ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 241–245

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Application of the quartz crystal microbalance to the monitoring of *Staphylococcus epidermidis* antigen–antibody agglutination

K.D. Pavey [a,*], Z. Ali [b], C.J. Olliff [c], F. Paul [a]

[a] *Novel Methods Group, CASS, Smithkline Beecham Pharmaceuticals, NFSP North, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b] *Division of Biotechnology and Chemical Sciences, University of Teeside, Middlesborough, Cleveland TS1 3BA, UK*
[c] *School of Pharmacy and Biomolecular Sciences, University of Brighton, Lewes Road, Brighton, Sussex BN2 4GJ, UK*

Received 9 May 1998; received in revised form 20 August 1998; accepted 31 August 1998

Abstract

The change in solution properties due to the agglutination of an antigen with its specific antibody has previously been used as a marker of infection. This method has been modified to allow the binding activity between species to be followed using the frequency response of a quartz crystal microbalance (QCM). The Bayston agglutination plate assay for *Staphylococcus epidermidis* has been modified to allow the electrode of a QCM to act as a direct sensor for the change in solution properties as agglutination occurs. Antibody and antigen were introduced to the crystal surface and the agglutination process was followed as a change in crystal resonant frequency. Serum, known to be infected with the organism, gave a titre of 3.9×10^{-2}% v/v (-118 Hz, ± 12 SD, $N=9$) matching that given by triplicate plate assay. Uninfected serum gave no frequency changes at this concentration, yielding a titre of 2.5×10^{-2}% v/v again matching the plate titre ($N=3$). Infected serum gave responses 40 times faster then those of the uninfected serum. The piezoelectric quartz crystal method gave a positive or negative diagnosis in <15 min compared with the 24 h required for the plate assay.

Keywords: Quartz crystal microbalance (QCM); *Staphylococcus epidermidis*; Agglutination; Rapid diagnosis

1. Introduction

A convenient method for the determination of bacterial infection within the body is the visible coagulation of antigen with its antibody, within a serum sample. Specific antigens are placed in microtitre plate wells in suitable buffers. Serum samples suspected of containing antibodies to these antigens, raised by immune response, are added to and mixed within these wells and then serially diluted. These plates are incubated for 24 h, during which time areas of visible coagulated material form, the dilution one before a full spot of coagulant, yielding the titre for the sample. The

* Corresponding author. Tel.: +44-1279-627895; Fax: +44-1279-627896..
E-mail address: karl_d_pavey@sbphrd.com (K.D. Pavey)

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00026-6

technique allows a specific determination, with no cross-contamination [1], which can reliably identify immune responses to bacterial infection, thereby identifying the species present in the bloodstream. However, the incubation step is relatively slow, which can delay the administration of the correct treatment. The technique also requires conjecture as to the nature of the infection before analysis, to allow use of the correct antigen.

Alternatively, a wide range of bacterial antigens is required, greatly adding to the complexity and volume of laboratory work required. A reduction in the analysis time would allow a greater number of species to be checked within a given time period.

The process of agglutination yields an increase in the viscosity and density of the supporting buffer solution, prior to the sedimentation of solid material onto the surface of the well. If this change in solution properties, preceding precipitation could be recorded, it could lead to decreased diagnosis periods.

Quartz crystal microbalances (QCM), are transducers capable of sub-microgram mass measurement in vacuo [2] and gases [3]. They have more recently been shown to be capable of the measurement of changes in solution properties [4]. The sensing element of the QCM is the change in resonant frequency of a vibrating piezoelectric quartz sheet. A drop in frequency indicates increased pressure on the crystal electrodes, binding to the surface, or the increase in solution properties such as solution viscosity and density. Therefore, it is proposed that the changes which occur during the early stages of agglutination could be followed if the assay were carried out upon the sensor surface.

Much of the early work involving antibody studies with QCM involved the binding of the material to the electrode surface [5]. Fixing species to metal surfaces can be time consuming and can potentially limit access to specific binding regions, therefore a system which could monitor binding without the need for surface entrapment could be useful.

The QCM technique has shown itself capable of this type of measurement. Huramatsu et al. [6] showed that the gelation of fibrinogen upon contact with thrombin could be followed in real time without the need to bind either species to the crystal surface, whilst Shi-hui et al. used a similar technique when looking at the interactions of platelet factor 3 [7]. To increase the frequency response with small species, several groups have used antibody-bearing latex beads in order to increase the change in solution properties at the surface [8–10], however this process still involves entrapment upon surfaces. The binding of an antibody to a specific bacterial antigen should be capable of producing a measurable change in solution parameters, without the need to use an external support.

Staphylococcus epidermidis is a Gram positive, class 1 pathogen commonly found in the natural skin flora [11]. In everyday life the organism is essentially harmless, however its pathogenic nature can appear, if it is allowed to come into contact with open incisions, whilst carrying out routine surgery [12], at catheter penetration sites or within prosthetic devices [13].

An immune response to the organism produces antibodies which are released into the bloodstream. Humans naturally carry a basal load of this antibody due to minor infection caused by skin cuts and ingestion. Patients with colonisation present a greatly increased antibody loading. Bayston describes an assay for *S. epidermidis* based upon the agglutination of this organism in serum with its antigen, in a microtitre plate [14]. This assay has been modified to allow it to be carried out within a well, formed upon the upper electrode of a 14 MHz QCM. Tests have been carried out using inoculated and uninfected serum from both rabbits and humans and the results compared with those from plate assays.

2. Experimental

2.1. Materials and instrumentation

Sörenson's phosphate buffer (0.2 M, pH 7.2), was prepared from Analar grade reagents (BDH Chemicals, Dorset, UK) in distilled water, sterilised by autoclaving at 121°C for 60 min.

14 MHz AT cut crystals furnished with gold electrodes were purchased from Piezoproducts (Portsmouth, UK), and modified using butyl rubber 'O'-rings attached using silicone sealant (Dow Corning 739), to give a well on one electrode, in which the assay was performed (Fig. 1, volume ≈ 40 μl). The crystal was connected to a parallel oscillator circuit [15]. Output from the crystal was monitored using a frequency counter capable of sub-single hertz resolution (Fluke 6685, Fluke, UK).

2.2. Antigen preparation

Antigen was prepared by inoculating blood agar plates with *S. epidermidis* SIIA and incubating for 48 h at 37°C. The organisms were then scrapped from the plate and suspended in 30 ml of Sörenson's buffer. Washing of the cells was achieved by twice centrifuging ($3000 \times g$, 15 min), and resuspending in buffer (30 ml).

Following the second washing the pellet was resuspended in 10 ml Sörenson's buffer. The suspension was then autoclaved at 121°C for 60 min before being diluted with buffer to give an absorption value of 0.890 at 490 nm.

2.3. Serum preparation

Serum was obtained from both *S. epidermidis* inoculated (sample), and noninfected rabbits (control) and from the whole blood of two healthy humans, by twice centrifuging ($3000 \times g$, 15 min, 4°C), whole blood in 50 ml plastic tubes and collecting the supernatant.

2.4. Bayston plate agglutination assay

Sörenson's buffer (200 μl), was pipetted into the first well of a 12-well wide, round bottomed microtitre plate. All other wells received 100 μl of buffer. Serum (20 μl), was added, by pipette, to the first well and mixed thoroughly. A 100 μl volume of this mixture was double diluted along the row by pipette, giving a range of dilutions from $0.05–9.8 \times 10^{-5}$ %v/v. Prepared antigen (100 μl), was added to each well, the tray shaken gently by hand then incubated at 37°C for 1 h, followed by 4°C for 24 h.

The highest dilution giving agglutination (full spot), is taken as the endpoint, the titre being the reciprocal of that dilution.

2.5. Piezoelectric agglutination assay

A mixture of Sörenson's buffer and antigen (40 μl, 50/50), was placed into the well on the surface of the piezoelectric crystal. The frequency was allowed to stabilise (2 min) and the frequency response over a 10 min period was recorded.

To the antigen and buffer on the crystal, solutions of serum diluted with Sörenson's buffer and antigen were added using GC syringes (1.0 and 10.0 μl), so that, as in the plate assay, the final antigen concentration remained constant at each dilution, and a range of serum concentrations equal to those in the plate assay were obtained. For the mass upon the crystal to remain equal throughout the experiment, calculated volumes of liquid were systematically removed from the well before the next injection of serum, buffer and antigen was made. At each concentration step,

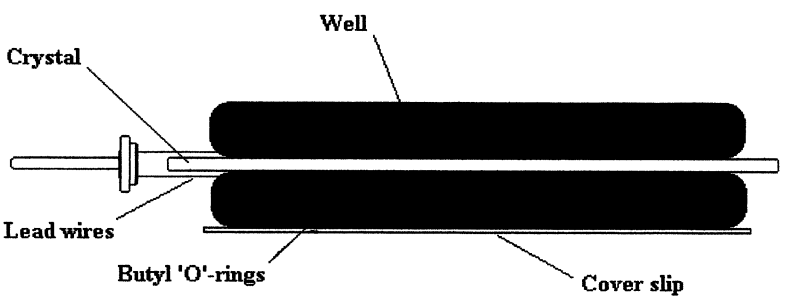

Fig. 1. Encased crystal holder design.

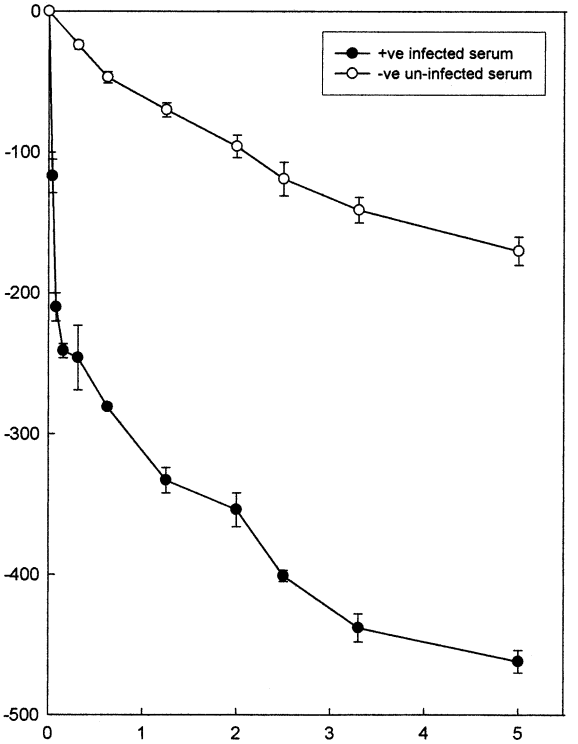

Fig. 2. Plot of cumulative frequency change versus serum concentration for both positive (infected) and negative (control), rabbit serum ($N = 6$, $\pm$ SD). Y-axis title: cumulative frequency decrease (Hz), X-axis title: serum concentration (% v/v).

frequency responses were recorded for a 10 min period.

The piezoelectric assay was carried out using both the infected and noninfected rabbit serum samples. Six replicates of each experiment were carried out. Triplicate plate assays were carried out alongside the piezoelectric assay.

Assays were also carried out in triplicate, on the serum samples taken from the two human volunteers carrying no obvious signs of infection, using both the piezoelectric method and the plate assay.

3. Results and discussion

Plots of cumulative frequency change over the 10 min period as a function of percentage serum concentration are given in Fig. 2, with each point being the mean of six experiments and error bars representing $\pm$ SD. The plot shows that at a concentration of 3.9×10^{-2}% v/v, there is a distinct decrease in cumulative frequency of 240 Hz (SD = 5 Hz, $N = 6$), for the infected rabbit serum. Identical titre values were obtained from triplicate plate assays. At the same dilution, the noninfected rabbit serum gave a frequency decrease of 24 Hz (SD = 3 Hz, $N = 6$).

Student's t-tests confirm that the mean of the frequency decreases at concentrations of both 3.1×10^{-3} and 5.0% v/v, are significantly different between infected and uninfected serum ($P = 0.05$, $N = 6$).The frequency dropped by rates of 2.7×10^{3} and 0.7×10^{2} Hz s^{-1} for the inoculated and uninfected sera respectively, at a concentration of 3.1×10^{-3}% v/v.

Assays of human serum samples by piezoelectrics gave frequency decreases of $\approx 400 \pm 50$ Hz at a concentration of 2.5% v/v, which was confirmed by triplicate plate assay. The literature gives a range of titres for positive, that is patients with colonised shunts, equal to concentrations between 0.3 and 1.95% v/v and for normal, uninfected children of 5–2.5% v/v [16]. Both the plate assay and piezoelectric assay gave results falling within the literature range of values or below, indicating greater sensitivity, as well as agreeing well with one another. The correlation coefficient for the plot of titre end point, for the piezoelectric assay versus that of the plate assay gave a straight line with a correlation coefficient of 0.998 and slope of 1.00.

The plate assays were, however, incubated for a period of 24 h, whereas results for the titre were obtained in < 2 h using the QCM technique, which is over 40 times faster than the plate method. For preliminary investigation of infection, a dilution to $\approx 5 \times 10^{-3}$% v/v of sample serum with appropriate antigen can produce a positive or negative diagnosis of < 10 min.

4. Conclusions

The piezoelectric detection of the titre of agglutination gives a faster response than the plate agglutination method, with equivalent resolution,

allowing a swift, specific identification of infection to be made and a decrease in the time before correct treatment can be administered.

This procedure also verifies that the changes in viscosity/density resulting from an agglutination reaction can be followed by means of the QCM system, enabling titre and reaction rates to be determined when required, as the process could be monitored in real time. It also shows that the agglutination process is proceeding long before it becomes visible and can be followed within this period.

The method highlighted here has great potential for the swift diagnosis of infections caused be *S. epidermidis*, and could potentially be modified for diagnosis of other organisms using a similar process.

Acknowledgements

The authors wish to thank Jackie Rogers (University of South West England), for instigating the inoculation programme and for helpful discussion. K. Pavey wishes to thank E.P.S.R.C and SmithKline Beecham for funding under the CASE award scheme, Award # G77.

References

[1] R. Holt, J. Clin. Pathol. 22 (1969) 475–482.
[2] G. Sauerbrey, Z. Physik 155 (1959) 206–222.
[3] J. Hlavey, G.G. Guilbault, Anal. Chem. 49 (13) (1978) 1890–1899.
[4] M. Thompson, G.K. Dhaliwal, C.L. Arthur, G.S. Calabrese, IEEE Trans. Ultrason. Ferroelectr. Freq. Control UFFC-34 (1987) 127–135.
[5] M. Plomer, G.G. Guilbault, B. Hock, Enzyme Microb. Tech. 14 (1992) 230–235.
[6] H. Muramatsu, E. Tamiya, M. Suzuki, I. Karube, Anal. Chim. Acta. 217 (1989) 321–326.
[7] S. Shi-Hui, N. Li-Hua, S.Z. Yao, Microchem. J. 51 (1995) 351–359.
[8] S. Kurosawa, E. Tamara, N. Kamo, F. Ohta, T. Hosokawa, Chem. Pharm. Bull. 38 (5) (1990) 1117–1120.
[9] M. Muratsugu, S. Kurosawa, N. Kamo, Anal. Chem. 64 (1992) 2483–2487.
[10] H.O. Ghourchian, N. Kamo, Anal. Chim. Acta 300 (1995) 99–105.
[11] T.D. Brock, M.T. Madigan, in: Biology of Microorganisms, 5th ed., Prentice Hall Publishing, New Jersey, USA, 1988.
[12] J.A. Black, D.N. Challacombe, B.G. Ockenden, Lancet 2 (1965) 921–924.
[13] J. Yu, M. Montelius Nordman, M. Paulsson, I. Gouda, O. Larm, L. Montelius, A. Ljungh, Biomaterials 15 (10) (1994) 805–814.
[14] R. Bayston, Develop. Med. Child Neurol. 13 (25) (1971) 135–140.
[15] C. Barnes, Sensors and Actuators A 29 (1991) 59–69.
[16] R. Bayston, J. Clin. Path. 25 (1972) 718–720.