleiotropic molecule that modulates numerous targets. It binds to as many as 33 different proteins including thioredoxin reductase, cyclooxygenase-2 (COX-2), protein kinase C (PKC), 5-lipoxygenase and tubulin. The various molecular targets modulated by this agent include transcription factors, growth factors and their receptors, cytochromes and genes regulating cell proliferation and apoptosis. It has been shown to have antioxidant, anti-inflammatory and antimutagenic properties (Ammon, 1993; Rao et al., 1995; Shishu and Kaur, 2008).

![Curcuma longa L.](image)

Figure 7. Curcumin (bis-α,β-unsaturated β-diketone) a hydrophilic polyphenol derived from the rhizome of the herb *Curcuma longa* L.

The multitargeting ability of curcumin is probably the key to its therapeutic potential. Curcumin has been shown to protect against different cancers including leukaemia and lymphoma, gastrointestinal cancers, genitourinary cancers, breast cancer, ovarian cancer, head and neck squamous cell carcinoma, cancer, melanoma, neurological cancers and sarcoma, in in vitro studies, including cancerous cell lines. List of curcumin products available in the market are listed in Table 4. Extensive list of products in the market indicates the popularity it has gained with the masses, inspite of the fact that BA of curcumin is ≤ 1%. From this it may be concluded that the launch of a suitably designed curcumin formulation...
improved BA and thus a promise for enhanced activity/effects has a great potential market.

However, there is little or no evidence of in vivo anticancer potency of curcumin, especially for organs like brain although a few reports demonstrate curcumin-evoked apoptosis, in vitro in brain tumour cells (Dhandapani et al., 2006).

Table 4. Marketed products* of curcumin

<table>
<thead>
<tr>
<th>Marketed Product</th>
<th>Dosage Form</th>
<th>Manufacturer/ Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocurcumax™ (BCM-95®, Biocurcumin®)</td>
<td>Softgels</td>
<td>Arjuna Naturals, India (<a href="http://www.arjunanatural.com">www.arjunanatural.com</a>)</td>
</tr>
<tr>
<td>Cur-500</td>
<td>Capsules</td>
<td>Indsaff, India (<a href="http://www.indsaff.com">www.indsaff.com</a>)</td>
</tr>
<tr>
<td>Curamin®</td>
<td>Capsule</td>
<td>Euro Pharma, USA (<a href="http://www.curamin.com">www.curamin.com</a>)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Tablets</td>
<td>Care Pharma, India (<a href="http://www.carepharma.net">www.carepharma.net</a>)</td>
</tr>
<tr>
<td>Curcumin C3 Complex®</td>
<td>Capsule</td>
<td>Ageless Cures LLC, USA (<a href="http://www.carepharma.net">www.carepharma.net</a>)</td>
</tr>
<tr>
<td>Curcumin Extreme</td>
<td>Capsule</td>
<td>Euro Pharma, USA (<a href="http://www.curamin.com">www.curamin.com</a>)</td>
</tr>
<tr>
<td>Green Tea Extract+Curcumin C3</td>
<td>Caplets</td>
<td>Ageless Cures LLC, USA (<a href="http://www.carepharma.net">www.carepharma.net</a>)</td>
</tr>
<tr>
<td>MEGA Curcumin C3 w/TIME RELEASE®</td>
<td>Caplets</td>
<td>Ageless Cures LLC, USA (<a href="http://www.carepharma.net">www.carepharma.net</a>)</td>
</tr>
<tr>
<td>N-Curcusorb (Nano-Curcumin)</td>
<td>Powder</td>
<td>Konark Herbals and Health Care, India</td>
</tr>
<tr>
<td>Nutmeric</td>
<td>Natural turmeric spread with almonds</td>
<td>Spread Health Foods, USA, (<a href="http://www.spreadhealthfoods.com">www.spreadhealthfoods.com</a>)</td>
</tr>
<tr>
<td>Organic Turmeric</td>
<td>Capsule</td>
<td>Genceutic Naturals, USA (<a href="http://www.genceutic.com">www.genceutic.com</a>)</td>
</tr>
<tr>
<td>Rheumax®</td>
<td>Capsule</td>
<td>Unijules Life Sciences Ltd., Ind (<a href="http://www.unijules.com">www.unijules.com</a>)</td>
</tr>
<tr>
<td>Super Bio-Curcumin</td>
<td>Capsule</td>
<td>Life Extension, USA (<a href="http://www.iherb.com">www.iherb.com</a>)</td>
</tr>
<tr>
<td>Super Curcumin w/Bioperine®</td>
<td>Time Release Tablets</td>
<td>Ageless Cures LLC, USA (<a href="http://www.carepharma.net">www.carepharma.net</a>)</td>
</tr>
<tr>
<td>Triple Strength Curcumin</td>
<td>Capsule</td>
<td>SVN, Canada (<a href="https://www.svncanada.com">https://www.svncanada.com</a>)</td>
</tr>
<tr>
<td>Turmeric Extract</td>
<td>Capsule</td>
<td>Vitamin Shoppe, North Bergen (<a href="http://www.vitaminshoppe.com">www.vitaminshoppe.com</a>)</td>
</tr>
<tr>
<td>Turmeric Force</td>
<td>Softgels</td>
<td>SVN, Canada (<a href="https://www.svncanada.com">https://www.svncanada.com</a>)</td>
</tr>
<tr>
<td>Turmeric or Curcumin Capsules</td>
<td>Capsule</td>
<td>Krishna Herbal Company, India (<a href="http://www.krishnaherbals.com">www.krishnaherbals.com</a>)</td>
</tr>
<tr>
<td>Ultra-Pack Curcumin C3 w/Bioperine®</td>
<td>Capsule</td>
<td>Ageless Cures LLC, USA (<a href="http://www.carepharma.net">www.carepharma.net</a>)</td>
</tr>
</tbody>
</table>

*All products are being marketed as dietary supplements not as prescription drugs
Review of Literature

A major hurdle to the use of curcumin as a therapeutic agent is its poor BA (Al. al., 2007). The main reasons (Figure 8) for its reduced BA include low activity, poor absorption, high rate of metabolism, low activity or inactive metabolic products and/or its rapid elimination and clearance from the body.

![Curcumin](image)

Figure 8. Problems associated with the multitargeted molecule curcumin

CHEMISTRY OF CURCUMIN

Chemically, curcumin is a 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxyphenyl)-(1E,6E) or diferuloylmethane, which exhibits keto-enol tautomerism having a predominant keto (diketonic) form in acidic and neutral solutions and enol form in alkaline medium. It is a yellow-orange powder insoluble in water but soluble in ethanol, methanol, dimethylsulfoxide and acetone (Tc and Karlsen, 1985). Curcumin has a melting point of 183°C, a molecular formula of C_{21}H_{20}O_{6} and a molecular weight of 368.37 g. It gives brilliant yellow hue at pH < 4 and red at pH > 7. The fact that curcumin in solution exists primarily in its enolic form (Tomren et al., 2007) has an important bearing on its radical-scavenging and antioxidant properties. The stability of curcumin in aqueous media improves at high pH (> 7). However, it is stable at acidic pH but unstable at neutral and basic pH.
Most curcumin (>90%) is rapidly degraded within 30 minutes of placement in phosphate buffer systems of pH 7.2 (Tonnesen and Karlsen, 1985). The ability of antioxidants such as ascorbic acid, N-acetyl-L-cysteine (NAC) and glutathione (GSH) to prevent this degradation suggests that an oxidative mechanism is at work.

In contrast, one of curcumin’s major metabolites tetrahydrocurcumin (THC) is quite stable at neutral or basic pH (Pan et al., 1999b) and still possesses antioxidant activity (Murugan and Pari, 2006; Pari and Murugan, 2007; Somparn et al., 2007). Curcumin is soluble in 0.1 M sodium hydroxide, although it remains stable for only 1–2 hours in alkaline solutions. In comparison, curcumin is more stable in cell culture medium containing 10% fetal calf serum and in human blood, with ≤20% of curcumin being decomposed within 1 hour, and after incubation for 8 hours, about 50% of curcumin still remaining (Wang et al., 1997). Based on mass and spectrophotometrical analysis, trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal was tentatively identified as a major degradation product, while vanillin, ferulic acid and feruloylmethane were identified as minor degradation products (Wang et al., 1997).

Since curcumin decomposes rapidly in serum-free medium, precautions or suitable modifications must be made during its formulation and storage. In addition, the biological effects caused by the degradation products of curcumin, especially vanillin, must also be taken into consideration. Vanillin, a naturally occurring flavouring agent, has been reported to inhibit mutagenesis in bacterial and mammalian cells. It may act as an antimutagen by modifying DNA replication and DNA repair systems after cellular DNA damage caused by mutagens occurs. Vanillin is also a powerful scavenger of superoxide and hydroxyl radicals. Degradation of curcumin is however slow at pH 1–6 (Wang et al., 1997), as normally encountered in the stomach where pH varies from 2 to 4.

PRECLINICAL PHARMACODYNAMIC STUDIES WITH CURCUMIN

Most of the chronic illnesses are caused by dysregulated oxygen metabolism and inflammation. Inflammation and oxidative stress have been found to play a major role in various neurodegenerative disorders including Alzheimer’s disease,
depression, cerebral ischemia, cancer, various cardiovascular (CVDs), metabolic, and even psychological diseases (Dantzer et al., 2008; Park et al., 2007).

Tumor necrosis factor (TNF-α) is known to be a potent mediator of inflammation in most diseases, and this effect is regulated by the activation of a transcription factor, nuclear factor (NF)-κB. Whereas TNF is the most potent NF-κB activator yet described, the expression of TNF-α is also regulated by NF-κB (Aggarwal et al., 2007b; Sethi et al., 2008a). Besides TNF, NF-κB is activated by most of the other inflammatory cytokines also. Interestingly, most mediators of inflammation that have been identified up to now are also regulated by NF-κB, including inflammatory cytokines, chemokines, adhesion molecules, enzymes, and kinases. Thus, NF-κB and NF-κB-regulated gene products have been closely linked with most chronic illnesses.

Role of curcumin in neurodegeneration

Neurodegenerative diseases are discernible by site-specific premature and slow death of certain neuronal populations (Mizuta et al., 2006). They are multifactorial in nature and engross complex mechanisms due to the multiple pathogenic events involved. One of the most common reasons assigned to neurodegenerative disorders include unbalanced overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) resulting in oxidative and nitrosylative stresses which induce neuronal damage and subsequently neuronal cell death, either by apoptosis or necrosis. According to free radical theory of aging, an elevation in ROS and RNS, damages neural membranes accompanied by the concomitant decline in cognitive and motor performance in the elderly population, even in the absence of neurodegeneration (Farooqui and Farooqui, 2009).

Dietary supplementation with various polyphenolics, including resveratrol, ginkosides, curcumin, ferulic acid, carotenoids, flavonoids, and omega-3 fatty acids is reported to exert a beneficial effect for restoring optimal neuronal communication (Joseph et al., 2007; Lau et al., 2007). Curcumin, also described as 'Indian gold' has an exceptional safety profile and with its pleiotropic action, holds potential for neuroprotective efficacy, considering its significant anti-inflammatory, antioxidant, and anti-protein-aggregate activities.
Alzheimer’s disease (AD)

AD is reported to affect 10% of people over the age of 65 and 50% of people over the age of 85 with an increased incidence in women. AD affects 15 million people worldwide including a high infliction rate of 30% in American population. To this, National Institute of Health predicts, if the current trend continues, there will be more than 8.5 million AD patients by the year 2030 in USA alone (Cole et al., 2007). Various studies and research results indicate a lower incidence and prevalence of AD in India. The prevalence of AD among adults aged 70-79 years in India is 4.4 times less than that of adults aged 70-79 years in the United States. This is attributed to the consumption of spices, including curcumin, by the major population of the Indian sub-continent.

Curcuminoids are proven to have strong antioxidant action as demonstrated by the inhibition of the formation and propagation of free radicals. It decreases the low-density lipoprotein oxidation and the free radicals that cause the deterioration of neurons, not only in AD but also in other neurodegenerative disorders such as Huntington’s and Parkinson’s disease (Mishra and Palanivelu, 2008). Curcumin has powerful antioxidant and anti-inflammatory properties; according to the scientists, these properties help ease Alzheimer's symptoms which are majorly caused by oxidation and inflammation (Frautschy et al., 2001).

Lipophilic nature of curcumin, helps it to cross the BBB and bind to the plaques formed during AD. Curcumin is reported to show amyloid-beta (Aβ) 40 aggregation inhibition activity and it destabilizes the Aβ polymer. In in vitro studies, curcumin not only inhibits its aggregation but also disaggregates the A beta polymer to form fibrillar Aβ 40. Curcumin given to APPswe/PS1dE9 mice for 7 days was found to cross the BBB as demonstrated by multi-photon microscopy and it reduced the existing senile plaques (Garcia-Alloza et al., 2007). Curcumin-derived isoxazoles and pyrazoles are reported to bind to the Aβ peptide and inhibit amyloid precursor protein (APP) metabolism (Narlawar et al., 2008).

In a recent study, improvement in cortical and hippocampal dependent learning in the transgenic mice, was reported after administration of the medical food cocktail containing curcumin for 6 months rendering their performance
indistinguishable from non-transgenic controls. Further, they showed that the improvement in learning and memory, resulted in reduction of soluble Aβ, including Aβ oligomers which was linked to cognitive functioning (Parachikova et al., 2010).

Role of curcumin in aluminium induced toxicity is well elicited from the literature reports. Latter is documented to be a suitable model mimicking Alzheimer’s like symptoms. A study demonstrated that curcumin exerts a protective effect against aluminium-induced ageing-related changes by modulating the extent of oxidative stress (by upregulating the activities of antioxidant enzymes) and by regulating the activities of Na(+) K(+) ATPase, PKC and AChE (Sharma et al., 2009). Another study, investigated the effect of curcumin treatment at a dose of 30 mg/kg/day against aluminium neurotoxicity in young and old animals. They proved that curcumin treatment attenuates the Al-induced alterations at biochemical, behavioral and ultrastructural levels. Further, they indicated the ability of curcumin to bind to redox active metals and cross the BBB thus playing a crucial role in preventing Al-induced neurotoxicity (Sethi et al., 2009). Protective effects were achieved after chronic administration of curcumin (6 months) at a dose of 100 mg/kg, with a significant improvement in memory retention. Further, attenuation of oxidative damage, acetylcholinesterase activity and aluminium concentration in aluminium treated rats (p≤0.05) was also reported (Kumar et al., 2009a). It may however be noted that all these studies indicate protective effect (i.e administration of curcumin starts before or simultaneously post induction of Al toxicity) and that too invariably after its chronic use. Latter may be due to a poor build up of curcumin within brain both due to a poor systemic BA and also a limited brain permeability, eventhough curcumin is reported to cross BBB.

A very recent study illustrates the preparation and characterization of curcumin loaded nanoparticles with a very high affinity for Aβ1-42 fibrils. Using surface plasmon resonance experiments it was demonstrated that the liposomes exposing the curcumin derivative (maintaining the planarity) have extremely high affinity for Aβ1-42 fibrils (1-5 nM), likely because of the occurrence of multivalent interactions, whereas those exposing non-planar curcumin did not bind to Aβ1-42 (Mourtas et al., 2011).
Depression is a severe neurological disorder characterized by a depressed or irritable mood, with a decreased interest in pleasurable activities. It is estimated that, 15-20% of the world population suffers from depression at a particular time. Despite the availability of various antidepressants, it is still not possible to treat 20-30% of the depressed patients due to the side-effects associated with these agents. Therefore, it is of utmost importance that efficacious and safe alternative drug therapies be identified for the treatment of depression (Kulkarni and Dhir, 2010).

Antidepressant activity of curcumin has been reported in different animal models. Effectiveness of curcumin in forced swim test (FST) and chronic unpredictable stress has been reported (Bhutani et al., 2009). The antidepressant activity of curcumin is due to its modulating effect on the release of serotonin and dopamine. Curcumin at a dose range of 10-80 mg/kg, i.p. demonstrated anti-immobility action in FST during a 6 min period. The maximum anti-immobility effect was observed at 90 min of its administration and at doses of 40 and 80 mg/kg it also reversed the reserpine-induced behavioral despair in mice (Kulkarni et al., 2008).

The antidepressant effects of *Curcuma longa* have also been investigated in behavioral despair tests in mice (Yu et al., 2002). In a study by Xu et al. (Xu et al., 2005a) curcumin treatment at 5 and 10 mg/kg (p.o.) significantly reduced the duration of immobility in both the tail suspension and FST. Their findings suggested the involvement of central monoaminergic neurotransmitter system, as a result of increase in level of serotonin and dopamine in antidepressant-like effects of curcumin. Another study demonstrated that curcumin inhibits the activity of monoamine oxidase (MAO) in C6 glial cells; MAO plays a central role in several psychiatric neurological disorders, including clinical depression and anxiety (Mazzio et al., 1998b). Further, there is also an evidence that MAO inhibitor-induced increase in monoaminergic neurotransmission can alleviate clinical depression (Dar and Khatoon, 2000).

Xu and his co-workers have also demonstrated that acute and chronic curcumin administration significantly decreased immobility time in behavioral despair tests, suggesting the role of central monoaminergic neurotransmitter system in the
anti-depressive effects of curcumin (Xu et al., 2005b). Further, the same group of researchers showed the antidepressant effect of curcumin in a pilot study and confirmed that acute administration of curcumin (1, 2.5, 5 and 10 mg/kg, p.o.) significantly decreased the immobility time in the FST.

Another animal model of depression is unpredictable chronic stress. A study reported that curcumin at doses of 20 and 40 mg/kg reversed the immobility caused as a result of chronic stress in stressed mice (Bhutani et al., 2009). Animals challenged with chronic unpredictable stress demonstrate lower levels of norepinephrine, serotonin and dopamine in the brain. Chronic administration of curcumin did not affect depleted norepinephrine levels but restored levels of serotonin and dopamine (Bhutani et al., 2009). In another study, curcumin produced beneficial effects in stressed rats by effectively improving the low sucrose consumption induced by chronic unpredictable stress in rats. The studies further demonstrate the participation of adenylyl cyclase (AC) and cyclic adenosine monophosphate (cAMP) pathway in the antidepressant activity of curcumin (Li et al., 2009b).

The antidepressant activity of curcumin has also been explored by combining it with various other agents. Curcumin enhanced the anti-immobility effect of sub-effective doses of fluoxetine (selective serotonin reuptake inhibitor), bupropion (dopamine reuptake inhibitor) and venlafaxine (dual reuptake inhibitor of serotonin and norepinephrine) (Kulkarni et al., 2008). In another study combination of piperine (2.5 mg/kg, i.p., 21 days), a bioavailability enhancer, with curcumin (20 and 40 mg/kg, i.p., 21 days) showed significant antidepressant effects as compared to free curcumin (Bhutani et al., 2009).

Parkinson’s disease (PD)

Another prevalent, age-related neurodegenerative condition is Parkinson’s disease (PD), a degenerative disorder of the central nervous system. It results from the death of dopamine-containing cells in the substantia nigra, a region of the midbrain. In western populations, significant age-related loss of pigmented neuromelanin-bearing neurons commonly occurs in this region, but symptoms of PD do not manifest until 60–80% neuronal loss has already taken place.
PD is associated with elevated oxidative damage, including auto-oxidative dopamine breakdown and related semiquinone metabolism to superoxide, as well as monoamine oxidase production of hydrogen peroxide (Cole et al., 2007). Effectiveness of low doses of curcumin to inhibit dopamine toxicity in vivo have been reported (Luo et al., 1999).

**Cerebral ischemia**

Oxidative damage and free radical generation are documented to increase in the animal brain after cerebral ischemia/reperfusion injury (Babu and Srinivasan, 1997). Curcuma oil (500 mg/kg i.p.) given 15 min before 2 h middle cerebral artery occlusion, followed by 24 h reflow in rats, significantly diminished the infarct volume, improved neurological deficit and counteracted oxidative stress (Rathore et al., 2007). A study conducted at Nanjing Medical University (China), showed that, a single injection of curcumin (1 and 2 mg/kg, i.v.) after focal cerebral ischemia/reperfusion, in rats, significantly diminished the infarct volume, improved neurological deficit, decreased mortality and reduced the water content in brain (Jiang et al., 2007).

The cytoprotective activity of curcumin is reported in in vitro and in vivo models of cytotoxicity by various research groups. Curcumin showed protective effect in isoproterenol induced myocardial ischemia in rats (Nirmala and Puvanakrishnan, 1996) and reduced renal damage and inflammation in renal ischemia reperfusion injury (Jones and Shoskes, 2000). Luo and co-workers (Luo et al., 1999) showed that curcumin inhibited activation of AP1 and NF-κB, upregulation of c-fos, c-jun and phosphorylation of c-jun proteins. Curcumin has been found to inhibit JNK, PKC, iNOS, and COX-2 enzymes (Huang et al., 1991) and is reported to show a membrane stabilizing activity (Nirmala and Puvanakrishnan, 1996).

Administration of 100 and 300 mg/kg of curcumin i.p. 60 min after middle cerebral artery occlusion (MCAO) was shown to significantly diminish the infarct volume, and improve neurological deficit in a dose-dependent manner (Zhao et al., 2008).

**Huntington’s disease and other neurodegenerative diseases including ageing**

These diseases are manifested by extended C-terminal CAG repeats coding for polyglutamine, which result in formation of protein aggregates. Resemblance of
curcumin to congo red and its homologue chrysamine G explains its anti-amyloid-binding protein properties which is also extended to other protein-misfolding diseases with a β-pleated sheet, including the polyglutamine diseases like Huntington’s disease (HD) (Caughey et al., 2003). Evidence for a protective effect in an HD transgenic model has been followed by a pilot curcumin clinical trial with HD patients at UCLA (www.newsroom.ucla.edu). Marie-Charcot Tooth disorder is another example of a similar protein-misfolding neuropathy and curcumin is reported to protect against this disorder in vitro and in vivo in a transgenic model (Zhu et al., 2006).

Further evidence for an impact of curcumin on ageing brain has been recently produced in aging rats, where chronic curcumin treatment was shown to result in reduced lipid peroxidation and accumulation of the age-pigment lipofuscin and to increase the antioxidant defense enzymes glutathione peroxidase and superoxide dismutase as well as sodium potassium ATPase, which normally declines with age (Kang et al., 2006). Curcumin resembles another biphenolic antioxidant, resveratrol, that is believed to have antiaging activity via induction of sirtuins and histone deacetylase (HDAC) activation, so ability of curcumin to limit histone acetyltransferase (HAT) and promote neurogenesis (Bala et al., 2006) might also impact longevity, promoting a sirtuin-like effect on HAT-regulated transcription. These results support the hypothesis that curcumin might slow normal ageing of the brain and presumably other tissues in which age-related oxidative damage is an issue.

**Role of curcumin in cancer**

Although cancers are characterised by the dysregulation of cell signalling pathways at multiple steps, most current anticancer therapies involve the modulation of a single target. The ineffectiveness, lack of safety and high cost of monoblock therapies have resulted in a trend towards developing multitargeted molecules for cancer control. Many plant-based products, however, accomplish multitargeting naturally and, in addition, are inexpensive and safe compared to synthetically designing such agents. The current paradigm for most of the treatments is to either combine several ‘smart drugs’ or design drugs that modulate multiple targets (multitargeted therapies), formally referred to as ‘dirty drugs’ (Figure 9).
The recent success of a number of promiscuous agents has led many researchers to investigate the rationale and potential of openly unspecific agents. Some authors advocate the development of ‘magic shotguns’ rather than ‘magic bullets’ as a more realistic and potentially successful approach to tackle a disease of such complexity as cancer (Roth et al., 2004). This line of thinking is also starting to prevail in other medical disciplines. In a review by Roth and co-workers (Roth et al., 2004), authors discussed the use of selective versus nonselective drugs for CNS disorders. Since in most cases multiple molecular lesions or signalling pathways are involved in the pathogenesis of CNS disorders, the authors conclude that attempts to develop more effective treatments for diseases such as schizophrenia and depression by discovering drugs selective for single molecular targets, the ‘magic bullets’ concept, have been largely unsuccessful. They hypothesise that selectively designing nonselective drugs that interact with several molecular targets will lead to new and more effective medications for a variety of CNS disorders. However, because pharmaceutical companies are not usually able to secure intellectual property rights to plant-based products, the development of plant-based antican therapies has not been prioritised.

Figure 9. Dirty drugs with their multiple targeting potential are gaining interest.
Review of Literature

Intensive studies on the action mechanisms of curcumin in various biological systems have indicated that it employs multiple antitumour-promoting pathways (Aggarwal et al., 2007b). It is conceivable that the molecular mechanism of action of curcumin is quite complicated and dispersed. The locations of targets of its action vary from genome (DNA) level, to the messenger (RNA) level, to the enzyme (protein) (Aggarwal et al., 2007b). The action of curcumin may proceed simultaneously or sequentially through these different levels. It appears that when any essential component of a signal transduction pathway is rendered hyperactive or autonomous, it may acquire the ability to drive the cell into unchecked proliferation leading to tumour promotion. Curcumin may attenuate or suppress the hyperactivity of these components of signal transduction and maintain simultaneously the normal cell function (Aggarwal et al., 2007b).

The various molecular targets (Figure 10) modulated by this agent include transcription factors, growth factors and their receptors, cytokines, enzymes and genes regulating cell proliferation and apoptosis (Aggarwal et al., 2009; Roy et al., 2002). The diversity of the biological actions of curcumin in mammalian species was recently emphasised by a noteworthy study demonstrating its beneficial effects in mice homozygous for a complete knockout of a gene linked with cystic fibrosis (Egan et al., 2004).

In vitro anticancer activity of curcumin by induction of apoptosis

Curcumin has been shown to exert a fascinating array of pharmacological effects in cells in vitro at physiologically attainable and at supraphysiological concentrations.

In a very recent study curcumin encapsulated in hydrophobically modified starch (HMS) revealed enhanced in vitro anticancer activity as compared to free curcumin due to its enhanced solubility by about 1670-folds (Yu and Huang, 2010). Further, a study revealed the efficacy of rubusoside-solubilized curcumin against human colon, breast, and pancreatic cancer cell lines using various cell viability assays (Zhang et al., 2011). Another study reported the involvement of mitochondria and caspase cascades in the modulation of apoptosis and cell cycle arrest in curcumin-treated NPC-TW 076 human nasopharyngeal carcinoma cells. Their
findings revealed that mitochondria and AIF caspase-3-dependent pathway vital role in curcumin-induced G2/M phase arrest and apoptosis of NPC-cells in vitro (Kuo et al., 2011).

Figure 10. Modulation of various molecular targets by curcumin

Still another study revealed the role of curcumin in ameliorating cisplatin-induced apoptosis of human embryonic kidney (HEK) 293 cells in vitro with cisplatin or oxaliplatin (Waly et al., 2011). Curcumin has been shown to suppress the proliferation of a wide variety of tumour cells, including B-cell non-Hodgkin lymphoma (Abe et al., 1999; Han et al., 1999; Piwocka et al., 1999), colon and epidermal carcinoma (Korutla and Kumar, 1994). Furthermore, curcumin suppresses the proliferation of various breast tumour cell lines such as B16 melanoma and MDA-MB-231. A few studies on the anticancer activity of curcumin against uterine...
have also been reported. Curcumin exhibits anticancer effects in various lung cancer cells through a variety of molecular targets.

At the cellular level, curcumin derivatives inhibit farnesyl protein transferase (FPTase), in A549 cells. The anticancer effect of curcumin in murine thymoma cells was found to be due to the blocking of IL-1 signalling by the inhibition of the recruitment of the IL-1 receptor-associated kinase. A study showed that curcumin could prevent tumour-induced thymic atrophy in thymic T cells, leading to the neutralisation of tumour-induced oxidative stress and the restoration of NF-κB activity and the re-education of the TNF-α signalling pathway, resulting in thymic protection (Kumar et al., 2000). Furthermore, curcumin was also described as an effective agent (Arbiser et al., 1998), explaining its chemopreventive effect at the level of tumour promotion. This phenomenon could be explained by vascular endothelial growth factor (VEGF) and angiopoietin 1 and 2 inhibition in Ehrlich ascites tumor (EAT) cells, by VEGF and angiopoietin 1 inhibition in NIH 3T3 cells and by inhibition of the tyrosine kinase Flk-1/KDR (VEGF receptor-2) in human umbilical vein endothelial cells (Gururaj et al., 2002). It also displays an inhibiting effect on human telomerase reverse transcriptase expression, reducing telomerase activity in MCF-7 cells (Ramachandran et al., 2002).

In vitro cellular experiments have shown that short-term treatment with curcumin inhibits epidermal growth factor receptor (EGFR) kinase activity and epidermal growth factor-induced tyrosine phosphorylation of EGFR in A431 cells and depletes cells of Her2/neu protein. Curcumin is also extremely potent at degrading intracellular HER2 and disrupting its tyrosine kinase activity (Tikhomirov and Carpenter, 2003). Curcumin has also been shown to induce apoptosis in acute T-cell leukaemias by inhibiting the phosphatidylinositol 3 kinase/Akt pathway and to induce G2/M arrest and nonapoptotic autophagic cell death in malignant glioma cells by abrogating Akt and extracellular-regulated kinase signalling pathways (Aoki et al., 2007).

Effects of curcumin are also apparently mediated through its inhibition of various other serine/threonine protein kinases. Curcumin completely inhibits the activity of several protein kinases including phosphorylase kinase (PKC), cytosolic
protamine kinase, autophosphorylation-activated protein kinase and pp60c-src tyrosine kinase. Other investigators have shown similar suppression of phorbol-12-myristate-13-acetate-induced activation of cellular PKC by curcumin (Shishodia et al., 2007b).

Most inflammatory stimuli typically activate one of the three independent mitogen-activated protein kinase (MAPK) pathways leading to activation of the p44/42 MAPK (also called ERK1/ERK2), Jun N-terminal Kinase (JNK) or p38 MAPK pathway, respectively. Curcumin can apparently inhibit all of these pathways directly or indirectly, thus providing evidence of its potent anti-inflammatory and anticarcinogenic effects (Shishodia et al., 2007b).

The ability of curcumin to induce apoptosis in cancer cells without cytotoxic effects on healthy cells contributes to the understanding of the anticancer potential of curcumin. This spice is described to efficiently induce apoptosis in various cell lines including HL-60, K562, MCF-7 and HeLa (Karunagaran et al., 2005; Roy et al., 2002). Curcumin also leads to apoptosis in scleroderma lung fibroblasts without affecting normal lung fibroblasts (Tourkina et al., 2004). Woo and co-workers (Woo et al., 2003) suggested that the induction of Caki (human kidney carcinoma cells) programmed cell death by curcumin is activated by Akt dephosphorylation, Bcl-2, Bcl-xL and inhibition of apoptosis protein inhibitor, as well as cytochrome c release and caspase 3 activation. Later, this was confirmed by the results of Bush and co-workers (Bush et al., 2001), Anto and co-workers (Anto et al., 2002) and Pan and co-workers (Pan et al., 2001) studying caspase 3 activation in melanoma and HL-60 cells. Caspase activation by curcumin was described to be blocked by heat shock proteins (HSP), which do not influence cytochrome c release (Rashmi et al., 2003). Jana and co-workers (Jana et al., 2004) demonstrated that curcumin inhibits proteasome activity in mice, potentially leading to induction of apoptosis through caspase 9 activation. In another study (Jiang et al., 1996), the induction of apoptosis by curcumin (30 μM) was shown to depend on the origin and malignancy of cell lines. It appears that the typical apoptosis can only be induced in immortalised mouse embryo fibroblast NIH 3T3, erbB2 oncogene-transformed NIH 3T3, mouse sarcoma 180, human colon cancer cell HT29, human kidney cancer cell 293 and human hepatocellular carcinoma HepG2 cells but not in primary cultures of mouse...
embryonic fibroblast C3H 10T1/2, rat embryonic fibroblast or human foreskin fibroblast cells (Jiang et al., 1996). Treatment of NIH 3T3 cells with the PKC inhibitor staurosporine, the tyrosine kinase inhibitor herbimycin A or arachidonic acid metabolism inhibitor quinacrine induces typical apoptosis. These findings suggest that blocking the cellular signal transduction in immortalised or transformed cells might trigger the induction of apoptosis by curcumin. They also demonstrated that curcumin (3.5 μg/ml) induces apoptosis in human promyelocytic HL-60 cells (Jiang et al., 1996).

The apoptosis-inducing activity of curcumin occurred in a dose- and time-dependent manner. Flow cytometric analysis showed that the hypodiploid DNA peak of propidium iodide-stained nuclei appeared 4 hours after treatment with 7 μg/ml curcumin. The apoptotic effect of curcumin was not affected by cycloheximide, actinomycin D, ethylene glycol tetraacetic acid (EGTA), W7 (calmodulin inhibitor), sodium orthovanadate or genistein whereas an endonuclease inhibitor, ZnSO₄, and a proteinase inhibitor, N-tosyl-l-lysine chloromethyl ketone, could markedly abrogate curcumin-induced apoptosis. The antioxidants NAC, l-ascorbic acid, α-tocopherol, catalase and superoxide dismutase all effectively prevented curcumin-induced apoptosis (Kuo et al., 1996).

Zheng and co-workers (Zheng et al., 2002) explored the apoptosis-inducing effects of curcumin in human ovarian tumour A2780 cells. They found that curcumin could significantly inhibit the growth of ovarian cancer cells by inducing apoptosis through upregulation of caspase 3 and downregulation of expression of NF-κB.

**Preclinical pharmacodynamic studies**

Prevention of cancer in the colon, skin, stomach, liver, lung, duodenum, soft palate and breasts of rodents following oral administration of curcumin has been extensively reported. In particular, the effects of dietary curcumin (0.05–2.0%) on colorectal carcinogenesis have been demonstrated in both carcinogen-induced and genetic rodent models. Curcumin inhibited carcinogenic initiation, as reflected by decreased levels of adducts induced by benzo[a]pyrene or by aflatoxin B1 (Kawamori et al., 1999; Soni et al., 1992). In the azoxymethane-induced rat colon cancer model, dietary curcumin (0.8%) reduced the number of aberrant crypt foci to
one-half compared with control (Volante et al., 2005). In intestinal cancer induced in mice by azoxymethane, oral curcumin (2000 ppm) treatment for 14 weeks produced a significant increase in the apoptotic histological index when compared to controls (Samaha et al., 1997). Genetic models, such as the multiple intestinal neoplasia (e.g., Apc\textsuperscript{Min}) mouse, permit the study of the inhibition of promotion phase of carcinogenesis. Curcumin interfered with adenoma formation in the Apc\textsuperscript{Min} mouse, which harbours an adenomatous polyposis coli gene mutation and is a model of the human disease familial adenomatous polyposis (Luongo et al., 1994). When administered in the diet at 0.1% and 0.2% for the animals lifetime, a significant decrease in adenoma number was observed compared to control animals (Milibedzka et al., 1910; Perkins et al., 2002). This was accompanied by downregulation of the expression of the enzyme COX-2 and attenuation of tissue oxidative status, as reflected by the levels of the oxidative DNA adduct pyrimido-[1,2\textit{a}]purin-10(3H)-one-2-deoxyguanosine.

Chemopreventive effects of curcumin along with its effects on the initiation or post-initiation phase of N-nitrosomethylbenzylamine-induced oesophageal carcinogenesis in male F344 rats are also reported (Ushida et al., 2000). In another similar study in rodents, curcumin was able to inhibit the development of N-methyl-N-nitro-N-nitroso-guanidine-induced stomach cancer (Ikezaki et al., 2001), an effect that may be mediated in part by an ability to suppress the proliferation of \textit{Helicobacter pylori} (the major pathogen in human gastric cancer) (Mahady et al., 2002).

Effects of curcumin (daily dose of 100 mg/kg) in an animal (Wistar rat) model of N-nitrosodiethylamine (DENA)-initiated and phenobarbital (PB)-induced hepatocarcinogenesis were investigated by Sreepriya and Bali (Sreepriya and Bali, 2005). The investigators reported that curcumin prevented the reduction of defensive hepatic GSH antioxidant activity, decreased lipid peroxidation and minimised the histological alterations induced by DENA/PB (Sreepriya and Bali, 2006). In another study, investigators found that the administration of curcumin and its synthetic analogue to nicotine-treated Wistar rats over a period of 22 weeks enhanced biochemical marker enzyme and lipid profiles (Kalpana et al., 2005). Other in vivo studies have investigated the effects of curcumin on tumour angiogenesis and on COX-2 and VEGF biomarkers in hepatocellular carcinoma cells implanted in nude
mice (Yoysungnoen et al., 2006). A group of researchers demonstrated that systemic administration of curcumin for 6 consecutive days to rats bearing the highly cachectic Yoshida AH-130 ascites hepatoma significantly inhibited tumour growth (Busquets et al., 2001). Antitumoral and inhibitory effects of curcumin were reported for melanoma cells (Odot et al., 2004) and melanoma lung metastasis in mice (Menon et al., 1995). A study in human breast cancer xenograft model of nude mice bearing the human-derived MDA-MB-435 breast tumour showed a decrease in the load of breast cancer metastases and concomitant suppression of NF-κB, COX-2 and MMP-9.

Curcumin, a multitargeting molecule, when applied topically at a concentration of 3–10 mol, 5 minutes prior to the application of carcinogen, inhibits chemical carcinogenesis of the skin (Conney, 2003; Goel et al., 2007). In this series of studies, tumour initiation was induced by benzo[a]pyrene or 7,12-dimethylbenz[a]anthracene (DMBA) and tumour promotion was induced by 12-O-tetradecanoylphorbol-13-acetate. Potential mechanisms of these effects were considered to involve inhibition of arachidonic acid-induced inflammation, inhibition of hydrogen peroxide formation and inhibition of ornithine decarboxylase activity/transcription, the last of which is a rate-limiting step in polyamine biosynthesis (Conney, 2003; Goel et al., 2007). In a recent study reported by us, curcumin entrapped in elastic vesicles (curcumin-EV) was significantly effective in controlling UV-induced lesions even at a dose as low as 1–3 μmol. At a dose of 10 μmol there was a complete alleviation of symptoms, when curcumin-EV were applied before and after UV exposure (Agrawal and Kaur, 2010).

It should also be noted that chemically induced carcinogenesis was not attenuated by curcumin in several instances. For example, dietary curcumin (500 ppm) did not affect prostate carcinogenesis in rats exposed to 3,2-dimethyl-4-aminobiphenol or 2-amino-1-methylimidazo[4,5-b]pyridine (Imaida et al., 2001). Considering that the major part of the curcumin is unabsorbed from GIT, it may be concluded that sufficient amount of curcumin is available for local action in the GIT upon oral administration. This probably explains its suitability for the treatment of gastric cancers as indicated by several studies including clinical trials and also its failure to treat other systemic cancers, in vivo.
Suppressing brain tumours by curcumin

Primary malignant brain tumours represent a heterogeneous group of diseases. They probably develop through accumulation of genetic alterations that permit cells to evade normal regulatory mechanisms and escape destruction by the immune system. In addition to inherited alterations in crucial genes that control the cell cycle, such as TP53, the chemical, physical and biological agents that damage DNA are suspected potential neurocarcinogens (Wrensch et al., 2002). However, detailed investigations may be required before a more comprehensive picture of the natural history and pathogenesis of brain tumours is clear. There is intensifying interest in understanding the cause of brain tumours because the prognosis for patients with glioblastoma and other tumour types remains grim. A clinical report from the Mass General Hospital (Roelfsema et al., 2006) states that a large number of brain tumours are caused by metastatic invasion of cancer cells, including melanoma, from other parts of the body.

Direct targeting of specific cellular and molecular alterations is a strategy in the development of cancer therapeutics (Dhandapani et al., 2007). Development of diet-derived chemopreventive agents is an emerging area of cancer research. Many diet-derived compounds show promising anticancer activities in epidemiologic as well as experimental studies (Karmakar et al., 2006). The therapeutic efficacy of curcumin in various human malignant glioblastoma cells has been established (Ambegaokar et al., 2003), and curcumin was found to inhibit the NF-κB signalling pathways in these cell lines (Dhandapani et al., 2007; Karmakar et al., 2007; Nagai et al., 2005). Numerous other mechanisms, like the induction of HSP (Kim et al., 2005b; Woo et al., 2005), TNF-related apoptosis-inducing ligand-induced apoptosis (Gao et al., 2005), inhibition of glucose-6-phosphate transporter (G6PT) gene expression (Belkaid et al., 2006), the activation of both receptor-mediated and mitochondria-mediated proteolytic pathways (Karmakar et al., 2006), the induction of histone hypoacetylation leading to apoptosis in a poly (ADP-ribose) polymerase (PARP)- and caspase 3-mediated manner (Kang et al., 2006b), the inhibition of the inhibitor of growth protein 4 (ING4) signalling pathway (Liu et al., 2007) and the induction of nonapoptotic autophagic cell death (Aoki et al., 2007; Shinojima et al., 2007) have also been established. Furthermore, curcumin was found to sensitise
glioma cells to several chemotherapeutic agents and to radiation therapy (Dhandapani et al., 2007).

Apart from the above-mentioned mechanisms, several other mechanisms of action by which solubilised curcumin acts, in vitro, have been elaborated by a group of researchers (Purkayastha et al., 2009). They observed that the solubilised curcumin causes activation of proapoptotic enzymes caspase 3/7 in human oligodendroglioma (HOG) and lung carcinoma (A549) cells, and in mouse tumour cells N18 (neuroblastoma), GL261 (glioma) and B16F10. A simultaneous decrease in cell viability was also revealed by MTT (3-(4,5-dimethylthiazolyl-2)-diphenyltetrazolium bromide) assays. Further examination of the B16F10 cells showed that curcumin effectively suppresses cyclin D1, P-NF-κB, Bcl-xL, P-Akt, VEGF, which explains its efficacy in blocking proliferation, survival and invasion of the B16F10 cells in the brain. Taken together, solubilised curcumin effectively blocks brain tumour formation and also eliminates brain tumour cells. Another study elaborating the usefulness of the developed curcumin nanoparticles for malignant glioma therapy showed that curcumin loaded nanoparticle were effectively transported into the cells by endocytosis and localized around the nuclei in cytoplasm. It was observed that curcumin nanoparticles showed higher apoptotic effect against rat C6 glioma cell lines (Shao et al., 2011).

Curcumin recently entered phase I clinical trials for the treatment of high-grade gliomas, and recent emerging literature suggests multiple beneficial effects of curcumin in glioma cells, including inhibition of cellular growth, invasion and angiogenesis (Dhandapani et al., 2007). The usefulness of solubilised curcumin as a prophylactic against brain cancer via intravenous or intracranial routes has been reported by a team of scientists (Purkayastha et al., 2009). They demonstrated the nontoxic nature of their formulation against normal brain cells. Furthermore, they demonstrated that tail-vein injection or, more effectively, intracerebral injection of curcumin through a cannula block tumour formation in mice that had already received an intracerebral bolus of mouse melanoma cells (B16F10). Although the above results are conclusive of the fact that the solubilised curcumin when utilised judiciously could prove to be highly efficacious for treatment of brain tumours, the invasive nature of the treatment (intracerebral injection) can be highly discouraging for long-term or frequent use (Purkayastha et al., 2009).
In another in vivo study, curcumin significantly decreased the incidence of radiation-induced pituitary tumours in rats (Aggarwal et al., 2007a). In the subcutaneous xenograft model of glioblastoma cells, curcumin inhibited tumour growth significantly and induced autophagy. In another study, when the brain tumours reached 50–70 mm$^3$ in volume, intratumoral injections of curcumin (100 mg/kg in dimethyl sulfoxide (DMSO)/phosphate-buffered saline) were administered every 24 hours for 7 days. Evaluation of the effect was performed on day 16 of the initial curcumin treatment. An approximately threefold decrease in mean tumour volume was observed in the curcumin-treated group compared to the controls (Purkayastha et al., 2009).

COMBINATORIAL PREVENTION BY CURCUMIN

Curcumin has shown its effectiveness not only when administered alone, but also when combined with several other agents. A current study showed the effectiveness of a formulated product ‘Collect’ which consisted of curcumin, green tea and selenomethionine on colon cancer. ‘Collect’ was found to inhibit the growth of colon cancer cells, by induction of apoptosis and inhibition of aberrant crypt foci (ACF) development. Furthermore, it was found to augment the growth inhibitory effect of 5-acetyl salicylic acid (ASA) in vivo (Aroch et al., 2010).

The enhanced therapeutic efficacy as a result of improved BA after combining curcumin with piperine (Super curcumin) was reported by Shoba et al (Shoba et al., 1998). The turmeric extract in ‘Super Curcumin’ with BioPerine® (95% piperine) has been standardized to 95% curcuminoids and the latter are enhanced with the thermo-nutrient BioPerine® in order to ensure optimal quality, potency, and bioavailability (Shoba et al., 1998).

In a very recent study by Bishnoi et al., co-administration of curcumin (25 and 50 mg/kg, i.p., 21 days) with piperine significantly enhanced the effect of curcumin at a dose of 25 mg/kg and prevented all the behavioural, cellular, and neurochemical changes associated with the chronic administration of haloperidol (Bishnoi et al., 2010). In another study, oral pretreatment of curcumin (80 mg/kg b.w), piperine (50 mg/kg b.w), and curcumin (80 mg/kg b.w) + piperine (50 mg/kg b.w), respectively, for 5 days, significantly reduced the frequency of micronucleated polychromatic...
erythrocytes and the percentage of chromosomal aberrations in the bone marrow of hamsters. Their results suggested that cucumin and piperine in combination have a potent antigenotoxic effect as compared to either agent alone in DMBA-induced genotoxicity in golden Syrian hamsters (Balakrishnan et al., 2008).

A US Patent 20070093457, disclosed a composition comprising an effective amount of curcumin and at least one non-steroidal anti-inflammatory drugs (NSAID) for the treatment of cancer and inflammatory diseases and disorders. Main intent of the inventors was to reduce the dose of NSAID by simultaneous or step-wise administration of curcumin (Arber et al., 2007).

Topical application of curcumin (10 mmol) 3 times weekly to the buccal pouch of Syrian golden hamsters has demonstrated inhibition of DMBA-induced oral carcinogenesis (Li et al., 2002). In this study of ‘combinatorial chemoprevention’, the effect of topical curcumin appeared to be enhanced by the concomitant consumption of green tea (6 mg tea solids/ml) for 18 weeks (Li et al., 2002). Studies combining curcumin with other chemopreventive agents have also shown augmented growth inhibitory effects or cytotoxicity in in vitro cell lines (Aggarwal et al., 2005; Bava et al., 2005).

Interestingly, certain rodent studies have suggested a potential for curcumin to confound unwanted detrimental effects of cytotoxic anticancer drugs. For example, curcumin administered to rats by gavage (100 or 200 mg/kg daily for 7 days) ameliorated chromosomal mutations induced by cyclophosphamide in the bone marrow (Shukla et al., 2002).

Aggarwal and co-workers (Aggarwal et al., 2005) appraised the chemosensitising effect of curcumin in combination with paclitaxel on breast cancer metastases to the lung. Other researchers have examined the effects of curcumin on human breast cancer (MDA-MB-231) cells in an immunodeficient mouse model of metastasis and observed that the number of lung metastases significantly decreased after intercardiac injection of curcumin, thus providing a clear demonstration of curcumin as a promising agent for dietary chemoprevention of metastases (Bachmeier et al., 2007). Authors investigated the effect of curcumin alone and in combination, against several cancers and found that (i) the combination of curcumin
and gemcitabine inhibits pancreatic cancer growth in nude mice by inhibiting NF-kB-regulated gene expression, cell proliferation and angiogenesis (Kunnumakkara et al., 2007); (ii) the combination of curcumin and docetaxel is effective against human ovarian cancer in nude mice (Lin et al., 2007); (iii) curcumin can suppress the growth of glioblastoma in rodents (Aoki et al., 2007; Zanotto-Filho et al., 2011); and (iv) curcumin sensitises colon cancers in nude mice to oxaliplatin (Li et al., 2007a). In addition, other recent studies have shown that curcumin sensitises prostate cancers to chemotherapeutics and radiation by downregulating expression of the MDM2 oncogene (Li et al., 2007b). Together, these in vitro and vivo studies clearly suggest anticancer potential of curcumin, when administered either alone or in combination with currently employed chemotherapeutic agents or radiation.

PHARMACOKINETIC STUDIES IN ANIMALS

Wahlstrom and Blennow (Wahlstrom and Blennow, 1978) were the first to examine the uptake, distribution and excretion of curcumin in Sprague-Dawley rats. Negligible amounts of curcumin in blood plasma of rats after oral administration of 1 g/kg of curcumin showed that curcumin was poorly absorbed from the gut. Pan and co-workers (Pan et al., 1999) investigated the pharmacokinetic properties of curcumin in mice. They found that after intraperitoneal (i.p) administration of curcumin (0.1 g/kg) to mice, about 2.25 µg/ml of curcumin appeared in the plasma during the first 15 minutes. One hour after administration, the levels of curcumin in the intestine, spleen, liver and kidneys were 177, 26, 27 and 7.5 µg/g, respectively. Only traces (0.41 µg/g) were observed in the brain at 1 hour. Perkins and co-workers (Perkins et al., 2002) examined the pharmacokinetics of curcumin in a Min/+ mouse model of familial adenomatous polyposis (FAP) using either dietary curcumin or a single dose of radiolabelled curcumin given via i.p route and showed that irrespective of the dose, traces of curcumin were present in the plasma, which were at levels near the limit of detection (5 pmol/ml). A 10 mg/kg i.v dose of curcumin administered to rats gave a maximum serum curcumin level of 0.36 ± 0.05 µg/ml, whereas a 50-fold higher curcumin dose administered orally gave only 0.06 ± 0.01 µg/ml maximum serum level in rat (Yang et al., 2007). An oral curcumin dose of 1 g/kg in rats produced a maximum serum curcumin level of 0.5 µg/ml after 45 minutes of curcumin dosing (Murugan and Pari, 2006). While a maximum serum curcumin
concentration of 6.5 ± 4.5 nM was reached 0.5 hours after oral dosing of curcumin (Marczylo et al., 2007). More recently, curcumin (0.1 g/kg) administered to mice was found to undergo metabolic reduction to dihydrocurcumin and THC, which were further converted to monoglucuronide conjugates (Kumar et al., 2009a). These studies clearly suggest the effect of route of administration, on achievable serum levels of curcumin. Furthermore, it may be concluded that curcumin shows highly unreliable kinetics and the results of no two studies seem to match with one another. This could be due to variable sensitivity of the methods of analysis being employed, and also due to the highly unstable and fast metabolisable nature of curcumin, such that detection of both free curcumin and also its metabolites is difficult and unreliable.

Another study, evaluated the tissue distribution of curcumin using tritium-labelled drug. The study found that radioactivity was detectable in blood, liver and kidney following doses of 400, 80 or 10 mg of [3H] curcumin (Ravindranath, 1981). With 400 mg, considerable amounts of activity were detectable in tissues even 12 days after dosing (Ravindranath, 1981). The percentage of curcumin absorbed (60–66% of the given dose) remained constant regardless of the dose, indicating that administration of more curcumin does not result in higher absorption (Ravindranath and Chandrasekhara, 1980). It was also indicated that in rats, there is a dose-dependent limitation to bioavailability.

PHARMACOKINETIC STUDIES INCLUDING CLINICAL TRIALS IN HUMANS

The pharmacokinetic studies of curcumin in humans have shown a profile very similar to that observed in the animal studies, with some variations. Low achievable plasma and target tissue concentration of curcumin in most of the pilot studies are suggestive of its extensive metabolism (Aggarwal et al., 2006; Anand et al., 2007; Pan et al., 1999). In a phase I trial, plasma and urine concentrations of curcumin in patients who had ingested 3600 mg curcumin orally were 11.1 nmol/l and 1.3 μmol/l, respectively (Sompam et al., 2007). Curcumin concentrations in colorectal tissues of patients on this dose were 7.7–12.7 nmol/g, while levels in the liver were below the limit of detection (Sharma et al., 2007). In another study, peak plasma concentrations of curcumin 1–2 hours after oral dosing reached 0.41–1.75 μM in patients receiving 4–8 g curcumin (Sharma et al., 2001b).
A similar study in healthy human volunteers reported $C_{\text{max}}$ values of $2.30 \pm 0.26$ and $1.73 \pm 0.19$ µg/ml after 0.25–72 hours with 10 and 12 g doses, respectively (Vareed et al., 2008). However, healthy volunteers who ingested 2 g pure curcumin powder after fasting showed less than 10 ng/ml curcumin in their plasma 1 hour after intake (Shoba et al., 1998). In the same study, coingestion of curcumin with 20 mg of piperine appeared to increase the bioavailability of curcumin by 2000%.

In a study of oral curcumin, patients with preinvasive malignant or high-risk premalignant conditions of the bladder, skin, cervix, stomach or oral mucosa received 0.5–8.0 g curcumin by mouth daily for 3 months (Cheng et al., 2001). Plasma curcumin concentrations were found to peak 1–2 hours after intake and gradually declined within 12 hours. The 8 g/day dose resulted in a peak serum concentration of $1.75 \pm 0.80$ µM. When administered orally in micronised form with orange juice at doses of 50–200 mg to 18 healthy volunteers, no curcumin was found in the plasma at or above the limit of quantification (~0.63 ng/ml) (Ruffin et al., 2003).

In a clinical phase I dose escalation study using a standardised oral Curcuma extract comprising mainly of curcumin, doses up to 180 mg of curcumin per day were administered to patients with advanced colorectal cancer for up to 4 months, without having achieved any detectable systemic bioavailability or any observable toxicity (Sharma et al., 2001b). In a follow-up study in 15 patients with advanced colorectal cancer refractory to standard chemotherapy, curcumin in the form of ‘curcuminoids C3’ (Sabinsa Corp., - NJ 20 Lake Drive, East Windsor, NJ 08520, USA, 90% curcumin) was consumed orally for up to 4 months at doses between 0.45 and 3.6 g daily (Sharma et al., 2004). Oral consumption of 3.6 g of curcumin per day resulted in levels of drug and glucuronide/sulfate conjugates in plasma near the limit of detection (5 pmol/ml). Curcumin and its conjugates were also detected in 24 hours urine collections. In the six patients who had consumed 3.6 g curcumin. Urinary levels (in µM) varied between 0.1 and 1.3 for curcumin, between 0.019 and 0.045 for curcumin sulfate and between 0.21 and 0.51 for curcumin glucuronide (Sharma et al., 2004).
Exploratory studies have also been performed in patients undergoing surgical intervention for colorectal cancer who consented to have their tissues analysed for research purposes (Garcea et al., 2005; Kuttan et al., 1987). Twelve patients with confirmed colorectal cancer received oral curcumin at 0.45, 1.8 or 3.6 g/day for 7 days prior to surgery. The levels of agent-derived species were determined in blood and colorectal tissue obtained at the time of surgical resection. The mean concentrations of curcumin in normal and malignant colorectal tissue of patients who had ingested 3.6 g curcumin daily were 12.7 and 7.7 nmol/g tissue, respectively (Garcea et al., 2005). The studies further explored the pharmacology of curcumin administered in capsules at daily doses ranging from 0.45 to 3.6 g for up to 4 months. This time, the effect of curcumin on leucocytes was measured in terms of three potential biomarkers: glutathione S-transferase (GST) activity, malondialdehyde deoxyguanosine adduct levels and prostaglandin E2 (PGE2) production ex vivo. In a comparison of inducible PGE2 production immediately before and 1 hour after dosing on days 1 and 29, the highest dose (3.6 g) elicited significant decreases (62% and 57%, respectively). Consequently, the investigators chose the 3.6 g dose for further evaluation in a phase II trial in cancers outside the gastrointestinal tract. Curcumin sulfate and curcumin glucuronide were also found in the intestinal tissue taken from these patients however trace levels of curcumin were detected in peripheral blood samples.

Compatible with the preclinical data presented earlier, these preliminary results in humans suggest that a daily dose of 3.6 g curcumin achieves measurable levels in colorectal tissue with negligible distribution of the parent drug outside of the gut. When 12 patients with liver metastases from colorectal cancer received oral curcumin (0–3.6 g) daily for 7 days prior to hepatic surgery, curcumin was not found in liver tissue resected 6–7 hours after the last dose of curcumin, whereas trace levels of products of its metabolic reduction were detected (Garcea et al., 2005). Levels of curcumin and glucuronide and sulfate conjugates in the low-nanomolar range were found in blood samples taken 1 hour after the last dose. The results of this pilot study suggest that doses of oral curcumin required to produce hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans.
Curcumin has potential as palliative therapy for cancerous skin lesions. In a study by Kuttan and co-workers (Kuttan et al., 1987), curcumin efficacy was evaluated in 62 patients when it was applied as either an ethanol extract of turmeric or an ointment to external cancerous skin lesions. Regardless of the application, curcumin provided remarkable symptomatic relief that was in many cases relatively durable (lasting several months) and in all cases (except for a single adverse reaction in one subject) extremely safe.

Apparently, curcumin can also safely exert chemopreventive effects on premalignant lesions. In a prospective phase I dose escalation study, Cheng and co-workers examined the safety (Cheng et al., 2001), efficacy and pharmacokinetics of curcumin in 25 patients with a variety of high-risk precancerous lesions (recently resected urinary bladder cancer \( n = 2 \), arsenic Bowen’s disease of the skin \( n = 6 \), uterine cervical intraepithelial neoplasms (CIN) \( n = 4 \), oral leukoplakia \( n = 7 \) and intestinal metaplasia of the stomach \( n = 6 \). Curcumin was administered to the first three patients at a starting dose of 500 mg/day for 3 months and, if no grade 2 or higher toxicities were observed, the dose was successively increased to 1000, 2000, 4000, 8000 and finally 12,000 mg/day. Curcumin was found to be nontoxic at doses of 8000 mg/day or lower, reaching peak serum concentrations at 1–2 hours (0.51 ± 0.11 \( \mu \)M at 4000 mg, 0.63 ± 0.06 \( \mu \)M at 6000 mg and 1.77 ± 1.87 \( \mu \)M at 8000 mg) and being gradually eliminated (principally through nonurinary routes) within 12 hours. Although frank malignancies occurred despite curcumin treatment in one patient each with CIN and oral leukoplakia, a remarkable number of patients (i.e., one patient with recently resected bladder cancer, two with oral leukoplakia, one with intestinal metaplasia of the stomach, one with CIN and two with Bowen’s disease) showed histological improvement in precancerous lesions (Cheng et al., 2001).

At least 12 active clinical trials of curcumin are ongoing in the United States, Israel and Hong Kong. Curcumin is being used alone in most of these trials and in combination with quercetin or sulindac in one of the studies. Meanwhile, chemopreventive trials of curcumin in hepatocellular carcinoma, gastric cancer and colon cancer are ongoing in Japan. In United States, several randomised and nonrandomised phase I/II trials (http://www.Clinical-Trials.gov) are investigating its effects on a range of human malignancies (e.g., colorectal cancer, aberrant crypt
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foci, FAP, pancreatic cancer, multiple myeloma, Alzheimer’s disease, myelodysplastic syndrome (MDS) and psoriasis) when given alone or in conjunction with other natural substances or NSAIDs.

In a phase II study using unformulated curcumin, free curcumin was barely detectable in 19 patients who were administered 8 g of curcumin daily. However, a trend towards changes in blood markers was observed, suggesting that circulating plasma curcumin levels may not reflect tumour tissue curcumin levels (Lao et al., 2006). Two phase II trials are interrogating the effects of curcumin in advanced pancreatic cancers. An Israeli trial is investigating the combined effects of curcumin and gemcitabine in patients with advanced or metastatic adenocarcinomas of the pancreas, while an exploratory clinical trial in the United States is testing the efficacy of curcumin alone in patients with unresectable or metastatic pancreatic cancers (http://www.Clinical-Trials.gov).

Another Israeli clinical trial is investigating the clinical efficacy of curcumin alone or in combination with coenzyme Q_{10} in patients with MDS. At M.D. Anderson Cancer Center, a pilot trial of curcumin alone or in combination with bioperine (a black pepper extract) is under way in patients with asymptomatic multiple myeloma. In a recent study in healthy volunteers, the solid lipid curcumin particles (SLCP) demonstrated increased bioavailability of curcumin as compared to an unformulated 95% curcuminoids extract at 650 mg dose. To what degree the enhanced bioavailability is a result of increased absorption or is due to reduced conversion of free curcumin to conjugates (when incorporated into SLCP) is still not clear, because in this study the samples were not pretreated with glucuronidase. Various researchers report two to threefold increase in curcumin absorption by simply dissolving or mixing curcumin in different types of lipids (Liu et al., 2006; Maiti et al., 2007; Marczylo et al., 2007; Vareed et al., 2008) and formulations containing lipids and emulsifiers have shown significant effects in an in vivo colitis model (Yadav et al., 2009).

To summarise the data from pilot and phase I clinical studies performed with curcumin, it appears that low systemic bioavailability following oral dosing is consistent with the findings in preclinical models presented earlier. Efficient first-pass and some degree of intestinal metabolism of curcumin, particularly glucuronidation
and sulfation, might explain its poor systemic availability when administered via the oral route.

DOSE–EFFECT RELATIONSHIPS

As on today, there exists only scarce data on dose–response relationship for any biomarker/enzyme with pharmacological relevance to curcumin efficacy in humans or in animals. In a very recent study by Lin et al. on mice, mechanisms for the modulatory effects of low-dose and high-dose curcumin on morphine tolerance were discussed. The authors report that even though curcumin itself is a neuroprotectant and low doses of the compound serve to attenuate morphine tolerance, high-doses of curcumin might cause neurotoxicity and aggravate morphine tolerance by inhibiting the expression of anti-apoptotic cytokines and neuroprotective factors. They concluded saying that the effect of curcumin on morphine tolerance may be biphasic, and thus must be used cautiously (Lin et al., 2010). Furthermore, curcumin also shows toxic effects in in vitro studies, at high doses (Rattan, 2008). A single oral dose of 20 mg of curcumin appeared to induce contraction of the gall bladder as adjudged by ultrasound scanning in human volunteers, compared to an amylum placebo (Raysid and Lelo, 1999). High doses of curcumin used as food additive exhibited diarrhea and related side effects in healthy volunteers. Two potential surrogate biomarkers of the efficacy of curcumin were evaluated in the blood of patients with advanced colorectal cancer who received up to 180 mg of curcumin per day for up to 4 months (Plummer et al., 2001). In three patients on 36 mg of curcumin daily, lymphocytic activity of GST was decreased with time to reach 41% of control (untreated) on day 29 of treatment. This decline was not observed at the higher dose levels and was not reproduced in a subsequent study at higher doses in patients with the same disease. Similarly, oxidative DNA adduct, pyrimido-[1,2] purin-10(3H)-one-2-deoxyguanosine (M1dG) levels in the blood leucocyte, were not much affected by curcumin intake, although interesting observations were made regarding GST isoenzyme genotypes and baseline leukocytic M1dG adduct levels (Plummer et al., 1999; Zhang et al., 1999).

The effect of curcumin described in an ex vivo assay developed using blood from healthy volunteers was associated with a daily oral dose which furnished plasma levels in the 10–8 μM range in patients with advanced colorectal cancer.
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(Sharma et al., 2001a). This concentration of curcumin is less than a hundredth of that shown in vitro to elicit an effect in blood or colon cells (Ireson et al., 2001). Blood was taken immediately predose or 1 h postdose on days 1, 2, 8, and 29 of treatment with 3.6 g of curcumin daily (Sharma et al., 2004). Following the addition of acetylsalicylic acid (200 μM) to eliminate COX-1 activity, whole blood was incubated for 24 h in the presence (Plummer et al., 2001) of lipopolysaccharide (LPS, 10 μg/mL). In the same trial, oral administration of curcumin did not impact on nonstimulated PGE$_2$ levels in leukocytes, nor did doses of 0.45–1.8 g daily alter LPS-induced PGE$_2$. In contrast, consumption of 3.6 g of curcumin daily affected LPS-induced PGE$_2$ levels. When values obtained immediately predose or 1 h postdose on days 1, 2, 8, and 29 were pooled for the six patients consuming this dose, PGE$_2$ levels observed postdose were 46% lower than those measured immediately predosing. The difference reached significance on days 1 and 29 of treatment but not on day 2 or day 8; this discrepancy could not be explained scientifically by the study investigators (Sharma et al., 2004). Although these results tentatively suggest that consumption of 3.6 g of curcumin daily was linked with inhibition of PGE$_2$ induction in blood taken postdose compared to blood taken predose, overall time-dependent trends were not identified and dose–response was not demonstrated for this biomarker. It may be noted again that ex vivo assay levels showed high interindividual and high intraindividual variability in the PGE$_2$ levels (Plummer et al., 2001).

In parallel with studies in which potential changes in blood taken from patients with advanced colorectal cancer were analyzed, exploratory clinical investigations have also been performed in patients undergoing operations for resectable colorectal cancer in whom colorectal and hepatic tissues have been analyzed to study potential pharmacodynamic effects (Garcea et al., 2005; Garcea et al., 2004). Twelve patients with confirmed colorectal carcinoma received oral curcumin at 0.45, 1.8, or 3.6 g/day for 7 days prior to surgery. Ingestion of 3.6 g of curcumin daily for 1 week affected M1dG levels in patients’ colorectal tissue, it did not decrease COX-2 protein expression in this tissue (Garcea et al., 2004). Interestingly, M1dG adduct levels were 2.5-fold higher in malignant colorectal tissue than normal colorectal mucosa. Whereas administration of curcumin did not affect M1dG levels in normal colorectal mucosa, it caused a 58% decrease in adduct levels in malignant colorectal tissue. A
similar study of hepatic tissue with the same oral dosing regimen suggested that the levels of curcumin attained in normal and malignant liver tissues were insufficient to exert biological activity (Garcea et al., 2005). This variation in observations both with respect to variable tissue concentrations, pharmacological effects and indirect effects on suitably defined biomarkers with varying doses of curcumin, point towards a dose-dependent response of curcumin. Earlier we indicated dose-dependent limitation of curcumin to bioavailability.

SIGNIFICANCE OF CONCEPT OF NEUROHORMESIS

Hormesis is defined operationally as response of cells or organisms to an exogenous or intrinsic factor (chemical, temperature and psychological challenge) in which it induces stimulatory or beneficial effects at low doses and inhibitory or adverse effects at high doses. A range of examples of neurobiological processes and responses to environmental factors that exhibit biphasic-dose responses, the signature of hormesis is reported (Calabrese and Baldwin, 2002). ‘Neurohormesis’, is defined as the adaptive process by which neurons (and hence nervous systems and organisms) respond to a moderate level of stress by enhancing their ability to resist a more severe stress that might otherwise be lethal or cause dysfunction or disease.

Nerve cell networks are the “first responders” to environmental challenges—they perceive the challenge and orchestrate coordinated adaptive responses that typically involve autonomic, neuroendocrine, and behavioural changes (Arumugam et al., 2006). In addition to direct adaptive responses of neurons to environmental stressors, cells subjected to a stressor, produce and release molecules such as growth factors, cytokines, and hormones that alert adjacent and even distant cells to the impending danger. The discovery that some molecules (e.g., carbon monoxide and nitric oxide) and elements (e.g., selenium and iron) that are toxic at high doses play fundamental roles in cellular signaling or metabolism, suggesting that during evolution, organisms (and their nervous systems) co-opted environmental toxins and used them to their advantage. Neurons also respond adaptively to everyday stressors, including physical exercise, cognitive challenges, and dietary energy restriction, each of which activates pathways linked to the production of neurotrophic factors and cellular stress resistance proteins.
The development of interventions that activate hormetic signaling pathways in neurons is a promising new approach for the prevention and treatment of a range of neurological disorders, and is another key area where the role of curcumin is presently being explored. Recent findings have elucidated hormetic mechanisms of action of curcumin using cell culture and animal models of neurological disorders. Examples of hormesis pathways activated by phytochemicals include the transcription factor Nrf-2 which activates genes controlled by the antioxidant response element, and histone deacetylases of the sirtuin family and forkhead family of protein (FOXO) transcription factors. Such hormetic pathways stimulate the production of antioxidant enzymes, protein chaperones and neurotrophic factors. In several cases neurohormetic phytochemicals have been shown to suppress the disease process in animal models relevant to neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, and can also improve outcome following a stroke. Numerous examples of the hormetic response of curcumin are illustrated in literature. One of these discusses the protection provided by dietary supplementation with curcumin against synaptic dysfunction and cognitive deficits in a model of traumatic brain injury (Wu et al., 2006a). In another study, in a transgenic mouse model of Alzheimer's disease, dietary supplementation with curcumin (160–5000 ppm) decreased the accumulation of amyloid β-peptide, and markers of oxidative stress and inflammation in the cerebral cortex (Lim et al., 2001). Curcumin also exhibits anti-depressant-like effects in animal models of depression such as the forced swim test (Xu et al., 2005b). Curcumin treatment was also effective in reducing neuronal apoptosis and improving functional outcome in an animal model of stroke (Wang et al., 2005). Curcumin can directly protect cultured neurons against death induced by oxidative insults (Scapagnini et al., 2006). However, the typical expression of hormesis, the biphasic dose response, of curcumin has also been reported in a study by Ali and Rattan (Ali and Rattan, 2006). In this, they demonstrated that curcumin treatment (up to 1 μM for 24 h) increased chymotrypsin-like activity by 46% compared to that in untreated keratinocytes. However, higher concentrations of curcumin were inhibitory, and at 10 μM the proteasome activity decreased to 46% of its initial value. Furthermore, the preincubation of human keratinocytes at 43°C for 1 h, followed by 24-h treatment with 3 μM curcumin, led to
an increase in heat-shock protein (hsp70 and hsp90) levels by 24% and 19%, respectively, and the effect was sustained at concentrations up to 10 μM. On the other hand, the level of the small hsp27 was unaffected by curcumin concentrations of 0.3–1 μM, while it decreased by 34% at 10 μM. In other studies curcumin doses above 10 μM have been reported to have anti-inflammatory and anti-cancer effects in experimental studies (Moos et al., 2004; Rashmi et al., 2003), while at lower doses curcumin stimulates proteasome activity, enhances HSP induction after heat shock and stimulates sodium pump activity (Ali and Rattan, 2006; Rattan and Ali, 2007).

**THERAPEUTIC STRATEGIES FOR BIOAVAILABILITY ENHANCEMENT OF CURCUMIN**

Unfortunately, the poor solubility and absorption, rapid metabolism and elimination, and poor in vitro and in vivo stability of curcumin result in a poor bioavailability of this interesting polyphenolic compound. Probable ways explored by researchers to overcome these problems are discussed below (Figure 11). One of the major means that have been explored to improve the bioavailability of curcumin is use of adjuvant therapy. Traditionally, turmeric was delivered orally as a crude emulsion in oil or milk, conceivably because of the hydrophobic nature of its bioactive constituents such as curcumin and turmeric oil. In view of the traditional concept, nanoparticles, liposomes, micelles and phospholipid complexes of curcumin have been developed and found to provide better permeability and resistance to metabolic processes (Shishu and Maheshwari, 2010).

**Adjuvants**

One of the most commonly used adjuvant to improve bioavailability of curcumin, is piperine, which is a well-known inhibitor of hepatic and intestinal glucuronidation (Antony, 2008). Oral bioavailability of curcumin was enhanced in rats as well as humans when combined with piperine. In rats, 2 g/kg of curcumin alone produced a maximum serum curcumin level of 1.35 ± 0.23 μg/ml at 0.83 hours, whereas concomitant administration of piperine (20 mg/kg) led to 154% enhancement in the bioavailability of curcumin. However, undetectable or very low curcumin concentrations were observed in the serum after administration of 2 g
curcumin to humans. Simultaneous administration of piperine, however, pro-
2000% increase in bioavailability (Majeed et al., 1999; Shoba et al., 1998).

Another study showed 48% increase in brain uptake of curcumin within
5 minutes when combined with piperine. However, the uptake in other organs
was found to be significantly improved by piperine in this study, and this can be expl-
ained by the poor solubility of piperine in 10% ethanolic saline (injection medium us-
ed in the study) (Ryu et al., 2006). The glucuronidation-inhibiting effect of piperine
(Singh et al., 1998) and the established lesser activity of curcumin glucuronides
(Iresi et al., 2001) indicate that inhibition of glucuronidation by piperine may be the
mechanism by which piperine increases the bioavailability of curcumin.

The synergistic inhibitory effect of curcumin and genistein against pest
induced cell growth of oestrogen-dependent breast carcinoma cell lines (MCF-7)
also been reported (Verma et al., 1997).
Curcumin uptake by rat skin after topical application of a curcumin hydrogel demonstrated that eugenol and terpineol could enhance curcumin absorption by 2.2- and 2.5-fold, respectively, at 8 hours after topical application on rat skin. The above studies are suggestive of the fact that these adjuvants have the ability to enhance the bioavailability of curcumin (Fang et al., 2003). Another study indicates that epigallocatechin-3-gallate, a component of green tea, could counteract certain undesirable activities assigned to curcumin (Balasubramanian and Eckert, 2004) demonstrating that the activity of curcumin can be modulated by simultaneous administration of other agents.

**Self-microemulsifying drug delivery system**

Self-microemulsifying drug delivery system (SMEDDS) is emerging as one of the most interesting approaches to improve the solubility, dissolution and oral absorption of poorly water-soluble drugs (Zhang et al., 2008). SMEDDS is an isotropic mixture of oil, surfactant, cosurfactant and drug substance, which can form a microemulsion under the conditions of gastrointestinal fluid and gastrointestinal motility after oral administration. The resultant microemulsion with a particle size less than 100 nm and an improved solubility of hydrophobic drug(s) can enhance absorption across the gastrointestinal tract (Patel and Sawant, 2007).

Very recently, an improvement of 1213% in the oral bioavailability of curcumin is reported, after formulating it into SMEDDS as compared with curcumin suspension (Wu et al., 2011). A similar pharmacokinetic study in rats dosed with liquid and pelleted SMEDDS showed 14 and 10 fold increased absorption of curcumin in plasma as compared to the aqueous suspension of curcumin (Setthacheewakul et al., 2010).

Further, Cui and co-workers (Cui et al., 2009) reported that formation of curcumin-loaded SMEDDS enhances the absorption of curcumin not only due to spontaneous formation of a microemulsion in the gastrointestinal tract but also because the surfactants present therein may reduce the interfacial surface tension, thus enhancing penetration of curcumin into the epithelial cells. Furthermore, presence of lymphatic tissues such as Peyer’s patches and microfold cells in the rat
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Intestine combined with the fact that the microemulsion droplets produced from SMEDDS in the perfusion can be uptaken via the lymphatic tissues (Narang et al., 2007), absorption of curcumin via these lymphatic tissues cannot be ignored.

Liposomes, micelles and phospholipid complexes

Envisaging the suitable and promising properties of liposomes, a variety of studies of their use as a carrier system for curcumin have been documented specially in the alleviation of carcinogenesis in various models (Agashe et al., 2011; Li et al., 2007a; Li et al., 2005; Pandelidou et al., 2011).

Efficacy of nanoliposomes of curcumin to cross the BBB was illustrated very recently. Improved cellular uptake, of nanoliposomes (NLs) covalently coupled with monomer or tandem dimers of apolipoprotein E (ApoE)-derived peptides (residues 141-150), by cultured RBE4 brain capillary endothelial cells at various densities indicated the potential of the synthesized nanoliposomes for brain targeting (Re et al., 2011). Another study showed efficacy of the developed lyophilised liposomal curcumin against colorectal cancer cell lines in a short-term and in a long-term (clonogenic) assay. Their results demonstrated that liposomes improve the activity of curcumin especially in the long-term assay and the liposomal formulation was found to be more potent against HCT116 and HCT15, cell lines which express the MDR phenotype (Pandelidou et al., 2011). Still another study reported a novel curcuminoid 4-[3,5-bis(2-chlorobenzylidene-4-oxo-piperidine-1-yl)-4-oxo-2-butenoic acid] or CLEFMA formulated with cyclodextrins to result in a liposomal formulation and showed its higher efficacy as an anti-proliferative in lung adenocarcinoma H441 cells as compared to naturally occurring curcumin (Agashe et al., 2011).

In a study on preclinical anticancer activity of a liposomal curcumin formulation in colorectal cancer, the efficacy of liposomal curcumin was compared with that of oxaliplatin, a standard chemotherapeutic agent for colorectal cancer (Li et al., 2007a). A synergism was established between liposomal curcumin and oxaliplatin at a ratio of 4:1 in LoVo cells in vitro. Significantly better tumour growth inhibition was observed in Colo205 and LoVo xenografts, in vivo, with liposomal curcumin than that with oxaliplatin. The study established the comparable or greater
growth inhibitory and apoptotic effects of liposomal curcumin in relation to oxaliplatin both in vitro and in vivo in colorectal cancer (Li et al., 2007a). Earlier the authors demonstrated the efficacy of liposomal curcumin to inhibit pancreatic carcinoma growth in humans (Li et al., 2005).

Ruby and co-workers (Ruby et al., 1995) also reported the antitumour and antioxidant activities of neutral unilamellar liposomal curcuminoids in mice. Nevertheless, in vivo preclinical studies to establish the improved bioavailability of this liposomal curcumin over free curcumin are still warranted. Kunwar and co-workers (Kunwar et al., 2006) evaluated the in vitro cellular uptake of liposomal curcumin and albumin-loaded curcumin. They found that liposomal vehicle is capable of loading more curcumin into cells than either human serum albumin or aqueous DMSO, and lymphoma cells showed a preferential uptake of curcumin compared to lymphocytes.

In a recent study the pharmacokinetics of a liposomal encapsulated curcumin preparation in rats showed a much higher bioavailability and better absorption as compared to that of free curcumin (Takahashi et al., 2009).

Both the micellar and phospholipid complexes have the ability to improve the gastrointestinal absorption of natural drugs, leading to higher plasma levels and hence an improved bioavailability. The intestinal absorption of curcumin and micellar curcumin formulation with phospholipid and bile salt evaluated using an in vitro model consisting of everted rat intestinal sacs suggested its biological transformation during absorption. A very recent study reported on the formation of curcumin-loaded MPEG-PCL (Cur/MPEG-PCL) micelles. The study showed that encapsulation of curcumin within MPEG-PCL micelles improved the half life and area under curve (AUC) of curcumin in vivo. Further, inhibition of angiogenesis by Cur/MPEG-PCL micelles on transgenic zebrafish model was also illustrated. Cur/MPEG-PCL micelles were also established as excellent aqueous system for i.v administration with an ability to inhibit the growth of colon carcinoma by inhibition of angiogenesis and direct killing of cancer cells (Gou et al., 2011). Another study revealed that the in vitro intestinal absorption of curcumin increased from 47% to 56% when the micellar formulation was used (Suresh and Srinivasan, 2007). In a pharmacokinetic study by
Ma and co-workers (Ma et al., 2007), a 60-fold higher biological half-life for curcumin was achieved with polymeric micellar curcumin in rats as compared to curcumin solubilised in a mixture of dimethylacetamide (DMA), polyethylene glycol (PEG) and dextrose. Phospholipid complex formulations of several natural drugs, such as silymarin (Kuttan et al., 1987) and dolichol (Kimura et al., 1989), have been found to show improved bioavailability. Liu and co-workers (Liu et al., 2006), demonstrated a significant improvement in curcumin bioavailability after oral administration of curcumin-phospholipid complex. They showed that curcumin-phospholipid complexes (corresponding to 100 mg/kg of curcumin) could achieved a plasma curcumin level of 600 ng/ml at 2.33 hours after oral administration as opposed to that of free curcumin (100 mg/kg) which showed a plasma concentration of 267 ng/ml only after 1.62 hours of oral dosing. About a 1.5-fold increase in curcumin half-life in rats was found in this study for the curcumin-phospholipid complex over free curcumin. The above studies indicate that the curcumin-phospholipid complex can significantly increase the circulating levels of presumably active curcumin in rats (Liu et al., 2006). Similar study by Maiti and co-workers (Maiti et al., 2007) showed a threefold increase in aqueous solubility and a better hepatoprotective effect of a curcumin-phospholipid complex compared to free curcumin. In an attempt to increase the aqueous solubility of hydrophobic drugs, Letchford and co-workers (Letchford et al., 2007) showed a $13 \times 10^5$-fold increase in curcumin solubility in a polymeric micellar formulation containing methoxy poly(ethylene glycol)-block-polycaprolactone diblock copolymers. The enormous increase in the solubility of curcumin in the above-said micelle makes it a promising formulation to be explored further.

A US patent Application 20090131373 disclosed that the phospholipid complexes of curcumin, prepared in protic solvents provide a higher systemic level of curcumin than unformulated curcumin (Giori and Franceschi, 2009).

**Nanoparticulate delivery system(s)**

Nanoparticle-based delivery systems are known to be suitable for highly hydrophobic agents like curcumin, circumventing the pitfalls of poor aqueous
solubility. This approach has been used to deliver natural products such as coenzyme Q\textsubscript{10} (Gao et al., 2005), estradiol (Mittal et al., 2011) and ellagic acid (Sonaje et al., 2007) and chemotherapeutic agents such as paclitaxel (Dong et al., 2009) and doxorubicin (Azarmi et al., 2006). Very recently Tsai and co-workers showed that curcumin when formulated into nanoparticles showed a 22-fold increase in oral bioavailability as compared to conventional curcumin when measured in rat plasma (Tsai et al., 2011).

In another study, PLGA loaded curcumin nanoparticles have been shown to result in a two times higher bioavailability than free curcumin in plasma of mice administered curcumin nanoparticles at a dose of 2.5 mg/kg (Anand et al., 2010). Furthermore, silk fibroin-derived curcumin nanoparticles have been reported to exhibit higher efficacy against breast cancer cells (Gupta et al., 2009b). Same authors also describe a biodegradable curcumin nanoparticulate formulation based on poly(lactide-co-glycolide) (PLGA) and a stabiliser PEG-5000 that exhibits enhanced cellular uptake and increased bioactivity in vitro and superior bioavailability in vivo in comparison to free curcumin (Gupta et al., 2009b).

A pharmacokinetic study performed with the nanoparticulate curcumin showed a mean curcumin concentration of 25 µg/ml in serum after 1 h of nanoparticulate curcumin administration in mice (i.v; 4 mg/ml). While serum availability of native curcumin administered at the same dose was as low as 0.02 µg/ml (Mohanty and Sahoo, 2010). A study by Bisht and co-workers (Bisht et al., 2007) reported the synthesis, physicochemical characterisation and cancer-related application of a polymer-based nanoparticle of curcumin called ‘nanocurcumin’ with less than 100 nm size. Nanocurcumin showed in vitro activities similar to that of free curcumin in pancreatic cell lines. Free curcumin and nanocurcumin also inhibited the activation of transcription factor NF-κB, and reduced the steady-state levels of proinflammatory cytokines, IL and TNF-α. However, the authors neither determined the in vivo effect of nanocurcumin in mice nor its biodistribution to show any potential increase in the efficacy of nanocurcumin over free curcumin in vivo. In another study, curcumin-loaded PLGA nanoparticles were prepared by emulsion-diffusion-
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evaporation method (Shaikh et al., 2009). The authors found a significant increase (p ≤ 0.001) in the AUC value of the nanoparticulate formulation in comparison to free curcumin suspension and curcumin with piperine, indicating the improved bioavailability with the nanoparticulate systems. A comparison of relative bioavailability exemplifies 26 times enhancement in the bioavailability of nanoparticulate formulation in comparison to free drug. In furtherance with the above, they also report T_{max} to be 2 hours with a sustained release attained up to 48 hours in plasma in comparison to free curcumin, where there were no detectable levels beyond 6 hours (Shaikh et al., 2009).

SLN loaded with curcuminoids for topical application have also been developed and characterised (Tiyaboonchai et al., 2007). In fact, there was no improvement in the in vitro release profile of curcumin from SLN in comparison to free curcumin (70% release in 12 hours by SLN versus 90% release in 8 hours by pure curcumin). However, the light and oxygen sensitivity of curcuminoids was markedly reduced by incorporating curcuminoids into this unique type of formulation. An in vivo study with healthy volunteers revealed improved efficiency of a topical application cream containing curcuminoid-loaded SLN over that containing free curcuminoids (Tiyaboonchai et al., 2007).

Transferrin-mediated solid lipid nanoparticles of curcumin (Tf-C-SLN) have been formulated recently to increase the photostability, and enhance the anticancer activity of curcumin against MCF-7 breast cancer cells (Mulik et al., 2010). The results indicated a considerable increase in cytotoxicity, ROS and cell uptake with Tf-C-SLN compared to curcumin solubilized surfactant solution (CSSS) and curcumin-loaded SLN (C-SLN) suggesting the targeting effect. Flow cytometric studies revealed an enhanced anticancer activity with Tf-C-SLN as compared to CSSS and C-SLN. Study indicates the potential of Tf-C-SLN in enhancing the anticancer effect of curcumin in breast cancer cells in vitro (Mulik et al., 2010).
SESAMOL

Sesame seeds (*Sesamum indicum*, Linn, Pedaliaceae) have long been categorized as a traditional health food in Japan and other East Asian countries. They were employed in the ancient Chinese medicine as energy boosters and to prevent ageing (Budowski, 1950; Charak, 1981; Fukuda, 1994). The core of the multifaceted aspects of sesamum species is its antioxidant status. The composition of sesame seed indicates the presence of antioxidants like lignophenols, carboxyphenols, bisepoxy lignans like sesamin (Figure 12) and sesamolin, and water-soluble lignan glycosides (sesaminol triglucoside and sesaminol diglucoside) in addition to 50% oil and 25% proteins. Sesamol (Figure 13) is present in traces in sesame oil but is readily formed by the hydrolysis of sesamolin present naturally in the oil (Fukudu et al., 1994). This natural antioxidant is responsible for the long shelf life of sesame oil. Sesame seeds are prone to rancidity but not the oil as it has the natural preservative, sesamol. Other lipid-soluble antioxidants isolated from sesame seeds, include sesaminol (Figure 14) (Katsuzaki et al., 1994), sesamolinol (Osawa et al., 1985), p1 (Katsuzaki et al., 1993) and pinoresinol (Figure 15) (Katsuzaki et al., 1992). Sesamin, a major lignan in sesame seeds has recently been reported to act as one of the major precursor of mammalian lignans (Liu et al., 2006). It is converted by intestinal microflora to enterolactone (Figure 16) which possess estrogenic activity (José et al., 2005).

Sesame oil is used in cooking, soaps, pharmaceuticals, paints, etc. However, large doses of the oil can cause abortion and obesity. Several studies have shown that sesamol can act as a metabolic regulator and possesses chemopreventive, antimitogenic, hepatoprotective and anti-ageing properties (Kapadia et al., 2002; Kaur and Saini, 2000; Sharma and Kaur, 2006). Sesamol was found to be a very effective inhibitor of lipid peroxidation of rat liver microsomes (Uchida et al., 1996). Ingestion of sesame seeds has also been reported to benefit postmenopausal women by improving blood lipids, antioxidant status, and possibly sex hormone status (Wu et al., 2006b). The authors indicate that these effects are probably due to the presence of sesamin in the sesame seeds.
Chemically, sesamol is 3,4-methylenedioxyphenol (Figure 13). It is sparingly soluble in water and is posted under the category of irritant. An oral bioavailability of 35.5 ± 8.5 % is reported for sesamol in Sprague Dawley rats. Conjugated metabolites of sesamol are widely distributed in rat tissues, with the highest concentrations in the liver and kidneys and the lowest in the brain (Jan et al., 2008).

‘Open sesame’

Sesame (Sesamum indicum Linn.) belongs to family pedaliaciae and is one of the oldest cultivated plants in the world. It was a hugely prized oil crop of Babylon
and Assyria at least 4000 years ago. Today, India and China are the world’s producers of sesame. Upon ripening, sesame capsules split, releasing the hence the famous phrase, “OPEN SESAME” (Figure 17). We however rephrase sentence to open research on sesame in terms of developing and establishing an important antioxidant obtained from this plant for its therapeutic various disorders ranging from inflammation, neuroprotection and arthritis.

Figure 17. Photograph of plant *Sesamum indicum*

**PHARMACOLOGY OF SESAMOL**

Sesamol is a very effective inhibitor of lipid peroxidation of rat liver microsomes (Osawa et al., 1987) and rat brain homogenates (Thiraviam 2009). It has been shown by us to exhibit antimutagenic activity against *Salmonella typhimurium* TA100 and TA102: Latter was attributed to its antioxidant activity (Kaur and Saini, 2000).

Sesamol has also been found to exert chemopreventive effect in the skin two-stage carcinogenesis and the Epstein-Barr virus early antigen ac induced by the tumor promoter 12-O-tetradecanoylphorbol 13-acetate Further, anti-cancer activity of sesamol was also evaluated in the brine cytotoxicity assay as well as on the stable 1,1-diphenyl-2-picrylhydrazyl (DPP
radical scavenging bioassay. The in vivo assay results showed that, sesamol offered 50% reduction in mouse skin papillomas at 20 weeks after promotion with TPA. Further, sesamol was found to show remarkable cytotoxic activity in the brine shrimp lethality assays as well as profound free radical scavenging activity in the DPPH bioassay at the highest concentrations tested (4000 μg/ml). Thus, sesamol was established to be a potent chemopreventive agent (Kapadia et al., 2002).

Effects of sesamol in atherosclerosis have been reported recently. A modified form of sesamol; INV-403 was found to improve vascular function, reduce systemic and plaque oxidative stress, and inhibit NF-κB activation. Additionally, the in vitro experiments in cultured endothelial cells revealed effects of INV-403 in reducing IκBα phosphorylation via inhibition of IκB kinase 2 (IKK2) (Ying et al., 2010).

Effect of sesamol on the lipopolysaccharide (LPS)-induced inflammatory response is also reported. Sesamol was found to inhibit serum TNF-α, IL-1β and nitrite production in LPS-treated mice, and inhibited LPS-induced inducible nitric oxide synthase (i-nos) expression in mouse leukocytes. Further, sesamol also downregulated TNF-α, IL-1β, and nitrite production as well as i-nos expression in LPS-treated RAW 264.7 cells. In addition, inhibition of LPS-induced NF-κB translocation and inhibitor (I)κB-α phosphorylation in RAW 264.7 cells by sesamol was also reported. The authors assigned the involvement of the above discussed mechanisms of sesamol to result in down regulation of LPS-initiated inflammatory response (Chu et al., 2010). The same group of researchers had previously reported attenuation of septic hypotension by alternating cytokine production by peroxisome proliferator-activated receptor (PPAR) activation after the onset of systemic inflammatory response by sesamol. The LPS-associated blood pressure decrease was inhibited by administration of sesamol. Further, it was also found to decrease the LPS-induced TNFα and IL-1β production and enhanced the IL-10 production in serum and the PPAR activation in white blood cells after the onset of systemic inflammation (Chu et al., 2009).

In another study, sesamol was found to dose-dependently inhibit LPS from binding to LPS binding protein. It significantly decreased the release of TNF-α and IL-1β in LPS-challenged peritoneal macrophages in in vitro medium and in the serum.
of LPS-challenged rats and also significantly reduced the mortality rate in mice given a lethal dose of LPS. From the above observations it was concluded by the authors that sesamol can be an effective agent against endotoxemia induced mortality (Hsu et al., 2009).

Efficacy of sesamol in osteoarthritis (OA) was shown in a very recent report. Sesamol significantly attenuated TNF-α- and IL-1β-induced gelatinolysis and expression of matrix metalloproteinases (MMP)-9 in a concentration-dependent manner in SW1353 cells. Furthermore, both MMP 1 and 13 stimulated by phorbol myristate acetate (PMA) were inhibited by sesamol. On the other hand, the NF-κB signaling activation through IκB-α degradation was restored by sesamol under TNF-α or PMA stimulation. Furthermore, this bioactive compound exerted the reduction on phosphorylation of ERK1/2 or p38 MAPKs after either PMA or IL-1β stimulation. The study also showed that sesamol attenuates destructive factor expression in vivo, providing a potential strategy for the chondroprotective therapy in OA (Lu et al., 2011).

Sesamol has been reported to inhibit the excessive production of nitric oxide in the lipopolysaccharide/gamma-interferon stimulated C6 astrocyte cells (Soliman and Mazzio, 1998). It also inhibited the formation of mutagenic/carcinogenic imidazoquinoline type heterocyclic amines through the unstable free radical Maillard intermediates (Kato et al., 1996). Inhibition of the development of pre-neoplastic hepatocytic foci formation in F344 rats by sesamol is also reported (Hagiwara et al., 1996). Sesamol showed protective effect against carbon tetrachloride (CCl₄) - induced liver injury in rats (Ohta et al., 1994).

Photoprotective effect of sesamol on UVB-radiation induced oxidative stress in human blood lymphocytes with increasing concentration of sesamol (1, 5 and 10 μg/ml) has been reported (Prasada et al., 2005). The study showed increased lipid peroxidation and disturbance in antioxidant defense in UVB-irradiated lymphocytes. However, pretreatment with sesamol resulted in significant reduction in thiobarbituric acid reactive substances (TBARS) a marker of lipid peroxidation. Also, a dose-dependent increase in antioxidants like reduced glutathione (GSH), superoxide
dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was observed in sesamol pretreated UVB-irradiated lymphocytes. The authors concluded that 10 µg/ml dose of sesamol was effective to result in the normalization of the UVB induced lipid peroxidation, indicating the photoprotective effect of sesamol in irradiated lymphocytes (Prasad et al., 2005). A study in our lab highlights anti-ageing properties of sesamol. Biochemical and histopathological investigations confirmed the effectness of sesamol in preventing photodamage (lesions, ulcers and changes in skin integrity) due to chronic UV exposure (Sharma and Kaur, 2006). The radioprotective ability of sesamol at various doses viz., 0, 10, 25, 40, 50, 70 and 100 mg/kg body weight (bw), administered intraperitoneally 30 min prior to 9.5 Gy whole-body gamma-irradiation was reported in Swiss albino mice. The study showed alleviation of the radiation-induced decrease in endogenous antioxidant enzymes (GSH, GST, catalase) and the increase in lipid peroxidation by pretreatment with sesamol [50 and 100 mg/kg bw] at all the monitored post-irradiation intervals however no protection was observed at a dose less than 25 mg/kg bw (Parihar et al., 2006).

Neuroprotective role of sesamol has been explored in numerous studies in the literature. A very recent study showed the protective effect of oral administration of sesamol (20 mg/kg bw) on aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and oxidative stress biomarkers in the brain tissue of normal and experimental animals. Glioma induced rats showed a significant increase in the activities of various enzymes and TBARS. The levels of ascorbic acid, tocopherol, reduced glutathione and activities of superoxide dismutase, catalase, glutathione peroxidase and Na⁺-K⁺ ATPase were significantly decreased in the brain tissues. Treatment with sesamol restored the above mentioned changes in glioma induced rats. These preliminary findings were suggestive of neuroprotective effect of sesamol against glioma induced changes in the rat brain (Narasimhan et al., 2011).

Another study evaluated the potential of sesamol treatment against 3-nitropropionic acid (3-NP)-induced cognitive impairment and oxidative damage in striatal, cortex and hippocampal regions of the rat. 3-NP significantly impaired
memory performance as assessed in Morris water maze and elevated plus maze and induced oxidative stress, which was significantly attenuated by sesamol (5, 10 and 20 mg/kg) pre-treatment (Kumar et al., 2010). Neuroprotective role of sesamol was also shown in an earlier study (Hou et al., 2006).

In another study, increased BBB permeability observed in streptozotocin (STZ) induced diabetic rats was attenuated by sesamol (S) post-treatment (VanGilder et al., 2009). In this study, sesamol, was administered to streptozotocin (STZ)-induced diabetic rats to examine the role that oxidative stress plays on BBB structure and function. Experiments were conducted at 56 days after STZ injection. Results of the study demonstrated that the increased BBB permeability observed in STZ-induced diabetic rats was attenuated in STZ + S rats to levels observed in control. Further, treatment with sesamol reduced the negative impact of STZ-induced diabetes on tight junction protein expression in isolated cerebral microvessels. Oxidative stress markers were elevated in STZ treated rats as compared to the control group. STZ + S displayed an improved antioxidant capacity which led to a reduced expression of superoxide and peroxynitrite and reduced lipid peroxidation. In conclusion, their study showed that sesamol treatment enhanced antioxidant capacity of the diabetic brain and led to decreased perturbation of hyperglycemia-induced changes in BBB structure and function. Another study showed the effect of sesamol on thermal and mechanical hyperalgesia, allodynia, oxidative-nitrosative stress, inflammation, and apoptosis in streptozotocin (STZ)-induced experimental diabetes. Chronic treatment with sesamol (2, 4, and 8 mg/kg body weight; po) for 4 weeks starting from the 4th week of STZ injection significantly attenuated behavioural, biochemical, and molecular changes associated with diabetic neuropathy. Further, it was also reported that diabetic rats treated with the combination of insulin-sesamol produced more pronounced beneficial effect as compared to treatment with insulin alone. The major finding of the study was the cumulative effect of combination of sesamol with insulin which not only attenuated the diabetic condition but also reversed associated neuropathic pain (Chopra et al., 2010).
Role of sesamol in Huntington’s disease is also reported. It was shown that sesamol (5, 10, and 20 mg/kg) pre-treatment significantly improved body weight, locomotor activity, motor coordination, and attenuated oxidative damage in different regions of rat brain. Significant improvement in mitochondrial enzymes in all regions of the brain after treatment with sesamol was also observed (Kumar et al., 2009).

A very recent study revealed the role of pre-treatment with sesamol in alleviating Alzheimer’s disease like symptoms. The authors reported a marked improvement in cognitive impairment, as a result of reduced acetylcholinesterase activity, TNF-α levels and attenuation of oxidative-nitric stress in brain of intracerebroventricular (i.c.v.)-streptozotocin treated rats. They demonstrated that administration of l-arginine (125 mg/kg i.p.), a nitric oxide donor, alone to i.c.v.-streptozotocin treated rats accentuated behavioral and biochemical deficits and also abolished the protective effect of sesamol (8 mg/kg). I-NAME (N(G)-nitro-l-arginine methyl ester); (10 mg/kg i.p.), a non-specific NOS inhibitor significantly restored all the behavioral and biochemical indices in i.c.v.-streptozotocin rats. Moreover, combination of I-NAME with sub-effective dose of sesamol (4 mg/kg) potentiated its protective effect. Their findings demonstrated the effectiveness of sesamol in preventing i.c.v. streptozotocin-induced cognitive deficits by modulating nitrergic signaling and oxido-inflammatory cascade (Misra et al., 2011). Earlier the same group of authors reported on the therapeutic potential of sesamol in diabetes-associated cognitive decline in rats. They elaborated that chronic treatment with sesamol (2, 4 and 8 mg/kg; p.o.) significantly and dose-dependently attenuated cognitive deficit, reduced acetylcholinesterase, oxidative stress and inflammation in diabetic rats (Kuhad and Chopra, 2008).

Still another study investigated the renoprotective potential of sesamol. They demonstrated that pretreatment with sesamol significantly reduced the serum creatinine and blood urea nitrogen levels, lipid peroxidation, restored levels of reduced glutathione and increased total renal nitric oxide levels. Further, it also attenuated the increased TNF-α levels and restored the normal morphology of the kidneys thus highlighting the renoprotective potential of sesamol in oxidative renal pathologies (Gupta et al., 2009a).