# Detection of Glucose and Related Analytes by Biosensors: A Fractal Analysis

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## Introduction

Physical activity and type of diet have been the key factors influencing the development of diabetes (Tuomilehto 2001). Studies conducted recently indicate that one in three Americans born in the year 2000 will suffer from diabetes (Narayan et al., 2003). Biosensors combine the exquisite selectivity of biology with the processing power of modern micro-electronics and opto-electronics to offer new powerful analytical tools with major applications in medicine, environmental diagnostics, and in the food and processing industries.

Pei et al. (2004) very recently indicate that diabetes is amongst the most prevalent and costly diseases in the world. In the year 2004 these authors estimated approximately 17 million people in the United States have diabetes. This is roughly 6.2 % of the population. Yonzon et al. (2004) further indicate that there are 16 million prediabetics in the United States. The American Diabetic Association (2003) indicates that the economic estimated annual cost of diabetes is $132 billion. There have recently been news reports that indicate that diabetes is reaching epidemic proportions.

According to the World Health Organization (WHO) the number of diabetics will double worldwide from 150 million to 300 million by the year 2025 (Newman et al., 2004). This represents a doubling of the number of diabetics in about 20 to 22 years. As expected, a considerable amount of research has been done and effort been spent in detecting glucose levels, which are critical in this disease (Henry, 1998; Thundat et al., 1994; Thundat et al., 1995; Shrestha et al., 2001).

In this manuscript, we re-analyze using fractal analysis the diffusion-limited binding data of glucose and insulin measurements (Leegsma-Vogt et al., 2004) on biosensor surfaces. Fractal analysis has been used previously to analyze the diffusion-limited analyte-receptor reactions occurring on heterogeneous biosensor surfaces (Butala et al., 2003a; Butala et al., 2003b; Sadana, 2003). Values of the binding rate coefficient, k and the fractal dimension, $D_f$, are provided. The fractal dimension, $D_f$, is a quantitative measure of the degree of heterogeneity on the surface. An increase in the value of the fractal dimension on the surface indicates an increase in the degree of heterogeneity on the sensor chip surface.

**Theory**

**Single-fractal analysis**

***Binding rate coefficient*:** Havlin (1989) indicates that the diffusion of a particle (analyte [Ag]) from a homogeneous solution to a solid surface (e.g. receptor [Ab]-coated surface) on which it reacts to form a product (analyte-receptor complex; (Ab.Ag)) is given by:

$$\mathbf{(Ab.Ag)} \approx \begin{cases} \mathbf{t}^{(3-D_{f,bind})/2} = \mathbf{t}^{p} & \mathbf{t < t_c} \\ \mathbf{t}^{1/2} & \mathbf{t > t_c} \end{cases} \qquad \mathbf{(1)}$$

Here $D_{f,bind}$ is the fractal dimension of the surface during the binding step. $t_c$ is the cross-over value. Above the characteristic length, $r_c$, the self-similarity of the surface is lost and the surface may be considered homogeneous. Above time, $t_c$, the surface may be considered homogeneous, since the self-similarity property disappears, and 'regular' diffusion is now present. For a homogeneous surface where $D_f$ is equal to 2, and when only diffusional limitations are present, $p = ½$ as it should be.

***Dissociation Rate Coefficient* :**The diffusion of the dissociated particle (receptor [Ab] or analyte [Ag]) from the solid surface (e.g., analyte [Ag]-receptor [Ab]) complex coated surface) into solution may be given, as a first approximation by:

$$\mathbf{(Ab.Ag)} \approx -\mathbf{t}^{(3-D_{f,diss})/2} = \mathbf{t}^{p} \qquad (\mathbf{t > t_{diss}}) \qquad \mathbf{(2)}$$

Here $D_{f,diss}$ is the fractal dimension of the surface for the dissociation step. This corresponds to the highest concentration of the analyte-receptor complex on the surface. Henceforth, its concentration only decreases. The dissociation kinetics may be analyzed in a manner 'similar' to the binding kinetics.

**Dual-fractal analysis**

***2.2.1 Binding rate coefficient***

Sometimes, the binding curve exhibits complexities and two parameters (k, $D_f$) are not sufficient to adequately describe the binding kinetics. This is further corroborated by low values of $r^2$ factor (goodness-of-fit). In that case, one resorts to a dual-fractal analysis (four parameters; $k_1$, $k_2$, $D_{f1}$, and $D_{f2}$) to adequately describe the binding kinetics. The single-fractal analysis presented above is thus extended to include two fractal dimensions. At present, the time ($t = t_1$) at which the first fractal dimension 'changes' to the second fractal dimension is arbitrary and empirical. For the most part it is dictated by the data analyzed and the experience gained by handling a single-fractal analysis. A smoother curve is obtained in the 'transition' region, if care is taken to select the correct

number of points for the two regions. In this case, the analyte-receptor complex is given by:

$$(Ab.Ag) \approx \begin{cases} t^{(3-D_{f1,bind})/2} = t^{p1} & (t < t_1) \\ t^{(3-D_{f2,bind})/2} = t^{p2} & (t_1 < t < t_2) = t_c \\ t^{1/2} & (t > t_c) \end{cases} \quad \textbf{(3)}$$

**Results**

The fractal analysis will be applied to the binding of glucose (Leegsma-Vogt et al., 2004). Leegsma-Vogt et al. (2004) have recently presented the potential of biosensor technology in clinical monitoring and in experimental research. They do emphasize that for continuous *in vivo* monitoring of patients very little data is reported. For example, Rhemberg-Boom (1999) describes a biosensor device and ultrafiltration sampling for the continuous *in vivo* monitoring of glucose. Leegsma-Vogt et al. (2004) emphasize that biosensors may be used for the continuous online monitoring of glucose and lactate which would help facilitate therapeutic interventions when need be. Figure 1 shows the oral glucose tolerance test (OGGT) administered by Leegsma-Vogt et al. (2004) with glucose and insulin measurements. Probes placed at different locations measured plasma insulin (Figure 1a), plasma glucose (Figure 1b), adipose tissue interstitial glucose (Figure 1c), and connective tissue interstitial glucose (Figure 1d).

Figure 1a shows the binding and dissociation of insulin in plasma. A dual-fractal analysis is required to adequately describe the binding kinetics. A single-fractal analysis is adequate to describe the dissociation kinetics. The values of (a) the binding rate coefficient, k and the fractal dimension, $D_f$ for a single-fractal analysis, (b) the binding rate coefficients, $k_1$ and $k_2$, and the fractal dimensions, $D_{f1}$ and $D_{f2}$ for a dual-fractal analysis, and (c) the values of the dissociation rate coefficient, $k_d$ and the fractal dimension in the dissociation phase, $D_{fd}$ for a single-fractal analysis are given in Table I. Note that as the fractal dimension value increases by a factor of 2.47 from $D_{f1}$ equal to 0.6827 to $D_{f2}$ equal to 1.6852, the binding rate coefficient increases by factor of 4.92 from $k_1$ equal to 1.0232 to $k_2$ equal to 5.0388. An increase in the degree of heterogeneity on the probe surface leads to an increase in the binding rate coefficient.

Figure 1b shows the binding and dissociation of glucose in plasma. A dual-fractal analysis is required to adequately describe the binding kinetics. A single-fractal analysis is adequate to describe the dissociation kinetics. The values of (a) the binding rate coefficient, k and the fractal dimension, $D_f$ for a single-fractal analysis, (b) the binding rate coefficients, $k_1$ and $k_2$, and the fractal dimensions, $D_{f1}$ and $D_{f2}$ for a dual-fractal analysis, and (c) the values of the dissociation rate coefficient, $k_d$ and the fractal dimension in the dissociation phase, $D_{fd}$ for a single-fractal analysis are given in Table I.

Figure 1c shows the binding and dissociation of adipose tissue interstitial glucose. A dual-fractal analysis is required to adequately describe the binding kinetics. A single-

fractal analysis is adequate to describe the dissociation kinetics. The values of (a) the binding rate coefficient, k and the fractal dimension, $D_f$ for a single-fractal analysis, (b) the binding rate coefficients, $k_1$ and $k_2$, and the fractal dimensions, $D_{f1}$ and $D_{f2}$ for a dual-fractal analysis, and (c) the values of the dissociation rate coefficient, $k_d$ and the fractal dimension in the dissociation phase, $D_{fd}$ for a single-fractal analysis are given in Table I. Note that as the fractal dimension value increases by a factor of 3.31 from $D_{f1}$ equal to 0.5720 to $D_{f2}$ equal to 1.891, the binding rate coefficient increases by factor of 8.88 from $k_1$ equal to 0.0545 to $k_2$ equal to 0.4841. Once again, an increase in the degree of heterogeneity on the probe surface leads to an increase in the binding rate coefficient.

On comparing the binding rate coefficient values for glucose in plasma and in the adipose interstitial tissue one notes that the binding rate coefficient values, $k_1$ and $k_2$ are higher in the interstitial adipose tissue than in the plasma. As expected, the corresponding values of the fractal dimensions are also higher.

Figure 1d shows the binding and dissociation of connective tissue interstitial glucose. A single-fractal analysis is adequate to describe the binding and the dissociation kinetics. The values of (a) the binding rate coefficient, k and the fractal dimension, $D_f$ for a single-fractal analysis, and (b) the values of the dissociation rate coefficient, $k_d$ and the fractal dimension in the dissociation phase, $D_{fd}$ for a single-fractal analysis are given in Table I.

Figure 2a and Table I show the decrease in the dissociation rate coefficient, $k_d$ with an increase in the fractal dimension in the dissociation phase, $D_{fd}$. For the data presented in Table I and for glucose and insulin present in plasma and in interstitial adipose and connective tissue, the dissociation rate coefficient, $k_d$ is given by:

$$k_d = (0.0939 \pm 0.0614) D_{fd}^{\,-1.583 \pm 0.753} \qquad (4)$$

Note that the data for glucose and insulin are plotted together. The dissociation rate coefficient, $k_d$ exhibits close to a negative one and one-half order dependence on the degree of heterogeneity ($D_{fd}$) that exists on the biosensor surface.

Figure 2b and Table I show the increase in the ratio of the binding and the dissociation rate coefficient, $k_1/k_d$ with an increase in the ratio of the fractal dimensions, $D_{f1}/D_{fd}$. For the data presented in Table I and for glucose and insulin present in plasma, and for glucose in interstitial adipose tissue and in interstitial connective tissue, the ratio of the binding and the dissociation rate coefficient, $k_1/k_d$ is given by:

$$(k_1/k_d) = (5.149 \pm 1.912)(D_{f1}/D_{fd})^{1.371 \pm 0.1036} \qquad (5)$$

The ratio of the binding and the dissociation rate coefficient, $k_1/k_d$ exhibits an order of dependence between first and one and one-half order (equal to 1.371) on the ratio of the fractal dimensions, $D_{f1}/D_{fd}$ that exists on the biosensor surface.

## Conclusions

A fractal analysis is used to model the binding and dissociation kinetics of connective tissue interstitial glucose, adipose tissue interstitial glucose, insulin and other related analytes on biosensor surfaces. The analysis provides insights into diffusion-limited analyte-receptor reactions occurring on heterogeneous biosensor surfaces. The fractal analysis provides a useful lumped parameter(s) analysis for the diffusion-limited reaction occurring on a heterogeneous surface via the fractal dimension and the rate coefficient. It is a convenient means to make the degree of heterogeneity that exists on the surface more quantitative.

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**Figures**

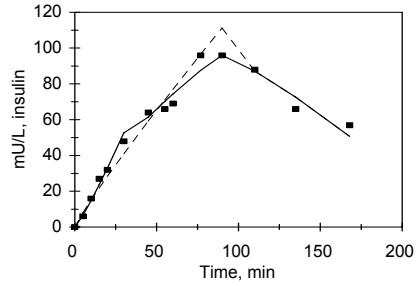


Figure 1a

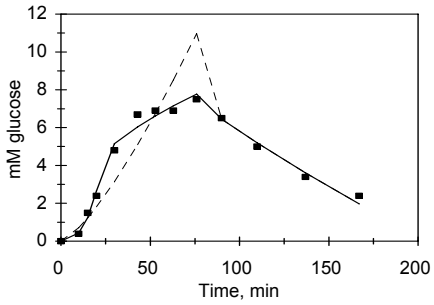


Figure 1b

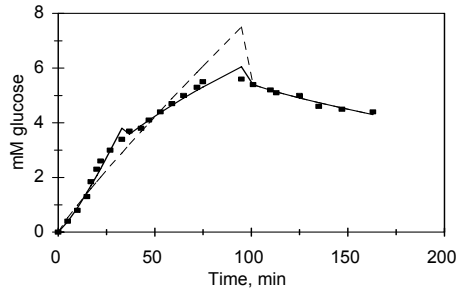


Figure 1c

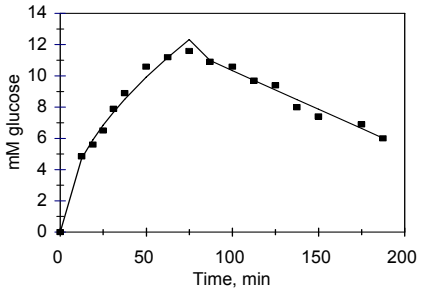


Figure 1d

Figure 1: (a) Binding and dissociation of insulin in plasma in the oral glucose tolerance test [16]
(b) Binding and dissociation of glucose in plasma
(c) Binding and dissociation of adipose tissue interstitial glucose
(d) Binding and dissociation of connective tissue interstitial glucose

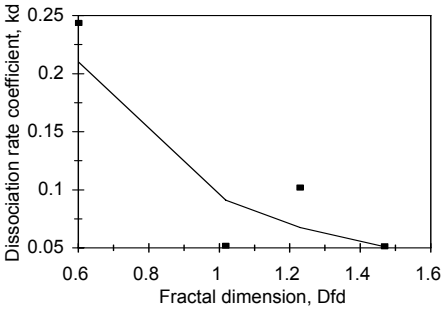


Figure 2a

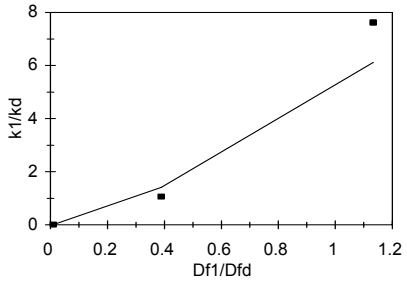


Figure 2b

Figure 2: (a) Decrease in the dissociation rate coefficient, $k_d$ with an increase in the fractal dimension for dissociation, $D_{fd}$
(b) Increase in the ratio of binding and dissociation rate coefficient $k_1/k_d$ with an increase in the ratio of the fractal dimensions $D_{f1}/D_{fd}$

**Tables**

TABLE Ia: Binding Rate Coefficients for Glucose in Plasma, in Connective Tissue, and in Adipose Tissue, and Insulin in Plasma (Leegsma-Vogt et al., 2004)

| Compound | Location | k | $k_1$ | $k_2$ | $k_d$ |
|---|---|---|---|---|---|
| Insulin | Plasma | 1.8557±0.334 | 1.0232±0.1309 | 5.0388±0.3671 | 0.2436±0.0875 |
| Glucose | Plasma | 0.0329±0.0154 | 0.00101±0.0002 | 1.1480±0.0974 | 0.1019±0.0103 |
| Glucose | Interstitial adipose tissue | 0.1246±0.0242 | 0.0545±0.0063 | 0.4841±0.0164 | 0.0513±0.0056 |
| Glucose | Interstitial connective tissue | 1.220±0.067 | na | na | 0.0519±0.0081 |

TABLE Ib: Fractal Dimensions for Glucose in Plasma, in Connective Tissue, and in Adipose Tissue, and Insulin in Plasma (Leegsma-Vogt et al., 2004)

| Compound | Location | $D_f$ | $D_{f1}$ | $D_{f2}$ | $D_{fd}$ |
|---|---|---|---|---|---|
| Insulin | Plasma | 1.1804±0.116 | 0.6827±0.175 | 1.6852±0.160 | 0.602±0.6334 |
| Glucose | Plasma | 0.3128±0.402 | 0 | 2.1168±0.227 | 1.2298±0.136 |
| Glucose | Interstitial adipose tissue | 1.200±0.111 | 0.5720±0.136 | 1.891±0.0456 | 1.4696±0.101 |
| Glucose | Interstitial connective tissue | 1.9284±0.066 | na | na | 1.0193±0.146 |