Review Paper

The counter ion: expanding excipient functionality

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ABSTRACT

It is increasingly evident that drug delivery mechanisms involving an assembly of molecules, or changes in their conformation, are often counter ion dependent, and may therefore be conveniently modulated by counter ion selection. Such mechanisms include supramolecular assembly, topological changes or ion pair formation to penetrate cell membranes and improve transfection efficiency, self association to achieve ratiometric release and interfacial force manipulation, and salt bridge engineering for spatial and temporal control of drug delivery. Counter ions serve as useful tools in pharmaceutical manufacturing processes, such as, imparting enantioselectivity, enhancing conventional and biocatalytic reaction rates, controlling the precipitant product's physico-chemical characteristics (such as morphology, particle size and surface area), imparting conductivity to polymers, in the bottom up synthesis of nano and micro electromechanical devices, enhancing protein refolding yields, and as selectable components of ionic liquids. When combined with other 'carrier system' approaches, counter ion modulation has the potential to significantly improve drug targeting and delivery. Our failure to recognize and understand its utility, coupled with a historically dismissive *weltanschauung*, has so far prevented us from unlocking its full potential.

KEY WORDS: Counter ion, drug delivery, excipient, ion-pair, drug targeting, self assembly, drug release

INTRODUCTION

Ionizable excipients (or molecules) dissociate into two (or more) ions in solution. Historically, the smaller of the two ions has been designated as the counter ion. Excluding the literature on pharmaceutical salts and ion assisted analysis, where the function of the counter ion is well understood and its importance recognized, the prevailing *zeitgeist* has treated the counter ion as part of the 'background', an uninteresting, yet inseparable part of ionic molecules. Such a view is not entirely unjustified, especially if the counter ion is easily exchangeable prior to, during and after dissolution, and the only value it adds is the ease of handling of pharmaceutical materials and excipients. It is not surprising, therefore, that studies in the literature that seek to methodically understand the mechanism of the action of the counter ion are sparse. In contrast, studies that merely report counter ion effects as being secondary in influencing or achieving another primary objective, are numerous, and are found in a large number of scientific journals, thus testifying to the universality of columbic forces, and to the need for a consolidated review.

Emerging evidence, however, has demonstrated that the counter ion is an extremely important facet of pharmaceutical systems, especially in drug product manufacturing processes and drug delivery. Since drug delivery systems have the

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ability to modulate the degree of ionization of incorporated molecules (including excipients), even in a medium of high dielectric constant, excipient counter ions can be 'tuned' to dissociate spatially, and temporally, in order to impart desired characteristics. Excipient counter ions (and excipients) that are not readily exchangeable can be designed, and will 'stick', or remain ion-paired, to the active pharmaceutical ingredient (API) for a considerable period of time, even when ionized and dissolved. Such capability, in turn, modulates drug efficacy, receptor engagement, cell or nuclear penetration, and changes in protein conformation as a function of the specific *type* of counter ion.

The focus of this review will be on applications in API and drug product manufacture and drug delivery. It will not cover reviews on pharmaceutical salt development (1), neither does it cover the use of counter ions in analytical chemistry (2). Both fields have been extensively covered previously in the literature.

If the mechanism by which counter ions influence the manufacture and delivery of medicinal agents can be determined, the knowledge can be used to pair appropriate counter ions (i.e. excipients containing these counter ions) with APIs so as to optimize drug delivery. Such mechanisms stem from properties related to the electronic configuration of the counter ion such as polarizability, kosmotropic or chaotropic character (3) (particularly as it relates to the Jones-Dole coefficient (4)), hydrophilicity (5), hydration, size (6), molar conductivity (7), electron density, chirality and steric properties. Counter ion specificity has also been shown to be dependent on the degree of protonation of selected paired amphiphiles (8). The counter ion contribution to the delivery influencing properties of the resultant API-counter ion pair cannot be attributed to a single function of the counter ion, rather these functions are inextricably intertwined. However, the contribution of some functions relative to others may be capable of being empirically determined (9).

MEMBRANE PENETRATION

Several counter ion-assisted passive transport mechanisms have been proposed which allow APIs to cross cell membranes in order to reach therapeutic targets. These include:

1 Ion-pairing to hydrophobic counter ions in order to overcome the increase in free energy required to insert a highly charged species into the low dielectric medium of the internal lipophilic phase of the phospholipid bilayer membrane (10).

2 The scavenging of anionic counter ions by alkylguanidinium cations of polyarginine residues in cell penetrating peptides, which can be considered as a specific instance of mechanism (1).

3 Phospholipolysis catalyzed by (counter ion facilitated) cationic groups on APIs and subsequent micellar budding and translocation between different bilayer membranes (11, 12).

4 Change in the amphiphilicity of an API or excipient with pH (13).

The above does not represent three distinct mechanisms of translocation across membranes, rather, translocation may occur using all three mechanisms in combination, albeit to differing degrees.

Potential drug delivery agents such as cell penetrating peptides (CPPs) consist of a disproportionately large number of arginine residues. Attempts to understand this "arginine magic" have recently revealed the critical role of counter ions in influencing the 'dynamic biphilicity' of the oligoguanidinium-counter ion complexes (14). It turns out that the insufficient acidity of the guanidinium cation (pK_a = 12.5) renders it incapable of releasing protons for the minimization of charge repulsion (as can occur

with the more acidic lysine cations, $pK_a = 10.5$). As a result, intramolecular charge minimization of the proximal guanidinium groups in polyarginine can only occur via counter ion scavenging (15). With the 'right' hydrophobic counter ions in place in the cell membrane, or administered as part of the formulation (the so called 'translocation catalysts' such as stearic acid, phosphatidylglycerol, cholesterol sulfate or pyrene butyrate), CPP delivery to live cells is significantly increased. Furthermore, 'direct' cytosolic delivery (as opposed to endocytic uptake) of large proteins may be possible by this mechanism due to the hydrophobic counter ion mediated diffusion through the cell membrane.

Membrane penetrating agents based on polyguanidino-oxanorbornene (PGON) have been designed that show specific selectivities. PGON-counterion complexes can serve as transporters whose selectivity can be modulated potentially by the choice of counterion (16).

The carboxylates of higher arenes, such as pyrene, coronene or fullerenes were found to be good activators of membrane translocation of CPPs (17). Since fullerenes have been used in clinical studies to deliver imaging agents, as well as packaged molecules, fullerene carboxylates can potentially be used as excipients to enhance CPP delivery to live cells. As an example, the molecule penetratin (a CPP that can scavenge the counter ion activator fullerene carboxylate) can be ion paired to arginine residues on the protein in order to enhance translocation.

It has been proposed that, at physiological pH, the proton released from the weakly acidic quaternary amine of cationic amphiphilic APIs, triggers the acid catalyzed ester hydrolysis of phospholipids. The resulting lysophospholipid's propensity is to form micellar structures (as opposed to bilayer membranes or liposomal structures) and thus to separate from the membrane bilayer in which it was initially incorporated (before hydrolysis). These micellar membrane fragments form mixed micelles with the cationic amphiphile and thus carry the API to other membranes thereby facilitating the transportation of the drug between, and across, cellular membranes. It has been shown that the choice of the counter ion for the API exerts a significant effect on the hydrolytic activity of the API, and consequently on its ability to traverse bilayer scaffolds via a degradative mechanism.

Ideally, an API or excipient should be able to penetrate the cell and escape from the endosome/lysosome. The number of protonatable groups on the counter ion caused an increase in the amphiphilicity of lysine based surfactants when the pH was decreased below the pK_a, as would be expected when inside the acidic environment of the lysosome. Surfactants with lysinium counter ions were therefore more effective as lysosomotropic agents than those with a tris counter ion (18). An increased number of protonatable groups could also function as a "proton sponge", causing a compensatory influx of water leading to lysosome rupture (19).

AGGREGATIVE MODULATORS

Photosensitizers, such as the porphyrins and the cyanines, are used clinically in the treatment of cancers and age related macular degeneration. These molecules, which typically possess a flat extended π electron system, are also prone to aggregation into highly ordered so-called J (edge-to-edge) and H (face-to-face) aggregates. Aggregation generally has a detrimental effect on photosensitizing ability (20), the maximal reaction with ground-state oxygen usually occurs with the monomeric species. The nature of the counter ion has been shown to affect the aggregation state of porphyrins usually in a Hofmeister series (lyotropic series) dependent manner (21-24). The choice of counter ion thus may have a profound effect on the efficacy of these compounds when used for Photo Dynamic Therapy. A similar mechanism of aggregative modulation has been postulated for 'ratiometric' drug release (see below).

DRUG RELEASE

It has been found that antineoplastic drug combinations exhibit synergism against tumor cells at certain specific molar ratios, while exhibiting no synergy, or even antagonism, at others. Achieving such 'ratiometric dosing' (25) becomes more difficult when multiple drugs are packaged into delivery vehicles, such as liposomes, because the release (from the liposome) is dependent on the relative thermodynamic properties of the individual drugs which may not lead to synergistic drug concentrations in the vicinity of the tumor cells. It has been shown that certain metals and/or excipients can modulate the aggregative behavior of encapsulated drug(s) such that ratiometric dosing can be obtained (26). The aggregation state of Irinotecan, co-encapsulated in liposomes along with floxuridine, could be modulated by copper gluconate and triethanolamine such that it demonstrated a slower release resulting in *in-vivo* synergistic concentrations of the two encapsulated drugs (27-28).

Order of magnitude differences in release rates, and synergistic increases in drug loading, can be observed when different inorganic or organic acidic counter ions are used during the processing of multivesicular liposomes using DepoFoam® technology (29).

SALT BRIDGES

The ion-pair(s) contributing to salt bridges in folded proteins are not excipients. However, judicious placement of such counter ions (which consist of charged amino acid residues) in engineered proteins or protein-polymer structures can modulate conformational change dependent drug delivery. In this context, the engineered protein or protein-polymer structure can be designated as an excipient whose drug delivery mechanism is counter ion dependent.

Vascular endothelial growth factor (VEGF)-laden, photopolymerized (mutant)calmodulin-PEG(acrylate) microspheres could be induced to trigger growth factor release upon incubation with the ligand, trifluoperazine (30). The ligand was shown to induce a conformational change in the calmodulin which caused a significant decrease in hydrogel volume which was, in turn, accompanied by VEGF release. In addition to achieving temporal control over growth factor delivery, the magnitude of the volume change, and hence, the amount of VEGF released, could be controlled by varying the cross-linking time and the ligand identity (31).

The principle of photoisomerization of a flexible chromophore to disrupt a salt bridge between the chromophore and its counter ion, namely another (oppositely charged) amino acid residue on the (rhodopsin) protein, appears to be generic to visual proteins (32). The resulting conformational change brings about phototransduction. By virtue of the counter ion in the protein not being mobile, only photon induced (not thermally induced) isomerization can occur (33, 34).

Based on the above examples, tumor cell receptor specific mutant protein microspheres could, conceivably, be designed to release therapeutic agents (such as VEGF antagonists) based on some generic tumor micro-environment specific triggers such as, altered pH, oxygen content, glucose utilization etc. These triggers would act to change the carrier-protein conformation by disruption of engineered salt bridges thereby releasing the drug payload. Investigators have demonstrated that pH dependent disruption of salt bridges is a plausible mechanism to modulate drug release (35-37).

As a corollary, salt bridges which have been disrupted due to mutations in the native protein can be restored so that receptor binding activity for the protein is restored. By contrast, salt bridge disruption(s) in a mutated receptor, which confers known altered sensitivity to a family of pharmacophores targeting that receptor, can determine the choice of pharmacophore for maximal efficacy.

An example of the former is the Apo-E2 isoform, where the Arg158-Cys mutation disrupts a salt bridge network, thereby altering the surface charge presented by Apo-E to its receptor (38). It may be possible to design compounds that bind to the Cys158 site so as to restore the native salt bridges. These are the so called pharmacoperones or pharmacological chaperones (indoles or quinolones possessing a piperidine ring or a secondary amine) which form ligand-mediated salt bridges to act as surrogates for the disrupted ones (39). Excipients such as Good's buffers could conceivably replace these pharmacoperones because they contain a piperazine ring and two centers of charge contained in the same molecule. It is interesting to speculate if salt bridges in mutant proteins could be restored by drug delivery systems containing Good's buffers. This proposal is not as far-fetched as it appears because these buffers have application in the study of protein conformation, and inexplicable or disparate results have not generally been attributed to differences in buffer composition (40).

An example of the latter is the epidermal growth factor receptor (EGFR), which represents a validated pharmacological target in various cancers. It has been found that a mutation in the EGFR gene, which disrupts the ion-pair between residues E884 and R958, results in altered kinase inhibitor sensitivity (41). Therefore knowledge of target kinase mutations can aid in the selection of kinase inhibitors for maximal efficacy.

The V510D mutation increases the stability of the ΔF508-cystic fibrosis transmembrane conductor receptor by promoting nuclear binding domain1-trans membrane domain 2 interactions, likely through the formation of a salt bridge with Arg1070 in ICL4 (42). Excipients, again either derived from the Good's buffers, or related molecules, could serve as a surrogate salt bridge between the 510 and 1070 peptide locations.

ELECTROSTATIC EFFECTS

Reactions in non aqueous media using enzymes as catalysts have many advantages. Many substrates are more soluble in organic than in aqueous solvents, enzymes are generally more thermostable in organic media and product recovery is easier in these easily evaporated solvents or, if carried out in polar ionic liquids, via supercritical processing (43). Furthermore, non-aqueous bioprocessing allows for tuning of enzyme selectivity by modification of solvent composition rather than the enzyme itself. In addition, the propensity of counter ions to associate strongly with charges in the enzyme, or its substrates, in media of low dielectric constant provides access to more tunable factors for better control of reactions simply by changing the counter ion (44). This includes the so called 'pH- (45, 46) or counter ion- (47) memory effect' as well as the 'bell-shaped effect' (48, 49). The one significant disadvantage of non-aqueous bioprocessing, the decrease in reaction rates, can be improved by the careful choice of counter ion in crystal-liquid two-phase systems (see below).

A wide variety of polyelectrolyte polymers can function as drug carriers (50, 51). While electrostatic interactions are obvious in such systems (and will not be discussed here due to the vast body of literature that already exists), the following example illustrates an atypical, yet important aspect of electrostatic interactions of counter ions with excipients that can influence efficacy. It has been demonstrated that *Vibrio cholerae* strongly adheres to starch (52). Various clinical studies have shown significant improvement in symptoms when starch based oral rehydration solutions (ORS) were administered to patients (53). Adhesion between starch and bacteria has been shown to be influenced by non-specific electrostatic forces, such as salt concentration, under simulated gastro intestinal tract (GIT) conditions (54). Therefore, starch based ORS should be formulated such that the electrolyte counter ion concentration to starch ratio is not detrimental to bacterial adherence, and

therefore does not decrease the effectiveness with which the causative organism is removed from the GIT.

IONIC LIQUIDS

Ionic liquids (ILs) are generally defined as salts that have melting points below 100°C. They were originally studied as chemical solvents, but have since been used in applications such as electrochemical devices (55), biomass conversion (56), protein stabilizers (57) and anticancer therapeutic agents (58). In the pharmaceutical context, ionic liquids exist in the liquid phase at room temperature. Although most solid pharmaceutical salts circumvent the problems of low solubility of the free base or acid, some problems exist, such as polymorphic conversion of the active pharmaceutical ingredient (API), reduced ability to cross biological membranes, spontaneous crystallization of amorphous forms, and hygroscopicity.

The ability of many APIs to form ionic liquids at room temperature depends on the choice of counter ion. Usually, these counter ions are bulky organic moieties with the ability to diffuse charge and disrupt crystalline network formation. Many of these counter ions are known excipients or, generally recognized in pharmaceutical formulations, as safe (GRAS) moieties. Examples include the acesulfamate ion paired with the antimuscarinic API, propanthelene and the docusate ion paired with the anesthetic, lidocaine (59). Unlike solid pharmaceutical salts, which necessarily must affect fixed paired physico-chemical attributes usually in a gain-loss fashion, ILs' can be 'tuned', by the choice of an appropriate counter ion, so that the attribute gain-loss pairing occurs by choice (60). For example, ILs can be designed to not only improve the aqueous solubility of the API, but also its ability to cross biological membranes. In addition, the counter ion itself can be an API which may act synergistically with, may counteract the side effects of, of may add another functionality to the original drug molecule. Such 'dual functionality' applications exploit the full potential of ILs for pharmaceutical uses.

Ionic liquids can be used to dissolve substances otherwise soluble only in water or in the 'classical' polar aprotic solvents such as dimethylformamide (DMF), dimethylacetamide (DMA) or dimethylsulfoxide (DMSO). This has the advantage that such substances can be synthesized or modified reproducibly with increased yields. For example, sulfated glycosaminoglycans were capable of being dissolved in imidazolium benzoate ionic liquids (61). The activity and stability of enzymes can be improved by design of ionic liquids whereby the cation contains one or multiple hydroxyalkyl groups (62). Interestingly, this investigation was based on the prior observation that inclusion of the hydrophilic lyoprotective excipient tris(hydroxymethyl) aminoethane could increase enzyme activity in ionic liquids (63).

The chirality of the counter ion of an IL may significantly alter its properties. For example, 1-alkylimidazolium salts of L-lactate demonstrated significantly lower MIC values against gram positive and gram negative bacteria, as well as, fungi compared to the DL-lactate counterparts (64).

MORPHOLOGY AND SURFACE CHARGE DENSITY

The transfection efficiency of genetic material delivered in cationic lipid complexes has been shown to be dependent in part on the lipid-DNA morphology (65, 66), which in turn, can be modulated by the lipid structure and composition. Chiral excipients, such as tartaric acid, are capable of modifying the topology of structures formed by certain amphiphilic carriers such as the Gemini® surfactants (67). Gemini® surfactants with chiral tartarate counter ions form 'twisted ribbons' (68) whose pitch can be modulated by varying the stoichiometry of the chiral excipient to amphiphilic carrier (69). The more favorable morphology of such structures may enable

better transfection efficiencies of complexed DNA.

Polynorbornene methyleneammonium cations paired with the counter ions, lactobionate or acetate, demonstrated significantly greater gene transfection than when paired with chloride. The hydrophilicity and the size of the counter ion, as well as, the relative size of the DNA-counter ion particles affected the gene transfer efficiency (70).

Mixing a cationic Gemini® surfactant with dimyristoyl phosphatidylcholine (DMPC) demonstrated a stoichiometry dependent reorganization in the headgroup region of the liposome bilayer which correlated with the magnitude of DNA compaction, and DNA transfection efficiency (71). The avidity with which different counter ions interacted with the Gemini® surfactant was proportional to the magnitude of condensation of the monolayer (72). Another study demonstrated a modest decrease in transfection with increased inductive electron withdrawal from ammonium ion headgroups of cationic lipids (73) suggesting that this may be a mechanism by which the electronegativity of the counterion influences transfection efficiency.

Linear charge density differences, as well as differences in helix structure, differentiate counter ion condensation on the A-form helix double stranded RNA (dsRNA) versus the B-form helix on double stranded DNA(dsDNA) (74). These changes in counter ion spatial distribution cause repulsion between RNA helices to vanish at lower ionic strength. Optimum counter ion conditions for packaging single interfering RNA (siRNA) are therefore likely to differ from those used to condense DNA for efficient cellular penetration.

The ability of an anionic surfactant to induce apoptosis in cells decreased as the size of the counter ion was increased in the order lithium > sodium > lysine > tris(hydroxymethyl)aminomethane (75). Counter ion specific modulation of the antimicrobial activity of amphiphilic hydrogelators has also been demonstrated (76).

SELF-ASSEMBLY

Self assembled structures with nanoscale dimensions are important in the fabrication of artificial ion channels, nanowires, biosensors, scaffolds and micro- and nano- electromechanical devices (MEMS and NEMS). Considerable economic and technological hurdles must be overcome to be able to artficially fabricate these structures on an industrial scale. In contrast, spontaneous, thermodynamically downhill, 'bottom-up', chemical fabrication of such self assembled structures is elegant and reproducible. In many instances, precise control of the process of self assembly, as well as the final structure of the supramolecular product, is mediated by the counter ion (77, 78) in combination with other tunable factors.

Cyclic peptides with alternating D and L residues stack on top of each other to form nanotubes which can be designed to selectively penetrate bacterial membranes. The nanotube characteristics can be varied by varying the amino acid sequence and their number on the cyclic peptide. In addition, because supramolecular assembly is dominated by non covalent interactions, it is reasonable to assume, and the literature suggests (79, 80), that charged species in the environment around the cyclic peptides will determine topological isomerism. Controlling the release of a suitable counter ion containing excipient at the site of infection (along with administration of suitable cyclic peptides) could thus increase selectivity and sensitivity of the formed nanotubes. The reader is referred to a review (81) of self assembling peptide nanotubes for more applications in drug delivery.

MANUFACTURING PROCESSES

The synthesis of single enantiomer APIs requires a process whereby the formation of one of the enantiomers is highly preferred. Such enantioselective synthesis has been

possible using chiral catalysts, usually comprising a chiral group bound to a central metal atom. It has recently been shown that an achiral catalyst, in the presence of its chiral counter ion, can confer greater enantio-selectivity in certain reactions than would otherwise have been possible using only the chiral catalyst (82-84). Going a step further, chiral catalysts coupled to their chiral counter ions demonstrated synergy in enantioselection. Thus, a small library of chiral counter ions has the potential to produce a wide range of enantioselective cationic catalysts. In addition, certain counter ions can impart stereoselectivity to glycosylation reactions by preferential coordination with the oxacarbenium cation on one side of the anomeric carbon, thereby hindering a nucleophilic attack on that side (85).

The electrochemical polymerization of electrically conducting polymers in the presence of different counter ions leads to significant changes in their impedance and charge storage capacity, properties important for signal fidelity and charge delivery respectively in the fabrication of neural implants and bio-stimulation devices (86). Electrochemical doping with different counter ions can result in differing redox properties of the doped polymer, a phenomenon known as iono-chromism (87). Biotin has been used as a counter ion dopant in a conducting polymer, polypyrrole, in an *in vitro* electrically stimulated nerve growth factor release system (88).

'Tunable wettability' is a phenomenon which is capable of making polymer surfaces hydrophobic or hydrophilic via several triggers such as dopants, pH, ionic strength and the presence of specific counter ions (89). The polymer surface can be cycled between the two states multiple times (90). Replacing the Cl^- counter ions in the hydrophobic polymer Poly[2-(methacryloyloxy)-ethyl-trimethylammonium chloride] (PMETAC) brushes with SCN^-, PO_4^{3-} and ClO_4^- anions promoted a dramatic change in the wetting properties of the substrate (91). Such a phenomenon could be used for the periodic release of drugs by associated periodic release of a suitable counter ion in its vicinity.

The induction time for the deposition of silicate on cationic surfactant templates to form mesoporous silica is a function of the counter ion of the added salt. It increases according to the Hofmeister series, reflecting the binding strength of the counter ion to the surfactant (92). The induction time can potentially control the pore structure, wall thickness and particle size (93) of the manufactured mesoporous silica particles. Hydroxyapatite nanoparticles, whose morphologies are counter ion dependent, can be manufactured via hydrothermal synthesis (94). Silver sols could be prepared by the reduction of silver salicylate with ascorbic acid which were stable at concentrations two orders of magnitude greater than those prepared by the reduction of silver nitrate. The increased stability at higher concentration was attributed to the differences in redox potential between the nitrate and salicylate anions, and an expansion of the diffuse layer boundary away from the surface of the reduced silver particle in the presence of the latter (95).

The manufacture of recombinant proteins commonly involves the solubilization of insoluble protein aggregates from inclusion bodies by denaturing agents. These denatured proteins then must be refolded back to their native conformation *in vitro*. Various organic cations such as derivatives of N, N'-substituted imidazolium, N-substituted pyridinium, tetraalkylated ammonium and tetraalkylated phosphonium ions, combined with different counter anions such as chloride, acetate, diethyl phosphate, ethyl sulfate, hexyl sulfate, p-toluenesulfonylate and dimethyl phosphate making up the ionic liquids, have been investigated as refolding solvents. While no generalizations could be drawn, the nature of the counter-anion significantly influenced the ability of the ionic liquid to refold various proteins (96).

Hydrophobic ion pairing (97) can be used to decrease the aqueous solubility of protein APIs.

This makes it possible to dissolve both the hydrophobic polymers (used as a polymeric matrix for controlled release) and the hydrophobic ion paired protein API in the same solvent. Loading efficiencies and protein denaturation/degradation are subsequently maximized and minimized respectively upon formation of drug loaded polymer micro-spheres when in contact with water and processed further.

A limitation of the nature of biocatalytic processes is that product yields are low because of biocatalyst inhibition at high substrate concentration. When the substrate is ionizable, i.e. capable of being protonated or deprotonated, the choice of a metal counter ion can dictate the solubility of the (metal) complexed substrate, or the solubility of the product, thereby decreasing substrate or product inhibition of the biocatalyst. This principle is widely used to improve product yields in commercialized bioprocesses (98, 99). The substrate counter ion has been shown to have a significant effect on biocatalyst activity depending on the stability constant (ratio between the undissociated or counter ion complexed substrate to the dissociated substrate) (100).

CONCLUSION

Charge separation, association or modulation is a powerful tool in drug delivery because columbic interaction constitutes a universal mechanism for cellular communication. Drugs can be delivered to specific cellular targets if their en-route charge attributes can be optimized for each barrier they have to cross or optimized depending on the cellular membrane phospholipid composition. A purely speculative example would be the alteration of the pitch of the 'twisted ribbon' of a Gemini surfactant carrying its DNA payload by controlled release of tartaric acid in its vicinity so as to effect optimum transfection depending on the composition of the cell membrane of the targeted tissue or organ.

Counter ions are more than passive bystanders in pharmaceutical manufacturing and development. They influence diverse processes including solubility, drug delivery and manufacturability. Counter ion incorporation into excipient salts, amphiphilic surfactants and ionic liquids may be a plausible avenue by which their unique characteristics may be utilized.

Can excipients comprising the appropriate counter ions play a role in the suppression of drug efflux pumps to decrease multi drug resistance toward cytotoxic drugs? Can specific counter ions induce or enhance the lysosomotropic effect of excipient amphiphiles? Can DNA or siRNA be predictably made to condense to a smaller hydrodynamic volume with enhanced charge screening by cationic lipids or amphiphiles in the presence of specific counter ions? Could a model be constructed to predict the solubility and stability of APIs in an ionic liquid composed of specific counter ions? Can specific counter ionic dopants change the properties of drug loaded biocompatible polymer surfaces in response to externally applied or physiological stimuli, and can they do this predictably and reproducibly multiple times? These are some questions the answers to which will require an understanding of fundamental mechanisms and first principles by which counter ions act. It is hoped that this review will act as an impetus for more research in this area, and play a role in bringing counter ion into the scientific limelight.

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