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Original Research Article

Interaction of ferrous gluconate with dextromethorphan, guaifenesin and caffeine and its application in bio-studies

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Abstract: UV spectroscopy shows that dextromethorphan, guaifenesin and caffeine bind to ferrous gluconate, causing their spectra to shift. From this change, it is calculated that the binding energy is in the order of caffeine > guaifenesin ≈ dextromethorphan. This is consisternt with the electrochemical/potentiometric data obtained in a previous study which detected electrons emitted from these drug-iron complexes if they were irradiated by UV light or connected to solutions having different electrochemical potentials. Based on the result of this study, ferrous gluconate is more useful for the rapid screening of the drug-heme or drug-iron interactions in biological/pharmacological systems than free (unbound) iron. It may also find utility in energy production, bio-sensing and photo-electronics.
Keywords: Dextromethorphan; Guaifenesin; Caffeine; Ferrous gluconate; Binding; UV spectroscopy

INTRODUCTION

The heme group consists of a ferrous (Fe^{2+}) ion that is chelated by four nitrogen atoms in a heterocyclic porphyrin ring (Scheme 1). It is a cofactor in many biological proteins such as hemoglobin, cytochrome and peroxidase, which contain additional ligands (usually histidine, cysteine, oxygen and water) for the ferrous ion above or below the porphyrin ring to satisfy an octahedral structure with six co-ordination bonds (Scheme 1). Due to its importance in drug metabolism, the interaction of the heme group in proteins with drug molecules has been studied extensively. However, many of these studies had to deploy sophisticated instrumentation or computer modeling software because the complex protein environment where the heme group is embedded in interfered with the analysis of simple equipment [1-4], and for the same reason, it is difficult to elucidate detailed mechanisms of the interaction at the atomic level. Therefore, it is desirable to come up with a simplified model to study the home-drug interactions. Besides the metalloproteins mentioned above, many other proteins are also capable of binding to transitional irons, perhaps in a looser manner, by the ionic side-chains of certain amino acids [5-8]. It is possible that the binding of drug molecules to these proteins involves a bridge of metal ions between the duos. Therefore, it will make sense to study the iron-drug interaction in which iron is in a chelated state as opposed to be in the free form.

Caffeine is a radio sensitizing agent used in radiation therapy of cancer. It was found that caffeine can improve the result of the therapy by affecting the DNA damage-responsive cell cycle and inhibiting certain protein kinas activities [9-11]. However, the mechanistic reason of these processes is not completed understood. In this study, a hypothesis is proposed that the anti-cancer effect of caffeine is possibly due to its ability to produce cancer-suppressing free radicals by irradiation of the caffeine-iron complexes in the body. It has been shown that free radicals can induce cytotoxicity that causes cell apoptosis [12].

In a previous study that was aimed for energy production and photo-electronics research, the author found that dextromethorphan, guaifenesin and caffeine (Scheme 1) can bind to ferrous gluconate in high concentration (as in semi-solids) and the resulting complexes can emit electrons if they were irradiated by UV light or wired to solutions having different electrochemical potentials such as acid solutions or hydrogen peroxide [13]. No such phenomenon was observed if ferrous gluconate alone was irradiated or connected into a battery setup. This observation is particular interesting because these three drugs were not known to interact with free iron. Apparently, when iron was in a chelated form as in ferrous gluconate, its affinity for a second ligand (the drug molecules) increased. In addition, the structure of ferrous gluconate resembles to the home group in metalloproteins with four oxygen atoms chelated to the ferrous ion in a planar square (Scheme 1). The bonding energy of these coordination bonds is roughly equivalent to three

imidazole nitrogen atoms bound to iron [14]. The drug molecules possibly interacted with the ferrous ion by occupying the two pyramid points of the octahedron. This structural feature makes ferrous gluconate a good model compound to simulate the heme chemistry in biological/pharmacological systems.

In the present study, the binding of dextromethorphan, guaifenesin and caffeine to ferrous gluconate is demonstrated in dilute solutions by UV spectroscopy, which supports the electrochemical/potentiometric data obtained in the previous study.

Experimental

Set 1: 3 moM of ferrous gluconate and 0.3 mM of dextromethorphan separately was dissolved in water. The UV spectra of the two solutions were taken from 200 nm to 600 nm. Then the two solutions were mixed at a ratio of 1:1 v/v to obtain the third solution containing 1.5 mM of ferrous gluconate and 0.15 mM of dextromethorphan. UV spectrum of the third solution was acquired and compared with the summation spectra of the former two solutions for any shift of wavelength and change in absorbance.

Set 2: Dextromethorphan was replaced with caffeine and the same procedures for Set 1 were repeated.

Set 3: Dextromethorphan was replaced with guaifenesin with its concentration increased to 0.6 mM. Then the same procedures for Set 1 were repeated.

RESULTS AND DISCUSSION

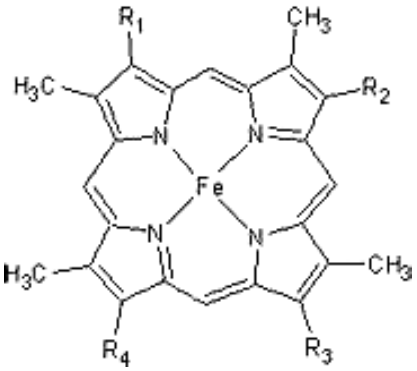
Figure 1 shows the UV spectra of ferrous gluconate and dextromethorphan in pure solutions and in a mixture solution. When the two pure solutions were unmixed, the λ_{max} of ferrous gluconate was 320 nm and the λ_{max} of dextromethorphan was 278 nm. However, when the two pure solutions were mixed, the λ_{max} of ferrous gluconate shifted toward a longer wavelength of 323 nm with the absorbance increased by 5.75%. On the other hand, the λ_{max} of dextromethorphan in the mixture solution shifted toward a shorter wavelength of 277 nm with the absorbance increased by 5.48%. The absorption peak of ferrous gluconate was relatively broad with a flat top, and, therefore, it is easier to visualize the red shift by looking at the wavelength at 95% maximum absorbance of the summation spectrum which shifted from 335 nm to 343 nm, a difference of 8 nm. These data suggests that ferrous gluconate and dextromethorphan complexed together and the bond energy of the new co-ordination bond formed between iron and dextromethorphan is probably equivalent to the red shift of the λ_{max} and increased absorptivity of the new species. Nonetheless, the blue shift of the λ_{max} of dextromethorphan was very small (1 nm), which suggests that the interaction of the two substances was quite week such that it did not affect the electronic density of dextromethorphan.

Figure 2 shows the UV spectra of ferrous gluconate and caffeine in pure solutions and in a mixture solution. When the two pure solutions were unmixed, the λ_{max} of ferrous gluconate was 317 nm and the λ_{max} of caffeine was 272 nm. However, when the two pure solutions were mixed, the λ_{max} of ferrous gluconate shifted toward a longer wavelength of 318 nm with the absorbance increased by 10.61%. The wavelength at 95% maximum absorbance of the summation spectrum shifted from 334 nm to 349 nm, an increase of 15 nm. Interestingly, the λ_{max} of caffeine did not change before and after the two pure solutions were mixed. But the absorbance at 272 nm increased slightly by 3.14%. Again, we see a bigger effect of the complexation of the two substances on the ferrous gluconate absorption peak than the drug peak. Moreover, caffeine had a much higher increase in absorptivity and longer red shift than dextromethorphan upon complexation probably because the binding of caffeine to iron was stronger than the binding of dextromethorphan to iron due to the heterocyclic double ring structure of caffeine. According to literature, the chelating activity of caffeine to free iron (unbound) is only 6% [15]. Here we see a much larger chelating activity of caffeine to iron that was bound to gluconate. The chelating activity is expected to be even higher if the concentration of caffeine is increased.

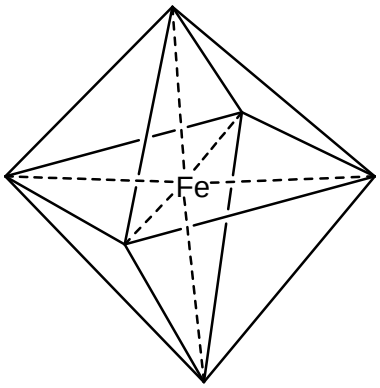
Figure 3 shows the UV spectra of ferrous gluconate and guaifenesin in pure solutions and in a mixture solution. When the two pure solutions were unmixed, the λ_{max} of ferrous gluconate was 319 nm and the λ_{max} of guaifenesin was 273 nm. However, when the two pure solutions were mixed, the λ_{max} of ferrous gluconate shifted toward a longer wavelength of 323 nm with the absorbance increased by 13.85%. The wavelength at 95% maximum absorbance of the summation spectrum shifted from 337 nm to 353 nm, an increase of 16 nm. Conversely, the λ_{max} of guaifenesin in the mixture solution shifted toward a shorter wavelength of 272 nm with the absorbance increased by only 4.26%. For the third time, we see a bigger effect of the complexation of the two substances on the ferrous gluconate peak than the drug peak, indicating it is a weak interaction. It should be noted in the Set 3 samples, the concentration of guaifenesin was twice higher than dextromethorphan in Set 1 samples, and this is the reason the extent of the red shift and increase in absorptivity of the new species in Set 3 is more than the one of dextromethorphan in Set 1 sample. If the concentration of guaifenesin is reduced to the same level as dextromethorphan, the complexation effect on the spectra is probably comparable. So to rank order of the strength of binding of the three drugs to ferrous gluconate, caffeine > guaifenesin ≈ dextromethorphan. This result also suggests that the binding site of

dextromethorphan to iron is possible the methyl phenyl ether group instead of the tertiary amine group because guaifenesin also has a methyl phenyl ether group. Amine groups usually have higher binding capacity to iron than oxygen containing groups [14] that may result in bigger shift of the UV spectrum. This postulation is consistent with the published computational data of the binding of dextromethorphan to CYP 2D6 which was thought to be the reason why O-demethylation is the more dominant metabolic pathway of dextromethorphan over N-demethylation [4].

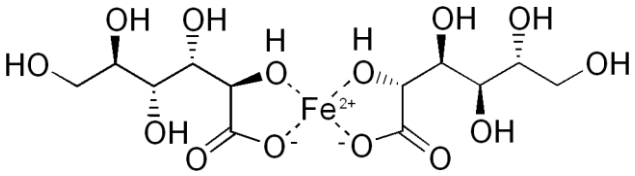
Scheme 1



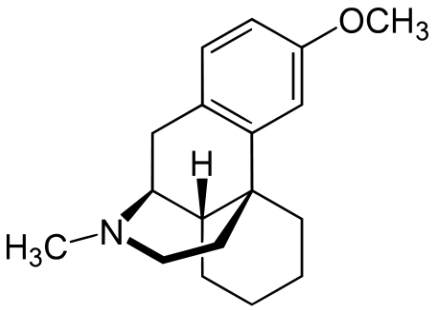
Heme



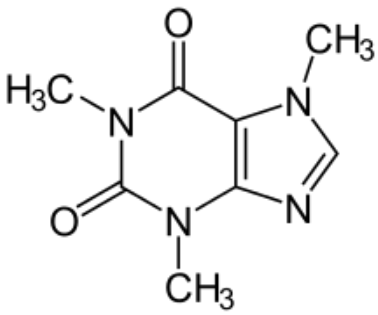
Octahedron of iron chelates



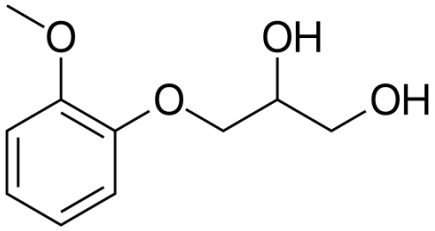
Ferrous gluconate



Dextromethorphan



Caffeine



Guaifenesin

Scheme-1: Structures of heme, the octahedron of iron chelates, ferrous gluconate, dextromethorphan, caffeine, guaifenesin

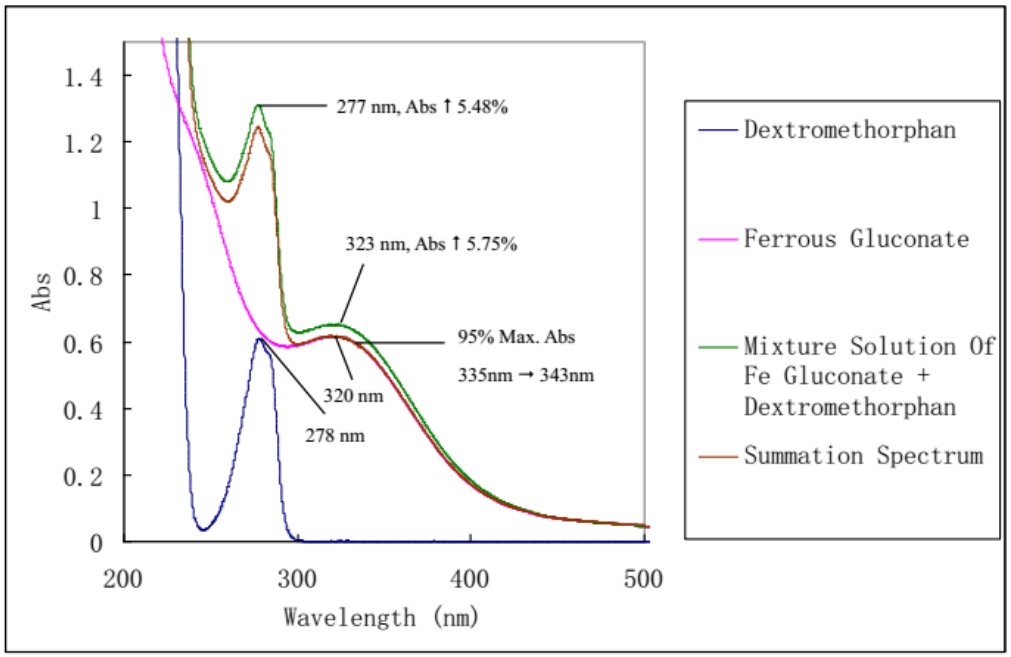


Fig-1: UV spectra of the solutions of pure ferrous gluconate (3 mM) and dextromethorphan (0.3 mM) with the summation spectrum of the two pure solutions and the spectrum of their mixture (1:1 v/v)

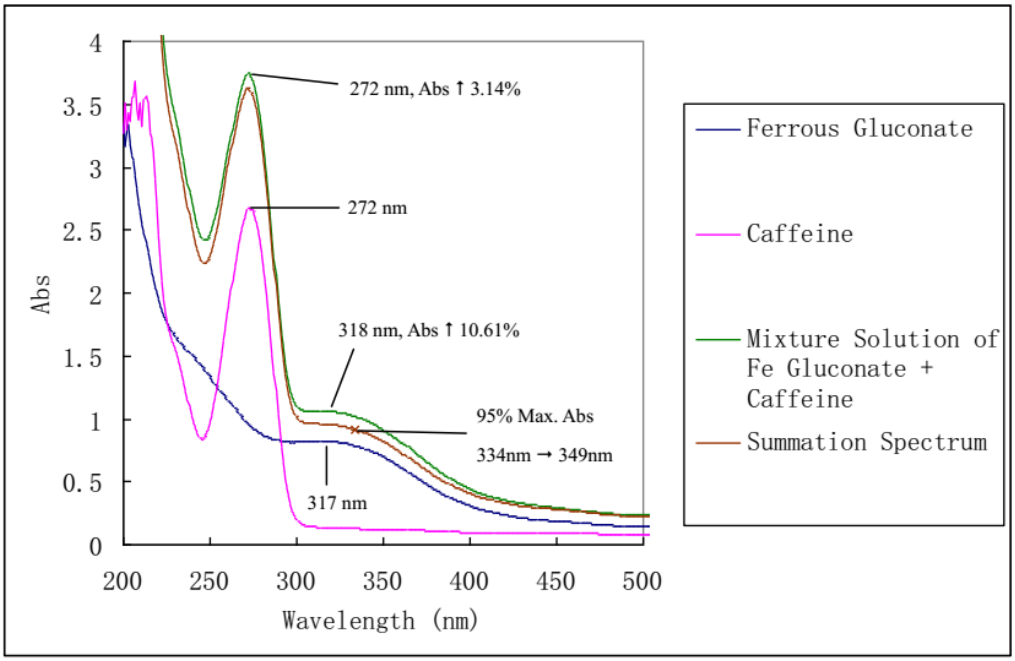


Fig-2: UV spectra of the solutions of pure ferrous gluconate (3 mM) and caffeine (0.3 mM) with the summation spectrum of the two pure solutions and the spectrum of their mixture (1:1 v/v)

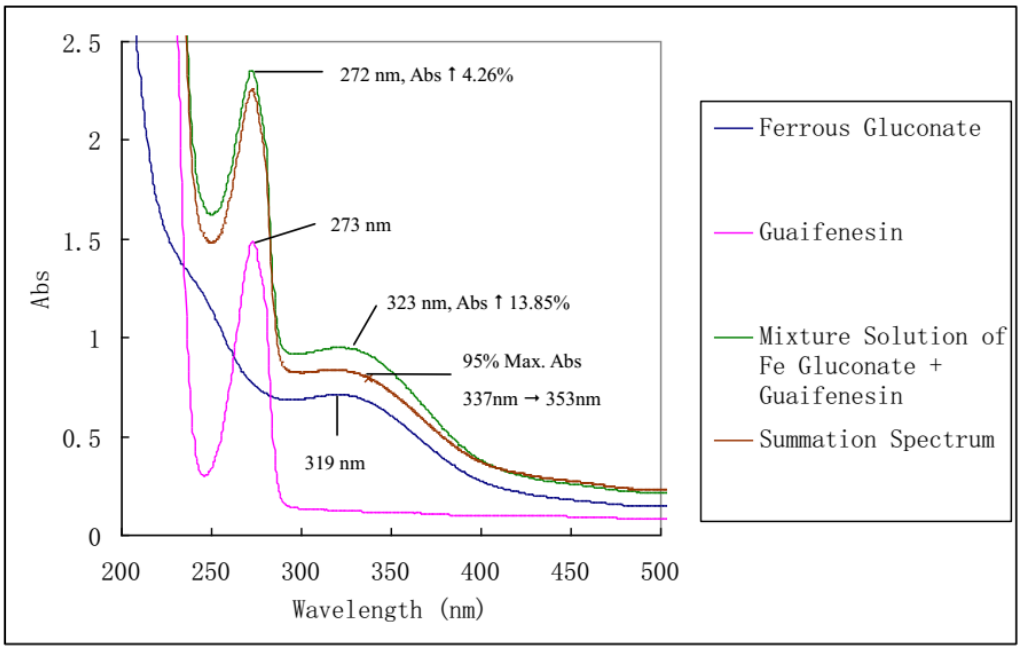


Fig-3: UV spectra of the solutions of pure ferrous gluconate (3 mM) and guaifenesin (0.6 mM) with the summation spectrum of the two pure solutions and the spectrum of their mixture (1:1 v/v)

In a previous study, the author showed that when aspirin bound to ferrous gluconate with its carboxyl groups in the salicylic and the acetic acid parts of the molecule, a decomposition reaction took place which generated several different products with the hydroxyl groups of gluconate subsequently, accompanying a big change in their UV spectra (a dramatic reduction of the ferrous gluconate peak and a 9 nm blue shift of the aspirin peak) [16]. These changes of spectra were not observed in any the three sets of experiments in this study, which suggests that dextromethorphan, guaifenesin and caffeine did not react with ferrous gluconate like aspirin because of the difference in the functional groups that bind to iron. The heme moiety has been tried to be used as a biosensor. It was noted that heme itself was ineffective for this application because of its low solubility; it must be chemically modified so that it can be attached to electrodes or coupled to protein molecules [17]. Ferrous gluconate, however, is quite soluble in water and can be prepared in a wide range of concentrations which allows direct measurement of its electrochemical and spectral properties, and this is an advantage of using it in biosensing.

CONCLUSION

UV spectroscopy shows that dextromethorphan, guaifenesin and caffeine bind to ferrous gluconate with the binding energy in the order of caffeine > guaifenesin ≈ dextromethorphan. This is in agreement with the electrochemical/potentiometric data obtained in the previous study. Based on the result of this study, ferrous gluconate is useful for the simulation of the drug-heme or drug-iron interactions in biological/pharmacological systems.

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