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Modeling and Simulation of the Autocatalytic Kinetics of Haemoglobin SS Polymerization: Onset of Polymerization

Edith Egbimhanhu Alagbe[1], Alfred Akpoveta Susu[2], Adedoyin Owolabi Dosunmu[3]

[1]Department of Chemical and Polymer Engineering, Lagos State University, Lagos, Nigeria
[2]Department of Chemical Engineering, University of Lagos, Lagos, Nigeria
[3]Department of Hematology, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria
Email: alfredasusu222@hotmail.com

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Abstract

We report a fresh and simpler approach to the modelling of the kinetics of the polymerization of Hb SS in sickle cell patients that couples the kinetics and the hydrodynamics of blood flow in mechanistic understanding of the process. The well-known two-step autocatalytic reaction scheme was used for the polymerization reaction with the assumption of simpler first-order reaction scheme for each stage. In addition, the forces acting on a particle in motion were also introduced to account for compelling settling of the red cells that lead to vessel occlusion (vaso-occlusion). A first attempt on the prediction of vessel blockage was made using this novel model. The time for the onset of the polymerization reaction was derived from hydrodynamic considerations and kinetics while the kinetic rate constants were obtained from the autocatalytic nature of the reaction. Experimental data for model validation were obtained from recruited SS patients and *in vitro* data of Hofrichter. Over 100 volunteers were recruited for participation in this work but less than 40% met the inclusion criteria. Participants were of age range 13 - 43 (with a mean of 26 ± 8 years) for SCD patients and 18 - 43 (with a mean of 28 ± 7 years) for control participants. Blood indices and Transcranial Doppler (TCD) test parameters of all participants were the principal parameters used for model validation. Constant k_2/k_1 ratios was obtained for individual *in vivo*/*in vitro* system. This ratio is unique for any individual, independent on protein sequence and also suggests the degree of expression of the symptoms of Sickle Cell Disease (SCD) with higher values reflecting greater propensity to pain crisis. Delay time, t_D, was found to have an inverse relationship with the kinetic constant for the residual reaction, k_1. Therefore, long delay times calculated, offer insight on why SCD patients are not in perpetual crises because enough time is provided the cells to escape microcirculation while keeping the residual reaction at the minimum. Sensitivity analysis was carried out to obviate the limitations encountered in the course of the work. Results showed the onset of occlusion to be most sensitive to the diameter of the blood vessel.

Keywords

Vaso-Occlusion, Hb SS Polymerization Kinetics, Residual and Secondary Reactions, Hydrodynamics of Blood Flow, Onset of Hb SS Polymerization

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1. Introduction

The sickle haemoglobin was identified as the first protein to cause disease (Pauling L, Itano HA, Singer SJ, Wells IC, 1949) [1]. The reason for this defect is the replacement of a negative glutamic acid (Glu) molecule with a hydrophobic valine (Val) molecule on the two beta subunit of the haemoglobin. While the former is able to make hydrogen bond and dominate the interactions in which they participate, the latter avoids contact with water (in the blood, in this case) and pack against each other (Petsko GA, Ringe D, 2004) [2].

Sickle cell disease (also known as Sickle Cell Anemia) is characterized by a molecular hemoglobin defect which causes the polymerization of deoxygenated hemoglobins and results in reduced erythrocyte flexibility, deformation and numerous rheological effects. Sickle cell anemia produces an abnormal type of hemoglobin called hemoglobin S (Hb S), which has less oxygen-carrying capacity. SCD is a blood disorder characterized by abnormal hemoglobin in the red blood cell. The red blood cell, RBC, has a diameter of about 7 μm and due to their flexibility are able to pass through capillaries of ≤3 μm diameter. In the case of SCD, the red blood cells have lowered deformability due to repeated sickling (polymerization) and unsickling (melting) processes and eventually become irreversibly sickled and have to be removed from circulation. This happens frequently and accounts for the anemia characteristic of the disease. Occlusion of vessels is a common phenomenon as these sickled cells co-operate to form aggregates that later block the vessels.

Sickle haemoglobin polymerization appears to be precise with respect to location and directionality of growth. Usually, thermal fluctuations provide the energy required for molecules of sickle hemoglobin that are competent to form long fibers, to assemble into small aggregates. This leads to the spontaneous formation of transient species of various sizes. At some critical size (called the nucleus), each additional molecule lowers the energy of the aggregate. The formation of nuclei is thus characterized by molecular fluctuations, and the time to form a nucleus is inherently stochastic.

All of the kinetic findings can be explained by a novel nucleation mechanism which postulates that the first polymer in a given solution volume forms via a simple homogeneous nucleation mechanism. This polymer grows by the addition of monomers to the end. The lateral surface of the growing oligomers may also serve as a template for the nucleation of new polymers (Heterogeneous nucleation).

Therapeutic options available from kinetic studies and currently in use in some cases, are: In particular, the discovery of the enormous concentration dependence of the rate of polymerization suggested decreasing the intracellular haemoglobin concentration as a new approach to the treatment of sickle cell disease; Decreasing the intracellular haemoglobin concentration (by crowding) with non-polymerizing agents like fetal haemoglobin Hb F or normal Hb AA (as currently used in Stroke Prevention Trial in Sickle Cell Anemia, STOP). A decrease in the intracellular haemoglobin concentration of only 10% - 15% has been predicted to have some therapeutic effect as evidenced in blood exchange therapies; Increasing the oxygen affinity so that the concentration of molecules capable of polymerization will be grossly decreased; Drugs that will prevent cellular dehydration.

2. Methodology

A sample size of 30 was obtained using the formula (FAO, 1990; Magnanni, Robert, 1997; UNICEF, 1995): [3]-[5]

$$n = \frac{t^2 p(1-p)}{m^2} \tag{2.1}$$

where:

n = required sample size.

t = confidence level at 95% (Standard value of 1.96).

p = Estimated prevalence of variable under investigation (SCD) in the project area.

m = Margin of error at 5% (standard value of 0.05).

2.1. Study Area and Population

Lagos metropolis was used as study area for this project. It was a cross sectional observational study. The study population was sickle cell patients within the age of 13 years and 45 years and Controls with hemoglobin AA and AS (age and sex matched) attending the paediatric and adult hematology clinics of the Lagos State Univer-

sity Teaching Hospital. Written consent were obtained from patients and their parents (for ages that are less than 18 years) after due explanation.

2.2. Research Procedures

Participants were invited to the clinic for recording of history, physical examination, laboratory and TCD tests. Thereafter, study questionnaires were filled and signed by all participants. The exclusion criteria was used to further filter out eligible participants for the Research study. Inclusion criteria were that Hb SS patients in the study must be in steady state, that is, patients that are not in any form of crisis. The controls were obviously normal Hb AA and Hb AS individuals with age and sex matching those of the sickle cell patients while Exclusion criteria were patients and controls with any comorbid chronic infection and obvious vascular abnormality.

Blood indices and Transcranial Doppler on the Anterior Carotid Artery, ACA; Internal Carotid Artery, ICA and Middle Cerebral Artery, MCA were carried out in all 21 SCD and 10 Control participants.

3. Model Development

3.1. Autocatalysis of the Hb SS Polymerization

If A = hemoglobin, Hb; B = oxygen and C = Hb. O_2 polymer, we present the autoctatlytic scheme of (Boudart M., 1968) [6] and (Susu AA, 1997; Susu AA, Kunugi T, 1980) [7] [8] to obtain:

$$A + B \rightarrow C \tag{3.1}$$

$$A + B \xrightarrow{C} C \tag{3.2}$$

In terms of reaction rate, the equations are represented as:

$$r_1 = -\frac{\mathrm{d}C_A}{\mathrm{d}t} = k_1 C_A \tag{3.3}$$

$$r_2 = -\frac{\mathrm{d}C_A}{\mathrm{d}t} = k_2 C_A C_C \tag{3.4}$$

The overall reaction rate, r_0 becomes:

$$r_0 = r_1 + r_2 = r_0 = k_1 C_A + k_2 C_A C_C \tag{3.5}$$

In terms of fraction converted, Equation (3.5) becomes,

$$r_0 = -\frac{\mathrm{d}C_{A0}(1-f)}{\mathrm{d}t} = k_1 C_{A0}(1-f) + k_2 C_{A0}(1-f) C_{A0} f \tag{3.6}$$

Rearranging and setting $k = k_2 C_{A0}$ and $\rho = k_1/k$ gives: (3.7)

$$\frac{\mathrm{d}f}{\mathrm{d}t} = k(f+\rho)(1-\mathrm{f}) \tag{3.8}$$

Setting the second derivative of Equation (3.8) to zero gives the point of inflection for the function and the maximum rate occurs at $f = 1/2$

Also, integrating Equation (3.8) with $f = 0$ at $t = 0$, we have;

$$f = \rho \frac{\mathrm{e}\exp\left[(1+\rho)kt\right] - 1}{1 + \rho\exp\left[(1+\rho)kt\right]} \tag{3.9}$$

Taking ln of both sides and precipitating out $t_{1/2}$ at the point of maximum rate, where $f = 0.5$, Equation (3.9) becomes:

$$t_{1/2} = \frac{1}{(1+\rho)k} \ln\left(\frac{1+2\rho}{\rho}\right) \tag{3.10}$$

From Equation (3.10), the rate constants for the reaction can be obtained.

3.2. Model for Settling Velocity of the RBC

The total amount of force exerted on a particle can be summarised as:

Force due to Acceleration, F_a = Gravity Force, g—Buoyancy Force, F_b—Drag Force,

$$F_d \tag{3.11}$$

Therefore, from Equation (3.11), for a particle to settle (attain its terminal velocity), F_b must be equal to F_d. So, for many spheres, it is represented as:

$$F_b = gV_p\left(\rho_p - \rho\right)\left(1-\phi\right) = F_d = \phi S\frac{1}{2}\rho u_s^2 \tag{3.12}$$

where ρ_P = density of particle = MCHC value from clinical analysis, ρ = density of fluid (serum), g = gravitational constant, V_p = volume of particle = Actual red cell volume, $\phi = \frac{NV_p}{V}$ = hct, S = drag co-efficient, u_S = settling velocity. Settling velocity from Equation (3.12) is:

$$u_S = \sqrt{\frac{2\left(\rho_P - \rho\right)\left(1-\phi\right)gv_P}{S\phi\rho}} \tag{3.13}$$

3.3. Prediction of Time of Vaso-Occlusion in the Vessel

For a fluid flowing through a pipe, the Poiseuille equation (Charm SE, Kurland GS, 1974) [9] is used to describe the plasma velocity, u_P, such that:

$$\frac{\mathrm{d}u_P}{\mathrm{d}t} = \frac{\pi}{8}\left(\frac{R^4}{\mu_0}\right)\left(\frac{P_1 - P_2}{L}\right)$$

Separating variables here and integrating between limits yields:

$$\int_{u_s}^{u_p}\mathrm{d}u_p = \frac{\pi R^4\Delta P}{8\mu_0 L}\int_0^{t_{1/2}}\mathrm{d}t \tag{3.14}$$

Therefore,

$$t_{1/2} = \frac{8\mu_0 L u_S}{\pi R^4\Delta P}\left(u_p - u_s\right) \tag{3.15}$$

The time for the onset of pain threshold in sickle cell patients is given by Equation (3.15).

4. Results and Discussion

4.1. *In Vitro* Results

For the *in vivo* data, with the assumption that onset of critical occlusion time corresponds to the time of maximum reaction, the k values (and corresponding slopes) at the points of inflection and occlusion time were computed for all arteries under investigation from Equations. (3.13), (3.44) and (3.15) using MatLab programming. The results obtained are shown in **Table 1**.

For all data presented here, $t_{1/2}$ = time to reach 50% conversion, min; S = slope at the point of inflection (that is, at 50% conversion) = Number of nuclei formed per time per volume, $\mathrm{min}^{-1}\cdot\mathrm{L}^3$; t_D = delay time, min; $K = k_2C_{A0}$, $\mathrm{gL}^{-1}\cdot\mathrm{min}^{-1}$, k_1 = rate constant for the residual (homogeneous) reaction, min^{-1}, k_2 = rate constant for the heterogeneous polymerisation, min^{-1}.

For *in vitro* data, relevant data like the values of slope, time to reach 50% conversion and delay times were extracted from the curves of previous works of (Hofrichter J, Ross PD, Eaton WA, 1974) [10] from calometric and optical birefringence measurements of the time course of sickle cell polymerization. Values of the slope and time to reach 50% conversion were inserted into Equations. (3.13) and (3.15). MATLAB programming was used to obtain the values of k_1 and k_2 at different temperatures. The results obtained are presented in **Table 2** and **Table 3**.

Table 1. Comparing *k* values in both sides of the skull (subscript 1 and 2 = Residual and secondary rate constants respectively).

	LEFT						RIGHT					
	ACA		ICA		MCA		ACA		ICA		MCA	
CODE	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2
101	14.392	39.539	279.901	768.959	22.219	61.042	35.981	98.848	279.901	768.959	44.438	112.083
102	25.052	68.262	365.358	995.527	34.809	94.848	75.155	204.781	292.314	796.495	13.924	37.939
103	23.889	65.991	371.711	1026.83	19.67	54.336	95.555	263.963	371.711	1026.83	17.703	48.903
104	43.615	118.519	282.702	768.213	161.594	439.113	87.23	237.038	339.34	922.119	32.32	87.826
105	36.463	101.007	425.498	1178.67	50.662	140.35	36.463	101.007	425.498	1178.67	33.776	93.562
106	15.353	42.53	946.227	2067.11	28.444	78.792	76.766	212.649	298.491	826.845	14.222	39.396
107	18.821	54.083	366.035	1051.82	19.371	55.664	18.821	54.083	366.035	1051.82	21.793	62.622
108	27.857	78.692	565.001	1596.05	32.256	91.117	27.857	78.692	541.781	1530.46	51.608	145.786
109	17.053	48.583	331.657	944.89	26.326	75.004	21.316	60.728	829.141	2362.23	31.592	90.006
110	94.617	265.035	115.005	322.141	21.91	61.373	23.653	66.255	368.079	1031.03	21.654	60.654
201	21.57	59.42	397.688	1095.56	25.263	69.594	20.454	56.347	318.182	876.535	55.088	151.756
202	118.948	338.882	462.574	1317.87	55.088	156.944	59.474	169.441	462.574	1317.87	21.186	60.359
203	147.273	419.58	400.304	1140.47	23.835	67.905	34.308	97.743	400.304	1140.47	22.486	64.062
204	NA	NA	NA	NA	NA	NA	42.481	119.329	330.41	928.117	80.126	225.072
205	14.421	42.291	336.453	986.666	32.049	93.985	17.299	50.731	280.397	822.28	15.669	45.95
206	16.916	48.608	411.123	1181.39	17.41	50.028	21.144	60.759	328.899	945.111	35.203	101.157
207	26.603	78.939	517.332	1535.11	35.203	104.459	44.339	131.569	517.332	1535.11	196.132	581.994
208	21.331	56.882	331.796	884.788	19.759	52.69	28.441	75.843	331.796	884.788	21.512	57.364
209	27.094	75.26	316.147	878.187	25.097	69.713	20.32	56.447	316.147	878.187	22.107	61.408
210	39.777	118.032	464.202	1377.46	55.268	164.001	39.777	118.032	464.202	1377.46	18.854	55.947
211	18.319	55.177	356.307	1073.21	18.854	56.789	18.319	55.177	356.307	1073.21	NA	NA

Table 2. Predicted occlusion time, delay time and kinetic constants using calorimetric data (Hofrichter J, Ross PD, Eaton WA, 1974) [10].

T, ˚C	Slope @f = 0.5	$T_{1/2}$, min	t_D, min	*K*	k_1	k_2	k_2/k_1
15.9	0.006	925	307.692	0.024	0.024	0.103	4.29
16.3	0.006	835	307.692	0.024	0.024	0.103	4.29
17	0.007	675	181.818	0.028	0.028	0.1202	4.29
17.8	0.006	512.5	142.857	0.024	0.024	0.103	4.29
18.7	0.004	325	117.647	0.016	0.016	0.0687	4.29
19.6	0.009	100	100.000	0.036	0.036	0.1545	4.29
20.3	0.048	85	40.000	0.192	0.192	0.824	4.29

Table 3. Predicted occlusion, delay time and kinetic constants birefringence data (Hofrichter J, Ross PD, Eaton WA, 1974) [10].

T, ˚C	Slope @f = 0.5	$T_{1/2}$, min	t_D, min	K	k_1	k_2	k_2/k_1
18	0.002	250	142.857	0.008	0.008	0.0343	4.29
20	0.016	96.25	62.500	0.064	0.064	0.2747	4.29
22.5	0.019	16.25	11.976	0.076	0.076	0.3262	4.29
25	*0.173*	*5.75*	7.692	0.692	0.692	2.97	4.29
30	3.467	0.85	0.930	13.868	13.868	59.5193	4.29

4.2. Discussion

The most striking result obtained is the constant *k* ratios for both *in vivo* and *in vitro* model validation. The *k* ratios show by how much the secondary nucleation is faster than the primary nucleation. For *in vitro* polymerization, the *k* ratios gave a constant value of 4.29 irrespective of the monitoring technique used. However, having constant *k* ratios from *in vitro* experiments suggest that the mechanisms of nucleation deduced sheds light on the *in vivo* polymerization of HbS in SCD patients. The k ratios for each SCD patient in all the arteries investigated was observed to be constant also. The severity of the symptoms and presentation of sickle cell disease can therefore be predicted with the *k* ratios. The *k* ratios, therefore, are indicative of patients' susceptibility to more frequent occlusive events/periodic pain cycles but seems to be heightened by higher hematocrits as postulated by Stuart (Stuart MJ, Nagel RL., Oct, 2004) [11].

No single parameter from the blood indices can be traced to the results obtained but a combination of the data from both the blood indices and Transcranial Doppler appears useful. Although blood flow velocities observed could not be immediately linked directly with any of the parameters measured yet small SD (red cell sizes) coupled with short vessel lengths gave very low velocities and the Transcranial Doppler (TCD) results showed more turbulence in the flow of SCD patients as compared to the control participants. Velocities of blood flow in the arteries were at least 1.5 times the values recorded in the control participants, which is at least 50% higher than for the control participants.

Since the delay time appears to be critical in the pathophysiology of sickle cell disease, SCD, it can be prolonged by either increasing the ability of the red cells to oxygenate or increasing the crowding agents, like Hb F, in the red cell.

5. Conclusions

The major finding in this study is the constant k_2/k_1 ratios peculiar to individuals participating in the *in vivo* data collection and the *in vitro* literature data. This ratio suggests an independence on the environment and reaction path and also, gives insight into the degree of expression of the symptoms of SCD with higher values reflecting greater propensity to pain crisis. Comparing the *in vivo* and *in vitro* results, higher rate constant ratios were obtained for data collected for *in vivo* patients. This may be largely due to the temperature differences in the two cases.

Therefore, it is recommended that the constancy of the model generated k_2/k_1 ratios with the diameter of the blood vessel be used in the pain management of SCD patients.

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