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Review article

Exploring the use of novel drug delivery systems for antiretroviral drugs

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ABSTRACT

Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral (ARV) drug therapy, thereby improving the management of patients with HIV/AIDS. This paper provides a comprehensive review of the various ARV delivery systems that have been developed for achieving sustained drug release kinetics, specifically targeting drugs to the macrophages, brain and gastric mucosa, and for addressing formulation difficulties such as poor solubility, stability and drug entrapment. Studies on the potential of systems for alternative routes of ARV drug administration, i.e., transdermal, buccal and rectal, are also highlighted. The physico-chemical properties and the *in vitro*/*in vivo* performances of various systems such as sustained release tablets, ceramic implants, nanoparticles, nanocontainers, liposomes, emulsomes, aspasomes, microemulsions, nanopowders and Pheroid™ are summarised. Further studies that remain to be undertaken for formulation optimisation are also identified. This review highlights the significant potential that novel drug delivery systems have for the future effective treatment of HIV/AIDS patients on ARV drug therapy.

1. Introduction to HIV/AIDS

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), commonly referred to as HIV/AIDS, constitute one of the most serious infectious disease challenges to public health globally, and has had a crippling effect in certain parts of the world especially in Sub-Saharan Africa [1–3]. There are currently 33.2 million people living with HIV/AIDS globally. Of this total number, an overwhelming 22.5 million people are HIV positive in Sub-Saharan Africa specifically, representing 67.8% of the global number [3]. Interventions such as AIDS counselling, educational tools and antiretroviral drug therapy have contributed to transforming HIV infection from a fatal to a manageable chronic infectious disease [4]. Despite the availability of these measures, the above statistics indicate that much remains to be accomplished as the number of newly reported HIV infections still remains unacceptably high.

There are currently two known species of HIV, viz., HIV-1 and HIV-2, with their respective subspecies. HIV-1 is the globally common infection while HIV-2 is more prevalent in West Africa, and takes a longer time to develop into immunodeficiency from infection than HIV-1 [5,6]. HIV infection in the human body results mainly from integration of the viral genome into the host cell for the purpose of cell replication, and AIDS is the advanced stage of the disease caused by HIV infection. The virus infects the host cell by binding the viral gp120 protein to two transmembrane receptors, i.e., CD4+ and either of the two chemokine receptors, CCR5 and CXCR4 [7]. HIV infects macrophages and T-helper lymphocytes (CD4+); but the defining feature of AIDS is the depletion of CD4+ cells. T-tropic viruses prefer to replicate in T cells, while M-tropic viruses prefer the macrophage. Of the HIV-1 viruses, M-tropic types predominate in the brain [8].

The viral genome contains three structural genes – *gag, pol* and *env* – and six regulatory genes – *tat*, *rev*, *nef*, *vif*, *vpr* and *vpu* [5]. The virus utilizes some of these genes to maximise its production using host cell resources. DNA microarray studies have implicated HIV encoded Nef protein in this process [9], and humans infected with the *nef*-deleted form of HIV have remained disease free for several years [10]. Interestingly, HIV has been referred to as a "master regulator" of cellular gene expression [9] as a means to augment expression of its own genome. An understanding of these processes is critical to developing novel therapeutic strategies for the suppression or elimination of the virus.

The immunopathogenesis of HIV/AIDS has been previously amply documented; from the time of infection to the end stage of the disease [5]. The end stage of the disease may be characterised by a spectrum of diseases [11] including opportunistic infections (such as *Pnuemocystis carinii* and *Mycobacteruim tuberculosis)*, dementia and cancer [6,11]. In addition to macrophages, lymph nodes, bone marrow, spleen and lungs, the CNS represents one of the most important anatomical sites of the virus after infection. This causes

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significant neuronal damage and loss that often leads to HIV associated dementia [12]. Without treatment, HIV-1 infection is nearly uniformly fatal within 5–10 years [11].

2. HIV/AIDS drug therapy and its current limitations

Although the development of drugs for HIV infection has undergone substantial progress, numerous uncertainties persist about the best way to manage this disease. Reports addressing this aspect have appeared in the literature [13]. At present, the different ARVs are classified under categories such as nucleoside reverse transcriptase inhibitors (NRTI), nucleotide reverse transcriptase inhibitors (NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and more recently fusion and integrase inhibitors [14]. Table 1 [15–19] lists the various drugs under the different classes, the available dosage forms as well as their half-lives and bioavailabilities. These drugs are administered as combined therapy as in the case of highly active antiretroviral therapy (HAART) [20]. Among the newer classes of drugs under investigation are the assembly and budding inhibitors [21], as well as the zinc finger inhibitors [22]. Virus assembly and disassembly are particularly attractive candidate processes for antiviral intervention. HIV-1 capsid (CA) protein and human cyclophilin A (CypA) play important roles in these processes, which consequently make them attractive targets of high priority [23].

Although ARV drug therapy has contributed significantly to improved patient/disease management, its current use is associated with several disadvantages and inconveniences to the HIV/AIDS patient. Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-life for several ARV drugs is short, which then requires frequent administration of doses leading to decreased patient compliance [24]. A major limitation is that HIV is localised in certain inaccessible compartments of the body such as the CNS, the lymphatic system and within the macrophages. These sites cannot be accessed by the majority of drugs in the therapeutic concentrations required; and the drugs also cannot be maintained for the necessary duration at the site of HIV localisation [25]. These subtherapeutic drug concentrations and short residence time at the required sites of action contribute significantly to both the failure of eliminating HIV from these reservoirs, and the development of multidrug-resistance against the ARVs [26]. The severe side effects associated with ARV therapy can therefore be attributed to the subsequent large doses essential for achieving a therapeutic effect, due to the inadequate drug concentrations at the site of action, and/or the poor bioavailability of several ARV drugs. These drugs also suffer from physico-chemical problems such as poor solubility that may lead to formulation difficulties [27,28]. Strategies currently being investigated to overcome these limitations include the identification of new and chemical modification of existing chemical entities, the examination of various dosing regimens, as well as the design and development of novel drug delivery systems (NDDS) that can improve the efficacy of both existing and new ARV drugs. More specifically, in the past decade there has been an explosion of interest in the development of NDDS for the incorporation of ARV drugs as a way of circumventing the problems described above and optimising the treatment of HIV/AIDS patients. To the best of our knowledge, the last review paper on NDDS for ARV drugs appeared in 1993 [28]. There have since been significant advancements of the systems described in that paper, and further new NDDS for ARV drugs have since emerged in the literature. The purpose of this paper is therefore to present a comprehensive review of the various NDDS, including studies on alternative routes of administration that have emerged for ARV drugs. This will identify the progress that has been achieved both for the technological development of these delivery systems, and their clinical potential for overcoming the limitations associated with current ARV therapy. This review will also enable the identification of future studies that remain to be undertaken for its optimisation and ultimately its commercialisation.

3. Novel drug delivery systems for ARV drugs

3.1. Sustained release/bioadhesive/enteric coated matrix tablets

Sustained drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimisation of drug related side effects due to controlled therapeutic blood levels instead of

Table 1
Examples of antiretroviral drugs, their commercially available dosage forms, bioavailabilities and half-lives

Name and class of drug	Dosage form [15–19]	F (%)* [15–19]	Half-life (h) [15–19]
Zidovudine(NRTI)	Capsule, liquid	60	1.1
Lamivudine(NRTI)	Tablet, liquid	86	3–6
Didanosine (NRTI)	Tablet, capsule (EC), liquid	30–40	1.3–1.6
Zalcitabine(NRTI)	Tablet	85	1–3
Stavudine(NRTI)	Capsule, powder for reconstitution	80	1–1.6
Abacavir(NRTI)	Tablet, liquid	83–100	1–2
Emtricitabine(NRTI)	Capsule	93	10
Tenofovir NtRTI	Tablet	25–39	17
Nevirapine (NNRTI)	Tablet, syrup	>90	25–30
Efavirenz(NNRTI)	Tablet, capsule, solution	42–80	40–50
Delavirdine(NNRTI)	Tablet	85	5.8
Etravirine (NNRTI)	Tablet	Unknown	30–40
Amprenavir(PI)	Capsule, solution	No data	7–10
Indinivir(PI)	Capsule	65	1.2–2
Saquinavir(PI)	Tablet, capsule	Erratic, 4	1.5–2
Nelfinavir(PI)	Tablet, powder	20–80	3.5–5
Ritonavir (PI)	Tablet, capsule, liquid	65	3–5
Atazanavir(PI)	Capsule	No data	7
Darunavir(PI)	Tablet	37	15
Enfuvirtide (Entry and FI)	Powder for subcutaneous injection	84.3	3.8
Maraviroc (Entry and FI)	Tablet	23–33	14–18
Raltegravir (II)	Tablet	No data	9

NRTI, nucleoside reverse transcriptase inhibitors; NtRTI, nucleotide reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; FI, fusion inhibitors; II, integrase inhibitors, F, Bioavailability.

oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered [29,30]. Bioadhesive drug delivery systems are designed for prolonged retention on the mucosa to facilitate drug absorption over a prolonged period of time by interacting with mucin [31]. Hence, the combination of both sustained release and bioadhesive properties in a delivery system would further enhance therapeutic efficacy. ARVs such as didanosine (ddI) would be an ideal candidate for sustained drug release due to its short half-life of 1.3–1.6 h, necessitating frequent administration of doses, as well as its severe dose dependent side effects [24]. In an attempt to improve the oral absorption of ddI by delivering it over a prolonged period of time as well as prolonging retention on the mucosae, Betageri et al. [32] prepared a sustained release bioadhesive tablet formulation of ddI, containing Polyox WSRN-303, Carbopol 974P-NF and Methocel K4M as polymeric matrix materials. Hydrogel forming tablet formulations with 10% and 30% Polyox WSRN-303 were able to extend the release of ddI (Fig. 1), while 30% Methocel K4M was required for extending the drug release in other formulations. Preparations with Carbopol 934P prevented complete release of ddI from the tablet during the test period, and the authors attributed this to drug–polymer interactions. The bioadhesivity also increased with an increase in polymer concentration. These researchers concluded that a single polymer could be used for the preparation of hydrogel matrix ddI tablets designed to provide both sustained release and bioadhesivity. However, while a single polymer may provide both bioadhesivity and sustained drug release, it has since become well recognised in the literature, via various *in vitro* drug release and bioadhesivity tests during formulation studies, that simultaneous optimisation of both these properties may require the blending of various polymers [33–35] for both single and multiple unit systems. These systems remain to be investigated for their clinical applicability.

ddI controlled release matrix tablets containing methacrylic (Eudragit RSPM) and ethylcellulose (Ethocel 100) polymers have also been prepared by Sanchez-Lafuente et al. [36]. The ddI 500 mg tablets (5, 10 or 15%w/w) were prepared by direct compression, and comprised Eudragit® RSPM and Ethocel® 100 in varying ratios (75/25, 50/50 and 25/75 w/w). The physical characteristics in terms of weight, thickness and diameter confirmed the excellent compactibility properties of these polymers with ddI, which allowed for direct compression in the absence of other excipients. The drug release studies showed that varying polymer ratios could modulate the release of ddI as a result of the swelling properties of Eudragit® RSPM and plastic properties of the hydrophobic Ethocel® 100. Since these two polymers showed potential for modulating drug release, the subsequent study by this group focused on the use of a statistical experimental design for formulation optimisation as well as for identifying and quantifying the effects of formulation variables on drug release. Therefore, a Doehlert design was applied to evaluate the influence of variables and possible interactions among such variables on ddI release from the directly compressed matrix tablets based on the blends of the two insoluble polymers, Eudragit® RSPM and Ethocel® 100 [37]. The drug content and the polymers had the most significant effect on drug release, while the compression force had no significant effect. The optimum formulation conditions identified in the studied experimental design for a formulation with optimum drug release were Eudragit–Ethocel ratio of 83/17 (w/w) and a drug content of 13%w/w. The experimental values obtained from the optimised formulation highly agreed with the predicted values, thereby validating the mathematical model used in the preparation of ddI tablets.

ddI also undergoes acid degradation in the gastric medium [38]. An enteric coated matrix tablet formulation that combines sustained drug release, bioadhesivity and an enteric coating to resist acid degradation to maximise therapeutic efficacy has also been reported. Deshmukh et al. [39] reported the preparation of enteric coated, sustained release bioadhesive matrix tablets of ddI comprising Polyox, WSRN-303 and Methocel K4M with hydroxypropylmethylcellulose phthalate (HPMCP 5.5). The formulation was shown to be resistant to dissolution in 0.1 N HCl but dissolved within 10 min in PBS, pH 7.4. Furthermore, the stability of the formulation for 6 months at varying storage conditions was confirmed. Permeation studies on the matrix tablets showed that Polyox WSRN-303 containing tablets demonstrated higher ddI permeability across live intestinal tissue compared with conventional tablets.

While the above tablets sought to provide sustained drug release, bioadhesion and resistance to gastric acid degradation, a possible limitation could be the fact that it would still undergo extensive first pass degradation since it is meant for oral administration.

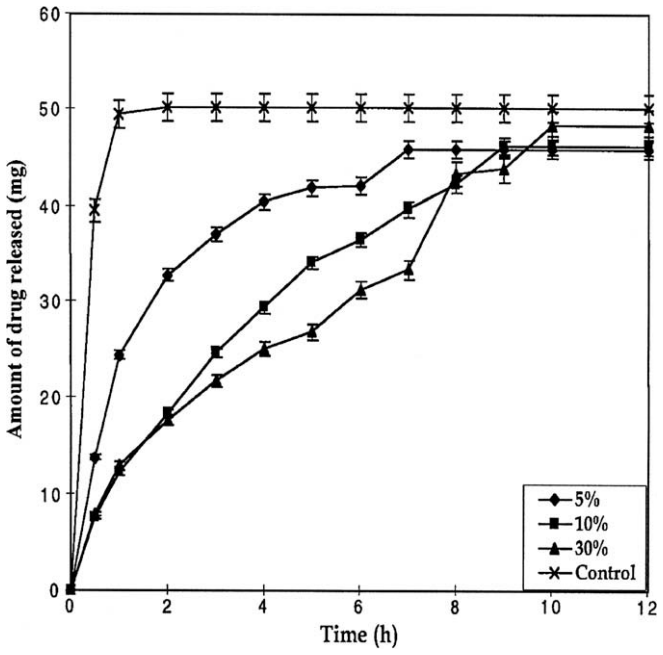

Fig. 1. Effect of Polyox WSRN-303 on the release of ddI from tablets (reproduced from Betageri et al. [32]).

3.2. Ceramic implants

Attempts have been made in the literature to explore the use of ceramic implants to modulate the release of antiretroviral drugs. Due to the adverse effects of AZT associated with oral and intravenous administration, Benghuzzi et al. [40] in early *in vivo* studies investigated the release of deoxynucleoside thymidine, the normal counterpart of azidothymidine (AZT), by means of alumino-calcium-phosphorous oxide (ALCAP) ceramic implantable capsules in rats. The results showed that thymidine could be released from the ALCAP ceramic capsules in a sustained manner for a minimum duration of 120 days. Based on the results with thymidine, they subsequently concluded that these implantable capsules could be considered for the delivery of AZT. Consequently, in a follow-up study [41], AZT was loaded into tricalcium phosphate (TCP) and ALCAP ceramic capsules. They showed that the rate of release of AZT from TCP capsules was lower than from ALCAP capsules. Fig. 2 confirms the sustained release of AZT from TCP ceramic capsules over 26 days when loaded with 20, 40 and 60 mg AZT.

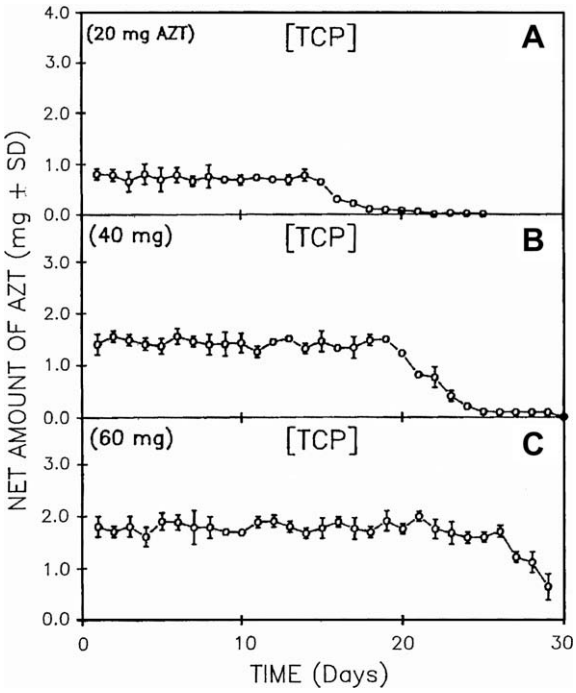

Fig. 2. Release of AZT from TCP ceramic capsules (reproduced from Benghuzzi et al. [41]).

To further control release, Nagy and Bajpai [42] extended this *in vitro* study by preparing a TCP ceramic delivery system containing thymidine and AZT by determining the effect of sesame seed oil or wheat germ oil on their release. Ceramic capsules were prepared by pressing 1 gram of <38 μm beta-TCP particles with or without the stipulated quantity of thymidine or AZT in a 10 mm die at a load of 4000 lbs in an electric hydraulic press. They found that sesame seed oil and wheat germ oil (Vitamin E) could delay the release of thymdine and AZT from TCP drug loaded capsules. Further, incorporation of thymidine or AZT in the form of a compressed pellet also retarded its release from the TCP ceramic capsules prepared with oil treated ceramic particles. The above studies were extended to an *in vivo* study later [43]. Three ceramic devices were implanted subcutaneously in Sprague–Dawley rats for 2 weeks. The *in vivo* studies showed that oil saturated TCP and AZT devices as well as the AZT pellet inserted in an oil saturated TCP shell device were able to retard AZT release at a significantly lower rate than the AZT and TCP untreated devices. These authors concluded that the treatment of ceramic devices with oil decreased the release rate and prolonged the delivery of AZT. The inclusion of wheat germ into another ceramic device, hydroxyapatite (HA) composite, was also able to deliver AZT for prolonged periods *in vitro* [44].

A subsequent *in vivo* study by Benghuzzi [45] compared the release of AZT from two commonly studied ceramic implants, i.e., TCP and HA. Sterilised drug loaded ceramics containing AZT in three dosages (40, 60 and 90 mg) were inserted under the skin of rats using standard surgical techniques. The data from this study showed that AZT release rates from TCP ceramic implants (30 mg = 2.38 ± 0.23 ng/mL, 60 mg = 4.64 ± 1.03 ng/mL and 90 mg = 11.92 ± 2.35 ng/mL serum AZT) were significantly higher than from HA ceramic implants (30 mg = 0.84 ± 0.05 ng/mL, 60 mg = 2.40 ± 0.83 ng/mL and 90 mg = 6.41 ± 1.24 ng/mL serum AZT). The authors concluded that TCP and HA ceramic implants could be considered effective for delivering AZT in quantities required for providing physiological responses *in vivo*. The sustained drug release profiles obtained indicated that large fluctuations of AZT concentrations in the blood stream and tissues, as with conventional routes of administration, could be eliminated using ceramic drug delivery systems.

While ceramic implants were actively studied between 1990 and 2000, there appears to be no further work since reported for ARV containing ceramic implants.

3.3. Liposomes

Liposomes, ranging in size between 25 nm and several microns, are microscopic vesicles that comprise one or more phospholipid bilayers which surround an aqueous core. They are prepared from natural or synthetic phospholipids and cholesterol, and may also additionally include other lipids and proteins. The aqueous core facilitates the entrapment of hydrophilic drugs, while hydrophobic drugs are bound to or incorporated in the lipid bilayer. When administered, liposomes are recognised as being foreign, and are immediately taken up by cells of the mononuclear phagocytic system (MPS). Since the HIV virus localises in these cells, liposomes therefore represent a suitable drug delivery system for targeting ARVs into infected cells; and thus have the potential of improving the efficacy of drugs and reducing side effects [46–48].

The effect of liposomal encapsulation of AZT in mice was determined in early studies [49,50]. Unlike injections of free AZT, liposomal encapsulated AZT showed no bone marrow toxicity with normal erythrocyte and leukocyte profiles. Also, enhanced localisation in the liver, spleen and lung was found with the AZT liposomes. Liposomal encapsulated AZT further reduced haematopoietic toxicity and resulted in enhanced antiretroviral activity in mice. Liposomal formulations have also been prepared for administration of AZT by the transdermal route [51]. The optimised liposomal formulation showed a transdermal flux of 98.8 ± 5.8 μg/cm^2 across rat skin as compared to 5.72 ± 0.3 μg/cm^2 for the free drug, and this should contribute to an improved bioavailability. These liposomes for the transdermal route were also able to target the RES organs more effectively.

Liposomes containing ddI were initially studied by Harvie et al. [52]. They found that the elimination plasma half-life of 112 and 83 nm liposomal ddI was 46 and 14 times higher than that of the free drug, respectively. They also reported efficient targeting of lymph nodes and macrophage-rich tissue with these conventional liposomes. In a subsequent study, they were able to extend further the ddI half-life in plasma from 3.9 h for conventional liposomes to 14.5 h by incorporating it into sterically stabilised liposomes. Following intravenous injection, the majority of the sterically stabilised liposomes also concentrated in the spleen with a peak level at 24 h (Fig. 3) [53].

Apart from AZT and ddI, zalcitabine (ddC) has also been investigated for encapsulation into liposomes by Makabi-Panzu et al. [54,55]. The ddC loaded liposomes were more rapidly taken up by the mouse macrophage cell line than the free ddC. They also reported that a high intracellular uptake of ddC was facilitated by the anionic nature of liposomes. To be pharmacologically active, dideoxynucleosides such as ddC must be phosphorylated into 5′-triphosphates by cellular kinases. Since some cell types have a low ability to phosphorylate these compounds, administration of the phosphorylated form of the drug would be most suitable. However, this would not be feasible as cell membranes are impermeable to the phosphorylated form, and phosphatases present in body fluids hydrolyse nucleotides into the corresponding nucleosides [56]. To overcome this limitation and to obtain site specific delivery, the antiviral effects of ddC and ddC-triphosphate (ddC-TP) and liposome encapsulated ddCTP (L(ddCTP)) were established and compared in cultured, human monocyte macrophages infected with HIV-1 [57]. ddCTP was dephosphorylated before entering the cells, while L(ddCTP) remained stable over days. These preparations

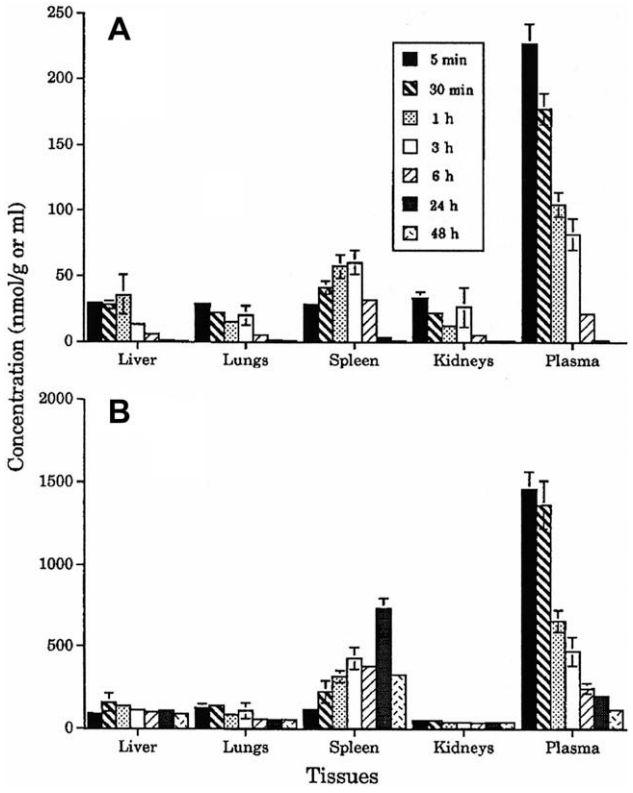

Fig. 3. Plasma and tissue distribution of ddI ([^{3}H]ddI) (A) and liposomal lipids ([^{14}C]DPPC) (B) from sterically stabilised liposome-encapsulated ddI after the administration of a single intravenous dose (3 mg of ddI per kg) to rats. Values are the means obtained for four to six animals per group per time point (reproduced from Harvie et al. [53]).

were also able to inhibit replication at nanomolar drug levels. Data obtained from liposome encapsulated ddCTP in a murine acquired immunodeficiency syndrome (MAIDS) model have also showed reduced proviral DNA in cells of the MPS in both spleen and bone marrow [58].

Liposomes have also been explored for the encapsulation and delivery of newly synthesized prodrugs. Lalanne et al. [59] synthesized two novel glycerolipidic ddI conjugates as prodrugs to avoid hepatic first pass metabolism. Liposomal formulations (1160 ± nm) of the prodrugs displayed antiviral activity and showed promise as formulations for enhancing drug bioavailability. Due to the low entrapment efficiency and high leakage of AZT from liposomes [48], AZT-myristate (AZT-M) has been synthesized as a prodrug and investigated for its potential for liposomal encapsulation. A high entrapment efficiency of 98% was achieved with higher plasma AZT being achieved with the AZT-M liposomes as compared to free AZT solution. Higher concentrations of AZT in organs of the RES and brain were also found with the liposomal preparation. This study could have been enhanced if AZT-M liposome preparations were compared not only with free AZT, but also with AZT entrapped liposomes. Prodrug liposomal preparations therefore offer the opportunity of not only more efficient targeting but also improved drug action and formulation processing.

In addition to liposomes having PEG chains attached to its surface, for increasing circulation time *in vivo* [60,61], active targeting of HIV infected cells can also be obtained by using liposomes that have surface attached ligands that specifically promote receptor interaction at the site of targeting [47] as well. Using the antibody, H-2-K(k), for Fc-mediated targeting, Betageri and Burrell [62] showed that the lipid composition of ddI-triphosphate liposomes influenced conjugation of antibodies and also retention of the

Table 2
Area under the curve for free and immunoliposomal indinavir in tissues after a single subcutaneous administration in mice (Reproduced from Gagne et al. [63])

Tissue	Immunoliposomal indinavir	Free indinavir	Ratio immunoliposomal/ free indinavir
Cervical lymph nodes	523.2	7.6	68.8
Brachial lymph nodes	617	4.9	126.0
Mesenteric lymph nodes	192.8	6.4	30.1
Inguinal lymph nodes	144.5	4.1	35.2
Popliteal lymph nodes	134.2	4.5	29.8
Liver	733.3	35.0	21.0
Spleen	211.3	5.3	39.9
Plasma	77.8	2.3	33.8

encapsulated drug. Sterically stabilised immunoliposomes containing grafted anti-HLA-DR antibodies were effective in enhancing the concentrations of indinavir (INV) in all tissues leading to a 21- to 126-fold increased accumulation as compared to the unencapsulated drug (Table 2) [63]. Also, immunoliposomal INV was as efficient as the free agent to inhibit HIV-1 replication in cultured cells. Lectin receptors, which act as molecular targets for sugar molecules, are found on the surface of cells of the mononuclear macrophage system (MPS), and have also been included in the strategy to improve site specific drug delivery. Using a mannose binding protein, concanavalin A, maximum cellular drug uptake occurred when mannosylated liposomes containing stavudine (D4T) were used [64]. Other sugar molecules used for liposomal formulations to target cells of the MPS include galactosylated D4T and AZT liposomes [65,66]. Together, these studies confirmed enhanced targeting to tissues rich in galactose specific receptors, and confirmed their potential of providing sustained drug release characteristics. Slepushkin et al. [67] have also reported that synthetic peptides can bind specifically to HIV infected cells. The potential of various ligands for active targeting of ARV loaded liposomes has therefore been confirmed, and shows potential for formulation optimisation.

In addition to targeting liposomes to the phagocytic system, other areas in the body have also been of interest. Kompella et al. [68] evaluated the effect of neutral liposomes on corneal and conjunctival permeability of ddI. While the liposomal formulations were able to encapsulate ddI and permeate through the rabbit conjunctival mucosa, the permeability coefficient, initial flux and tissue levels of ddI at the end of the transport study were actually lower in the presence of liposomal formulations. These neutral liposomes failed to enhance the corneal or conjunctival transport or uptake of ddI.

One of the disadvantages of liposomes is the poor stability in terms of drug retention and poor encapsulation. When assessing the stability of ARVs incorporated into liposomes, Betageri [69] found that lipid composition influenced encapsulation and retention of ddI-triphosphate (ddITP); and that its retention in the DMPC:CHOL liposomes was maximum when stored at 4 °C.

A novel liposomal formulation, i.e., "emulsomes" for sustained and targeted delivery of AZT to the liver has recently been described by Vyas et al. [25]. Emulsomes are a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. In addition to demonstrating a retarded drug release profile (12–15% after 24 h), studies in rats showed better uptake of the emulsomal formulations by the liver cells. We agree with the researchers that this proposed cationic emulsome-based system shows excellent potential for intracellular hepatic targeting.

Liposomes have clearly been more extensively investigated for their *in vitro* and *in vivo* properties than other NDDS for ARV delivery. A greater number of drugs and prodrugs have been encapsulated, and additional formulation optimisation techniques and *in*

vivo evaluations have been undertaken. These studies highlight and underscore the potential benefits of liposomes for improving ARV drug therapy.

3.4. Nanoparticles

Drug encapsulated nanoparticles are solid colloidal particles that range from 10 to 1000 nm in size [70]. Based on their size and polymeric composition, they are able to target drug to specified sites in the body, and have also shown potential for sustained drug delivery [71]. Nanoparticles have also been explored for improving the formulation and efficacy of drugs with physicochemical problems such as poor solubility and stability [72]. They are being increasingly investigated for targeted delivery of ARVs to HIV infected cells and to achieve sustained drug release kinetics. Their encapsulation into such systems may provide improved efficacy, decreased drug resistance, the reduction in dosage, a decrease in systemic toxicity and side effects, and an improvement in patient compliance.

Cells of the mononuclear phagocytic system (MPS), such as the monocytes/macrophages (Mo/Mac), act as a reservoir for the HIV virus [73]. Therefore, drug treatment of HIV infection should involve targeting drugs to these cells in addition to the lymphocytes. Several studies involving ARV loaded nanoparticles for targeting to the macrophages have consequently emerged. In an early preliminary study, Schafer et al. [74] prepared AZT loaded polyalkylcyanoacrylate (PACA), polymethylmethacrylate (PMMA) and human serum albumin (HSA) nanoparticles. This study confirmed uptake of the nanoparticles into macrophages isolated from HIV infected patients. The same group also later prepared and confirmed the potential of human serum albumin and poly(hexylcyanoacrylate) nanoparticles loaded with the nucleoside analogues, AZT and ddC for the targeting of macrophages. These *in vitro* studies were also undertaken using macrophages isolated from the peripheral blood of healthy blood donors and transmission electron microscopy [75]. Saquinavir (SQN) and ddC have also been loaded into poly(hexylcyanoacrylate) nanoparticles [76] by emulsion polymerization. While ddC showed no superiority to an aqueous solution of the drug in terms of reducing the HIV-1 antigen production, a significantly higher efficacy was observed for SQN loaded nanoparticles as compared to its aqueous solution. An *in vivo* study in rats to investigate the oral delivery of AZT bound to hexylcyanoacrylate nanoparticles for delivery to the reticuloendothelial cells was undertaken by Löbenberg, Araujo, and Kreuter [77]. The area under the curve (AUC) of [^{14}C] AZT in the liver was 30% higher when the drug was bound to nanoparticles than after administration of the solution. Higher AZT levels were also found in the blood and brain when nanoparticles were used as compared to the control solution. In an *in vivo* study a year later using the intravenous route instead, they showed that AZT concentrations were up to 18 times higher in organs of the RES if the drug was bound to nanoparticles as compared to unbound AZT [78]. Surface modification of nanoparticulate systems with hydrophilic groups such as polyethylene glycol has been shown to influence the biodistribution of nanoparticles [79]. Using THP-1 human monocyte/macrophage (Mo/Mac) cell line, Shah and Amiji [80] showed that a significantly higher percentage of the administered dose of nanoparticles was internalized within the cells when SQN was incorporated into poly(ethylene oxide)-modified poly (epsilon-caprolactone) nanoparticles (200 nm). Also, intracellular SQN concentrations were significantly higher when administered in the surface-modified nanoparticles as compared to its aqueous solution. A possible limitation of this study is that while aqueous solutions of SQN were compared to SQN PEG-modified nanoparticles, a comparative study with surface-unmodified SQN nanoparticles was not performed. This would have provided greater insight to the contribution of PEG specifically for ARV delivery. Most recently, the uptake of AZT loaded poly(lactic acid)–poly(ethylene glycol) nanoparticles by polymorphonuclear leukocytes *in vitro* was shown to be dependent on PEG and its ratio in the polymer [81].

Since the HIV virus can migrate to, multiply and localise in the CNS causing several neurological disorders, targeting of ARV drugs to the brain has become a significant goal for drug therapy. The blood–brain barrier (BBB) prevents access of ARVs to the brain due to the tight endothelial cell junctions of the brain capillaries and the presence of efflux transporters on the cell surface [81]. Nanoparticulate systems promote drug delivery in the brain, since they may gain entry by means of endocytosis/phagocytosis and are also moved away from the vicinity of efflux pumps [82,83]. Kuo [84] therefore loaded D4T into polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) nanoparticles for brain targeting. Drug loading of the nanoparticles (59.5–149.2 nm) was inversely proportional to particle size, and was also affected by freeze-drying and preservation as it influenced particle size. Similar to other studies [85], they also found pH to be critical, since variation in pH value of the loading medium from pH 7.2 led to a reduction in the loading efficiency of D4T. Kuo and Chen [86] then evaluated the effects of size of PBCA and MMA-SPM nanoparticles and alcohol on the permeability of AZT and lamivudine (3TC) across the BBB using blood–brain-microvascular endothelial cells model (BMEC). Both loading efficiency and permeability of AZT and 3TC decreased with an increase in the particle size of the two polymeric carriers. While PBCA nanoparticles increased the BBB permeability of AZT and 3TC 8- to 20- and 10- to 18-folds, respectively, the MMA-SPM nanoparticles led to a significant 100% increase in the BBB permeability of both drugs. A 4–12% enhancement in the BBB permeability of the two drugs with 0.5% ethanol was attributed to temporary unfolding of tight junctions among BMECs upon treatment with alcohol. In a subsequent paper, these authors compared the transport of D4T, delavirdine (DLV) and SQV across the *in vitro* BBB using (PBCA), (MMA-SPM) and also solid lipid nanoparticles (SLNs) [83]. These various polymeric systems investigated enhanced permeability of the drugs with higher permeabilities being reported with smaller particle sizes. In their most recent paper, Kuo and Kuo [87] showed that exposure to an electromagnetic field (EMF) could further enhance drug permeability across the BBB. The potential of SLNs for targeted brain delivery of another ARV, atazanavir, has also recently been confirmed [88].

More recently, a novel approach was proposed by Dou et al. [89,90]. They postulated that the mononuclear phagocytes, as the principal reservoir for viral dissemination, could also serve as a transporter of antiretroviral drugs themselves, since they are responsible for dissemination of HIV, i.e., macrophages can enter into tissues that limit entry of many ARV drugs. In these two papers, they describe a macrophage-based nanoparticulate system as a carrier itself for indinavir (INV). A nanoparticle indinavir (NP-INV) formulation was prepared and packaged into bone marrow-derived macrophages (BMMs). The effects of this drug carrier on drug distribution and disease outcomes were assessed in immune competent and human immunodeficiency virus type 1 (HIV-1) infected humanised immune-deficient mice [89]. Significant lung, liver and spleen BMMs and drug distribution were observed. This initial study also reported reduced numbers of virus infected cells in plasma, lymph nodes, spleen, liver and lung as well as CD4(+) T-cell protection when the NP-IDV BMMs were administered to HIV-1 challenged humanised mice. Later, a similar NP-INV formulation was prepared with Lipoid E80 [90]. They reported sustained drug release from the macrophages. The administration of NP-INV, when compared to equal drug levels of free soluble INV, also significantly blocked induction of multinucleated giant cells, production of reverse transcriptase activity in culture fluids and

cell associated HIV-Ip24 antigens after HIV-1 infection. This study proved that the use of a macrophage-based NP delivery system has potential for the treatment of HIV-1 infections.

The use of ligands on nanoparticles for receptor-mediated targeting has just been reported in the literature [91,92]. Since macrophages contain various receptors such as mannosyl and galactosyl, Jain et al. [91] prepared mannosylated gelatine nanoparticles (MN-G-NP) (248–325 nm) (Fig. 4) with a drug encapsulation of 40.2–48.5%. Via fluorescence and *ex vivo* studies using alveolar macrophages from rats, they showed a 18.0 and 2.7 times higher uptake by the macrophages from MN-G-NPs as compared to the free drug and uncoated G-NPs (Fig. 5).

The use of nanoparticles for targeting other areas such as the gastrointestinal mucosa and associated lymphoid tissues has also been reported by Dembri et al. [93]. As compared to the drug solution, AZT loaded isohexylcyanoacrylate nanoparticles were able to efficiently concentrate AZT in the intestinal mucosa. They also found that the nanoparticles were also able to control the release of free AZT.

Solid lipid nanoparticles (SLNs) are prepared from lipids that remain in a solid state at room and body temperature. Heiati et al. [94] initially prepared SLNs consisting of AZT-palmitate (AZT-P) and trilaurin (TL) as the solid core with dipalmitoylphosphatidylcholine (DPPC), and a mixture of DPPC and dimyristoylphosphatidylglycerol (DMPG). Their study concluded that the loading of AZT-P was proportional to the concentration of phospholipids content, and was independent of the amount of trilaurin used. Phospholipids with transition temperatures below 37 °C increased drug release. In a subsequent study, coating the SLNs with a PEG layer on its surface further increased the levels of AZT in the blood, since PEG creates a steric barrier that reduces particle uptake, thereby prolonging circulation [95]. They also found that the SLN–PEG nanoparticles were able to decrease the drug release rate in plasma as compared to SLN particles without PEG. The studies by this research group confirmed that surface modification with PEG could be used for controlling drug release and the pharmacokinetic behaviour of SLNs.

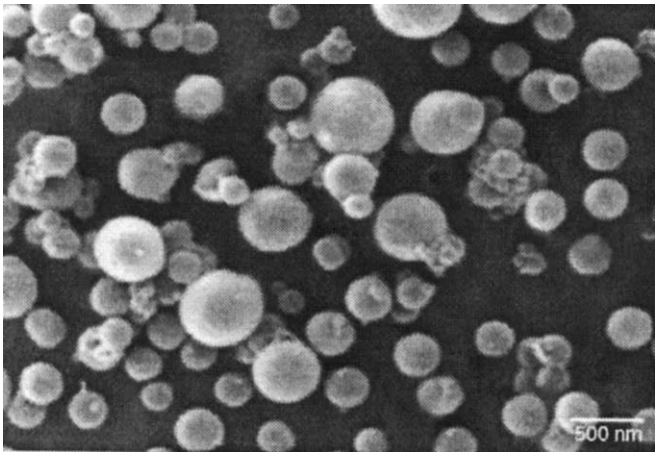

Fig. 4. Scanning electron micrograph of ddI loaded mannose coupled gelatin nanoparticles (30,000×) (reproduced from Jain et al. [91]).

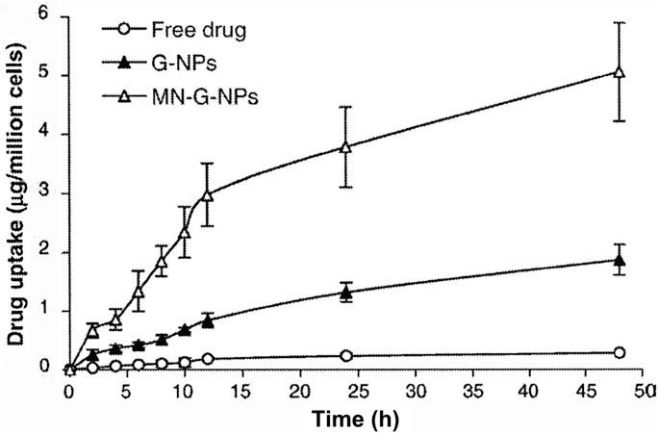

Fig. 5. Drug uptake from ddI containing mannosylated gelatin nanoparticles by alveolar macrophages at different time points at 37 ± 2 °C (reproduced from Jain et al. [91]).

While the majority of studies have focused on targeted delivery of ARVs with nanoparticles, some studies have also focused on modifications to its preparation to enhance drug loading and decrease toxicity; and also to increase its absorption by facilitating pH-sensitive drug release. Boudad et al. [96] prepared SQN loaded poly(alkylcyanoacrylate) nanoparticles and showed that incorporation of cyclodextrins enhanced the entrapment of SQN. Studies on the Caco-2 cell line showed that incorporation of cyclodextrins with nanoparticles decreased cytotoxicity when compared to blank and SQN loaded nanoparticles. The ability of cyclodextrins to mask to some extent the cytotoxic effects of the aliphatic alcohols originating from the hydrolytic degradation of the polymers was proposed as a possible reason for this effect. The oral bioavailability of a poorly water soluble HIV-1 protease inhibitor (CGP 70726-Novartis) was also enhanced when incorporated into pH sensitive nanoparticles prepared from poly(methacrylic acid-co-ethacrylate) copolymer Eudragit L100-55 [72].

The surge of interest in nanoparticulate systems for ARV therapy has led to several drugs being studied for its incorporation. These *in vitro*/*in vivo* studies clearly confirm the ability of nanoparticles to enhance the therapeutic efficacy of ARVs, as well as addressing formulation problems.

3.5. Nanocontainers

Dendrimer-based systems have also been explored for the concept of ARV targeting. Dendrimers are characterised as being synthetic, highly branched, spherical monodispersed macromolecules. Due to their unique architecture and macromolecular characteristics, they have emerged as an important class of drug carrier for targeted delivery [97,98]. Hence, not surprisingly, they have just been reported for targeting of ARV drugs. Recently, Dutta et al. [99] prepared poly(propyleneimine) (PPI) dendrimer-based nanocontainers for targeting of efavirenz (EFV) to Mo/Mac. Fifth generation PPI dendrimer, *t*-Boc-lycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimers (MPPI) were synthesized and used to prepare "nanocontainers". Like a dendritic box, these molecules act as closed containers of nanoscopic size containing the entrapped drug, and are therefore called nanocontainers. The drug entrapment efficiency of the nanocontainers varied, with the mannose conjugated dendrimer being 47.4%, followed by that of the PPI dendrimer (32.15%) and *t*-Boc-glycine conjugated dendrimer (23.1%). While the PPI dendrimer released the drug by 24 h, the dendrimer-based nanocontainers of *t*-Boc glycine and mannose conjugated dendrimers prolonged the release rate up to 144 h. The authors found significant increase in cellular uptake of EFV by Mo/Mac with nanocontainers of the mannose conjugated dendrimer being 12 times higher than that of free drug and 5.5 times higher than those of *t*-Boc-glycine conjugated dendrimer. Further, PPI showed a very high toxicity on HEPG2 cells while TPPI and MPPI had negligible toxicity (Fig. 6). These differences were attributed to the free terminal amino groups in PPI which is masked in MPPI and TPPI. This study therefore showed that mannosylated

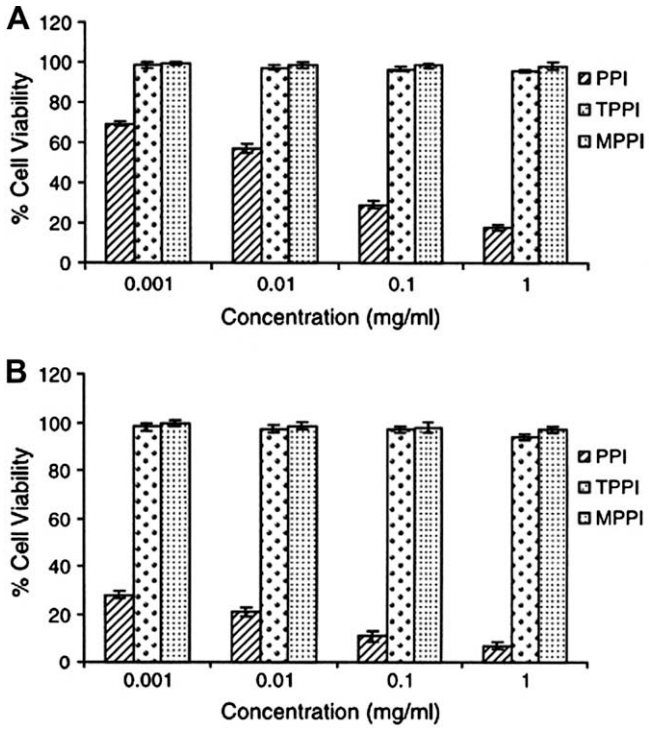

Fig. 6. Cytotoxicity of poly(propyleneimine) (PPI) dendrimer and its nanocontainers, *t*-Boc-lycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimers (MPPI) (a) after 24 h and (b) after 48 h of incubation for targeting of efavirenz (EFV) to Mo/Mac (values = mean ± SD, $n = 3$) (Reproduced from Dutta et al. [99]).

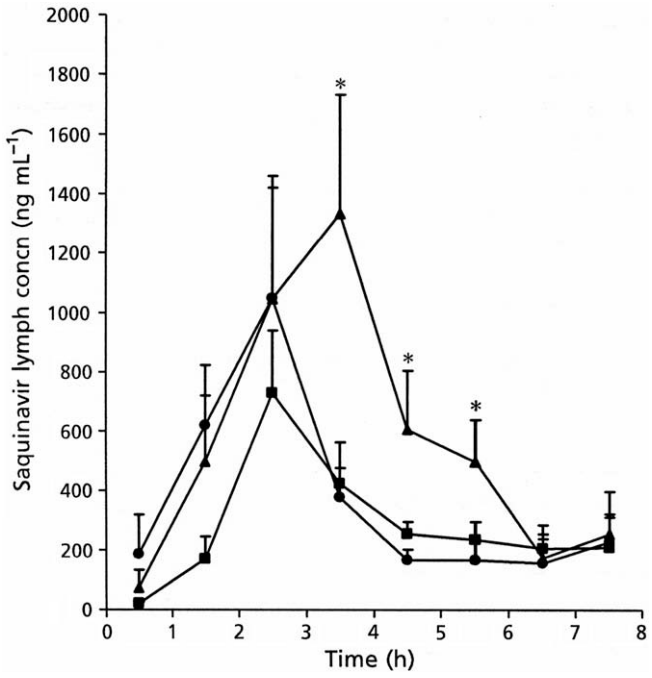

Fig. 7. Concentration of SQN in intestinal lymph versus time (mean ± SE, $n \geqslant 5$). SQN (5 mg) was administered intraduodenally to anaesthetized rats in a cremophor–oleic acid mixed micellar formation (closed circle), a TPGS–oleic acid mixed micellar formulation (closed circle) or as an oleic acid microemulsion (closed triangle) (Reproduced from Griffin and O'Driscoll [100]).

PPI dendrimers could be an effective carrier system for targeted delivery of EFV and possibly other ARVs.

3.6. Micelles and microemulsions

Microemulsions have been studied for ARV drug delivery as an approach to redirect the absorption of ARV from the portal blood to the HIV-rich intestinal lymphatics, thus enhancing the bioavailability of drugs that undergo extensive first pass metabolism and have poor oral bioavailability. Three formulations of SQN containing oleic acid have been studied [100] for targeted intestinal lymphatic transport using rats as the *in vivo* model: cremophor–oleic acid mixed micelles, D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)–oleic acid mixed micelles and an oleic acid microemulsion. The extent of lymphatic transport from the lipid vehicles was 0.025–0.5% of the dose administered. The microemulsion generated higher and more prolonged mesenteric lymph concentrations than the micellar formulations (Fig. 7). The systemic bioavailability was estimated to be 8.5% and 4.8% for the cremophor mixed micelle and the microemulsion, respectively. Since the cremophor mixed micelles produced higher bioavailability than TPGS mixed micelles, the researchers concluded that the nature of the surfactant can influence biodistribution of the drug between lymph and plasma.

3.7. Nanopowders

Most recently, nanopowders have been used as a delivery system for oral administration to enhance the dissolution rates of poorly soluble drugs. Tween 80/poloxamer 188 stabilised nanosuspensions of the hydrophobic ARV, loviride, were prepared by media milling, and sucrose co-freeze-dried to obtain solid nanopowders [101]. Morphological characterisation showed plate like structures in the nanopowder which was different from the morphology of untreated loviride crystals (Fig. 8). Loviride showed higher dissolution rates in nanosized products than in their respective physical mixtures, i.e., the amount of drug released after 15 min was 104.2% for the nanopowder prepared from freeze-drying with sucrose, 58% for the freeze-dried nanosuspension without sucrose, 54.8% for the physical mixture containing sucrose, 14.5% for the physical mixture without sucrose and 64.7% for the pure untreated loviride (Fig. 9). The addition of sucrose also further enhanced the dissolution rates. Caco-2 experiments revealed a significantly higher transport of loviride from the nanopowder formulation as compared to the physical mixture and the untreated loviride. Nanopowders were able to increase the dissolution rate due to its high surface area while sucrose had an additional enhancing effect due to its disintegrant properties.

3.8. Suspensions

Since studies with INV in HIV positive patients have indicated that drug concentrations in lymph node mononuclear cells were about 25–35% of mononuclear cells in blood, in a proof of concept study, Kinman et al. [102] showed that association of INV with lipids could enhance localisation in lymphoid tissues and also reduce the viral load. This was accomplished by preparing lipid associated complexes in suspension for subcutaneous injection to HIV-2287-infected macaques. They showed that INV concentrations in both peripheral and visceral lymph nodes were 250–2270% higher than plasma as compared with <35% with soluble lipid-free drug administration in humans. Also, administration of the INV–lipid complexes reduced significantly the viral RNA load and increased CD4 T cell number concentrations (Fig. 10).

3.9. Transdermal delivery

The advantages offered by drug administration via the transdermal route include avoidance of first pass effect and/or GI

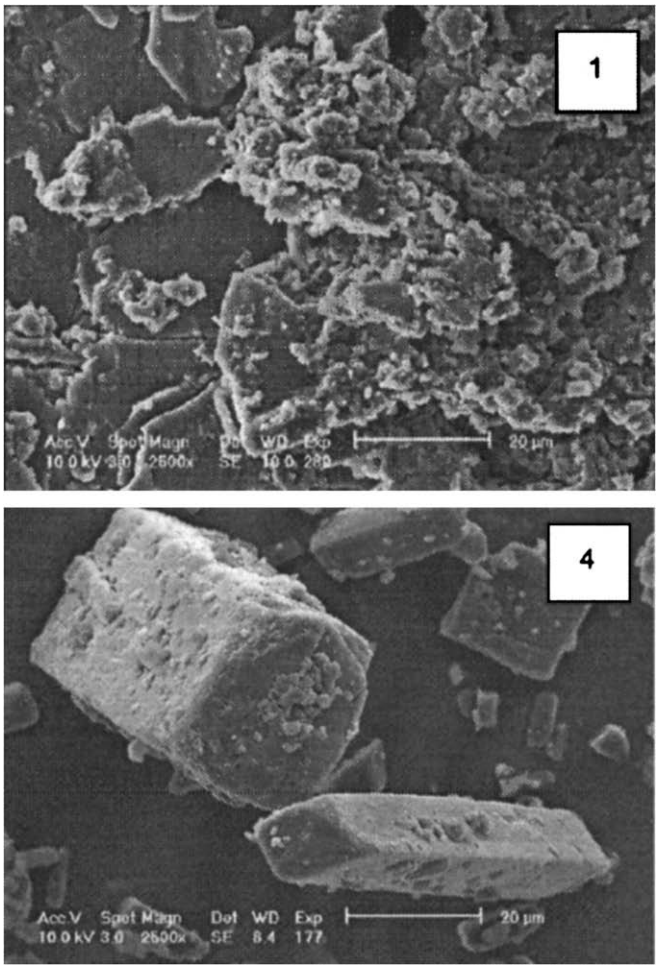

Fig. 8. Scanning electron micrographs of (1) nanopowder and (4) untreated loviride crystals (reproduced from Van Eerdenbrugh et al. [101]).

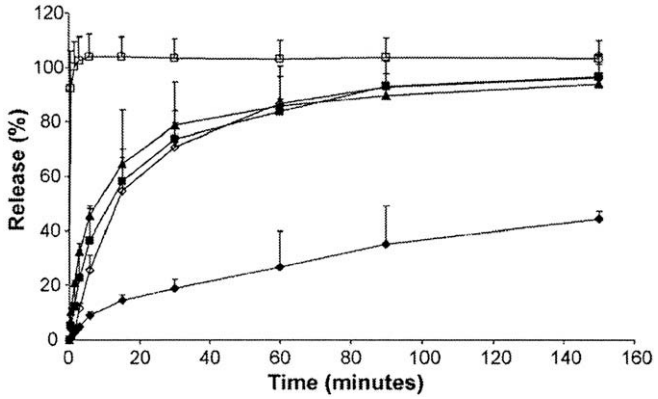

Fig. 9. Dissolution profiles: freeze-dried nanosuspension without sucrose (open diamond), physical mixture without sucrose (closed diamond), nanopowder (open square), physical mixture with sucrose (closed square), untreated loviride (closed triangle) (reproduced from Van Eerdenbrugh et al. [101]).

degradation, reduced fluctuations in plasma drug concentrations, excellent targeting of the drug for local effect as well as improved patient compliance [103,104]. The potential of ARVs for transdermal administration has therefore been extensively reported. The various transdermal permeation studies with ARV drugs specifically in terms of the focus/foci of the particular investigation and

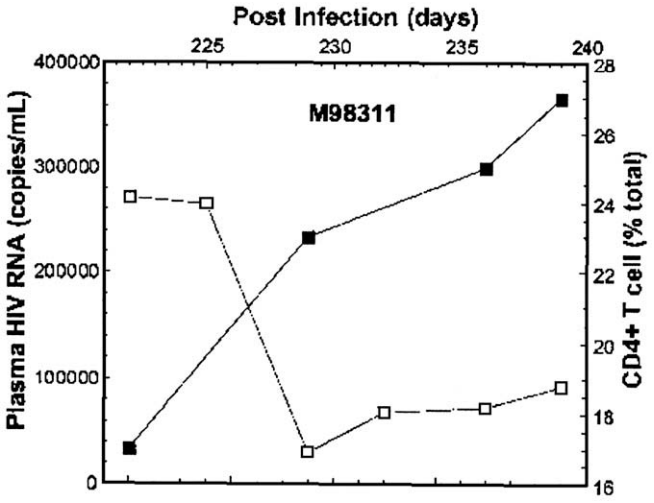

Fig. 10. Changes in plasma viral load of one HIV-2_{287}-infected macaques at 25 weeks postinfection and treated with 10 daily 20 mg/kg (SC) doses of lipid associated indinavir over 14 days (reproduced from Kinman et al. [102]).

the main outcomes of the study are summarised in Table 3. The most commonly investigated drug thus far for transdermal delivery has been the AZT, although there are some studies that have also investigated ddC and ddI for transdermal delivery. One of the limitations of transdermal delivery of drugs is poor skin/percutaneous penetration/absorption of drugs. Hence, the majority of ARV transdermal studies have focused on permeation enhancement investigating, *inter alia*, various chemical enhancers, types of vehicles (solvents/cosolvents), as well as iontophoresis and anodal current application. Table 3 identifies specifically the various penetration enhancers and vehicles that have been specifically investigated thus far. These various permeation enhancement variables either alone or in combination have been found to be beneficial in promoting ARV drug permeation through the skin.

In addition to comparative permeation enhancement studies with drug solutions, some studies have developed and evaluated transdermal delivery systems of an ARV drug. Gels containing AZT [105,106] and AZT patches using a gum matrix [107,108] have been developed. Both were found to be capable of facilitating ARV permeation, and the gel formulations were also found to be more stable than drug solutions. One of the first vesicular carriers to be studied for transdermal delivery of AZT was aspasomes [109]. These are vesicles formed from ascorbyl palmitate (ASP) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Fig. 11 shows that aspasomal AZT (ASP-AZT) was able to significantly enhance transdermal permeation of drug as compared to the AZT solution. Although lower than ASP-AZT, the higher drug permeation of ASP-AZT dispersion as compared to AZT free drug solution showed that ascorbyl palmitate had skin permeation enhancing properties. An elastic liposomal formulation of AZT has also enhanced transdermal flux, provided sustained drug release and improved site specificity of the drug [51]. Pheroid™ is a patented submicron emulsion which has been shown to entrap, transport and deliver several pharmacological compounds for enhanced therapeutic action [110,111]. Pheroid™ comprises essential and plant fatty acids, i.e., ethyl esters of the essential fatty acids, oleic, linolenic and linoleic acids, which are emulsified in water and saturated with nitrous oxide. As shown in Table 2, oleic acid is an effective permeation enhancer due to its kinked structure that briefly disrupts the packed formation of the intercellular lipids [112]. Recently, the use of Pheroid™ was investigated for its potential to enhance the transdermal permeation of ddC, 3TC and

Table 3
Summary of transdermal delivery studies on ARVs

ARV drugs	Focus/foci of study	Summary of main findings	Reference
AZT	Investigated effect of *N*-methyl-2-pyrrolidone (MP) as a penetration enhancer and ethylene–vinyl acetate copolymer membrane for controlled-release	Permeation of AZT was significantly enhanced and plasma concentration of AZT maintained for 10 h after the application of MP controlled-release transdermal system	[120]
ddI	Explored transfollicular absorption route for ddI and investigated effect of penetration enhancers, i.e., azone and propylene glycol. Determined the pharmacokinetics of ddI after topical application	Systemic bioavailability in high and low follicular density rats was similar indicating unimportant role of the transfollicular route for ddI. Transdermal delivery of ddI exceeded the oral bioavailability and was further increased by pre-treatment with absorption enhancers	[121]
AZT	Investigated the effect of *t*-anethole, carvacrol, thymol, linalool and L-menthol. Determined the *in vivo* performance of AZT gel formulation	Transport of AZT was optimum with 5% enhancer concentrations. *In vitro* studies produced higher amount and rate of AZT transport than *in vivo* studies	[106]
ddC ddI AZT	Determined stability profiles of drugs in solution when in contact with hairless rat skin and identified the degradation mechanisms of ddC and ddI	AZT was found to be stable for 30 h at 37 °C. ddC and ddI degraded by bacterial and ddI by cutaneous enzyme-degradation mechanisms. ddC was stabilised with thimerosal or gentamicin, while ddI was stabilised with *para*-chloromercuricbenzoic acid	[122]
ddC	Investigated the effects of ethanol/water and ethanol/tricaprylin cosolvents and other permeation enhancers such as oleic acid and *N*-methyl-2-pyrrolidone	Permeation rate across human cadaver skin was significantly lower than across hairless rat skin. Enhancement of ddC permeation using 1%v/v of oleic acid in ethanol/water (60:40) cosolvent was 4–5 times higher than target rate of 0.14 mg/cm^2/h to maintain the therapeutic blood level	[123]
AZT	Determined drug release from AZT patches made from Karaya gum through excised hairless mouse skin and also investigated the effect of enhancers	Thickness of gum matrix and enhancers such as propylene glycol, oleic acid and sodium dodecyl sulphate influenced drug release from patches. Permeation was best enhanced with propylene glycol/oleic acid/sodium dodecyl sulphate ternary system	[108]
ddC ddI AZT	Investigated effects of ethanol/water and ethanol/tricaprylin as cosolvent systems and oleic acid as permeation enhancer on permeation rate of each of the drugs alone	Permeation rates of AZT, ddC and ddI increased with ethanol/water and ethanol/tricaprylin cosolvent systems. Addition of oleic acid to the ethanol/water system enhanced permeation but did not with the ethanol/tricaprylin system. Permeation rates reached the target for required therapeutic levels with ethanol/water (60:40) containing oleic acid at 1.0%v/v	[124]
DdC DdI AZT	Investigated effects of ethanol/water and ethanol/tricaprylin as cosolvent systems and oleic acid as permeation enhancer on the simultaneous skin permeation of the three drugs together using hairless rat skin	Permeation rates of AZT, ddC and ddI increased with ethanol/water and ethanol/tricaprylin. Addition of oleic acid in ethanol/water (80:20) significantly increased permeation but not in the ethanol/tricaprylin (50:50) solvent	[125]
ddC ddI AZT	Compared the skin permeation rates of ddC, ddI and AZT, alone or in combination with various compositions of ethanol/water and ethanol/tricaprylin cosolvent systems, across human cadaver and rat skins	Human cadaver skin permeation rates of the drugs alone, or in combination were lower than the rat skin. The addition of oleic acid at 0.3–1% v/v increased permeation rate of all three drugs. 5% v/v oleic acid increased permeation rate of ddC and ddI in combination and saturated in ethanol/water (80:20)	[126]
ddC ddI AZT	Compared permeation rates of drugs. Permeation enhancing effects of ethanol/water systems and oleic acid were investigated	Permeation increased as volume fraction of ethanol increased. For ddC, ddI and AZT, addition of oleic acid (>2.0%w/v) in ethanol/water (70:30) further enhanced skin permeation rate. Enhancement for hydrophilic drugs was greater than for lipophilic drugs	[127]
AZT	Investigated transdermal flux of AZT using iontophoresis and propylene glycol/oleic acid. Effect of flux enhancement by iontophoresis was also investigated using a karaya gum matrix formulation of AZT and compared with AZT solution	Enhancement of transdermal flux by iontophoresis was smaller with the karaya gum matrix containing AZT. The iontophoretic flux from AZT solution increased about 4- to 5-fold. Penetration enhancers increased the passive flux 2- to 50-fold and worked synergistically with iontophoresis	[107]
AZT	Investigated permeation of AZT using penetration enhancers such as menthol, cineole, linolenic acid, oleic acid, in combinations of cineole or menthol with either oleic acid or linolenic acid or anodal current application	Permeability enhancing properties of the penetration enhancers were in the order of linolenic acid > menthol > oleic acid > cineole > vehicle. Combination of cineole and oleic acids enhanced permeation. Simultaneous application of the current with menthol and cineole significantly increased AZT permeation	[128]
AZT	Compared permeation of a AZT gel formulation including penetration enhancers (menthol and oleic acid) with solutions	Gel formulation was found to be more stable than solutions. There was no retardation in permeability of AZT in the gel formulation across the rat skin compared to the AZT solution. Combination of penetration enhancers at 2.5% w/w enhanced permeation	[105]
AZT	Investigated effects of binary vehicles [ethanol/water; isopropyl alcohol/water; polyethylene glycol/water; and ethanol/isopropyl myristate (IPM)], penetration enhancers [*N*-methyl-2-pyrrolidone (NMP); oleic acid; and lauric acid] and polymer [microporous polyethylene (PE) membrane] on permeation	Ethanol/IPM (50/50, v/v) demonstrated highest transdermal flux. Use of vehicle and enhancer combinations (ethanol/IPM 20/80 plus 10% NMP and ethanol/IPM 30/70 plus 10% NMP) resulted in increased AZT solubility as well as high AZT flux values, when compared to vehicles without enhancers	[129]
AZT	Investigated permeation of AZT across human cadaver skin and the effect of terpenes [L-menthol and 1, 8-cineole] on phase behaviour and molecular organization of a model Stratum Corneum (SC) lipid system	Terpenes enhanced permeation of AZT by transforming SC lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered hexagonal subcell packing. Terpenes caused disruption/alteration in the barrier property of SC and enhanced permeation of AZT more than ethanol and water	[130]
AZT	Evaluated the formation and transdermal permeation properties of aspasomes containing AZT	Proportion of cholesterol affected drug release rate with maximum retardation achieved with 45 mol% of cholesterol. Aspasomes had better antioxidant activity than ascorbic acid. Asposomal AZT enhanced transdermal permeation of the drug	[115]
AZT	Evaluated use of elastic liposomes for transdermal delivery of AZT	Elastic liposomes enhanced transdermal flux, provided sustained drug release and improved site specificity of AZT.	[51]
3TC ddC *N*-acyl-3TC esters	Determined the *in vitro* transdermal permeation of ddC, 3TC and synthesized 3TC esters through human epidermis with or without Pheroid™ as drug delivery system	Drugs with higher aqueous solubilities displayed greater transdermal flux values both in PBS and in Pheroid™. Transdermal flux values of drugs in Pheroid™ were lower than in PBS	[113]

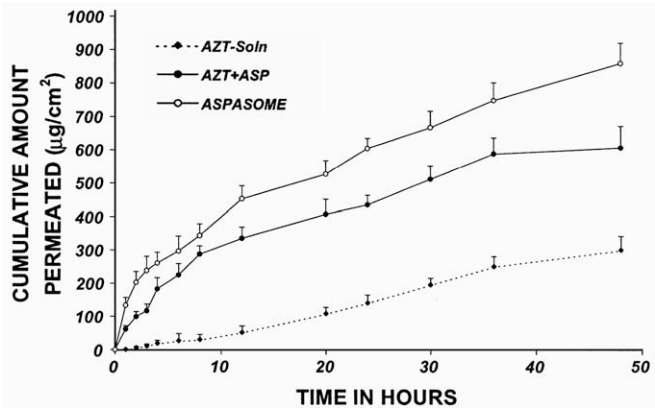

Fig. 11. *In vitro* permeation profiles of AZT across excised rat skin following treatment with various systems, i.e., aspasomal AZT (ASPASOME); AZT-ASP dispersion (AZT+ASP), free AZT solution (AZT-Soln) (reproduced from Gopinath et al. [109]).

several *N*-acyl lamivudine esters [113]. However, while the drugs were shown to be entrapped in the Pheroid™, the transdermal flux of the drugs in Pheroid™ was lower than in PBS. Hence, the Pheroid™ delivery system showed no practical advantage in terms of its transdermal application.

The various transdermal delivery studies with drugs such as ddI, ddC and AZT using various animal models such as the skin of rat, mouse, pig and human cadaver have confirmed the potential of ARV drugs for transdermal delivery.

3.10. Buccal delivery

Delivery of drugs via the buccal mucosa has received increased attention in the literature as an attractive alternative to the traditional oral and other conventional routes of drug administration. Use of the buccal mucosal route presents several advantages, such as the bypass of first pass hepatic metabolism and avoidance of gastrointestinal enzymatic degradation, thereby increasing the bioavailability of drugs [114]; higher permeability than that of the other routes such as the skin [115]; larger surface area for drug application, and good accessibility compared to other mucosal surfaces such as nasal, rectal and vaginal mucosa [116]. ARV drugs may therefore benefit from buccal mucosal administration instead of traditional oral administration.

Studies investigating the feasibility of the systemic buccal delivery of anti-HIV drugs have emerged. Shojaei et al. [117] initially investigated the use of a safe and effective permeation enhancer, i.e., menthol, on the buccal permeation of ddC. This study showed that the *in vitro* transbuccal permeation of ddC increased significantly in the presence of 1-menthol with an enhancement factor of 2.02 and a t_{lag} of 6 h. The permeation enhancement was not concentration dependent as no significant difference was observed between the permeation enhancement of ddC in the presence of 0.1, 0.2 and 0.3 mg/mL of 1-menthol [117]. Later, Xiang et al. [27] also studied the feasibility of transbuccal delivery of ddC using McIlvaine buffer solution (IMB). Their study focused on identifying the major permeation barrier within the epithelium of the buccal mucosa, the influence of sodium glycodeoxycholate (GDC) as a permeation enhancer as well as the histological effects of ddC on the buccal mucosa. These researchers reported that the basal lamina layer within the epithelium of buccal mucosa acted as an important barrier to the permeation of ddC. They also found that the permeability of ddC was significantly enhanced by GDC up to 32 times (Fig. 12). Histological studies revealed that the basal lamina remained intact, and no nucleated cell leakage was found within 24 h. These studies also showed that the thickness of epithelium was greatly reduced after buccal tissues were immersed in IMB solution for 12 and 24 h, and no difference was observed between the tissue samples incubated in the IMB and ddC IMP solutions. These two research groups concluded that transbuccal delivery is a potential route of administration of ddC, and hence for enhancing antiretroviral drug therapy.

Unlike the transdermal route, the buccal route for ARV permeation potential has not been comprehensively investigated. The reported studies to date have focused only on two different permeation enhancers, and no studies on the formulation and assessment of buccal delivery systems of ARVs could be found.

3.11. Rectal delivery

The rectal route has also been considered for effective delivery of ARV drugs that undergo first pass hepatic metabolism and/or extensive GI degradation. Two studies were found to have been reported in the literature. Sustained-release AZT suppositories were prepared [118] using hydroxypropyl cellulose (HPC), and were assessed in rats. It was found that AZT suppositories at 10 mg/kg maintained constant plasma levels above 1 µM for more than 6 h, and they subsequently proposed suppositories as an alternative drug delivery system for AZT (Fig. 13). A further study of rectal

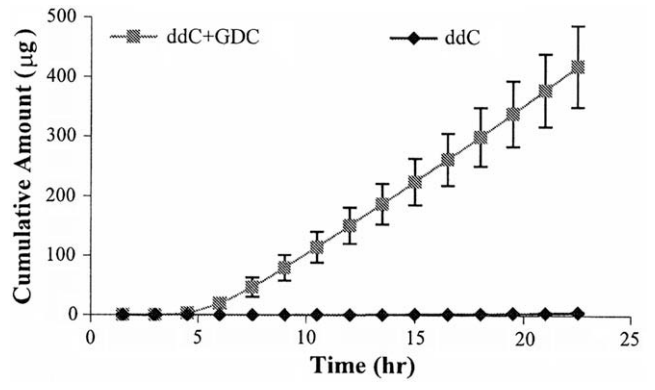

Fig. 12. Cumulative amount of ddC permeating through the porcine buccal mucosa without GDC (closed triangle) and with co-administration of GDC (closed square). Data are presented as means ± SD (n = 3) (Reproduced from Xiang et al. [27]).

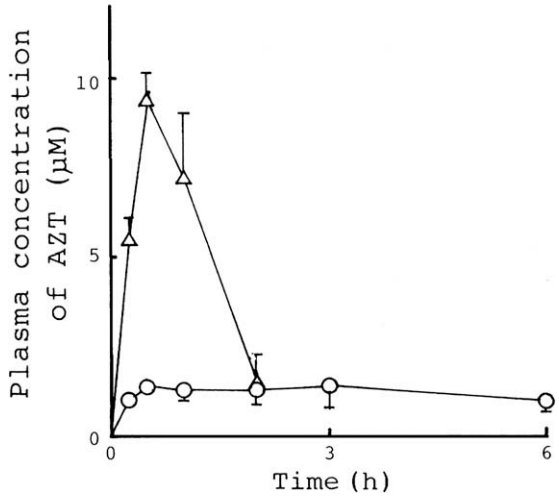

Fig. 13. Plasma concentration–time profiles following the administration of AZT suppositories: conventional (open triangle) and sustained release (open circle) (Reproduced from Kawaguchi et al. [118]).

administration of AZT [119] showed that the drug was considerably absorbed after rectal administration, with a pharmacokinetic profile that resembled that of a sustained-release delivery device. No further studies on this approach have since been identified in the literature. The work in this area appears to be limited, most probably due to patient inconvenience, as well as to the fact that HIV/AIDS patients often suffer from diarrhoea.

4. Conclusions and future studies

Despite significant advances that have been made in understanding the mechanism of HIV infection and in identifying effective treatment approaches, the search for optimum treatment strategies for AIDS still remains a major challenge. Results presented in this review indicate that novel drug delivery systems clearly present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral drug therapy. The use of such systems began in the early 1990s but it is only within the past 5 years that there appears to be a sudden surge of interest and publications in the use of novel drug delivery systems for ARV drugs. While several novel drug delivery systems have been investigated for ARV delivery, recently there appears to be greater interest and advancement in the use of liposomes and nanoparticles as compared to other systems. While the clinical potential for several NDDS has been reported from *in vitro* and animal studies, there is the lack of data on formulation optimisation and detailed physico-chemical/mechanical characterisation of these NDDS. Since HIV/AIDS treatment involves combination drug therapy, the potential of these novel drug delivery systems for simultaneous loading of various drug combinations needs to be investigated. While the potential of alternate routes of ARV drug administration such as transdermal and buccal has been confirmed, the design and development of drug delivery systems for these routes specifically are currently lacking. Correlations between the performances of these systems with their permeation potential need to be established. Although various papers report efficacy studies under *in vitro* conditions including experimental animal studies, there is the significant lack of data on the clinical applicability (human *in vivo* studies) and toxicity of these preparations. These therefore need to be extensively explored. Based on the complexity of the disease and the formulation optimisation and evaluation studies required, multidisciplinary research would be essential for eventual commercialisation of NDDS containing ARV drugs.

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