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Selective and stable adhesion of Yeast cells to Boronate-containing Polymer Grafted to glass Supports

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ABSTRACT

A series of sorbents containing various amounts of borate groups were prepared by chemical immobilization of P-aminoethylphenylboric acid on a copolymer- treated organosilane spacer glass plates. It was found that baker's yeast cells selectively adhered to these supports. Yeast cells stained with procion red HE-3B formed denser and more stable layer on the copolymer-grafted supports compared onto supports modified with phenylboric acid via a organosilane spacer. Adhesion of yeast cells to boronate-containing polymer fixed on solid support in presence of different concentration of fructose, a sugar with high affinity to boronates, was studied.

Keywords: Sorbent; Copolymer; Yeast; Affinity

INTRODUCTION

The ability of boronate to form complexes with hydroxyl groups in carbohydrates is exploited in boronate affinity chromatography [1-5]. During the last decade the use of boronic acid-substituted polymers for the separation of diol-containing molecules such as nucleosides, nucleotides, catecholamines, carbohydrates and transfer RNA has been well documented [6-12]. The vicinal diols of these substances are able to form reversibly cyclic boronate esters with the boronate anion at high pH, and the formation of these complexes is dependent on pH, ionic strength, temperature and, in the case of nucleic acid components, on the structure of the base.

The interaction is not only specific for carbohydrates, as any compound containing hydroxyl groups in a suitable geometry will form a moderately stable complex with the boronate. As a consequence, boronate chromatography can, for example, be used for the separation of glycoproteins, nucleosides and catechol compounds [13-15]. A variety of other functional groups, such as α-hydroxycarboxylic acids, aromatic α- hydroxy acids and amides can also interact with boronates. These functional groups can be found in compounds such as lactic acid, salicylic acid, salicylamide and steroids [16, 17].

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Although boronate chromatography was introduced in the 1970s, the number of successful applications in the purification of glycoproteins is still limited. One explanation of this may be the formation of protein-boronate complexes which reduce the capacity and purification efficiency of the method. Boronate chromatography would be a much more powerful tool in glycoprotein purification if it were possible to facilitate the process of interacting of boronate ligands with the carbohydrate moieties of glycoproteins by selectively preventing the interaction with protein moieties.

This work aims to demonstrate the feasibility of using composite sorbents with immobilized phynylboronic acid for adhesion of baker's yeast cells and also compare adhesivity of the grafted boronate-containing copolymers support and direct immobilized boronic acid on the support via organosilane.

EXPRIMENTAL

Materials

1,4-Dioxane , sodium hydroxide , sodium carbonate , sodium bicarbonate , sodium chloride , hydrochloric acid , and acetic acid were products of Merck (Darmstadt , Germany). 3-Aminophenylboronic acid hydrochloride ,N,N-dimethylacrylamide , 3-mercaptopropyltrimethoxysilane , 3-glycidoxypropyltrimethoxysilane and aluminum oxide were from Aldrich (Steinheim , Germany). 2,2′-Azobis (2-methylpropionittrile) was purchased from Acros (New Jersey, USA). Acryloyl chloride was purchased from Fluka Chemica (Buchs Switzerland).

1. Pre-treatment and washing of glass plates

20 pieces of glass plates (0,9×3,6 cm) were placed into 4 M NaOH for two days and washed with distilled water . Then, the glass plates were placed into 4 M HCl for two days, washed with distilled water and dried under vacuum in a desiccator over dry calcium chloride.

2. Modification of glass plates with 3-glycidoxypropyltrimethoxy silane

(GPTMS) and immobilization of *phenylboronic acid (PBA)*

a) Preparation of γ-glycidoxypropyl-silyl supports (GPTMS-glass)

The first step was modification of glass plates with 3-glycidoxypropyltrimethoxy silane (GPTMS). GPTMS solution (5%) in 1,4-dioxane was prepared (50 ml) by adding 2.5 ml GPTMS to sufficient amount of 1,4-dioxane . Modification was carried out in the boiling solution of GPTMS in a conical flask for ca. 16 h . Then, the glasses were washed with 1,4-dioxane for several times and dried under vacuum in a desiccator over dry calcium chloride .

b) Hydrolysis of epoxy groups GPTMS-glasses in to diols

GPTMS-glass plates were divided in to two parts:

The first part was treated with diluted hydrochloric acid pH 2.4 for 24 h. at room temperature [4]. After hydrolysis the plates were rinsed with water.

The second part of glasses were used for modification with PBA (section 2c).

c) Preparation of modified glass plate with PBA (PBA-GPTMS-glass)

The next step was immobilization of PBA on GPTMS-glass plates. 600 mg 3-aminophenylboronic acid hydrochloride was dissolved in 15 ml water, then (0.5 M) sodium bicarbonate buffer pH=9 was added dropwise to the solution until the pH was adjusted to 8.5. The GPTMS modified glass plates were put into the solution and shaken on a rocking table for 24 h at ambient temperature. Then the glass plates were washed with distilled water and stored in water. [1]

3. Synthesis of 3-acrylamidophenylboronic acid (AAPBA)

1.72 g (10mmol) 3-aminophenylboronic acid was dissolved in 20 ml 2 M NaOH (40 m mol) and cooled to 2°C in an ice bath. 1.6 ml (20mmol) acryloyl chloride was added dropwise to the solution of 3-aminophenylboronic acid under intensive magnetic stirring for 10-15 min. 2M hydrochloric acid was added dropwise to the reaction mixture in order to adjust its pH to ca. 1 and precipitate the product , which was separated by filtration on the sintered glass filter (Schott, Duran.No.3) and washed on the filter by 50 ml chilled distilled water . The precipitate was dissolved in 40 ml distilled water on heating to ca. 60 ° C and crystallized as needles for over night at 8°C. The substance was filtered off on Munktell No. 3 paper filter , washed with chilled distilled water and dried under vacuum in a desiccator over dry calcium chloride .[2]

4. Modification of glass plates with (3-mercaptopropyl) trimethoxy silane

(MPTMS) and graft copolymerization of AAPBA with – N,N-dimethyl acrylamide (DMAA-AAPBA copolymer)

a)Preparation of mercaptopropyl-modified glasses (MPTMS-glasses)

The first step was modification of glass plate with (3-mercaptopropyl)trimethoxy silane (MPTMS). MPTMS solutions (5%) in 1,4-dioxane (50 ml) were prepared by adding 2.5 ml MPTMS silane to sufficient amount of 1,4-dioxane . Chemical modification was carried out in the boiling solution for 24 h. Then, the glasses were washed several times with 1,4-dioxane and dried under vacuum in a desiccator over dry calcium chloride .

Flash chromatography

A column (2.5 × 0.9 cm) containing 1.6 g aluminum oxide was used for removal impurities from 3 ml N,N dimethylacrylamide(DMAA). These impurities play inhibiting role in polymerization reaction .This amount of DMAA was applied to the column dropwise and came down by its gravity.

b) Preparation of glass plates grafted with DMAA-AAPBA copolymer

The next step was grafting the *DMAA-AAPBA copolymer* on the mercaptopropyl modified glasses. Glass plates, modified by GPTMS, were placed into the degassed polymerization mixture (20 ml ethanol , 431.7 mg AAPBA , 2.07 ml N,N dimethylacrylamide (DMAA) , and 53.7 mg 2,2′-azobis(2methyl-propionitrile)) for 5 h at 75° C with nitrogen bubbling. After polymerization, the glass plates were washed with ethanol and placed in water.

5. Modification of glass plate by poly N,N-dimethyl acrylamide (poly DMAA)

Preparation of glass plates grafted by poly N, N-dimethylacrylamide

The glass plates modified with MPTMS (see section 4a) were placed into the degassed polymerization mixture (20 ml ethanol , 2.07 ml N,N dimethylacrylamide (DMAA) , and 53.7 mg 2,2′-azobis (2methyl-propionitrile)) for 5 h at 75° C with nitrogen bubbling. After polymerization the glass plates were washed with ethanol and placed in water.

6. Staining of yeast cells by Procion Red HE - 3B

Yeast cell suspension (20 mg / ml suspension, 100 ml) in 0.15 M NaCl was heated for 40 min. at 70° C. Then cells were washed twice by centrifugation (3700×g, 15 min) and re-suspended in 2 ml distilled water. The suspension of yeast cell and 0.03 g of Procion Red was mixed, 0.3 g NaCl added and mixed up on a rocking table for 24 hours. The next step was addition of 0.03 g Na_2CO_3 followed by suspending of cells for 36 hours on rocking table. Then cells were washed with water by centrifugation (3700 ×g, 5min) until the washings become colorless [3]. The cells were re-suspended and stored in 0.5 M sodium bicarbonate buffer, pH 9.05.

7. Precipitation of the stained yeast cells on the chemically modified glass plates

A chemically modified glass plate was washed with 0.5 M sodium bicarbonate buffer pH 9.05 and placed into the suspension of stained yeast cells (2g) in 20ml buffer. The stained yeast cells were allowed to precipitate on the modified glass for 2h. Then the glass was washed with buffer in order to remove non-attached stained yeast cells from the glass. The amount of stained yeast cells on the modified glass was quantified by UV - VIS spectrometry (λ_{max}=590), the glass plate was placed vertically in a 1 cm cuvette. After attainment to the constant absorbance, the cuvette containing the plate was swung up side down for 2 times and the buffer solution was decanted. The fresh buffer was added in it and the amount of attached stained yeast cells on the glasses was quantified again. This experiment was repeated seven times for PBA-GPTMS-glass, three times for hydrolyzed GPTMS - glass, three times for MPTMS-glass, six times for DMAA-AAPBA-glass, three times for DMAA-glass and three times for non-modified glass plates.

8. Regeneration of DMAA-AAPBA-grafted glasses

0.15 M Acetic acid solution pH 3.33 was prepared by adding acetic glacial acid to water down to pH to 3.33. In order to regenerate DMAA-AAPBA-grafted glasses, we put the plates into the prepared solution at given period of time (0.5 – 24 h), after that the plates were stored in distilled water before the next cycle.

Results and Discussion

In the present investigation we would like to study adhesion of yeast cells to non-modified glasses , glasses containing PBA attached via GPTMS (PBA – GPTMS – glasses) and glasses containing grafted DMAA – AAPBA copolymer (DMAA –AAPBA –glasses) .

At first we prepared glasses modified with GPTMS and then attached PBA to them. For increasing the measuring sensitivity, yeast cells were stained with Procion Red HE-3B. The yeast cells were allowed to precipitate on the non-modified glass plates ,the modified glass plates with hydrolyzed GPTMS and PBA-GPTMS for 2h , then the plates were photometered in 1 cm cuvette at λ_{max}=590 . Fig.1 illustrates the absorbance of stained yeast cells adhered to non-modified glasses , glasses modified with hydrolyzed GPTMS and PBA-GPTMS- glasses . The arrows show the moments of swinging the cuvette containing a cell-covered plate and rinsing the plate with fresh buffer .

Fig. 1 demonstrates preferential adhesion of the stained yeast cells to PBA-GPTMS-glasses compared to non –modified glasses and hydrolyzed GPTMS-glasses

with diol functions. This indicates a well-expressed interaction between the PBA-glasses and the cells. The

layer of adhered cells was not stable, however, and largely decomposed after 2 or 3 rinsing with fresh buffer solution.

In the second step, we modified the glasses by MPTMS and grafted DMAA-AAPBA copolymer to the modified glass plates. After precipitation of yeast cells on the plates, absorbance of 590 nm was measured to quantify the yeast cells adhered to the glasses .The arrows in Fig. 2 show the moments of swinging the cuvette containing plate and rinsing the plate with fresh buffer. Because the sodium bicarbonate buffer could not elute the yeast cells from the glass plate modified by the copolymer, 0.1 M solution of fructose in the buffer was used to remove the yeast cells from the plates. Elution of the yeast cells by 0.1 M fructose indicates the specific character of interaction between the cell carbohydrates and boronic acid groups of the grafted copolymer. It is noteworthy that population of DMAA-AAPBA-glass with the stained yeast cells is denser and more stable than that of PBA-GPTMS-glass, see Fig. 4. This difference can be ascribed to higher accessibility of the PBA-ligands attached to the polymer grafts, and / or to higher amount of these ligand groups.

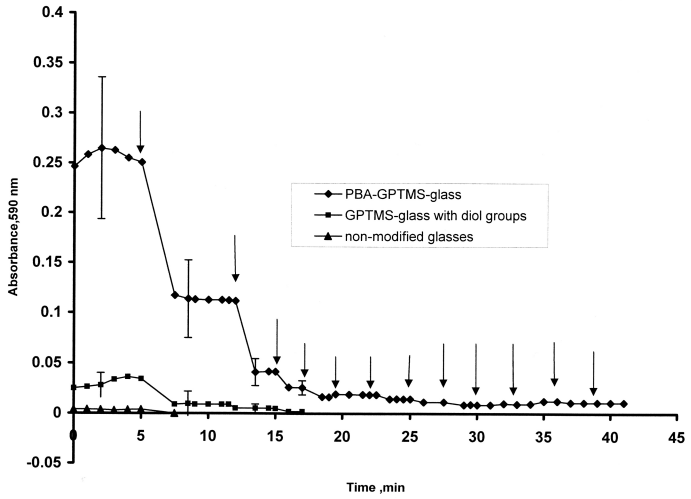

Fig.1. Comparison of absorbance of stained yeast cells adhered to PBA-GPTMS-glasses (♦), GPTMS- glasses with diol- groups (□) and non-modified - glasses (Δ). The arrows indicate moments of cuvette swinging and rinsing the plates with fresh buffer.

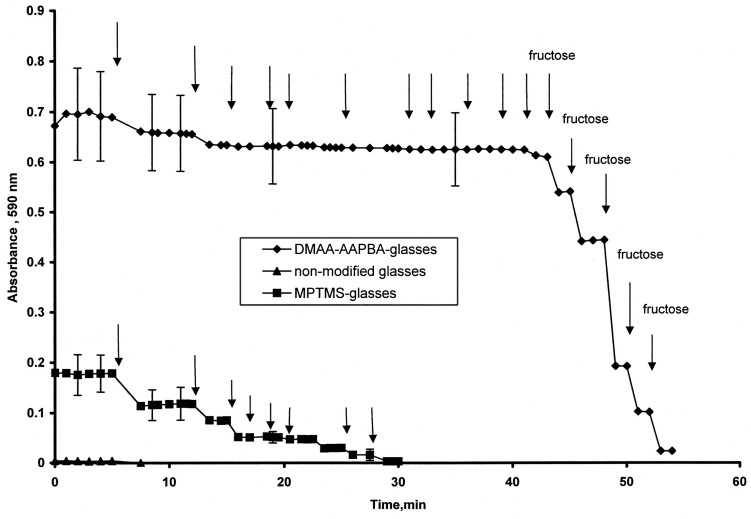

Fig.2. Comparison of DMAA-AAPBA-glasses (♦), the glasses modified with (3-mercaptopropyl)trimetoxy silane (□) , and non-modified glasses (Δ) . The arrows indicate the moments of swinging and replacing fresh buffer in cuvette containing the plates. For detachment of the yeast cells from polymer grafted glasses, 0.1 M fructose solution in 0.5 M sodium bicarbonate buffer was used.

The difference between the glass plates grafted with polymer containing PBA groups and the same polymer without PBA groups was also investigated (Fig.3). Although the plates modified with MPTMS have a slight ability to bind the yeast cells , the attachment of poly DMAA suppresses this ability.

In order to remove fructose from DMAA-AAPBA-glass plates and regenerate them for the next experiment, we put the glass plates in 0.157 M acetic acid solution (pH=3.33) for 24, 18 and 6 h and changed the solution every 6 h and used the plates again and compared the results with previous results. There were no differences between the plates. We reduced the time of regeneration in the acetic acid solution (pH 3.33) to 4 , 2 , 1 and 0.5 h before the next cycle and compared the result with the 6 h-plate (Fig.5) . So, putting the plates in the acetic acid solution for 0.5 h is acceptable manner for regeneration of the DMAA-AAPBA-glass plates after each cycle of cell adhesion and detachment.

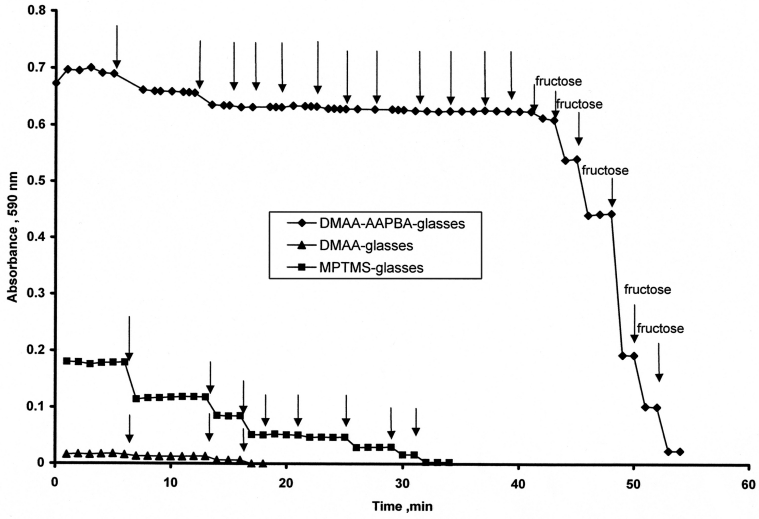

Fig.3. Comparison of DMAA-AAPBA-glasses (♦) with glasses modified with (3-mercaptopropyl)trimetoxy silane (□) , and glasses modified with poly DMAA (Δ) . The arrows indicate 3moments of cuvette swinging and rinsing the plates with fresh buffer . For detachment of yeast cells from polymer - grafted glasses , 0.1 M fructose solution in 0.5 M sodium bicarbonate buffer was used.

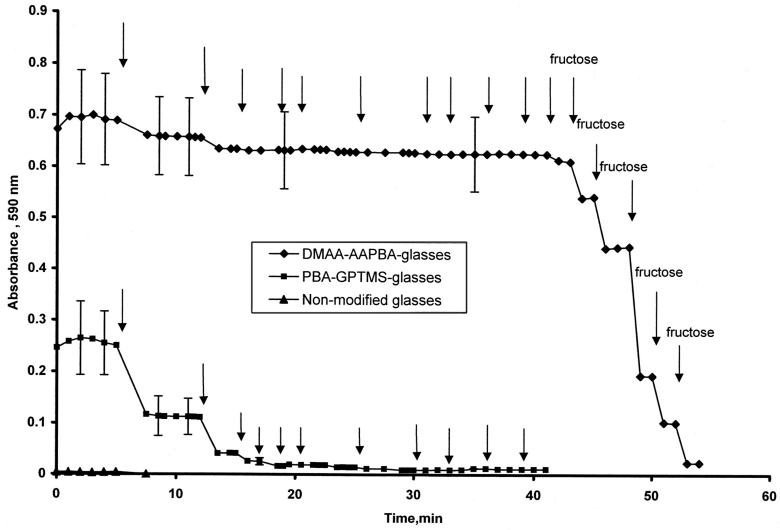

Fig. 4. illustrates the difference between non-modified glasses, PBA-GPTMS-glasses and DMAA-AAPBA glass plates. This figure shows that the amount of yeast cells, which adhere to the polymer grafted glass plates, is much higher than that observed with PBA-GPTMS-glass. Moreover, the amount of the yeast cells on the PBA-GPTMS-glass goes to zero after few washings with fresh buffer, whereas the absorbance profile describing the cell adhesion to the polymer-grafted glass shows that even at much longer swinging and numerous washings with the fresh buffer the amount of yeast cells on the polymer-grafted glass plates remains constant. Detachment of the yeast cells needs 0.1 M fructose in the rinsing buffer.

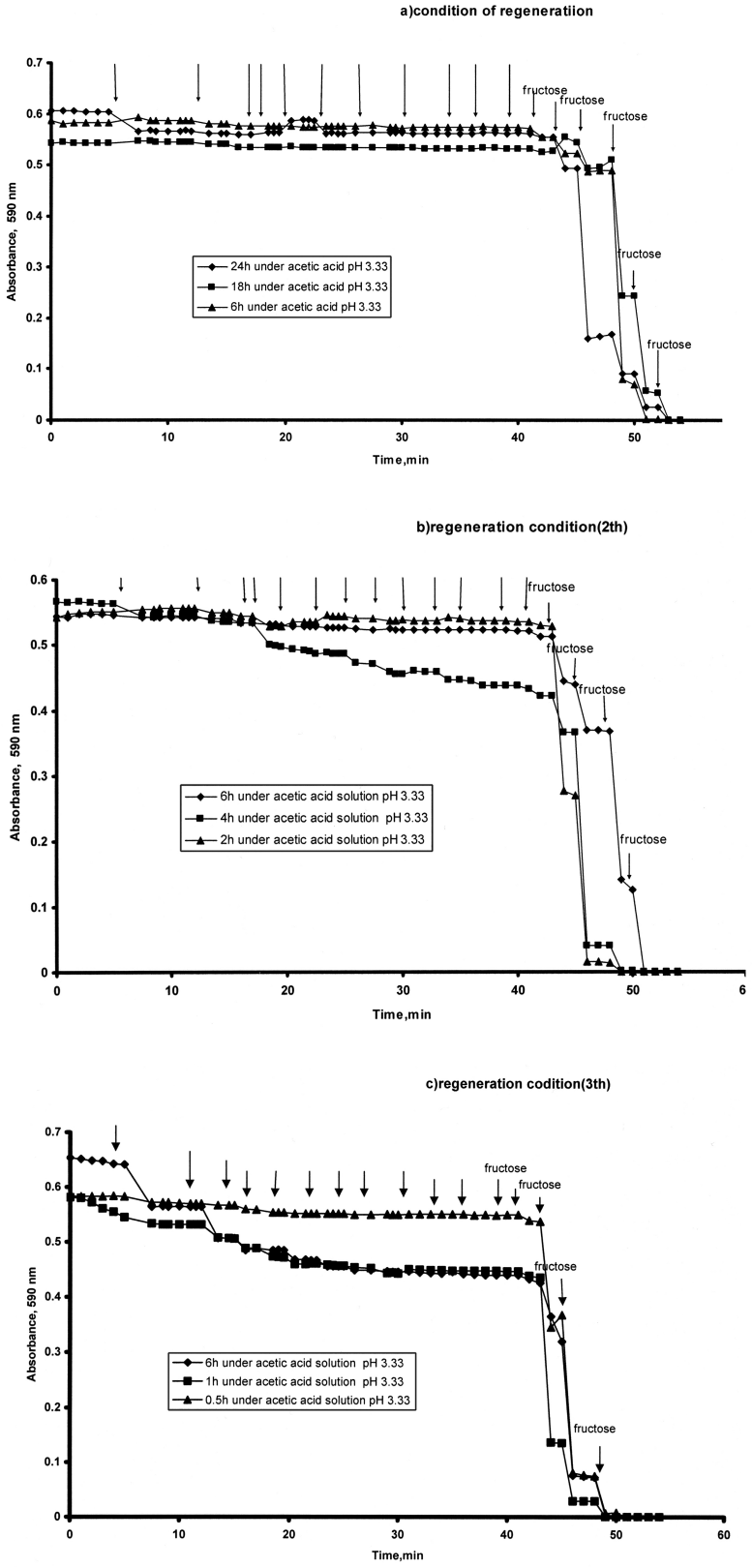

Fig. 5. For regeneration condition, each plate was placed in diluted acetic acid solution (pH=3.33) for given time (0.5 – 24 h) in order to remove adhered fructose from the plate before it is used in a new cycle. The arrows indicate moments of cuvette swinging and rinsing the plates with fresh buffer. For detachment of yeast cells from polymer grafted glasses, 0.1 M fructose solution in 0.5 M sodium bicarbonate buffer was used.

Effect of fructose concentration on the detachment of the adhered yeast cells was also studied. As follows from Fig. 6, the higher concentration of fructose (0.08 M and 0.1 M) results in the faster cell detachment, while the detachment process becomes slower at concentrations of fructose lower than 0.05M. At fructose concentration of 5 mM the complete detachment of cells was not possible.

Adhesion of yeast cells in the presence of different concentration of fructose was also investigated (Fig. 7). Because of the strong complexation between fructose and PBA, increase of fructose concentration in the system causes decrease in attachment of yeast cells on the surface of DMAA-AAPBA-glass plates. At concentration 0.01 M fructose and higher, yeast cells can not adhere to the DMAA-AAPBA-glass plates.

CONCLUSION

We have prepared glass plates grafted with boronate-containing copolymer of N,N-dimethylacrylamide. The stained yeast cells exhibited much higher affinity to DMAA-AAPBA-grafted glasses compared to non-modified glasses and the glasses containing PBA immobilized via the self-assembled layers of 3-GPTMS. Although the latter sample (PBA-GPTMS-modified glass) exhibited certain affinity towards the yeast cells, the stability of the cell layer was relatively low so that only few runs of rinsing with 0.5 M bicarbonate buffer solution (pH 9.0) resulted in disintegration of the layer. It is noteworthy that population of DMAA-AAPBA-glass with the stained yeast cells is denser and more stable than that of PBA-GPTMS-glass plates. The cell adhesive properties of DMAA-NAAPBA-glasses were certainly due to the pendant PBA groups attached to the grafted polymer chains.

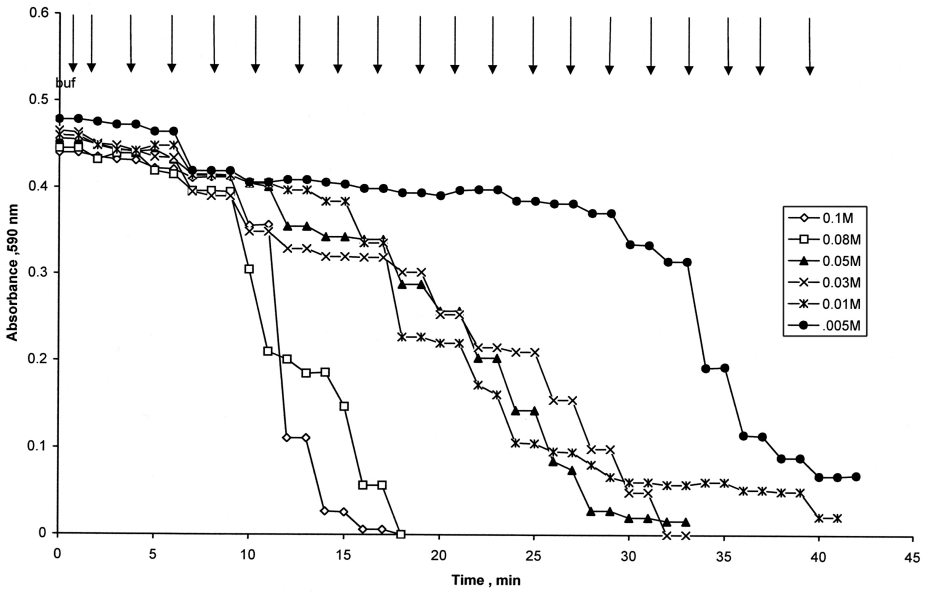

Fig. 6. Rinsing the adhered yeast cells on glass plates with fructose solution in 0.5 M sodium bicarbonate buffer pH 9.05 at different concentration of sugar . The first arrow indicates the moment of cuvette swinging and rinsing the plates with fresh 0.5 M sodium bicarbonate buffer pH 9.05 and the other arrows indicate moments of cuvette swinging and rinsing the plates with fresh fructose solution for detachment of yeast cells from DMAA-AAPBA-glass plates.

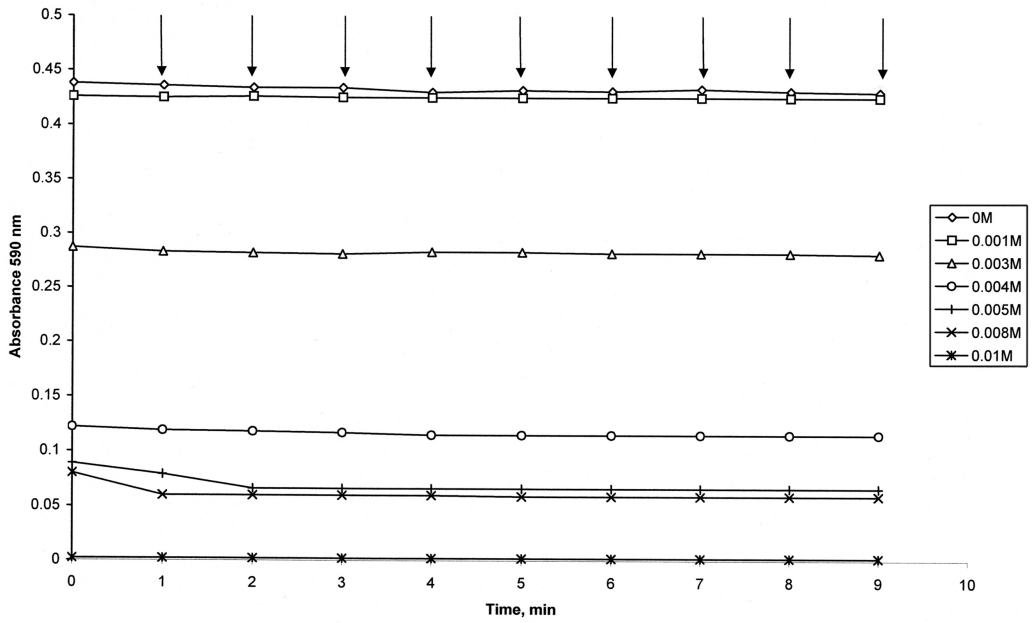

Fig. 7. Adhesion of yeast cells to the DMAA-AAPBA-glass plates in the presence of different concentration of fructose in 0.5 M sodium bicarbonate buffer, pH 9.05 . The arrows indicate moments of cuvette swinging and rinsing of the plates with fresh buffer.

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