Rheology of Dispersions of Collagen Fibrils Manufactured from Waste Corium for Environmental and Biochemical Applications

Snehanjani Shivakumar

Chemical Engineering, Manhattan College

Manhattan College

New York City, USA

Gennaro Maffia

Chemical Engineering, Manhattan College

Manhattan College

New York City, USA

Abstract—**Rheology of collagen dispersions were examined as a function of time. Further rheological studies were done using admixtures like Vitamin B12 (VB12) and Blue Dextran (BD) in the dispersion. The dispersion of collagen matrices is non-Newtonian in nature and it exhibits pseudoplastic behavior. The flow of dispersion is governed by the power law with one of its parameters decreasing with increasing rate of shear and time. But this parameter also reaches almost a constant value exactly in two weeks. The other parameter is independent of time and is always below unity indicating that these dispersions have pseudoplastic characteristic. Also Collagen dispersions had lower viscosity with the admixture than without. Hence admixtures do have an effect on the rheology over period of time.**

Collagen; pseudoplastic; rheology; admixture; non-Newtonian; freeze dried; rate of shear; shear stress; power law; shear thinning; ball milling; Newtonian ; cross linking ; Vitamin B12; Blue Dextran; Nanofibrils

I. INTRODUCTION

Collagen is a fundamental structural protein present in all animals. After certain processing steps collagen forms into a bundle of fiber which has similar appearance to a rope. Collagen is not soluble in water but due to its surface charge chemistry it can hold upto hundred times its mass in water. This unique surface charge characteristic make Collagen most useful in biotechnological and environmental applications. The Collagen dispersions were made by using raw Bovine Hide Corium. Once these collagen dispersions are made, they can be frozen and freeze dried. The new freeze dried material retains its overall properties of the original frozen material. But the new cryodessicated material has 99% void space and so it is 99% porous and the remaining material is just spongy aerogel structure with controllable pore size, excellent mechanical properties and density as low as one thousandth of water. Also, the collagen dispersions can be cross linked which will secure its shape, pore size and skeleton structure.

These collagen dispersions have several applications in environmental and biotechnological fields. The most beneficial environmental application is the use of Collagen in aid to filtration process. Collagen dispersion carrying positive charges have affinity to the negative charges of the polar water molecule thereby helps in agglomeration of the suspended solid particles in sludge or waste water and inert suspensions.

Covalent bonds between the neighboring collagen molecules are developed which makes the collagen dispersion not to retain any more water. The collagen dispersion is now sterile due to cross linking as well and hence this cross linked collagen material can be used in cell cultures especially for organ production, skin replacements etc.

The rheology plays a vital role in determining the characteristics of the Collagen Dispersions. Rheology of Collagen Dispersions verifies that these dispersions are non-Newtonian in nature. The extent to which it is non-Newtonian is mainly governed by the power law fluid parameters. Equation "1" mentioned below is the power law that regulates the flow of collagen dispersion.

$$\tau_{xz} = A \, (dv_z/dx)^B \qquad (1)$$

τ_{xz} [=] Shear Stress ($N.m^{-2}$)

A [=] Power law parameter

dv_z/dx [=] Rate of shear in (min^{-1})

B [=] Power law parameter

In equation "1", A and B are the power law parameters in which A depends on time and B carries the pseudoplasticity nature. These criteria determine the non-Newtonian characteristics of collagen dispersion.

II. MATERIALS AND METHODS

A. Bovine Corium Processing

The Collagen dispersion is made from raw fibrillar type *I* bovine corium as a starting material. Corium is the dermis layer of the hide which is very rich in connective tissue which has high collagen content. The micrograph of the bovine hide collagen before milling is illustrated in "Fig. 1" below.
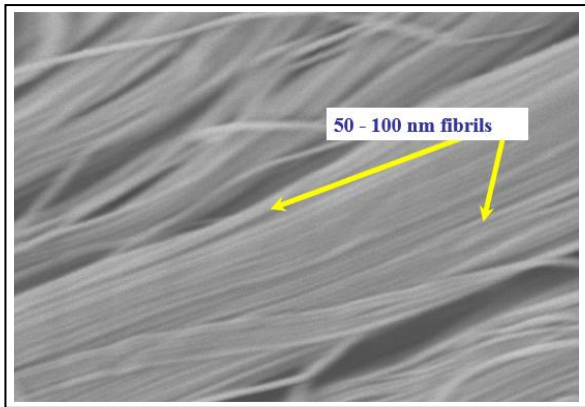
Figure 1. *Collagen nanofibrils before milling*

It can be seen from "Fig. 1" that these nanofibrils are not well dispersed. In order to make it dispersed it is initially mixed with small volume of water undergoes ball milling with zirconium media for 7-10 days. The resultant dispersed collagen paste is then strained, washed and centrifuged at low temperatures. After the centrifugation process, the floating oils and fats are decanted. This method is repeated until no floating oils or fats are found above the clear phase of the dispersion. Now the collagen nanofibrils have dispersed and it can be seen in "Fig. 2".
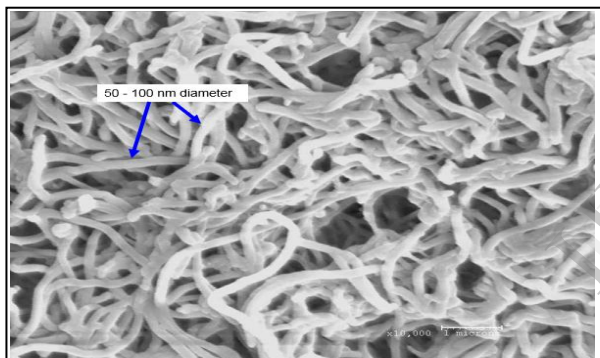

Figure 2. *Collagen nanofibrils after milling*

The viscous phase which contains most of the collagen is mixed in a solution of organic acid such as acetic acid or citrus acid. It is then blended into solution because of the need to form dispersion and also for the thickening of the formed dispersion paste. The end dispersed collagen has better physical properties thereby increasing the efficiency in its applications.

B. Dispersion thinning via Rheology with dependence on time

The shear thinning of collagen dispersion was studied by obtaining the varying viscosity to the increase in shear rate. This was done using the thermo scientific viscotester 550 for different time intervals of 0.7 percent collagen dispersion. The shear stress ($\mathfrak{T}$) was calculated by multiplying the viscosity (μ) with the shear rate (dv_z/dx) as shown below in "2".

$$\mathfrak{T}_{xz} = \mu \, (dv_z/dx) \tag{2}$$

Then a graph using log of shear rate data and the log of shear stress data was plotted. The slope of this graph was the power law parameter B and the exponent of the intercept gave the parameter A in "1". The thinning process and the nature of the parameters were closely monitored for two weeks when the power law parameter A slowly started not to change at the end of the second week.

C. Shear thinning via admixtures

The rheology of collagen dispersions when admixtures were introduced was studied by loading Vitamin B12 (B12) and Blue Dextran (BD) into the dispersion. Vitamin B12 and Blue Dextran were used due to their molecular weights being 1355.37 *g/mol* and $2e^6$ *g/mol*. The loading of B12 and BD was done by weighing 42.0 grams to 42.5 grams of the collagen dispersion. This was then added with 0.01 grams of B12 and BD. The shear rate and shear stress data was obtained using the viscometer and this was done for different percentages of collagen content. The results were plotted to finally infer that the viscosity lowered with the admixtures than without.

III. RESULTS AND DISCUSSION

For a non- Newtonian fluid, the power law parameter A in "1" should either increase or decrease with increasing rate of shear. Collagen being non- Newtonian has its parameter A decreasing with increasing rate of shear. Also the deviation of parameter B from unity indicates the extent to which the fluid deviates from its Newtonian behavior. Parameter B for collagen dispersions were less than 1 for all time periods experimented indicating that collagen dispersion is pseudoplastic in nature. "Table 1" illustrates the change in the parameter A and B with the dependence of time.

TABLE I. CHANGE IN POWER LAW PARAMETERS WITH DEPENDENCE ON TIME

Parameters	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
B	0.402	0.392	0.405	0.401	0.402	0.398	0.397
A	7.224	7.099	7.094	6.897	6.867	6.568	6.507
	8th day	9th day	10th day	11th day	12th day	13th day	14th day
B	0.406	0.408	0.409	0.429	0.442	0.420	0.421
A	6.352	6.219	6.061	5.151	3.648	3.648	3.647

From "Table 1", it is clear how the parameter A reduced starting from the first day and started not to have any changes as time increased from the first day of monitoring to almost the fourteenth day.

Also parameter B seemed to have very minimal changes over time, indicating that time may not affect B. This time independence of B was investigated further by making some statistical analysis presented in "Table 2". It is clear from "Table 2" that with 95% confidence level, the value of parameter B changing with time lies between 0.401 and 0.417. Parameter B lies between 0.409 and 0.008 higher or lower confidence. This range is very small and so it can practically be said that parameter B is independent of time.

TABLE II.   STATISTICAL ANALYSIS FOR TIME INDEPENDENCE OF PARAMETER B

Statistical Analysis for parameter B	
Mean	0.409
Standard Error	0.003
Median	0.405
Mode	0.402
Standard Deviation	0.013
Sample Variance	0.000
Kurtosis	0.974
Skewness	1.166
Confidence Level (95.0%)	0.007

"Fig. 3" illustrates the change in parameter A over a period of two weeks. Here again it is easily seen that as time increases, A decreases but levels out close enough to after two weeks. At day 1, A is 7.2 and it decreases to 3.6 on the fourteenth day. Also, the graph indicates a polynomial curve where A decreases and then starts to stay constant.
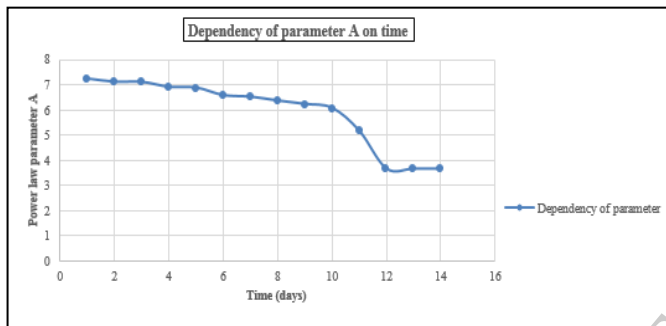

Figure 3.   *Dependency of parameter A on time*

Parameter B of collagen dispersions is less than unity ranging from 0.39 to 0.42 throughout the study for different time periods indicating that the collagen matrices undergoes shear thinning.

Rheology of collagen dispersion was further examined by adding admixtures to the pseudoplastic fluid. "Figure 4" depicts the change in viscosity with and without the admixture VB12. The blue line indicates the collagen without VB12 and the red line indicates with VB12.
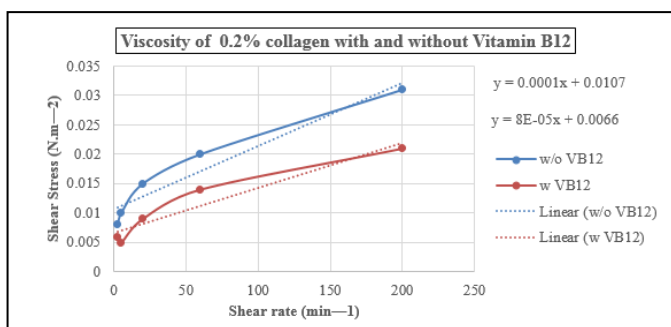

Figure 4.   *Viscosity of 0.2% collagen with and without Vitamin B12*

In "Fig. 4", it can be seen that the collagen with the VB12 had lower viscosity of 8e−5 (N.s.m^{-2}) compared to when it did not contain the VB12 loaded into it which had a viscosity

of 0.0001 (N.s.m^{-2}). With increasing rate of shear, VB12 acts as an agent aiding in shear thinning process of the fluid.

"Fig. 5" also denotes similar results using BD as the admixture with 0.2% collagen. The blue line indicates the collagen without BD and the red line indicates with BD. The collagen with the BD loaded into the dispersion had lower viscosity 6e−5 (N.s.m^{-2}) compared to when it did not contain BD which had a viscosity of 0.0001 (N.s.m^{-2}). Here again, BD acts as an agent aiding in shear thinning process of the fluid with an increasing rate of shear.
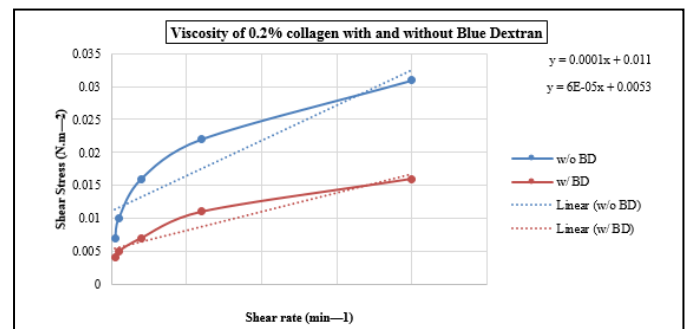

Figure 5.   *Viscosity of 0.2% collagen with and without Blue Dextran*

On comparing the change in viscosity of 0.2% collagen with VB12 and with BD, it can be inferred that a greater decrease in viscosity was observed with VB12 than with BD. Reason could possibly be because VB12 has a similar structure to that of porphyrin, but with one of the connecting methylene groups removed. So, interactions with the central cobalt are possible. This might also be due to the fact all metal ions tend to be out of plane in porphyrin ring, and might be available for reaction with collagen. But the actual cause to this decrease in viscosity has still not been studied thoroughly.

On the whole, when the rheology was investigated for 0.3, 0.4, 0.5 and 0.6 percent collagen content, the viscosity of collagen with the admixtures was lower than the collagen without the admixtures. Implying no matter how concentrated the dispersion is with the collagen, admixtures always had an effect in the rheology over a period of time.

IV.   CONCLUSION

Collagen is the basic protein found in all animals. It is made from type *I* bovine hide corium. This corium is milled with zirconia alloy for almost 10 days in ball milling to get the desired collagen paste. The collagen paste is then mixed into solution with an organic acid thereby forming dispersed collagen. Due to its surface charge chemistry, the ability to retain the structural properties of the original frozen material even after being freeze dried and having 99% void space after being freeze dried, collagen practically can be used for applications in biotechnology, cosmetics, environmental, catalysis and biomedical field.

Studies on rheology of collagen dispersion have been made to comprehend the nature of its non- Newtonian behavior. It is concluded from this study, that collagen dispersions are pseudoplastic in nature following the power law equation in "1". The power law parameter A is time dependent for the chosen dispersion with 0.7% collagen content. Also, parameter A was decreasing with increase in

rate of shear and time but it does level out to almost being constant in exactly two weeks Parameter B which is not a function of time was less than unity denoting the deviation from Newtonian behavior verifying that the dispersions are pseudoplastic in nature. The pseudoplastic nature of the matrix is revealed by these parameters. Hence the power law that collagen dispersions follow is concluded in "3". In "3" (t) is denoting the dependency of parameter A on time, whereas B does not depend on time and it is a function of shear rate.

$$\tau_{xz} = A\,(t)*\,(dv_z/dx)^{\,B} \qquad (3)$$

Also the rheology was examined by introducing admixtures like VB12 and BD. The viscosity of the collagen matrix that was loaded with the admixture was lower than the viscosity of the matrix which was not loaded with the admixture. Moreover, VB12 caused a greater decrease in the viscosity than BD. Hence admixtures do affect the rheology of collagen dispersions over a period of time.

Finally, with further studies on rheology, the accurate reasons as to why admixtures like VB12 and BD decreased the viscosity when loaded into the collagen matrix can be figured.

ACKNOWLEDGMENT

V. REFERENCES

[1] R. B. Bird, E. W. Stewart and N. E. Lightfoot, Transport Phenomena, Wisconsin: John Wiley & Sons, 1960.

[2] G. J. Maffia, "Using untreated raw fibrillar type I corium as the starting material; milling to unravel fibers; washing and straining; centrifuging; repeating these steps until not fats are present; and mixing with an acid". United States of America Patent US6660829 B1, December 2003.

[3] G. J. Maffia, "Porous metallic structures". United States of America Patent US8329091 B2, December 2012.

[4] W. Friess, "Effects of processing conditions on the rheological behavior of collagen dispersions," *European Journal of Pharmaceutics and Biopharmaceutics,* vol. 51, no. 3, p. 259–265, May 2001,.

[5] D. Quemada, "Rheology of concentrated disperse systems II. A model for non-newtonian shear viscosity in steady flows," *Rheologica Acta ,* vol. 17, no. 6, pp. 632-642, 1978.

[6] K. S. Sorbie, P. J. Clifford and E. R. W. Jones, "The rheology of pseudoplastic fluids in porous media using network modeling," *Journal of Colloid and Interface Science,* vol. 130, no. 2, p. 508–534, July 1989.

[7] M. Guedda and Z. Hammouch, "Similarity Flow Solutions of a Non-Newtonian Power-law Fluid," *International Journal of Nonlinear Science,* vol. 6, no. 3, pp. 255-264, 2008.

[8] D. S. Malkus, J. A. Nohel and B. J. Plohr, "Dynamics of shear flow of a non-Newtonian fluid," *Journal of Computational Physics,* vol. 87, no. 2, p. 464–487, April 1990.

[9] M. M. Cross, "Rheology of non-Newtonian fluids: A new flow equation for pseudoplastic systems," *Journal of Colloid Science,* vol. 20, no. 5, p. 417–437, June 1965.

[10] D. M. Knapp, V. H. Barocas, A. G. Moon, K. Yoo, L. R. Petzold and R. T. Tranquillo, "Rheology of reconstituted type I collagen gel in confined compression," *Journal of Rheology,* vol. 41, no. 51, 1997.

[11] J. C. Reed and A. B. Metzner, "Flow of non-newtonian fluids— correlation of the laminar, transition, and turbulent-flow regions," *AICHE Journal,* vol. 1, no. 4, pp. 434-440, JUN 2004.