Determination of the casein isoelectric point

THEORY

Clustering of colloidal particles to make larger macroscopic units is called coagulation. Coagulation of colloidal protein solutions occurs by disrupting solvation shell. In an aqueous solution protein macromolecules form colloidal particles, having two basic functional groups, amino group ($-NH_2$) and carboxyl group ($-COOH$). By a self-interaction or due to the pH of the surrounding medium a protein molecule may act in three different ionic forms, as a cation (NH_3^+-R-COOH), anion (NH_2-R-COO^-) or amphion (NH_3 + -R-COO^-).

Amphion, which outwardly appears to be an electro-neutral molecule, has minimal ability to attract solvent molecules, which in great part leads to removal of hydration shell, due to electrostatic attraction of opposite charges macromolecules form tangles and coagulation of colloidal solution occurs.

The acidity of the solution, expressed by a certain pH value, at which the concentration of a given protein amphion is at highest value, is called the isoelectric point. At this pH, the protein solution is the cloudiest, which can be used to determine the approximate value of the isoelectric point only by visual comparison.

TASK

- Observe casein coagulation in dependence on the pH of acetate buffer and empirically determine its isoelectric point.

EQUIPMENT AND CHEMICALS

- Thermometer; pH meter; 50ml volumetric flask; 3× 100ml volumetric flask; 9× test tube; graduated pipette (1, 10 ml); pipette adapter; 2× beaker; spoon; weighing boat; glass rod; tube; stand; 1M CH_3COOH; $CH_3COONa \cdot 3H_2O$ (M_r = 136,08); casein.

PROCEDURE

- Into 100ml volumetric flask, prepare 1M solution of sodium acetate trihydrate by weighing appropriate amount of solid substance on analytical scales.
- Weigh 0.2 g of casein, then add it to a beaker with 5 ml of prepared 1M CH_3COONa. Dissolve it by heating at 50 °C in water bath for 10 – 15 minutes, while gradually mixing it with glass rod. Then pour prepared solution into 50ml volumetric flask and fill it up to the mark by distilled water.

- In two 100ml flasks prepare 0.1M and 0.01M solutions of acetic acid by dilution of the stock solution (1M CH_3COOH).
- According the table below prepare acetic acid solutions of different concentrations into nine test tubes. Additions of each solution are in ml.
- Then to each tube, add 1 ml of casein solution and measure the pH of the solution. After 5 minutes find out at what pH coagulation was the most distinctive. This pH corresponds to the isoelectric point of casein.

Test tube number	1	2	3	4	5	6	7	8	9
H_2O	8.4	7.7	8.8	8.5	8.0	7.0	5.0	1.0	7.4
0.01M CH_3COOH	0.6	1.3	–	–	–	–	–	–	–
0.1M CH_3COOH	–	–	0.2	0.5	1.0	2.0	4.0	8.0	–
1M CH_3COOH	–	–	–	–	–	–	–	–	1.6

PROTOCOL

- Calculation of dilution/sample weight for all sample solution preparation.
- Measured values of pH and empirically determined value of the isoelectric point.

Measurement of body surface temperature

THEORY

Clinically important manifestation of life, among the others, is body temperature. Center for regulation of body temperature is located in the hypothalamus and it is set to a temperature of 36.5°C. Temperature measurement can be made only indirectly, i.e. by comparing certain phenomena dependent on temperature and it is feasible to contact or noncontact method.

Into **contact thermometry** we can include devices based on the variation of resistance of a metallic conductor in dependence on the temperature. For low temperature difference electrical resistance increases approximately linearly according to the relationship: $= R_0 \, (1 + \alpha \cdot \Delta t)$,

where R_0 is the resistance at t_o temperature, α is the temperature coefficient of el. resistance, which changes accordingly to the value t_o and it is characteristic for a given substance, and Δt is the temperature difference: $\Delta t = t - t_0$.

Another type of body surface temperature measurement is **non-contact thermometry** using an infrared thermometer. This type of detection is based on the detection of infrared radiation that the human body emits in a wavelength range from 5 to 15 microns.

TASK

- Compare the infrared thermometer and resistance thermometer in terms of speed and precision by measuring the surface temperature of the body.

EQUIPMENT AND CHEMICALS

- Infrared thermometer; resistance thermometer.

PROCEDURE

- Perform practical measurement on one of the participants in the exercise, at the following locations: volar (inner) side of the forearm, dorsal (outer) side of the forearm, palm, back of the hand, face and ear – always on the right and left side of the body; forehead, chin.
- Switch the contact resistance thermometer on by pressing the on/off and attach the measuring tip to the measured body part. Write down measured value of resistance and find matching temperature in conversion table. Estimate the temperature **to one decimal place**. With infrared thermometers we measure from a distance of 0.5 cm. Record the measurement results in the table.

PROTOCOL

- Summary table of all the measured values.

Data / Appl. Sheet	Platinum resistance temperature sensors Pt100 (Pt1000) Relation Temperature vs. Resistance According to IEC751/ITS 1990	CE
Document No.: P-TSIEC751/CE 990907	Application: General Purpose	

ºC ITS-90	0	1	2	3	4	5	6	7	8	9	10
-200	18.52										
-190	22.83	22.40	21.97	21.54	21.11	20.68	20.25	19.82	19.38	18.95	18.52
-180	27.10	26.67	26.24	25.82	25.39	24.97	24.54	24.11	23.68	23.25	22.83
-170	31.34	30.91	30.49	30.07	29.64	29.22	28.80	28.37	27.95	27.52	27.10
-160	35.54	35.12	34.70	34.28	33.86	33.44	33.02	32.60	32.18	31.76	31.34
-150	39.72	39.31	38.89	38.47	38.05	37.64	37.22	36.80	36.38	35.96	35.54
-140	43.88	43.46	43.05	42.63	42.22	41.80	41.39	40.97	40.56	40.14	39.72
-130	48.00	47.59	47.18	46.77	46.36	45.94	45.53	45.12	44.70	44.29	43.88
-120	52.11	51.70	51.29	50.88	50.47	50.06	49.65	49.24	48.83	48.42	48.00
-110	56.19	55.79	55.38	54.97	54.56	54.15	53.75	53.34	52.93	52.52	52.11
-100	60.26	59.85	59.44	59.04	58.63	58.23	57.82	57.41	57.01	56.60	56.19
-90	64.30	63.90	63.49	63.09	62.68	62.28	61.88	61.47	61.07	60.66	60.26
-80	68.33	67.92	67.52	67.12	66.72	66.31	65.91	65.51	65.11	64.70	64.30
-70	72.33	71.93	71.53	71.13	70.73	70.33	69.93	69.53	69.13	68.73	68.33
-60	76.33	75.93	75.53	75.13	74.73	74.33	73.93	73.53	73.13	72.73	72.33
-50	80.31	79.91	79.51	79.11	78.72	78.32	77.92	77.52	77.12	76.73	76.33
-40	84.27	83.87	83.48	83.08	82.69	82.29	81.89	81.50	81.10	80.70	80.31
-30	88.22	87.83	87.43	87.04	86.64	86.25	85.85	85.46	85.06	84.67	84.27
-20	92.16	91.77	91.37	90.98	90.59	90.19	89.80	89.40	89.01	88.62	88.22
-10	96.09	95.69	95.30	94.91	94.52	94.12	93.73	93.34	92.95	92.55	92.16
0	100.00	99.61	99.22	98.83	98.44	98.04	97.65	97.26	96.87	96.48	96.09
0	100.00	100.39	100.78	101.17	101.56	101.95	102.34	102.73	103.12	103.51	103.90
10	103.90	104.29	104.68	105.07	105.46	105.85	106.24	106.63	107.02	107.40	107.79
20	107.79	108.18	108.57	108.96	109.35	109.73	110.12	110.51	110.90	111.29	111.67
30	111.67	112.06	112.45	112.83	113.22	113.61	114.00	114.38	114.77	115.15	115.54
40	115.54	115.93	116.31	116.70	117.08	117.47	117.86	118.24	118.63	119.01	119.40
50	119.40	119.78	120.17	120.55	120.94	121.32	121.71	122.09	122.47	122.86	123.24
60	123.24	123.63	124.01	124.39	124.78	125.16	125.54	125.93	126.31	126.69	127.08
70	127.08	127.46	127.84	128.22	128.61	128.99	129.37	129.75	130.13	130.52	130.90
80	130.90	131.28	131.66	132.04	132.42	132.80	133.18	133.57	133.95	134.33	134.71
90	134.71	135.09	135.47	135.85	136.23	136.61	136.99	137.37	137.75	138.13	138.51
100	138.51	138.88	139.26	139.64	140.02	140.40	140.78	141.16	141.54	141.91	142.29
110	142.29	142.67	143.05	143.43	143.80	144.18	144.56	144.94	145.31	145.69	146.07
120	146.07	146.44	146.82	147.20	147.57	147.95	148.33	148.70	149.08	149.46	149.83
130	149.83	150.21	150.58	150.96	151.33	151.71	152.08	152.46	152.83	153.21	153.58
140	153.58	153.96	154.33	154.71	155.08	155.46	155.83	156.20	156.58	156.95	157.33
150	157.33	157.70	158.07	158.45	158.82	159.19	159.56	159.94	160.31	160.68	161.05
160	161.05	161.43	161.80	162.17	162.54	162.91	163.29	163.66	164.03	164.40	164.77
170	164.77	165.14	165.51	165.89	166.26	166.63	167.00	167.37	167.74	168.11	168.48
180	168.48	168.85	169.22	169.59	169.96	170.33	170.70	171.07	171.43	171.80	172.17
190	172.17	172.54	172.91	173.28	173.65	174.02	174.38	174.75	175.12	175.49	175.86

Pt1000 = Pt100 x 10

Determination of rate constant of solid substance dissolution

THEORY

The prepared sample of magnesium carbonate is a model of a sparingly soluble substance in water. Magnesium carbonate is chemically tri hydrate with chemical formula $MgCO_3 \cdot 3H_2O$. Since it is a substance poorly soluble in water, but the strong electrolyte, it is possible to observe substance dissolution by measuring the electric conductivity G of the resulting aqueous solution of magnesium carbonate in dependence on time.

Dissolution rate constant k of solid substance can be defined in equation:

$$\ln \frac{c_s}{c_s - c_t} = k \cdot t \, ,$$

where c_s is concentration of saturated solution; c_t is solution concentration in time t; t is dissolution time in minutes.

Because the electrical conductivity of strong, slightly soluble electrolytes is proportional to the concentration, the equation can be rewritten into relationship:

$$\ln \frac{G_s}{G_s - G_t} = k \cdot t \, ,$$

where G_s is the conductivity of a saturated solution; G_t is the conductivity of the solution at time t; t is the dissolution time in minutes.

Graphical expression of dependence:

$$\ln \frac{G_s}{G_s - G_t} = f(t)$$

is a line – the value of its slope indicates rate constant k.

Value of G_s for saturated sodium carbonate solution is **165 µS**.

TASK

- Construct a graph of the dependence of $\ln \frac{G_s}{G_s - G_t}$ on time and determine the value of $MgCO_3$ dissolution rate constant from the slope of linear regression.

EQUIPMENT AND CHEMICALS

- Conductometer; electromagnetic stirrer; beaker; basket; stand; $MgCO_3$ sample; saturated $MgCO_3$ solution.

PROCEDURE

- Weigh 100 mg of $MgCO_3$ powder into the basket and attach the basket into the holder on the stand.
- Pour 350 ml distilled water into the 400ml beaker, put the magnetic stirrer inside and place the beaker on the electromagnetic stirrer. Set rotations per minute at half of maximum, and let it unchanged throughout the whole measurement in order to maintain constant diffusion layer.
- Turn the conductometer on and press the measuring unit button 199.9 μS. Place the probe into a beaker and at first measure the conductivity of purified water itself (G_0 value). Then immerse the basket with $MgCO_3$ under the water level and measure the conductivity at 5, 10, 15, 20, 25 and 30 minutes after that.
- After finishing the work with conductometer probe, wash it with distilled water and dry it with celullose wadding.

PROTOCOL

- Table with columns: time *t*, solution conductivity value G_t, $\ln \frac{G_s}{G_s - G_t}$ value.
- A graph of the dependence of $\ln \frac{G_s}{G_s - G_t}$ on time and the determination of the value of $MgCO_3$ dissolution rate constant including units as a slope of linear regression line.