Effect of Solution on the isoelectric point of collagen

Guide professor: Yunqiao Ding

Email address: 673659149@qq.com

Student name: Mengmeng Sui

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Qilu University of Technology

Target and significance:

My experiment mainly focusing on what kinds of effects collagen have when environment changed, the environment including buffer, pH and ionic nature. Using a variety of testing methods to search collagen's morphological change, as well as the essential reason that environmental impact to collagen structure, stability, mechanical properties, biological properties(crosslinking effect).

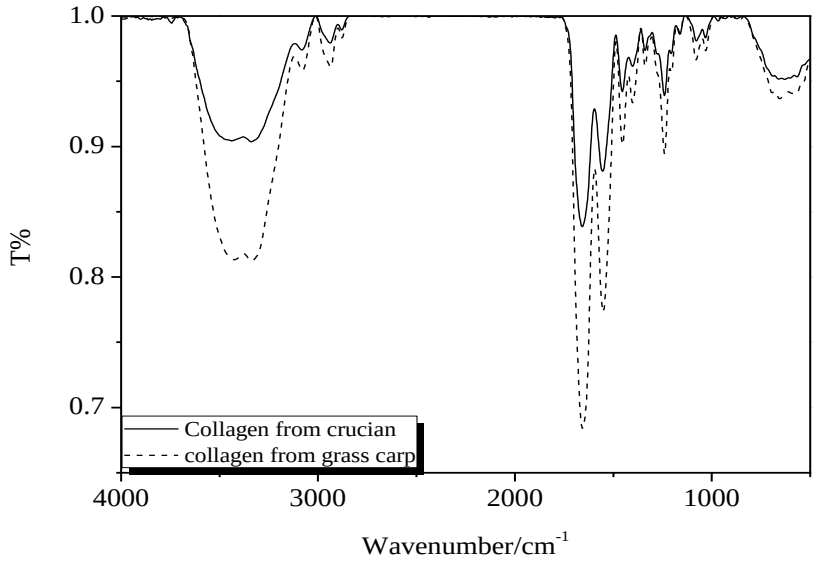
By studying the structure of collagen to presume the location of a number of diseases; By studying self-assembly of collagen laying a solid foundation for the development of new biomaterials; researching of Chromium can help researchers find better pollution-free leather additives for leather tanning industry.

Works and results:

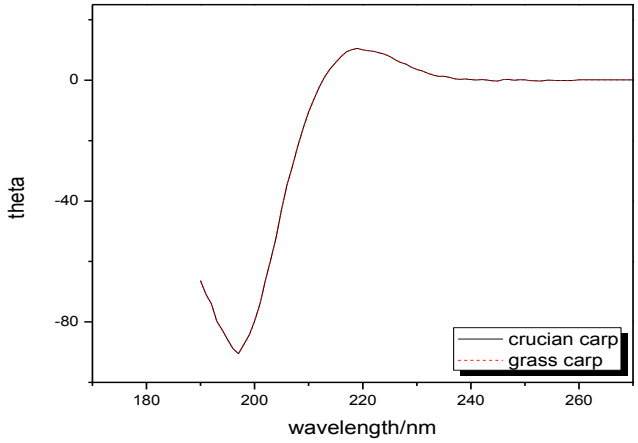
Part1.

The collagen I used is extracted from crucian and grass carp by student Chenggong Yang.

Using infrared spectrometer to Characterize collagen with amid band. Circular Dichroism is used to characterize the integrity of collagen triple helix.



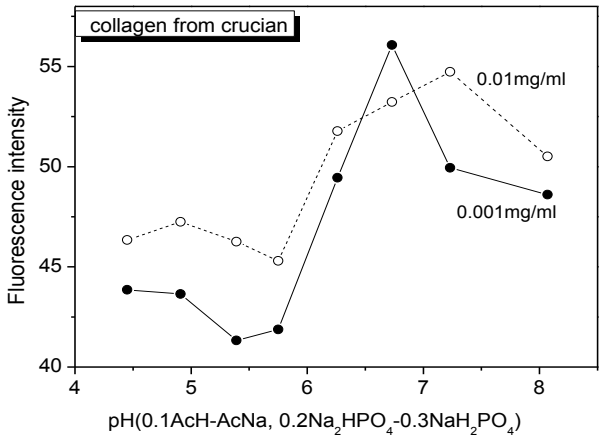
Pic1-1：crucian carp/grass carp collagen-IR



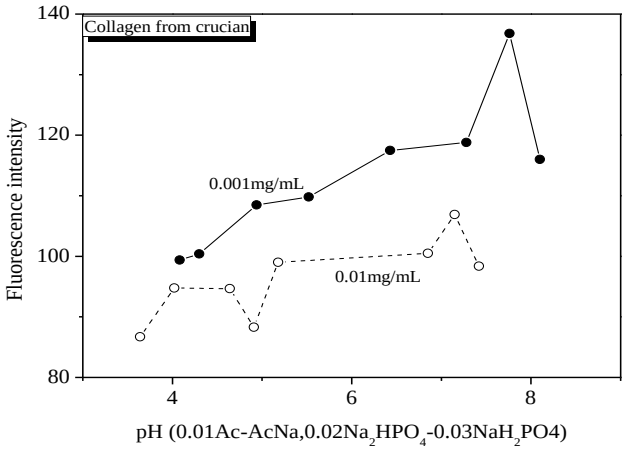
Pic1-2：crucian carp/grass carp collagen-CD

Part2.

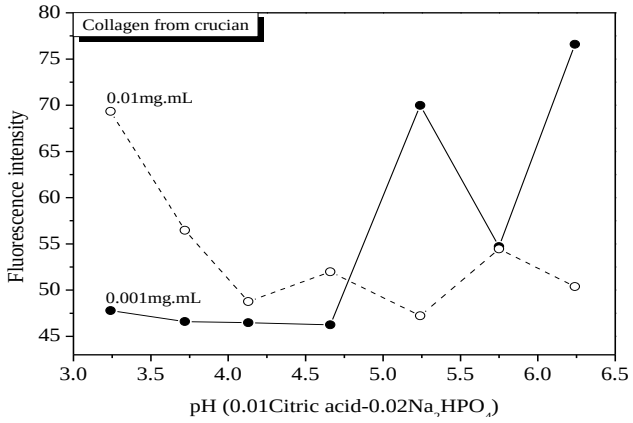
In the first part, I used Fluorescence Spectroscopy detect fluorescence intensity of collagen in different concentration and buffer.



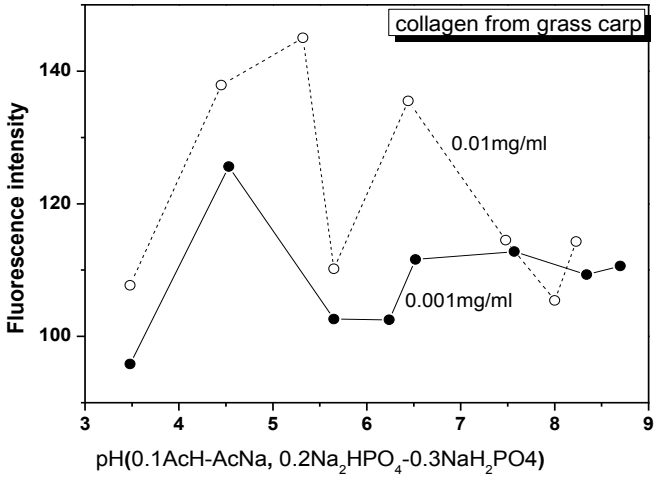
Pic1-3: AcH(0.1M)-AcNa(0.1M)/[Na_2HPO_4(0.2M)-NaH_2PO_4(0.3M)](buffer)-crucian collagen



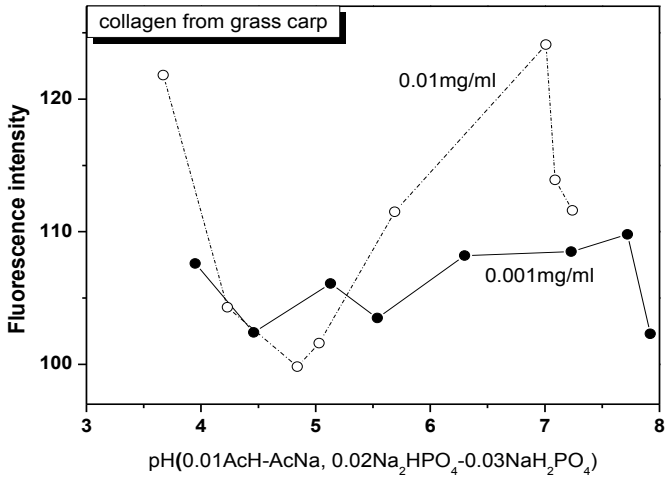
Pic1-4: AcH(0.01M)-AcNa(0.01M)/[Na_2HPO_4(0.02M)-NaH_2PO_4(0.03M)](buffer)-crucian collagen



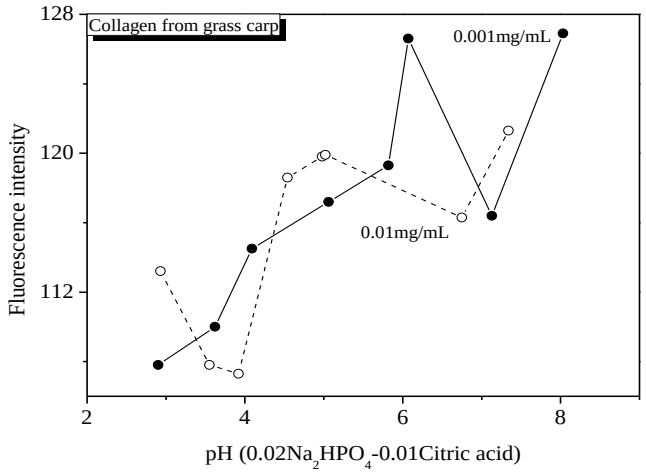
Pic1-5: citric acid（0.01M）- Na_2HPO_4（0.02M）(buffer)- crucian collagen



Pic1-6: AcH(0.1M)-AcNa(0.1M)/[Na_2HPO_4(0.2M)-NaH_2PO_4(0.3M)](buffer)- grass collagen



Pic1-7: AcH(0.01M)-AcNa(0.01M)/[Na_2HPO_4(0.02M)-NaH_2PO_4(0.03M)](buffer)- grass collagen



Pic1-8: citric acid（0.01M）- Na_2HPO_4（0.02M）(buffer)- grass collagen

Typically, the higher the concentration of collagen, the fluorescence intensity should be stronger, but a small range of reversion can be found according to the experimental data. The fluorescence intensity has relation with both collagen solution and pH as well as concentration of buffer, at the same time, salt ions in buffer also produce a specific effect on the fluorescence intensity of collagen, so this experiment will be explored in the next part : salt ions effect on collagen solutions.

Part3.

I used 15 kinds of slats in this part of experiment(CH3COOH、CH3CONa、NaH2PO4、Na2HPO4、NaCl、MgCl、CrCl、CaCl2、CH3COOK、Ca(CH3COO)2、Mg(CH3COO)2、CH3COONa、Cr2(SO4)3、K2SO4、Na2SO4、MgSO4、Cr(CH3COO)3、KCl、CrO3)

Then I used Fluorescence Spectroscopy detect fluorescence intensity of collagen in different situation.

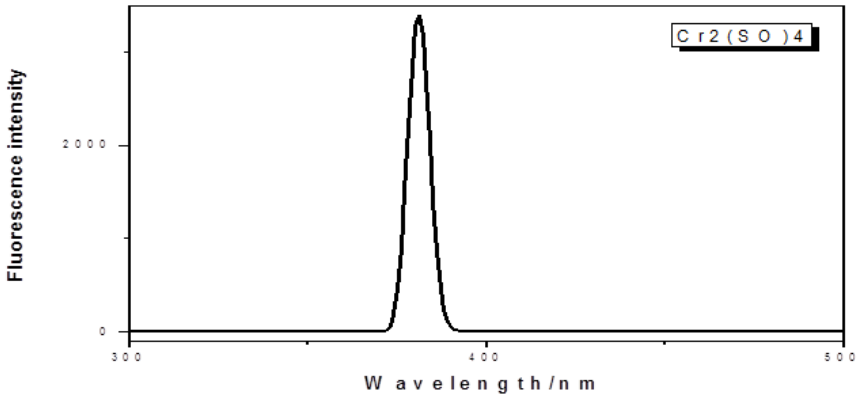
. Table1-1: The pH and FL in salts(40mmol/l)- crucian collagen(0.05mg/ml)

The pH of the collagen solution	3.14	
The pH of collagen-salty solution and FL	pH	FL
NaCl	2.96	438.4/5.692
Na_2SO_4	3.34	436.8/5.745
$NaCH_3COO$	4.74	438.4/5.737
KCl	3.11	438.4/6.441
K_2SO_4	3.36	436.6/6.773

KCH_3COO	4.75	437.8/6.740
$MgCl_2$	2.97	439.2/4.935
$MgSO_4$	3.25	436.2/5.029
$Mg(CH_3COO)_2$	3.34	436.8/5.745
$CaCl_2$	2.79	437.2/7.384
$CaSO_4$		
$Ca(CH_3COO)_2$	4.84	438.8/6.383
$CrCl_3$	2.78	
$Cr_2(SO_4)_3$	2.90	
$Cr(CH_3COO)_2$	3.80	

After adding salts, there are 4 ~ 6nm blue shifts in the collagen solution, and the absorption intensity is greatly reduced, which illustrates the structure of the collagen had changed after the metal ions is added. The reason that varying degrees for the decrease in fluorescence intensity is that there are differences in the way the metal ion binding with collagen.

There is fluorescence quenching in Cr(III). Transition metal ion chromium (III) is a very good fluorescence quencher may be related to fluorescence quenching in collagen molecules carboxyl complexation formation.



Pic1-9 fluorescence intensity of $Cr_2(SO)_4$

Summary:

There are some elements have special effect to collagen's isoelectric point as well as fluorescence intensity, such as buffer, pH and salty ions. They affect amount of application function of collagen. This essay mainly use fluophotometer to measure fluorescence intensity of collagen in different conditions. The result shows that there is a reversion of a certain pH in fluorescence intensity under ultra dilute solution.

Collagen's medification happens during adding slats, and there is a coordination reaction between Tyr and metal ions. 4~6nm blue shift in fluorescence intensity happened during it, and the intensity becomes lower dramatically.

Future work:

The topic of this thesis is 'Effect of Solution on the isoelectric point of collagen', but indeed, professor Ding and I found it was more meaningful to focus a small part of it, so I mainly focusing about 'the effect of solution on Spectroscopic Properties of collagen', so in the next research, I will fulfill the whole experiment.

Lacking of time could not allow me to complete the whole experiment and get the final reasons for those experimental phenomena, so in the next experiment, we are focusing on why those phenomena happen.

Relating to the current equipment, the sensitivity cannot detect sample solution in very dilute state, so in the next study, we are going to study the theoretical calculation and simulation methods, obtained under special circumstances change in fluorescence intensity of collagen conclusions.

In our experiment, it also lacks the structure of collagen and collagen extracted salt solution analysis (such as CD spectroscopy, atomic force microscopy, etc.), rheological testing. So we will fulfill them in the future.