Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1156 (2007) 213–219
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Analysis of melatonin using a pH- and temperature-responsive aqueous chromatography system

Eri Ayano [a], Yusuke Suzuki [a], Masahito Kanezawa [a], Chikako Sakamoto [a], Yuko Morita-Murase [a], Yoshiko Nagata [a], Hideko Kanazawa [a,*], Akihiko Kikuchi [b], Teruo Okano [c]

[a] *Department of Physical Pharmaceutical Chemistry, Kyoritsu University of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan*
[b] *Faculty of Industrial Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*
[c] *Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawadacho, Shinjyuku-ku, Tokyo 162-8666, Japan*

Available online 26 January 2007

Abstract

A new method for the qualitative and quantitative analysis of an intracerebral hormone, such as melatonin, has been proposed, utilizing newly designed copolymers that include ion-exchange groups. These copolymers responded to both the temperature and the pH, and the copolymers were modified with cross-linked hydrogel applied onto aminopropyl silica beads. The products were evaluated as HPLC packing materials for a pH- and temperature-responsive chromatography. The property of the surface of the stationary phase was altered from hydrophilic to hydrophobic, and from charged to non-charged by changes in both the temperature and the pH. In the chromatographic system, we investigated how to change the retention of melatonin by varying the temperature. A pH- and temperature-responsive chromatography is expected to be useful for the separation of pharmaceuticals and biomolecules.

Keywords: Poly(*N*-isopropylacrylamide); HPLC; pH- and temperature-responsive chromatography; Melatonin; Intracerebral hormone

1. Introduction

Recently, the research of various polymers has been widely carried out, and investigations on the structure and physical properties in response to external signals play important roles in various fields, such as drug-delivery systems [1,2], cell-culture dishes [3], cell sheets [4] and bioconjugates [5]. The structures and functions of these materials, termed 'intelligent materials', are controlled by their response to the surrounding conditions, such as the pH [6], electric field [7], light [8], chemical species [9] and temperature [1,3,10]. Recently, the research of a uniformly sized molecularly imprinted polymer (MIP) is also being widely carried out [11]. A temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the intelligent materials that exhibit a thermally reversible phase transition in response to temperature changes across a lower critical solution temperature (LCST) of 32 °C in aqueous solution [12].

In water, the polymer chains of PNIPAAm hydrate and expand below the LCST, while they dehydrate to form a compact, insoluble conformation above it. We recently reviewed a new hydrophobic chromatography in an aqueous mobile phase using PNIPAAm-modified silica as a column packing material [13–20]. The chromatography is based on reversible changes in the hydrophilic/hydrophobic properties of PNIPAAm-grafted surfaces in response to changes in the temperature. This HPLC system is very simple, because elution can be controlled merely by adjusting the temperature, and only aqueous solutions are used as the mobile phase. Using this system, we achieved the separation of steroids [13,14,17], peptides [14–16,20], proteins [15] and environmental pollutants [18,19] using temperature-responsive chromatography. Temperature is known to play a significant role in biomolecules and chiral separation, but its influence on the separation of small molecules in conventional reversed-phase HPLC is much less important. The slope of the van't Hoff plots on the PNIPAAm-modified column is negative in the hydrophobic substances, opposite to those observed for conventional chromatography. This provides additional evidence that the interaction between hydrophobic substances and temperature-responsive surfaces becomes stronger at elevated temperature. Additionally, on the PNIPAAm-modified column,

* Corresponding author at: Department of Physical Chemistry, Kyoritsu University of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan. Tel.: +81 3 5400 2657; fax: +81 3 5400 1378.
E-mail address: kanazawa-hd@kyoritsu-ph.ac.jp (H. Kanazawa).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.01.063

a deviation from linearity was found between the ln k values and $1/T$. Interestingly, the slope of the van't Hoff plots of each analyte on the PNIPAAm-modified column changed markedly at the LCST boundary. This corresponds to a phase transition of the polymer modified on the surface.

Temperature-responsive chromatography is an analytical method with a small load on environmental pollution, because no organic solvent is used in the mobile phase. We have designed and synthesized a novel pH- and temperature-responsive polymer (stimuli-responsive polymer) which responds to the temperature and other environmental stimuli, such as changes in the pH [21–24]. They showed hydrophilic/hydrophobic phase transitions in response to temperature changes, and the transition temperatures were affected by the pH [25].

We mainly examined the separation of hydrophobic substances using temperature-responsive chromatography with a sole aqueous mobile phase. In general, biologically active substances, such as peptides, proteins and nucleic acids, can be separated by reversed-phase chromatography, ion-exchange chromatography systems and their combinations, since they have both charge and a hydrophobic nature. It is thought that there are effects, not only concerning the difference of hydrophilic/hydrophobic, but also in the separation of the charge in such substances. We constructed a novel separation carrier that could modulate two properties of hydrophobic and electrostatic by controlling the temperature.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a famous pineal hormone synthesized from L-tryptophan via serotonin (5-hydroxytryptamine) and *N*-acetylserotonin (*N*-acetyl-5-hydoroxytryptamine) [26,27], is a chemical modulator of the biological clock of vertebrates [28], and is clinically used to treat jet lag [29] and sleep disorders [30]. Fig. 1 shows that melatonin is synthesized from L-tryptophan via serotonin. The synthesis and secretion of melatonin fluctuate in a circadian rhythm that is entrained to a 24 h light/dark cycle [31]. It is now widely accepted that melatonin is an endogenous mediator of photoperiodic information, and a molecular component of the circadian timekeeping system. In addition, melatonin is reported to be effective against many diseases, such as cancers [32], Alzheimer's disease [33] and depressive syndrome [34]. Therefore, melatonin determination is important for the diagnosis of rhythm disorders, for the research of new biologically active substances, and for estimating the effect of medicines. L-Tryptophan is one of the building blocks of protein, but unlike some amino acids, L-tryptophan is considered to be essential because the body cannot manufacture its own. L-Tryptophan plays many roles in animals and humans alike, but, perhaps most importantly, it is an essential precursor to a number of neurotrans-mittters (serotonin being one of the most important) in the brain. As such, L-tryptophan is the only substance that can be converted into serotonin. Since serotonin, in turn, is converted into melatonin, which has been shown in several good studies to assist in sleep, L-tryptophan clearly plays an instrumental role in balancing mood and sleep patterns. L-Tryptophan may also be of some benefit in the treatment of some psychiatric disorders. Usually, in the isocratic elution of samples containing solutes with a wide range of polarity, it is sometimes difficult to achieve

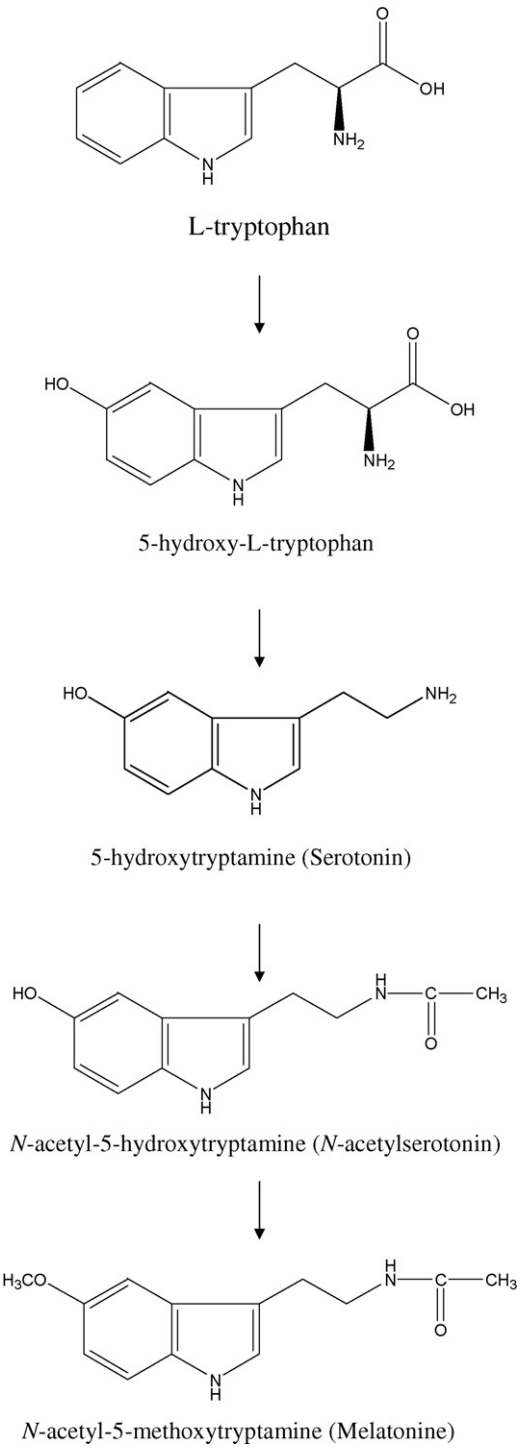

Fig. 1. Melatonin synthesized from L-tryptophan via serotonin.

the desired resolution in a reasonable time. It may be necessary to use gradient elution where volumes of an organic solvent, the composition of the mobile phase, or other properties of the solvent (e.g., pH or ionic strength) are changed during separation [35]. In such analysis, retention behavior changes by increasing temperature are not observed.

The purpose of the present contribution is to describe the design of new stationary phases for the effective separation of bioactive compounds. In this study, we utilized pH- and