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Recent Advances in NDDS (Novel drug delivery systems) for delivery of Anti-HIV 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. Currently available Anti-HIV drugs can be classified into three categories: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Most of these drugs bear some significant drawbacks such as relatively short half-life, low bioavailability, poor permeability and undesirable side effects. Efforts have been made to design drug delivery systems for antiHIV agents to: a) reduce the dosing frequency, b) increase the bioavailability and decrease the degradation/metabolism in the gastrointestinal tract, c) improve the CNS penetration and inhibit the CNS efflux, and d) deliver them to the target cells selectively with minimal side effects. 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 and buccal 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.

Keywords: HIV/AIDS; Antiretroviral drugs; Novel drug delivery systems

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INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), caused by Human immunodeficiency virus (HIV), is an immunosuppressive disease that results in life-threatening opportunistic infections and malignancies[1]. HIV infection is one of the major threats to human health due to the lack of relevant vaccine and drugs to cure AIDS.

Highly active anti-retroviral therapy (HAART) strategy involves the use of combination anti-retroviral agents for synergistic therapeutic outcomes. With the adoption of HAART, the average survival of HIV/AIDS patients has increased from less than 1 year to over 10 years [1, 2]. Despite the success of HAART in the clinics, HIV/AIDS therapy is far from optimal. One of the major problems in the chronic treatment is the fact that the viral particles are able to reside in cellular and anatomical sites in the body following replication and remain viable even when there are adequate drug concentrations in the blood [3, 4]. Examples of cellular reservoirs include T-lymphocytes, monocytes, and macrophages, while the major anatomical reservoirs include central nervous system (CNS), lymph nodes, liver, spleen, lungs, and the genitals [5]. Poor drug availability in the cellular and anatomical reservoirs is affected by expression of efflux transporters (e.g, P-glycoprotein), presence of drug metabolizing enzymes (e.g, cytochrome P-450), poor permeability properties, non-targeted distribution, and rapid clearance. The reduced bioavailability and short residence of anti-retroviral agents at these viral reservoir sites have profound impact on the clinical management of the disease. The overall consequence is that upon discontinuation of therapy or when drug resistance develops, HIV is able to re-seed the systemic circulation and continue to propagate the infection.

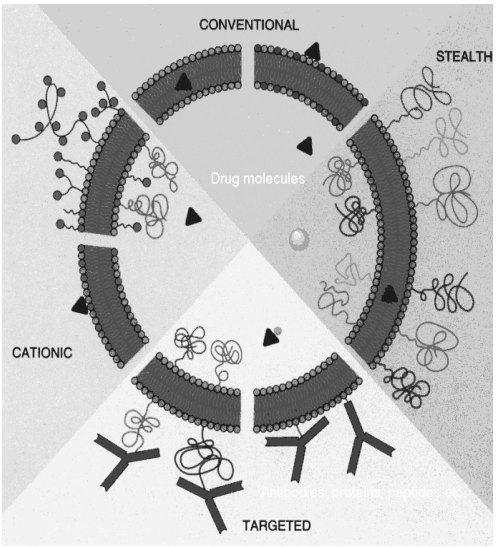
A new research that addresses from simple organoleptic or technological problems to more complex issues involving the targeting of specific tissues and organs has emerged. With the aim to reduce dosing frequency, to improve the compliance of the existing pharmacotherapy and to target viral reservoirs, the design of novel drug delivery systems is becoming complementary to new drug discovery.

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name niosomes, liposomes, nanoparticles, mucoadhesives, transdermal patches and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g, pH- or temperature-sensitive), and even targeted (e.g, by conjugating them with specific antibodies against certain characteristic components of the area of interest).
With the aim to reduce dosing frequency and to improve the compliance of the existing pharmacotherapy, the design of novel drug delivery systems (NDDS) is becoming complementary to new drug discovery. The goal of the present review is to describe state-of-the-art ARV NDDS and to thoroughly discuss the challenges in the development of medicines with enhanced biopharmaceutical properties. Several published reviews discussed different NDDS designed to optimize the delivery of ARV drugs Contrary to this, the goal of the present article is to highlight the drawbacks that, based on to their common molecular features, characterize the different families of ARV drugs and the qualities that

seriously hamper effective pharmacotherapy. Thus, a comprehensive overview of the different NDDS approaches is presented for each ARV group.

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.



The effect of liposomal encapsulation of AZT in mice was determined in early studies [6,7]. 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 [8]. The optimised liposomal formulation showed a transdermal flux of 98.8 ± 5.8 lg/cm2 across rat skin as compared to 5.72 ± 0.3 lg/cm2 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.[9]. 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 [10].

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. [11]. 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.

To overcome low water solubility and intestinal absorption, poorly water soluble indinavir and saquinavir were also loaded into phosphatidylcholine and phosphatidylglycerol liposomes.

For the last decades, topical delivery of drugs by liposomal formulations has evoked a considerable interest. Despite intensive research, results of the interaction of liposomes with skin are contradictory [12]. Recently, it became evident that traditional liposomes are of little or no value as carriers for transdermal drug delivery, because they do not deeply penetrate skin, but rather remain confined to upper layers of the stratum corneum [13]. Confocal microscopy studies showed that intact liposomes were not able to penetrate into the granular layers of the epidermis. The possible mechanisms by which traditional liposomes could improve skin delivery of drugs have been extensively studied and reviewed. Recent approaches in modulating drug delivery through skin have resulted in the design of two novel vesicular carriers, deformable liposomes and ethosomes.

(Transfersomes®) are the first generation of elastic vesicles introduced by Cevc et al. and were reported to penetrate intact skin carrying therapeutic concentrations of drugs, but only when applied under non-occluded conditions [14]. They consist of phospholipids and an edge activator. An edge activator is often a single chain surfactant that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers.

Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Orally administered zidovudine, however has strong side effects. Therefore an adequate zero order delivery of zidovudine is desired to maintain expected anti-AIDS effect. A study was carried out where the zidovudine was encapsulated, in recently developed novel vesicular carrier ethosomes, for its enhanced transdermal delivery and results were compared with liposomes. Ethosomes of zidovudine were prepared and characterized in vitro and in vivo. The effect of different formulation variables on skin permeation of zidovudine was studied using locally fabricated Keshry-Chien type of diffusion cell. To understand the mechanism of better skin permeation of ethosomes, vesicle skin interaction study was carried out. To confirm the better skin permeability of ethosomes, fluorescence microscopy using rhodamine 123 as fluorescence probe was performed. Results were compared with those obtained after administration of liposomes and hydroethanolic and ethanolic solution of drug. The optimized ethosomal formulation showed transdermal flux 78.5 plus minus 2.5 miu g/cm2/h across the rat skin as compared to 5.2 plus minus 0.5 miu g/cm2/h for control hydroethanolic solution of drug, and 7.2 plus minus 0.6 miu g/cm2/h for ethanolic drug solution. Vesicle skin interaction study showed that ethosomes affected the ultra structure of the stratum corneum, distinct regions with lamellar stacks derived from the vesicle were observed in intercellular spaces of the stratum corneum. These lamellar

stacks disrupted the organization of the skin bilayers leading to increased skin permeability. This was further confirmed by fluorescence microscopy. Finally, it can be concluded from the study that complex lipid molecule, ethosomes can increase the transdermal flux, prolong the release and present an attractive route for the sustained delivery of zidovudine. Our results indicate that the ethosomal system may be a promising candidate for transdermal delivery of number of problematic drug molecules [15].

The efficacy of PEG grafted liposomes carrying epitopes on their surface proved to be a more effective adjuvant in the elicitation and prolongation of immune response for antigenic epitopes. Since the antigenic epitope was hapten, it could not elicit the immune response, targeted to the immune system on the surface of the liposomes [16] .The epitope was incorporated onto the liposome surface by conjugating them with phosphatidylethanolamine and incorporation of the resulting epitopes-PE conjugates in the formulation of liposomes. The liposomes carrying epitopes elicited the immune response to a great extent and lasted for more than 12 months. Further, the liposomes carrying epitopes on their surface were sterically protected by shielding with methoxy-poly(ethylene glycol) of mass 20 kDa [17] .Methoxy-poly(ethylene glycol)-N-succinimide carbonate was prepared by converting methoxy-poly(ethylene glycol) into its N-succinimide carbonate, an electrophilic derivative [18,19] . This form of the polymer reacts readily with the amino group of phosphatidyl ethanolamine [20,21] .

This modified phosphatidylethanolamine has a PEG chain grafted onto the backbone by hydrolytically stable urethane (carbamate) linkage [18,19].The PEG grafted phosphatidylethanolaminewas used in the formulation of epitopes carrying liposomes and grafted on their surface. The PEG grafted epitopes carrying liposomes showed about two times higher immune response and prolonged persistence of antibodies than that of liposomes carrying epitopes without PEG moieties.

Nanoparticles

Drug encapsulated nanoparticles are solid colloidal particles that range from 10 to 1000 nm in size [22]. 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 [23]. Nanoparticles have also been explored for improving the formulation and efficacy of drugs with physicochemical problems such as poor solubility and stability [24]. 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.

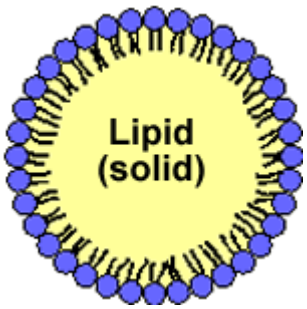
Cells of the mononuclear phagocytic system (MPS), such as the monocytes/macrophages (Mo/Mac), act as a reservoir for the HIV virus [25]. Therefore, drug treatment of HIV infection should involve targeting drugs to these cells in addition to the lymphocytes. Several studies involving ARV loaded nanoparticles for targeting to the macrophages have consequently emerged. In an early preliminary study, Schafer et al. [26] 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.

An in vivo study in rats to investigate the oral delivery of AZT bound to hexylcyanoacrylate nanoparticles for delivery to the reticuloendothelial cells wasundertaken by Löbenberg, Araujo, and Kreuter [27]. The area under the curve (AUC) of [14C] 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.

Numerous technological approaches aiming to improve the effectiveness of the treatment by targeting different cellular and anatomical viral reservoirs are being pursued. In this framework, the use of nanoparticles has arguably become the most attractive research avenue for targeting monocytes/macrophages [28] the CNS [29] and [30] and the gastrointestinal tract [31]. Nanocarriers display a number of advantageous features: (i) poorly water soluble or unstable drugs can be hosted within the particle to attain improved solubility and stability under physiological conditions; and (ii) they are up-taken by phagocytic cells. In early studies, Lobenberg and co-workers developed AZT-loaded hexylcyanoacrylate nanoparticles using bis(2-ethylhexyl) sulfosuccinate sodium as a surfactant and evaluated their biodistribution upon peroral and intravenous administration in rats [32] and [33]. The oral administration resulted in higher plasma levels than the free drug in solution and a more effective delivery to the cells of the reticuloendothelial system (RES). Moreover, following IV injection, drug concentrations were found to be up to 18-fold higher in the cells of the mononuclear phagocyte system with the drug -loaded nanoparticles as opposed to the control solution.

For brain-targeting delivery, solid lipid nanoparticles (SLNs) exhibited the typical advantages of nanoparticulate carriers with excellent biocompatibility (34). Moreover, SLNs could extend the half-life of pharmaceuticals in blood and the scale-up feasibility of SLNs was high in practical manufacturing [35]. SLNs were also capable of entrapping anti-tumor FUdR and carrying FUdR into the central nervous system [36] . By pharmacokinetic analysis, SLNs were concluded to be a qualified colloidal transporter for the delivery of camptothecin into the brain, the heart, and the reticuloendothelial system [37] . Furthermore, positively charged carriers were beneficial to the loading of drugs [38] and to the cellular uptake via electrostatic interactions .



Cationic solid lipid nanoparticles (CSLNs) with entrapped saquinavir (SQV) were fabricated by microemulsion method. Here, CSLNs were stabilized by polysorbate 80, and the lipid phase contained cationic stearylamine (SA) and dioctadecyldimethyl ammonium bromide (DODAB) and nonionic Compritol 888 ATO (CA) and cacao butter (CB). Properties of the present pharmaceutical formulations including the entrapment efficiency, the release kinetics, and the distribution of SQV in CSLNs were analyzed. The results indicated that a mixture of SA and DODAB and a mixture of CA and CB were beneficial to the entrapment efficiency of SQV. However, an increase in the content of cationic lipids insignificantly affected the entrapment efficiency of SQV when the weight percentage of SA and DODAB was greater than 1% during

emulsification. Also, the rate of SQV released from CSLNs with lipid cores of a mixture of CA and CB was slower than that of pure CB. The temporal variation in the released SQV suggested that the carriers could be sustained delivery systems with no apparent initial burst. Hence, the current CSLNs could carry SQV for the improved medication of individuals infected by human immunodeficiency viruses [39].

Permeability of stavudine (D4T), delavirdine (DLV), and saquinavir (SQV) across the blood–brain barrier (BBB) by incorporation of polybutylcyanoacrylate (PBCA), methylmethacrylate-sulfopropylmethacrylate (MMA-SPM), and solid lipid nanoparticles (SLNs) were investigated. The loading efficiency (LE) of the three drugs on PBCA and MMA-SPM was on the order of D4T > DLV > SQV (hydrophilic order). Since MMA-SPM bore strong fixed charge, LE of D4T on MMA-SPM was larger than that on PBCA. The reverse was true for DLV and SQV. Moreover, LE decreased with an increase in the particle size. The order of the entrapment efficiency (EE) of the three drugs in SLNs followed SQV > DLV > D4T, indicating SLNs as efficient carriers for hydrophobic drugs. Also, EE increased with the SLN diameter. The permeability of the three drugs enhanced about 12–16 folds on PBCA, 3–7 folds on MMA-SPM, and 4–11 folds in SLNs. For DLV and SQV, the order of permeability promotion was PBCA > SLNs > MMA-SPM; for D4T, PBCA > MMA-SPM > SLNs. Through the present BBB model, the three carriers were demonstrated as constructive colloidal drug delivery systems [40] .

Transdermal delivery

To optimize the drug bioavailability, minimize the pill burden and prevent taste-related avoidance, several research groups have explored the potential of the transdermal (TD) route for the delivery of NRTIs. In a pioneering work, Seki et al. investigated the *ex vivo* permeation of AZT from a 12 mg/mL solution in isopropyl myristate containing 20% N-methyl-2-pyrrolidone as a penetration enhancer through rat abdominal skin [41] . Plasma levels around 1 µM were found 1–2 h after administration. Kim and Chien investigated the effect of different vehicles and skin permeation enhancers on the skin absorption of AZT, didanosine and zalcitabine or a three-drug combination solubilized in ethanol/water or ethanol/trycaprylin using hairless rat and human cadaver skin models [42] .The permeation rate for each drug (or the combination of drugs) was similar for both solvent systems.

To overcome side effects of orally-administered AZT, ethanol-rich vesicles (ethosomes) [43] were evaluated for TD delivery [44]. An optimized formulation displayed a transdermal flux of 78.5 µg/cm^2/h across rat skin. A more limited flux was observed when the drug was solubilized in hydroalcoholic and aqueous solutions with values of 5.2 and 7.2 µg/cm^2/h, respectively. In addition, findings suggested that the vesicles affected the structure of the stratum corneum resulting in a permeability increase. Lamivudine-loaded ethosomes were employed to elucidate whether the absorption mechanism of the drug involved intracellular or intercellular delivery [45].

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. [46] in early in vivo studies investigated the release of deoxynucleoside Attempts have been made in the literature to explore the use of ceramic 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.

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 oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered [47,48]. 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 [49]. 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 [50]. 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.

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 [51,52] for both single and multiple unit systems.

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. 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 .

Cyclodextrin complexation

To improve drug solubility and dissolution, a recent study investigated the preparation of different efavirenz inclusion complexes with β-cyclodextrin (β-CD), hydroxypropyl β-CD (HPβCD), and randomly methylated β-CD (RMβCD). With β-CD, the apparent solubility of the drug increased linearly as a function of the CD concentration in the 0.002–0.3 M range, suggesting the formation of a complex with a 1:1 molar ratio. HPβCD and RMβCD showed a similar trend up to a CD concentration of 0.008 M. At higher concentrations, a 1:2 or higher molar ratios were observed. X-ray analysis showed that in the β-CD binary systems, the drug was crystalline. In contrast, HPβCD and RMβCD led to the formation of totally amorphous complexes, though a small amount of crystalline free drug was found in physical mixtures and kneaded samples. DSC experiments supported these findings.

The low aqueous solubility of saquinavir restricts absorption upon oral administration and biovailability is extremely low (4–10% depending on the formulation used). Aiming to improve the solubility of saquinavir in water, the complexation of the drug with different cyclodextrins has been pursued. Boudad et al. produced a hydroxypropyl-β-cyclodextrin–saquinavir inclusion complex [53]. The apparent solubility was increased up to 400-fold at pH 7.

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 [54] 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 . The systemicbioavailability 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.

REFERENCES

[1] Frezzini C, Leao JC and Porter S. J Oral Pathol Med 2005 ; 34:513–531.
[2] Holtgrave DR. J STD AIDS 2005; 16: 777–781.
[3] Schrager LK and D'Souza MP. J Am Med Assoc 1998; 280: 67–71.
[4] Chun TW, Davey R, Ostrowski M, Engel D, Mullins J, Lane C and Fauci A. Nat Med 2000; 6 :757–761.
[5] Vyas TK, Shah L and Amiji MM. Expert Opin Drug Deliv 2006; 3 : 613–628.
[6] Phillips NC, Skamene E, Tsoukas C. J Acquir Immune Defic Syndr 1991;4: 959–966.
[7] Phillips NC, Tsoukas C. Blood 1992;79: 1137-1143.
[8] J Subheet, Tiwary AK, Jain NK. Curr. Drug Deliv. 2006;3:157–166.

[9] Harvie P, Desormeaux A, Gagne N, Tremblay M, Poulin L, Beauchamp D, Bergeron MG. Aids 1995; 9: 701–707.
[10] Harvie P, Desormeaux A, Bergeron MC, Tremblay M, Beauchamp D, Poulin L, Bergeron MG. Antimicrob Agents Chemother 1996;40: 225–229.
[11] Vyas SP, Subhedar R, Jain S. J Pharm Pharmacol 2006; 58: 321–326.
[12] Kirajavainen M, Monkkonen J, Saukkasaari M, Valjakka-Koskela R, Kiesvaara J. J Control Release 1999; 58: 207-214.
[13] Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. J Control Release, 2000a; 65: 403-418.
[14] Cevc G and Blume G. Biochim Biophys Acta 1992; 1104: 226-232.
[15] Jain S, Umamaheshwari RB, Bhadra D, Jain. Ind J Pharm Sci 2004; 66: 72-81.
[16] Friede M, van Regenmortel MHV, Schuber F. Anal Biochem 1993; 211: 117–22.
[17] Lasic DD, Papahadjopoulos D. Science 1995; 267: 1275–6.
[18] Abuchowski A, Kazo G, Verhoest C. Cancer Biochem Biophys 1984;7: 175–8.
[19] Matsuda Y, Noro S, Sato N, Sakurada N, Fujita N, Ishizaki T et al. Eisei Shikenjo Hokoku 1991;109: 107–10.
[20] Sehgal D, Vijay IK. Anal Biochem 1994;218: 87–91.
[21] Staros JV, Wright RW, Swingle DM. Anal Biochem 1986; 156:220–2 .
[22] Panyam J, Labhasetwar V. Adv Drug Deliv Rev 2003; 55: 329–347.
[23] L Brannon-Peppas, JO Blanchette. Adv Drug Deliv Rev 2004; 56: 1649–1659.
[24] F De Jaeghere, E Allemann, F Kubel, B Galli, R Cozens, E Doelker, R Gurny. J Control Release 2000; 68 : 291–298.
[25] Weiss RA. Science 1993; 260: 1273–1279.
[26] Schafer V, von Briesen H, Andreesen R, Steffan A.M, Royer C, Troster S, Kreuter J, Rubsamen-Waigmann H. Pharm Res 1992; 9 : 541–546.
[27] Löbenberg R, Araujo L, Kreuter J. Eur J Pharm Biopharm 1997; 44: 127–132.
[28] H Von Briesen, P Ramge and J Kreuter. AIDS Rev 2000; 2: 31–38
[29] Nath A and Sacktor N. Curr Opin Neurol 2006; 19: 358–361.
[30] Ronaldson PT, Persidsky Y and Bendayan R. Glia 2008; 56:1711–1735.
[31] Dembri A, Montisci M-J, Gantier JC, Chacun H and Ponchel G. Pharm Res 2001; 18: 467–473.
[32] Löbenberg R and Kreuter J. AIDS Res Hum Retroviruses 1996; 12: 1709–1715.
[33] Löbenberg R, Araujo L and Kreuter J. Eur J Pharm Biopharm 1997; 44:127–132.
[34] Kuo YC, Kuo CY. Int J Pharm 2008;351:271–281.
[35] Bargoni A, Cavalli R, Caputo O, Fundaro A, Gasco MR, Zara GP. Pharm Res 1998; 15: 745–750.
[36] Wang JX, Sun X, Zhang ZR. Eur J Pharm Biopharm 2002; 54: 285–290.
[37] Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. J Control Rel 1999; 59: 299–307.
[38] Kuo YC. Int J Pharm 2005; 290, 161–172.
[39] Kuo Yung-Chih, Chen Hung-Hao. Int J Pharm 2009;365:206-213.
[40] Kuo Yung-Chih and Su Fu-Lung. Int J Pharm 2007; 340:143-152.
[41] Seki T, Kawaguchi T, Juni K, Sugibayashi K and Morimoto Y. J Control Release 1991; 17: 41- 47.
[42] Kim DD and Chien YW. Drug Dev Ind Pharm 1996; 22:1047–1054 .
[43] Godin B, Touitou E. Crit Rev Ther Drug Carr Syst 2003; 20: 63–102.
[44] Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ind J Pharm Sci 2004; 66: 72-81.
[45] Jain S, Tiwary AK, Sapra B, Jain NK. AAPS PharmSciTech.2007;8:111.

[46] Benghuzzi HA, Barbaro RM, Bajpai PK. Biomed Sci Instrum 1989; 25: 169–177.
[47]Gates KA et al. Pharm Res 1994; 11: 1605–1609.
[48] Banaker UV. Am Pharm 1987; 2: 39–48.
[49] Kamath KR, Park K. Mucosal adhesive preparations. Boylan (Eds.), Encyclopedia of Pharmaceutical Technology, Marcel Dekker, New York, 1994, 133–163.
[50] Li X.L, Chan WK. Adv Drug Deliv Rev 1999; 39: 81–103.
[51] Munasur AP, Pillay V, Chetty DJ, Govender T. Int J Pharm 2006; 323: 43–51.
[52] Perugini P, Genta I, Conti B, Modena T, Pavanetto F. Int J Pharm 2003; 252: 1–9.
[53] Boudad H, Legrand P, Lebas G, Cheron M, Duchêne D, Ponchel G. Int J Pharm 2001; 218: 113-124.
[54] Griffin BT, O'Driscoll CM. J Pharm Pharmacol 2006; 58: 917–925.