Elsevier Editorial System(tm) for Analytica Chimica Acta
Manuscript Draft

Manuscript Number: ACA-08-1743R2

Title: AN OPTICAL SENSOR FOR THE DETERMINATION OF DIGOXIN IN SERUM SAMPLES BASED ON A MOLECULARLY IMPRINTED POLYMER MEMBRANE

Article Type: Full Length Article

Section/Category: SENSORS & BIOSELECTIVE REAGENTS

Keywords: Molecularly imprinted polymer; affinity membrane; digoxin; organic sample analysis.

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AN OPTICAL SENSOR FOR THE DETERMINATION OF DIGOXIN IN SERUM SAMPLES BASED ON A MOLECULARLY IMPRINTED POLYMER MEMBRANE

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Abstract

This paper reports the synthesis and testing of a molecularly imprinted polymer membrane for digoxin analysis. Digoxin-specific bulk polymer was obtained by the UV initiated co-polymerisation of methacrylic acid and ethylene glycol dimethacrylate in acetonitrile as porogen. After extracting the template analyte, the ground polymer particles were mixed with plasticizer polyvinyl chloride to form a MIP membrane. A reference polymer membrane was prepared from the same mixture of monomers but with no template. The resultant membrane morphologies were examined by scanning electron microscopy. The imprinted membrane was tested as the recognition element in a digoxin-sensitive fluorescence sensor; sensor response was measured using standard solutions of digoxin at concentrations of up to 4×10^{-3} mg L^{-1}. The detection limit was 3.17×10^{-5} mg L^{-1}. Within- and between-day relative standard deviations RSD (n= 5) were in the range 4.5-5.5% and 5.5-6.5% respectively for 0 and 1×10^{-3} mg L^{-1} digoxin concentrations. A selectivity study showed that compounds of similar structure to digoxin did not significantly interfere with detection for interferent concentrations at 10, 30 and 100 times higher than the digoxin concentration. This simply manufactured MIP

membrane showed good recognition characteristics, a high affinity for digoxin, and provided satisfactory results in analyses of this analyte in human serum.

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1. Introduction

Digoxin is a glycosylated steroid-like drug derived from the leaves and seeds of *Digitalis lanata* (the foxglove). It is one of the most commonly prescribed cardiac glycosides in the treatment of congestive heart failure, atrial fibrillation and atrial flutter [1, 2]. However, digoxin has only a small therapeutic range of concentrations (0.5 to 2.0 ng mL^{-1}) and its use requires strict monitoring of blood levels to minimize toxicity [3, 4].

Different techniques have been used to determine concentrations of digoxin in blood and urine, including radioimmunoassay (RIA) [5], the enzyme-multiplied immunoassay technique (EMIT) [6], fluorescence polarization immunoassay (FPIA) [7], high performance liquid chromatography (HPLC) assay with RIA [8] or fluorescence detection and LC/MS [9] or LC/MS/MS assay [10]. Some of these methods are relatively tedious and have been largely replaced by RIA which is more practical. However, the use of radionuclides also presents problems, and although this method is sensitive and commonly used in clinical and non-clinical studies, it is reported to have specificity problems; cross-reactions with digoxin metabolites and endogenous digoxin-like substances are known to occur [11-13]. HPLC avoids such interference, but is often insufficiently sensitive to quantify digoxin when present in

small amounts. New, rapid, easy-to-use, selective and sensitive methods for digoxin analysis are therefore required.

Combining highly selective recognition of biomolecules with the stability of cross-linked polymers, molecularly imprinted polymers (MIPs) offer an alternative for the analysis of digoxin concentrations. Traditionally, the MIPs used in separation and sensor technology [14, 15] have been obtained by bulk polymerisation [16], precipitation [17] or suspension polymerisation [18]. However new MIP formats are being developed that avoid the limitations of the traditional approach; certainly, MIP membranes have attracted much interest in recent years [19]. MIPs show inherent porosity, which makes them suitable for use in numerous applications. For example, they may be used as novel separation devices, as highly sensitive and selective chemical sensors [20], as drug delivery systems with molecular recognition, and in biomimetic membranes.

The aim of the present work was to produce an optical sensor for digoxin determination, using a MIP membrane as the support for a fluorescence reaction. This membrane was characterised and its digoxin recognition capacity evaluated. To test the affinity and selectivity of this membrane, cross reactivity studies were performed with codeine, morphine and heroin. The membrane returned satisfactory results in digoxin concentration analyses of human serum samples.

2. Experimental

2.1. Chemicals and instruments

Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) were obtained from Merck (Darmstadt, Germany), 2-2´-azobisisobutyronitrile (AIBN) from Fluka (Buchs, Switzerland), FITC-digoxin (10 μmol L^{-1}) from MicroPharm (Newcastle

Emlyn, Carmarthenshire, UK). Digoxin (95%), digitoxin (97%) and dibutyl phthatale and polyvinyl chloride (PVC) from Sigma Aldrich (Madrid, Spain). All solvents used (acetonitrile, methanol and tetrahidrofurane) were of analytical grade and purchased from Scharlab (Barcelona, Spain).

An ultraviolet lamp (Vilber Lourmat CN-6T) was used to induce the polymerisation process. Template extraction was performed using a Soxhlet extractor system. Imprinted and control polymers were ground in a glass mortar (Aldrich, Madrid, Spain) and then passed through CISA standard sieves (355-600 μm) (Afora, Madrid, Spain). A Hitachi model S-3000N scanning electron microscope (SEM) was used to characterise the morphology of the polymer membranes. Fluorescence measurements were made on a LS-5 Perkin-Elmer spectrofluorimeter, employing a 10×10 mm quartz glass cell (Hellma; Jamaica, NY, USA). The textural characterization of MIP was made using a Micromeritics ASAP 2010 equipment (Norcross, USA).

2.2. MIP binding ability

Before preparing the MIP membranes, the recognition ability of the imprinted polymer was examined in batch fluoroligand binding assays, using non-imprinted polymer (NIP) as a control. Binding assays were performed in either organic acetonitrile (ACN) or aqueous phosphate buffer solution (PBS, pH= 7.5) solvents. In both binding assays the polymer particles (20 mg) were added to 900 μL of solvent containing 100 μL of a solution of FITC-digoxin (10^{-5} μmol L^{-1}). The mixture was stirred and incubated over night at room temperature. The supernatant containing the non-binding FITC-digoxin was analysed by fluorescence spectroscopy at 496 nm (λ_{em}= 517 nm). The concentration of conjugated digoxin in solution was determined by reference to calibration curves previously plotted for both solvents. The amount of bound FITC-

digoxin was calculated from the difference between the concentrations added and the conjugated digoxin content of the supernatant. The imprinting factor (α), representing the degree of imprinting achieved, was calculated as the ratio of MIP-bound conjugated digoxin to NIP-bound conjugated digoxin. The partition coefficient (k) was calculated as the ratio between the amount of FITC-digoxin binding to the polymer (C_p) and the concentration of this in the solution (C_s), according to the method described elsewhere [21].

2.3. Preparation of digoxin-imprinted polymeric membranes

Imprinted polymer was prepared by bulk polymerisation using digoxin as the template molecule (2×10^{-3} mmol), functional monomer (MAA; 2 mmol), crosslinker (EDMA; 10 mmol), and an initiator (AIBN; 6×10^{-2} mmol), all dissolved in 10 ml ACN in a 20 mL glass test tube. The pre-polymerisation mixture was bubbled with nitrogen for 10 min, and polymerised for 24 h at 10ºC by UV light at 365 nm. The bulk polymer obtained was ground in a mechanical mortar and passed trough a 355-600 μm mesh sieve. The template was extracted in a Soxhlet apparatus with MeOH:ACN (50:50 v/v) (80 mL) over a period of 20 h. The NIP, used as the control, was prepared by the same procedure but omitting the template molecule [22].

MIP membranes were prepared by adding 500 mg of polymer particles to 6 mL THF solution containing 200 mg of PVC and 1 mL of dibutyl phthatale plasticizer. The mixture was poured into a Petri dish (6 cm diameter) and the organic solvent slowly evaporates at room temperature for 2 days. NIP membranes were prepared by the same procedure but using NIP in place of MIP.

2.4. Morphological analysis

The surface morphology of the resultant membranes was examined using a Hitachi S-3000N SEM. Samples of dry membranes were sputtered with a gold-palladium layer to avoid electrostatic charges and to improve image resolution. Scanning of the entire samples was performed before micrographs were taken.

2.5. Serum sample preparation

Serum samples from digoxin-treated patients were supplied by the Puerta de Hierro Hospital (Madrid) (stored at 4ºC until use). Prior to analysis, 900-1500 μL of ACN aliquots were added to 650 μL sample aliquots and these mixtures centrifuged at 3500 rpm for 30 min (i.e., until total protein precipitation was achieved). For serum analysis, 130 μL of FITC-labelled digoxin at 0.23 mg L^{-1} were added to 1300 μL of the samples and competitive assays performed using the piece of MIP membrane. Each synthesised membrane was divided in four pieces being the lifetime of each 12 assays. The life time of the membrane-based sensor was measured as the maximum number of assays carried out for each piece of membrane without appreciable loss of the binding capacity of MIP, which is connected with the decrease of the analytical signal. As well, a sensor is not considered stable or useful when the analytical signal decreases a 20 per cent with respect to the first assays done (which belong to the maximum signal). Taking into account that each synthesized membrane is divided in four parts, the total life time for the whole membrane is about 50 assays.

2.6. Digoxin assay protocol

The digoxin concentrations of the serum samples were determined by a heterogeneous competitive fluorescence assay using the MIP membrane. A calibration

curve was first plotted for a 0-4×10^{-3} mg L^{-1} digoxin range. Digoxin standard solutions were prepared using the corresponding volume of a digoxin solution (0.5 mg L^{-1}) recently prepared in 0.1 M ACN/Na_2CO_3 (50/50 v/v) at pH= 8.0. 200 μL of FITC-digoxin (0.23 mg L^{-1}) were added to all standards and ACN up to 2000 μL. Data for the competitive calibration curve were obtained by incubating 1200 μL of each digoxin

standard solution on the MIP membrane for 10 min in a glass precipitation vessel. The membrane containing the fluorescent complex (FITC-digoxin) bound to the MIP was removed and then placed in a cell in the spectrofluorimeter, and the fluorescence measured. The MIP membranes were regenerated by three successive washings with 1.5 mL MeOH/ACN (90:10). Serum samples were analysed following the same procedure.

3. Results and Discussion

Flexible molecularly imprinted polymer membrane was synthesised using a self support a digoxin-MIP. The structure and properties of the MIP on the membrane, determine the membrane recognition characteristics. Figure 1 shows SEM micrographs revealing the internal morphology of the synthesised MIP and NIP membranes. MIPs have inherent porosity, defined as the volume of voids or interstices per unit mass of dry material. Pores on the nanometer scale are called micropores (<2 nm in diameter) or mesopores (2 to 50 nm), whereas those of larger size are called macropores (0.05-10 μm) or superpores (10-1000 μm). The textural characterisation of the digoxin-MIP was accomplished by nitrogen gas adsorption at 77 K [23-25]. Figure 2 shows the nitrogen adsorption isotherms characteristic of the MIP. The specific surface area of the polymer was calculated from the nitrogen adsorption data by the Brunauer-Emmett-Teller (BET) method [24]. The external surface area (S_{ext}) and the micropore volume (V_1) were calculated by the t-plot method [25], and the pore size distributions and total pore

volumes of the MIP (V_p) by the Functional Theory of Densities model (DFT) [23]. The specific surface area (BET) was found to be 31.8 $m^2\ g^{-1}$; the total volume of pores was 0.194 $cm^3\ g^{-1}$. The MIP showed a micropores volume of 0.011 $cm^3\ g^{-1}$, and a mesopores volume of 0.086 $cm^3\ g^{-1}$.

To determine the recognition capacity of MIP, batch binding assays were performed in PBS (pH= 7.5) and ACN. Table 1 shows the amount of FITC-digoxin bound to the MIP and NIP in terms of specific uptake (the percentage of rebinding to the MIP minus the percentage for the NIP). The quantities bound by the MIP in ACN were higher than in PBS (Table 1). The amount of analyte bound by the NIP was practically nil compared to the MIP, indicating that the presence of the template in the imprinting process imparts recognition capacity.

Dibutyl phthalate was used as a modifier-elastifier in the formation of the membranes, and its quantity, plus that of the PVC added, was optimised. Good accessibility to selective cavities in a highly cross-linked digoxin polymer is thought to be extremely important in selective membranes to be used as sensors. The optimum incubation time for the membrane was also studied (5, 10 and 15 min); a 10 min incubation was found to offer the best results. Membranes were ready for use in new assays after three consecutive washes with 1.5 mL MeOH:ACN (90:10).

Sensitivity was established via a competitive calibration curve, using digoxin standards concentrations in a working range up to 4×10^{-3} mg L^{-1} with 0.23 mg L^{-1} of conjugated digoxin. The normalized fluorescence signals were expressed in terms of B/B_o, where B is the peak of the fluorescence complex at different standard digoxin concentrations, and B_o is that of the control sample. Figure 3 shows the calibration curve obtained. The experimental points fitted a quadratic polynomial curve, the equation for which was: $\%B/B_o= 6\times10^6\ C^2 - 46784\ C + 102$ (r= 0.992). The detection

limit obtained with five replicates of a 0 mg L^{-1} digoxin standard solution containing 200 μL of FITC-digoxin at 0.23 mg L^{-1} concentration, expressed as the least detectable dose (LDD) of analyte, was 3.17×10^{-5} mg L^{-1}. The dynamic range of the assay, defined as the analyte concentrations that inhibit the maximum signal by 80% and 20%, was between 4×10^{-4} and 2.7×10^{-3} mg L^{-1}. The within- and between-day reproducibility of the results returned by the MIP membrane was studied for 0 and 1×10^{-3} mg L^{-1} digoxin concentrations (n=5). The within-day RSD obtained were 4.8 and 5.4%, and the between-day RSD 5.9 and 6.4%, for a blank solution and a digoxin concentration of 1×10^{-3} mg L^{-1} respectively.

Interference was determined by performing cross-reactivity reactions involving heroin, morphine, codeine and digitoxin, and calculating the I_{50} values. The interference studies were carried out for concentrations of interfering (heroin, codeine and morphine) 100 times higher than digoxin analyte in standard calibration curve (0 to 4×10^{-3} mg L^{-1}), so the concentrations tested for these compounds were in a range from 0 to 0.4 mg L^{-1}. For digitoxin two calibration curves carried out from 0 to 0.04 and between 0 and 0.12 mg L^{-1}, respectively. No interference was detected for concentrations 100 times higher than that of the digoxin analyte; and in the case of digitoxin, the results showed that digitoxin at 10 times higher does not produce cross reactivity at 50%, which means that MIP has specific binding sites for digoxin. (Fig. 4).

Digoxin serum samples from treated patients were analysed using the proposed protocol, and the results compared with the hospital reference method (microparticle enzyme immunoassay, MEIA) using the AxSYM Digoxin II assay kit (Abbot Laboratories). The RSD obtained was <10%, with no significant differences observed between the values obtained by the two methods (95% CI). The total assay time was 13

min (including regeneration time). The life of the MIP membrane was approximately 50 assays.

4. Conclusions

The present work reports a new type of polymeric membrane with selective recognition sites for digoxin. The membrane was used as a sensor for the rapid, inexpensive and sensitive detection and determination of digoxin in serum samples. This work is the first research in MIP-membrane for digoxin and it is directed to development routine binding assay for clinical use.

The molecular recognition properties of MIPs are largely associated with the size and shape of the template compound. The partition coefficients obtained in ACN were higher for the MIP than the NIP. This difference in k indicates that the molecular imprinting procedure produces cavities with affinity for digoxin.

The high affinity and selectivity of the MIP membrane produced, together with its sensitivity, its simple and inexpensive preparation, and its 50-assay working lifetime, suggest it to could be useful new sensor for determining the digoxin concentrations of serum samples. The studies carried out are going to direct in a future investigation towards the development of a kit assay for digoxin routine analysis. Taking into account the results obtained, the MIP-based membrane seems useful to apply to the development of this new kit.

Acknowledgements

The project was funded by the Spanish Ministry of Science and Technology (project CTQ 2006-15027/PPQ) and the *Universidad Nacional de Educación a Distancia* via the *Plan de Promoción de la Investigación en la UNED*. The authors

thank the Puerta de Hierro Hospital for supplying the serum samples and Adrian Burton for revising the English manuscript.

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TABLE 1

Percentage uptake and partition coefficients for the MIP and NIP in ACN and PBS.

	Partition coefficient (k)				Percentage uptake						Imprinting factor (α)	
	Assay 1		Assay 2		Assay 1			Assay 2			Assay 1	Assay 2
	MIP	NIP	MIP	NIP	MIP	NIP	Specific	MIP	NIP	Specific		
ACN	2.5	0.35	2.9	0.38	70.7	26.0	44.7	74.5	27.3	47.2	2.72	2.74
PBS	0.12	0.06	0.10	0.05	10.5	5.9	4.6	9.3	5.4	3.9	1.78	1.70

TABLE 2

Results of human serum samples analysis.

Sample	MIP membranes (10^{-4} ng μL^{-1})	Reference method (10^{-4} ng μL^{-1})
1	11.0 ± 0.6	10.0 ± 0.5
2	8.6 ± 0.4	9.0 ± 0.5
3	15.2 ± 0.8	15.0 ± 1.0
4	6.3 ± 0.3	6.0 ± 0.3

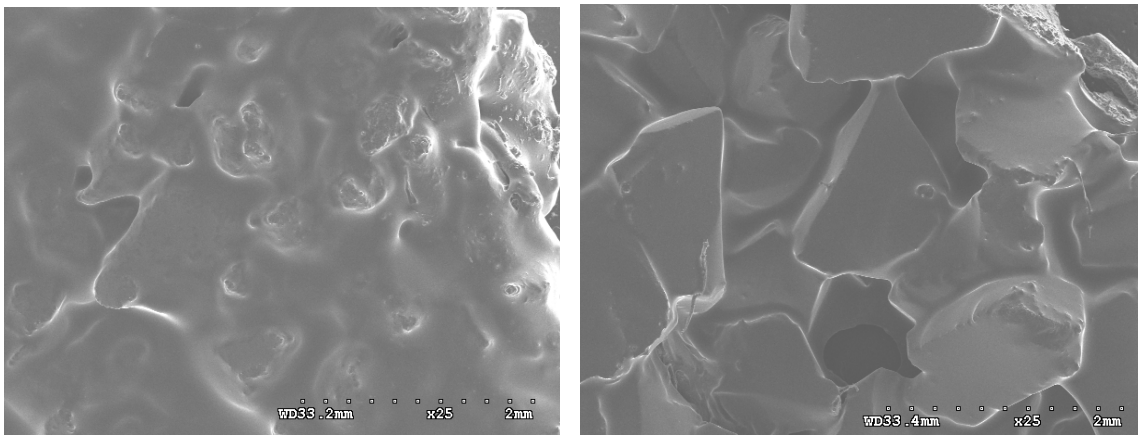
FIGURES LEGENDS

Fig. 1. SEM micrographs of membrane samples: 1a. Non-imprinted membrane (NIP). 1b. Membrane imprinted with digoxin (MIP).

Fig. 2. Adsorption-desorption isotherm for N_2 at 77 K obtained for the digoxin-MIP.

Fig. 3. Calibration curve for the MIP membrane obtained by plotting the normalized sign against the digoxin concentration.

Fig. 4. Calibration curves for four interfering compounds to digoxin analysis at concentrations for heroin, codeine, morphine 100 times higher than digoxin analyte; and 10, 30 times higher for digitoxin.



1a. 1b.

Fig. 1.

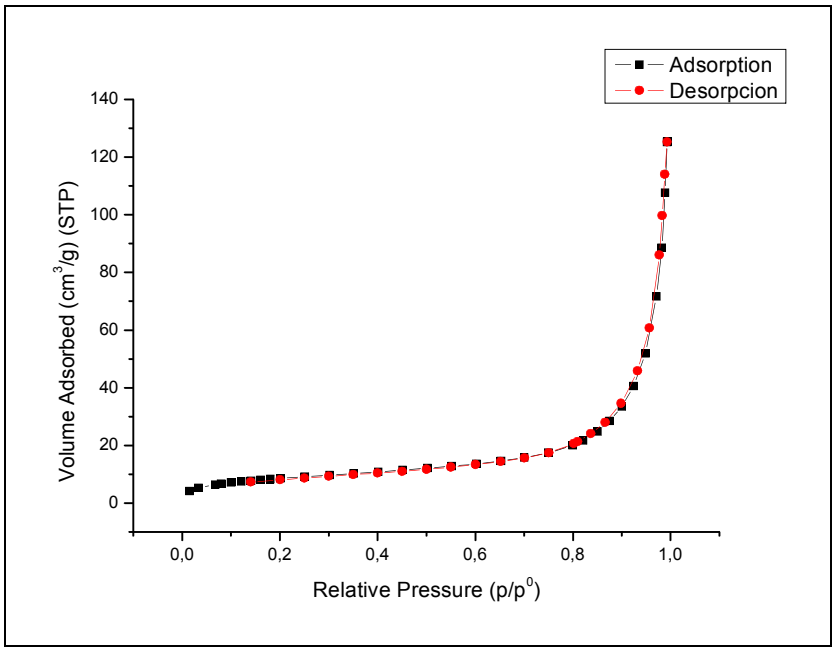

Adsorption
Desorpcion
Volume Adsorbed (cm3/g) (STP)
Relative Pressure (p/p0)
0
20
40
60
80
100
120
140
0,0
0,2
0,4
0,6
0,8
1,0

Fig. 2.

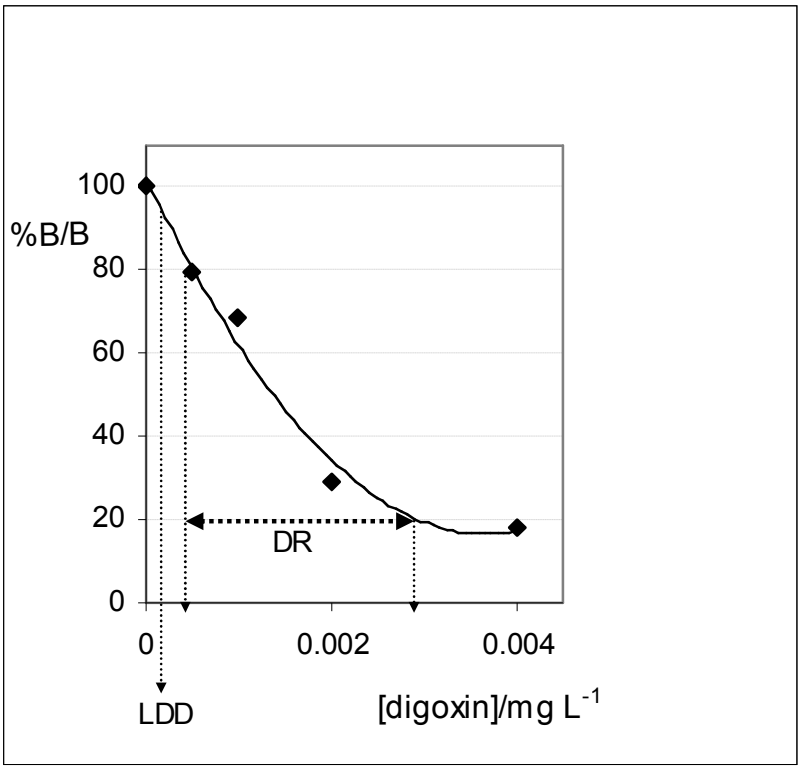
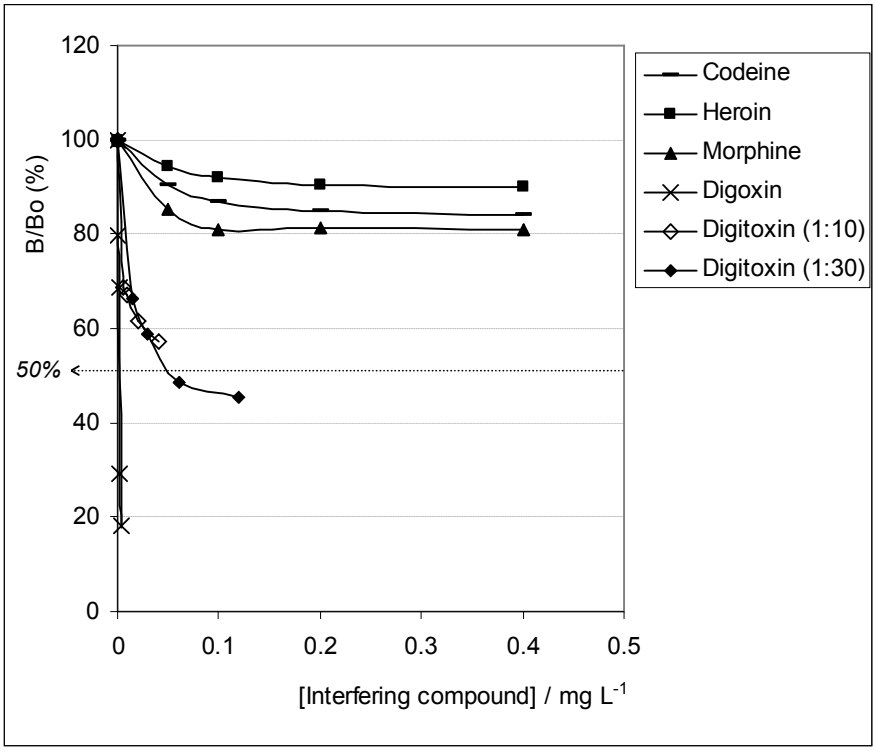


Fig. 3.


120
100
80
60
50%
40
20
0
B/Bo (%)
0
0.1
0.2
0.3
0.4
0.5
[Interfering compound] / mg L-1
Codeine
Heroin
Morphine
Digoxin
Digitoxin (1:10)
Digitoxin (1:30)

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2.2. MIP binding ability

Before preparing the MIP membranes, the recognition ability of the imprinted polymer was examined in batch fluoroligand binding assays, using non-imprinted polymer (NIP) as a control. Binding assays were performed in either organic acetonitrile (ACN) or aqueous phosphate buffer solution (PBS, pH= 7.5) solvents. In both binding assays the polymer particles (20 mg) were added to 900 μL of solvent containing 100 μL of a solution of FITC-digoxin (10^{-5} μmol L^{-1}). The mixture was stirred and incubated over night at room temperature. The supernatant containing the non-binding FITC-digoxin was analysed by fluorescence spectroscopy at 496 nm (λ_{em}= 517 nm). The concentration of conjugated digoxin in solution was determined by reference to

calibration curves previously plotted for both solvents. The amount of bound FITC-digoxin was calculated from the difference between the concentrations added and the conjugated digoxin content of the supernatant. The imprinting factor (α), representing the degree of imprinting achieved, was calculated as the ratio of MIP-bound conjugated digoxin to NIP-bound conjugated digoxin. The partition coefficient (k) was calculated as the ratio between the amount of FITC-digoxin binding to the polymer (C_p) and the concentration of this in the solution (C_s), according to the method described elsewhere [21].

2.3. Preparation of digoxin-imprinted polymeric membranes

Imprinted polymer was prepared by bulk polymerisation using digoxin as the template molecule (2×10^{-3} mmol), functional monomer (MAA; 2 mmol), crosslinker (EDMA; 10 mmol), and an initiator (AIBN; 6×10^{-2} mmol), all dissolved in 10 ml ACN in a 20 mL glass test tube. The pre-polymerisation mixture was bubbled with nitrogen for 10 min, and polymerised for 24 h at 10ºC by UV light at 365 nm. The bulk polymer obtained was ground in a mechanical mortar and passed trough a 355-600 μm mesh sieve. The template was extracted in a Soxhlet apparatus with MeOH:ACN (50:50 v/v) (80 mL) over a period of 20 h. The NIP, used as the control, was prepared by the same procedure but omitting the template molecule [22].

MIP membranes were prepared by adding 500 mg of polymer particles to 6 mL THF solution containing 200 mg of PVC and 1 mL of dibutyl phthatale plasticizer. The mixture was poured into a Petri dish (6 cm diameter) and the organic solvent slowly evaporates at room temperature for 2 days. NIP membranes were prepared by the same procedure but using NIP in place of MIP.

2.4. Morphological analysis

The surface morphology of the resultant membranes was examined using a Hitachi S-3000N SEM. Samples of dry membranes were sputtered with a gold-palladium layer to avoid electrostatic charges and to improve image resolution. Scanning of the entire samples was performed before micrographs were taken.

2.5. Serum sample preparation

Serum samples from digoxin-treated patients were supplied by the Puerta de Hierro Hospital (Madrid) (stored at 4ºC until use). Prior to analysis, 900-1500 μL of ACN aliquots were added to 650 μL sample aliquots and these mixtures centrifuged at 3500 rpm for 30 min (i.e., until total protein precipitation was achieved). For serum analysis, 130 μL of FITC-labelled digoxin (0.2 μmol L^{-1}) were added to 1300 μL of the samples and competitive assays performed using the MIP membrane.

2.6. Digoxin assay protocol

The digoxin concentrations of the serum samples were determined by a heterogeneous competitive fluorescence assay using the MIP membrane. A calibration curve was first plotted for a 0-4×10^{-3} mg L^{-1} digoxin range. Digoxin standard solutions were prepared using the corresponding volume of a digoxin solution (0.5 mg L^{-1}) recently prepared in 0.1 M ACN/Na_2CO_3 (50/50 v/v) at pH= 8.0. 200 μL of FITC-digoxin (0.2 μmol L^{-1}) were added to all standards and ACN up to 2000 μL. Data for the competitive calibration curve were obtained by incubating 1200 μL of each digoxin standard solution on the MIP membrane for 10 min in a glass precipitation vessel. The membrane containing the fluorescent complex (FITC-digoxin) bound to the MIP was removed and then placed in a cell in the spectrofluorimeter, and the fluorescence

measured. The MIP membranes were regenerated by three successive washings with 1.5 mL MeOH/ACN (90:10). Serum samples were analysed following the same procedure.

3. Results and Discussion

Flexible molecularly imprinted polymer membrane was synthesised using a self support a digoxin-MIP. The structure and properties of the MIP on the membrane, determine the membrane recognition characteristics. Figure 1 shows SEM micrographs revealing the internal morphology of the synthesised MIP and NIP membranes. MIPs have inherent porosity, defined as the volume of voids or interstices per unit mass of dry material. Pores on the nanometer scale are called micropores (<2 nm in diameter) or mesopores (2 to 50 nm), whereas those of larger size are called macropores (0.05-10 μm) or superpores (10-1000 μm). The textural characterisation of the digoxin-MIP was accomplished by nitrogen gas adsorption at 77 K [23-25]. Figure 2 shows the nitrogen adsorption isotherms characteristic of the MIP. The specific surface area of the polymer was calculated from the nitrogen adsorption data by the Brunauer-Emmett-Teller (BET) method [24]. The external surface area (S_{ext}) and the micropore volume (V_1) were calculated by the t-plot method [25], and the pore size distributions and total pore volumes of the MIP (V_p) by the Functional Theory of Densities model (DFT) [23]. The specific surface area (BET) was found to be 31.8 $m^2\ g^{-1}$; the total volume of pores was 0.194 $cm^3\ g^{-1}$. The MIP showed a micropores volume of 0.011 $cm^3\ g^{-1}$, and a mesopores volume of 0.086 $cm^3\ g^{-1}$.

To determine the recognition capacity of MIP, batch binding assays were performed in PBS (pH= 7.5) and ACN. Table 1 shows the amount of FITC-digoxin bound to the MIP and NIP in terms of specific uptake (the percentage of rebinding to the MIP minus the percentage for the NIP). The quantities bound by the MIP in ACN

were higher than in PBS (Table 1). The amount of analyte bound by the NIP was practically nil compared to the MIP, indicating that the presence of the template in the imprinting process imparts recognition capacity.

Dibutyl phthalate was used as a modifier-elastifier in the formation of the membranes, and its quantity, plus that of the PVC added, was optimised. Good accessibility to selective cavities in a highly cross-linked digoxin polymer is thought to be extremely important in selective membranes to be used as sensors. The optimum incubation time for the membrane was also studied (5, 10 and 15 min); a 10 min incubation was found to offer the best results. Membranes were ready for use in new assays after three consecutive washes with 1.5 mL MeOH:ACN (90:10).

Sensitivity was established via a competitive calibration curve, using digoxin standards concentrations in a working range up to 4×10^{-3} mg L^{-1} with 0.2 μmol L^{-1} of conjugated digoxin. The normalized fluorescence signals were expressed in terms of B/B_o, where B is the peak of the fluorescence complex at different standard digoxin concentrations, and B_o is that of the control sample. Figure 3 shows the calibration curve obtained. The experimental points fitted a quadratic polynomial curve, the equation for which was: $\%B/B_o = 6\times10^{6}\ C^2 - 46784\ C + 102$ ($r = 0.992$). The detection limit obtained with five replicates of a 0 mg L^{-1} digoxin standard solution containing 0.2 mg L^{-1} FITC-digoxin, expressed as the least detectable dose (LDD) of analyte, was 3.17×10^{-5} mg L^{-1}. The dynamic range of the assay, defined as the analyte concentrations that inhibit the maximum signal by 80% and 20%, was between 4×10^{-4} and 2.7×10^{-3} mg L^{-1}. The within- and between-day reproducibility of the results returned by the MIP membrane was studied for 0 and 1×10^{-3} mg L^{-1} digoxin concentrations (n=5). The within-day RSD obtained were 4.8 and 5.4%, and the between-day RSD 5.9 and 6.4%, for a blank solution and a digoxin concentration of 1×10^{-3} mg L^{-1} respectively.

Interference was determined by performing cross-reactivity reactions involving heroine, morphine and codeine (structures analogues to that of digoxin) at concentrations up to 0.4 mg L^{-1}, and calculating the I_{50} values. No interference was detected for concentrations 100 times higher than that of the digoxin analyte.

Digoxin serum samples from treated patients were analysed using the proposed protocol, and the results compared with the hospital reference method (microparticle enzyme immunoassay, MEIA) using the AxSYM Digoxin II assay kit (Abbot Laboratories). The RSD obtained was <10%, with no significant differences observed between the values obtained by the two methods (95% CI). The total assay time was 13 min (including regeneration time). The life of the MIP membrane was approximately 12 assays.

4. Conclusions

The present work reports a new type of polymeric membrane with selective recognition sites for digoxin. This membrane was used as a sensor for the rapid determination of digoxin in serum samples. The molecular recognition properties of MIPs are largely associated with the size and shape of the template compound. The partition coefficients obtained in ACN were higher for the MIP than the NIP. This difference in k indicates that the molecular imprinting procedure produces cavities with affinity for digoxin.

The high affinity and selectivity of the MIP membrane produced, together with its sensitivity, its simple and inexpensive preparation, and its 12-assay working lifetime, suggest it to could be useful new sensor for determining the digoxin concentrations of serum samples.

Acknowledgements

The project was funded by the Spanish Ministry of Science and Technology (project CTQ 2006-15027/PPQ) and the *Universidad Nacional de Educación a Distancia* via the *Plan de Promoción de la Investigación en la UNED*. The authors thank the Puerta de Hierro Hospital for supplying the serum samples.

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TABLE 1

Percentage uptake and partition coefficients for the MIP and NIP in ACN and PBS.

	Partition coefficient (k)				Percentage uptake						Imprinting factor (α)	
	Assay 1		Assay 2		Assay 1			Assay 2			Assay 1	Assay 2
	MIP	NIP	MIP	NIP	MIP	NIP	Specific	MIP	NIP	Specific		
ACN	2.5	0.35	2.9	0.38	70.7	26.0	44.7	74.5	27.3	47.2	2.72	2.74
PBS	0.12	0.06	0.10	0.05	10.5	5.9	4.6	9.3	5.4	3.9	1.78	1.70

TABLE 2

Results of human serum samples analysis.

Sample	MIP membranes (10^{-4} ng μL^{-1})	Reference method (10^{-4} ng μL^{-1})
1	11.0 ± 0.6	10.0 ± 0.5
2	8.6 ± 0.4	9.0 ± 0.5
3	15.2 ± 0.8	15.0 ± 1.0
4	6.3 ± 0.3	6.0 ± 0.3

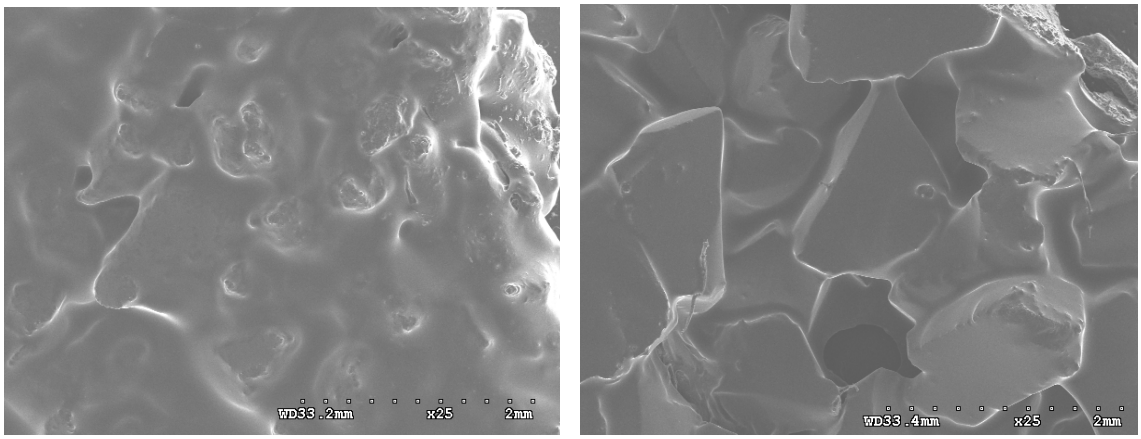
FIGURES LEGENDS

Fig. 1. SEM micrographs of membrane samples: 1a. Non-imprinted membrane (NIP). 1b. Membrane imprinted with digoxin (MIP).

Fig. 2. Adsorption-desorption isotherm for N_2 at 77 K obtained for the digoxin-MIP.

Fig. 3. Calibration curve for the MIP membrane obtained by plotting the normalized sign against the digoxin concentration.

Fig. 4. Calibration curves for three compounds structurally analogous to digoxin at concentrations 100 times higher than the digoxin analyte.



1a. 1b.

Fig. 1.

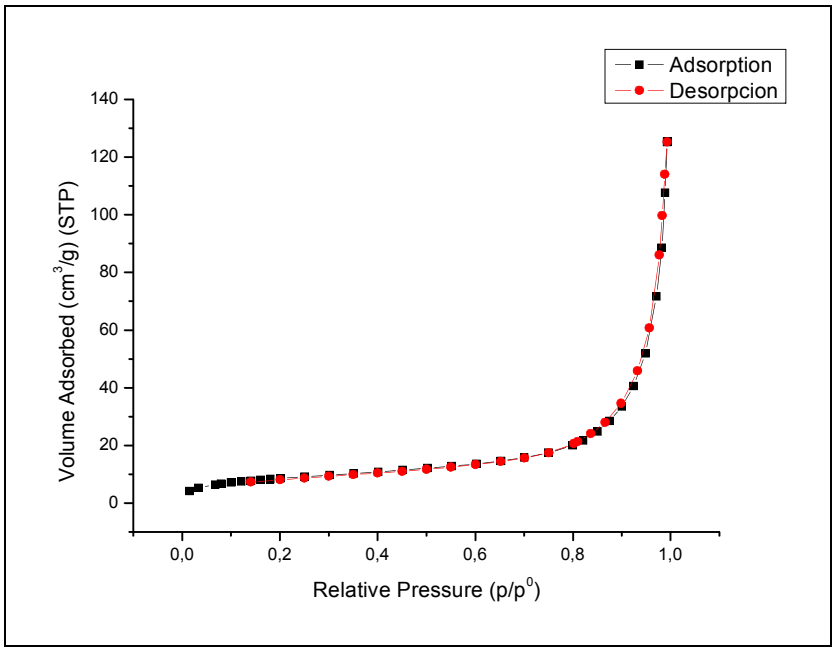

Adsorption
Desorpcion
Volume Adsorbed (cm3/g) (STP)
Relative Pressure (p/p0)
0
20
40
60
80
100
120
140
0,0
0,2
0,4
0,6
0,8
1,0

Fig. 2.

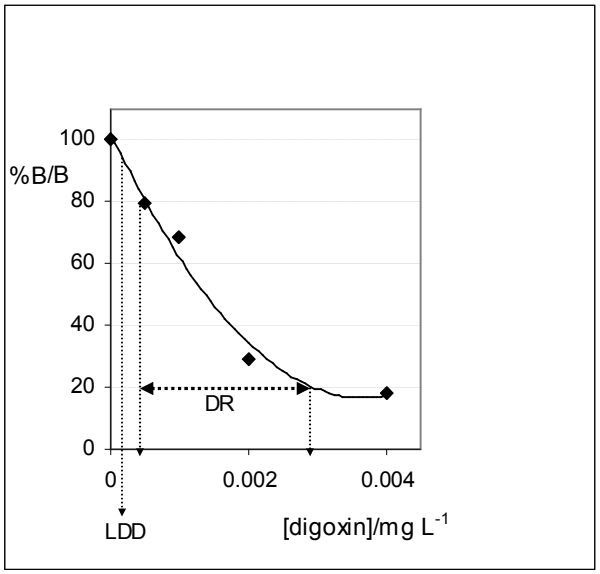


Fig. 3.

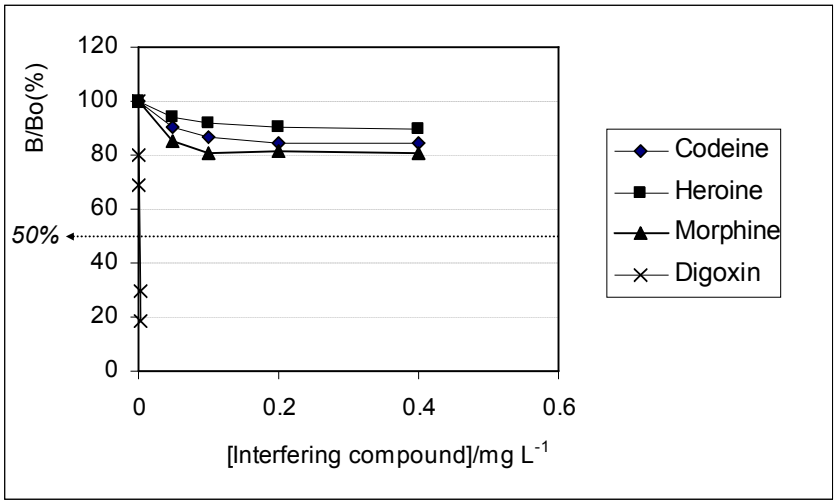

120
100
80
60
40
20
0
B/Bo(%)
50%
0
0.2
0.4
0.6
[Interfering compound]/mg L-1
Codeine
Heroine
Morphine
Digoxin

Fig. 4.

8. Figure

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