Studies on Growth Patterns of Amino Acids Admixed with Glucose in Gel and Blood Media: Reduction in Blood Glucose by Amino Acids

SHOBHNA DORTHY FREDRICK[A, B], NAMITA RANI AGRAWAL [A*] AND ISHWAR DAS [B]

[a] Chemistry Department, St. Andrew's College, Gorakhpur-273001 (INDIA)
[b] Chemistry Department, D.D.U Gorakhpur University, Gorakhpur-273009 (INDIA)
Corresponding author: dr.namitachem@gmail.com

Abstract

Non – equilibrium growth morphologies of glucose, L-alanine, L-histidine and glucose containing L- alanine and L- histidine were developed in aqueous and blood medium. The medium conditions for amino acid- glucose system showed remarkable differences in their morphologies. L- alanine crystallized in the form of fractal and L-histidine as dendrite in the aqueous medium whereas L- alanine and L-histidine crystallized in the form of mosaic and tree- type structures respectively in the blood medium . Fractal dimension (D) of the growth patterns of L- alanine and its reaction product with glucose in aqueous medium was calculated by box-counting method and found to be 1.72 $\pm$ 0.01 and 1.47 $\pm$ 0.01 respectively. Interaction between amino acid and glucose was also evident by UV- Visible spectral studies, which showed red and blue shifts for L-alanine-glucose and L-histidine- glucose systems respectively. Melting point and electrical conductivity were also decreased with increase in amino acid concentration. Powder x- ray diffraction studies revealed the formation of nanosized reaction products with average crystallite size in the range 54-66 nm. Glucose level in the blood sample in presence of amino acids was measured and found to decrease with amino acid concentration for both the systems.

***Key words**: Growth morphologies; fractal- dimension; amino acids, glucose.*

Introduction

There is considerable interest in the study of non - equilibrium growth patterns in chemical and biological systems [1-13]. Pattern formation occurs in a variety of contexts with implications in different areas of science. Patterns of different shapes may be observed depending on control parameters. It is observed that the morphology of the crystal strongly depends on the distance of the formation conditions from thermodynamic equilibrium [14]. Different kinds of geometrical growth present in nature are far from equilibrium and have attracted attention for a longer time.

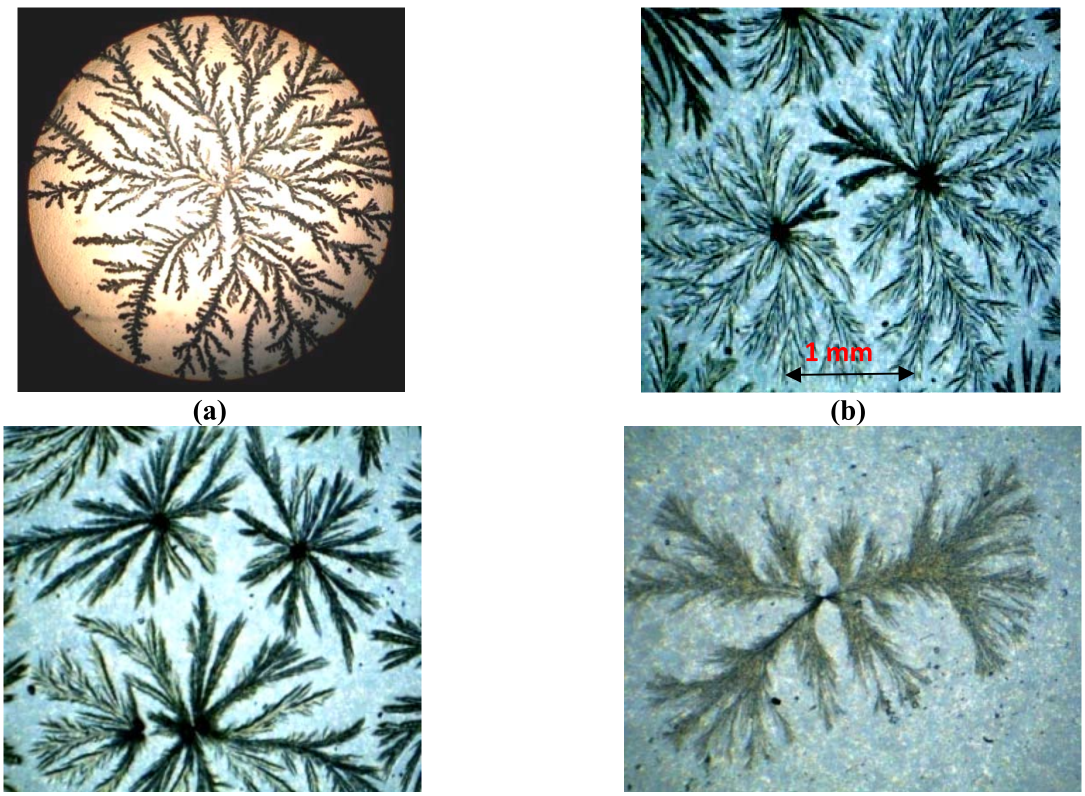
Amino acids were traditionally classified as nutritionally essential or non-essential for animals and humans. [15]. Amino acids under appropriate conditions enhance insulin secretion from primary islet cells and β- cell lines[16,17]. The study of intermolecular interaction plays a key role in the development of molecular science. Interaction of carbohydrates with proteins plays a key role in a wide range of biochemical processes [18]. Studies on the carbohydrate- protein interaction are very important in the field of immunology, biosynthesis, pharmacology and medicine [19]. Protein- carbohydrate interaction is also evident by the host-pathogen recognition [20], signal transduction [21] and cell- adhesion [22].The thermodynamic interaction between protein and carbohydrate is also reported [23-35]. Effect of blood doping on habits and coagulation of cupric chloride dendrites grown in aqueous solutions was studied by Shibata et al [36]. They reported the surface structure of cupric chloride grown from aqueous solutions without and with doping of human blood and observed remarkable differences. The study between amino acid and analogous compounds containing amino group are indispensable basic components in biology and possibly take part in sensing process [37] .The Millard reaction initiated by non- enzymatic glycation of amino group by sugars has been studied for its potential role in aging and the complication in diabetes [38, 39].

In the present communication, non equilibrium growth patterns of aqueous solutions of L-alanine, L-histidine and their solutions admixed with glucose in different proportions will be carried out in aqueous and blood media and characterized by powder X ray diffraction, UV- Visible spectral studies, fractal dimension calculation, melting point, electrical conductivity measurements and estimation of glucose level in the chicken blood samples.

Experimental Work

Materials

D-Glucose (GR, S.Merck) , L-alanine , (S. d fine-chem.), L-histidine (S.d fine-chem.) ,agar- agar (Difco, bacteriological grade) were used as such. Fresh chicken blood was also collected in EDTA voids.



(a) (b) (c) (d)

Fig.1 Two dimensional growth patterns of (a) L-alanine (0.05M) and (b -d) L-alanine containing glucose in different proportions 0.02, 0.04 and 0.05M respectively on micro slides at 25$\pm$ 0.1^0 C. All the solutions were admixed with 0.2% agar- agar.

Procedure

Growth morphologies of glucose, L-alanine, L-histidine and glucose containing L- alanine and L-histidine were developed at different experimental conditions. For this purpose 1 mL of aqueous solution of glucose (0.05M containing 0.2 % agar- agar) and an amino acid (L-alanine or L-histidine) was admixed with glucose, spreaded over micro- slides and kept in an incubator at 25^o C to crystallize. In one experiment, concentrations of L-alanine and L- histidine were kept constant and the concentration of glucose was varied in the range 0.01 to 0.05 M. In another experiment, the concentration of glucose was kept constant and concentration of L-alanine was changed. The slides were dried and observed under 'OLYMPUS' Polarized Light Microscope and micro photographs were taken. Results are shown in Figs. 1-3. Fractal dimension (D) of the growth patterns of L-alanine and its reaction product with glucose in 1:1 molar ratio was calculated. The fractal dimension of pictures was determined by box-counting method as employed by Das et al [12] (Fig. 4). The log-log plots of N (r), the total number of boxes inside a circle of radius (r) versus r was made. The fractal dimension was given by the slope of the best fitted straight line using least square method of analysis.

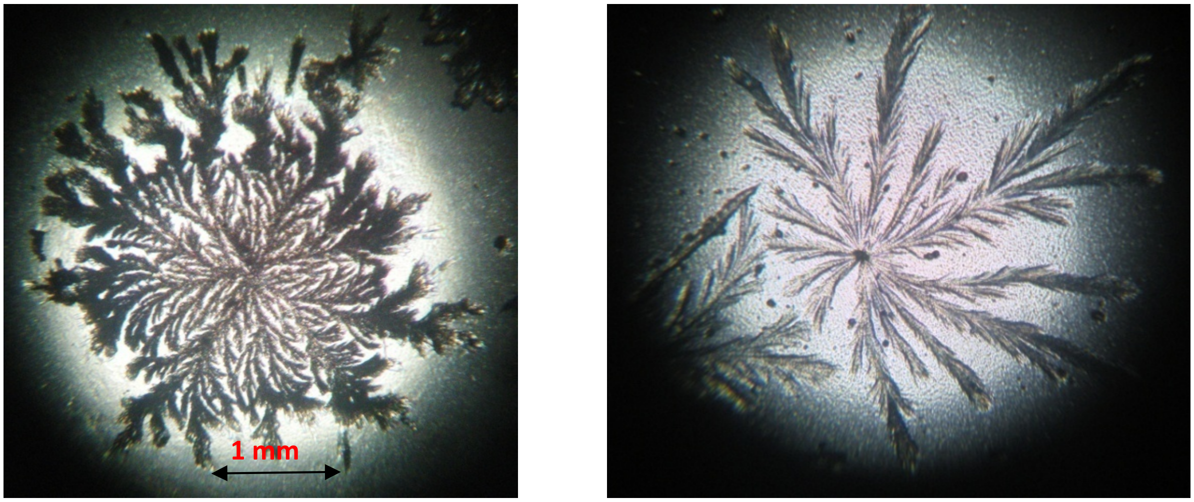
To study the interaction of L-alanine and L-histidine with glucose, UV- Visible spectra were taken using Hitachi Spectrophotometer. Results are shown in the Fig. 5.

X- ray diffraction patterns of glucose, L-alanine, L-histidine, L-alanine- glucose product and L-histidine- glucose reaction products were taken using CuK_{α} radiation. Results are recorded in the Table 1.

Table: 1 Powder X-ray diffraction data for Glucose, L-alanine, l- histidine and their reaction products.

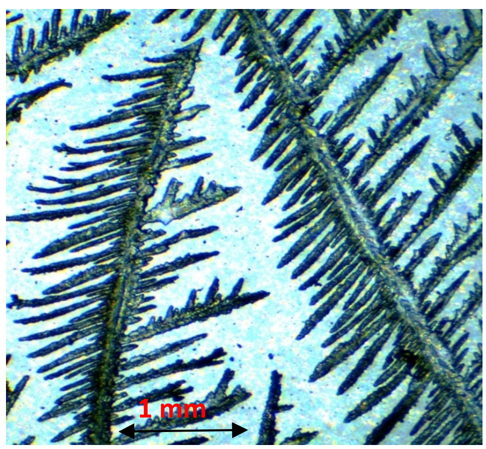
Glucose		L-alanine		Reaction Product		Average crystallite size(nm)	L-histidine		Reaction Product		Average crystallite size (nm)
2Θ	I/I_0	2Θ	I/I_0	2 Θ	I/I_0		2 Θ	I/I_0	2 Θ	I/I_0	
19.80	**100**	**20.607**	**100**	**20.4**	**100**		**20.688**	**100**	**26.116**	**100**	
20.25	**89**	**19.526**	**91.9**	**32.694**	**74.7**		**23.664**	**57.7**	**33.032**	**38.4**	
22.87	**61**	**19.989**	**73.9**	**34.47**	**45.2**		**18.61**	**26.9**	**32.538**	**33.6**	
28.38	**40**	**28.065**	**50.7**	**29.17**	**44**		**30.311**	**20.7**	**23.466**	**29.2**	
12.78	**40**	**22.571**	**50.2**	**30.271**	**43.5**	**54.3**	**30.684**	**15.4**	**22.81**	**26.8**	**66.5**
9.19	**23**	**32.905**	**43.6**	**20.763**	**38.4**		**37.304**	**15.2**	**24.868**	**25.7**	
41.59	**20**	**33.905**	**39.9**	**36.813**	**22.5**		**41.358**	**14.5**	**36.948**	**25.2**	
25.51	**20**	**30.484**	**32.4**	**42.704**	**19.1**		**41.451**	**13.4**	**28.09**	**17.3**	
18.43	**19**	**41.14**	**32.2**	**43.237**	**14.4**		**39.488**	**13.1**	**30.258**	**14.2**	
31.33	**17**	**36.65**	**31.6**	**36.151**	**9.7**		**33.231**	**12.5**	**43.811**	**12.7**	

Melting points of amino acids (L- alanine and L-histidine) and amino acids admixed with glucose in different proportions were also measured. Results are shown in the Fig. 6.

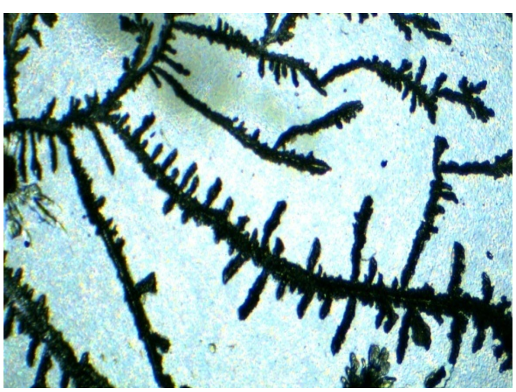


(a) **(b)**

Fig.2 Two dimensional growth patterns of glucose (0.05M) admixed with 0.01 and 0.02 M L-alanine respectively at 25$\pm$ 0.1^{0}C. All the solutions contained 0.2% agar-agar.



(a)



(b) **(c)**

Fig.3 Two dimensional growth patterns of (a) L-histidine (0.05M) and (b-c) L-histidine (0.05M) admixed with glucose in 0.03M and 0.04M respectively at 25$\pm$ 0.1^0 C. All the solutions contained 0.2% Agar-agar**.**

Electrical conductivity of aqueous solutions of amino acid on addition of glucose at different volume was measured for observing the interactions between them. The results obtained are shown in the Fig. 7. Glucose level in the blood sample was measured with Accu-check Glucometer (India). Results are shown in Fig. 8. Surface microstructures of chicken blood, L-alanine, L-histidine and amino acid- glucose reaction products in chicken blood were developed and observed under microscope. Results are shown in the Fig. 9.

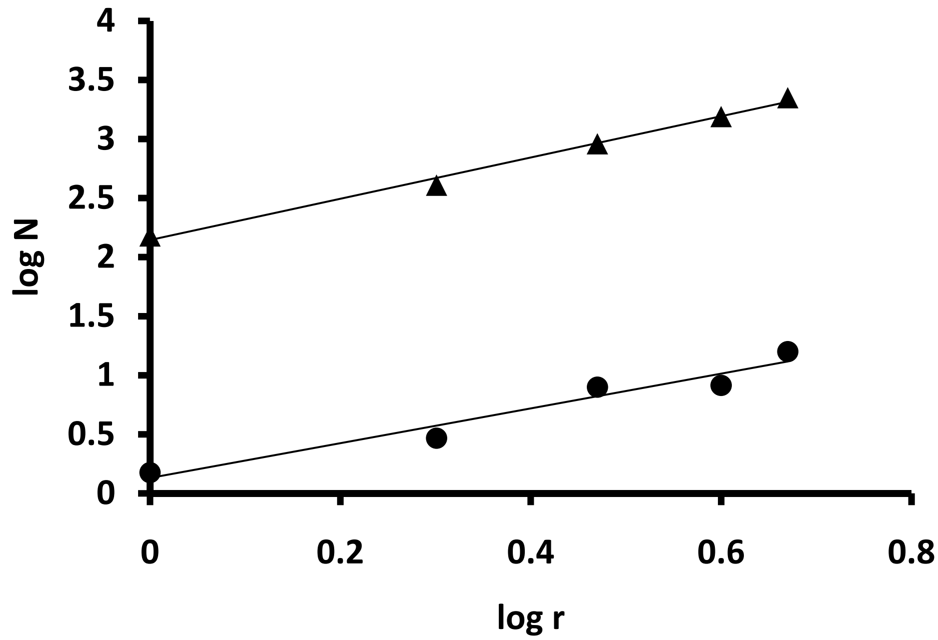
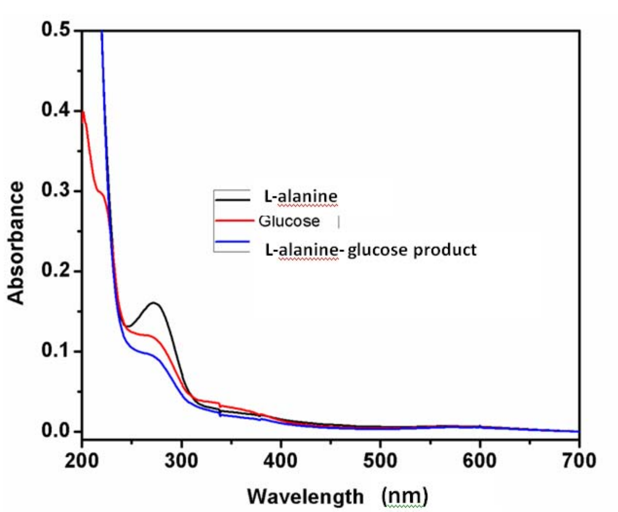
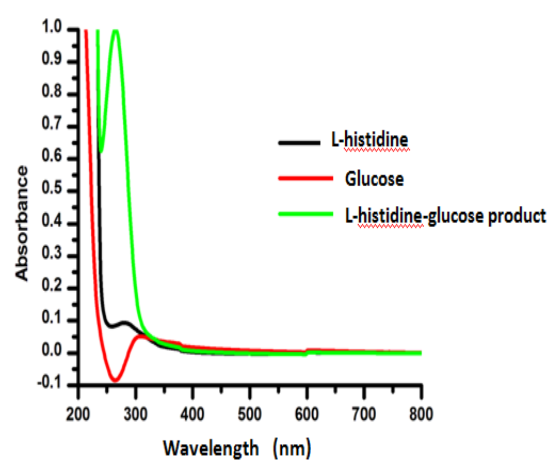


Fig.4 Plots between log N and log R for L-alanine (▲) and L-alanine containing glucose (●)
Conditions: (L- alanine 0.05M), (glucose concentration 0.04M) and 0.2 % agar-agar.

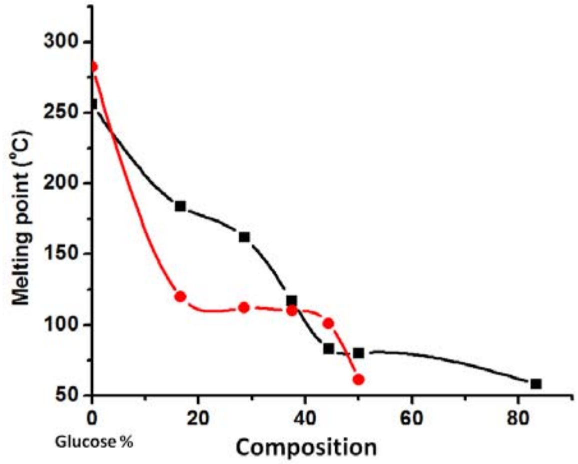


(a)



(b)

Fig.5 Absorbance spectra of (a) L-alanine, glucose and the reaction product (1:1 molar ratio) and (b) L-histidine glucose and the reaction product (1:1 molar ratio).



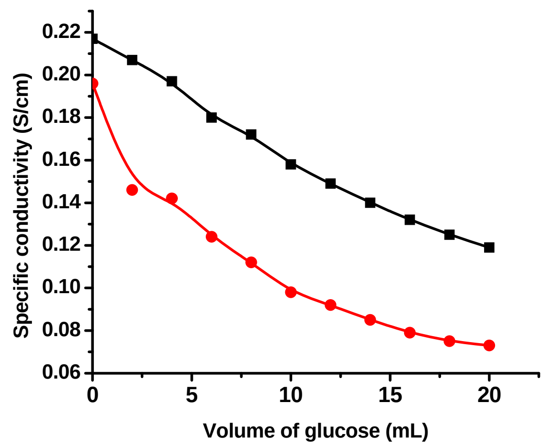


Fig.6 Plots showing melting point of L- alanine (■) and L- histidine (●) in presence of glucose in different proportion. Conditions: [Amino acid]= 0.05M, [Glucose] = 0.05M.	**Fig.7** Dependence of specific conductivity of amino acid on volume of glucose. Conditions: [Amino acid] = 0.05 M, [Glucose] =0.05M.

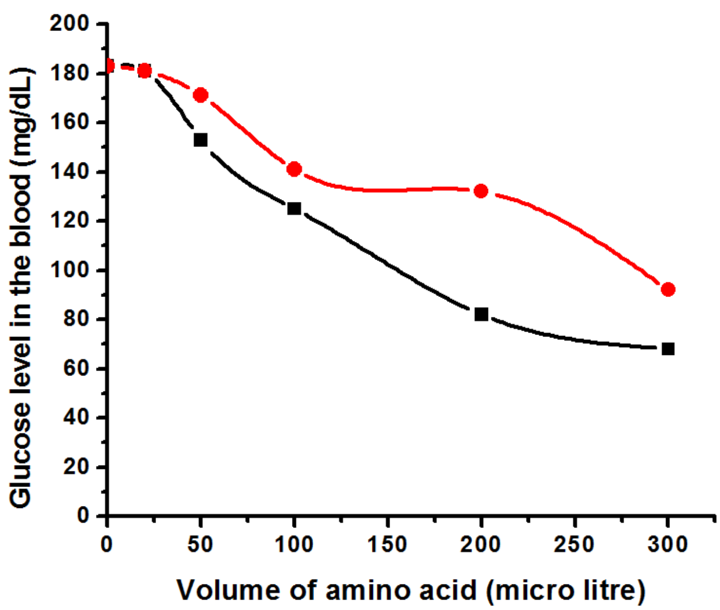


Fig.8 Dependence of glucose level in the blood sample on volume of amino acid. (■) L-alanine and (●) l -histidine .Condition: [L- alanine] =0.1M, [L-histidine]= 0.1M and [Glucose] = 0.1M.

Results and discussion

Amino acids (L-alanine, L-histidine), glucose and amino acid containing glucose in different proportions crystallized two dimensionally from thin film of aqueous solutions containing 0.2% agar-agar .L-alanine crystallized as fractallike growth patterns with dimension 1.72 $\pm$ 0.01 (Fig. 1a). The fractal dimension was found to be independent of L-alanine concentration while in case of L-histidine, dendritic structure was observed. It was observed that on addition of glucose, fractal dimension morphology was changed from 1.72 $\pm$ 0.01 to 1.47 $\pm$ 0.01 as shown in Fig. 1 and 4 respectively. L-alanine admixed with glucose in equimolar ratio showed spherulite type structure (Fig. 1e).

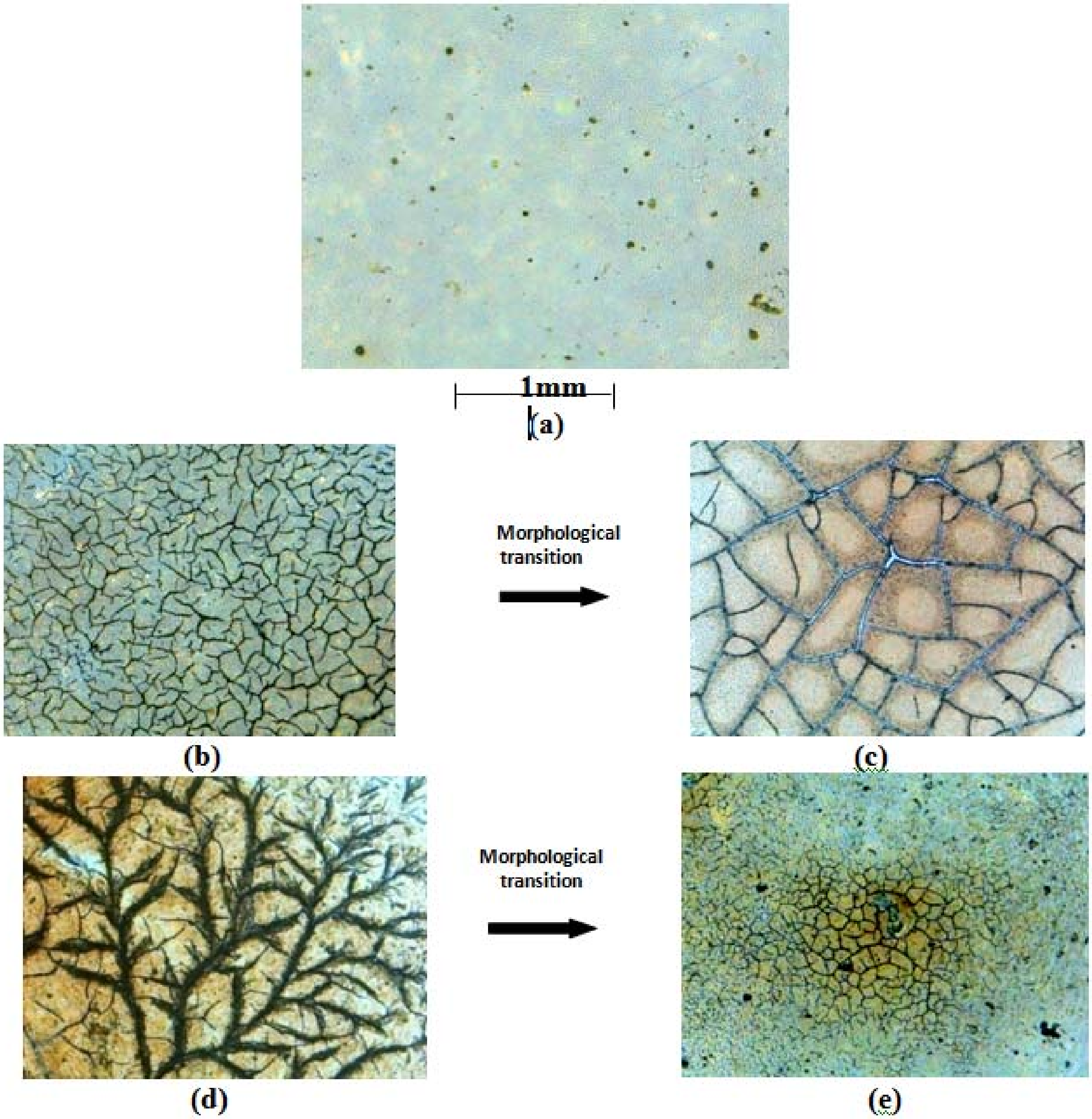
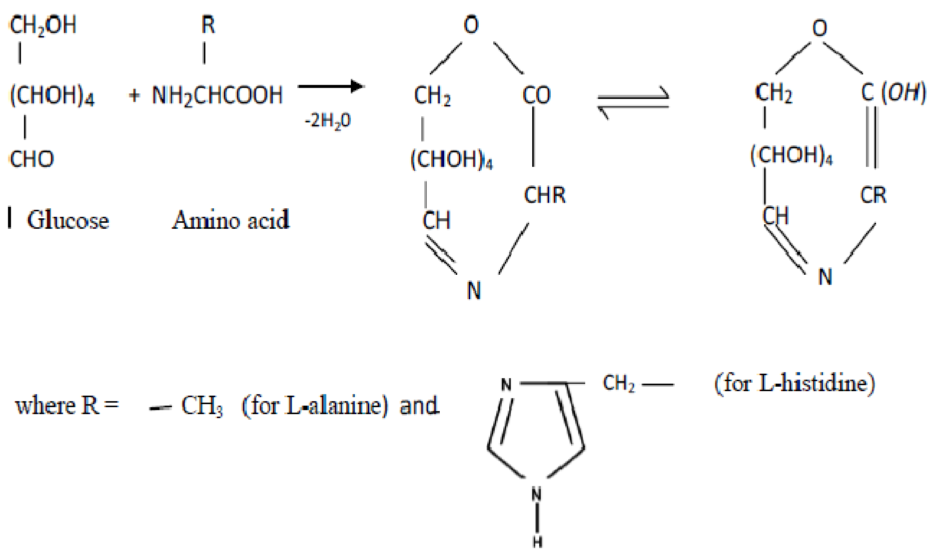


Fig.9 Microphotographs of (a) Pure chicken blood , (b-e) crystallization patterns of L- alanine, L-alanine containing glucose, L-histidine, L-histidine containing glucose in 1:1 molar ratio respectively in chicken blood at 25$\pm$ 0.1^{0}C. Conditions: [L-alanine] =0.1M, [L-histidine] =0.1M and [Glucose] =0.1M.

Morphological changes were also observed with the variation of L- alanine concentration and keeping the concentration of glucose fixed (0.05 M) (Fig.2). It was observed that at low L-alanine concentration (0.01M), the morphology was dense fractal. On increasing the concentration of L-alanine (0.02M), the fractal morphology was open.

Two dimensional growth patterns of L-histidine and L-histidine containing glucose in different proportions are shown in Fig. 3. L-histidine crystallized in the form of dendrite with side branches having acute angle. The morphology of L-histidine resembles with the leaves of *Bryophytes* family (Like Fern). On addition of glucose in L- histidine, the growth morphology changed to dendrite with side branches at right angle were observed. Further the melting point of amino acids decreased with increase in glucose concentration as shown in Fig. 5.

Electrical conductivity of amino acid was measured at different volume of glucose as shown in Fig.6 and found to decrease with increase in glucose concentration. Usually aqueous solution of amino acids is ionized and can act as acid or base due to the formation of Zwitter ion (H_3N^+--CH(R) –COO^-). The knowledge of acid base properties of amino acids is extremely important in understanding many properties of the protein. This decrease in the electrical conductivity of an amino acid (L- alanine and L-histidine) on increasing the glucose concentration shows the neutralization process. The decrease in electrical conductivity on addition of glucose was explained on the basis of Scheme I.



Scheme I

UV- Visible spectra of glucose, amino acid and the reaction products when glucose was admixed with amino acids in 1:1 molar ratio are shown in Fig. 7. It was found that there is a shift in the λ_{max} value of L-alanine- glucose system towards the longer wave-length which shows Red- shift while L-histidine – glucose system showed a shift towards the shorter wave-length side (Blue –shift) .

Powder x- ray diffraction data for glucose, L- alanine, and amino acid glucose reaction products in 1:1 molar ratio are recorded in Table 1. Results indicate that the reaction products have lines different from their individual reactants. The average crystallite size (t) of amino acid- glucose reaction products were calculated using Debye- Scherrer formula, $t = \frac{0.9\lambda \times 360^0}{\beta.Cos\theta.2\pi}$

where λ= wave-length of x-ray radiation .The value of λ for CuK_α is $_{1.5418\ A,}$ β =Full^o width half maximum, Θ = Bragg's angle. The average crystallite size was found to be 54 and 66 nm for L- alanine- glucose and L- histidine reaction products respectively. Glucose level in the blood sample was measured on periodic addition of amino acid (L-alanine and L- histidine) in the blood. Results are shown in Fig. 8. L- alanine is found to be more effective in reducing glucose level in blood as compared to L-histidine. It is due to the shorter alkyl chain length of L-alanine. L-histidine is found to be less effective due to the presence of imidazole ring. The morphological studies, change in fractal dimension, decrease in melting point, electrical conductivity, UV- Visible spectral studies and powder X- ray diffraction studies elucidate the interaction between L-alanine and L-histidine with D- glucose in aqueous media.

Blood containing amino acids 0.1M (L-alanine , L-histidine) and (0.1M) glucose were also crystallized two dimensionally in chicken blood medium. Micro slide of pure blood was also prepared for comparison. Microphotographs are shown in Fig. 9. A remarkable difference in morphology was observed on addition of amino acids (L-alanine and L-histidine) in the blood. For pure blood no morphologies was seen while in case of L- alanine in blood, thin branched structure was observed. Blood containing L- alanine- glucose reaction product in 1:1 molar ratio, dark orange colored thick rectangular disc (mosaic) like structure was observed. (Fig 1c)

Microphotographs of blood containing L- histidine and blood containing L-histidine- glucose reaction product showed different morphological transition as shown in Fig. 9 d. It resembles thick tree- like structures .On addition of L-histidine containing glucose in 1:1 molar ratio in the blood sample, tree- like to mosaic structures was observed. Thus from the above discussions, we found that individual amino acids showed different morphologies. Totally different morphological transitions were observed in all the cases. It confirms the interaction of amino acids and glucose (carbohydrate source) in the blood sample also.

Conclusion

Surface microstructures of glucose, L-alanine, L-histidine and glucose containing L-alanine and L-histidine were developed in aqueous and blood media. L-alanine crystallized in the form of fractal with fractal dimension 1.72 $\pm$ 0.01 and was independent of initial L- alanine concentration. The fractal dimension was reduced to 1.47 $\pm$ 0.01 for L-alanine- glucose reaction product. L-histidine crystallized as dendrite in the aqueous medium and showed morphological change on addition of glucose. Surface microstructures of blood containing L- alanine and L- alanine- glucose reaction products showed morphological transition from thin branched $\longrightarrow$ mosaic whereas for L-histidine and its reaction product with glucose transition in morphologies from tree- like $\longrightarrow$ mosaic was observed. Such a two-dimensional morphological transitions and decrease in glucose level are due to interaction between amino acid and glucose. It was also established by shift in U.V-Visible spectra, change in PXRD patterns, decrease in melting point and specific conductivity.

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