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REVIEW

Nanosuspensions: a new approach for organ and cellular targeting in infectious diseases

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Abstract Most of the infectious diseases depending upon the stage of infection need treatment at least for a month to several years e.g. tuberculosis, acquired immunodeficiency syndrome. Medication by oral administration is of choice in all treatments, but unfortunately use of large doses resulting from drug poor solubility and bioavailability leads to toxicity. The problem of solubility should be solved in easiest way to reduce dose and to increase bioavailability thereby decreasing the unnecessary exposure of other organs than the targeted one without increasing the cost of treatment. Nanosuspensions offer a simple, easy and cost effective solution to solve all above issues, without exposing body to extra drug dose. They can also serve as industrially relevant formulation approach by overcoming problems associated with other drug delivery systems like low entrapment, polymer toxicity, biocompatibility and stability issues. In this paper, applications of nanosuspensions are discussed in detail focusing particularly treatment of infectious diseases. Furthermore, potential of them in targeting at organ, cellular and subcellular levels is debated.

Keywords Poor solubility · Nanosuspensions · Bioavailability · Infectious diseases · Cellular and organ targeting · Intracellular drug delivery

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Introduction

Nanosuspensions as delivery system

Delivery of the drug to the target site at right time to achieve desired therapeutic response remains as benchmark in designing novel drug delivery systems. Nanocarriers are increasingly investigated for their potential in treatment of various diseases with reduced side effects. Nanocarriers (50–1,000 nm) because of their higher ratio of surface area to volume improve pharmacokinetic and biodistribution pattern. They allow surface modification to allow specific targeting, fast release or extended release properties (Davis et al. 1986; Panagiotou and Fisher 2011).

It takes almost 10–20 years for chemical entity to enter into market and almost $803–810 million. It is very disappointing when most active compounds resulting from high throughput screening, combinatorial chemistry and proteomics are highly lipophilic (Müller and Akkar 2004). Poor solubility may hinder drug effectiveness and alter biodistribution pattern independent of route of administration. As a result, the pharmaceutical companies have to spend lot of time, effort and money to improve solubility and increase bioavailability. Lipophilic nature of active helps in transport across biological membranes and play crucial role in exerting biological activity. Thus, newly developed drugs and some marketed drugs are poorly soluble in aqueous and most of the time simultaneously in organic media creating major obstacle to their formulation development and eventually in their clinical application. Approximately 40 % of the drugs on the market exhibit poor solubility; in addition, around 50 % of all new molecular entities (NME's) shows some degree of polymorphism, sometimes giving rise to significant variation in

bioavailability between different polymorphic forms. The sales of molecules with sub optimal (<50 %) oral bioavailability and poor water solubility accounted for approximately $57 billion in 2000 (Fig. 1). Sometime poor bioavailability issues compel formulator to opt for intravenous route instead of the oral route or to abandon the project during development itself. Several factors other than particle size like crystalline structure, surface area, degree of hydration of crystal, amount of surfactant used, presence special coating, etc. affect the bioavailability and ultimately its therapeutic performance.

Various techniques have been applied to overcome solubility problems like solubilization in surfactant solution use of cosolvents, pH adjustment and complexation (Ali et al. 2009; Kesisoglou et al. 2007; Patravale et al. 2004). Among other solubilization techniques, melt extrusion technology has been used for improving the performance of various drugs. However, large amount of additives limit their use in overcoming solubility issue concerning of safety. Whereas amount of drug included in cyclodextrins is limited by the volume of the cyclodextrin complex (Rabinow 2004). An alternative in attempts to overcome these obstacles is the formulation of the drugs as nanosuspensions. Nanosuspensions are sub-micron colloidal dispersions of discrete particles in presence of surfactants, polymers or a mixture of both. The nanosuspensions can be used to formulate compounds that are insoluble in water, oils and organic media. Nanosuspensions can be produced using two types of technique (i) bottom-up processes (controlled precipitation/crystallization) and (ii) top-down processes (nanonizing).

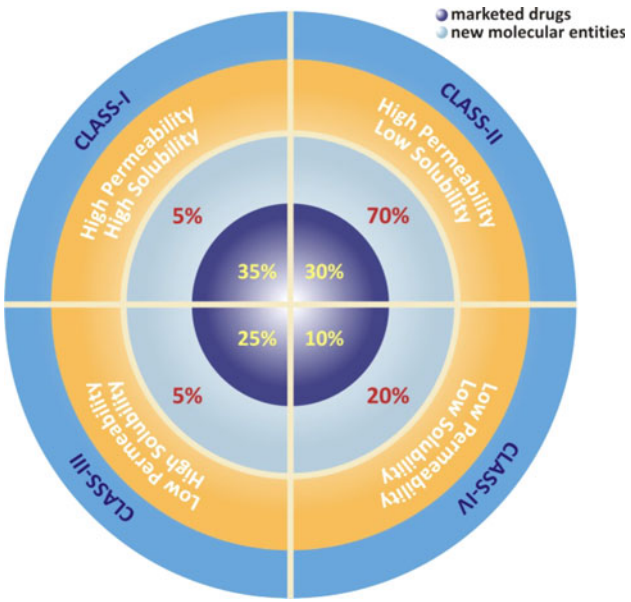

Fig. 1 Biopharmaceutical classification of poorly soluble drugs in proportion of marketed and upcoming new molecular entities

Production of nanosuspensions

Two basic approaches are involved in production of nanocrystals, large-size drug powder either to be reduced in size, e.g. by mechanical attrition or by precipitating the drug in presence of solvent-anti solvent. However, the combination techniques, combining a pre-treatment with a subsequent size reduction step are also being employed (Arunkumar et al. 2009).

Bottom-up—precipitation methods

Sucker developed the "hydrosols" (List and Sucker 1988; Suker and Gassmann 1994). This technology is based on classical precipitation principle where drug is dissolved in an organic solvent and subsequently precipitated by mixing with a non-solvent resulting in production of crystalline drug nanoparticles. Strict control of production process, avoidance of crystal growth, drug solubility issues in organic solvent and solvent residues are major limitations. Complexity of process hinders the clinical application of this technology. Auweter et al. (1998), developed amorphous nanoparticles of the active. Theoretically, a particle being in the nano range and at the same time amorphous is suitable, highest increase in saturation solubility can be seen (Auweter et al. 2000). However, a risk of re-crystallization is of critical concern. Other bottom-up process includes controlled crystallization during freeze drying (de Waard et al. 2008, 2009).

Top-down technologies

Bead/pearl milling The NanoCrystal® technology uses a bead milling to achieve particle size reduction (Liversidge et al. 1992). Mixture of milling media, dispersion medium (generally aqueous surfactant solution) along with drug is milled. Shear forces generated by the movement of the milling beads leads to breaking of particle ultimately size reduction. Depending upon the need, smaller or larger coated milling pearls of ceramics (cerium or yttrium stabilized zirconium dioxide), stainless steel, glass or highly crosslinked polystyrene resin-coated beads can be selected. Erosion of milling bead during the milling process is a well known problem of this technology. The milling time can last several days. Scaling up of nanosuspensions with a pearl mill is possible, but there is a certain limitation in size of the mill due to its heavy weight. The NanoCrystal® technology has successfully expanded the use of nanosuspensions for oral, inhalation, intravenous, subcutaneous and ocular delivery (Merisko-Liversidge and Liversidge 2008). This is an important technology and can be produced at industrial batch size and five FDA-approved products support this.

High pressure homogenization It includes three different processes viz. microfluidizer technology (IDD-P™ technology) (Haynes 1992), piston-gap homogenization either in water (Dissocubes® technology) (Müller et al. 1992) or alternatively in water-reduced/non-aqueous media (Nanopure® technology) (Müller et al. 2001).

The Microfluidizer technology is based on the jet stream principle which leads to particle collision, shear forces and cavitation forces because of frontal collision of two fluid streams in a Y-type or Z-type chamber under pressure. Relatively high numbers of cycles (50–100 passes) are required to obtain desired particle size reduction (Keck and Müller 2006).

The Dissocubes® technology was developed by Müller and co-workers by employing piston-gap homogenizers (e.g. APV Gaulin/Rannie homogenizers) later acquired by SkyePharma. In this technique, a drug dispersed in an aqueous surfactant solution is forced by a piston under pressure (typically 1,500–2,000 bar) through a tiny gap (e.g. 5–20 μm). The resulting high streaming velocity of the suspension causes an increase in the dynamic pressure. This is compensated by a reduction in the static pressure below the vapour pressure of the aqueous phase; hence water starts boiling forming gas bubbles. When these gas bubbles collapse the liquid leaves the homogenization gap. The mean size of bulk population obtained depends on the homogenizer pressure, number of homogenization cycles and hardness of drug (Müller et al. 2000). The Nanopure® technology uses the piston-gap homogenizer for production of nanocrystals in non-aqueous liquids, like oils, melted PEG, or water reduced media (e.g. glycerol–water, ethanol–water mixtures), which are subjected to homogenization at low temperatures.

Combination technologies The NanoEdge™ technology uses precipitation step followed by subsequent annealing step of high pressure homogenization (Kipp et al. 2001). Theoretically, the annealing step prevents the growth of the precipitated nanocrystals. A biggest disadvantage of this technique is the use of organic solvents. In case of large scale production relatively large amounts of solvent need to be removed which makes production more tricky and expensive. The smartCrystal® technology is a club of various combination processes. Special feature of the processes like H69 and H96 is the ability to produce crystals <100 nm (a range practically not accessible by high pressure homogenization alone). Spray-drying or lyophilization of the drug solution leads to a powder more susceptible to be broken in the subsequent high pressure homogenization step. The smartCrystal® technology is considered as the second generation of drug nanocrystals (Keck et al. 2008).

Unique features of nanocrystals

As a result of diminution of particle (surface area ↑) saturation solubility increases and dissolution velocity which eventually results in bioavailability increase (Müller et al. 2000; Shegokar and Müller 2010). Similar to other nanoparticles (Ponchel et al. 1997), nanosuspensions also show an increased degree of adhesiveness to mucosal membranes and other tissues. Nanosuspensions may induce changes in the crystalline structure, increasing the amorphous fraction in the particle or even forming amorphous particles (Kocbek et al. 2006). Special feature of this outstanding nanonization technology is easiness during formulation preparation and during production. The size reduction leads to an increased surface area and thus according to the Noyes–Whitney equation (Noyes and Whitney 1897) to an increased dissolution velocity. This increase can also be explained by Kelvin equation. A low dissolution velocity always results in low saturation solubility. Saturation solubility is a function of the particle size, it increases with decreasing particle size <1000 nm. The increased saturation solubility may result in increased concentration gradient between gut lumen and blood, consequently higher absorption. For *i.v.* administration, nanocrystals should possess size as small as possible so that pharmacokinetics of a solution is mimicked. For targeting drug to specific site in body e.g. to the brain or to other organs/tissues, the drug nanocrystals should possess a certain size to delay or to enhance dissolution. The particle size optimization gives chance to reach the blood–brain barrier (BBB) for internalization by the endothelial cells of the BBB targets in the body or other target (Kreuter et al. 1995).

Decrease in particle size shows increase in adhesiveness to surface as well. There is a distinct increase in adhesiveness of ultra fine particles compared to coarse powders (Stieß 1995). This adhesiveness of small drug nanoparticles can be exploited for improved local, oral and dermal delivery of poorly soluble drugs. Adhesive property can be further improved by coating the surface of nanocrystals with polymers, like chitosan and Carbopol. Another special feature of nanosuspensions is the absence of Ostwald ripening because of uniform particle size created by the homogenization/milling process ensures its long term physical stability as an aqueous suspension. Nanocrystals can be surface modified either to increase mucoadhesive nanosuspensions for oral application or with selective surfactant/polymer for site-specific targeting after intravenous injection (e.g. targeting to the brain, bone marrow etc.). This innovative technology can be combined with traditional dosage forms, e.g. pellets or tablets. For further reading readers are requested to refer other interesting reviews published (Müller et al. 2011; Peltonen and

Hirvonen 2010; Van den Mooter et al. 2008; Zhang et al. 2008).

The scope of this review is to explore applications of nanocrystal or nanosuspension in chemotherapy of infectious diseases. The production details of nanosuspension technology and the recent developments in treatment and prevention of infectious diseases focusing individual examples of significant interest are discussed.

Organ and cellular targeting in infectious diseases

Infectious diseases

According to world health organization, Infectious diseases are any disease which is spread through contact with infected person or through air (miasmatic) and caused by pathogenic microorganisms, e.g. bacteria, viruses, parasites or fungi. Gromashevsky classification divides infectious diseases according to location of infection in body due to microbes into intestinal infections, blood infections, respiratory infections and skin infections. Each is further classified as anthroponoses and zoonoses e.g. in intestinal classification group of anthroponoses mainly include infectious like bacterial infections and amoebic dysentery, cholera, viral hepatitis A. Typhoid fever, poliomyelitis, helminthiasis, while brucellosis, leptospirosis, salmonellosis, botulism, etc. are classified under heading of zoonoses. Four of the top ten causes of death in world are infectious diseases like HIV/AIDS, malaria, tuberculosis, influenza and diarrhea. Infectious pathogens can be transmitted by mucosal route which include, respiratory, GI (e.g. cholera, shigella, salmonella, *E. coli*. etc.), ocular (e.g. *Chlamydia trachomatis* infection) and urogenital linings, or through blood or cutaneous route vector transmitted diseases like leishmaniasis, dengue and malaria enter in host. Figure 2 shows classification of diseases according to causative agent.

Targeted drug delivery

In various viral (Marsh and Helenius 2006) and non viral infectious diseases drug targets are localized in cellular and subcellular (e.g. cytosol, endosomes, lysosomes, Golgi complex, mitochondria, nucleus, endoplasmic reticulum) levels (Gruenberg and van der Goot 2006). Beside extensive research in field of nanotechnology only few reports are available for cellular or intracellular targeting and mainly highlights that the drug targeting to these sites is really challenging. Majority of research work is focused on enhancing bioavailability or organ targeting. In general, drug delivery systems are developed to overcome formulation hurdles but to achieve efficient therapeutic response it is important to be aware of the type of the drug, site of action, and targeting required at organ or at complex cellular level. In this case, surface modification or ligand binding can facilitate transport of drug across cell organelles e.g. plasma membrane is ideal target for delivering enfuvirtide (HIV fusion inhibitor) for which surface modification of carrier system is ideal. Targeting plasma membrane is promising as HIV attacks raft domains at membrane to enter in T cell, newly published study showed that HIV virus fusion occurs in endosomes which is membrane bound vesicle formed by cleft in plasma membrane during endocytosis process (Miyauchi et al. 2009), this suggests that endosomes could serve as best target (Sheff 2004) for fusion inhibitors (Ingenito et al. 2008) and in protozoal diseases (Ferguson 2000; Murphy et al. 2006). Viruses and pathogen often use raft induced budding by making use of lipid and protein components in the raft to enter into cells by activating clustering of raft components. Several anticancer drugs have been successfully targeted to cellular components using surface ligand like transferrin, folate, Vitamin B12 and lipoproteins which has affinity towards cognate receptors (which are overexpressed in malignant tumors). Another important subcellular target in infectious disease is mitochondria; it was found that viral (HIV) RNA preferentially located in mitochondria in relatively high concentrations to that of cytoplasm and nucleus (Somasundaran et al. 1994) (Table 1, 10).

Detailed understanding of this regulation mechanism is important for formulation scientist to pave the way for the movement of developed nanoparticles in the body without accumulating at undesired sites. It is also equally important to understand the blood flow rates and volumes of various organ and tissues. It is well known that cellular uptake is greater for nanoparticles compared to microparticles (Desai et al. 1997; Kanni 2006). This uptake is also governed by the type and the location of tissue. The drug can cross blood capillaries and other cell layers either by transcellular or paracellular route. The tight junctions mostly control the paracellular transport for e.g. the diffusion of large molecules may not be feasible, but the migration of white cells is allowed. Formulation development would be more effective when developed keeping in mind ultimate target organ and basic cellular mechanism involved. In general, lipophilic drugs which exhibit a high passive transcellular transport across the intestinal epithelial cell membranes may also exhibit comparative transport across sinusoidal canalicular cell membranes of hepatocytes. A biological membrane has tens of several kinds of transporter molecules. Sufficient knowledge of the "target" and related cellular processes and transporters might help in achieving the desired drug levels at the site of action. Generally most of the target sites are accessible through either microcirculation by blood capillaries or pores present at various surfaces and membranes. For passive drug

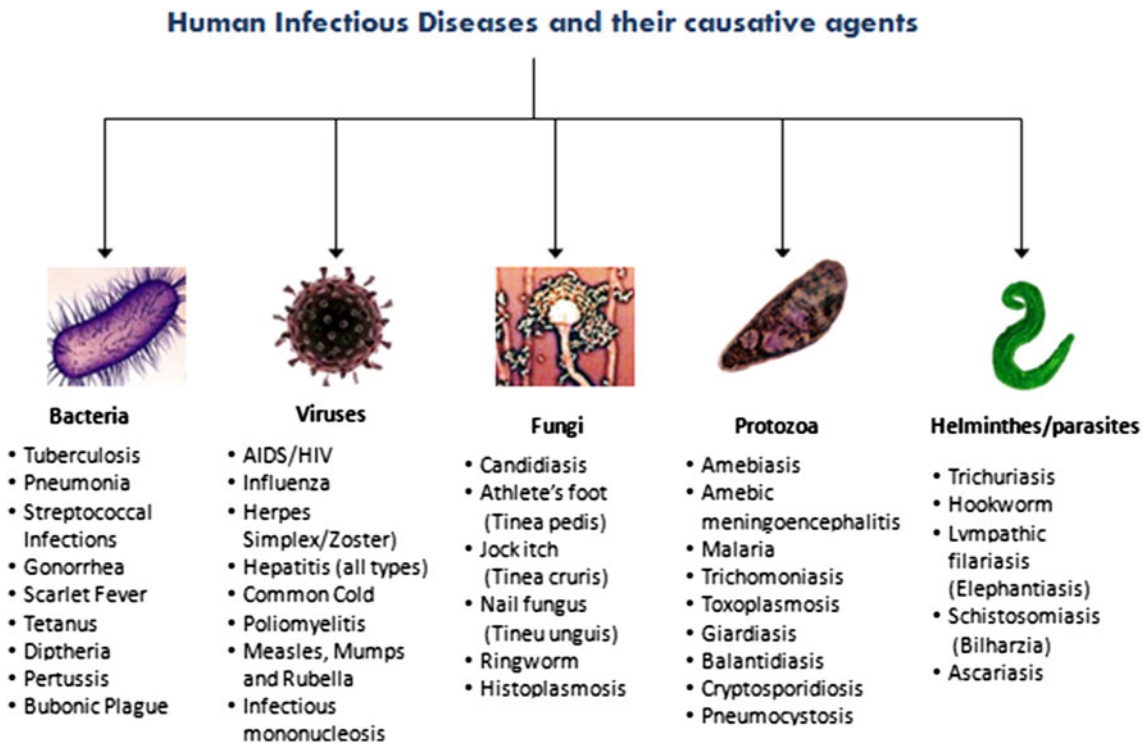

Fig. 2 Classification of infectious diseases based on their causative agents. The common infectious diseases include TB, pneumonia, HIV/AIDS, malaria, influenza, hepatitis, candidiasis and lymphatic filiariasis

delivery, the physicochemical properties and geometric features of drug formulations are crucial parameters. In this case, nanoparticles remain in the vasculature when they are narrower than 2,000 nm and for the effective transport the recommended size must be smaller than 300 nm.

Nanotechnology has made noteworthy developments including targeting of drug nanoparticles to specific cells, tissues and crossing of BBB. They are also used as imaging agent for diagnosis of cancer and cardiovascular diseases. Other developments include carbon nanotubes, gene delivery using nanoparticles, and the use of quantum dots in elucidating gene trafficking. Nanoparticles represent a potential approach for targeting disease site at altered drug levels by reducing frequency of administration, time of treatment and adverse reaction. On the other hand, nanosuspensions provide us a unique tool for formulation of drugs that comes under BCS class II and IV. Since harsh chemicals and solvents are usually avoided during nanoparticle processing, the risk of toxicity from these sources is typically not a concern. Poorly soluble drugs need more than 20 mg/kg/day dose in order to achieve a therapeutic response in humans. In infectious diseases like HIV/AIDS and tuberculosis, it is more important to cure the disease or extend the life span than patient compliance. Various techniques are used to increase solubility such as prodrug approach, salt synthesis, use of surfactant, oily dispersions, complexation, solid dispersions, spraying drying and hot melt extrusion etc. (Garad 2004). Various drug delivery systems were studied for the effective delivery of anti-microbial drugs in different diseases (Zhang et al. 2010a). In some infectious diseases, dual targeting is desired i.e. inert compound (e.g. excipient) has activity in addition to drug and attacks at multiple target sites simultaneously. Some practical applications of cellular carriers include drug loaded RBCs, macrophages, lymphocytes for treating diseases.

In following sections, four major infectious diseases are discussed one by one in detail, focusing mainly on their present mode of treatment and the research done using nanosuspensions as carrier system.

HIV/AIDS infection and chemotherapy

AIDS is a deadly infectious disease caused by a lentivirus called human immunodeficiency virus (HIV, 100–150 nm). Open lifestyle, lack of education, insufficient medical facilities and poverty are the major reasons for the spread of this disease mainly in younger population. An estimated, 40 million people worldwide are living with HIV/AIDS and 24.8 million people have died of AIDS. Around 60,000 people are becoming infected each day and 68 million will die of AIDS by 2020. It is stated that around 32.2 million people are infected worldwide in 2010 (http://www.avert.org/worldstats.htm). Presently two types of HIV viruses are known, HIV-1 (most prevalent) and HIV-2 (less prevalent and has only 40 % genetic identity with HIV-1).

Table 1 Lists different delivery systems studied for selective drugs mainly used in infectious diseases

Drugs	Delivery systems	Target disease/infection caused by
Doxorubicin	Liposome	*Candida* spp., *Aspergillus fusarirum*
Amphoterecin B		Fungal infections, Visceral leishmaniasis
Polymyxin B		*P. aeroginosa* (pneumonia)
Ampicillin		*Salmonella typhimurium*, *Micrococcus luteus*
Benzyl penicillin		*Staphylococcus aureus*
Ciprofloxacin		*Salmonella dublin*
Gentamicin		*Brucella* spp., *Klebsiella pneumoniae*, *Mycobacterim avium*
Streptomycin		
Vancomycin/teicoplanin		Methicillin resistant *Staphylococcus aureus*
Netilmicin		*Bacillus subtilis*, *E. coli*
Amikacin		Gram negative bacteria
Zidovudine, stavudine, didanosine, indinavir, zalcitabine		HIV/AIDS
Primaquine, quinine, choroquine, artemether artesunate		*Plasmodium falciparum*
Pentosam, glucantime, paromomycin		Leishmaniasis
Benznidazol, etanidazole, nitroemidazole		Chagas disease
Gliadin	Polymeric nanoparticles	*Helicobacter pylori*
Ciprofloxacin		*Staphylococcus*, *Bacillus anthracis*
Amphoterecin B arjunglucoside		*Leishmania denovani*, *Candida albicans*, *Leishmania denovani*
Rifampicin, isoniazid, pyrazinamide, ethambutol		*Staphylococcus aureus*, *Mycobacterium avium* and *M. tuberculosis*
Ampicillin		*Listeria monocytogenes*
Saquinavir		HIV/AIDS
Primaquin and ampicillin		*Leishmania donovani*, *Salmonella typhae*
Primaquine		*Plasmodium falciparum*
Zidovudine, lamvudine		HIV/AIDS
Nifurtimox, nitroimidazole, allopurinol, benznidazole		Chagas disease
Tobramycin	SLN/lipid nanocapsules/lipid drug conjugates	*Pseudomonas aeruginosa*
Rifampicin, isoniazid, pyrazinamide		*Mycobacterium tuberculosis*
Ciprofloxacin hydrochloride		Gram −ve and +ve bacteria, mycoplasma
Clotrimazole		Fungal infections caused by yeast, aspergilla dermatophytes
Ketoconazol, miconazol nitrate, econazole nitrate		Fungal infections
Quinine dihydrochloride		Malaria (cerebral malaria)
Artemether		Malaria (*P. bulgheri*)
Atazavir, stavudine, delaviridine, squinavir, indinavir		HIV/AIDS
Suramin, pentacarinat, melarsoprol, ornidyl		African trypanosomiasis
Amphotericin		

Table 1 continued

Drugs	Delivery systems	Target disease/infection caused by
Sulfamethoxazole	Dendrimers	*Streptococcus*, *Staphylococcus aureus*, *Haemophilus influenza*
Niclosamide		Tapeworm
Silver salts, nadifloxacin, prulifloxacin		Bacterial infections (gram −ve, gram +ve)
Artemether		*Plasmodium falciparum*
Chloriquin phosphate		
Efavirez, Lamvudine		HIV/AIDS
Dihydroartemisinin	Nanosuspension	*Plasmodium falciparum*
Indinavir, rilpivirine, loviride		HIV/AIDS
Nevirapine		HIV/AIDS
Halofantrine	Nanocapsules	*Plasmodium falciparum*
Quinine		
Triclosan		
Zidovudine		HIV/AIDS
Benznidazole, itraconazole, albaconazole		Chagas disease
Rifampicin		Tuberculosis
Ritonavir	Micelles	HIV/AIDS
Efavirenz		HIV/AIDS
Linolenic and linoleic acids		Malaria/*Plasmodium berghei*
Hydroxymethylpyrazinamide, isoniazid and rifampin		Tuberculosis
5-Nitro-2-furfurilylidene benzhydrazide		Chagas disease

HIV is best known for infecting the T cells and macrophages of the immune system. It is estimated that nearly 99 % of viral replications occur in CD4 cells and lymphoid tissues (Blankson et al. 2002; Pomerantz 2002; Turriziani et al. 2010), this means macrophages are of major concern as lymphocytes composes only 2 % in circulation at a particular time. The half life of infected and virus replicating CD4 + T cells is approximately 1–2 days, while virus can be present in reservoirs up to several years. Other potential tissue reservoirs of HIV exist mainly as anatomical sanctuaries and a small pool of infected long-lived memory T lymphocytes. HIV-1 latency in long-lived cell populations e.g. memory T lymphocytes, macrophages (in the brain, kidney, liver, lungs, GALT, lymph nodes, genital organs, thymus and spleen), bone marrow, dendritic cells and follicular cells poses a hurdle to eradication because the current antiviral combination treatments fail to eliminate the integrated proviruses from the resting cells. These reservoir sites protect the virus for a prolonged period in active or latent form, thereby saving it from immune reactions and biological elimination pathways. A viral replication in macrophages and T cells, and viral persistence in several tissue compartments, such as the CNS e.g. cells like mononuclear and perivascular macrophages, microglia are not readily accessible to current therapies. HIV virus enters the CNS via the blood cerebro-spinal fluid barrier or by crossing the blood brain barrier (Trojan horse approach) which results in several neurological complications like AIDS dementia and mania. The risk and severity of these complications is higher in paediatric patients (Gendelman et al. 1997; Gonzalez-Scarano and Martin-Garcia 2005). For the effective treatment of AIDS dementia complex and viral suppression (Kasongo et al. 2011), it is very important for the antiretroviral drugs to reach the CNS by crossing the BBB and/or BCSFB at sufficient concentration by taking advantage of specific surface processes like solute and membrane transport, receptor mediated endocytosis and paracellular transport. A number of approaches can be used to achieve this aim, like drug property modification, pharmacological disruption of BBB, inhibition of ABC transporters (e.g. p-glycoprotein, multiple isoforms of MRP, ABCG2), hyper osmotic opening of BBB, ultrasound or microbubble approach, change in route of administration, and efficient nanoparticulate drug delivery (surface modified with cell penetrating peptides, tuftsin, ApoE)(Wong et al. 2010). It has been also kept in mind that the delivery of substances from blood to the brain is mainly dependent upon molecular size, lipid solubility, binding capacity to specific transporters and surface charge (Panagiotou and Fisher 2011).

Various drugs from nucleoside reverse transcriptase inhibitors (NRTI's) e.g. zidovudine, didanosine and stavudine, non-nucleoside reverse transcriptase inhibitors (NNRTI'S) e.g. nevirapine, efavirez and delavirdine, protease inhibitors (PI) e.g. sequinavir, ritonavir, lopinavir and indinavir, integrase inhibitor, entry (fusion) inhibitors e.g. enfuvirtide, and other inhibitor includes e.g. dicaffeoyltartic acid, L-chicoric acid, nonapeptoid CGP 64222, zinc figure inhibitor and budding inhibitor are used in HIV chemotherapy (Table 2). A combination approach (combing two or three classes) of highly active antiretroviral therapy (HAART), is used to overcome bioavailability and drug resistance issues and increasing clinical efficiency (Temesgen et al. 2006). Over the time it was realised that the continuation of HAART is insufficient to achieve viral suppression besides that it results in severe side effects, liver dysfunction, multi drug resistance, drug–drug interaction and other metabolic complications (Este and Cihlar 2010). Other factors affecting insufficient drug therapy is due to extensive first pass metabolism, short half life, gastric degradation, large dose administration, poor solubility of drugs, cytotoxicity etc. Carrier systems like liposomes, bioconjugates, dendrimers polymeric carriers, micelles and nanoparticles have been studied (Gupta and Jain 2010). Either direct targeting to cells by intravenous administration, exploring lymphatic, systemic or cerebrospinal circulation for effective reservoir targeting (das Neves et al. 2010). Some scientists have even explored the transdermal route for targeting using liposomes. Superior levels of zidovudine encapsulated liposomes were observed in plasma and RES organs compared to hydrophilic ointment in rats (Jain et al. 2008). Microbicide approach to target HIV mucosal sites is widely studied (du Toit et al. 2010; Shegokar and Singh 2007). One successful example is VivaGel, a dendrimer-based microbicide gel which is in clinical trials.

Application of nanosuspensions in HIV chemotherapy is in the introductory stage. Nanosuspensions (50–1,000 nm) are easily engulfed by the host cell, where they can release the drug to kill the virus by interrupting various intracellular infections. This can be achieved either by passive or active targeting. Nanocrystals can easily escape bioelimination processes if engineered wisely. They can also be produced in variable sizes depending on the target cells e.g. for macrophages (300–600 nm), or intracellular delivery (100–200 nm). Coating of nanocrystals with pH dependant polymers, ligands or proteins offers great potential for site specific targeting. To our knowledge only a handful of reports are published for the delivery of antiretrovirals using nanocrystals.

Shegokar et al. (2011b) prepared nevirapine nanosuspensions to target potent cellular and anatomical HIV reservoirs. Aqueous nanosuspensions were prepared by HPH at 1,500 bar to get a mean particle size of 460 nm. Nevirapine solubility in water was increased by four folds. The developed formulation showed excellent plasma compatibility when tested in vitro. Phagocytic uptake studies revealed significant accumulation in rat peritoneal macrophages. Furthermore, the surface of nanosuspension was modified using polysaccharide, serum albumin and polyethylene glycol by simple physical adsorption at 1 % w/w concentration. In vitro adsorption of plasma proteins

Table 2 List of drugs available in market and presently used in chemotherapy of HIV/AIDS

Drugs	Company	Dose	Solubility	BCS class
Anti-retrovirals				
Efavirenz	Bristol Myers Squibb, Gilead	50–200	0.0099	II
Lamvudine	–	100–150	33	III
Nevirapine	Boehringer Ingelheim, Aurobindo, Prinston Inc., Mylan Labs	200	0.0099	II
Abacavir	VIIV healthcare, Mylan Pharms Inc.	20–600	22.89	III
Didanosine	Aurobindo Pharma, Barr, Matrix Labs Ltd.	25–100	10	III
Stavudine	Hetero Drugs, Aurobindo Pharma, Cipla Ltd., Mylan	15–40	83	III
Tenofovir	Gilead Sciences	40–300	13	III
Zidovudine	Ranbaxy, Roxane, Hetero Drugs Ltd., Cipla Ltd., Teva Pharms	100–300	10	III
Acyclovir	Watson Labs, Apotex Inc., Dava Pharms Inc., Ranbaxy, Baxter Hlthcare, Dr Reddys Labs Ltd., Wockhardt, Sandoz	200–800	1	III (WHO) IV (UK/JP)
Ribavirin	Zydus Pharms USA, Sandoz, Three Rivers Pharms	200	33	III
Indinavir	Merck Sharp Dohme Ltd., Cipla Ltd., Aurobind, Ranbaxy, Genixpharma	200–400	0.0149	II
Lopinavir	Abbvie, Aurobindo Pharma	130	0.0099	II
Nelfinavir	Agouron, Cipla Ltd.	250–625	0.0099	II
Ritonavir	Abbvie, Ranbaxy, Dr. Reddys	30–100	0.0099	II
Saquinavir	Hoffmann La Roche, Cipla Ltd.	200	1	I

on nanocrystal surface was studied using Pathfinder® technology which enables to predict plasma protein adsorption pattern in vitro and thereby predicting in vivo distribution. Protein accumulation study on surface nanocrystals showed adsorption of various immunoglobulins (Shegokar and Singh 2011a). The results obtained from cellular uptake studies and 2D PAGE protein adsorption pattern collectively showed that developed formulation has promise for targeting MPS rich organs. The amount of drug accumulated inside macrophages of nanocrystals was significantly higher for polysaccharide and albumin coated nanocrystals than that of uncoated nanosuspension. Cytotoxicity studies conducted in murine macrophages confirmed a dose dependant increase in toxicity. In vivo distribution of technetium labelled bare and surface modified formulations was studied using gamma imaging and tissue distribution analysis in rats. As predicted, the uncoated formulation was significantly accumulated in spleen, liver, lung and heart which represent potential HIV reservoirs. The radioactivity was comparatively higher for albumin coated nanosuspension followed by polysaccharide coated nanosuspension than that of uncoated nevirapine nanosuspension. Only albumin coated nanosuspension showed accumulation in brain exhibited the promise in treating brain dementia. Toxicity studies, both acute and repeated dose toxicity showed formulation is safe to administer even at doubled therapeutic dose without any severe toxicity profile (Shegokar et al. 2011c). After successful in vitro and in vivo results, scale up studies was carried out for nanosuspension using bead milling (Bühler PML-2, Bühler AG, Switzerland) and high pressure homogenization (Avestin C50, 2 kg). The particle size obtained was similar to that of lab scale production, while milling resulted in superior diminution of crystals to get a particle size of 150 nm (Shegokar and Singh 2011b). X-ray diffractograms showed that the particles are still crystalline in nature. Short and long term stability studies revealed significant increase in particle size for samples stored at room temperature and refrigeration, but at 40 °C, particle sizes were found to be stable. However, lyophilization was recommended to ensure stability of the formulation (Shegokar and Singh 2012). This study shows the successful use of simple easy, scalable nanosuspension approach for targeting potential cellular and organ HIV reservoirs.

In another separate work, loviride (NNRTI) which acts by blocking the action of HIV's reverse transcriptase. Nanosuspensions of loviride was produced at lab scale using milling techniques, a mean particle size of 264 ± 14 nm was obtained after 4 h of milling. Loviride milled nanosuspension showed higher dissolution profiles when compared with coarse powder. Freeze drying in the presence of sucrose as cryoprotectant was done to obtain loviride nanopowder of a size 560-590 nm. In an experiment conducted on Caco-2 cell at end of 120 min, loviride nanopowder showed superior drug amount transported to cells (1.59 ± 0.02 μg) as compared to the physical mixture which contained sucrose and untreated drug (0.93 ± 0.01 μg) and the coarse drug (0.74 ± 0.03 μg) (Van Eerdenbrugh et al. 2007). Attempts were also made for down scaling of loviride and other compounds (Van Eerdenbrugh et al. 2009).

In other study, Indinavir (IND) nanosuspension (1.6 μm) stabilized with lipoid E80 was prepared using HPH. Nanosuspensions were tested in monocyte-derived macrophages (MDM) pre infected by HIV-1ADA. Indinavir nanosuspension showed comparative suppression profile of 99 % as that of native drug (indinavir sulfate) of 97 % when analysed by reverse transcriptase activity, in contrast to cells treated as control which demonstrated cytopathic effects when tested by MTT assay. Prepared IND-nanosuspensions were further loaded into BMM cells (bone marrow derived macrophages). Nanosuspensions were co-cultured with BMM cells for 12 h and administered intravenously to mice. Single photon emission computed tomography imaging, HPLC analysis and histology observations together exhibited higher drug loaded BMM cell and drug distribution. After two weeks post administration, indinavir levels in tissues like lung, liver, spleen, and kidney were ≥50 μM and also similar levels were found in sera (Dou et al. 2006).

An attempt was made to develop depot nanosuspensions formulation of rilpivirine also called TMC278. Drug nanosuspension (200 nm) was administered via intramuscular or subcutaneous route to rats and dogs. A dose-dependent sustained release of TMC278 over 2 and 6 months in rats and dogs, respectively was observed. Initial higher peak plasma levels and faster wash-out of drug was observed for both animals after intramuscular administration while stable plasma-concentration profile for more than 6 weeks in dogs after subcutaneous administration was noted. After the IM injection, TMC278 levels in the lymph nodes exceeded the plasma levels by more than 100-fold at the end of 30 days (van't Klooster et al. 2008). This shows the potential of nanosuspension to target HIV virus in reservoirs likes lymph nodes and free virus circulating in blood.

Nanosuspension approach was used to improve the suboptimal bioavailability of BMS-488043 by using media milling. The polystyrene beads 500 μm were used as milling media. Spray drying and flash evaporation were employed to prepare amorphous intermediates. In vivo pharmacokinetic study was conducted for tablet (micronized BMS-488043) and capsules (containing amorphous drug or nanosuspension) in beagle dogs. A 4.7 fold increase in C_{max} and 4.6 fold in AUC_{0-24} were observed for crystalline nanosuspension in fasted state. The AUC_{0-24} of nanonized drug was comparatively lower than that of amorphous coprecipitates (Fakes et al. 2009).

The chemotherapy of HIV/AIDS is very multifaceted involving long dosing regimens which contains mainly combination therapy. The costs, drug resistance, serious side effects, poor drug biodistribution, and variable pharmacokinetic patterns are observed for most of the antiretroviral drugs beside their poor solubility (Amiji et al. 2006). Therefore, an effective targeted drug delivery is desirable which can be cleared easily from the circulation, able to recognize target, reach in time at target site in ample concentration, able to deliver (multiple) drug in a controlled manner and cost effect. Nanosuspensions offer lots of advantages for the delivery of antiretrovirals by overcoming their poor solubility and thereby reducing dose. This could be achieved by engineering or modifying the surface of the carrier system (Gunaseelan et al. 2010). These systems are still in initial developments, surface modification with suitable ligand could achieve organ specific delivery. Surface coating of nanosuspensions with pH sensitive polymer can facilitates the activation of enzymes in lysozyme for cargo degradation. Almost 40 different hydrolytic enzymes are present in lysozyme which mediates controlled degradation of macromolecules intracellularly. Encapsulation of nanocrystals with suitable signalling material might facilitate cellular uptake to the desired destinations. Being discrete particles, they are easily engulfed by fluid phase endocytosis while larger ones are phagocytosed and delivered to phagosomes. The irregularities of particles (nanometer length scale) surface can play an important role in adhesion. In case of nanosuspension, this property could be explored for drug release in particular segment of GI track. Nanosuspensions can deliver much larger amounts of drug in a smaller volume than any other drug delivery systems. The mucoadhesive nature of nanosuspensions can be useful for the mechanical filtration occurring in lung and can be explored for new route of drug delivery.

Malaria

Malaria still remains a global problem particularly in sub Saharan Africa and other many parts of Asia. Malaria infection is commonly accompanied by coma, multi organ failure, severe anaemia and metabolic acidosis (Buffet et al. 2011; Engwerda et al. 2005; Totino et al. 2010). *Plasmodium falciparum* majorly causes 95 % of malaria deaths with high mortality rate of 1–3 %, due to the development of drug resistant by strains. In human, malarial infection starts with a bite of Anopheles mosquitoes infecting sporozoites (*P. falciparum* and *P. vivax*). These sporozoites replicate rapidly in hepatocytes forming merozoits which are then released into the circulation. These merozoits further enter and multiply within erythrocytes at approx. rate of four fold per day infecting large population of RBCs within short time. Finally the sexual forms of the parasite are ingested in the blood of Anopheles as a bite of the infected person, this chain completes the cycle of malaria parasite.

Spleen plays an important role in malarial infection, as parasitized RBCs are removed by blood circulation and undergo breakdown in spleen. Spleen is the main lymphoid organ and a blood filtration unit, white pulp of spleen plays a vital role in the recirculation of immunocompetent lymphocytes. The reticular endothelial cells also play important role in this recirculation process, globins and heme get recycled while porphyrin is degraded to bilirubin which is further conjugated by the liver and excreted in the gut. RBCs are passed through 2–3 micron apertures of splenic sinusoids, while rigid cells are phagocytosed. Other components of RBCs are removed during splenic circulation and related immune responses are generated. High levels of TNFα, INF-γ, IL-6 and IL-1 are noted in children with severe malaria cases (Banchereau et al. 2000; Lanzavecchia and Sallusto 2001). Marginal zone and red pulp of the spleen are two important sites for the removal of infected erythrocytes in malarial infection (Angus et al. 1997; Chotivanich et al. 2002). Species like *Plasmodium ovale* and *Plasmodium malariae* produce dormant liver parasites and long lasting infections, respectively. This is very essential information for delivering the drug to right organ especially in case of patients with a very high parasitaemia. Phagocytosis function of macrophage is reduced in malaria infection. This could be due to haemonozoin, where crystalloid polymer of haem accumulates during the erythrocytic cycle, inhibiting process of phagocytosis (Urban and Roberts 2002).

Presently more than 35 countries are involved in malaria phase control, elimination and prevention of reintroduction. Insufficient research and development in chemotherapy added malaria to the category of neglected diseases. This lack of knowledge or research could be due to the complex parasitic life cycle, drug resistance, presence of other immune infections like HIV. Other reasons includes, multiple malarial strain infection, higher dose combination therapy, severe side effects (like arrhythmia and neurotoxicity) and lastly poor patient compliance due to inadequate access to therapy. Vaccine development is still in clinical trials (Kester et al. 2009; Mettens et al. 2008). The occurrence of drug resistance by parasites could be also due to the use of ineffective dosage forms which are not able to deliver drug at sufficient concentrations. It is observed that parasites develop resistance mainly at low drug concentration in presence of high parasitic count (Newton et al. 2006). Malaria therapy is in an urgent need of an effective drug delivery system which can deliver the drug to the target site, lowering the dose and reducing the toxic effects (Table 3). The main aim of malarial therapy is

Table 3 List of poorly soluble drugs presently available in market for treatment of malaria

Drugs	Company	Dose (mg)	Aq. solubility (mg/ml)	BCS class
Anti-malarials				
Chloroquine	Natco Pharma Ltd., Sanofi Aventis US, Sandoz, Zydus Pharms USA Inc.	100	0.10	II
Atovaquone-	Glaxosmithkline	100	1	I
Artemether–lumefantrine	Novartis	20—arte 120—lume	NF 1	I
Mefloquine	Sandoz, Roxane, Barr			
Quinine	Ar Holding Co Inc.	300	0.526	II
Quinidine	Lilly, Watson Labs, Sandoz, Avanir Pharms	250	0.14	I
Clindamycin (used in combination with quinine)	Dow Pharm Sciences, Sanofi Aventis Us, Aurobindo Pharma, Ranbaxy, Zydus Pharms USA, Nycomed US	600	50	I
Artesunate	Neros Pharmaceuticals, Adva Care Pharma	50–200	NF	II
Pyrimethamine	Glaxosmithkline LLC	25	0.0099	II

NF not found

to deliver a sufficient quantity of the drug to the intracellular compartments of infected RBCs, liver cells and reticulo-endothelial cells. To achieve this drug should be able to cross cellular membrane barriers which include, host cell membrane, parasitoophorous vacuolar membrane, plasma membrane of the parasite, reticulum membrane and organelle membrane (Biagini et al. 2005).

During the infection by plasmodium, membrane permeability of infected RBCs increases for low molecular weight solutes. These channels are also referred as new permeability pathways. New drug delivery systems can be developed and transported to RBCs taking advantage of these specific pathways generated after 12–16 h of the plasmodium invasion. On the other hand, for selective targeting of anti-malarials, one can take advantage of the parasite-encoded erythrocyte choline carriers which are involved in choline uptake by infected erythrocytes. In addition, interruption of de novo biosynthesis pathway in plasmodium and organelle apicoplast could serve as a new target in malaria chemotherapy. To get details of the new activities of anti malarial compounds e.g. targeting, nanotechnology used and their mechanism of action can be found in several reviews (Becker and Kirk 2004; Biagini et al. 2005; Santos-Magalhaes and Mosqueira 2010; Valderramos and Fidock 2006). PEGylated systems are more desirable in malarial treatment as they can stay in blood stream for a prolonged period of time which allows more drug interaction with infected RBCs and parasite membranes. The presence of malaria parasite in spleen and liver needs organ targeting at an effective dose. Various attempts have been made using liposomes (AlAngary et al. 1996; Hasan et al. 2011), polymeric nanoparticles (Gaspar et al. 1992; Rodrigues et al. 1994), stealth and surface modified nanoparticles (Agrawal et al. 1987; Mertins et al. 2010; Zhang et al. 2011b) to target malarial sites in the body passively and actively.

Very limited data are available on the preparation of nanosuspensions and in vivo evaluation for malaria treatment. Lumifantrine (Pka = 9), is a drug of choice in multidrug resistant malaria and in cerebral malaria treatment. However, due to low solubility it has severe bioavailability problems. Gahoi et al., (Gahoi et al. 2012) prepared lumifantrine nanocrystals stabilized by HPMC E3 and Tween 80 by milling technique to overcome solubility related issue. The six hours milling was sufficient to reduce particle size (d(v)50 %) from 72 μm of coarse powder to 0. 251 μm of nanosuspensions for above composition. Further the lumifantrine nanosuspension was spray dried on lactose 150 M. The nanosuspension at dose of 0.1 ng/ml resulted in IC50 of 175 times less than for coarse drug at concentration of 17.5 mg/ml when tested for its in vitro antimalarial activity against *Plasmodium falciparum*. The dose for nanonized lumifantrine was 42 times lower than that of standard used i.e. choloquine at 4.2 ng/ml concentrations. When tested in Swiss mice infected with *Plasmodium yoelii nigeriensis*, the parasitaemia was cleared on 18th day for lumifantrine spray dried powder at done of 60 mg/kg, while it took 28 days for coarse drug to reduce it to only 60 %. Even reduced doses of lumifantrine spray dried powder were effective in reducing parasitaemia than micron sized drug. The mean survival time of more than 28 days was observed for all doses of lumifantrine nanopowder, while it was more than 24 days for coarse drug at lower dose of 15 mg/kg. The results are promising and can easily overcome bioavailability issues of poorly soluble drug lumifantrine by simply converting it into nanosuspension. Increased saturation solubility and dissolution velocity are the main reasons for reduction in doses required to reduce parasitaemia.

In another separate study, aqueous nanosuspensions of poorly soluble drug dihydroartemisinin (DHA) were prepared in the presence of polymer and/or surfactant. At different weight ratios binary (drug/polyvinyl pyrrolidone K30 and also drug sodium cholate) and/or ternary mixtures (drug/PVP K30/NaDC) were prepared and finely ground using vibrating rod mill. Formed ground powder was dispersed in water to form nanosuspension. It was found that ternary ground mixtures did not give superior nanosuspension in terms of particle size reduction and recovery of drug nanoparticles as compared to the nanosuspension prepared using binary mixture. Nanosuspension prepared from ternary mixtures showed excellent stability as compared to nanosuspensions prepared using binary mixtures of DHA/NaDC ground mixtures. Grinding time and amount of PVP K30 and NaDC showed marked effect on particle size reduction. The DHA nanosuspensions showed higher in vitro antimalarial activity against *Plasmodium falciparum* than microsuspensions. The findings showed that co-grinding of DHA with PVP K30 and NaDC can be used as a promising method for the preparation of DHA nanosuspension (Chingunpitak et al. 2008).

Kakran et al., prepared artemisinin nanocrystals (100–360 nm) using evaporative precipitation technique to enhance the dissolution rate of the drug. Fabrication of nanocrystals was closely monitored statistically for effects of drug concentration and solvent to antisolvent ratio on the physical, morphological and dissolution properties of the drug. The dissolution of precipitated drug nanocrystals was increased to great extent as compared to the original coarse drug powder. A percent dissolution surface-response model elucidated the significant effect of drug concentration and solvent to antisolvent ratio on dissolution. The highest dissolution percentage was found to be 75.9 %, at the drug concentration of 15 mg/ml and solvent to anti-solvent ratio (v/v) of 1:20 (Kakran et al. 2010). In another work, quercetin nanocrystals (300–500 nm) were developed for malarial chemotherapy using HPH, milling and cavi precipitation method to overcome poor solubility (Kakran et al. 2012).

Similar method was discussed by Gao et al., in comparison with the HPH to evaluate their feasibility to form a chemically stable quercetin nanosuspension. Both techniques showed similar particle size and zeta potential values, but differences in thermal behaviour (by DSC) and crystallinity (by XRD) were observed. The crystalline-to-amorphous phase transition was observed for the precipitated quercetin powder, while the initial crystalline state of drug was maintained throughout the HPH process. The dissolution profile showed superiority in enhancing drug solubility and dissolution rate of quercetin using the evaporative precipitation method than the HPH process. Both techniques showed feasibility in the production of chemically stable nanosuspension (Gao et al. 2011).

Microparticles of artemisinin were prepared in order to enhance its solubility using modified 4-fluid nozzle spray dryer. The effect of the process variables (inlet temperature and feed concentration) on the physical properties and dissolution rate of the spray dried artemisinin was studied by applying factorial design. The SEM analysis showed that the particle size of the spray dried particles was affected by the inlet temperature and feed concentration. It was observed that with increasing inlet temperature and feed concentration, the crystallinity of spray dried particles was slightly decreased. Significant enhancement in the dissolution of spray dried particles was observed as compared to commercial artemisinin. The highest dissolution achieved was 117.00 $\pm$ 5.15 g/ml at a drug feed concentration of 10 g/l and inlet temperature of 140 °C (Baboota et al. 2005).

Several new analogues are being synthesized for malarial chemotherapy which needs effective delivery system, among them, tafenoquine, a synthetic analogue of primaquine that is very effective in inhibiting all stages of parasites including exo-erythrocytic, erythrocytic asexual forms and sporogonic development stages. It is developed by Glaxo-SmithKline Pharmaceuticals and the Walter Reed Army Institute of Research. Another example is artelinic acid and both are in various clinical trials (Milhous 2001; Peters 1999). Presently available antimalarial drugs and newly synthesized promising chemical entity definitely need a potential carrier system to achieve the goal of arresting malaria parasite growth. Nanosuspensions offer a solution for poor solubility, dose reduction, improved pharmacokinetic and biodistribution profile. The detailed understanding of parasite, target organ, barriers in targeting would further explore the uses of nanocrystals in malaria chemotherapy. Infected RBCs, spleen and liver are major sites of interest for delivering drug at required concentration to reduce the load of parasite. Nanocrystal as a drug delivery in treating malaria is still in its infancy and this can be seen from the few published reports and lack of in vivo data. This drug delivery approach offers much promise for single and multiple drug delivery of antimalarials.

Tuberculosis

Another important infectious disease with highest mortality rate is tuberculosis which is caused by two species of *Mycobacterium* i.e. *tuberculosis* and *bovis*. The biggest problem with current therapies is high dose, low patient compliance, the side-effects associated with the long duration of therapy and drug resistance. Literature provides sufficient information on anti-tubercular drug targeting to primary infection site (Khuller et al. 2004; Pandey and Khuller 2005, 2006). Recently, a new respirable form of rifampicin was invested with improved stability and

Table 4 Drugs presently used in chemotherapy of tuberculosis

Drugs	Company	Dose (mg)	Aq. solubility (mg/ml)	BCS class
Anti-tuberculars				
Isoniazid	Rimifon® (Roche), Cotinazin® (Pfizer) Ditubin® (Schering), Nydrazid® (Bristol-Mayers Squibb)	30–300	100	III
Rifampicin	Rifadin® (Sanofi-Aventis), Abrifam® (Abbott), Rifaprodin® (Almirall), Rimactan® (Novartis)	60–300	1–2	IIa
Pyrazinamide	Zinamide® (Merck & Co.), Pezetamide® (Hefa-Frenon), Pyrafat® (Fatol)	150–500	10	III
Ethambutol HCl	Myambutol® (Dura Pharmaceuticals), Etibi, Tibutol	100–400	10	III
Streptomycin	Sesquisulfate-AgriStrep® (Merck & Co.), Streptobrettin® (Norbrook)	500	>20	N/A i.v. or i.m.
Pyrazinamide	Zinamide® (Merck & Co.),Pezetamide® (Hefa-Frenon), Pyrafat® (Fatol)	150–500	10	III
Rifabutin	Mycobutin® (Pfizer)	150	0.19	II
Clofazimine	Lamprene® (Novartis)	100	0.01	II
Ethionamide	Trecator® (Wyeth), Nisotin®, Trescatyl® (M & B), Aetina®, Ethimide®, Iridocin® (Bayer)	500	0.1	II
Clarithromycin	Biaxin® (Abbott), Clathromycin® (Taisho), Klaricid® (Abbott), Naxy® (Sanofi Winthrop), Veclam® (Zambon)	500	0.00033	II
p-Aminosalicylic acid	Paser® (Jacobus), Rezipas® (Bristol-Mayers Squibb)	500	1.7	N/A
Cycloserine	Closina®, Farmiserina® (Farmitalia), Micoserina®, Oxamycin® (Merck & Co.), Seromycin® (Lilly)	500	100	IV/II
Amikacin	Sulfate-Amiglyde-V® (Fort Dodge), Amiklin®, BB-K8®, Biklin® (Bristol-Mayer Squibb), Lukadin® (San Carlo), Mikavir® (Salus), Novamin® (Bristol-Mayer Squibb)	1,000	NF	III
Kanamycin A	Kantrex® (Bristol-Mayer Squibb)	1,000	NF	N/A i.v. or i.m.
Capreomycin	Capastat® (Dista), Capastat sulphate® (Eli Lilly)	1,000	Soluble in water	N/A i.v. or i.m.
Lavofloxacin	Cravit® (Daiichi), Levaquin® (Ortho-McNeil), Tavanic® (Aventis), Quixin® (Santen)	500	Sparingly soluble in water	N/A i.v. or i.m.
Motifloxacin	Actimax® (Sankyo), Actira® (Bayer), Avelox® (Bayer)Octegra® (Bayer), Proflox® (Esteve), Vigamox® (Alcon)	400	NF	NF
Gatifloxacin	Tequin® (Bristol-Mayers Squibb), Zymar® (Allergan)	400	60 mg/ml at pH 4	NF
Linezolid	Zyvox® Zyvoxid® (Pfizer)	400	3 mg/ml	NF

NF not found

aerodynamic profile (Son and McConville 2011). Recently published review articles discussing present status of nanoparticle research in tuberculosis can be referred for more details (Gelperina et al. 2005; Griffiths et al. 2010; Shegokar et al. 2011a) (Table 4). Nanosuspensions can solve solubility and targeting associated issues, thereby, improving drug pharmacokinetic and lung deposition. For inhalation targeting, nanosuspensions can be directly converted to nanopowder using spray dryer or lyophilization and directly filled in inhalator without affecting their particle sizes (100–800 nm). This will allow deposition of nanocrystals in deeper layers of lung.

In this direction, Reverchon et al. prepared rifampicin amorphous submicronized particles with particle size

ranging between 400 nm to 3 μm by employing supercritical carbon dioxide-assisted atomization (Reverchon et al. 2002; Reverchon and Della Porta 2003). Effects of different solvents on rifampicin particle size and percentage of drug degradation was studied. This nanoparticle production approach has a promise in targeting drug to lung locally. The micronization using dimethyl sulfoxide (DMSO) at 40 °C showed mean diameters between 400 and 1,000 nm at a low pressure of 120 bar. Increase in particle size was observed when pressure was lowered, e.g. at 90 and 110 bar and mean diameters were 2.5–5 μm as spherical single microparticles. These changes in morphology could be due to modification of the high pressure vapour and liquid equilibria of the prepared ternary system (rifampicin–DMSO–CO_2) with respect to the behaviour of the binary system. The increase in the concentration of drug in liquid increased the mean particle size and particle size distribution range.

Clofazimine, a riminophenazine compound is mainly used for treating *Mycobacterium avium* infection. However, the limited use of clofazimine is due to its poor solubility and low bioavailability. Clofazimine nanosuspensions were prepared using HPH to get particle size of 385 nm. Intravenous administration to mice infected with *M. avium* resulted in a considerable reduction of bacterial loads in the liver (72.5 mg/kg tissue), spleen (81.4 mg/kg tissue) and in lungs (35.0 mg/kg tissue) (Peters et al. 2000). Drug amount in these organs reached concentrations higher than the minimal inhibitory concentration required for most *M. avium* strains. Nanocrystals showed comparable concentration profile to that of clofazimine encapsulated marketed liposomes. This technology can also be extended to the Clofazimine derivative like riminophenazine.

Global Data recently published a new report by on "Tuberculosis Therapeutics Market-Pipeline Assessment and Market Forecasts to 2017". They confirmed that presently available tuberculosis (TB) chemotherapy is based on generic drugs which include WHO approved DOTS and DOTS-Plus regimes. This report provides important information on the global tuberculosis therapeutics. According to them the "global tuberculosis therapeutics market will decline by 2.8 % during the next seven years to reach $79 m by 2017" (Globaldata 2010). The clinical trial pipeline contains promising 36 new entities e.g. TMC 207, GSK M72, PA-824 and AERAS-402/Crucell Ad35 from leading firm like TB Alliance, Aeras, GSK Biologicals, Sanofi Aventis and others (Globaldata 2010; Shegokar et al. 2011a). Obviously these drug need effective oral or inhalation therapy with improved formulation characteristics. Very limited data are available on use of nanocrystals for targeting lung, local lymph nodes and macrophages where mycobacterium accumulates during infection and in years of dormancy. Nanosuspension would serve effective, simple and promising approach to treat TB. To determine the effectiveness of nanonization technique in tubercular chemotherapy, sufficient formulation developments and in vivo evaluations are required to be done in this direction.

Chagas disease

Chagas disease is another neglected disease belonging to a group of tropical diseases, mainly found in Latin America Africa and Asia. It affects nearly 15–20 million people mainly in southern California, Argentina and Chile. According to current estimates, approximately 50,000 people die annually and more than 200,000 people get infected every year causing an economic loss over $ 6.5 billion (Strosberg et al. 2007). Chagas disease is caused by a hemoflagellate flagellate protozoan *Trypanosome cruzi* (*T. Cruzi*) which is transmitted by several *Hematophagous reduviidae* bugs to human either by contamination of faeces of bugs, during organ or blood transplant operations, congenital transmission and ingestion of contaminated food. It also causes an opportunistic infection among immune compromised patients e.g. HIV infected people. Until now three morphogenetic forms of the parasite are known viz. extracellular non dividing form (trypomastogotes), replicative form (epimastigotes) and amasmastigotes (in midgut of reduviids). Limited data are available on effective treatments not only due to the lack in vitro and in vivo screening protocols but also the lack of biomarkers for treating parasitaemia. To increase the effectiveness of treatment, the identification and validation of parasite targets is very important (Romanha et al. 2010). In chronic conditions, the parasite resides in the nervous system (autonomic), reticulo-endothelial cells and digestive muscles. The amastigotes are particularly found in glial cells and skeletal muscles, cardiac muscles and smooth muscles. Because of this, Chagas disease is related to many cardio vascular problems like cardiac arrhythmia, apical aneurysm, congestive heart failure and sudden cardiac death and this disease is referred as "silent killer". Table 5 lists various drugs screened for the treatment of Chagas disease. Currently, two drugs are used to treat this infection viz. Nifurtimox and benznidazole, since 45 years they have been the first choice drugs. Although many natural and synthetic compounds have been studied only a few of them advanced to clinical trials. However, ample vaccine research findings to prevent *T. cruzi* have not been developed. Both the drugs are not effective in chronic conditions and have low efficacy and severe side effects leading to discontinuation of the treatment in most cases.

Nifurtimox is the drug of choice for chagas chemotherapy and is described practically in soluble in water (Library 2011). It is a nitrofurane derivative antiprotozoal

Table 5 List of different drugs used in chemotherapy of chagas disease

Drug	Company	Solubility/BCS class	Dose
Nifurtimox 3-methyl-4-[(5-nitrofurfurylidene)amino] thiomorpholine1, 1-dioxide	Bayer Healthcare	Practically insoluble in water/ Class III	250 mg (8–10 mg/kg/day)/ 90 days
Benznidazole *N*-benzyl-2-nitro-1-imidazole-acetamide	Roche Passed Rights To Govt. Of Brazil Pharmaceutical Laboratory Of Pernambuco State (LAFEPE)	Practically insoluble in water/BCS III	100 mg
Posaconazole	Schering-Plough	1.20e−02 g/l/BCS II	400–800 mg (20 mg/kg)
Thioridazina	Novartis Pharma Corp, Sandoz Inc., Teva Pharma-Ceuticals, USA, Wockhardt, Ivax Pharmaceuticals Inc., Mutual Pharma-Ceutical Co Inc., Mylan Pharma-Ceuticals Inc., Par Pharmaceutical Inc., Watson Laboratories Inc., West Ward Pharmaceutical Corp.	0.0336 mg/l poorly soluble	100–600 mg (80 mg/kg/ day)
Itraconazole	Sandoz Inc., Ortho Mcneil Janssen Pharmaceuticals Inc., Stiefel Laboratories Inc	1.8 μg/ml/BCS II	200–400 mg
Ravuconazole	Bristol-Myers Squibb	Poor water solubility/BCS II	Phase II/50–200 mg
D0870	Zeneca Pharmaceutical	Poorly soluble	50–70 mg
Albaconazole (UR-9825)	Uriach & Company, Spain	Poorly soluble	50–70 mg
TAK-187	Takeda Chemical Company	Poorly soluble	100 mg
ES700 & ER-119884	Eisai Company Ltd	Poorly soluble	200 mg
Allopurinol	Prometheus, Glaxo smithkline	0.48 mg/ml/BCS IV	100–800 mg
Ibandronate	Hoffmann La Roche Inc.	Poorly soluble	60–100 mg
Pamidronate	Novartis Pharma-ceuticals Corp	Poorly soluble	
	Cipla Ltd., Generamedix Inc.		
	Sun Pharma Global Inc.,		
	Teva Parenteral Medicines Inc.		
Risedronate	Warner Chilcott Co LLC, Teva Pharma-Ceuticals USA, Procter & Gamble	Poorly soluble	5–100 mg

NA not available

drug which shows anti *T. cruzi* activity due to bioreduction of the nitro-group and dependence on redox cycling with oxygen. In combination with buthionine sulfoximine it displayed increased toxicity towards various form of *T. cruzi* (epimastigote, trypomastigote, and amastigote). It reacts with the nucleic acids of the parasite and inhibits a parasite-specific antioxidant defence enzyme called trypanothione reductase. In liver it undergoes nitroreduction involving cytochrome P-450 and P-450 reductase. Bayer is the only producer of nifurtimox for the chemotherapy of Chagas disease. Bayer HealthCare joined hands with the World Health Organization (WHO) to fight against Chagas disease. In 2011, Bayer has signed an extension of 5 years with WHO to fight the parasitic infection which was effective from April 2012. Bayer HealthCare has also promised to double its initial donation of 2.5 million Lampit^{TM} tablets (nifurtimox) to a total of 5 million by 2017.

Gonzalez-Martin et al. prepared polyalkylcyanoacrylate nanoparticles loaded with nifurtimox for targeted delivery against *T. cruzi*. Nanoparticles (<200 nm) were prepared by an emulsion polymerization process. They found that in cultures of *T. cruzi* epimastigotes, the nanoparticles considerably increased trypanocidal activity compared with a standard solution of nifurtimox. In this study, pre-infected cell cultures with metacyclic forms of the parasite showed that only 2-h treatment with 0.001 % of the nanoparticles suspension reduced parasitaemia by 87–94 %. Electron-microscopy confirmed degeneration and lysis of cells suggesting that it is an apoptotic processes for intracellular amastigotes and free amastigotes treated with the nanoparticles (Gonzalez-Martin et al. 1998). A report by the same group studied trypanocidal activity of ethyl cyanoacrylate nanoparticles loaded with nifurtimox in comparison with the free drug against the parasite. The highest trypanocidal activity on cell culture-derived trypomastigotes was noted

for nifurtimox-loaded nanoparticles with a 50 % inhibitory concentration (IC50) which is 20 times less than the free drug. The drug loaded nanoparticles showed increased trypanocidal activity on intracellular amastigotes with an IC50 of 13 times less than that of free drug. The cytotoxicity in Vero cells of unloaded nanoparticles at low concentrations was almost similar to that of free drug (Sanchez et al. 2002).

Benznidazole is another traditionally used drug in chemotherapy of Chagas disease. It has a poor water solubility and a variable bioavailability. In one study, benzidazole microcrystals were prepared by solvent change precipitation. The process of microcrystal formation was optimized to yield small particle size with faster dissolution rate. The therapeutic efficiency of the formed microcrystals was studied in murine model of Chagas disease. They found that the microcrystals did not show any polymorphic change and resulted in smaller particle size. A significant improvement in the drug dissolution rate was observed for benznidazole microparticles and tablets as compared to coarse drug powder and the marketed product (Rochagan®). In vivo studies showed a significant increase in the therapeutic efficacy of the drug microparticles due to improved dissolution property (Maximiano et al. 2011). According to our knowledge, no work is published on nanosuspension for treatment of Chagas disease. However, some papers suggesting the use of liposomes and/or polymeric carriers have been found for benznidazole, nifurtimox and etanidazole. All these early reports show successful use of nanotechnology-based formulations. Nanosuspensions offer passive targeting during acute stages of infections with increased permeability due to nanometer size range, faster cellular uptake and desired interaction with cell membrane after coating with suitable pH dependant polymer or ligand. To know more about promising anti-Chagas drugs reader can refer some important published reviews and research paper (Araujo et al. 2000; Soeiro Mde et al. 2009; de Castro et al. 2011; Urbina 2001; Urbina and Docampo 2003).

Other infectious diseases

For the treatment of amoebiasis and yaws, arsthinol nanosuspensions were developed. HPH was used for the preparation of arsthinol nanosuspensions. Nanosuspensions were tested for their anti-leukaemic activity in NB4 acute promyelocytic leukaemia cells against a solution of pure arsthinol, arsthinol and melarsoprol. Arsthinol inhibited the growth of NB4 cells at a lower concentration resulting in IC50 of 0.78 ± 0.08 μmol/l in comparison to arsthinol solution with IC50 of 1.60 ± 0.23 μmol/l and melarsoprol solution with IC50 of 1.44 ± 0.08 μmol/l after 24 h. Arsthinol nanosuspension, showed IC50 of 1.33 ± 0.30 μmol/l after 24 h. Higher drug concentration of 2 μmol/g in bone marrow concentration was found posing their use in acute promyelocytic leukemia (Ajana et al. 2009)

In another study, surface coated atovaquone nanosuspensions were developed by Shubar et al. for the treatment of *Toxoplasmic encephalitis* caused by *Toxoplasma gondii* which is mostly found in immunologically challenged patients like reactivated HIV/AIDS patients and organ transplant patients. Current treatment available is associated with allergic reaction and hematologic toxicity. As an alternative treatment sodium dodecyl sulfate (SDS) and Poloxamer 188 coated atovaquaone nanosuspensions (440–470 nm) were prepared and compared with marketed micronized drug suspension Wellvone®. Nanonized drug showed improved levels in serum and brain tissues, with significantly lower levels of parasite load compared to untreated control and commercial product in *Toxoplasma gondii* infected mice. Similar results were obtained for murine model of reacted toxoplasmosis. SDS coated atovaquone formulation showed improved oral bioavailability and improved brain uptake and they can be used in the treatment of cerebral infection or encephalitis caused by *Toxoplasma gondii* (Shubar et al. 2011). The itraconazole nanosuspension at twice the dose and the rate of infusion showed improved efficacy and prevented the negative inotropic effect as compared with SPORANOX (marketed itraconazole) Injection in conscious dogs (McKee et al. 2010). In another study, itraconazole nanosuspensions (294 nm) stabilized by poloxamer 407 was prepared using milling technique at bench scale by employing magnetic stirrers. The effect of various parameters line stirring time and amount of beads was studied. The optimized nanosuspension was further lyophilized in presence of mannitol at 1: 1 ratio. Nanonized itraconazole showed 90 % release within 10 min while only 10 % release was obtained for coarse drug. In short, nanonization increased dissolution of drug by 9 folds and 5.3 folds over marked formulation (Nakarani et al. 2010a). Lemke et al. (2010) developed polysorbate 80 and sodium cholate coated amphotericin B nanosuspensions with showed improved delivery to brain. Nanosuspensions showed promising amebicidal activity against *Balamuthia mandrillaris* when tested in vitro and in vivo. Das and Suresh (2011) prepared amphotericin B nanosuspensions (150–290 nm) stabilized with Eudragit RS 100 by using solvent displacement technique for management of ocular fungal infections. The antifungal activity of amphotericin nanosuspension was almost same that of free drug solution when tested by disk diffusion method. When administered in Rabbits eye, the scores for conjunctival swelling, discharge, iris hyperemia and corneal opacity were zero. That means no sign or irritation was seen when applied topically, confirming that Eudragit stabilized amphotericin nanoparticles are completely safe for ocular use.

In one study, aphidicolin nanosuspension showed improved activity against extracellular promastigotes and

Table 6 Drugs presently used in chemotherapy for various pathological conditions and their respective BCS class

Drugs	Company	Dose (mg)	Solubility	BCS class
Anti-infective				
Cefditoren pivoxil	Cornerstone Therapeutics	100	0.0099	II
Sultamicillin	Morepen laboratories Ozay Pharmaceuticals	375–750	0.100	II
Levofloxacin	Aurobindo Pharmaceuticals Ltd., Dr Reddy's Labs Inc., Glenmark Generics, Lupin, Sandoz, Torrent Pharmaceuticals, Wockhardt	100–500	10	III
Ciprofloxacin	Bayer Healthcare, ACS Dobfar Info Sa, Baxter Healthcare, Claris Lifesciences, Akorn Inc.	100–750	10	III
Anti-fungals				
Fluconazole	Ivax Sub Teva Pharmaceuticals, Pfizer, Ranbaxy, Apotex Inc., Baxter Healthcare, Claris Lifesciences	50–200	1	III
Griseofulvin	Perrigo Co Tennessee, Ivax Sub Teva Pharmaceuticals, Actavis Mid Atlantic	125–250	0.0099	II
Iriconazole	–	100	0.0099	II
Albendazole	Glaxo smithkline LLC	400	0.0099	II
Mebendazole	Teva Pharmaceuticals	100–500	0.0099	II
Niclosamide	Taj Pharmaceutical Ltd. Hebei Kexing Pharmaceutical Co., Ltd.	500	0.0099	II
Praziquantel	Bayer Healthcare	150–600	0.100	II
Pyrantel	Cipla Ltd.	200–250	0.0099	II

Table 7 Poorly soluble drugs presently used in various topical (infectious/non infectious) pathological conditions

Drugs	Company	Route of administration	Solubility
Isotretinoin	Ranbaxy, Barr Pharmaceuticals, Cipher Pharmaceuticals, Steifel Laboratories Inc.	Oral/topical	Poor water solubility
Tacrolimus	Sandoz, Dr Reddys Labs Ltd., Watson Labs, Mylan, Astellas	Oral/topical	
Lutein	Amway, Bosch and Lomb	Oral/topical	
Griseofulvin	Antifungal	Systemic/topical	
Fluconazol	Pfizer	Oral/vagina topical application	Slightly soluble in water
Miconazol	Actavis mid Atlantic, Stiefel Labs Inc., Insight Pharmaceuticals	Topical/iv	Poor water solubility
Ketoconazol	Janssen Pharmaceuticals, Teva Pharmaceuticals, Stiefel Labs Inc.	Oral/topical	Insoluble
Amphotericin	Lifecare Innovations Private Limited/Bristol Mayer Squibb	Topically for corneal ulcers and keratitis/slow iv infusion	
Docosanol	Avanir Pharmaceuticals	Topical for treatment of Shingles/herpes simplex.	
Testosterone	Teva Pharmaceuticals, Auxilium Pharms, Abbvie Pharmaceuticals, Endo Pharmaceuticals	Topical	Practically insoluble

intracellular amastigotes of *Leishmania donovani* in murine macrophages (Kayser et al. 2001; Kayser 2000). In similar direction, oral Amphotherin B nanosuspensions (528 nm) were developed for the treatment of visceral leishmaniasis. Amphotericin B nanosuspension showed reduction in parasite load by 28.6 % in mouse model of visceral leishmaniasis compared to control due to because of narrow size ranges. The coarse drug did not show any reduction (Kayser et al. 2003). Bupravaquone nanosuspensions for treatment of *Pneumocystis carinii* pneumonia lung infections were developed and the effect of two different nebulization techniques based on jet and ultrasonic mechanism was studied (Hernandez-Trejo 2006; Hernández-Trejo et al. 2004, 2005). In another work, atovaquone nanosuspensions (279 nm) were produced using HPH. Prepared nanosuspension showed promising activities when tested in macrophages infected with the BK strain of *Toxoplasma gondii* and mice infected with ME49 strain of *Toxoplasma gondii*

Table 8 List of some important drugs presently used in chemotherapy of non infectious pathological conditions

Drugs	Company	Dose	Solubility	BCS class
Anti-neoplastic				
Bicalutamide	Accord Healthcare Inc., Actavis Totowa, Astrazeneca, Astrazeneca, Zydus Pharms USA Inc.	50–150	0.0049	II
Cyproterone	–	100	0.0099	II
Gefitinib	Astrazeneca	250	0.0099	II
Imatinib	Novartis	100–400	0.0489	II
Tamoxifen	Mylan, Watson Labs, Teva Pharmaceuticals	10–20	0.0099	II
Respiratory agents				
Ebastine	Nycomed and Almirall	10	0.0099	II
Zafirlukast	Astra Zeneca, Dr Reddy's	10–30	9.62e–04 g/l	–
Pranlukast	SmithKline Beecham	120	0.0099	II
Cadiovascular agents				
Atorvastatin	Pfizer	10–80	0.0099	II
Benidipine	–	2–8	0.0099	II
Candesartan Cilexetil	Astrazeneca	2–20	0.0099	II
Carvedilol	Apotex Inc.	2–20	0.0099	II
Cilostazol	Breckenridge Pharmaceuticals, Corepharma, Actavis Totowa, Otsuka, Sandoz	50–100	0.0099	II
Clopidogrel	Sanofi Aventis US	75	0.0099	II
Ethylicosapentate	–	300–600	0.0099	II
Ezetimibe	MSP Singapore	10	0.0099	II
Fenofibrate	Abbott Labs, Cipher Pharmaceuticals Inc., Lupin Atlantis, Ranbaxy, Skyepharma AG	50–200	0.0099	II
Furosemide	Wockhardt, Dava Pharmaceuticals Inc., Roxane, Sanofi Aventis US, Sandoz, Watson Labs	40	0.0099	IV
Hydralazine	Glenmark Pharmaceuticals Ltd., Heritage Pharms Inc., Par Pharm, Zydus Pharms USA	50	33	III
Irbesartan	Sanofi Aventis US	300	0.0099	II
Losartan	Apotex, Merck, Sandoz, Torrent Pharmaceuticals, Upsher Smith, Zydus Pharmaceuticals USA Inc., Teva Pharmaceuticals	100	100	III
Manidipine	–	20	0.0099	II
Nifedipine	Pfizer, Bayer Healthcare, Valeant Intl, Actavis	10	0.0099	II
Simvastatin	Abbott, Accord Hlthcare, Dr Reddys Labs Inc., Merck, Zydus Pharms Usa	5–80	0.0099	II
Spironolactone	Gd Searle LLC, Actavis, Elizabeth, Vintage	25	0.0099	II
Telmisartan	Boehringer Ingelheim	20–80	0.0099	II
Ticlopidine	Sandoz, Apotex, Teva	100	0.0099	II
Valsartan	Novartis	40–350	0.0099	II
Verapamil	Abbott, Glenmark Generics, Elan Drug, Par Pharm, Watson Labs	40–80	0.0099	II
Warfarin	Bristol Myers Squibb, Usl Pharma, Zydus Pharms USA	10	0.0099	II
Central nervous system				
Carbamazepine	Nostrum, Validus Pharms Inc., Novartis, Torrent Pharmaceuticals	100–400	0.0099	II
Celecoxib	GD Searle	100–400	0.0099	II
Chlorpromazine	Baxter Hlthcare, USL Pharma, Sandoz	100	0.0099	II

Table 8 continued

Drugs	Company	Dose	Solubility	BCS class
Clozapine	Azur Pharma Intl, Novartis, Ivax Sub Teva Pharmaceuticals	25–100	0.0099	II
Codeine	Wockhardt, Ortho Mcneil Janssen	30	1	III
Diclofenac	Sandoz, Novartis, Nycomed Us, Bausch And Lomb, Unique Pharmaceuticals Labs	25	0.100	II
Ethosuximide	Parke Davis, Teva Pharmaceuticals, Banner Pharmacaps	250	100	III
Ibuprofen	Abbott, Watson Labs Florida, Amneal Pharmaceuuticals, NY	200–400	0.0099	II
Ketoprofen	Heritage Pharms Inc Watson Labs Florida	25–50	0.0099	II
Lemotrigine	–	25–200	0.170	II
Levodopa	Orion, Impax Labs, Merck Sharp Dohme	5–500	1	II
Metaxalone	King Pharmaceuticals, Sandoz	400–800	0.1	II
Phenytoin	Baxter Healthcare, Strides Arcolab, Pfizer, Parke Davis	25–100	0.0099	II
Quetiapine	Astrazeneca	25–300	1	II
Sumatriptan	Glaxosmithkline, Par Pharm, Dr Reddys Labs Inc., Glaxosmithkline	25–100	100	III
Valproic acid	Abbott, Wockhardt, Sun Pharma Inds Inc.	200–500	1	II
Anti-diabetic				
Epalrestat	–	50	0.0099	II
Metformin	Bristol Myers Squibb, Heritage Pharma-ceuticals Inc., Zydus Pharms USA Inc., Dr Reddys Labs Inc., Andrx Labs LLC Merck	250–1,000	33	III
Pioglitazone	Takeda Global, Mylan	15–30	0.0099	II
Raloxifene	Lilly	60	0.1	II
Orlistat	Hoffmann La Roche	120	0.0099	II
Rebamipide	–	100	0.0099	II
Sulfasalazine	Pharmacia And Upjohn Vintage Pharms, Watson Labs	500	0.0099	II
Teprenone	–	50	0.0099	II
Ursodeoxycholic acid	–	100	0.0099	II
Hydroxyzine	Actavis Totowa, Invagen Pharms, Mutual Pharmaceuticals Northstar Healthcare, Sandoz	100	0.0099	II

(Schöler et al. 2001). For treatment of hepatitis, Oleanolic acid nanosuspensions (285 nm) with increased saturation solubility were developed. Nanosuspensions showed significantly enhanced hepatoprotective effect in rodent (Chen et al. 2005). Metronidazole-magnetite nanosuspension showed reduction in dose of drug with enhanced anthelmintic activity when studied on Indian earthworms (*Pheretima poi*) (Latha et al. 2009) and can be used in various GI tract infections. Tables 6, 7 lists some important chemotherapeutic agents used in various pathological conditions.

Application overview of nanosuspensions in non infectious diseases

Nanosuspensions (200–250 nm) of immunosuppressant drug size cyclosporine were formulated to overcome the toxicity caused by Cremophore EL 35 used in a commercial formulation i.e. Sandimmune® I.V. The in vivo studies showed that both formulations had comparable bioavailability (Nakarani et al. 2010b). In separate work, Nakarani et al. (2010b), prepared poloxamer 407 stabilized cyclosporine nanosuspension (213 nm) by using media milling. Cyclosporin nanosuspensions showed almost 5 fold increase in AUC over free drug and ~1.3 fold against marketed formulation when studied in albino rat. Puerarin which is used in myocardial diabetes, retinal artery obstruction and angina is rendered as nanosuspension (82 nm). The nanosuspension showed similar targeting efficiency to brain as that of nanoemulsion, while superior for liver and kidney following oral administration in mice. Oridonin nanosuspensions were investigated in vitro for their inhibitory effects in human prostatic carcinoma PC-3

Table 9 Subcellular targets for site specific drug delivery of chemotherapeutic agents

Organelles	Size (nm)
Ribosomes	25
Golgi complex	30–80
Secretary vesicles	100–1,000
Glycogen granules	10–40
Lipid droplets	200–5,000
Vaults	55
Lysozymes	500–1,000
Proteosomes	11
Peroxisomes	500–1,000
Mitochondria	500–1,000
Superfine filaments	2–4
Microfilaments	5–7
Thick filaments	15
Microtubules	25
Centrioles	150
Nuclear pores	70–90
Nucleosomes	10
Chromatin	1.9

cell line. Nanosuspension induced apoptosis and showed significant cell proliferation suppression and it is proposed as a promising candidate for treatment of androgen independent prostate cancer (Zhang et al. 2010b). Oridonin nanosuspension was checked for in vitro and in vivo anti tumor activity by Lou et al. in another study (Lou et al. 2009). Application of deacety mycoepoxydiene (Wang et al. 2011) and silybin (Zheng et al. 2011) in prostate cancer was also studied.

Nimodipine nanosuspension (300 and 650 nm) meant for intravenous use for treatment of senile dementia were produced by high pressure homogenization. Nanosuspension having size 650 nm were additionally coated with Tween 80. Biodistribution studies showed significant higher concentration in MPS rich organs for 650 nm nanosuspension compared to drug ethanol formulation and 300 nm nanosuspensions in mice. Furthermore, particle size and dissolution rate played important role in targeting brain for 300 nm particles compared to 650 nm suspensions (for both uncoated and coated with Tween 80) (Xiong et al. 2008). Camptothecin nanocrystals (200–700 nm, −40 mV) were prepared by sonication-precipitation method. Camptothecin nanocrystals having low cytotoxicity profile, higher cellular uptake in KB cell line further showed significant suppression (at inhibition rate of 65 %) of tumor growth in MCF-7 xenografted BALB/c mice at dose of 7.5 mg/kg compared water soluble salt moiety of drug (inhibition rate of 52 %). The tumor accumulation of drug was fivefold higher compared salt solution (Zhang et al. 2011a). Table 8 lists presently used dugs in chemotherapy of non infectious pathological conditions.

Intracellular drug delivery

All above studies shows potential of nanosuspension in drug delivery. They can be used for targeted delivery as their surface properties and in vivo behavior can easily be altered. The formulation versatility and easiness of industrial production enable the development of commercially viable nanosuspensions. The surface engineering of nanosuspensions by using various surface coatings can promote targeting. Kayser (2001) studied targeting of *Cryptosporidium parvum* (cryptosporidiosis) by using surface-modified mucoadhesive nanosuspensions of bupravaquone. A superior targeting was achieved for mucoadhesive bupravaquone

Table 10 Overview of disease specific target cells and respective receptors for HIV/AIDs, malaria, TB and chagas disease

Infectious disease	Target cell/s	Receptor
HIV/AIDS	Macrophages	Mannose-6-phosphate
		β Galactose
		α2 macroglobulin protease complex
	T4 cells	Galactose (low density), CD4, gp-120, transferrin, interleukin, oligosaccharide recognizing receptor, T8 lymphocyte-L-rhamnose
	Monocytes	Mannose-6-phosphate-terminated neoglycoproteins, beta glucan,
	Endothelia	Insulin, transferrin, glycoproteins
Malaria	Hepatocytes	Galactose (high density), high density lipoprotein, low density lipoproteins, IgA, transferrin, Fc receptor, lectin
	Kupffer cells	Mannose, low density lipoproteins, complement factors, galactose
	Endothelia	Insulin, transferrin, glycoproteins
Tuberculosis	Lung macrophages	Mannose and galactose receptors surfactant protein receptor
Chagas	Histiocytes, macrophages	Histamine receptors Mannose and galactose receptors

nanosuspension, because of their prolonged residence at the infection site. A 10-fold reduction in the infectivity score of *Cryptosporidium parvum* was noted as compared to the bupravaquone nanosuspensions without mucoadhesive polymers. Similarly, pulmonary aspergillosis can be easily targeted by using amphotericin B nanosuspensions instead of using stealth liposomes (Kohno et al. 1997). The surface properties of the nanosuspension can determine the qualitative and quantitative type of adsorption patterns of blood proteins (Blunk et al. 1993) thus, determining the subsequent fate of the injected particles in the body e.g. brain or bone marrow. The surface properties can be altered in such a way, that the desired proteins adsorb automatically on the nanoparticle surface (Gessner et al. 2006; Göppert and Müller 2003). This concept of "differential protein adsorption" was exploited for targeting nanocrystals to the brain (Table 9).

BBB is in close contact with the pericytes, actrocytes, neurons, and glial cells. The tight junctions that connect adjacent endothelial cells and physically restrict solute flux between the blood and the brain is limited only to lipophilic compounds. In various brain pathologic conditions like trauma, stroke, or diabetes mellitus, the alterations of the BBB permeability result in leakage of plasma components. In the case of brain tumors, the absence of tight junctions allows penetration and retention of drugs, due to enhanced permeability and retention (EPR) effect allowing intracellular delivery improved with tumor-specific targeting which generally restricted by normal BBB. Optimal delivery of drug nanoparticles to brain vasculature can be obtained by keeping them circulating in the blood, thereby avoiding peripheral distribution. Depending on their size, nanoparticle can cross the endothelium of brain capillaries by passive diffusion through normal BBB.

Several research teams found that surfactant-coated nanoparticles can deliver the drug to the brain (Alyautdin et al. 1998; Rao et al. 2008; Shegokar and Singh 2008). These methods are generally referred as "empirical" approaches as they do not have sound theoretical background and because the mechanisms for their uptake are still imaginary. Another way for transporting the drug to the brain is by absorptive mediated transcytosis or receptor mediated transcytosis. In absorptive-mediated transcytosis, cationized proteins (albumin, immunoglobulins) facilitate the absorptive-mediated endocytosis through the BBB by allowing electrostatic interaction between the positively charged proteins and the negatively charged plasma membranes. The main drawback is that besides their localization in brain they might also accumulate in liver, kidney, and/or lung tissues. The degree of cationization and nanoparticles size remain determining factors for assessing uptake by brain cells. In receptor-mediated transcytosis, transcytotic delivery of nanoparticle (<100 nm) possessing surface receptor recognizing ligand to brain cells take place via receptor. Relatively high brain specificity can be achieved by targeting over expressed receptors at the luminal side of brain compared to other organs e.g. receptors of transferrin, insulin, insulin-like growth factor (IGF), or low-density lipoproteins (LDL) (Olivier 2005; Terasaki and Pardridge 2000). Table 10 lists various cellular targets along with the receptors available on the surface of cells.

Drug nanosuspension can be engulfed faster than inert particle through the phago-lysosomes of the macrophages as these cells are capable of engulfing all types of biodegradable particles. The faster uptake of polymeric carriers may exert burden on the macrophages and ultimately reticuloendothelial system which may become overloaded by phagocytic activity, if the phagocytic overload is continued and heavy risk of reticuloendothelial blockage could occur (Pardeike et al. 2009). Nanosuspensions provided sustained release without peaks in delivery, thus minimizing the risk of toxic concentration of the drug in cell and ultimately to organ. Following i.v. administration of nanosuspension, injected nanoparticles diffuse quickly to yield pharmacokinetics equivalent to that for a solution formulation (Pace et al. 1999). The particle which is injected locally may dissolve slowly showing prolonged or extended drug release (Shegokar et al. 2010a). The reduction of particle size is essential for viable intravenous formulations so that they can substantially avoid blockage of capillary blood vessels.

Conclusion

The challenges in treating AIDS, malaria, TB, chagas and others can be reduced to certain limit by use of correct formulation type i.e. nanosuspensions. These diseases demand attention of pharma R & D's to invest resources for developing effective drug delivery systems. Many published reports confirmed that the nanocrystal would serve as an ideal industrially feasible option and can save several active compounds coming from high throughput screening. Intracellular drug targeting at therapeutic level is very essential for these pathogens which form latent sanctuaries at cellular and subcellular level. In particular, when developed smartly, the nanosuspension can release drug at target organ/cells slowly and maintain it for longer time, this will help to remove hidden viruses and bacteria. Other than discussed diseases, this technology can be effectively explored for treatment of other neglected diseases like human African trypanosomiasis (sleeping sickness), Elephantiasis, Kala-azar etc. which can be fatal without treatment.

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