Blood response to plasticized poly(vinyl chloride): Dependence of fibrinogen adsorption on plasticizer selection and surface plasticizer level

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The high level of plasticizer in plasticized poly(vinyl chloride) (PVC) ensures that plasticizer selection has an important influence on the suitability of PVC to function in blood-contacting applications. In this study, three types of plasticized PVC in sheet form, with di-(2-ethylhexyl)phthalate (DEHP), tri-(2-ethylhexyl)trimellitate (TEHTM) and n-butyryltri-*n*-hexyl citrate (BTHC) as plasticizer, were selected for assessment and single solute fibrinogen adsorption was utilized as an initial index of interactions with blood components. Fibrinogen adsorption behavior shows a strong dependence on the plasticizer selection, plasticizer level at the surface and the adsorption conditions, such as adsorption time and fibrinogen solution concentration. Results indicate that BTHC plasticized PVC possesses the lowest adsorption capacity in the three types of plasticized PVC, while TEHTM plasticized PVC seems to have the strongest reactivity in certain fibrinogen solution concentrations. The alteration of surface plasticizer level was achieved by a methanol-cleaning treatment with a variety of cleaning times and the fibrinogen adsorption on plasticized PVC decreases with the reduction of surface plasticizer level. The migration behavior of two phthalate esters (DEHP and TEHTM) was evaluated using UV-Spectrophotometer to determine the plasticizer level at the surfaces. In addition, the fibrinogen adsorption mechanism was examined with Freundlich adsorption modeling.

1. Introduction

The application of plasticized poly(vinyl chloride) (PVC) in medical device manufacture during the last 40 years has demonstrated its ability to satisfy the demanding requirements of the medical health care industry [1]. As one of the most commonly utilized biomaterials, plasticized PVC has dominated the market of biomedical materials for a long period due to its competitive physical properties, ease of fabrication and cost-effectiveness. At present, the biomaterials challenge is to promote a better utilization of existing biomaterials and the development of improved materials [2]. In the case of plasticized PVC as a blood-contacting biomaterial, this challenge requires an enhanced understanding of the nature of plasticized PVC, the consequences of plasticizer selection and, in particular, the surface characteristics.

Today, the most common plasticizer for PVC biomaterials is di-(2-ethylhexyl)phthalate (DEHP), which has been found to leach into blood during blood–polymer contact. As an improvement, tri-(2-ethylhexyl) trimellitate (TEHTM) is less extractable than DEHP because of its higher molecular weight (546 vs 390). It has been confirmed that a plasticizer is present at the plasticized PVC surface and the less extractable plasticizer, TEHTM, induces a more pronounced blood response because of its higher surface level [3, 4]. This is consistent with the view that the blood response to plasticized PVC is influenced by the plasticizer selection, the surface plasticizer level or surface composition and the nature of the plasticizer [5].

The objective of this study was to correlate the fibrinogen adsorption behavior on plasticized PVC with plasticizer selection. The study focused on three types of the plasticizer using DEHP, TEHTM and n-butyryltri-n-hexyl citrate (BTHC), and considered plasticizer surface level and the adsorption conditions. In addition, the adsorption mechanism was examined with Freundlich adsorption modeling.

2. Materials and methods

2.1. Materials

PVC plasticized with DEHP, TEHTM and BTHC (abbreviated as DEHP–PVC, TEHTM–PVC, and BTHC–PVC) was supplied by Ellay Inc., California, in flat sheet form. Cuprophan regenerated cellulose haemodialysis membrane (Akzo, Wuppertal, Germany) was selected as a reference control material. Human

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^{125}I-fibrinogen was purchased from Amersham Int. Inc., UK. DEHP, TEHTM plasticizers were provided by Hydro Polymers Ltd and used without further purification. Methanol was purchased from Sigma Chemical Ltd in HPLC grade.

2.2. *In vitro* fibrinogen adsorption

Protein-material contact was achieved by means of a modified 24-well incubation cell for adsorption at 25 °C [6]. Iodinated (^{125}I) human fibrinogen was reconstituted with 1 ml distilled water as a stock solution containing 0.65 mg/ml iodinated fibrinogen. For the adsorption experiment, 40 µl of the fibrinogen stock solution were added into a vial containing 20 ml of PBS buffer solution (0.05 M, pH = 7.4) and stirred at 25 °C. The initial fibrinogen solution concentration was 1.3 µg/ml. 1 ml of this solution was added into the well to contact with the material to be tested and incubated at 25 °C for the selected incubation time. The area of material in contact with protein solution was 2 cm^2. After the adsorption, protein solution was removed out and 1 ml of buffer solution was added for rinsing for 20 s. The material was taken out and placed in a vial and counted three times using a standard gamma well counter. Each type of sample was repeated three times for statistical analysis. The level of protein adsorption was calculated by dividing the retained radioactivity on the sample, corrected for background, by the specific activity of the original protein stock solution and the known area of the sample as shown in the following equation:

Amount of protein adsorption =
(radioactivity on sample cps)/(radioactivity of original stock solution cps/µg)/(area of sample cm^2)

2.3. Alteration of the plasticizer level at PVC surface by methanol solvent

This was achieved using the same modified 24-well incubation cell. 4 ml of methanol were added into the well in contact with one-side PVC surface and the contact area was 2 cm^2. After extraction for different times, the methanol was removed and the surface-cleansed PVC was tested for fibrinogen adsorption by the method described above. In addition, the surface of methanol treated sample was analyzed using Fourier transform attenuated total reflection infrared spectroscopy (ATR-FT-IR). ATR-FTIR analysis was performed on a Mattson 3000 FT-IR spectrometer, UNICAM. Spectra were recorded on an average of 25 scans at a resolution of 2 cm^{-1}. Slices of PVC samples with a smooth side were pressed against 45° zinc selenide (ZnSe). The sampling depth at 1000 cm^{-1} band is about 0.6 µm using ZnSe.

2.4. Migration behavior of DEHP and TEHTM plasticized PVC in methanol solvent

DEHP and TEHTM plasticized PVC samples (2.5 cm × 10 cm) were stored in 100 ml of methanol in a volumetric flask for different times. The DEHP and TEHTM plasticizer content in the extraction solution was monitored by UV-spectrophotometer at 274 and 290 nm, respectively [7] and the standard work plots for DEHP and TEHTM were carried out according to the method shown in Ref. [7]. Thus, the plasticizer level at surface can be evaluated from the migration results. This is discussed later.

2.5. Statistical analysis

Statistical analysis was performed with the Minitab package (version 8.0). Comparisons of different groups were carried out by analysis of variance. All statistically significant differences are reported at 95% confidence intervals ($p < 0.05$).

3. Results

3.1. Time dependence of fibrinogen adsorption to plasticized PVC

For obtaining the equilibrium adsorption time at which the adsorption reaches a steady level, the time dependence of fibrinogen adsorption on three types of plasticized PVC was investigated, as shown in Fig. 1.

From Fig. 1, it can be found that the levels of protein adsorption reach adsorption equilibrium after 20 min incubation. On this basis, the time of 20 min was selected for the adsorption investigation.

3.2. Plasticizer selection

The fibrinogen adsorption on three types of plasticized PVC with and without PBS buffer solution soaking overnight is shown in Fig. 2 in order to determine the influence of plasticizer selection on fibrinogen adsorption. Results indicate that there is no significant difference between DEHP and BTHC plasticized PVC ($p = 0.47 > 0.05$) but TEHTM plasticized PVC shows the highest binding ability for fibrinogen. For the hydrophilic Cuprophan control, an overnight soaking led to a reduction of fibrinogen adsorption ($p = 0.0001 < 0.05$), but for plasticized PVC no significant difference was found between soaking and not soaking as shown in the statistical analysis for DEHP–PVC ($p = 0.28 > 0.05$), TEHTM–PVC ($p = 0.30 > 0.05$) and BTHC–PVC ($p = 0.06 > 0.05$).

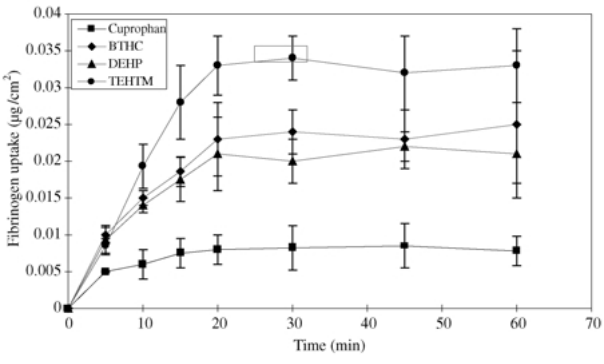

Figure 1 Time dependence of fibrinogen adsorption to plasticized PVC; fibrinogen concentration is 1.3 µg/ml.

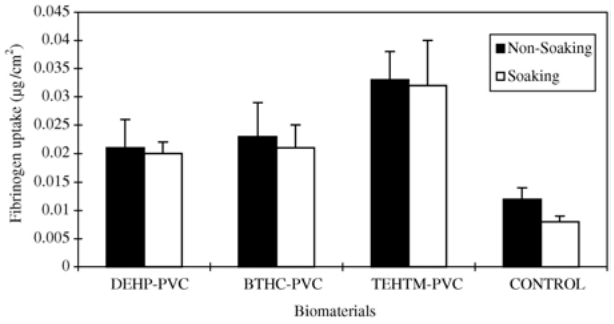

Figure 2 Influence of plasticizer selection of plasticized PVC onto fibrinogen adsorption; fibrinogen concentration is 1.3 μg/ml; adsorption time is 20 min ($n = 9$); soaking solution: pH 7.4/0.05 M PBS. There are no significant differences for plasticized PVC between PBS soaking and PBS non-soaking ($p > 0.05$).

This indicates that it is not necessary to soak PVC sheet overnight prior to protein adsorption because of the hydrophobic nature of the PVC surface.

3.3. Influence of fibrinogen solution concentration on fibrinogen adsorption

The adsorption isotherms of plasticized PVC in a range of fibrinogen concentration, from 0.0 to 2.0 μg/ml are shown in Fig. 3.

From Fig. 3, it can be seen that BTHC plasticized PVC possesses the lowest adsorption capacity when the fibrinogen concentration is lower than 1.3 μg/ml. For DEHP plasticized PVC, it can reach the maximum adsorption capacity at 0.6 μg/ml of fibrinogen concentration while the adsorption capacity for TEHTM plasticized PVC has a marked increase with the increase of fibrinogen concentration. There are significant differences for TEHTM plasticized PVC ($*p < 0.05$) at selected fibrinogen concentration compared to both DEHP and BTHC plasticized PVC, while at a certain point, there is no significant difference between DEHP and BTHC plasticized PVC. This indicates that fibrinogen concentration has a great influence on the adsorption pattern of plasticized PVC with different

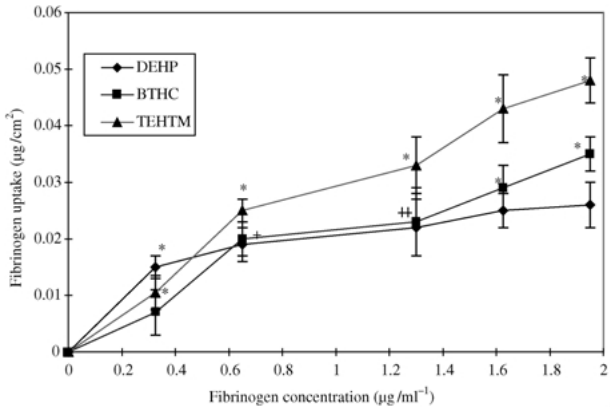

Figure 3 Influence of fibrinogen concentration onto adsorption on plasticized PVC, adsorption time is 20 min ($n = 9$). There are significant difference at any selected concentration for PVC–TEHTM compared to PVC–BTHC or PVC–DEHP ($*p < 0.05$). There is significant difference at 0.65 μg/ml ($+p = 0.036 < 0.05$) for PVC–BTHC compared to PVC–DEHP while there is no significant difference at 1.3 μg/ml ($++p = 0.47 > 0.05$) for it compared to PVC–DEHP.

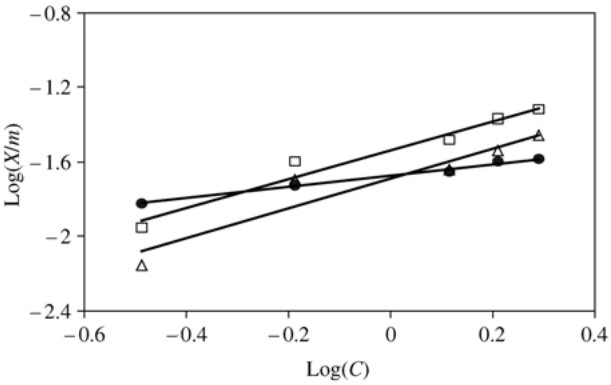

Figure 4 The Freundlich adsorption isotherms of plasticized PVC: □ PVC–TEHTM, • PVC–DEHP, △ PVC–BTHC .

TABLE I Freundlich physical adsorption parameters of plasticized PVC

No.	Adsorption parameters		
	$K_{25\,^\circ C}$	n	r*
DEHP–PVC	0.0257	0.2235	0.9716
TEHTM–PVC	0.0284	0.8194	0.9891
BTHC–PVC	0.0194	0.8708	0.9977

* r: regression coefficient.

plasticizers. From these results, the adsorption mechanism can be modeled with the Freundlich adsorption isotherm.

3.4. Freundlich adsorption modeling

According to Freundlich adsorption isotherm (see Equations 1 and 2), the modeling is achieved:

$$x/m = KC^n \tag{1}$$

where x/m is the fibrinogen up-take amount (mg/cm^2); K is the adsorption constant and n is the adsorption index; C is the corresponding fibrinogen concentration.

From Equation 1, the Freundlich adsorption isotherm can be derived to Equation 2:

$$\log x/m = \log K + n \log C \tag{2}$$

Plotting log x/m with log C, the Freundlich adsorption isotherms for plasticized PVC are shown in Fig. 4 and the adsorption parameters are presented in Table I.

Results indicate that these three types of plasticized PVC fit Freundlich adsorption isotherm resulting from the linear least-squares fits. DEHP–PVC shows the lowest adsorption index while BTHC–PVC has the lowest adsorption constant.

3.5. Migration behavior of phthalate plasticized PVC (DEHP–PVC and TEHTM–PVC)

Figs 5 and 6 demonstrate the DEHP and TEHTM migration behavior from plasticized PVC in methanol as detected by UV-spectrophotometer at selected intervals.

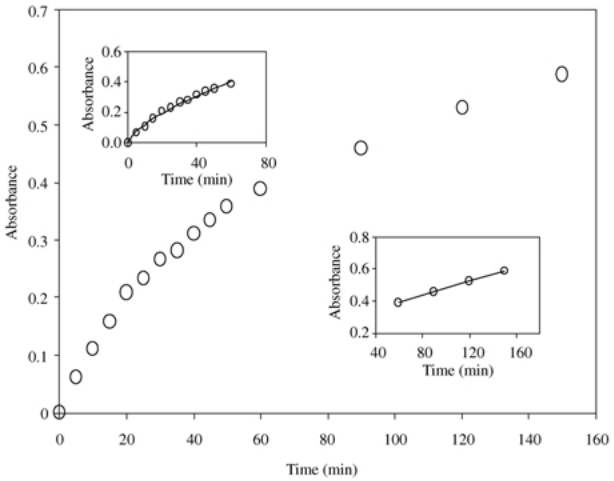

Figure 5 Migration behavior of DEHP from DEHP–PVC in methanol solvent.

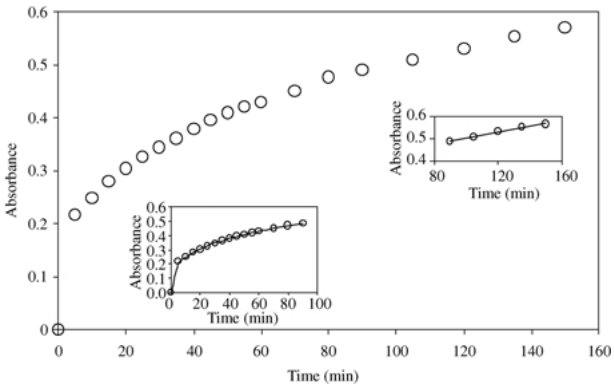

Figure 6 Migration behavior of TEHTM from TEHTM–PVC in methanol solvent.

Results indicate that the surface phthalate is removed by a surface dissolution process reflected in the figures as an asymptotically increased curve at the initial stage. This is followed by the bulk phthalate diffusion through the surface according to Fick's Law of diffusion, which is shown as linear. The data of DEHP migration from DEHP plasticized PVC for these two stages fit two formula: Dissolution stage: $Y = 0.02703X^{0.6596}$; Diffusion stage: $Y = 0.002237X + 0.2564$. For TEHTM plasticized PVC, dissolution stage: $Y = 0.1236X^{0.3047}$; Diffusion stage: $Y = 0.3647X + 0.001377$, in which, Y is UV absorbance for plasticizer while X is the time of the methanol treatment. The critical point at the crossing of the two lines is regarded as the completion of surface removal of phthalate by dissolution. At this point, the surface plasticizer level is considered to be zero and the extracted amount of plasticizer at this time represents the original surface plasticizer level. Based on this method, the surface plasticizer level at varied extraction time can be evaluated according to following Equation 3:

$$L_t = L_0 - L_{Et} \tag{3}$$

where, L_0 is the extracted amount of plasticizer at the critical point; L_{Et} is the extracted amount of plasticizer at time interval; L_t is the surface plasticizer level at time interval. Thus, the relevance of surface plasticizer level with methanol extraction time can be obtained as shown

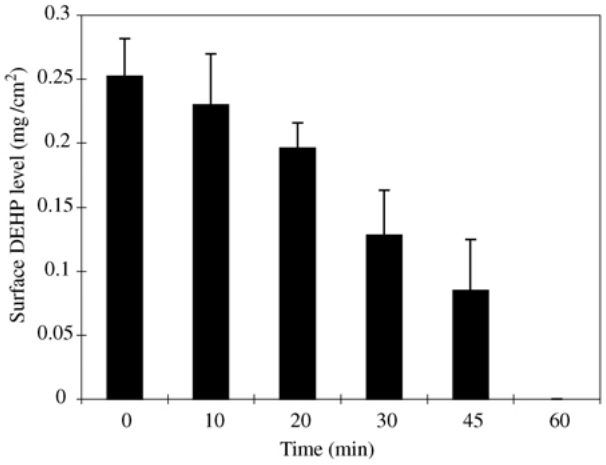

Figure 7 Relevance of surface DEHP level with methanol extraction time.

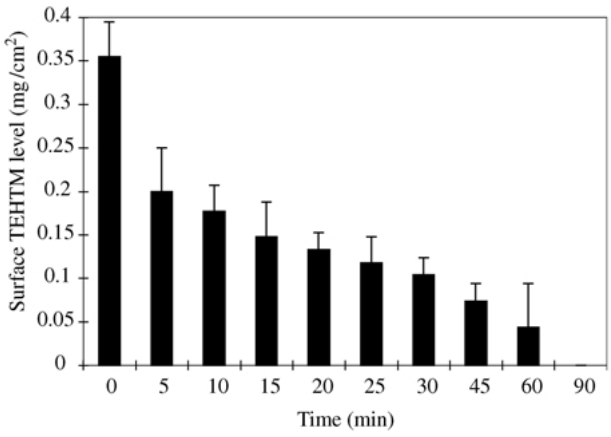

Figure 8 Relevance of surface TEHTM level with methanol extraction time.

in Figs. 7 and 8 and the critical time points are 90 min for TEHTM and 50 min for DEHP.

3.6. Relevance of surface plasticizer level with fibrinogen adsorption

Figs. 9 and 10 demonstrate the influence of DEHP and TEHTM surface level on the fibrinogen adsorption induced by plasticized PVC. Results indicate that, with an increase in surface plasticizer level, fibrinogen adsorption capacity increases linearly. For TEHTM, a long extraction time leads to an increase of adsorption, which might be attributed to surface roughness [5].

BTHC, a citrate type plasticizer, has no UV absorbed groups in its chemical structure. Therefore, the surface level cannot be measured by the above-mentioned technology. However, the relevance of fibrinogen adsorption with the extraction time (or surface cleaning time) is shown to have the similar pattern to those DEHP and TEHTM plasticized PVC. The pattern is shown in Fig. 11.

3.7. Surface analysis of plasticized PVC using ATR-FTIR

In this study, attenuated total reflection FTIR (ATR-FTIR) was employed for surface characterization of

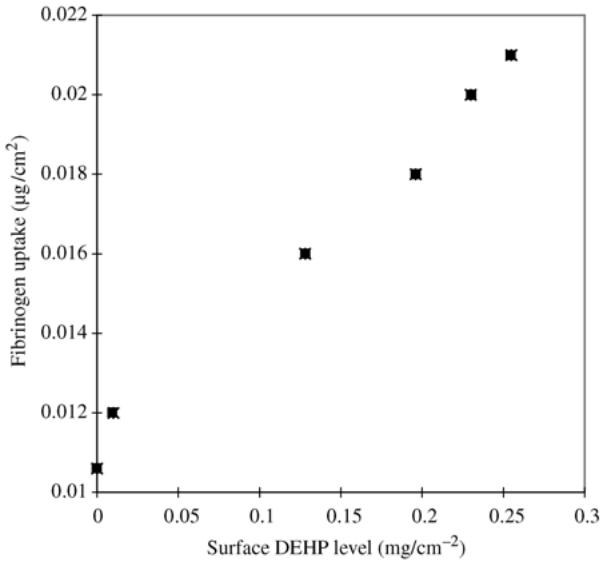

Figure 9 Relevance of surface DEHP level with fibrinogen adsorption on DEHP–PVC.

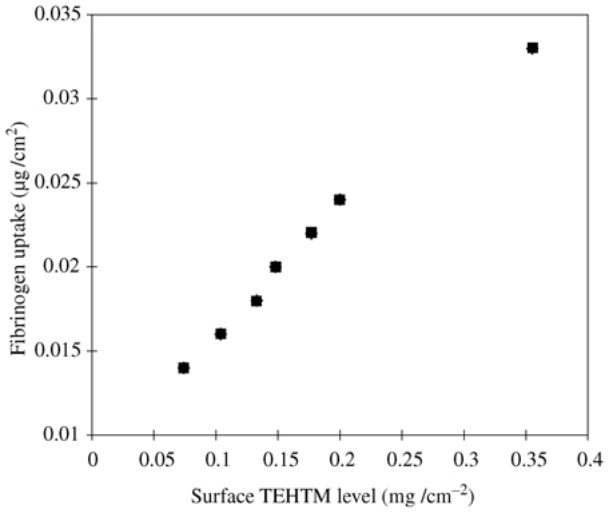

Figure 10 Relevance of surface TEHTM level with fibrinogen adsorption on TEHTM–PVC.

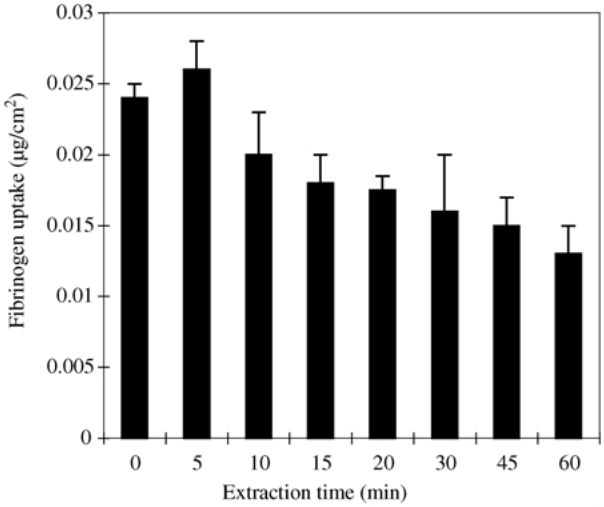

Figure 11 Relevance of extraction time on fibrinogen adsorption on BTHC–PVC.

plasticized PVC. From typical ATR-FTIR spectra as shown in Figs. 12–14, it can be seen that the reduced plasticizer surface level will lead to a reduced intensity at νC=O, νC–O and νC–H vibration absorption peaks for DEHP and TEHTM. For BTHC, the absorbance intensity of O–C = O and C–H at 1737 cm^{-1} and 2930 cm^{-1} is reduced with an increase in methanol surface treatment time. Since the plasticized PVC surface is dominated by plasticizer, the absorption bands of ATR-FTIR spectra of plasticized PVC are mainly assigned to plasticizer instead of PVC [5].

4. Discussion

Protein adsorption is an early event after contact of blood with foreign surfaces. The protein adsorption pattern has been found to correlate with surface properties of biomaterials [8]. For plasticized PVC biomaterials, plasticizer at the surface must play an important role for its blood compatibility, which can be evaluated with fibrinogen adsorption for an initial *in vitro* study.

4.1. The influence of plasticizer nature

In the selected three types of plasticizer, DEHP and TEHTM have the same chemical nature, with only a difference in molecular weight. However, BTHC, has a different chemical nature compared to DEHP and TEHTM. Fig. 15 shows the chemical structures of the three plasticizers.

From the fibrinogen adsorption results, it can be found that BTHC possesses the lowest adsorption capacity when the fibrinogen concentration is below 1.6 μg/ml. For TEHTM and DEHP, TEHTM plasticized PVC has a higher reactivity to bind fibrinogen but it is difficult to infer that this can be attributed to their different molecular weights. Similar results have been observed in DEHP and TEHTM plasticized PVC tubing previously and it was concluded that the higher blood response of TEHTM plasticized PVC was related to a higher surface level of TEHTM [4].

4.2. The influence of surface plasticizer level

For an enhanced understanding of the relationship between fibrinogen adsorption and surface plasticizer level, a surface cleaning treatment with methanol was utilized to achieve an alteration of surface plasticizer level which was determined using UV-spectrophotometer. It is clear from the experimental results that plasticized PVC with a cleaned surface, no matter which type of plasticizer it contained, has a similar low fibrinogen adsorption capacity about 0.010–0.014 μg/cm^2. With the increase of plasticizer level at the surface, fibrinogen adsorption capacity increases. For DEHP and TEHTM plasticized PVC, if the fibrinogen adsorption capacity is based on surface level, a similar result could be obtained no matter how long the surface was treated with methanol and what types of plasticizer were utilized (Table II).

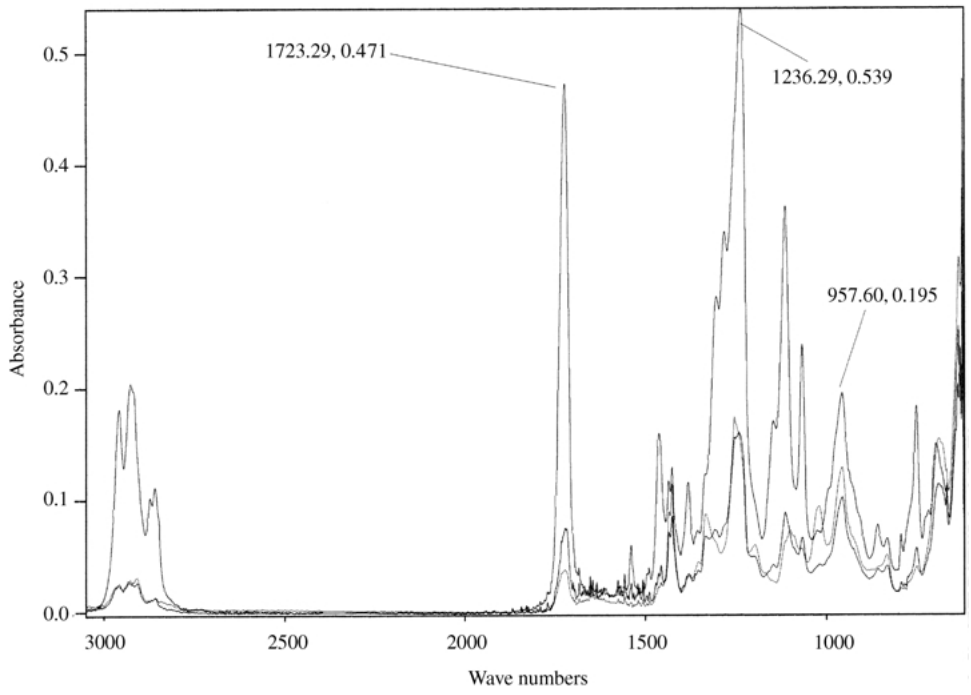

Figure 12 ATR-FTIR surface characterization of DEHP–PVC with increased methanol surface treatment time (0, 10 and 45 min).

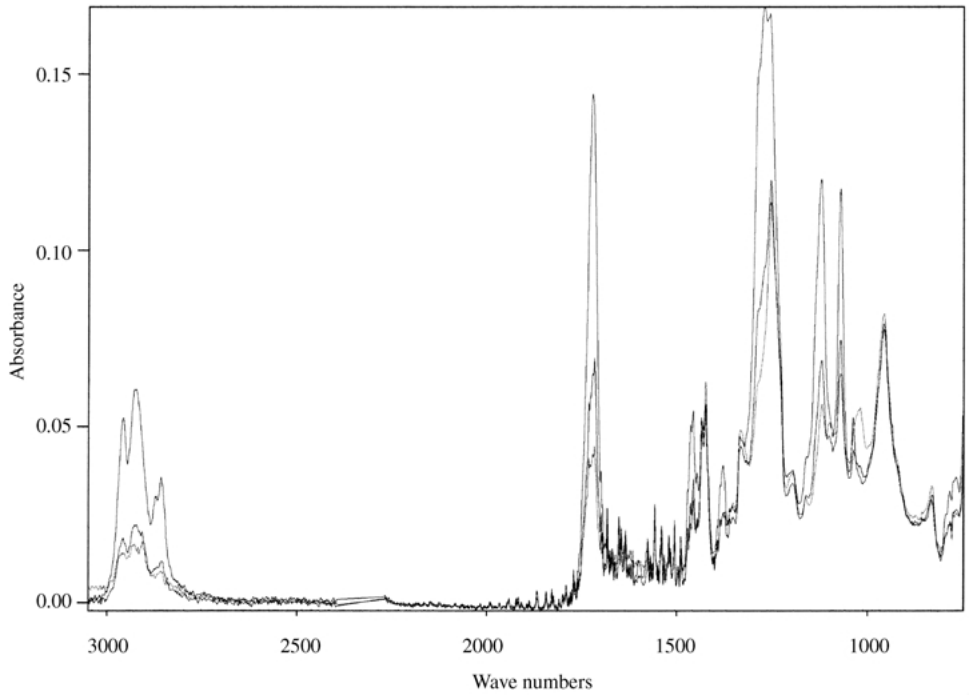

Figure 13 ATR-FTIR surface characterization of TEHTM–PVC with increased methanol surface treatment time (0, 10 and 90 min).

4.3. The migration behavior of phthalate plasticized PVC and the surface characterization of plasticized PVC

From Figs. 5 and 6, it also can be found that TEHTM plasticized PVC requires longer extraction time to reach the critical point than DEHP plasticized PVC. This indicates that TEHTM is less extractable than DEHP because of its higher molecular weight. Surface characterization of plasticized PVC using ATR-FTIR indicates that methanol surface treatment causes a reduced intensity of absorption bands at 2900–2880, 1720, 1122 and 1072 cm^{-1}, which resulted from a reduced DEHP, TEHTM and BTHC level. At the same time, the increase of intensity at 1425 and 958 cm^{-1} indicates the increase of PVC concentration at the surface. The ratio of peak integrated area at 1726 cm^{-1} to that at 958 cm^{-1} ($A_{1726\,cm^{-1}}/A_{958\,cm^{-1}}$) can be used as an index to check the surface distribution of PVC and plasticizer. In our study, it was found that after 30–45 min methanol treatment, a steady value of $A_{1726\,cm^{-1}}/A_{958\,cm^{-1}}$ was achieved, which indicates a clean surface is obtained. For TEHTM plasticized PVC, after 60 min of methanol treatment, the value tends to be steady, while it takes about 45 min to get a clean surface for BTHC plasticized PVC (Fig. 16).

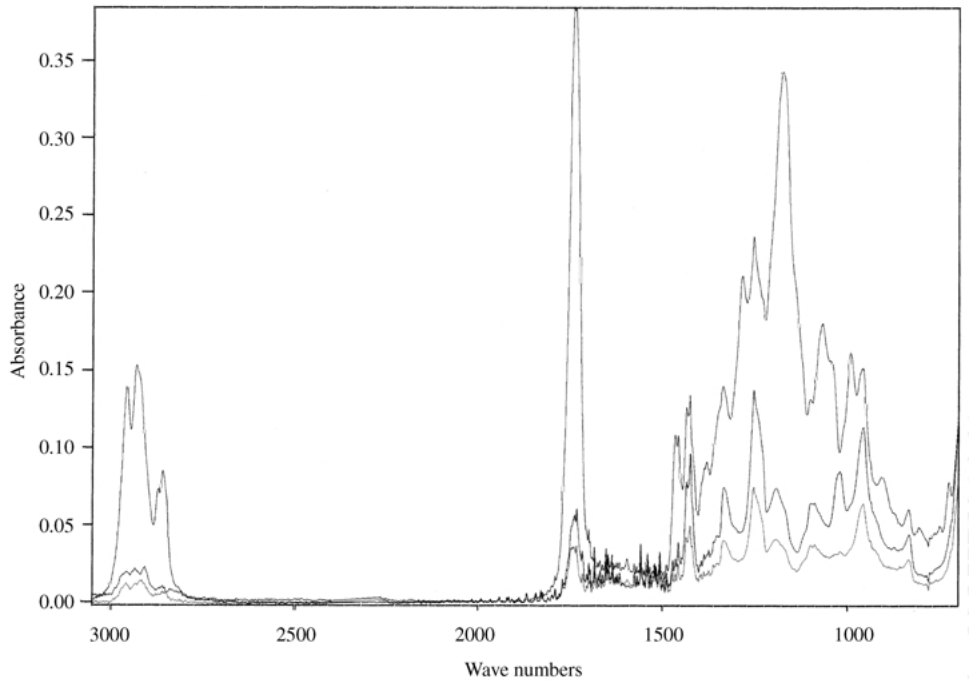

Figure 14 ATR-FTIR surface characterization of BTHC–PVC with increased methanol surface treatment time (0, 20 and 60 min).

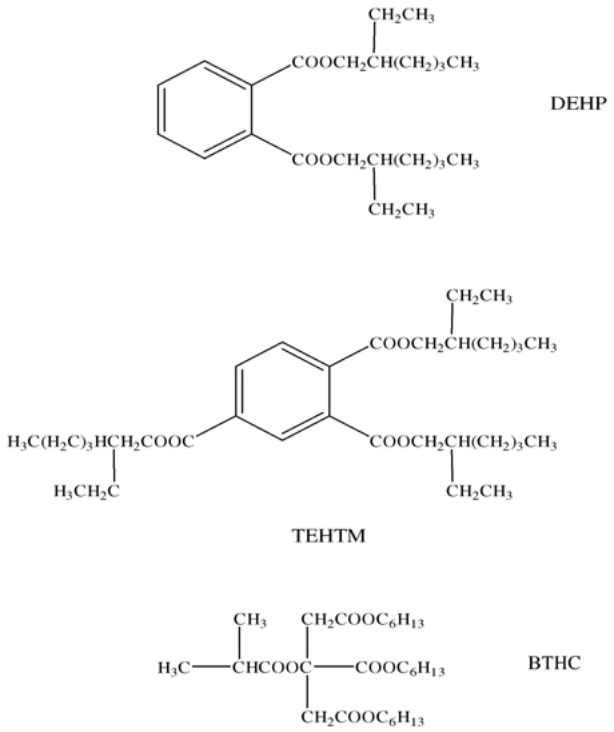

Figure 15 Chemical structures of three types of plasticizers.

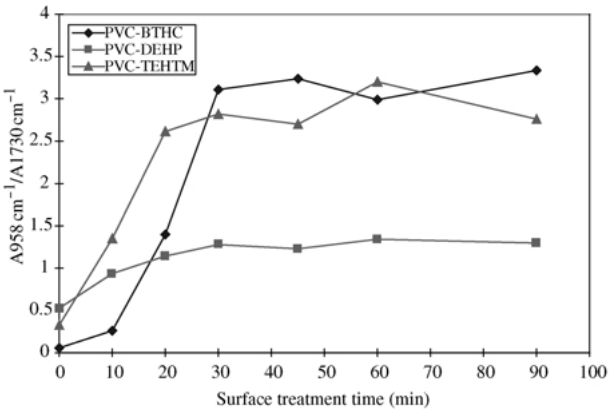

Figure 16 Correlation of $A_{958\,cm^{-1}}/A_{1726\,cm^{-1}}$ with methanol surface treatment time.

TABLE II Evaluation of influence of surface plasticizer level onto fibrinogen adsorption

	Extraction time (min)	DEHP–PVC	TEHTM–PVC
Fibrinogen uptake (μg/cm^2)	0	0.092	0.093
	10	0.089	0.133
Surface plasticizer level (mg/cm^2)	20	0.094	0.105
	30	0.117	0.120

4.4. The adsorption mechanism

The Freundlich adsorption modeling of fibrinogen adsorption on plasticized PVC reveals that the isotherms of adsorption are mono-molecular layer adsorption, which is in agreement with the adsorption isotherms of fibrinogen adsorption on glass and polyethylene [9]. However, it indicates that there is a different sites which causing different adsorption heat at the surface due to chemical distribution of plasticizer and poly(vinyl chloride) (PVC), which is quite different from the Langmuir isotherm of PVC [10]. The parameter α can be thought of as being indicative of the intensity of adsorption and the parameter K as being proportional to the capacity of adsorption [11]. The slope α is higher for fibrinogen adsorption on DEHP–PVC indicates the surface is of lower hydrophobicity than that of

TEHTM–PVC and DEHP–PVC while the highest K for TEHTM–PVC indicates its highest reactivity to fibrinogen.

5. Conclusions

The work presented here describes fibrinogen adsorption on three types of plasticized PVC in order to correlate this with plasticizer selection and surface properties. Results indicate that the plasticizer level at the PVC surface has a strong influence on fibrinogen adsorption. The migration behavior of DEHP and TEHTM was determined and a UV-spectrophotometer employed to evaluate the plasticizer level at PVC surface, which offers a straightforward simple approach for the measurement of surface phthalate plasticizer level. In addition, surface characterization of plasticized PVC using ATR-FTIR provides the evidence of the plasticizer surface level changes during methanol treatment. On the basis of these results, an enhanced understanding of plasticized PVC as blood-contacting biomaterial can be achieved in terms of the nature of plasticizer, the plasticizer surface level and protein adsorption conditions.

References

1. C. R. BLASS, *Med. Dev. Technol.* **3** (1992) 32.
2. J. M. COURTNEY, N. M. K. LAMBA, J. D. S. GAYLOR, C. J. RYAN and G. D. O. LOWE, *Artif. Organs* **19** (1995) 852.
3. X. B. ZHAO, P. WILL, H. Q. YIN, A. ESPOSITO and J. M. COURTNEY, *ibid.* **21** (1997) 253.
4. H. Q. YIN, N. M. K. LAMBA, J. D. S. GAYLOR, J. M. COURTNEY, C. R. BLASS and G. D. O. LOWE, *Int. J. Artif. Organs* **17** (1994) 433.
5. X. B. ZHAO, PhD Thesis, University of Strathclyde, UK (1999).
6. J. YU, N. M. LAMBA, J. M. COURTNEY, T. L. WHATELEY, J. D. GAYLOR, G. D. O. LOWE, K. ISHIHARA and N. NAKABAYASHI, *Int. J. Artif. Organs* **17** (1994) 499.
7. European Pharmacopoeia, P153 (1997).
8. J. L. BRASH and S. UNIYAL, *J. Polym. Sci. Polym. Symposium* **66** (1979) 377.
9. J. L. BRASH and V. J. DAVIDSON, *Thromb. Res.* **9** (1976) 249.
10. H. Y. K. CHUANG, W. F. KING and R. G. MASON, *J. Lab. Clin. Med.* **92** (1978) 483.
11. A. W. ADMSON, ''Physical Chemistry of Surfaces'', 3rd edn. (John Wiley, New York, 1976).

Received 2 July 2002
and accepted 4 February 2003