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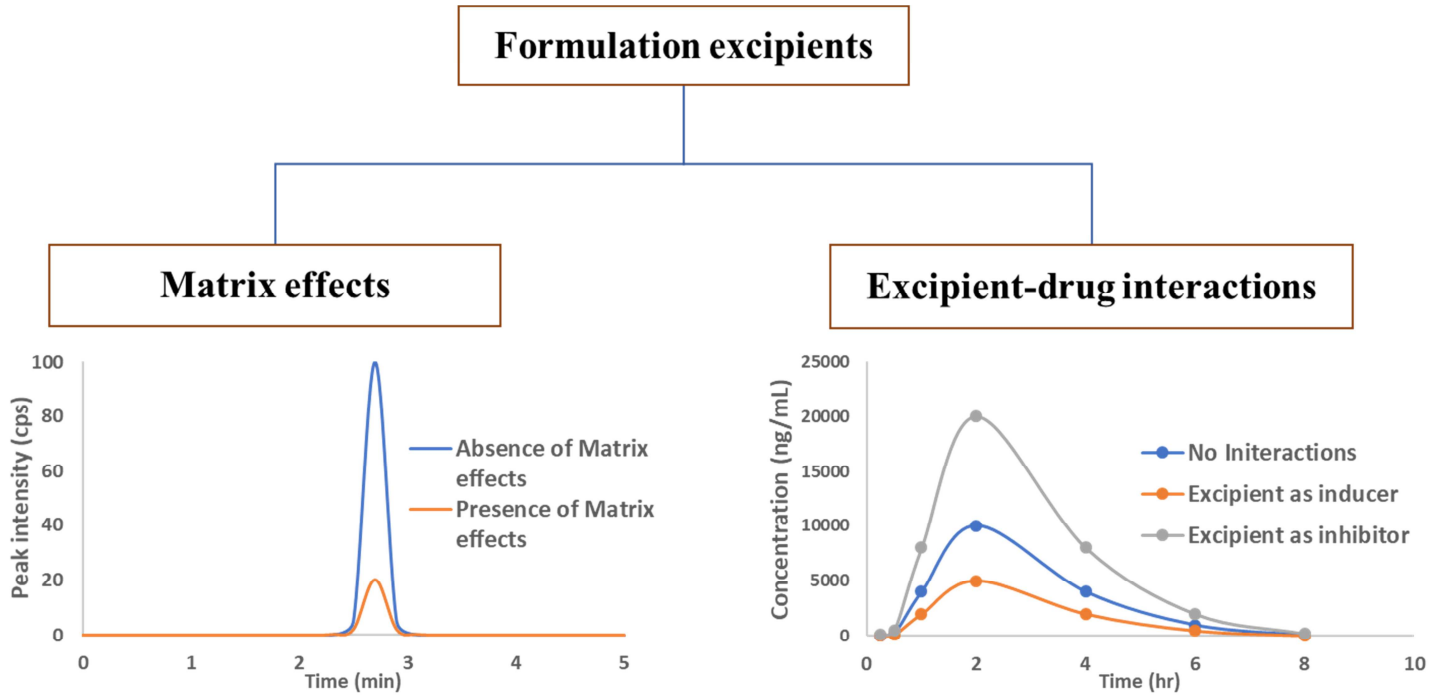
Formulation excipients
Matrix effects
Excipient-drug interactions
Peak intensity (cps)
100
80
60
40
20
0
0
1
2
3
4
5
Time (min)
Absence of Matrix effects
Presence of Matrix effects
Concentration (ng/mL)
25000
20000
15000
10000
5000
0
0
2
4
6
8
10
Time (hr)
No Initeractions
Excipient as inducer
Excipient as inhibitor

Three-dimensional aspects of formulation excipients in drug discovery: a critical assessment on orphan excipients, matrix effects and drug interactions

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ABSTRACT:

Formulation/pharmaceutical excipients play a major role in formulating drug candidates, with the objectives of ease of administration, targeted delivery and complete availability. Many excipients used in pharmaceutical formulations are orphanized in preclinical drug discovery. These orphan excipients could enhance formulatability of highly lipophilic compounds. Additionally, they are safe in preclinical species when used below the LD_{50} values. However, when the excipients are used in formulating compounds with diverse physico-chemical properties, they pose challenges by modulating study results through their bioanalytical matrix effects. Excipients invariably present in study samples and not in the calibration curve standards cause over/under estimation of exposures. Thus, the mechanism by which excipients cause matrix effects and strategies to nullify these effects needs to be revisited. Furthermore, formulation excipients cause drug interactions by moderating the pathways of drug metabolizing enzymes and drug transport proteins. Although, it is not possible to get rid of excipient driven interactions, it is always advised to be aware of these interactions and apply the knowledge to draw meaningful conclusions from study results. In this review, we would be comprehensively discussing a) orphan excipients that has wider applications in preclinical formulations, b) bioanalytical matrix effects and possible approaches to mitigate these effects, and c) excipient driven drug interactions and strategies to alleviate the impact of drug interactions.

KEYWORDS: Formulation excipients, preclinical, drug discovery, matrix effects, drug interactions, bioanalysis, pharmacokinetics, formulation development

1. INTRODUCTION:

The word *excipient* is derived from the Latin word *excipere*, meaning 'to except', which could be simply explained as 'other than' [1, 2]. Formulation/pharmaceutical excipients are basically everything other than the active ingredient. “Formulation excipients” terminology is often used in preclinical research space, as an alternate to “pharmaceutical excipients” termed in clinical arena. They are nothing but a refined list of pharmaceutical excipients used for preclinical in vivo studies. Pharmaceutical excipients are used to prepare a wide variety of dosage forms ranging from tablets, capsules, oral liquids, ointments, creams, gels, transdermal patches, injectable products, implants, suppositories and inhalers [3-9]. On the other hand, formulation excipients are used to prepare either suspensions or solutions for the ease of administration of drug candidates to the preclinical species (viz. mice, rat, rabbits, dogs, pigs and monkeys) in order to evaluate their efficacy, safety, toxicity and pharmacokinetic disposition. For preparation of clinical dosage forms, various pharmaceutical excipients such as diluents, disintegrants, glidants, lubricants, co-solvents, binders, granulating agents, compression aids, plasticizers, preservatives, complexing agents, lipids, polymers, surfactants, emulsifying agents, sweeteners, preservatives, thickeners, viscosity modifiers and formulation dependent pH buffers are used. However, in the case of preclinical formulations various excipients such as surfactants, emulsifying agents, co-solvents, complexing agents, lipids, polymers and formulation dependent pH buffers are routinely used. Apart from these routine formulation excipients, there are many pharmaceutical excipients which are not extensively used in developing preclinical formulations despite of their prerogative in the clinical setting. Taking above facts into consideration, we coined the term “orphan excipients” for this category of pharmaceutical excipients with the objective of bringing them to limelight and provide broader applications in drug discovery. Our review will focus on the key attributes of these orphan excipients, which might help in strategizing the current approaches being followed in preparing preclinical formulations.

In pharmacokinetic studies, following the administration of drug candidates to preclinical species, concentration of the analyte of interest is measured in the biological matrices (blood, plasma or tissues)

using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Introduction of highly sensitive and specific LC-MS/MS instruments has revolutionized the bioanalytical methodologies by offering high-throughput sample analysis [10-12]. Even though there exists different ionization techniques in LC-MS/MS, electrospray ionization (ESI) and atmospheric pressure chemical ionisation (APCI) are the most preferred ones because of the obvious advantages of robustness, speed, specificity and sensitivity [13]. However, ion suppression/enhancement effects that are frequently encountered in the analysis of biological samples outweigh these advantages. Despite of being a superior detection technique as compared to HPLC, the issues encountered with ion suppression/enhancement effects raises question on the authenticity of data generated using LC-MS/MS. Ion suppression/enhancement effects observed while using LC-MS/MS for bioanalysis can be broadly termed as "matrix effects". The "matrix" refers to all components in the sample other than analyte(s) of interest [14]. Matrix effects are defined as the "interference from matrix components that are unrelated to the analyte" and result in significant deviation in bioanalytical data which in turn questions the reliability of corresponding pharmacokinetic parameters of drug candidate. Matrix effect alters the sensitivity, reproducibility and challenges the reliability of analytical techniques. Although matrix effects occur because of various exogenous and endogenous components, one major area of concern is formulation excipients (an exogenous component) used in the preparation of preclinical formulations. In this review, we have discussed the impact of formulation excipients on the ionization of analytes, mechanisms by which formulation excipients causes matrix effects and possible alternatives to mitigate these effects.

Drug-drug interaction is defined as "a substance (perpetrator drug) which impacts the disposition of a second drug (victim) when administered together". Interactions can be synergistic or antagonistic or a new effect that neither produces. Drug–drug interactions (DDIs) are not desired as they increase the risk of adverse events and inevitably results in increased/decreased clearance or absorption of the affected drugs [15]. These changes can alter the safety and efficacy profile of a drug or its active metabolites in important ways. The science of DDIs involves the drug transporters and drug metabolizing enzymes that are ubiquitously present in major clearance (intestine, liver, brain and kidney) organs. Drug-drug

interactions can also be food-drug, formulation-drug and herb-drug type. As the subject of this review is focused on formulation excipients, we emphasized more on formulation-drug interactions. In general, excipient-drug interactions occur when excipients inhibit/induce enzymes that are actively involved in metabolism; inhibit/induce transporters involved in the uptake/efflux transport mechanism [16-23]. In this review, we have comprehensively discussed on the excipient mediated drug interactions shown by commonly used excipients, their impact on drug disposition and finally care to be taken while using these excipients. Even though excipient-drug interactions can be numerous, we have focused on the impact of excipients on drug disposition modulated by drug metabolizing enzymes and drug transporter proteins.

Overall, to our knowledge this will be a unique review that proposes the uncovered pharmaceutical excipients (defined here as orphan excipients) to be used in preclinical drug discovery; describes mechanism by which excipients cause matrix effects and offer possible remedies; presents excipient-drug interactions caused by the commonly used excipients and precautionary measures to be followed while choosing these excipients.

2. SCOPE:

We conducted a review of published formulation/pharmaceutical excipients, matrix effects caused by these excipients and excipient mediated drug interactions. Since there is ambiguity on the pharmaceutical excipients (privileged in a clinical setting) that can be used in preclinical formulations, this review primarily focuses on these excipients which we believe will help drug discovery scientists to understand the holistic picture of their applications in the preparation of these formulations. Additionally, knowledge on matrix effects and possible excipient-mediated drug interactions will also help in key decision making while choosing formulation excipients. The literature review was conducted using PubMed® search (NCBI 2016), SCIFINDER® and Google Scholar databases with specific key words such as preclinical, formulation excipients, excipient-drug interactions, CYP450 interactions, pharmaceutical excipients, and matrix effects to collect the related full-length articles and abstracts. The literature search covers the period until January 2019.

3. Preclinical safety and tolerability of orphan excipients:

Formulation selection in preclinical drug discovery comes with a broad range of options. Based on the route of administration, test articles must be either in solution or suspension form. Solution formulations are more desired for parenteral route (esp. intravenous) of administration, whereas, suspension formulations are handy for parenteral (except intravenous)/enteral routes. The objective of formulation vehicle selection is to provide the desired availability/ bioavailability of test article and should be as simple as possible with low toxicity. Formulation vehicle selection in early PK should also consider its suitability for late stage developments and strengths used should be within generally recognized as safe (GRAS) limits. Various conventional formulation selection approaches includes, pH adjustment, low concentrations of polymers/ suspending agents (methyl cellulose, carboxymethyl cellulose, hydroxypropyl methyl cellulose), low concentrations of solubilizing agents (cyclodextrins, polysorbate80, cremophor EL, solutol HS15), cosolvents (Dimethyl sulfoxide, dimethylacetamide, propylene glycol, ethanol, N-methyl-2-pyrrolidone, propylene glycol, PEG400, and PEG300), lipid based excipients (medium chain glycerides), nanosuspensions and solid dispersions (hydroxypropyl methylcellulose acetate succinate, PEG6000, polyvinylpyrrolidone) [24]. Irrespective of various combinations available, the simplest formulations are comprised of primarily aqueous solutions and suspensions. Solution and suspension formulations can be prepared using pH buffers, suspending agents, cosolvents and solubilizing agents.

For compounds insoluble in simple solution and suspension-based formulations, alternate strategies such as lipid, solid dispersion and nanosuspension based approaches might be considered. Lipid-based vehicles are primarily helpful in solubilizing highly lipophilic compounds [25-28]. Even though it is challenging to use these excipients when it comes to late stage development, nevertheless these formulation approaches will help in making early inroads to decide the fate of discovery projects.

Solid dispersion and nano suspension approaches although are effective in solubilizing highly lipophilic compounds, nonetheless are much difficult and labor intensive to be adopted in early drug discovery stages. Advantages of both of these strategies are extensively reported in the literature [29-33].

Pharmaceutical applications of commonly used pharmaceutical excipients is reported in the literature [34]. Few of these excipients are very rarely used as formulation excipients in drug discovery, termed here as "orphan excipients". If used in preclinical formulations, these orphan excipients could enhance druggability of new compounds [35]. Hence, we presume this review article will extend the vision of preclinical researchers to make best use of these orphan excipients. List of orphan excipients include cyclodextrins (captisol), cosolvents (glycerin), non-ionic surfactants (d-α-tocopherol, alpha-tocopherol-polyethylene glycol 1000 succinate (Vit E-TPGS), sorbitan monoesters, poloxamers, Softigen 767, polyoxylglycerides, Lauroglycol and Plurol), and water insoluble lipids (labrafac/labrafac lipophile). A brief overview of these orphan excipients is given below:

- **Captisol** is a polyanionic beta-cyclodextrin derivative with a sodium sulfonate salt separated from the lipophilic cavity by a butyl ether spacer group, or sulfobutylether (SBE). It helps in solubilizing neutral, cationic and anionic compounds. Captisol exhibits limited plasma protein binding, distributes to extracellular fluid and does not produce any pharmacological effects on the cardiovascular system; autonomic or somatic functions; respiratory capacity; or fluid or electrolyte excretion following intravenous administration. It is relatively safer than other cyclodextrins [36] and can be used at a concentration up to 40% w/v for both enteral and parenteral routes in preclinical as well as clinical species [34, 37].
- **Glycerin** occurs naturally in animal and vegetable fats and oils that are consumed as part of a normal diet. It is mainly obtained as a by-product from oils and fats used in manufacturing of soaps and fatty acids. It may also be obtained from natural sources by fermentation. Synthetic glycerin is prepared by the chlorination and saponification of propylene [38]. Glycerin is used in a wide variety of pharmaceutical formulations including oral, ophthalmic, parenteral, and topical preparations as a solvent/cosolvent, humectant/emollient, sweetening agent, plasticizer, antimicrobial preservative and

viscosity enhancer [39]. Higher LD_{50} values (4.1 g/kg in mouse, 12.6 g/kg in rats) in rodents makes it a suitable vehicle for usage in preclinical formulations [40].

- **Alpha-Tocopherol** is the orally bioavailable alpha form of the naturally-occurring fat-soluble vitamin E, with potent antioxidant and cytoprotective activities. It is a highly lipophilic compound, and is an excellent solvent for many poorly soluble drugs [34, 41, 42]. It is used as a non-ionic surfactant in oral and injectable formulations and as a plasticizer. The reported LD_{50} value for tocopherol is 7.5 g/kg in rat [43]. In general tocopherols are well tolerated, however, excessive oral intake of tocopherol may cause headache, fatigue, weakness, digestive disturbance, and nausea [38].
- **Alpha-tocopherol Polyethylene glycol 1000 succinate (Vitamin E-TPGS):** TPGS is the esterified product of vitamin E succinate with polyethylene glycol (PEG). 1000 denotes the molecular weight of PEG, and the final product is referred as TPGS1000, or simply TPGS. Vitamin E TPGS is a nontoxic, non-ionic surfactant that is used in many drug delivery systems [38]. TPGS is used as a P-gp inhibitor, solubilizer, and absorption/permeation enhancer. Vitamin E TPGS has been hypothesized to increase the bioavailability of certain drugs by enhancing the solubility of the API and by acting as a weak P-gp inhibitor [35]. LD_{50} value of Vitamin E-TPGS is reported to be >7 g/kg in rats [35].
- **Sorbitan monoesters** are a series of mixtures of partial esters of sorbitol and its mono- and dianhydrides with fatty acids. Sorbitan monooleate is a pharmaceutical excipient that has been used in cyclosporine formulations, Gengraf and Sandimmune [34]. Sorbitan esters are widely used in cosmetics, food products, and pharmaceutical formulations as lipophilic nonionic surfactants. They are mainly used as emulsifying agents in the preparation of emulsions, creams and ointments. Sorbitan esters produce stable water-in-oil emulsions and microemulsions. However, when used in combination with varying proportions of a polysorbates, they produce water-in-oil or oil-in-water emulsions, and their applications include self-emulsifying drug delivery systems for poorly soluble compounds [38]. Sorbitan esters are generally considered as nontoxic, nonirritant excipients. Various sorbitan monoesters used as excipients include, sorbitan di-isostearate, sorbitan dioleate, sorbitan monoisostearate, sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate, sorbitan

monostearate, sorbitan sesquiisostearate, sorbitan sesquioleate, sorbitan sesquistearate, sorbitan tri-isostearate, sorbitan trioleate, sorbitan tristearate. LD_{50} value of sorbitan monolaurate and sorbitan monooleate in rats is reportedly >33.6 g/kg and >31 g/kg, respectively [38].

- **Poloxamers** are nonionic polyoxyethylene-polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents [44-51]. Poloxamer polymers are prepared by reacting propylene oxide with propylene glycol to form polyoxypropylene glycol. Ethylene oxide is then added to form the block copolymer. The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic. Poloxamers are used as emulsifying, solubilizing, wetting and stabilizing agents. Poloxamers are used in a variety of oral, parenteral, and topical pharmaceutical formulations, and are generally regarded as nontoxic and nonirritant excipients. Various poloxamers used as excipients include Poloxamer 124, Poloxamer 188, Poloxamer 237, Poloxamer 338, and Poloxamer 407. LD_{50} value of Poloxamer 188 is >15 g/kg in mouse and >9.4 g/kg in rats [38].
- **Softigen 767 (PEG-6 Caprylic/Capric Glycerides)** is the ethoxylation product of medium chain partial glycerides whose fatty acids are derived from coconut and palm kernel oil. Softigen acts as surfactant, viscosity reducing, solubilizer, wetting and refatting agent. Softigen 767 was dosed at 120 mg/kg in male SD rats [52]. However, information on LD_{50} values is not available.
- **Polyoxylglycerides** are mixtures of monoesters, diesters, and triesters of glycerol, and monoesters and diesters of polyethylene glycols (PEG). Polyoxylglycerides act as dissolution enhancer; nonionic surfactants; emulsifying/ penetration/ solubilizing/ sustained-release agents [34]. They are nontoxic and nonirritant materials [38]. Various polyoxylglycerides include caprylocaproyl polyoxylglycerides (labrasol), lauroyl polyoxylglycerides (gelucire 44/14), linoleoyl polyoxylglycerides (labrafil M2125CS), oleoyl polyoxylglycerides (labrafil M1944CS) and stearoyl polyoxylglycerides (gelucire 50/13). LD_{50} values for gelucire 44/14, labrasol, and labrafil M1944CS in rats are 20 g/kg, 22 g/kg and >20 mL/kg, respectively [38, 43].

- **Lauroglycol** a nonionic water-insoluble surfactant used as co-surfactant in oral lipid-based formulations. It consists of propylene glycol mono- and di- esters of lauric (C12) acid, mainly composed of monoesters and a small fraction of diesters. Lauroglycol acts as a solubilizer of highly lipophilic compounds and enhances bioavailability. LD_{50} value for lauroglycol in rat was 2.003 g/kg/day [53].
- **Plurol (Polyglyceryl-3-diisostearate)** a nonionic water-insoluble surfactant used as co-surfactant in oral lipid-based formulations. It consists of polyglyceryl-3 esters of stearic (C18) acid, the diester fraction being predominant. It acts as a solubilizer for highly lipophilic compounds and enhances bioavailability. The LD_{50} of Polyglyceryl-3-Diisostearate in mice and rats is >2 g/kg, and >5 g/kg, respectively [54].
- **Labrafac/Labrafac Lipophile (medium chain triglycerides)** consist of a mixture of triglycerides of saturated fatty acids, mainly of caprylic acid and of capric acid. It acts as emulsifying agent, solvent, suspending and therapeutic agent. Medium-chain triglycerides have been used in a variety of pharmaceutical formulations including oral, parenteral, and topical preparations are essentially nontoxic and nonirritant materials [38]. Reported LD_{50} value in rat after oral administration is >10 mL/kg [43].

In summary, these orphan excipients have been extensively used as pharmaceutical excipients in clinical drug formulations. However, they have not been rigorously considered in preclinical formulations. With higher LD_{50} values for most of these excipients and also their diverse applications, they broaden the scope of formulatability of highly lipophilic compounds in a preclinical setting. Summary of list of orphan excipients and their LD_{50} values is presented in **Table 1**.

4. Excipients causing bioanalytical matrix effects:

Matrix components that exist in extracts/supernatants after sample preparation alter the ionization of compounds in mass spectrometry. This could be by ion suppression or ion enhancement. However, more typically ion suppression is encountered than enhancement. The process of ion

suppression/enhancement is in general referred as matrix effect and is the main subject of various published reviews [55-60]. Matrix effects show major impact on the bioanalytical results, when extracted components are differentially present between calibration and study samples. They occur from endogenous components, which include phospholipids; exogenous components such as mobile phase additives, co-administered drugs, metabolites, internal standards, formulation vehicles and plastic tubes [58-67]. As the current context is on formulation excipients, we discussed briefly on nature/mechanism/impact of formulation excipient-mediated matrix effects and possible strategies to counter these effects.

Dosing vehicles are generally used at high concentrations to solubilize highly lipophilic test articles [63, 64, 68]. This in turn can be instrumental in causing matrix effects, thereby questioning the reliability of preclinical pharmacokinetic parameters. This phenomenon has been reported by us in the past for various excipients such as PEG400, cremophor EL and solutolHS15 [58-60, 69-72]. Formulation vehicles could cause 80-90% ion suppression when administered in both oral and intravenous administration routes [63, 64, 73, 74]. Xu et al did not find any matrix effects following either intravenous or oral dosing of drug candidates formulated using 20% hydroxypropyl-β-cyclodextrin or 0.4% methyl cellulose, however, 50-80% ion suppression for early eluting compounds was observed in the presence of 0.1% tween 80 [73]. PEG400 resulted in 30-50% ion suppression for early eluting compounds in oral formulations [75]. In other instance, polymeric vehicles such as tween 80 and PEG400 were reported to cause significant ion suppression (>50%), when sample clean-up was minimal and analytes co-eluted with the vehicles [64]. In a separate study, PEG was reportedly present in large quantities in the blood collection tubes used for pharmacokinetic studies [76]. The effect of dosing vehicle excipients such as PEG400, propylene glycol, tween 80, and hydroxypropyl-β-cyclodextrin on the accuracy of LC-MS/MS measurements used in pharmacokinetic studies were reported in the literature [63].

Significant ion suppression in general is noticed with early sampling points in intravenous/ oral route of administration. This is due to higher concentration of excipients in the early sampling points. As excipients are eliminated from the body in due course of time, impact of ion suppression gradually

reduces [63, 64, 76]. Also, matrix effects are more pronounced, when samples are analyzed using ultrafast gradients and shorter elution times. These analytical conditions typically cause co-elution of analytes with excipients and imparts ion suppression. Interference from coeluting excipients is more difficult to address as polymeric components elute across a wide retention window, hovering in the intermediate retention time range [77]. When both analyte and excipient coelutes, ion suppression can occur, however, the exact mechanism by which matrix components causes matrix effects is not known. Matrix effects occur at the interface between the MS system and LC system [78]. These mechanisms are discussed in detail in the subsequent sections. The U.S food and drug administration (US FDA) guidance for industry on bioanalytical method validation insists upon the assessment of matrix effects during method validation for quantitative LC-MS/MS methods [79]. Schematic representation of the matrix effects and their impact on physiological concentrations is shown in **Fig 1**.

When it comes to ionization source, it is generally understood that electrospray ionization (ESI) is more prone to ion suppression effects than atmospheric pressure chemical ionization (APCI) [80]. In ESI, ion formation happens through a multistep process which involves both liquid and gas phases. Ionisation happens in liquid phase as LC effluent passes through a needle maintained at high voltage [81]. High voltages along with nebulizer gas assists in the formation of charged droplets that undergo coulombic explosion and solvent evaporation [82, 83]. It is in the process of ionization and evaporation, matrix components and analyte of interest compete with each other for charge and result in ion suppression [84]. Response saturation at higher concentrations of analyte is also a result of competition for charge mechanism [85, 86]. Furthermore, high concentrations of matrix components can limit access of the analyte to the surface of the droplet. In addition, species with hydrophobic moieties such as lipids as well as dosing additives such as tween 80 and PEG 400 have high surface activity and can thereby limit the number of analyte ions reaching the surface of the droplet, further suppressing the ionization efficiency of the analyte [73]. These matrix components can also affect the viscosity and surface tension of the droplet, resulting in less efficient spray formation and subsequent solvent evaporation leading to a decreased number of ions reaching the gas phase. Ion suppression is highly experienced with very polar compounds.

Polar compounds tend to accumulate in the aqueous phase of droplet instead of surface, which eventually results in lower surface activity and higher ion suppression [87]. When mobile phase consists of ion pairing agents, it results in the formation of neutral complexes with the charged analytes and causes ion suppression [88].

Once in the gas phase, the analyte ions are still susceptible to the influence of matrix components that also exist in the gas phase. Neutral matrix species may then compete for protons from the charged analyte based on their relative gas phase basicity through proton transfer reactions. Those components with higher gas phase basicity will remove a proton from the analyte, neutralizing its charge and cause decreased signal intensity [89]. APCI ionization consists of formation of ions from neutral species in gaseous phase, through reagent ions (which is termed as chemical ionization) generated from corona discharge needle. Hence, matrix effects that exist in liquid phase does not occur in APCI. Overall based on the principle of ionization, matrix effects are more predominant in ESI source than APCI source.

Several mechanisms have been proposed to explain matrix effects, but the exact process remains uncertain [78, 90]. Various mechanisms by which matrix components cause ion suppression are as follows:

- Charge competition between analyte and matrix components [91, 92].
- Change in droplet surface tension leading to formation of large droplets and insufficient desolvation [78, 87].
- Preferential ion evaporation due to matrix components gathering at droplet surface [14].
- Change in mass of analyte ion due to ion pairing and adduct formation [14].
- Co-precipitation with non-volatile matrix components [93].
- Gas phase deprotonation [14].

Reduction of matrix effects can be achieved by various strategies which includes decreasing the level of matrix components, improving chromatographic separation of interfering materials from the analyte, various sample preparation strategies, lower injection volumes, and even by simple dilution of

samples to reduce the overall concentrations of both analyte and co-extracted materials [65, 74]. Switching ionization sources will also help in mitigating the matrix effects. Matrix effects occurring in the early time point samples can be monitored, using another aliquot of the early time point samples analyzed at a higher dilution [74, 94]. If the two measurements with different dilution factors agree with each other, it indicates matrix effect is insignificant. If the two measurements for the early time points differ by more than a certain threshold (e.g., 30%), one may need to improve the method and reanalyze samples [74]. Additionally, it is worthwhile to use a combination of ultrafast liquid chromatography and microbore columns. This analytical feature helps in reducing matrix effects by increasing the resolution of analytes of interest from the interfering components [92]. Overall, these are various strategies that can be employed to mitigate the impact of matrix effects caused by excipients and a careful choice should be made based on the nature of matrix effects.

5. Excipients driven drug interactions:

Ideal excipient should not interfere with the pharmacological activity of test compound of interest. However, most of the excipients used in drug discovery, produce some sort of interactions. These interactions could be due to modulation of drug metabolizing enzymes/ drug transporters. For example, clinically achievable concentrations of PEG-300 caused almost complete inhibition of P-gp activity in both Caco-2 and MDR1-MDCK cell monolayers [95]. P-gp inhibition caused by polyethoxylated pharmaceutical excipients was also reported in the literature [96-98].

Similarly, cremophor EL enhanced the bioavailability of a P-gp substrate doxorubicin which was considered desirable from an efficacy perspective and resulted in the increased antitumor activity. On the contrary, the enhanced doxorubicin exposure resulted in higher cardiotoxicity [99]. Excipients such as PEG fatty acid esters, PEG stearates, poloxamers and polysorbates, showed higher P-gp inhibition potential. Researchers should be aware of this bearing on overall exposures and cautiously monitor for safety/ toxicity concerns [100]. The interaction potential of excipients (Polysorbate 80, Cremophor EL, and Solutol HS 15) on the intrinsic clearance (CL_{int}) of midazolam (MDZ) was investigated in rat

microsomes and hepatocytes. The above excipients caused a decrease in the intrinsic clearance of CYP3A substrate MDZ with the increase in concentrations [101]. This case study presented excipient-mediated inhibition of CYP3A isozyme, and altered clearance.

Impact of nine excipients (lactose, sodium lauryl sulfate, tween 80, HPMC, docusate sodium, EDTA, propylene glycol, PEG400, anhydrous cherry flavor) on the Caco-2 permeability of seven low permeable compounds was studied by Bhagwant et al [102]. Sodium lauryl sulphate and tween 80 increased apical to basolateral permeability of low permeable compounds. However, rest of excipients did not show considerable impact on the overall permeability of these compounds. On the other hand, PEG-cholecalciferol, polyethylene glycol succinate and TPGS increased the uptake of P-gp substrates by inhibiting the P-gp efflux process [96]. Increased permeability results in higher exposures and also triggers toxicity concerns. As discussed above, researchers should be precarious in understanding the modulation of exposures caused by these excipients and develop effective correlations to safety/toxicity.

Anderberg et al studied the effects of synthetic, anionic and nonionic surfactants on the monolayer integrity of epithelial cells, permeability, intracellular enzyme activity and cell morphology [103]. All surfactants exhibited concentration-dependent effects on intracellular enzyme activities, permeability, and morphology. The effects of the anionic surfactants were more pronounced than those of the nonionic surfactants. In a different study, selected excipients (imwitor 742, labrasol, cremophor EL, softigen 767, miglyol, solutol HS 15, sucrose monolaurate, TPGS, polysorbate 20, and polysorbate 80) were screened for their ability to enhance the absorption of digoxin and celiprolol in vitro [52]. It was concluded that these excipients/surfactants can modify the pharmacokinetics of orally administered drugs that are P-gp substrates.

Apart from P-gp inhibition, which results in enhanced cell permeability, excipients also alter the permeability by increasing elasticity and reducing the membrane viscosity [104, 105]. This altered morphology in Caco-2 cell model enhances the absorption of compounds by both paracellular and transcellular routes. Cremophor RH40 inhibits both CYP3A and P-gp in a concentration-dependent manner which explains the increase in bioavailability of P-gp substrates in vivo [20]. Similarly, tween 20

and pluronic P85 increased the mitoxantrone uptake in BCRP expressing cells, but these effects disappeared up on omission of excipients [106].

Coming to effect of excipients on drug metabolizing enzymes, when male Sprague-Dawley rats were fed 20% corn oil for 4 days following 2 days of fasting the hepatic P450s 1A2, 2B2, 2E1, and 3A were regulated positively but the level of pulmonary P450 2B1 was suppressed. This enhanced/ suppressed enzyme levels altered the drug disposition [107]. Impact of various excipients (DMSO, ethanol, propylene glycol, PEG, dimethyl acetamide, cyclodextrins, glycofurol, cremophor, solutol HS15) on altered drug disposition (metabolism, pharmacokinetics, renal elimination, absorption, distribution, hepatic blood flow) was extensively discussed in published reports [108]. In a separate study, researchers evaluated the effect of 23 commonly used excipients on CYP450 isoforms using recombinant CYP enzymes. It was concluded that several excipients have the potential to modify the pharmacokinetics of administered drugs [109].

Apart from CYP isoforms, impact of 25 excipients on hydrolyzing enzymes (human carboxylesterases) was studied [110]. Out of the excipients tested, surfactants significantly inhibited carboxylesterase (CES) activity. It was suggested that such inhibition should be taken into consideration during drug administration [110]. In addition, impact of 19 excipients on human PXR activation, and CYP3A4 mRNA expression in immortalized human liver cells (HepG2 and Fa2N4), human primary hepatocytes, and the intestinal LS174T cell models was determined. Pregelatinized starch, polysorbate 80, and hydroxypropyl methylcellulose decreased mRNA and protein expression across these models [111]. Additionally, effect of 22 pharmaceutical excipients on CYP3A4 was studied using midazolam as a probe substrate. The results showed that 15 of 22 (68.2%) tested excipients inhibited the activity of CYP3A isozyme by more than 50%, particularly the surfactants and polymers [112]. A summary of excipients causing drug interactions is represented in **Table 2**.

Overall, most of the promising excipients were reported to impact the drug disposition. As the usage of these excipients is inevitable, researchers should perform prior risk versus benefit analysis. However, while dealing with highly hydrophilic compounds aqueous formulations could be a better

choice [37]. Alternatively, pH adjustment might be employed as one of the strategies to solubilize highly lipophilic compounds. Both these approaches can be adopted keeping in view of the high solubility requirements in late stages of drug discovery [37]. Wherever possible, it is advisable to deploy more efforts in early stages of drug discovery to find out the right formulation vehicle. These early efforts in optimization could help to keep the formulation vehicle uniform throughout the course of project and support to generate unbiased results. Otherwise, when abrupt/ intermittent changes are made in the formulation, it becomes difficult to correlate the results.

6. CONCLUSION:

Pharmaceutical/formulation excipients are essential for formulating the new chemical entities/ drug candidates under preclinical investigation. Firstly, even though there exist many excipient recipes in pharmaceutical formulations, not all are used in preclinical drug discovery. In this review, we have emphasized more on these orphan excipients. Higher LD_{50} values of these excipients suggest, their safety in preclinical species. Additionally, orphan excipients are capable of solubilizing highly lipophilic compounds. Secondly, we have discussed excipients-mediated bioanalytical matrix effects, its underlying mechanisms and overall impact on the study outcomes. If not addressed, matrix effects will alter the bioanalytical results, which eventually lead to incorrect pharmacokinetic attributes of the compounds. Various strategies to mitigate matrix effects including sample preparation, sample dilution, chromatographic conditions and change of ionisation source were proposed. Finally, formulation excipients-mediated drug interactions impacting the drug metabolizing enzymes/drug transporters were discussed using numerous case studies. As usage of excipients is inevitable, researchers should always be cognizant of these interactions and outweigh risk versus benefit. It is also advised not to make abrupt changes in formulation vehicle at different stages of drug discovery. Rather, more efforts should be levied at early stages of the program to optimize suitable formulation vehicle (keeping in view of highly lipophilic compounds).

CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

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Table 1: Summary of list of orphan excipients and their LD_{50} values

Excipient	LD_{50} Value/ Max strength permissible	References
Vitamin E-TPGS	>7 g/kg (rat)	[35]
Captisol	Up to 40% w/v (rat, mouse)	[37]
Sorbitan monolaurate	>33.6 g/kg (rat)	[38]
Sorbitan monooleate	>31 g/kg (rat)	[38]
Alpha tocopherol	7.5 g/kg (rat)	[38]
Gelucire 44/14	20 g/kg (rat)	[38]
Labrasol	22 g/kg (rat)	[38]
Poloxamer 188	>15 g/kg (mouse) >9.4 g/kg (rat)	[38]
Glycerin	4.1 g/kg (mouse) 12.6 g/kg (rat)	[40]
Labrafil M1944CS	>20 mL/kg (rat)	[43]
Labrafac/ Labrafac Lipophile	> 10 mL/kg (rat)	[43]
Softigen 767	120 mg/kg (rat; tested dose)*	[52]
Lauroglycol	2.003 g/kg/day	[53]
Plurol	> 2 g/kg (mice) > 5 g/kg (rat)	[54]

***LD_{50} not available**

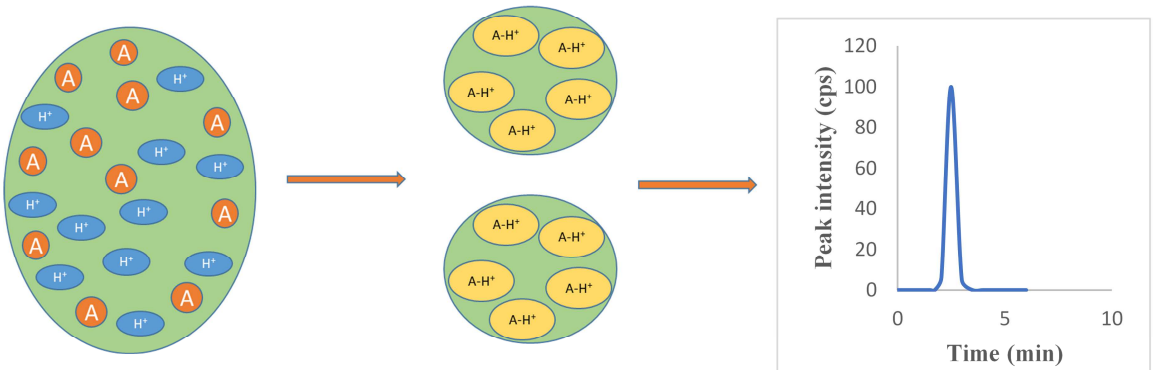
Table 2: Summary of formulation excipients-mediated drug interactions

Excipients	Mechanism of drug interaction	References
Cremophor RH40	CYP3A4 and Pgp inhibition	[20]
Imwitor 742, Labrasol, Cremophor EL, Softigen 767, Miglyol, Solutol HS 15, Sucrose monolaurate, TPGS, Polysorbate 20, and Polysorbate 80.	Pgp inhibition	[52]
PEG300	Pgp inhibition	[95]
PEG cholecalciferol, PEG succinate, and TPGS.	Pgp inhibition	[96]
Polyethoxylated pharmaceutical excipients	Pgp inhibition	[96-98]
Cremophor EL	Pgp inhibition	[99]
PEG fatty acid esters, PEG stearates, Poloxamers, and Polysorbates.	Pgp inhibition	[100]
Polysorbate 80, Cremophor EL, and Solutol HS15.	CYP3A4 inhibition	[101]
Sodium lauryl Sulphate, and Tween 80.	Pgp inhibition	[102]
Anionic surfactants (sodium dodecyl sulfate, sodium dioctyl sulfosuccinate); Nonionic surfactants (polysorbate 80 and polyoxyl 40 hydrogenated castor oil); Synthetic surfactants and bile acids (sodium taurocholate, sodium taurodeoxycholate, and sodium taurodihydrofusidate.	Enzyme activity, permeability, and cell morphology	[103]
Tween 20, and Pluronic P85.	BCRP Inhibition	[106]
	Hepatic CYP Induction	

Corn oil	(CYP1A2, 2B2, 2E1, 3A4); Pulmonary CYP Inhibition (CYP2B1)	[107]
DMSO, Ethanol, Propylene glycol, PEG, Dimethyl acetamide, cyclodextrins, Glycofurol, Cremophor, and Solutol HS15.	Absorption, distribution, metabolism, elimination, pharmacokinetics, and hepatic blood flow	[108]
PEG1000, PluronicF68/F127, Kollicoat, Polyvinlalcohol, Polyvinylpyrrolidone, Hydroxypropyl cellulose, Hydroxypropyl methylcellulose, Hydrolyzed gelatin, or Sodium carboxy methyl cellulose, Sodium deoxycholate, Sodium caprylate, Vit-EPEG, Sisterna 11, Sisterna 16, Cremophor, Solutol HS15, Tween 20, Tween 80, Brij 58, Hyamine, and Cetyltrimethylammonium bromide.	Recombinant CYP activity	[109]
Propylene glycol, Glycerin, Lactose, PEG 200, PEG 400, PEG 4000, PEG 6000, Microcrystalline cellulose, Carboxymethyl cellulose sodium, Poloxamer 188, Sodium lauryl sulfate, Hydroxypropyl cellulose, Povidone, Sodium alginate, Lecithin, Oleic acid, Triton X-100, Polyoxyl 35 castor oil (EL35), polyoxyl 40 hydrogenated castor oil (RH40), Tween 20 and Tween 80, Sodium bisulphite, Ascorbic acid, and Polyoxyl 40 stearate.	Carboxylesterase activity, CYP3A4 activity	[110, 112]
Citric acid, croscarmellose sodium CYP3A4 cytochrome	PXR activation, and	[111]

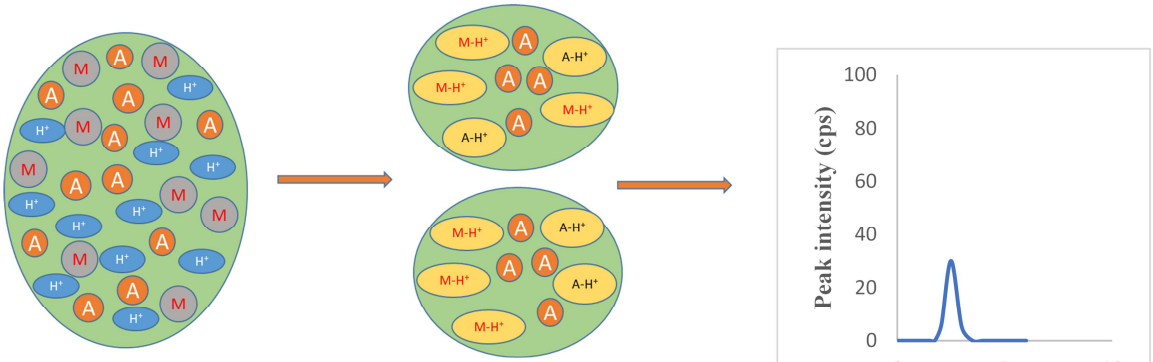
P450 3A4, dicalcium phosphate dehydrate, dimethyl sulfoxide, fumaric acid, hydroxypropyl methylcellulose, lactose, malic acid, microcrystalline cellulose, magnesium stearate, polyethylene glycol 3350, polysorbate-80 povidone, pregelatinized starch, propylene glycol sodium lauryl sulfate, sodium starch glycolate, sucrose, and Cross povidone.	CYP3A4 mRNA expression

a)

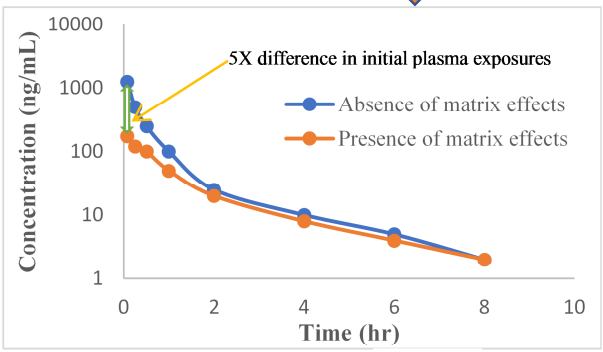


(i) (ii) (iii)

b)



(iv) (v) (vi)



(vii)

Figure 1: Schematic representation of matrix effects and their impact on physiological concentrations of drug candidates. a) In the absence of interfering components, conventional droplet is formed with only analyte ions acquiring charge (i), which further breaks down to mist of droplets (ii) and results in optimal peak response (iii); b) in the presence of interfering components, droplet formation is altered with only few analyte ions acquiring charge due to competition (iv, v), finally resulting in compromised peak response (vi) and eventually impacting the exposures (vii). "A" represents analyte, "M" represents matrix components, $A\text{-}H^+$ represents ionized analyte, $M\text{-}H^+$ represents ionized matrix components.

HIGHLIGHTS:

1) Promising but orphanized formulation excipients were unveiled and their LD50 values captured in preclinical species.
2) Matrix effects that arise as a result of formulation excipients were explicitly discussed with mechanisms and strategies to mitigate these effects were presented.
3) Excipient-drug interactions that arise as a consequence of usage of formulation excipients was briefed and possible alternatives offered.
4) To our knowledge this is a comprehensive review article covering three-dimensional aspects of formulation excipients.