The Importance of Chromatographically Purified Excipients

Excipient impurities can disrupt many properties when formulating a new drug product; chromatographically purified excipients help to ensure that a finished formulation is stable, reliable and safe.

In the not so distant past, pharmaceutical excipients were viewed as ‘hidden’ inactive ingredients that played a very small role in the safety and efficacy of active pharmaceutical ingredient (API) formulations. However, the thought process has changed; the pharmaceutical excipient has been gaining more respect as a functional component that contributes to overall improved pharmacological activity of a particular API. These once hidden ingredients are now being called upon by the pharmaceutical industry to aid in solubility, stability and efficacy to improve the bioavailability of today’s ever-changing active pharmaceutical ingredients.

One of the greatest challenges the chemical community continues to face is formulating a stable, reliable and safe product, along with maintaining the desired pharmacokinetics and pharmacodynamics of the API. The excipient can undergo oxidation, which is known to directly contribute to formulation difficulties when working with complex and highly reactive APIs. These difficulties can be directly correlated with the amount of impurities in a given excipient, which can include the following: free ethylene oxide and 1,4-dioxane; residual solvents such as ethylene glycol and diethylene glycol; secondary oxidation products such as formaldehyde, higher molecular weight aldehydes and peroxides; processing components such as residual catalysts and trace quantities of metals; and, lastly, high moisture content. Excipient impurities can and will accelerate the degradation of the API, destabilise emulsions, cause skin and cellular irritation (1,2) and contribute to gelatin cross-linking (3) in both soft and hard gelatin capsules – ultimately leading to a decreased efficacy of the drug active. Therefore, it is critical that an API’s exposure to these impurities is eliminated or, at the very best, minimised as much as is physically possible.

Through chromatographic purification of these excipients, it has been possible to eliminate many of the impurities that are normally present without altering the chemical structure of the excipient. Chromatographic purification effectively removes and reduces polar and oxidative impurities, namely: moisture, residual catalysts, peroxides and molecules with aldehyde functionality. The removal of polar impurities helps to reduce API interaction, while maintaining both the stability of the drug and the integrity of the finished formulation.

In order to better demonstrate the importance of excipient purity, four studies were conducted on chromatographically purified (CP) commonly used excipients compared with their standard compendial counterparts. These studies are presented in this review. The highlights of these studies illustrate how excipient purity affects cellular irritation, formaldehyde formation, gelatin cross-linking and topical API stability. The cellular irritation study involved the use of polysorbate 80. The formaldehyde formation and the gelatin cross-linking studies focused on the commonly used PEG 400, and the final study investigated topical API stability, with the excipient in this study being dimethyl isosorbide (DMI).

CELLULAR IRRITATION

Cellular irritation of excipients used in applications such as transdermal, parenteral, topical and ophthalmic applications can not only produce a profound effect on a well-orchestrated drug formulation, but also negatively impact the functionality of the formulation. However, with these sensitive areas of use, patient compliance will be a significant factor when evaluating the end product. Minimising the irritation potential of the excipients required in these applications will significantly improve this important factor. Determining cellular irritation of the CP polysorbate and the standard compendial form, a trans-epithelial permeability assay (TEP) was conducted (3). The polysorbates were formulated into a simple...
surfactant system and placed in direct contact with MDCK cells for 15 minutes, and the intercellular leakage was then measured. The results of the TEP assay showed a decrease in the irritation potential of CP polysorbate 80 by 40 per cent compared with its standard compendial counterpart (see Figure 1).

**GELATIN CROSS-LINKING**

Formaldehyde content in excipients has been shown to cross-link gelatin. As a result, encapsulation companies require low formaldehyde levels for excipients used in hard and soft gel capsule formulations. In the gelatin cross-linking study, gelatin used in the manufacture of soft gelatin capsules was cut into strips. Solutions were prepared containing one-part gelatin (one per cent w/v) in water (distilled/de-ionised, DD) and two parts CP PEG 400 and two parts pharmaceutical grade PEG 400, respectively. The different solutions were agitated for several minutes and permitted to stand for one hour. When centrifuged at 3,500rpm, a flocculant was observed in the gelatin/water/pharmaceutical PEG 400 vials, whereas the gelatin/water/CP PEG 400 vials remained clear. When the vials were then centrifuged at 10,000rpm for 10 minutes, a solid precipitate was observed in the gelatin/water/pharmaceutical grade PEG 400 vials, whereas the gelatin in water alone vials remained clear (see Figure 2). The supernatant liquid in each vial was then analysed by high performance liquid chromatography (HPLC). The HPLC analysis showed a distinct difference between the CP PEG 400 and pharmaceutical grade PEG 400 samples. The solid precipitate produced in the pharmaceutical PEG 400 samples was due to the formation of a higher molecular weight insoluble gelatin derivative via cross-linking. The remaining gelatin component in solution was greatly reduced and had a different composition compared with the original gelatin employed (one per cent w/v in DD water).

**FORMALDEHYDE DETECTION**

Formaldehyde not only causes cellular irritation but is also a highly reactive molecule that is known to directly contribute to undesired gelatin cross-linking in both soft and hard gelatin capsules. This gelatin cross-linking has been displayed in the aforementioned studies. The data, both numerical and visual, clearly emphasises the benefit and importance of formaldehyde detection and shows how a CP excipient is crucial to end-product drug formulation for optimal performance of the API. In order to measure formaldehyde content, vials of PEG 400 were incubated at 50°C for four weeks. The formaldehyde content was measured at zero and four weeks using 2, 4-Pentanedione (PDO) derivatisation (4). It was found that the CP PEG 400 formaldehyde values were more than 60 per cent lower at four weeks at 50°C, compared with the standard compendial grade of PEG 400 (see Figure 3, page 12). A similar formaldehyde detection study conducted on polysorbate 80 found that CP polysorbate 80 contained 81 per cent less formaldehyde compared with standard compendial grade polysorbate 80.

**API STABILITY**

As previously mentioned, the stability of many complex and reactive molecules in today’s chemical community is an absolute necessity with respect to an API’s desired pharmacological properties. If the API degrades, not only is the active unstable, but the integrity is lost, the overall efficacy of the drug is decreased and the bioavailability that once was, is now diminished.

To investigate the impact of excipient purity with regard to API stability, DMI was chosen as it is commonly used as a solvent for APIs. Chromatographically purified DMI containing one per cent w/w benzocaine was compared with standard grades of DMI containing one
per cent w/w benzocaine, all of which were incubated at 50°C for nine weeks. The samples were then analysed by HPLC after zero, two, four, six and nine weeks. After two weeks, 99 per cent benzocaine recovery was obtained with the CP DMI while the three lots of standard grade DMI achieved only 86, 98 and 88 per cent benzocaine recovery. By week nine, the amount of recovered benzocaine had further decreased to 78, 92 and 73 per cent in the three lots of standard grade DMI, whereas the CP DMI continued to achieve 99 per cent benzocaine recovery (see Figure 4).

CONCLUSION

The results of these studies clearly demonstrate the importance of excipient purity, the advantages conferred by chromatographic purification and how impurities can disrupt many properties while trying to design a successful end product formulation. Chromatographically purified excipients – ranging from oils to polysorbates and PEGs to dimethyl isosorbide – offer the pharmaceutical formulator a greater opportunity to develop more stable drug formulations. Excipient purity should be considered a critical factor when planning, designing and developing new drug products.

References


4. Purity of PEG 400 affects the stability of gelatin capsules, AAPS Poster Abstract, R6012, AAPS, Nashville, 6-10 November, 2005