

ADSORPTION AND RELEASING PROPERTIES OF BEAD CELLULOSE

A. Morales^{a,d}, E. Bordallo^b, V. Leon^b and J. Rieumont^{c*}

^a*Faculty of Chemistry and Pharmacy, Central University of Las Villas. Camajuani km 5 1/2 Villa Clara, Santa Clara 54830, Cuba*

^b*Research-Production Plant Cuba 9, Quivicán, Havana, Cuba*

^c*Faculty of Chemistry, Havana University, Ave. Zapata and G, Havana 10400, Cuba*

^d*Departament of Drug Design, Chemical Bioactive Center, Central University of Las Villas. Camajuani km 5 1/2 Villa Clara, Santa Clara 54830, Cuba*

Abstract The adsorption of some dyes on samples of bead cellulose obtained in the Unit of Research-Production “Cuba 9” was studied. Methylene blue, alizarin red and congo red fitted the adsorption isotherm of Langmuir. Adsorption kinetics at pH = 6 was linear with the square root of time indicating the diffusion is the controlling step. At pH = 12 a non-Fickian trend was observed and adsorption was higher for the first two dyes. Experiments carried out to release the methylene blue occluded in the cellulose beads gave a kinetic behavior of zero order. The study of cytochrome C adsorption was included to test a proteinic material. Crosslinking of bead cellulose was performed with epichlorohydrin decreasing its adsorption capacity in acidic or alkaline solution.

Keywords Bead cellulose, Adsorption, Isotherms, Kinetics of adsorption, Dyes

INTRODUCTION

Bead cellulose has usually been prepared from cellulose solutions of viscose^[1–4], calcium thiocyanate^[5], *N,N*-dimethyl acetamide^[6] or from cellulose derivatives soluble in organic solvents such as di or tri-acetate^[7]. Also, cellulose beads can be made from microcrystalline cellulose^[8] or cellulose fibre fragments^[9]. Cellulose used in fibrous or microgranular form is not suitable as the raw material for packing materials in chromatography or for carriers in sustained drug release^[10]. For the latter purpose the amount of drug that it is possible to load into a bead is proportional to the pore volume. Thus, it is essential to start with a material of high porosity for efficient drug loading. The question of what type of solvent should be used to swell the three-dimensional skeletal fibre system of the cellulose bead to improve the loading and how strong is the interaction of the drug with the cellulosic matrix are essential for a good performance. Also, chemical affinity is a disadvantage for fractionation by molecular sieving. There is strong evidence that adsorption centres usually consist of carboxylic groups even present at very low density^[11]. In this context the interaction of dyes with cellulose can serve as a guide. Methylene blue is a “direct” dye for polypeptides such as wool and silk but not for cellulose fibers. Alizarin red possesses the alizarin moiety that behaves as a mordant dye, needing a process to link the dye to the fiber, but also a sulfonic group. Congo red is a “direct” dye for cotton with amine and sulfonate groups, which can interact with the cellulose ether and hydroxyl groups^[12].

The aim of this study was to use cellulose beads obtained in the Research-Production Plant Cuba 9 from Cuban wood pulps and determine its sorption and releasing properties for some dyes in order to test it as a matrix for controlled release or for chromatographic purposes. Also, the adsorption capacity with cytochrome C was tested.

* Corresponding author: Jacques Rieumont, E-mail: jacques@fq.uh.cu

Received May 7, 2003; Revised November 6, 2003; Accepted November 17, 2003

MATERIALS AND METHODS

Chemicals

Methylene blue, congo red, alizarin red, epichlorohydrin, methylene dichloride and cytochrome C were used as received from suppliers. The Research-Production Plant Cuba 9 supplied bead cellulose. Phosphate buffered solutions were prepared by standard procedures.

These beads are spherical ranging between 40–200 μm in diameter as shown in the micrograph of Fig. 1.

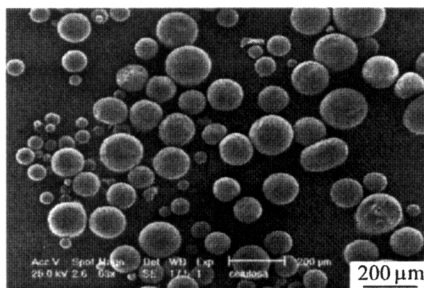


Fig. 1 Micrograph of cellulose beads

Spectrometric Determinations

All UV-V spectrometric measurements were carried out using a Jasco V-530 spectrometer coupled to a computer. Lambert-Beer plots gave the following absorption coefficients: methylene blue (51000) at 663 nm, alizarin red (2700) at 417 nm, congo red (15300) at 500 nm. All measurements were taken with less than 5% of error.

Isotherms

Samples of bead cellulose (0.500 g) Celef M-27 were put in several flasks containing 10 mL of dye at different concentrations. Flasks were under stirring for 48 h at room temperature. The amount of dye adsorbed was estimated from the initial and final spectrometric absorption.

Kinetics of Adsorption

Absorption was automatically recorded at a fixed wavelength. Cuvettes (1 cm thickness) containing 50 mg of cellulose beads were filled with dye solution of known concentration, stirred and absorption recorded at the maximum wavelength of the dye.

Kinetics of Release

A mixture of bead cellulose, dye and small amount of methylene dichloride were thoroughly stirred and the solvent evaporated. An amount of the dry material was put in a cuvette, filled with water at a fixed pH and the absorbance recorded with time.

Crosslinking Technique

A mixture of 25 mL of a 18% sodium hydroxide solution and 25 mL of isopropyl alcohol was added to a sample of 5 g of bead cellulose Celef M-27, previously dried at high vacuum. The mixture was stirred at room temperature for one hour and later 1 or 2 mL of epichlorohydrin was added. The mixture was heated at 60°C for 1 h, filtered, neutralized and thoroughly washed with water and ethanol, dried under high vacuum and kept under vacuum in a dessicator. The sphericity of particles was not changed by the crosslinking procedure when observed under the optical microscope.

Sedimentation Volume

A dried sample of bead cellulose Celef M-27 (0.500 g) was put into a centrifuge tube, filled with 8 mL of distilled water and left overnight. The volume occupied by the solid was reported as sedimentation volume.

Swelling

Centrifuge tubes used above were centrifuged several times for 10 min at 2250 r/min to constant weight. Swelling was reported as volume of water per gram of cellulose.

Percent of Porosity

Porosity was determined from the swelling measurement using the reported equation^[13].

$$\%P = \frac{w_{\text{agua}} \cdot \rho_{\text{agua}}}{(w_{\text{agua}} \cdot \rho_{\text{agua}}) + (w_{\text{celulosa}} \cdot \rho_{\text{celulosa}})} \cdot 100 \quad (1)$$

$\%P$ is the percent of porosity, w_{agua} is the weight of water, ρ_{agua} is the density of water, w_{celulosa} is the weight of cellulose and ρ_{celulosa} is the density of cellulose.

Cellulose density was taken from Ref. [14] as 1.62 g/mL and assumed to be equal for the bead cellulose.

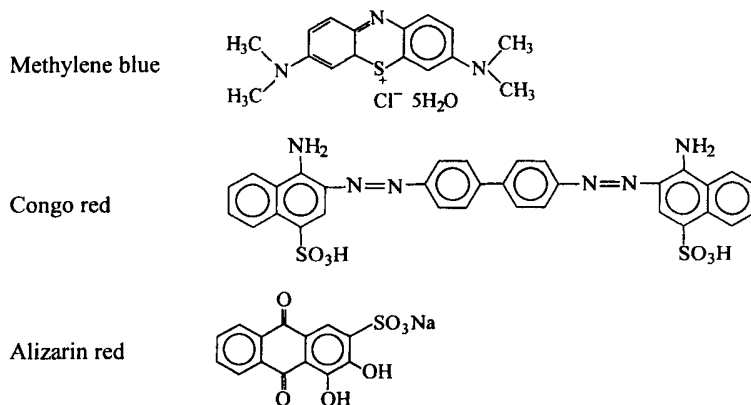
Morphological Analyses

Micrographs were performed by scanning electron microscopy (Jeol JSM-840 A Technics Co, Ltd, Tokyo) and the particles were previously metallized with gold.

RESULTS AND DISCUSSION

Isotherms for Dye-Bead Cellulose Systems

Structures of the three dyes used are the following:



In spite of different structures, the three dyes used gave quite good linear Langmuir-type isotherm plots at pH = 6 (see Eq. 2).

$$Q^{-1} = (CeBQm)^{-1} + Qm^{-1} \quad (2)$$

$1/Q$ (mg of dye adsorbed per gram of bead cellulose) versus $1/Ce$ (initial molar concentration of the dye) at pH = 6 and 25°C; Qm is maximum dye adsorption; B is isotherm constant

In Table 1 fittings and isotherm constants B and Qm are shown. Typically, this type of behavior occurs when dyes are adsorbed by ionic polymers or polymers containing polar groups^[15, 16]. Attempts to fit data to Freundlich-type isotherm did not succeed. Methylene blue, that is not a direct dye for cellulose, gave a lower saturation adsorption (Qm) as expected. The value of Qm expressed as carboxylic equivalent is about 6×10^{-6} equiv/g. For a cellulosic matrix with low adsorptivity that value should be less than 10^{-5} equiv/g^[5].

Adsorption Rate on Cellulose Beads

Adsorption was recorded over time by measuring the visible absorption at two different pH. In Fig. 2 and 3 the results for methylene blue and alizarin red are shown. A similar adsorption trend was observed in spite of different structure of these dyes. The first one possessing a phenazo-thionium chloride structure and the second one being an anthraquinone structure with a sulfonate group. At pH = 6 the linear plot of optical density (D_t/D_0)

versus $t^{0.5}$ indicates that the process is ruled by a pure Fickian diffusion and is not dependent on swelling kinetics^[15]. At pH = 12 the rate of adsorption is increased and a non-Fickian behavior is observed. At this pH some of the carboxylic groups of the cellulose beads become ionized and the volume chain conformation increases, making the adsorption easier because more groups are available for interaction. Furthermore, methylene blue adsorption at pH = 12 showed a linear trend after the non-linear part of the curve. It seems that the conformational changes that occur on swelling at the beginning are disturbing the rate but later the rate is again under diffusion control.

Table 1. Linear Langmuir plots*

Dye	$B \times 10^{-4}$	$Q_m \times 10^5$	R
Methylene blue	5.3	0.6	0.991
Congo red	1.6	2.3	0.985
Alizarin red	0.4	4.4	0.994

* Q_m and B isotherm constant, R : correlation coefficient

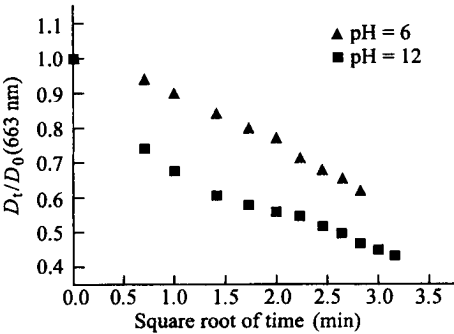


Fig. 2 Adsorption of methylene blue (Molar initial concentration 2.6×10^{-6}) in samples of 50 mg of bead cellulose without crosslinking
Plot of absorbance versus square root of time in minutes (D_t : absorbance at time t ; D_0 : initial absorbance).

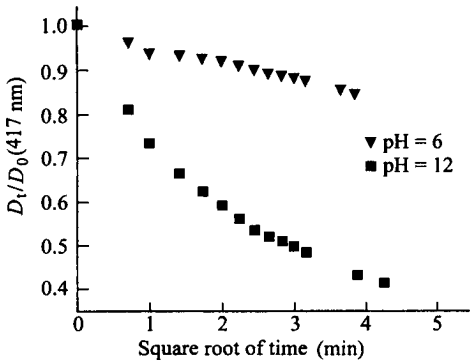


Fig. 3 Adsorption of alizarin red (Molar initial concentration 7.9×10^{-5}) in samples of 50 mg of bead cellulose without crosslinking
Plot of absorbance versus square root of time in minutes (D_t : absorbance at time t ; D_0 : initial absorbance).

A different adsorption pattern was observed for the more effective dye for cellulose *i.e.* Congo red (see Fig. 4). At pH = 12 the adsorption rate was slower than that at pH = 6 and both curves are linear indicating that conformational changes did not affect the linear trend. This dye has two amine and two sulfonate groups of opposite nature and its main interaction occurs with the ether linkage and hydroxyl groups of the cellulose structure which are more prone to ionization than carboxylic groups.

For the purpose of comparison a protein such as cytochrome C was used. This is a protein with iron-porphyrins prosthetic groups. Its isoelectric point is 10.65. It has a relative high content of lysine (α,ϵ -diaminocaproic acid)^[17] and a molecular weight of 12380 g/mol.

Cytochrome C behaved qualitatively very similar to Congo red (see Fig. 5).

In order to estimate quantitatively the differences between dyes, Fick's Second Law was considered

$$\frac{\partial C}{\partial X} = D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2} \right) \tag{3}$$

Its general solution^[18] assuming spherical geometry gives

$$\frac{m_t}{m_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left(\frac{-n^2 \pi^2 D_c t}{r_c^2} \right) \tag{4}$$

where n = integer, m_t = amount of permeant diffused at time t , m_∞ = amount of permeant diffused at time infinity, D_c = diffusion coefficient, r_c = particle radius, t = time, $\pi = 3.1416$.

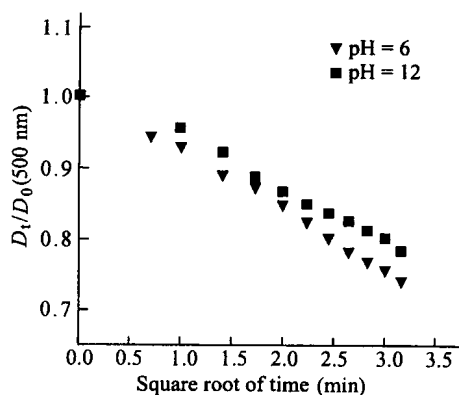


Fig. 4 Adsorption of congo red (Molar initial concentration 9.1×10^{-6}) in samples of 50 mg of bead cellulose without crosslinking

Plot of absorbance versus square root of time in minutes (D_t : absorbance at time t ; D_0 : initial absorbance).

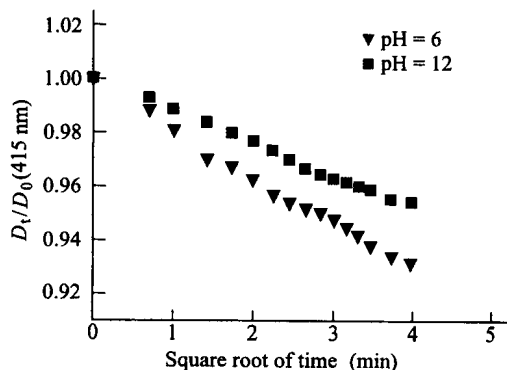


Fig. 5 Adsorption of cytochrome C (Molar initial concentration 5.0×10^{-5}) in samples of 50 mg of bead cellulose without crosslinking

Plot of absorbance versus square root of time in minutes (D_t : absorbance at time t ; D_0 : initial absorbance)

At short times ($m_t/m_\infty \leq 0.3$) this equation converges to

$$\frac{m_t}{m_\infty} \approx \frac{6}{\sqrt{\pi}} \left(\frac{D_c t}{r_c^2} \right)^{1/2} \quad (5)$$

m_t/m_∞ is the dye uptake. Linear plots of m_∞/m_t versus $t^{1/2}$ permit us to obtain the diffusional time constants (D/r^2) that are shown in Table 2.

Table 2. Diffusional time constants (s^{-1}) obtained from plots m_t/m_∞ versus $t^{1/2}$

Compound	$D/r^2 \times 10^4$		Coefficient of correlation	
	pH = 6	pH = 12	pH = 6	pH = 12
Alizarin red	2.5	3.2	0.994	0.973
Methylene blue	0.65	4.9	0.994	0.997
Congo red	1.48	1.09	0.997	0.992
Cytochrome C	1.1	0.79	0.986	0.994

At the pH range used these dyes did not change their colors^[19], thus their structure is the same at the two pH. It seems that the main differences at the two pH used is due to the fact that carboxylic groups of the beads are ionized at higher pH. The first two dyes strongly interact with them, in particular methylene blue, which possesses a positive charge.

Releasing

Methylene blue was chosen to test the release response of the cellulose beads because of its known low affinity for cellulosic materials. The releasing exhibited a "burst" at the beginning due to the dye that is on the surface or not occluded and comes out immediately. Later the dye occluded into the pores or the bound dye is released slowly. After the initially unstable behavior the trend is linear (see Fig. 6) indicating a zero order. Thus, the process is not purely Fickian.

Methylene Blue Adsorption on Crosslinked Bead Cellulose

A sample of bead cellulose Celef M-27 was crosslinked using 23% and 46% of epichlorohydrin (EPI) according to the technique described above. Differences between these crosslinked materials and the cellulose without

crosslinking are shown in Table 3. Actually the latter had not been subjected to the alkaline treatment to be crosslinked. Thus, a comparison is not fair. However, comparison between crosslinked celluloses indicates that a side reaction such as branching may take place in order to explain the higher swelling for the material treated with 46% of epichlorohydrin. Those branches would introduce higher hydrophilicity.

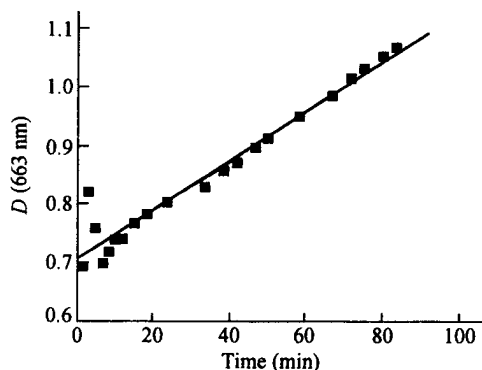


Fig. 6 Release of methylene blue trapped on bead cellulose without crosslinking
Plot of absorbance versus time in minutes

Table 3. Characterization of bead cellulose crosslinked with epichlorohydrin

Sample	Sedimentation volume (mL)	Swelling mL water/g cellulose	% of porosity
Bead cellulose without crosslinking	3.0	0.011	42.2
Crosslinked bead cellulose with 23% EPI	4.0	0.021	57.7
Crosslinked bead cellulose with 46% EPI	4.0	0.054	78

Adsorption of methylene blue on these materials is shown in Table 4. As a rule, adsorption is higher in alkaline solution than in acidic media as was discussed above for this dye. Furthermore, adsorption is lower for the more crosslinked material (46%) due to the trapping of carboxylic groups in the inner portion of the material.

Table 4. Adsorption of methylene blue on bead cellulose crosslinked with epichlorohydrin (EPI)% of adsorption at 17 min

Sample	% to pH = 5.8	% to pH = 12
Bead cellulose without crosslinking	14.6	17.2
Bead cellulose crosslinked with 23% EPI	18.3	32.9
Bead cellulose crosslinked with 46% EPI	8.5	13.6

* Samples of 19 mg of bead cellulose were used.

Table 5. Diffusion constants (D/r_c^2) for the adsorption of methylene blue on cellulose beads crosslinked with epichlorohydrin (EPI)

Crosslinked bead cellulose	$(D/r_c^2) \times 10^3$ s at pH = 6	$(D/r_c^2) \times 10^3$ s at pH = 12
23% EPI	1.49	2.40
46% EPI	0.88	1.94

The role of crosslinking can be evaluated quantitatively by measuring the diffusion time constants as was discussed above. Adsorption is higher at higher pH and crosslinking decreases it, as shown in Table 5. It seems that the carboxylate group is more available in alkaline solution but is hidden by crosslinking. It is to be noted that diffusion constants are greater than those shown in Table 2. Actually they can not be compared because

during crosslinking the material is treated by strong alkaline solution and severe changes could be induced in this material.

CONCLUSIONS

Samples of bead cellulose used exhibited a strong chemical adsorption of methylene blue, congo red and alizarin red because of the fit of the Langmuir type isotherm. Kinetics of adsorption is governed by diffusion in acidic solution and as a rule adsorption is higher for the first two dyes. Congo red and cytochrome C showed a similar adsorption trend. Experiments carried out to release the methylene blue occluded in the cellulose beads gave a kinetic behavior of zero order. Crosslinking decreased the adsorption capacity of this material.

REFERENCES

- 1 Peska, J., Stamberg, J., Hradil, J. and Ilavsky, M., *J. Chromatogr.*, 1976, 125: 455
- 2 Stamberg, J., Peska, J., Paul, D. and Philipp, B., *Acta Polym.*, 1979, 30: 734
- 3 Lenfeld, J., *Makromol. Chem.*, 1993, 212: 147
- 4 Okuma, S., 1991, U.S. Pat., 5064950
- 5 Kuga, S., *J. Chromatogr.*, 1980, 195: 221
- 6 De Olivera, W. and Glasser, W.G., *J. Appl. Polymer Sci.*, 1996, 60: 63
- 7 Motozato, Y. and Hirayama, C., *J. Chromatogr.*, 1984, 298: 499
- 8 Newton, J.M., Chow, A.K. and Jeewa, K.B., *Pharm. Technol. Int.*, 1992, 4: 52
- 9 Davidson, R., Nyqvist, H. and Ragnarsson, G., *Int. J. Pharm.*, 1993, 100: 49
- 10 Wolf, B., *Journal of Pharmaceutics.*, 1997, 156(1): 97
- 11 Porath, J., Janson, J. and Laas, T., *J. Chromatogr.*, 1971, 60: 167
- 12 Finar, I.L., "Organic Chemistry", ed. by Alhambra, Madrid, Spain, 1967, 2, p. 920
- 13 Peska, J., Stamberg, J., Hradil, J. and Ilavsky, M., *J. Chromatogr.*, 1976, 125: 455
- 14 Brandrup, J. and Immergut, E.H., "Polymer Handbook" (3rd. Ed.), Wiley & Sons, New York, 1989, p.V122
- 15 Naylor, T., "Polymer Properties", ed. by Allen, G., Pergamon Press, London, 1989, Ch. 20, p. 651
- 16 Kimura, I., Goncalves, A., Stolberg J., Laranjeira, M. and Favere, M., *Polímeros: Ciencia e Tecnología*, 1999, 3: 51
- 17 Fruton, J.S. and Simmonds S., "General Biochemistry", ed. by Habana, R., Cuba., 1963, p. 349
- 18 Rutven, D., "Principles of Adsorption and Absorption Processes", J. Wiley & Sons, New York, 1984, Ch. 6, p. 166
- 19 "Handbook of Chemistry and Physics," ed. by Weast, R.C., Chem. Rubber Publishing Co. USA, 1971, p. D106