THE INNOVATOR

from ANATECH LTD. "the leader in innovative histology chemicals" Winter 2007

New Mucicarmine Makes Your Job EZ

What do cactus, French spies and British Imperialism have to do with modern histology? They are all involved with why so many laboratories have had difficulty with their mucicarmine stains over the last few years. Weak staining and poor shelf life are common complaints. It didn't matter whether commercially prepared solutions were obtained or the stains were made up in the lab with fresh dye. Until the Fall of 2006, Anatech did not offer carmine-based stains, in part because of concerns over supply and quality of the dye powder. However, we were asked to look into this, given our success with similar problems involving Alcian blue, nuclear fast red and Congo red[1]. As usual, we started by exploring the history of the dye and subsequently discovered one of the most remarkable stories in our field. In the end, we found the answers and a whole new way to deal with carmine. We think you will enjoy what follows.

Figure 1.
Prickly pear cactus with cochineal bugs.

History

We hear a lot about geopolitical forces today, but little rivals the fierce competition for world markets as the 300 year long conflict among Spain, France and Great Britain[2]. It began shortly after the "discovery" of the New World in 1492. Spain was the naval powerhouse early in the Age of Exploration and took over most of Central and South America. France garnered a few islands in the Caribbean and Portugal managed to control a large portion of eastern South America which later became Brazil (a story of its own for another time, involving the dye brazilin).

In its New World territories, Spain found three treasures of enormous economic importance: gold, silver and a brilliant red dye called cochineal. All three were plundered from the native peoples and shipped in incredible quantities back to the Old World, almost exclusively on Spanish ships. Shipping records reveal that 50-160 metric tons of cochineal were shipped annually between 1575 and 1603[3]. Those numbers rose as merchant vessels grew into the magnificent 4-masted Tall Ships of the late 1700's and early 1800's.

It is easy to understand the gold and silver trade, but what about the dye? Why was it so important, and where did the native people get it in such quantity? Before 1500, Europe and the Middle East had only two good red dyes and each had its problems. Kermes was derived from tiny insects living in oak galls. It gave a brilliant red color, but was in such short supply that only the extremely wealthy could afford it; thus, it became an icon for the privileged religious and ruling classes. Meanwhile, the common folk used madder for their red colors. Madder, whose active ingredient is alizarin, was cheap and plentiful, but it could not produce really vibrant red hues and tended to fade with exposure to sunlight and repeated laundering.

When Spain introduced cochineal, and more particularly fabrics native-dyed with cochineal, the new red became the hottest European fashion color overnight. Demand kept the price high enough to ensure a steady flow, yet not so high as to be prohibitively expensive. Spain held the monopoly on the source and the shipping routes, but quite willingly sold the dye to its friends and enemies alike at elevated prices. Not to be outdone, Great Britain and France pursued different strategies to break the Spanish stranglehold on this curious commodity.

Cochineal was a great mystery. Actually, it held several secrets, one for 107 years, another for 277 years. As the dye flooded European markets, dyers and paint makers struggled to reproduce the brilliant tones characteristic of native fabrics and pigments. Even the Spanish overseers of the trade located on site failed to appreciate how the natives obtained the dye and produced the beautiful colors from it. Apparently few Spaniards bothered to learn Aztec, Incan and other native languages, so they had little real communication with the people who knew everything about cochineal. The sophisticated native cultures had learned long before that the dye had to be mixed with tin in order to bring out its full virtue and to keep it from fading. In 1607, the English alchemist Cornelius Drebble rediscovered this and immediately became rich by setting up a dyeworks using his newfound technology. Nevertheless, Spain still held the monopoly on supply, which continued to foment trouble between Spain and its European neighbors.

Britain resorted to trade blockades, hiring mercenaries so that the British navy could claim innocence—thus beginning the reign of pirates so romanticized in modern times. In reality, they were upper and middle class Englishmen ("privateers") who staffed their heavily armed ships with merchant marines, retired naval sailors and harbor riff-raff. They worked out of many of the Caribbean islands forming the Antilles, among which the Spanish merchant ships had to pass to enter the Atlantic Ocean. Pirated goods ended up in England, but despite the carnage, Spain managed to keep its hold on the market.

Meanwhile, France tried espionage. In 1777, the government hired a French physician and botanist, Nicholas-Joseph Thiery de Menonville[4], to get into Mexico, find out how cochineal was produced, steal its source material and bring it back to France. The obstacles were daunting. After 277 years, no one in Europe (and few Spaniards in the New World) knew where cochineal came from. Rumors abounded that it grew on trees, was extracted from bushes, or was taken from animals. To compound the difficulties, Mexican authorities were very reluctant to let non-Spaniards into the country for any reason. Thiery de Menonville got provisional approval to collect botanical specimens, but almost immediately was put under house arrest after fraternizing with the natives. Actually, he learned that cochineal was from parasitic insects found on prickly pear cactus cultivated for that purpose (Fig. 1). He escaped long enough to collect live cactus plants and left Mexico with them hidden in his botanical cases. Unfortunately, the plants, hence the insects, died before they reached France. The secret was out, however, and numerous other attempts were made to grow infected cactus in Europe and British-held India, all to no avail. Today, all cochineal comes from countries currently or formerly under Spanish control.

There is a third secret to cochineal. Centuries before the Conquistadors arrived, native people had artificially selected the best dye-producing insects and the best insect-producing plants. As there is a great deal of variation in soil, climate and manufacturing practices, each region of Central and South America produces a unique form of cochineal. Chemists can actually detect the region of origin by analyzing the dye.

Carmine

Cochineal is the raw material of carmine, comprised of the dried and ground bodies of cochineal insects. The active ingredient is carminic acid (Fig. 2, left), produced by extracting cochineal with water or alcohol. By itself it has little power as a dye, but when combined with a metal mordant, it acquires the ability to bind to textile fibers or to function as an artist's pigment. This new compound is carmine and most often the metal is aluminum (Fig. 2, right), although artists' pigments may contain barium. All three substances, cochineal, carminic acid and carmine,

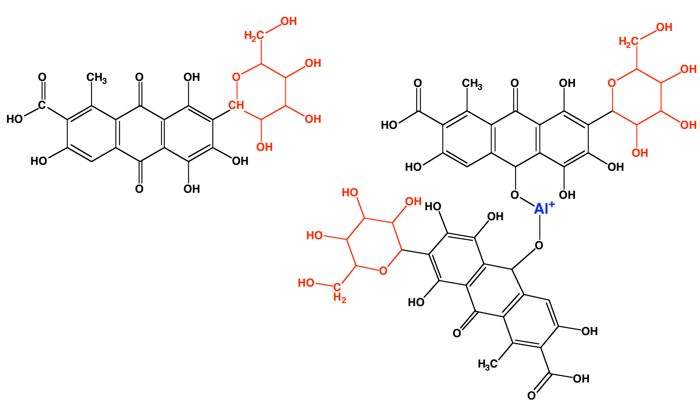

Figure 2.

Carminic acid (left) and carmine (right) molecules. The bond between the central chromophore (black) and the carbohydrate endgroup (red) is readily broken by heat.

are commonly found as colorants in red and pink foods (grapefruit juice, pie fillings, yogurt, candy), as you will discover by reading the labels for these products. Nothing wrong with a bit of bug juice in your diet!

The trouble with carmine

Today's problems with carmine stem from supply chain dynamics. Cochineal is produced by cottage industries in Mexico, Bolivia, Peru, Chile and the Canary Islands. Each has its unique biochemical characteristics induced by local climate, soil and agricultural practices. Each processing plant has its own proprietary method of extracting carminic acid and complexing it with aluminum. Most processors obtain cochineal from diverse geographical locations which change seasonally or according to the success of the harvest. As a result, quality is unusually variable, even for a natural dye. A recent study[5] of 81 samples of carmine certified by the Biological Stain Commission since the 1920's found two disturbing trends. First, the amount of variability in samples from 2000 to 2004 exceeded the variability from all previous decades. Second, samples from the first 4 decades (1920's-1950's) averaged higher in dye content than those from the next 54 years (1960's to 2004). Eight of the lowest 10 assays from all decades came from samples since 1990. Clearly, modern carmine is not what it used to be.

Nothing wrong with a bit of bug juice in your diet!

How does dye content relate to problems in staining or to shelf life? As dye content drops, impurities increase. This means more dye is needed to obtain an expected staining intensity. It also can affect solubility. Impurities of unknown composition can act as focal points for precipitation, bringing dye out of solution and further weakening an already problematic stain. Impurities may also interfere with staining directly by competing with carmine for attachment sites on tissue molecules.

You may be wondering why these changes were not detected earlier, especially by the Biological Stain Commission, since they certified the dye lots. Carmine was one of a few certified dyes that did not have an assay for purity included in the certification procedure. A new procedure is now available[5] and will be included in all future certification procedures for carmine[6].

Carmine has another problem: solubility. All certified lots are insoluble in water. Textbook procedures[7] for getting the dye into solution include fusing with aluminum chloride over a flame (Mayer's method), or by adjusting the pH drastically while boiling (Southgate used alkaline aluminum hydroxide then acidic aluminum chloride, while Best used potassium carbonate and ammonium hydroxide). Because carminic acid is heat labile[8] (as noted in Fig. 2), boiling is likely to split off a portion of the dye molecule. Just what effect this might have on subsequent dye-tissue interactions is problematic.

The new carmine

Anatech Ltd. has a new carmine that addresses all the problems previously discussed. We found a source of cochineal that is grown, extracted and made into carmine at one integrated facility. Only cochineal grown on site is used, eliminating much of the natural variation. Only one process is used in extraction and manufacturing, further reducing variability. To top it off, we have obtained a special, water-soluble version of the dye. This means that solutions can be made simply, without resorting to extremes of pH or heat. We call it EZ Carmine, and it is available to you the end user, and to vendors who make stain solutions for you. Ask for it specifically.

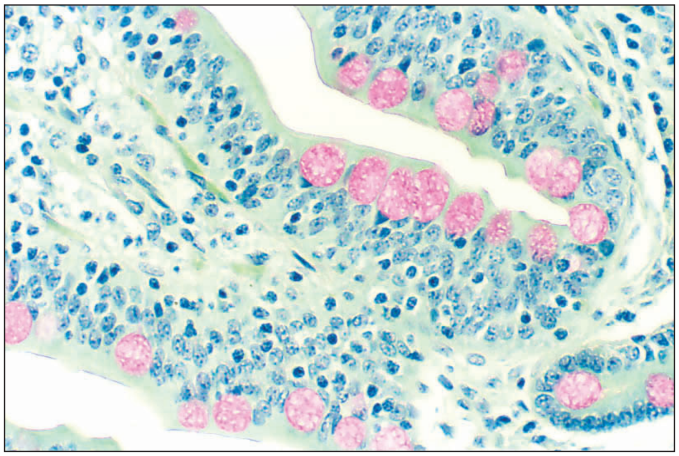

Figure 3.

Normal intestinal mucus stained with EZ Mucicarmine and EZ Green; 40x

To make life even simpler, we offer a mucicarmine solution in ready-to-use format, made with EZ Carmine. Not surprisingly, we call it EZ Mucicarmine.

Now a funny thing happened on the way to market with our new products. We discovered that the usual counterstain, metanil yellow (CI 13065) leaves a lot to be desired. The dye has rather poor solubility, so we set out to find a substitute. Consulting a color wheel, we immediately saw that green would be striking. So we used a new, Granny Smith apple green dye, Acid green 1 (CI 10020), to make a delightfully colored counterstain. Could we call it anything but EZ Green?

What does mucicarmine stain?

Most textbooks simply state that mucicarmine demonstrates mucins, but the stain is more selective than that. It actually parallels Alcian blue in its behavior, staining acidic mucin of epithelial origin (Fig. 3). Gastric mucus does not stain with either dye because it carries no ionic charge. Which should you use, red (mucicarmine) or blue (Alcian blue)? Either will work well, given quality dye, and Anatech provides both.

If you find this information helpful, please try one or more of the products mentioned here. All come with complete instructions, including suggested staining protocols. The dyes are accompanied by formulations for our stain solutions.

Product	Cat.#	Unit
EZ Carmine	872	25 gram jar
EZ Mucicarmine	871	1 quart
EZ Green	873	1 quart

References

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4 Thiery de Menonville N-J (1787). *Traité de la culture du nopal et de l'education de la cochenille dans les colonies Francaises de l'América*. Cap-Français. Subsequently translated as Travels to Guaxaca in 1777. In *A General Collection of the Best and Most Interesting Voyages and Travels*, Pinkerton J, ed., 1810-1814, volume 13:753-875. London.

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6 Dapson RW, Frank M, Kiernan JA (2007). Revised procedures for the certification of carmine (CI 75470) as a biological stain. *Biotechnic & Histochem*. 82: (in press).

7 Sheehan DC, Hrapchak BB (1980). *Theory and Practice of Histotechnology*. CV Mosby Company, St. Louis, pp. 167-169.

8 Green FJ (1990). *The Sigma-Aldrich Handbook of Stains, Dyes and Indicators*. Aldrich Chemical Comp., Milwaukee. p. 191.