Deep eutectic solvents as excipients for increasing the bioavailability of orally administered protein active pharmaceutical ingredients.

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Editorial

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We beat it out flat; we beat it back square; we battered it into every form known to geometry – but we could not make a hole in it.
"Three men in a boat." Jerome K. Jerome

To enable orally administered protein Active Pharmaceutical Ingredients (APIs) to permeate the intestinal membrane into the blood has so far remained an insurmountable challenge. Single digit bioavailability makes the cost of goods alone, a potent demotivator. Incremental progress is irrelevant from a commercial and competitive standpoint, an order-of-magnitude improvement is needed at a minimum, while a closer to two orders-of-magnitude may very well achieve the holy grail of obsoleting ambulatory IV administration altogether. Many would argue that the right tools, substances that significantly and transiently increase intestinal mucosa permeability on demand, have either not yet been discovered, or not been judiciously and intelligently used; not unlike the desperate measures to open a can without a can-opener, so poignantly depicted with 'hopeless and incurable veracity' by Jerome K. Jerome's hapless protagonists.

A mixture of substances that displays a melting point at defined molar ratios that is significantly lower than that of the pure substances alone is said to exhibit eutectic behavior. In some instances, the melting point of the mixture may be lowered to below room temperature, such that the mixture exists as a liquid at room temperature. As an example, the melting point of a 1:2 molar ratio of choline chloride (melting point at 302°C) and urea (melting point at 133°C) is 12°C. Deep eutectic solvents (DES) are composed of a quaternary ammonium salt that functions as a hydrogen bond acceptor in conjunction with one or more hydrogen bond donors such as polyalcohols, amides or organic acids. They can be formed from a variety of Generally Recognized as Safe (GRAS) designated substances and thus are more suitable for biopharmaceutical use than are ionic liquids. It has been proposed that the reason why simple compounds such as sugars, amino acids, choline and organic acids show up in considerable amounts in mammalian, plant and microbial cells is because they form natural deep eutectic solvents (NADES) that enable the biosynthesis, storage and recycling of water and lipid insoluble compounds and polymeric macromolecules in high concentrations. NADES are exemplified by maple syrup, honey and an equimolar mixture of sucrose, fructose and glucose; among others.

Therapeutic deep eutectic solvents (THEDES) have been described containing an API as one of the DES components. These systems provide modest increases in skin or intestinal mucosa permeability for

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transdermal systems and class III or IV BCS APIs respectively. Enteric coated capsules containing 10 U/Kg insulin dissolved in a choline-geranic acid eutectic solvent caused a decrease in blood glucose levels similar to that for 2 U/Kg s.c administered insulin. Although this represents only a 20% functional bioavailability when compared to the s.c formulation, it is significantly greater than that (2%) achieved by Novo-Nordisk using a gastro-intestinal permeation enhancement technology (GIPET©, containing excipient salts of C_8 and C_{10} fatty acids) oral formulation in a phase II trial. In the oral eutectic insulin formulation, tight junction integrity as measured by transepithelial electrical resistance (TEER) was comparable to that of sodium caprate while the mucous layer viscosity was significantly reduced from 10 mPa.s to 2.5 mPa.s. This indicates either a more effective or a more multifaceted mechanism(s) of permeability enhancement by eutectic solvents when compared to their amphiphilic surfactant counterparts.

Chemical permeation enhancers have been classified based on their mechanism of action. In a study to correlate molecular descriptors with the effectiveness of chemical permeation enhancers, those enhancers that extracted lipids from the stratum corneum were termed extractors, while those that partitioned into the bilayer were termed fluidizers, based on the FTIR change in absorbance of the methylene stretching peak originating from the fatty acids in the bilayer. The effectiveness of fluidizers correlated with the molecular descriptor of the logarithm of their octanol-water partition coefficient (log P), whereas that for extractors was proportional to the ratio of their hydrogen bonding to the square root of the cohesive energy density. The ratio of solubility of the protein API in the DES to its solubility in water (solubility enhancement ratio, SER) therefore would seem to dictate whether the DES need be a fluidizer or an extractor for maximum membrane permeability enhancement. A fluidizer DES would be expected to enhance permeability to a greater extent for an API with a larger SER, whereas an extractor DES would be expected to enhance permeability to a greater extent for an API with a SER close to unity. Therefore, an increase in SER is not a precondition for BCS class III APIs when formulated with extractor DES. On the other hand, BCS class IV APIs would need to be formulated in fluidizer DES with a high SER.

The eutectic state is marginally lower in Gibbs free energy than the individual substances because the magnitude of the decrease in enthalpy brought about by the formation of hydrogen bonds between the individual constituents is greater than the energy required to break lattice bonds (enthalpy of fusion). However, there seems to be a considerable activation energy barrier associated with DES formation. For example, many of the DES do not form unless heated significantly above body temperature for extended periods of time. Even small changes in aliphatic chain length or the presence of a hydroxyl group on the hydrogen bond donor organic acids or the organic cation, or changing the anion on the quaternary ammonium hydrogen bond acceptor molecule, cause considerable changes in the chain melting temperature of phospholipids within a bilayer in contact with DES, lipid extraction efficiency, SER or flux across membranes. The extent of the melting point depression of the DES is directly proportional to the number and magnitude of hydrogen bonds, although correlation is poor.

It is becoming increasingly evident, that drastic variation in membrane permeability improvement can be obtained for the same protein/API incorporated into different DES. Molecular descriptor models attempt to reduce recourse to rule of thumb and empirical data. These models can predict multiple optimum thermodynamic or computational DES combinations for a given protein, not all of which, however, may be *functionally* optimum. A judicious use of DES as permeability enhancing excipients for a given protein should start from generalized assumptions, first approximations and logical qualitative categorization, whose output should then serve as the input for thermodynamic and computational models. Such a scheme ensures the best functional pairing of API and DES and avoids the not infrequent pitfalls of the indiscriminate application of mathematical models, that are themselves non-discriminatory toward excipient function, that have plagued past chemical permeation enhancement

efforts on account of not being able to see the forest for the trees. One such scheme is attempted in Table 1. According to the classification model proposed here, BCS class III APIs/Proteins permeability would not be significantly increased by pairing them with fluidizers, nor by exclusively increasing their SER in extractor *or* fluidizer DES, nor by utilizing DES individual constituents that have (relatively) lower melting points, regardless of how well their molecular descriptors match those of the DES. Judicious selection and pairing with the API protein is essential so that this new class of emerging promising eutectic solvents, does not suffer the same fate - viz. that of relegation to permeability enhancement excipient oblivion – as that of previous chemical permeability enhancers.

There are aspects to the DES paradigm that need more investigation. For example, lactic acid is known to form DES with various amino acids in addition to quaternary ammonium salts. Recombinant GRAS designated lactic acid bacteria (LAB) are Gram positive organisms that are being investigated to deliver (recombinant) proteins upon oral ingestion. A particularly intriguing and tantalizing opportunity is being exploited by non-integrative plasmid transformed yogurt bacteria expressing the protein API. Anecdotal data indicates that the bioavailability of yogurt expressed protein is an order-of-magnitude greater than that of free protein alone. It is not unreasonable to suggest that the mechanism could be due to the lactic acid from the yogurt bacteria spontaneously forming a DES with amino acid or related components of the mucous in a fashion similar to that of NADES. If *in vivo* formation of DES could be achieved in close proximity to the intestinal mucosa by the lactic acid from recombinant LAB, the downstream processes of protein extraction, glycosylation, refolding, purification and formulation in manufacturing processes could be eliminated. Protalix Inc. has a variant on this scheme using carrot cells in clinical trials.

Ill-informed excipient choice decisions range from those made in an egregiously *ad hoc* manner based on the exigencies of the moment, funding, time, regulatory or supply chain constraints, to those decisions made based on thermodynamic or computational models unsuited to functionality and/or application. The input from sound qualitative generalizations subjected to conventional mathematical models, in conjunction with the inherent membrane interactivity of DES rectifies the latter. A judicious use of deep eutectic solvents may bring back oral protein administration from the brink of failure.

Table 1 Formulation selection for maximum membrane permeability

PROPERTY/ATTRIBUTE	BCS CLASS III APIs/PROTEINS	BCS CLASS IV APIs/PROTEINS
Nature of DES	Extractor	Fluidizer
SER a precondition for formulation	No	Yes
Structure of DES components	Increased number of functional groups capable of hydrogen bonding, lesser aliphatic chain length of organic acids, smaller molecules with lesser polarizable electrons and London Dispersion Forces	Decreased number of functional groups capable of hydrogen bonding, greater aliphatic chain length of organic acids, larger molecules with greater polarizable electrons and London Dispersion Forces
Difference between parents' and eutectic melting temperature	Parent molecules forming DES have (relatively) greater melting points	Parent molecules forming DES have (relatively) lesser melting points