

TABLE 1-continued

	Anion Component	Frequency Change(Hz)	Cation Component	Frequency Change(Hz)
Example 17	10 m M F A D/15 m M P S S	117	19 m M P D D A	29
Example 18	1 m M M B 29	-2	1.9 m M P D D A	10
Example 19	10 m M M B 29/15 m M P S S	214	19 m M P D D A	34
Example 20	15 m M P S S	83	10 m M M B 29/19 m M P D D A	75

TABLE 2

	Anion Component	Frequency Change(Hz)	Cation Component	Frequency Change(Hz)
Example 21	1 mg/mL G O D	2330	1 mg/mL P E I	52
Example 22	0.5 mg/mL G O D	2125	1 mg/mL P E I	50
Example 23	0.005 mg/mL G O D	670	1 mg/mL P E I	44
Example 24	0.5 mg/mL G O D/1 mg/mL P S S	482	1 mg/mL P E I	45
Example 25	0.05 mg/mL G O D/1 mg/mL P S S	468	1 mg/mL P E I	33
Example 26	1 mg/mL P S S	130	0.5 mg/mL G O D / 1 mg/mL P E I	382
Example 27	1 mg/mL P S S	149	0.05 mg/mL G O D / 1 mg/mL P E I	185
Example 28	1 mg/mL P S S	110	1 mg/mL P E I	40

Examples 21–28: Layering and Fixation of Proteins

The Examples 21–28 were carried out to study the applicability of the present invention for constructing films composed of proteins. The results are summarized in Table 2.

Examples 21 and 22 are given for films prepared by the conventional alternate layer-by-layer method, in which GOD enzyme was layered alternately with PEI. In these Examples there is observed a very large frequency change in each adsorption step, which corresponds to a simultaneous layering of 3 to 4 GOD layers, suggesting that the GOD was assembled in the form of multi-layered and associated molecules.

By contrast, with Examples 23 and 24 in which an admixed GOD/PSS solution was employed for the alternate layering with PEI and also with Examples 25 and 26 in which an admixed GOD/PEI solution was employed for the alternate layering with PSS, the frequency changes resulting from the GOD adsorption were found to be relatively small. At the same time, such frequency changes are significantly larger than those in Example 27 and 28 in which the alternate layering was conducted using PSS and PEI. Thus, it was verified that in the films of Examples 23 through 26 GOD molecules are layered and fixed without occurrence of the association of molecules, by premixing (admixing) GOD with polymer ions.

Examples 29–34: Enzyme Reaction Using the Thin Films

Examples 29–34 are to illustrate enzyme reactions carried out by immersing the films obtained as in the above-described Examples 21–27 in an aqueous solution containing glucose, POD and DA67 (a redox enzyme). As the reaction proceeded, DA67 was oxidized, resulting in an increased absorption at 665 nm. This spectral change with time is an indication of the enzyme activity. Thus, the relative enzyme activities per unit weight of the enzyme were determined for the respective cases, in which the enzyme activity of the film in Example 29 was employed as a standard. The results are given in Table 3.

TABLE 3

	Film employed	Relative Activity
Example 29	Example 21	1.000
Example 30	Example 22	0.998
Example 31	Example 23	0.995
Example 32	Example 24	1.958
Example 33	Example 25	7.407
Example 34	Example 26	7.284
Example 35	Example 27	67.44

In Examples 29 and 30 were used the films prepared by the conventional alternate layer-by-layer method, in which the film in Example 30 was prepared from a solution with a smaller GOD concentration as compared with the film in Example 29. There are observed no enhancement in the relative activity with Example 30. It is clearly indicated that the employment of an admixed solution will result in an enhancement in the enzyme activity, as seen in Examples 23 and 24 in which GOD/PSS admixed solutions were used in the alternate layer-by-layer assembling with PEI and also in Examples 25 and 26 in which GOD/PEI admixed solutions were used in the alternate layer-by-layer assembling with PSS.

It was also found that GOD of a smaller concentration admixed with the polymer ions produced a higher enzyme activity as can be seen from the comparison between Examples 31 and 32 and that between Examples 33 and 34. This fact may be reasoned that the less is the GOD proportion in the admixture, the more effectively the association of GOD molecules are suppressed and the diffusion of the substrate is facilitated resulting in the enhancement in the enzyme activity. It was further noted that the admixing of the enzyme with oppositely charged polymer ions in a solution led to a higher relative activity, as can be seen from a comparison between Examples 31 and 33 and also that between Examples 32 and 34. Thus, it was shown that admixing of an enzyme with a polymer ion which will exert a stronger interaction with the enzyme results in an increased activity due to the suppression of the association of the enzyme molecules.

Examples 35–37

The alternate layer-by-layer assembling on quartz crystal was carried out by immersing the precoated quartz crystal