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Data and Calculations for Experiment 17

Viscosity of DNA solutions

(1) pH of acidified buffer _____
(2) Efflux time of acidified buffer (t_0) _____ sec
(3) pH of acidified DNA solution _____
(4) Concentration of DNA solution _____
(5) Efflux time of acidified DNA solution _____ sec
(6) Efflux time of neutralized DNA solution at time of neutralization _____ sec
(7) 20 min. later _____ sec
(8) 40 min. later _____ sec
(9) 60 min. later _____ sec
(10) 80 min. later _____ sec
(11) 100 min. later _____ sec

pH dependence of the viscosity of DNA solutions

(12) pH of neutral buffer _____
(13) Efflux time of neutral buffer _____ sec
(14) pH of DNA solution in neutral buffer _____
(15) Efflux time of DNA in neutral buffer _____ sec

After addition of 1 drop of 0.1 M HCl

(16) pH of buffer _____
(17) Efflux time of buffer _____ sec
(18) pH of DNA solution _____
(19) Efflux time of DNA solution _____ sec

After addition of 1 drop of 0.1 M NaOH

(20) pH of buffer _____
(21) Efflux time of buffer _____ sec
(22) pH of DNA solution _____
(23) Efflux time of DNA solution _____ sec

After addition of 2 drops of 1 M NaOH

(24) pH of buffer _____
(25) Efflux time of buffer _____ sec
(26) pH of DNA solution _____
(27) Efflux time of DNA solution _____ sec

Tabulate your data on the pH dependence of relative viscosity

pH	η_{rel}
(3) _____	(5) / (2) _____
(14) _____	(15) / (13) _____
(18) _____	(19) / (17) _____
(22) _____	(23) / (21) _____
(26) _____	(27) / (25) _____

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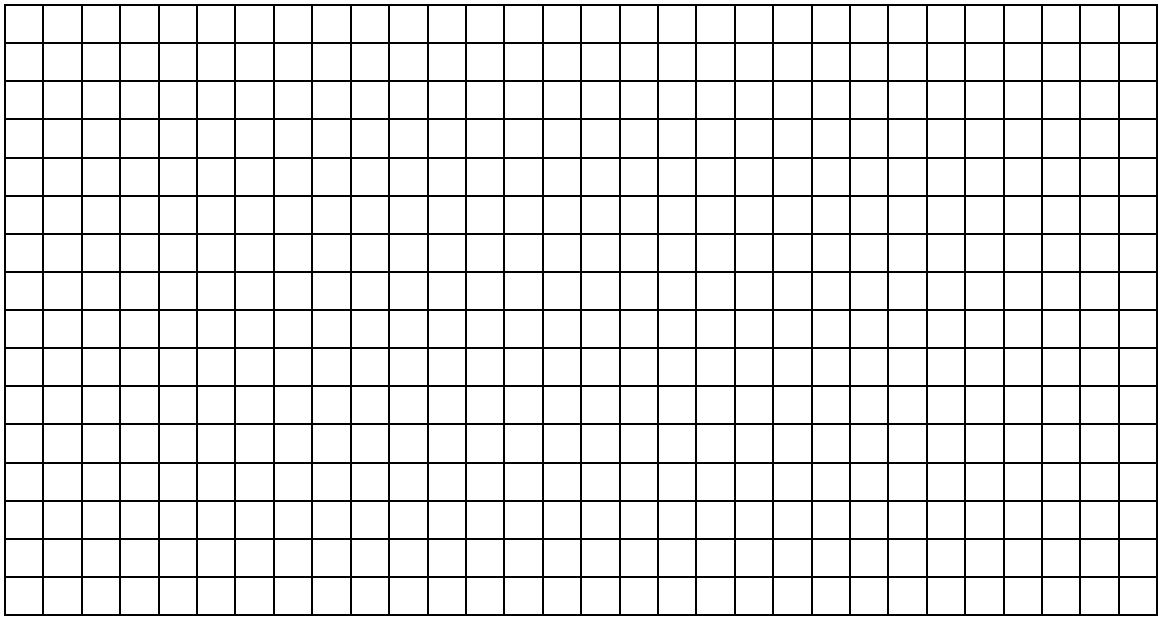
Questions

1. Plot your tabulated data: relative viscosity on the y–axis, and pH on the x–axis.



2. At what pH values did you observe helix-to-coil transitions?

3. Now plot your data on the refolding of DNA double helix (5) – (11) using Microsoft Office Excel®. Plot time on the x–axis (i.e., time after neutralization in min.) and the efflux times on the y–axis (in sec.). Make sure to include this graph with your report. See Experiment #18 for directions on using Excel®. Include the best-fitting line for the data points; *please note that this graph is NOT linear.*

4. Was there any indication that, upon neutralization of the denaturing acid, the DNA did refold into a double helix? Explain.

5. Compare the efflux time of the neutral DNA (15) to that of the denatured DNA 100 min. after neutralization (11). What does the difference between these two efflux times tell you regarding the refolding process?

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6. Calculate the intrinsic viscosity of your DNA at:

   a. Neutral pH = 2.3 × {log [(15) / (13)]} / (4) =

   b. Acidic pH = 2.3 × {log [(5) / (2)]} / (4) =

   c. Basic pH = 2.3 × {log [(27) / (25)]} / (4) =

   d. Neutral pH 100 min. after neutralization = 2.3 × {log [(11) / (13)]} / (4) =

7. A high intrinsic viscosity implies a double helix; a low intrinsic viscosity means a random coil. What do you think is the shape of the DNA after acid denaturation and subsequent neutralization? (See 6d above.) Explain your answer.