Synthesis and *in vitro* Modeling and Characterization of Self-Assembling Drug Conjugates for Targeted Medicinal Application

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Abstract: A number of conjugates tend to self-associate in a transient or permanent fashion and this has formed the basis of considerable intense scientific and commercial investigation over recent years. This article considers a variety of strategic formulations, their flaws and advantages. Working practices and groundbreaking developmental activities within the sphere of self-assembling drug conjugates are also reviewed.

Key Words: Smart colloids, encapsulation, surfactant, polymer, integration, nano-engineering.

1. INTRODUCTION

A large number of artificial and mimetic drug conjugates have been proposed and investigated [1-3]. The objective in such cases is targeted delivery of the drug, bio-mimicry, replication and through development of new materials the means to build intelligent targeting capabilities, which improve on natural methods that are currently available [4]. Organic conjugates of formulation aids, associated drugs and chemical entities such as technetium, ^{99}Tc, used in radio-therapy [5] are used ubiquitously in medicine for diagnosis and as surgical aids. Medicinal applications include chemotherapy [6-10] when isotopes can be used as light particle emitters and localized miniaturized thermal devices, some such applications were recently discovered for labeled gold particles. In the case of imaging, conjugated drug-carriers have been used in medicine for utilization in radio-opacity or magnetic resonance (spectroscopic) imaging applications [11, 12]. There are of course a plethora of therapeutic applications using conjugated systems that have been discussed and investigated widely, for example in anti-viral [13] and anti-fungal pharmaceutical formulations. A number of diverse polymeric drug-support systems have been suggested for use as bio-mimics and for the purposes of scaffold generation for application in tissue repair and osteo-integrative therapies [14-16].

Two of the most common methods that are currently used in diagnostic technologies include conjugation of antibodies to a specific surface and a second, which makes use of a drug conjugated to a self-assembling system to make a specific biosensor [17-19]. There are also the possibilities of using such nano-particulate systems with the intent of purposely creating drug-specific diagnostic biosensors to understand the uptake of drug particles based on highly specific surface "recognition" chemistry [20]. Lectin-functionalized multiple emulsions [4] are other novel examples where a targeting strategy and intelligent formulation fuse to produce a better and more specific product.

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A number of conjugates have been investigated for use as mimics and models of pro-drugs and an associated quantitative structure and activity relationship (QSAR) pharmacologic model [21, 22]. Conjugates have also been used directly in constructing pharmacologic models for use in mimicking cellular compartmentalization and this has been studied both for dendrimer [16, 23-26] and colloidal [4, 6, 25, 27-33] systems. Dendrimers are used widely in this field as they are polymers with a branch-like configuration [24], in the simplest scenario, but may also be constructed to form an array of architectures, many of which approximate to globules, spheroids or ellipsoidal cages and super-dense spheroids [11, 16]. Colloidal systems in this type of self-assembly are also referred to as condensed matter materials. They present themselves rather ideally as possible routes for "smart" or "designer" chemistry and its employment in routine drug delivery. Recently, this conjugation was mentioned in terms of a highly successful, pH-swellable specific structure [6] for delivery of doxorubicin; this also has potential for use in other smart dendrimer applications. Further synthetic chemistry work has continued in a vastly under-tested area of specific biological targeting. This alternative approach was reported recently for unparalleled ribosome binding using a nucleotide and nucleic acid intercalator conjugate, such as pyrene for delivery of chloramphenicol [34] and more generally for DNA-peptide conjugates [35].

2. CHEMICAL-ASSEMBLIES, PRO-DRUG FORMATS AND STRATEGIES

The schematic (Fig. (**1**)) depicts the current diversity of generalized synthetic conjugate structures used in the delivery of an active pharmaceutical ingredient (API). Conjugates may be classified according to the two distinct variants that are found, separated by the broken line in the Figure. These classifications are, incorporation of a drug on or within a vehicle or support and permanent covalent attachment. The mechanism of the former is customarily electrostatic attraction, physi-sorption or solubilization and can be considered to be both transient and variable in strength depending on the chemical environment and the latter form, which is largely invariable and fixed. The

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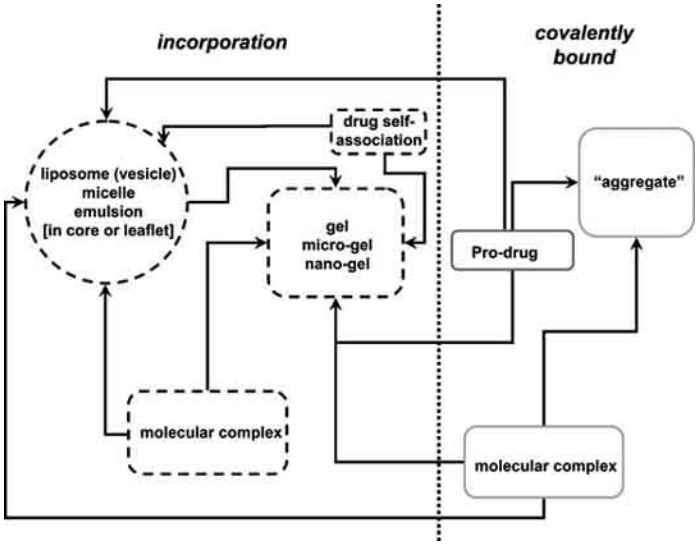

Fig. (1). Model illustrating the range and interrelationship of synthetic conjugate structures and their form. Broken textured features indicate "transient" soft condensed matter assemblies. The broken vertical line in the Figure separates species that exist primarily as incorporation or covalently bound (permanent) structures.

covalent linkage might typically be exemplified by disulphide or ester linkage and reverts to a transient system as a result of chemical or enzymic cleavage *in situ.* A working example in current use is the esterification of steroids to produce a pro-drug dispersed in an ointment for application to the skin (topical medicine). The ester bond in this conjugate is broken down by esterases in the *dermis* of the skin and produces free steroid. What is immediately clear when considering the form of the "pro-drug" (Fig. (**1**)) is the scope for a multitude of inter-related pro-drug entities and macro-molecular structures.

The drug incorporation strategy in most cases makes use of association colloids (0.1-200 nm) also referred to as nano-structures (liposomes and micelles) and coarse dispersions (micro-gels, emulsions and lipid-coated foams), which can as a general rule range from 100 nm to 5 μm in size. These miniature assemblies or molecules generally have some part of their structure in the colloidal range (1-100 nm). Some drugs such as polycyclic amphiphiles, like the antidepressant chlorpromazine and linear alkylated anesthetics such as lidocaine and tetracaine are able to self-associate to from micellar structures [36] or to stabilize emulsions much as any other surfactant might do.

The common feature of most association structures lies in the small units (molecules) used to form these aggregates. In many cases, the most successful simple surfactants are in the molecular weight range 0.1-5 kDa, such as polysorbate 20 (1.5 kDa) and the most successful amphiphilic polymers and peptides have a size distribution of 2–150 kDa, such as poloxamine 908 (22 kDa) and bovine serum albumin (67 kDa). Amphiphiles at the lower end of the size range have a tendency to reconfigure more effectively and have a greater degree of time-independent association. These polymers and surface-active agents because of their constitution and exposure of their hydrophilic and lipophilic domains possess the ability to bind and solubilize hydrophilic and hydrophobic portions of a large drug molecule or the entire drug molecule and retain them in a composite structure. Binding and protection of an encapsulated drug is and continues to be one of the most significant forms of drug conjugation. In a typical example, this is seen when surfactants associate in the simplest way to create a normal spherical micelle particle, having a hydrophobic core and a polar particle surface. Normal micelles are by far the most prevalent form of pharmaceutical nano-dispersion. A reverse micelle has a minute aqueous core but needs an apolar organic solvent environment to form and as a rule they are far less appropriate in most physiological applications. This process of micellization is used to great effect for increasing the longevity of the drug in aqueous solution with base sensitive drugs such as indomethacin. The drug might also be encapsulated within a small multi-lamellar vesicle or other forms of single-walled liposomes and lamellar liquid crystal bilayers [36]. These components could then be incorporated, in an intact form into coarse dispersions, such as a Pickering emulsion (an emulsion stabilized by solids), to give a more consistent dosed product.

2.1. Conjugation of the Drug

Drug delivery forms that work particularly well in many instances make use of assembling structures. Typically aggregates must accommodate moieties from conjugation of a number of organic entities [9, 15, 27, 37-39] with ease. A complex conjugate of particular activity has been described based on attachment to an immuno-responsive agent [9]. In this case it carried with it particular difficulties of component compatibility and obfuscation. Conjugation of organic moieties can take the form of linkage to polymers such as proteins [40, 41], PLGA (poly(lactic acid)-(glycolic acid)) [25], cyclodextrin [42], PEG-PCL (poly(ethylene glycol)–poly(caprolactone)) [26] and fibrin [43], for use in the stenosis of collapsed blood arteries. These biodegradable forms of a number of drug conjugates have and continue to

prove useful in avoidance of reticulo-endothelial system clearance and immune response. The nature of the conjugation of drug to a biocompatible polymer such as a poloxamer (poly(propylene oxide)-poly(ethylene oxide) block copolymer) and PEGylated lipid is one which is used to great effect in current pharmaceutical drug delivery systems [32, 44, 45]. Such an approach is not without difficulty but its advantages generally outweigh its drawbacks.

2.1.1. Conjugation Between Drugs

It is also possible to envisage a stage in drug administration where more convenient and purpose-driven conjugation between drugs is made possible and thus effectively two or more drugs are delivered to the site of interest contemporaneously. At present this is mainly made possible by the use of multiple encapsulation of drug in a colloidal nano-particle or *via* self-association between drugs that are "surfactant-like" in their physico-chemical characteristics, such as the anesthetic tetracaine. However, Kurtz and Scriba [39] do report on the successful dual action of drug-phospholipid conjugated pro-drugs which are degraded by pancreatic phospholipase A_2 [39]. In some cases, using the notion of reversible disulphide linkages [46] can prove advantageous to augment uptake of the drug, as the drug conjugate is delivered very close to the area of systemic interest in a stable inactive form and is reduced *in situ* to liberate the free drug. It is also possible to link pharmacologically active inorganic materials to organic compounds such as polyphosphazene [47]. An alternative approach is to link a drug to an inorganic material, in this way the inorganic material does not pose a significant pharmacologic threat. Only the extent and type of the chemical synthesis determines the conjugation number or extent of compound incorporation in the conjugate. It also determines the physico-chemical properties (Table **1**) of the conjugate, their effective use and size and provides a notion that the drug may be re-modified or further engineered.

2.1.2. Permanent Associations

These can be largely inorganic silicon-based preparations formed *via* covalent bonds (Table **1**), the most commonly used are silicates and aluminates and in many applications, dendrimers [24, 50] and latexes. In some cases, the bonds may be cleaved and the drug liberated *in situ* and this has the direct value of increasing the drug efficacy, in others the use of the drug and targeting agent is an integral feature of the applicability of the drug. Such an application may be in the use of imaging contrast agents and chemotherapeutic agents using radio-nucleides.

In many successful drug delivery systems the drug is simply conjugated to an inert material. As already stated, in most cases the inert material is frequently silica or magnetite. This area of drug delivery remains one which has been vastly underexploited other than for cytotoxic and anti-tumor applications (Table **2**) but it is clear that this field is likely to increase substantially in the future. One hopes that a broader diversity and greater extent of use of novel materials for appropriate pharmaceutical applications of covalent conjugates can be augmented in the near future. At present, doxorubicin a potent anti-tumor cytotoxic drug has been successfully immobilized onto magnetite, silica and polystyrene nano-spheres, respectively [50]. Large-scale discussion of this aspect of drug delivery lies outside the scope of this article. However, it is necessary to allude to its small but significant role within drug delivery and possibilities for cross-over with other methods. Table **2** shows opportunities for conjugation between solids (dispersed particles in aqueous environment) and drugs. Their appearances are likely to increase, based on the current number of variations and desire for new products that solve problems of scientific capability.

2.2. Self-assembling Behavior – Association Colloids

Soft matter composite assemblies (Fig. (**1**) – incorporation systems) are characterized by their dynamic nature of formation, disintegration and a re-formation by their individual components (surfactants/polymers). These assemblies may also include regular and irregularly-shaped particles and gels. This process of assembly takes place on a sub-nanosecond timescale, consequently the structures can appear to be near static. The driving force for formation of these association structures are the enthalpic and entropic

Table 1. Selection of Types of Conjugation and Potential Linkage Strategies of Drug to Supports

functionalization	use / application	reference
avidin-biotin(ylation)	specific	[48]
cross-linking via a bifunctional agent [a]	general	[1, 40, 41]
free radical copolymerization [b]	general	[24, 38]
galactosylation	specific	[49]
amide bride cross-linking [c]	general	[34]
succinic ester spacer linking drug to polymer [d]	general	[37]
amido-linked derivatives [e]	specific[#] / general	[13]
condensation-elimination [f]	specific[#] / general	[10, 39]

a-f, referred to in the text. [#] - specific use is dependent on conjugated moiety.

Table 2. Descriptions of a Range of Solid-State Self-Assembled Structures

application	support	drug	reference
anti-tumor	magnetite	doxorubicin[g]	[50]
	silica	doxorubicin	[51]
	polystyrene co-maleic acid	neocarzinostatin	[52]
	polyphosphazene	platinum(II)[h]	[47]
	hydroxy-propyl(methacrylic acid)	doxorubicin	[53]
transfection chemotherapeutic	lipid particle	plasmid[i]	[32, 35, 54]
	calcium phosphate	cisplatin	[55]
	Mylotarg™ humanized Monoclonal antibodies	N-acetyl-γ-calicheamicin	[56]

g-i, referred to in the text.

ordering of hydrophobic parts of the surfactant or polymer. For the purposes of drug incorporation, the assemblies (nano-structures) may be considered to be discrete intact units. A number of association colloids should be perceived as useful in terms of drug deposition and medicinal application (Table **2**). The tendency in current thinking and success of drug administration is toward the miniature, this is principally because such nano-structures (1-100 nm) have much greater efficacy, particularly in the domain of drug delivery [44]. A major factor involved in this efficacy is the role of diffusion-driven processes and related solubilization and re-solubilization and these are maximized as the surface area to volume ratio of such tiny particle is very large. It thus plays a key part in the kinetics that govern the process of drug passage from nano-particle core to site of interest in the tissues because of the similarly small dimensions of the cells (~20 μm) and cell membranes (~8 nm) concerned.

In some cases, such as use of a drug-polymer conjugate (Table **1**) or an association structure in an active coating for a chemical sensor, the dimensions of this layer or particle are also important in eliciting a maximal response. Once again, as with drug efficacy and QSAR prediction this is related to rapid kinetics of chemical reaction at the surface. A typical example of where kinetic performance might be is important is in the success or failure of the active portions of an analytical diagnostic apparatus such as a quantitative polymerase chain reaction micro-arrays. This type of array might have a sensitized portion of the surface with a dimension of around 20-100 μm^2 [19] and so the rate or extent of reaction becomes particularly important. These devices are used in point-of-care clinical testing and high throughput screening (HTS) on medicinal entities and an API as part of structure-activity prediction [57] and new drug development.

The most common forms of encapsulation of drugs and medicinal presentation is in the form of colloids such as, aggregates, micelles and vesicles (Table **3**). The core of the nano-particles and the diameter of the zone where most entrapment of drug occurs is approximately 5-80 nm if the particle diameter itself is about 100 nm. However, the encapsulation ratio of the drug in a particle can and has been found to vary widely between 20 and 70% in liposomal and nano-particle preparations [44]. It is therefore worth noting that care must be exercised to produce a consistent product in order to elicit a consistent pharmacologic response. For this reason alone some alternative methodologies are

Table 3. Association Units Made from the Self-Assembly of Molecules (Surfactants, Proteins and Amphiphilic Polymers)

name	component	number of units	diameter (nm) or basic dimension	nature of colloid (usual)
aggregate[j]	polymer	~100	1-10	dispersed in water [44]
micelle	surfactant	~100	1-10	dispersed in water or water dispersed in particle
vesicle[k]	surfactant or lipid	~5,000	1-1000	dispersed in water [6]
liquid crystal	surfactant	>>10,000	1-10	dispersed in water or water dispersed in layers
gel	polymer	enormous	μm-mm	dispersed in water or oil
sol	aggregate	enormous	μm-mm	dispersed in water or oil

j-k referred to in the text.

currently under consideration. Incorporation of the drug into the structure of the outer layers of the nano-particle and vesicle outer leaflet is one possible remedy and presents itself as a cutting-edge approach to drug delivery. In the vast majority of cases the dispersed nano-structures are based on aqueous or simple hydrocarbon solvents, for the purposes of biocompatibility (Table **3**), such as the micelle or reverse micelle, respectively. Problems arise when changing the polarity of the local environment as this can change the overall configuration of the self-assembly by altering the configuration of the components with respect to one another within the micelle, vesicle and lamellar liquid crystal gel.

The association colloids involved in contemporary nanotechnology applications include coarse dispersions (emulsions, solid particles and foams). The coarse colloids are favored in one sense because of their ease of consistent fabrication and ease of specifically engineering their molecularly-oriented surface structures and outer layers [58]. True colloids or nanoparticles generally focus on nano-emulsions [59], vesicles [60] (liposomes), micelles (including liquid crystals [61]) and amphiphilic macromolecules [33] or minor simple structures shown in Fig. (**2**). These particles are likely to find increasing exploitation in the near future. The principal determinants of particle configuration are the mechanical energy input into the dispersion and the energy of mixing between the components, however the shape of the component unit is also an essential consideration as the geometric form of the surface active unit can be pre-disposed to preferentially give certain colloidal structures.

The Figure (Fig. (**2**)) shows the relationship between a variety of types of self-assembly and self-associating structures (nano-particles). These aggregates are being used increasingly for novel synthetic, medicinal three-dimensional architectures and pharmaceutical formulations. A comprehensive review of the various types of nano-particle can be found in an excellent paper by Lawrence and Rees [62]. In the past ten years, a most remarkable change has occurred to the array and complexity of nano-particulate drug delivery systems and controlled drug-releasing gel matrices. Once "less favored" and on the margins of drug formulation science in parenteral and topical drug formulations, those based on liquid colloidal dispersed systems, now account for approximately 20% of all prescribed drugs and well over 60% in the area of chemotherapy.

One time popular emulsions, at 1-5 micron diameter, have been replaced routinely by nano-capsules, nano-emulsions (solid lipid nano-particles and perfluorinated gaseous aphrons), micelles, liposomes, liquid crystal phases and very small nanometer sized molecular conjugates and complexes (Fig. (**2**)). These molecular complexes are currently used in isolation but also increasingly to fabricate robust nanometer sized nano-gels and nano-capsules and in some cases aggregates contained within a gel. The obvious advantage of such association lies in the efficacious release profiles of the drug and the ability of the association structure to safeguard and protect the chemical moiety from harsh chemical and physiological environments. These conditions are sometimes needed to provide long term or longer term stability to the formulation. Harsh conditions that exist naturally in the body, such as the stomach can very easily degrade the drug even over relatively short timescales. The solubilization and encapsulation of photo- and chemo-labile drugs is one of the principal considerations to the pharmaceutical chemist.

Fig. (**2**) also shows the use of solid-phase quantum dots and solid-state micro-structures. These can have applications as diverse as short lifespan diode lasers, for use as nanometer sized robotic systems that are used in photodynamic radio- and phototherapy. Other possible applications include magnetic particles for tumor excision and location during surgical procedures. For the moment this field of exploration lies right at the edge of current research and know-how. It is also conceivable that in the future amalgamation of solid-state and soft condensed matter (self-assemblies) might be

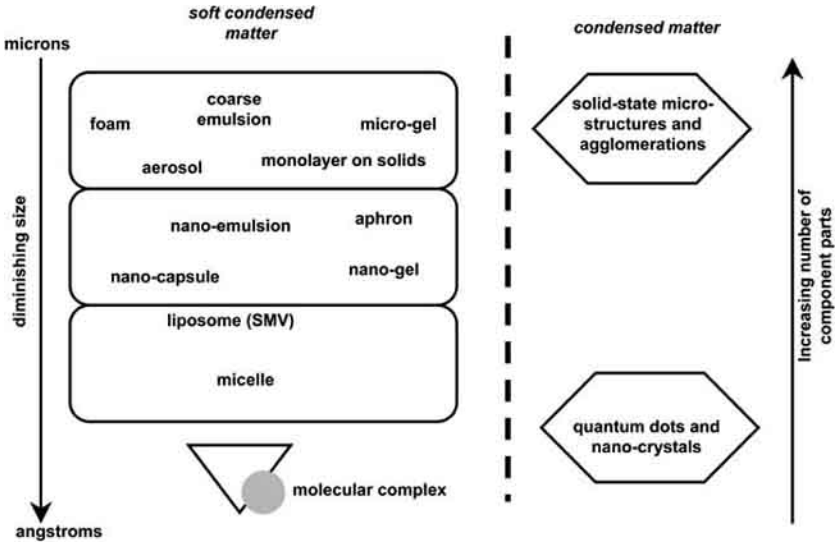

Fig. (2). The "family" of self-associating and self-assembling structures proposed or currently used in medicinal preparations. These can be compared to a molecular ruler, where a C-H bond length is approximately 1 angstrom (0.1 nm).

merged to give hybrid materials with better pharmacologic and medicinal properties.

2.2.1. Pro-drug Surface Active Molecules

The surfactants and polymers that make-up the association structures listed in Table **3** can be made from drug-polymer conjugates (Table **2**). These conjugated drugs can make good amphiphiles because the relative proportions of the molecule that are hydrophobic and hydrophilic can be modified. Amphiphilic drug conjugates, such as the novel phospholipid-methotrexate compound reported by Williams *et al*. [63] and others [15, 39, 40, 44] can form association structures such as micelles, liposomes and emulsions (Fig. (**1**)). Insightful and imaginative thinking in medicinal chemistry is at the forefront of breakthrough success in pharmaceutical therapy. Consequently, thoughtful synthetic chemistry and pro-drug design can lead to the production of truly novel and valuable materials.

2.2.2. Novel Polymers and Surfactants

At present, vast arrays of potential candidates are available for use as pro-drug or modified drug conjugates and many are clearly destined to fail. Krafft and Goldman [28] in a large list of ground-breaking papers have indicated the potential for use of fluorinated surfactants and fluorinated block copolymers as surface active agents. The next step will be to synthesize fluorinated polymer-drug conjugates on a large scale and undertake chemical and pharmacologic testing. Other areas that are currently under-exploited are listed in Table **2** and these include aspects of investigation such as the use of geometric peptides. Inert mineral architectures could produce pro-drugs that are ideal and insufficiently chemo- or heat labile to be damaged by processing conditions (such as a Pickering emulsion) and metabolism before reaching the target site of required activity (Table **2**). Success of the end product is related to an optimal quantity of intact drug, so the use of drug conjugation to inert material presents itself as a real possibility for mass production. There exist a number of possibilities for design and creation of new drug-surfactant mixtures, these are:

1. Conjugation of polymer-drug (unfortunately the form of the drug and specificity is unpredictable),
2. Conjugation of lipid-drug (unfortunately the exposure/ orientation of the drug is unknown),
3. Conjugation of inert-drug (unfortunately the drug accessibility is unknown and can depend on linker group length),
4. Conjugation of drug-drug (cleavage can liberate two intact drugs, a real advantage).

One outstanding positive feature of a new breed of conjugated surfactants [15, 23, 32, 39, 40, 44, 64] lies in their ability to form these self-associating structures and therefore provide a means of binding, solubilizing and encapsulating poorly soluble drugs like the non-steroidal anti-inflammatory drug indomethacin. Self association conjugates like inorganic templates lend themselves to easy mass production and this in turn favors take up and commercialization. At present a number of options are available to the drug designer, most currently make use of "solid" particles, micelles and aggregates that are comprised of amphiphiles. The relative success of these solid particles has been built around the ability to heat sterilize the finished product without losing potency. At present number of lipid-drug, lipid-polymer-drug and nano-structured lipidic carriers are used. These molecules should be capable of withstanding the rigors' of processing required to produce sterile medicines and of being produced cheaply in both small and more importantly large-scale manufacturing.

2.2.3. Nano-assemblies

Lipid-based drug delivery systems in the form of emulsions from triglycerides, micellar systems and vesicles have and continue to be used for drug-by-injection (parenteral) and ocular delivery purposes. These products are thermodynamically stable, therefore amazingly shelf-stable and frequently optically transparent and so suit their application ideally. Their effectiveness is largely related to their high surface area to volume ratio that allows the drug to diffuse easily from the particle interior where in most cases it is held. In some cases the drug may be isolated and bound to the surface of the nano-particle (Fig. (**2**); Table **3**). Vitamin E (Tocol®), solid lipid nano-particles, nano-suspensions, nano-emulsions and bi-continuous liquid crystal networks are all examples of design-flexible new classes of colloidal dispersions for use in implant, bio-mimicry and parenteral drug administration. Formulation of a new breed of drug often involves a two step strategy, first using pro-drugs in conjugate form and second, formulated in a specific nano-assembly. Preparation of drug-bearing micelles and micro-emulsions; the use of water soluble block co-polymers (such as PEG's, PEGylated lipids and polymers, and Pluronics®), phospholipids, antibody carrying micelles and lipid coated micro-bubbles all permit binding and co-solubilization of drugs. Nano-structured vehicles are also used for the purposes of gene therapy and direct nucleic acid deposition through DNA transfection to the cell nucleus. Such transfection has been reportedly used with success for cationic lipid assemblies that secure the negatively charged nucleic acid and using nano-emulsion particles [65].

A range of strategies are available to keep hold of the drug species within an association structure. This was discussed recently for fatty acid conjugates [32]. The strategies most commonly considered are:

1. Entrapment (unfortunately this can provide differential release rates),
2. Adsorption (unfortunately this can produce differential dosage rates, through minor differences in surface activity of the species concerned),
3. Dissolution (solubilization and release rate depend on drug hydrophobicity),
4. Encapsulation (unfortunately this can give difficult to predict release rates with the multiple partitioning kinetics involved),
5. Cross-linking (unfortunately this can result in a loss of functionality).

Entrapment usually takes the form of size-related inclusion within a matrix, typically this would be a hydrogel. The main disadvantage with this approach can be non-specific release profiles, but depends on the extent and nature of "sticky" binding domains on the substance; typically a polymer is used to entrap the API. Adsorption is generally considered to be the most flawed approach although some work [28] discusses an interesting way of maintaining and retaining the drug in a hydrophobic portion of a bubble surfactant layer or nano-emulsion surface *stratum*. Dissolution has a long and historical heritage of past and current use and is reported as being both suitable and robust in numerous applications by Washington [66] and Sonneville-Aubrun *et al*. [67]. However, encapsulation is a particular favorite in state-of-the-art formulations such as nano-shells and solid lipid nano-particles [59], because these mimic the bodies' own lipid particles, chylomicrons. There are, as always of course potency concerns with release profiles for solid particles (novel drugs) but these profiles can be engineered by using a complementary mix of low and moderate temperature melting lipids [68] that sequester the API and therefore control its release with their gel phase behavior. At present a multitude of drugs, such as Proprovan®, the intravenous anesthetic; paclitaxel, the chemotherapeutic and tocopherols are successfully delivered *via* drug encapsulation in nano-emulsion and nano-particle delivery systems.

3. SYNTHETIC CHEMISTRY

Generation of chemical entities has made use of a number of liquid and solid-state synthetic routes; these have been used to good effect in novel dendrimer synthesis [24, 69]. Solid phase synthesis has been used specifically to engineer poly(ethylene oxide) (PEO) – peptide block copolymers for drug delivery [70]. Such modified conjugate entities have the advantage of being able to circumvent the blood-brain-barrier (BBB). In some more recent investigations involving smart dendrimer capsules, cell toxicity issues have somewhat stifled comprehensive clinical study. This hurdle remains a bugbear of much inventive novel synthetic medicinal chemistry. However, despite restrictions in most medicinal chemistry applications dendrimer work has initiated a large swathe of patenting and intellectual property applications and shows great promise.

A muco-adhesive pharmaceutical preparation, which uses a chitosan hydrogel (Table **4**) provides a new and exciting polymer excipient for use in medicinal formulation and particularly for controlled release [71]. The medicinal chemistry community will eagerly await evaluative clinical experimentation to follow the potential for a diverse range of controlled and sustained release dosage form applications for this chitosan derivative. A recently developed methotrexate–lipoamino acid conjugate has a variable solubility which depends on the chain length of the lipid portion but that is also able to form multi-lamellar vesicles (~100 nm) when combined with dipalmitoyl-phosphatidylcholine (lecithin) at 37°C [63, 73]. This and other current investigation underway, seems a particularly interesting and clear-cut indicator of a move in the direction taken by contemporary drug development science. What seems very likely is the increased use of lipophilic entities as these can by themselves or when mixed in particular ratios with natural phospholipids form a series of unique liposomal drug delivery particles. The shape and alignment of lipophilic and lipophobic portions of the lipid conjugate is also important in terms of drug delivery formulation and this can be optimized to give particular types of particles and soft matter assemblies [59]. For example, cylindrical-shaped molecules tend to produce lamellar structures and conical molecules tend to favor production of liposomes or micelles [62]. This is an area of spectacular and widespread interest uniting physicists, chemists, biologists and medics with an interest in medicinal chemistry, nano-technology and pharmaceutics.

3.1. Improvements and Fitness-of-purpose

A number of diverse yet cogent strategies are available to increase the efficacy of the API and drug delivery form [6, 9, 10, 50, 56, 62]. The extent and applicability of various forms of pro-drug format varies considerably. Size reduction is one such widespread strategy and a second involves altering the solubility of the conjugate and which facilitates an increase in the encapsulation ratio of the drug within the vehicle. The use of new formulations and self-assembling molecular delivery forms can also be used to enhance the potency, specificity of targeting or success of the medicine. One other means is by increasing the circulation lifetime by disguising the particle in a "stealth" bonded coating and rendering it effectively invisible. In this case, such a particle avoids the reticulo-endothelial system and total, rapid (<6-12 hours) clearance by the liver [44]. However, this is not always appropriate or needed, for example in tumor therapy it might also be possible to make use of the enhanced permeability and retention effect that is commonly observed in tumorous tissue and associated with coarsening of cellular structure and vascularisation. Here large particulates are taken up by

Table 4. A Selection of Specimen, Current Synthetic Chemistry Routes for Creating Pro-Drug Conjugate Candidate Molecules

conjugate	application	reference
chitosan-thioethylamidine	muco-adhesive[l]	[71]
ascorbic acid-diclofenamic acid	brain drug delivery[m]	[72]
glucosyl-lipoamino acid-methotrexate	brain drug delivery[n]	[73]
biotin-phosmidosine-o-ethyl ester	anti-tumor[o]	[10]

l-o, referred to in the text.

the micro-porous tissue structure. A risk of non-specific undesirable cell-drug interactions and avoidance of their related combined side-effects, as far as possible, is always a principal consideration during directed drug delivery. It is desirable therefore, in most cases to choose an application methodology, which allows a minimal dose. Finally, there is the possibility to increase active uptake of the drug by conjugation to a monoclonal antibody. This type of targeting with the additional element of using a nano-assembly has been used with the so-called immuno-micelle [9]. This strategy has the distinct advantage of not wasting the drug and needing lower concentrations to achieve a targeted dose at a specific site. Much of the non-specific cell death and tissue damage associated with a chronic regime of chemotherapy is associated with a rather unfocused supply of the drug within the tissues concerned.

3.2. Stealth and Specific Liganding Strategies

The use of immuno-conjugation and biocompatible synthetic and derivatized natural biopolymers has proved useful in specific targeting [74, 75]. The immuno-micelle discussed by Patel *et al*. [9] has the advantage in providing a means of active targeting aligned with a drug delivery form, which is very effective at depositing its "payload." Use of biocompatible polymers that avoid systemic complement activation and consequent clearance by the reticulo-endothelial system have long been the mainstay of long-circulating particles. A typical example might be the PEG-phosphatidyl-ethanolamine conjugated amphiphile discussed by Lukyanov and Torchilin [44] used as a stealth formulation and in some instances with by-passing of the BBB. The commercial formulation is known as Doxil®, it uses doxorubicin as the bound chemotherapeutic drug. The formulation has been and continues to be used in cancer therapy with widespread success. The phospholipid moiety is used to co-solubilize the drug and the PEG (or PEO) portion of the conjugate-molecule confers the nano-particulate with a degree of biocompatibility. Active targeting is then also made possible by using a targeting agent such as biotin [10] or monoclonal antibody. Lectin-functionalized multiple-emulsions [4] and lectin-modified insulin bearing liposomes [60] have been used for active targeting of the cell surface. Further advances have been reported by Yamazaki *et al*. [75] who use application of a sugar chain to active drug delivery systems to step-up-the-pace of focused delivery by further mimicking the cell surface, thus rendering the drug delivery particle effectively "invisible." A number of cases of glyco-sylation of protein-drug conjugates [69, 76] have also been reported with positive results that relate to a similar anti-complement activation detection strategy. The generalized and simplified format of targeted drug delivery related to such specific therapy is presented in Fig. (**3**).

A simplified schematic of active targeting shown in Fig. (**3**) demonstrates a rationale for using a specific drug carrier in some therapeutic models. A number of other strategies do consider alternative drug uptake models. These do not conveniently fit with the subject of this review. In the model presented here, a specific pro-drug format can be used to permit the drug to by-pass the physical and energetic barrier that is the receptor-sensitized cell membrane. Once internalized a cascade of other events dictate the "free" or inhibited movement of the drug to the target site. This would be a key consideration in the smart engineering of the pro-drug molecule. Such intelligent drug delivery also has the

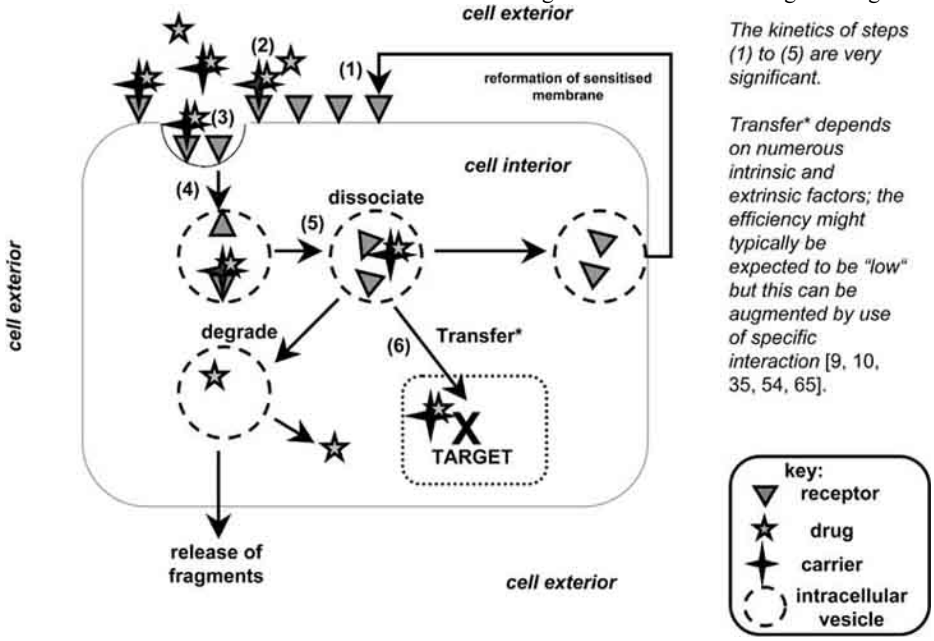

Fig. (3). Highly simplified cartoon showing receptor-mediated endocytosis of a drug-carrier complex. Stages 1-6 indicate the conventional route to delivery of the drug to target compartment or location. It is not guaranteed that for any one drug that stages 1-6 will occur naturally; this depends in part on the physico-chemical nature of the conjugated drug and to a significant extent on the barriers to movement of the drug within the cell. The carrier may be a polypeptide, block copolymer, dendrimer, micelle or other vehicles. The complex pathway by which the carrier acts has been simplified significantly for the purposes of clarity. A key is provided in the Figure itself.

advantage of limiting non-specific uptake, commonly a problem in passive targeting strategies and limiting non-specific cellular alterations, mutagenesis and cell death. The schematic shows a simplified six-step process for the drug to reach the target location but success in delivery necessitates a thorough knowledge of the metabolic and transport pathways open to the drug within the cellular compartment. This is an important consideration in the specific design of the drug moiety. However, an extensive discussion of the complex biochemistry of the cell lies outside the scope of this review.

The drug carrier "molecule", which can also be an encapsulator (for instance a micelle or immuno-micelle) or moiety attached by covalent means to the drug could be both a chemically inert substance or activated [74, 75] material such as cyclodexdrin or dendrimer [23] and used as the basis of the target drug entering the cell interior *via* the generated endosome [34, 45, 63, 77]. Appropriate internalization, endosomal or cytoplasmic activity within the inner compartment and metabolic degradation [53] of the drug-carrier association (Fig. (**3**), steps 4-6) allow a proportion of the "free" drug to be trans-located to the target site. It is then clear that the proportion of drug taken up by the target site is dictated by an extensive series of interconnected processes, one being the initial uptake of the drug-complex by a "fusogenic" process involving the cell membrane. This mode of uptake is now a common route used in gene therapy applications and DNA transfection using protein and PEG-lipid conjugated pro-drugs [32, 35]. The efficiency and effective recovery rate of the free drug is dependent on the kinetics and various permutations of the degradation and assimilation-uptake process. These properties can be modeled by suitable biochemical testing and QSAR prediction.

3.3. Post-platform Technology Modifications and Designer Engineering

3.3.1. Architectures and "Smart" Designs

Successful "smart design" has been related and presented as the mode by which the drug is conjugated within the self-assembly and investigated at depth by *in vitro* modeling of cell uptake, release, dissolution and chemical profiling of the drug species. Several authors have focused attention of the existence of nano-domains within the particle surface layer that effectively sequester or retain molecules of particular polarities. This retention is dependent on the chemical and amphiphilic nature of the stabilizing surfactant or drug [28, 58, 62]. Here, the emphasis is placed on chemically sculpturing the composition of the dispersion medium-dispersed particle interface such that the drug remains bound to the particle surface, whilst remaining available for release. It is expected that this frontier science is likely to become increasingly more relevant, robust, heavily scrutinized and commercially viable as the decade progresses. In one current exemplar, selected to demonstrate a case-in-point, surface modification of a polyester-fatty acid conjugate [48] is sought for use with ideal polymeric biocompatible and biodegradable supports. It may also fulfill part of the requirement for the mode of binding or complexation within an association structure.

3.3.2. Cross-linking and Molecular Complexation

Cross-linking of drugs and delivery in a pro-drug format can be achieved by using a now diverse group of linear bi-functional agents [1, 40, 41, 71, 72]. In the past glutaraldehyde was used as a typical bifunctional agent for cross-linking but has now in part been superseded by alternatives, including the use of "user friendly" enzymes. Later in the drug delivery process the covalent linkage forming the conjugate is removed and the drug is liberated. This strategy can be used to sculpt the nature of the adsorbed layer that gives the micelle or liposome its structure and could therefore be used to control the release profile of the drug. Recently, Saito *et al*., [46] discussed, with significant promise and obvious widespread applicability, the use of a pro-drug delivery system that uses reversible disulphide linkages which are activated by the target cell to release the drug. It is possible to conceive of a nano-particle assembled from such a system that is constituted from a protein-paclitaxel conjugate or bovine serum albumin conjugates [40, 41]. This has been reported for an experimental drug analogue 5-N-(octadecanoyl)aminofluorescein in lipid stabilized foam bubbles [58]. Here, interfacial complexation of the analogue proved more effective in the presence of a composite structure that was based on a co-solubilization layer. This will without doubt form the basis of a new generation of sequestered drug platform technologies for use in controlled and sustained release applications. In a recent paper supporting this notion the formation of fluorine-rich nano-domains on the surface of perfluorocarbon blood cell mimics was suggested as a means of binding hydrophobic drugs in future drug delivery nano-emulsions and micelles [28].

4. DRUG MONITORING AND DELIVERY

4.1. In Vitro *and* In Vivo *Assessment*

In vitro modeling has been used to-date in a vast array of investigations, primarily for HTS applications. Here the value of candidate molecules or drug delivery aggregates can be assessed clearly and equivocally without the risk of random or systematic error. The technique has also produced a series of novel diagnostic tools with which to examine and screen drug suitability [17, 18, 20, 58]. A number of commercially available diagnostic biosensors are available for HTS. These devices frequently make use of poly (dimethylsiloxane) and silicon-based micro-fluidic analytical techniques and micro-arrays. Two currently used examples include a Flow-Thru-Chips™ (Genelogic) and a GeneChip™ (Affymetrix) system for "omic" HTS. One of the limitations of such screening is the accurate processing of the accumulated data. This unfortunately now requires elegant supplementary chemometric and data management (LIMS) software and consequently rather extensive validation. In one of a large number of HTS therapeutic studies reported in 2005, Dalpiaz *et al*. [72], tested ascorbic acid and derivatized conjugates on neuro-active drugs *via* retinal pigment epithelial cells. In this investigation *in vivo* interaction of the pro-drug was increased by conjugation for one of three tested species with relatively minor structural differences. A substantial part of any *in vitro* testing the pro-drug would

need to show QSAR and to elucidate physico-chemical and mechanical characteristics. Ultimately, this would have a significant effect on purpose-built formulation of the API and the most successful form of presenting the drug.

4.2. Commercialization Opportunities

There has been a resurgence in interest in recent years of opportunities for extending the range targeted drugs and a diversification, based on varying pro-drug delivery strategies [1-3, 12]. This could be because in today's market place biopharmaceutical products can, in some cases approach 40-50% of commercially available products under license. It may also be compounded by the fact that synthetic biotechnology is no longer considered break-through "blue-sky" science, that biopharmaceutical manufacture is relatively commonplace and easy to undertake with many advantages over traditional synthetic routes and has been for the last ten years. Much of the commercialization interest is built around a desire for an ever more elegant and appropriately sophisticated means of very actively targeting drugs, which rely less on "chance" and are known to act through a clear series of metabolic routes.

Commercialization based on the platform modification itself and opportunities for designer-engineering provide an obvious a push for continual improvement. In this case, a kind of upsurge and augmentation in uptake increase the chances for commercialization. The most important and significant consequence of this increased activity are the resultant health benefits to the community [5]. At this point in time and as a scientific community taking a keen interest in the evolution of drug development technology we will have a wait, duration unknown, to see the consequences of scientific investment and the pace of increase in the tangible benefits for better drug delivery. What is certain is that imaginative and creative medicinal chemistry is driving innovation and through commercialization that benefits us all.

5. THE CURRENT STATE OF TECHNOLOGY – 2006

Drug delivery diversity is a key to the successful treatment of a range of complex pathologies. At present the range of media for self-assembled structures covers lipid coated bubbles, hydro- and organo-gel matrices, dendrimer capsules [23, 24], nano-emulsions and dispersed solid particles. Many of the drug delivery systems and formulations utilized in pharmaceutics at present are used in chemotherapy applications, for application in photo-dynamic therapy where light is generated that may kill the diseased cell. Photo-dynamic therapy often also employs ionizing radiation with encapsulated and conjugated α, β and γ–particle emitters [5]. Nano-particulate and pro-drug formats are used for cytotoxic applications in order to minimize systemic non-specific cellular damage and patient trauma. Nano-engineered drug delivery systems are also currently used for parenteral nutrition and most specifically in tumor therapeutics [5, 62]. In the case of biomedical imaging, quantum dots, such as cadmium sulphide nano-crystals and ferro-magnetic fluids are currently being used for imaging purposes. In a subtle application change the contrast agents used in positron emission tomography (PET) as part of "CT brain scans" use drug labeled with PET isotopes, such as radioactive iodine, ^{124}I [78]. These imaging agents are more often than not formulated to be encapsulated in coarse dispersions such as an emulsion or a colloidal nano-particle such as a micelle. Colloidal self-association systems, like gels and sols are now used ubiquitously to provide the growth and support structures for scaffolds and implants. This type of implantation is also now used routinely for osteo-integration and fabrication of artificial skin patches [14-16, 62].

Pharmacology, structure and activity prediction has been made possible and is used increasingly for HTS applications. This helps to screen out weak candidate molecules. Modeling of drug kinetic profile and activity based on a multi-factorial experimental design has been crucial to commercialization of novel drugs and drug formulations. The multi-factorial approach is largely based on simulation of metabolic pathways and physical properties of the API. In today's pharmacology lab HTS is often achieved by using cell models and these may be based on micro-fluidic and silicon-based bioreactors and biosensors, which mimic the cellular environment. Use of nano-structured materials is integral to the successful working of such state-of-the-art technologies. Chemico-medical diagnostics (biosensors) in 2006 are increasingly making use of conjugated drug-media complexes. These regio- and stereo-specific complexes are used to impart a selectivity and specificity to the chemical sensor detection apparatus. It is the nature of the conjugation and the formation of specific structural mosaics that is the basis for the biorecognition [58].

6. FUTURE DIRECTIONS, INITIATIVES AND INNOVATIONS

Point-of-care testing is likely to be the fastest growing part of clinical practice for prescribing healthcare professionals. This is closely related to HTS and a striving for better, faster and more detailed assessment of drug candidate suitability. The advantage of using nano-technology as the catalyst for improvement in drug formulation efficacy lies in the ability of small drug-loaded particles to give better performance. It provides a mechanism and a platform technology for continuing expansion and development, their simplicity minimizes the expense of drugs, which is normally a function of formulation development time and this reduces product cost that is ultimately of benefit to the customer. As the societal demands increase, further miniaturization with be required and in this instance nano-engineered drug conjugates will become increasingly important. As a consequence further expansion in the domain of nano-science, molecular synthetic engineering (nano-technology) will become more imperative. This demand will ultimately lead to increased requirements from nano-machining and molecular sculptures [79]. The major stumbling block of course is the acceptance of emerging technologies and the suitability and biocompatibility of engineered products. What is, however likely given previous scientific discoveries in this field is the dynamic nature of the drug development arena. As new technologies and discoveries are made and gain legal and social acceptance this will undoubtedly lead to establishment of new platform technologies.

REFERENCES

[1] Naik, S. S.; Liang, J.-F.; Park, Y. J.; Lee, W. K.; Yang, V. C. *J. Controlled Rel.*, **2005**, *101*, 35.
[2] Roy, I.; Gupta, M.N. *Chemistry Biology*, **2003**, *10,* 1161.
[3] Denny, W. A. *Eur. J. Med. Chem.,* **2001**, *36*, 577.
[4] Khopade, A.J.; Nandkumar, K.S.; Jain, N.K. *J. Drug Target.,* **1998**, *6*, 285.
[5] Sahoo, S.K.; Labhasetwar, V. *Drug Discov. Today,* **2003***, 8*, 1112.
[6] Hruby, M.; Konak, C.; Ulbrich, K. (2005) *J. Controlled Rel.,* **2005***, 103*, 137.
[7] Lippacher, A.; Müller, R. H.; Mäder, K. *Eur. J. Pharmaceutics Biopharm.,* **2002***, 53*, 155.
[8] Valliant, J.F.; Riddoch, R.W.; Hughes, D.W.; Roe, D.G.; Fauconnier, T.K.; Thornback, J.R. *Inorg. Chim. Acta,* **2001***, 325*, 155.
[9] Patel, V.F.; Hardin, J.N.; James J. Starling, J.J.; Mastro, J.M. *Bioorg. Med. Chem. Lett.,* **1995***, 5*, 507.
[10] Sekine, M.; Okada, K.; Seio, K.; Obata, T.; Sasaki, T.; Kakeya, H.; Osada, H. *Bioorg. Med. Chem.,* **2004***, 12*, 6343.
[11] Kostarelos, K. *Adv. Coll. Interface Sci*., **2003**, *106*, 147.
[12] Torchilin, V.P. *J. Controlled Release,* **2001***, 73*, 137.
[13] McKenna C.E.; Kashemirov, B.A.; Eriksson, U.; Amidon, G.L.; Phillip E. Kish, P.E.; Mitchell, S.; Kim, J.-S.; Hilfinger, J.M. *J. Organometallic Chem.,* **2005***, 690*, 2673.
[14] Kopecek, J. *Eur. J. Pharm. Sci.,* **2003***, 20*, 1.
[15] K. Ulbrich, K.; Subr, V.; Strohalm, J.; Plocová, D.; Jelínková M.; Ríhová, B. *J. Controlled Rel.,* **2000***, 64*, 63.
[16] Smith, D.K.; Hirst, A.R.; Love, C.S.; Hardy, J.G.; Brignell, S.V.; Huang, B. *Prog. Polym. Sci.,* **2005***, 30*, 220.
[17] Basak, A.; Bag, S.S.; Basak, A. *Bioorg. Med. Chem.,* **2005***, 13*, 4096.
[18] Mandal, S.; Phadtare, S.; Sastry, M. *Curr. Appl. Phys.,* **2005***, 5*, 118.
[19] Jianrong, C.; Yuqing, M.; Nongyue, H.; Xiaohua, W.; Sijiao, L. *Biotech. Adv.,* **2004***, 22*, 505.
[20] Hildebrand, A. *J. Coll. Interface Sci.,* **2002***, 249*, 274.
[21] Geldenhuys, W.J.; Lockman, P.R.; Nguyen, T.H.; Van der Schyf, C.J.; Crooks, P.A.; Dwoskin, L.P.; Allen, D.D. *Bioorg. Med. Chem.,* **2005***, 13*, 4253.
[22] Alonso, M.J. *Biomed. Pharmacotherapy,* **2004***, 58*, 168.
[23] Gillies, E.R.; Fréchet, J.M.J. *Drug Discov. Today,* **2005***, 10*, 35.
[24] Mackay, M. *Comptes Rendus Chimie,* **2003***, 6*, 747.
[25] Tansey, W.; Ke, S.; Cao, X.-Y.; Pasuelo, M.J.; Wallace, S.; Li, C. *J. Controlled Rel.,* **2004***, 94*, 39.
[26] Park, E.K.; Lee, S.B.; Lee, Y.M. *Biomaterials,* **2005***, 26*, 1053.
[27] Zovko, M.; Zorc, B., Novak, P.; Tepes, P.; Cetina-Cizmek, B.; Horvath, M. *Int. J. Pharmaceutics,* **2004***, 285*, 35.
[28] Krafft, M.P.; Goldmann, M. *Curr. Opin. Colloid Interface Sci.,* **2003***, 8*, 243.
[29] Ueda, K.; Furukawa, T.; Kawaguchi, Y.; Miki, Y.; Sakaeda, T.; Iwakawa, S. *J. Controlled Rel.,* **2004***, 95*, 93.
[30] Nakano, M. *Adv. Drug Delivery Rev*., **2000**, *45*, 1.
[31] Fukai, H.; Koike T.; Seneki, A.; Sonoke, S.; Seki, J. *Int. J. Pharmaceutics,* **2003***, 265*, 37.
[32] Song, L.Y.; Ahkong, Q.F.; Rong, Q.; Wang, Z.; Ansell, S.; Hope, M.J.; Mui, B. *Biochim. Biophys. Acta – Biomemb.,* **2002***, 1558*, 1.
[33] Cipollone, M.; De Maria, P.; Fontana, A.; Frascari, S.; Gobbi, L.; Spinelli, D.; Tinti, M. *Eur. J. Med. Chem.,* **2000***, 35*, 903.
[34] Johansson, D.; Jessen, C.H.; Pøhlsgaard, J.; Jensen, K.B.; Vester, B.; Pedersen, E.B.; Nielsen, P. *Biorg. Med. Chem. Lett.,* **2005***, 15*, 2079.
[35] Niemeyer, C.M. *Trends Biotechnol.,* **2002***, 20*, 395.
[36] Florence, A.T.; Attwood, D. In *Physicochemical Principles of Pharmacy*; Macmillan Press: London, **1998**, pp. 206-208.
[37] Cavallaro, G.; Licciardi, M.; Caliceti, P.; Salmaso, S.; Giammona, G. *Eur. J. Pharmaceutics Biopharm.,* **2004***, 58*, 151.
[38] Nan, A.; Croft, S.L.; Yardley, V.; Ghandehari, H. *J. Controlled Release,* **2004***, 94*, 115.
[39] Kurtz, M.; Scriba, G.K.E. *Chem. Phys. Lipids,* **2000***, 107*, 143.
[40] Dosio, F.; Brusa, P.; Crosasso, P.; Arpicco, S.; Cattel, L. *J. Controlled Release,* **1997***, 47*, 293.
[41] Tian, J.; Liu, J.; Hu , Z.; Chen, X. *Bioorg. Med. Chem.,* **2005***, 13*, 4124.
[42] Wang, N.; Wu, Q.; Xiao, Y.M.; Chen, C.X.; Lin, X.F. *Bioorg. Med. Chem.,* **2005***, 13*, 2667.
[43] Thomas, A.C.; Campbell, J.H. *J. Controlled Release,* **2004***, 100*, 357.
[44] Lukyanov, A. N.; Torchilin, V. P. *Adv. Drug Delivery Rev.,* **2004***, 56*, 1273.
[45] Ulbrich, K.; Etrych, T.; Chytil, P.; Jelínková, M.; Ríhová, B. *J. Controlled Rel.,* **2003***, 87*, 33.
[46] Saito, G.; Swanson, J.A.; Lee, K.-D. *Adv. Drug Delivery Rev.,* **2003***, 55*, 199.
[47] Song, R.; Kim, Y.J.J.J.I.; Jin, C.; Sohn, Y.S. *J. Controlled Rel., 105,* **2005**, 142.
[48] Fahmy, T.M.; Samstein, R.M.; Harness, C.C.; Saltzman, W.M. *Biomaterials,* **2005***, 26*, 5727.
[49] Managit, C.; Kawakami, S.; Nishikawa, M.; Yamashita, F.; Hashida, M. *Int. J. Pharmaceutics, 2003, 266*, 77.
[50] Mykhaylyk, O.; Kotzuruba, A.; Dudchenko, N.; Torok, G. *J. Magnet. Magnetic Mater.,* **2005***, 293*, 464.
[51] Scric, S.; Rupprecht, H.; Daca, J.; Smid-Korbar, J. *Int. J. Pharmaceutics,* **1993***, 99*, 21.
[52] Maeda, H. *Adv. Drug Delivery Rev*., **2001**, *46*, 169.
[53] Kovar, M.; Kovar, L.; Subr, V.; Etrych, T.; Ulbrich, K.; Mrkvan, T.; Loucka J. M.; Rihova, B. *J. Controlled Rel.,* **2004***, 99*, 301.
[54] Ambegia, E.; Ansell, E.; Cullis, P.; Heyes, J.; Palmer, L.; MacLachlan, I. *Biochim. Biophys. Acta – Biomemb.,* **2005***, 1669*, 155.
[55] Barroug, A.; Kuhn, L.T.; Gerstenfeld, L.C.; Glimcher, M.J. *J. Orthopaedic Res.,* **2004***, 22*, 703.
[56] Damle, N.K.; Frost, P. *Curr. Opin. Pharmacol.,* **2003***, 3*, 386.
[57] McNeil, S.E. *J. Leucocyte Biol.,* **2005***, 78*, 1.
[58] Sarker, D.K. *Curr. Nanosci*., **2005**, *1*, 1.
[59] Sarker, D.K. *Curr. Drug Delivery,* **2005***, 2*, 297.
[60] Zhang, N.; Ping, Q.N.; Huang, G.H.; Xu, W.F. *Int. J. Pharmaceutics,* **2005***, 294*, 247.
[61] Ito, T.; Sugafuji, T.; Maruyama, M.; Ohwa,Y.; Takahashi, T. *J. Supramol. Chem.,* **2001***, 1*, 217.
[62] Lawrence, J.; Rees, G.D. *Adv. Drug Delivery Rev.,* **2000***, 45*, 89.
[63] Williams, A.S.; Love, W.G.; Williams, B.D. *Int. J. Pharmaceutics,* **1992***, 85*, 189.
[64] Muller, R.H.; Keck, C.M. *J. Biotechnol.,* **2004***, 113*, 151.
[65] Wu, H.; Ramachandran, C.; Bielinnska, A.U.; Kingzett, K.; Sun, R.; Weiner, N.D.; Rossler, B.J. *Int. J. Pharmaceutics,* **2001***, 221*, 23.
[66] Washington, C. *Adv. Drug Delivery Rev.,* **1996***, 20*, 131.
[67] Sonneville-Aubrun, O.; Simonnet, J.-T.; L'Alloret, F. *Adv. Coll. Interface Sci.,* **2004***, 108-109*, 145.
[68] Garti, N.; Aserin, A.; Tiunova, I.; Fanun, M. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **2000**, *170*, 1.
[69] Di Stephano, G.; Lanza, M.; Kratz, F.; Merina, L.; Fiume, L. *Eur. J. Pharmaceutical Sci.,* **2004***, 23*, 393.
[70] Van Domeselaar, G.H.; Kwon, G.S.; Andrew, L.C.; Wishart, D.S. *Coll. Surfaces B :Biointerfaces,* **2003***, 30*, 323.
[71] Kafedjiiski, K.; Krauland, A.H.; Hoffer, M.H.; Bernkop-Schnurch, A. *Biomaterials,* **2005***, 26*, 819.
[72] Dalpiaz, A.; Pavan, B.; Vertuani, S.; Vitali, F.; Scaglinati, M.; Bortolotti, F.; Biondi, C.; Scatturin, A.; Tananelli, S.; Ferrarro, L.; Marzola, G.; Prasad, P.; Manfredini, S. *Eur. J. Pharm. Sci.,* **2005***, 24*, 259.
[73] Pignatello, R.; Di Guardo, L.; Puleo, A.; Puglisi, G. *Thermochim. Acta,* **2005***, 426*, 163.
[74] Rothbard, J.B.; Jessop, T.C.; Wender, P.A. *Adv. Drug Delivery Rev.,* **2005***, 57*, 495.
[75] Yamazaki, N.; Kojima, S.; Yokoyama, H. *Curr. Appl. Phys.,* **2005***, 5*, 112.
[76] Morphy, R.; Kay, C.; Rnakovic, Z. *Drug Discov. Today,* **2004***, 9*, 641.
[77] Sato, H.; Sugiyama, Y.; Tsuji, A.; Horokoshi, I. *Adv. Drug Delivery Rev.,***1996***, 19*, 445.
[78] Wallace, R.C.; Purnell, G.L.; Jones-Jackson, L.B.; Thomas, K.L.; Brito, J.A.; Ferris, E.J. *Neurotoxicol.,* **2004***, 25*, 533.
[79] Alper, J. In *Chemistry*, Spring Edition, American Chemical Society Press: USA, **2005**; pp. 23-27.

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