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Synthesis, characterization and release studies of monomer and copolymers based on 3-aminopyridine as a pharmaceutical precursor

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Abstract: The aim of the current study is to prepare controlled release formulations composed from 3-aminopyridine. This active molecule is modified by chemical grafting on monomer based on (m,p)-vinylbenzaldehyde and then copolymerized with dimethylacrylamide to get hydrosoluble systems. In these systems, the active agent is spaced out from copolymer chain by phenyl group. The obtained supports i.e. monomer and copolymers are characterized by FTIR, NMR (^{1}H, ^{13}C) and other techniques. The drug release from these formulations is studied and the values of release constants demonstrated that 3-aminopyridine release can be modified using these systems. Also, the effect of pH release media on the drug release is discussed.

Key words: 3-Aminopyridine, copolymer support, hydrolysis, drug delivery systems, diffusion.

Introduction

Significant advances have been made in the development of drug delivery systems in order to increase the drug efficacy. These devices can control the drug release and can be obtained by different techniques[1,2].

A drug delivery system can be a matrix of polymer incorporating the active agent; the drug can be dispersed in the polymer or covalently linked to polymer backbone[3-10]. In the latest technique, the linkage of the drug to the polymer can be obtained either by a chemical modification of the polymer or by grafting the drug onto a monomer which is polymerized or copolymerized. In this domain, the styrenic and acrylic polymers are widely used by the researchers[11, 12] and some parameters such as geometry, functional group and group spacer can modify the drug release. The linkage between drug and polymer can be realized by various functions such as amide, carbonate, ester or imine functions[5, 11, 13-15]. We are interested in imine linkage because it can be easily hydrolyzed in all range of pH media and allows a grafting of amine-terminated drug precursors.

In the present study, 3-aminopyridine (Am) is used as an active agent; this molecule is used in agrochemistry as intermediate, in pharmacy for medicine preparation and as colorant[16-18]. Also, the effect of 3-aminopyridine on the synaptic transmission was studied[19] and recently other research reported the efficacy and safety of aminopyridines for neurological deficits in adults with multiple sclerosis[20]. So, our research is intended to use

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polymeric supports able to control the release of aminopyridine in the defined periods in order to increase its efficacy and reduce the undesirable effects. In this field, S. Yokoyama and al. have studied the 3-aminopyridine release kinetics from fatty acid complex in acidic medium (pH=1.2)[21].

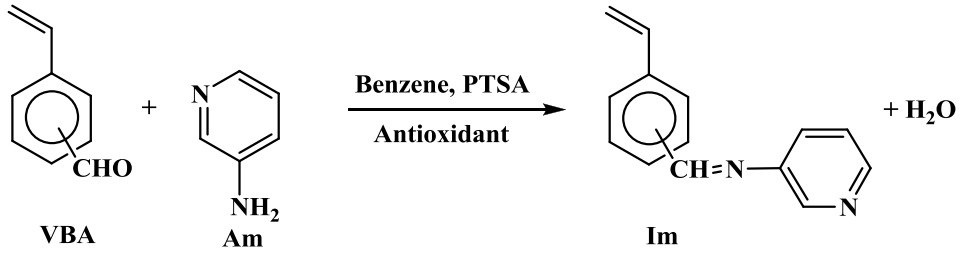
However, in the present paper, this active molecule is attached to m,p-vinylbenzaldehyde (VBA) as monomer "support" to get N-(m, p)-vinylbenzyliden-3-aminopyridine (Im). Then, in this formulation, the obtained monomer (Im) is copolymerized with N,N-dimethylacrylamide (DMA) in order to acquire hydrosoluble supports. In this case, the drug is attached to a monomer with an imine function and it is spaced out from the styrenic backbone by the phenyl group. The aim of the study is to modify the drug release using these new formulations; in fact, the drug release takes place after the hydrolysis of this Schiff base and it is controlled by diffusion. This type of linkage was used in other researches and the mechanism of the hydrolysis was largely discussed for different active agents[22-24]. Therefore, we have studied the release of 3-aminopyridine from the synthesized monomer and copolymer supports in heterogeneous media. This paper is also devoted to study the effect of the pH of release medium on the transfer of the active agent by two values of pH closed the most digestive tube pH (pH = 1,2 and 8).

Results and Discussion

Characterization results

a- Monomer characterization

The monomer support of 3-aminopyridine "Im" (scheme 1) is characterized by FTIR and ^{1}H NMR spectroscopy.



Scheme 1

First, the synthesized VBA is characterized by FTIR and the most important bands are: C=O at 1701 cm^{-1}, C-H (aldehyde) at 2731 cm^{-1} and 2824 cm^{-1}, C=C(aromatic) at 1604 cm^{-1} and C-H (vinylic) at 918 cm^{-1} and 989 cm^{-1}.

The infrared spectrum of Schiff base Im shows clearly the absence of C=O aldehyde band at 1701 cm^{-1} and the principal FTIR absorption bands are: C=N (imine) at 1624 cm^{-1}, C-H (vinylic) at 989–914 cm^{-1}, C-H (aromatic) at 3087 cm^{-1}, C=C (aromatic) at 1604, C=C and C=N of pyridine at 1575 and 1477 cm^{-1}, C-H of pyridine (out of plane bending) at 709 cm^{-1}, C-H of pyridine at 3006-3033 cm^{-1} (figure 1).

The ^{1}H NMR spectrum of monomer in deuterated dimethyl sulfoxide (DMSO) gives the following proton signals **δ** (ppm): 5,38-5,97 for C**H**$_2$= CH- (2d), 6,81-6,89 for CH$_2$=C**H**- (2t), 7,0-8,51(m) for C**H** phenyl and pyridin, 8,69-8,71 for C**H**=N- (mixture of meta and para compound).

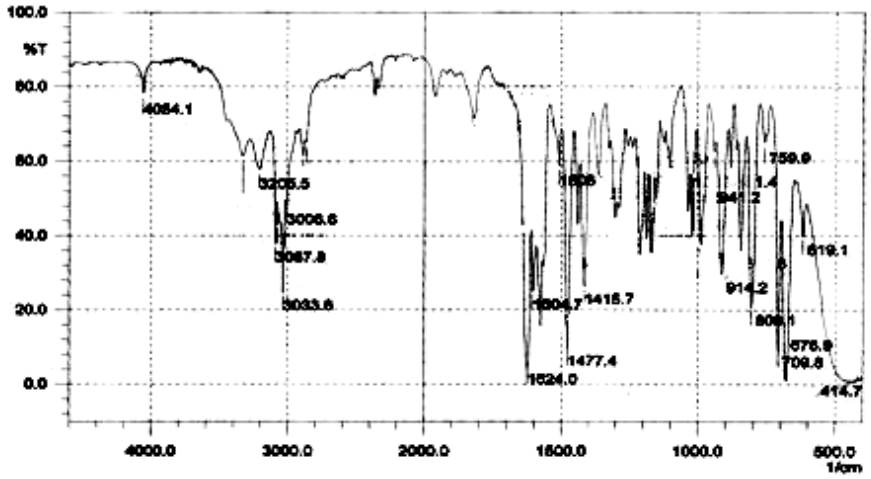
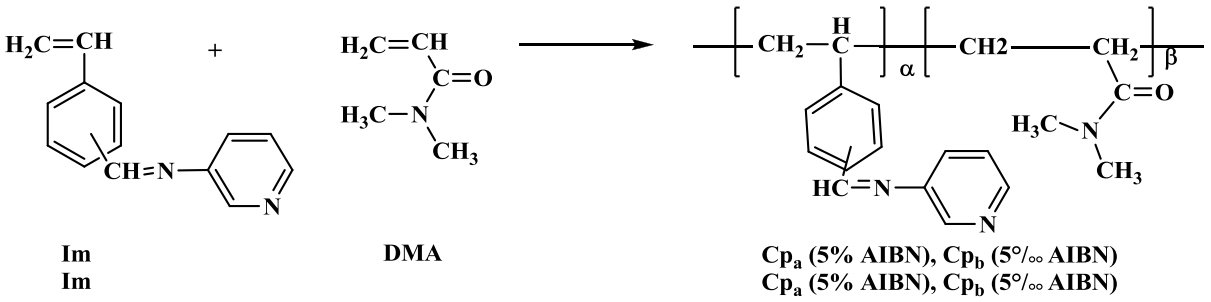


Figure 1. Infrared spectrum of monomer Im

b- Copolymers characterization:

The Scheme 2 shows the copolymerization's reaction of the obtained monomer with DMA where copolymers Cp_a and Cp_b are prepared respectively with 5% and 5°/$_{oo}$ of AIBN as initiator.



Im
Im

DMA

Cp_a (5% AIBN), Cp_b (5°/$_{oo}$ AIBN)
Cp_a (5% AIBN), Cp_b (5°/$_{oo}$ AIBN)

Scheme 2

The FTIR spectra of copolymers show the absence of vinylic band and the most important FTIR bands of copolymers are given in table 1.

Table 1: Principal IR bands of copolymers.

	ν (cm^{-1})	
	Cp_a	**Cp_b**
C-H (-CH and -CH_2-)	3020 - 2950	3040 - 2970
C-H (CH_3)	2920	2940
C=O (amide)	1660	1668
C=C (aromatic)	1600	1600

The ^{1}H NMR spectra of copolymers give the corresponding ^{1}H NMR signals resumed in table 2.

Table 2: Values NMR ^{1}H signals of copolymers.

	Cp_a **δ** (ppm)	**Cp_b** **δ** (ppm)
C**H**$_2$- and C**H**-	0,8 - 2	0,85- 1,60
C**H**$_3$-	2,9	3
C**H** phenyl and pyridin	7,1 – 8,03	7,08 – 8,4
-C**H**=N-	8,42	8,46
OC-C-**H**	4,25	4,0

We noticed that the signal of CH phenyl and pyridine are weak, this is due to the small incorporation ratio (β) in copolymers.
The figure 2 gives the NMR 2D (COSY et NOESY) spectra of copolymer Cp_a. We have not established Cp_b spectra because it has the same structure as Cp_a.

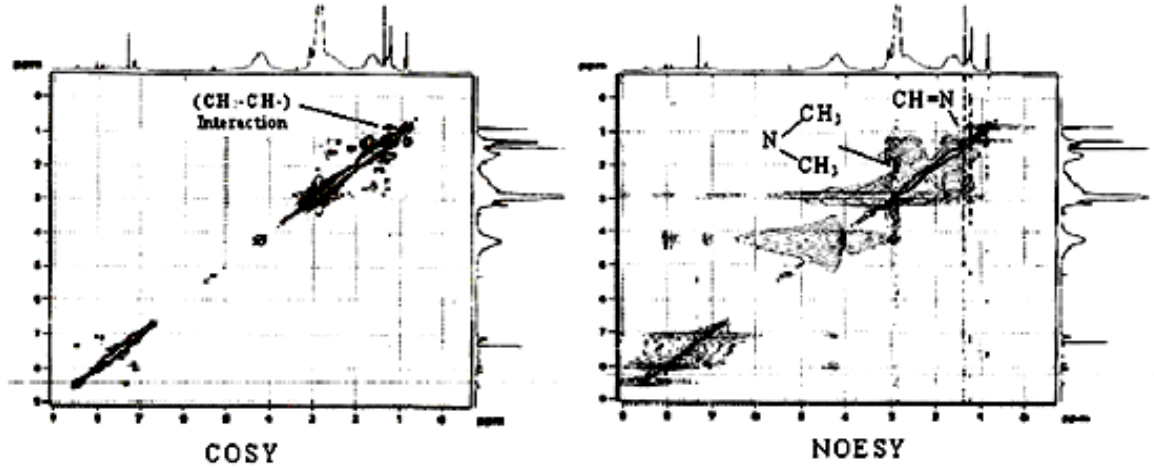


Figure 2. NMR 2D (COSY and NOESY) spectra of copolymer Cpa.

The ^{13}C NMR spectra of copolymers give the δ (ppm) values resumed in table 3. In these spectra, we noticed the absence of phenyl and pyridine signals for the reason of low percentage of these groups in copolymers (low value of β).

Table 3: Values of NMR ^{13}C signals of copolymers.

	Cp_a **δ** (ppm)	**Cp_b** **δ** (ppm)
C=O	175,08	175,13
CDCl$_3$-	77,04 – 77,89	77,03 – 77,88
CH$_3$	37,47	37,49
CH$_2$- and **C**H-	14,50 – 36,22	36,21 – 36,64

The incorporation ratios (α and β) in copolymers are (0,1394 and 0,8606) for Cp_a and (0,1059 and 0,8941) for Cp_b. They are calculated from the results of hydrogen microanalysis (8,15 % - 8,32 % for Cp_a and 8,33 % - 8,51 % for Cp_b).

The viscosity molecular weights (Mv) of copolymers are 3120 for Cp_a and 10260 for Cp_b. The glass transition temperatures (Tg) are 30 °C for Cp_a and 36 °C for Cp_b.

Release study

The kinetics of 3-aminopyridine delivery from a monomer and copolymers in the various release media at the different pH are illustrated in the following figures (figures 3 and 4).

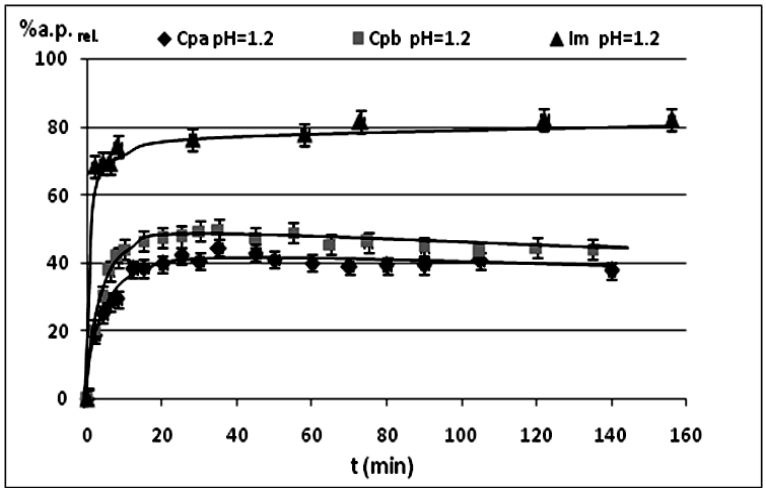


Figure 3. % of drug released as function of time in pH = 1,2 (37 °C, 500 rpm)

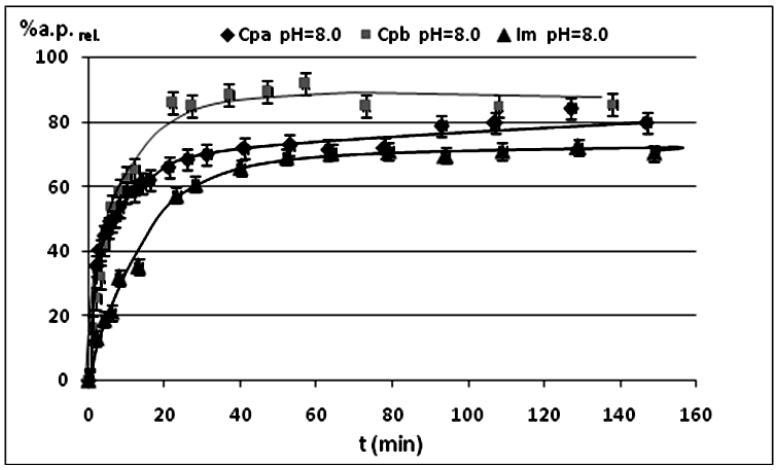


Figure 4. % of drug released as function of time in pH = 8 (37 °C, 500 rpm)

The results demonstrated that the release of 3-aminopyridine from the monomer Im is faster than the copolymers Cp_a and Cp_b in acidic medium. In fact, after 1hour, the empirical percentage of drug released from Im reaches 80 % however it is from 40 and 50 % for Cp_a and Cp_b. This remark is in agreement with theory because the release from copolymer includes additional stage which is the diffusion throughout macromolecular structure. Also, in general, high percentages of drug released are obtained because the hydrolysis of Schiff bases is rapid in acidic pH media[15].

In basic media, the results are inversed; the drug release became faster from the copolymers. As a matter of fact, the copolymers are hydrosoluble and the hydrolysis of Schiff base is slow down in alkaline pH. Consequently, the drug release from monomer which is insoluble in the release medium is slow.

These kinetics cannot be expressed by simple classical equations. The diffusional appearance of this delivery was demonstrated when the percentage of drug released was plotted as function of a square root of time. In fact, a linear relationship is observed for short times (figure 5).

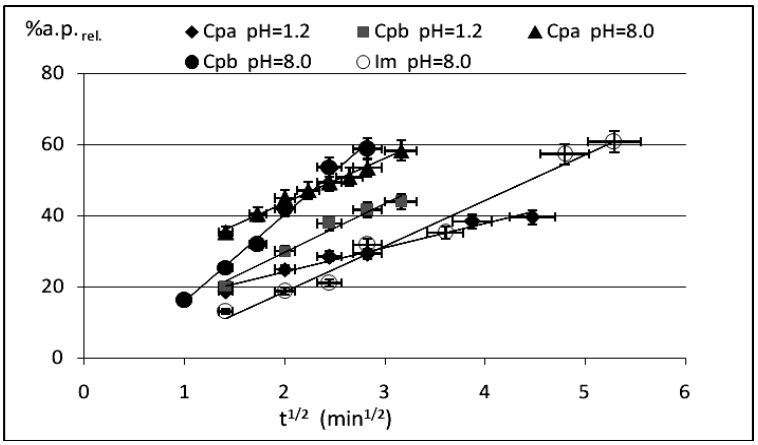


Figure 5. % of drug released as function of square root of time.

In this case, we can say that the release kinetics are controlled by diffusion according to Fick's laws[25] but we cannot calculate de diffusivities because we don't know the monomer or the copolymer dimensions. Nevertheless, we give in the following table (table 4) the values of release constants (k) or slopes resulting from the plot of % drug released as a function of a square root of time. This parameter which is called release constant was widely used by pharmaceutical researchers to describe the release rate[26, 27]. At pH = 1,2, and for a monomer Im, the release of 3-aminopyrine is fast and it doesn't obey the diffusion laws.

Table 4: Values of drug release constants from monomer and copolymers.

pH	Support	% a.p.= f ($t^{1/2}$)	R^2
pH=1,2	Im	-	
	Cp_a	$y = 6,81\ t^{1/2} + 10,55$	0,972
	Cp_b	$y = 13,79\ t^{1/2} + 1,99$	0,975
pH=8	Im	$y = 12,85\ t^{1/2} - 7,15$	0,978
	Cp_a	$y = 12,45\ t^{1/2} + 18,67$	0,988
	Cp_b	$y = 24,49\ t^{1/2} - 8,68$	0,988

The drug release constants of Cp_b are higher both in acidic and basic media. Certainly it is due to its high mass which includes the hydrophilic co-monomer "dimethylacrylamide". The presence of DMA permits a rapid hydrolysis of the imine linkage. Concerning the drug release from copolymers, it is observed a notable burst effect particularly for Cp_a. This effect is due inevitably to its low mass and the high percentage of monomer support of drug which can be on the macromolecule chain extremities.

Conclusion

In the present paper, 3-aminopyridine is modified by grafting on monomer based on (m,p)-vinylbenzaldehyde and copolymerization with dimethylacrylamide. The drug release

from these formulations is studied in acidic (pH = 1,2) and basic (pH = 8) media and the results demonstrated that 3-aminopyridine release is strongly affected by the copolymer supports. Then the results show the importance of the molecular mass of polymers on the drug release constant. Consequently, we can select a desired formulation on the base of the drug application. This study does not stop at this state and has a perspective which is the inclusion of the obtained formulations i.e. monomer and copolymer in other polymeric supports using other techniques principally microencapsulation in order to get a large domain of drug release modification.

Experimental Section

Chemicals

(m,p)-Vinylbenzaldehyde (VBA) was prepared from a mixture of (meta/para)-chloromethylstyrene : (60/40). 3-Aminopyridine at 98 % of purity from SIGMA. Benzene (99,8 %), Dioxane (98%) and diethyl ether (99 %) are provided from Prolabo and tetrahydrofuran (THF) from Labosi.

Preparation of monomer

a- Synthesis of (m,p)-Vinylbenzaldehyd (VBA)

VBA is prepared from a commercial mixture of meta/para:60/40 chloromethylstyrene (CMS), according to SOMMELET method[28]. 0,6 mol of hexamethylenetetramine (HMTA) is added to 0,3 mol of fresh distilled CMS which is placed in a flask equipped with refrigerant, and then 125 mL of acetic acid is introduced and diluted with 125 mL of water. After adding some spangles of 4-tertiobutylcathecol, the mixture is heated until reflux at 100°C during 2 hours under agitation. 100 mL of HCl is added to the mixture and heated for 15 min. The organic phase is extracted with ether oxide and washed several times with Na_2CO_3 solution at 10 % and with pure water until neutrality. This phase is dried with Na_2SO_4 and concentrated by rotavapor. The obtained green oil of VBA is distilled under vacuum (p = 1mmHg) and at 65 °C of temperature.

b- Synthesis of N-(m, p)-vinylbenzyliden -3-aminopyridine (Im)

The Schiff base (Im) is prepared according to the following stages (scheme 1): same quantities in mol of 3-aminopyridine and VBA are dissolved in benzene, in the presence of 4-tertiobutylcathecol and paratoluensulfonic acid (PTS) as an antioxidant and a catalyst successively, in a flask equipped with Dean-Stark apparatus. The mixture is heated until reflux at the azeotrope temperature (experimental T = 79,5 °C) in order to eliminate the produced water. After filtration, the organic phase is concentrated by rotavapor. Finaly, the brown oil of Schiff base is collected with 87 % of yield.

Synthesis of copolymers

The obtained monomer (Schiff base) is copolymerized with N,N-dimethylacrylamide at a ratio 10:90 of Im : DMA under nitrogen atmosphere, in Dioxane as solvent, at 65°C during 15 hours and in the presence of two percentages of initiator : 5 % or 5 °/₀₀ of AIBN (2,2-azo-bis-isobuthyronitrile). The obtained copolymers (Cp_a with 5 % of AIBN and Cp_b with 5 °/₀₀ of AIBN) are purified by re-crystallization in the Chloroform/Ether petroleum couple (scheme 2).

Characterization methods

Fourier transform infrared spectroscopy (FTIR): the infrared spectrun of monomer (Im) was registered on SCHIMADZU FTIR-8300 apparatus in NaCl glasses, The FTIR spectra of copolymers were registered on PERKIN-ELMER-197 spectrophotometer on dryed KBr disk.

The proton magnetic resonance (^{1}H NMR) spectrum of monomer was recorded using BRUCKER DRX 400 apparatus in deuterated dimethylsulfoxide $DMSOd_6$ as solvent.
The ^{1}H NMR spectra of copolymers were registered on Brûker AC-300 (300 MHz) at room temperature and in deuteriated Chloroform ($CDCl_3$).
Brüker AC-300 (300 MHz) apparatus was used for the carbon magnetic resonance (^{13}C NMR) analysis, $CDCl_3$ was used as solvent.

The viscosity molecular weights (M_v) of copolymers were obtained by viscosimetry method using an Ubbelhode viscometer at 25 °C and by applying Mark-Houwink law : $[\eta] = KM_v^{\ a}$ where [η] is intrinsic viscosity and the coefficients K and a are respectively $17{,}5.10^{-3}$ (mL/g) and 0,68 for poly (N,N-dimethylacrylamide) as reference polymer backbone in methanol as solvent[29].The experimental values of dynamic viscosities (cp) are 0,76 for Cp_a and 0,80 ± 0,02 for Cp_b and [η] (mL/g) are : 4,14 ± 0,01 for Cpa and 9,35 ± 0,03 respectively.

Thermograms of differential scanning calorimetry (DSC) are registered using a DSC 200 PC apparatus (6 mg of copolymer, 5 °C/min).

Drug dissolution tests:

Release experiments are conducted in closed flask, kept at 37 ± 0,5 °C and at controlled stirring rate of 500 rpm. At t = 0, 100 mg of powder of monomer or copolymer, are soaked in 100 mL of buffered aqueous solution at pH = 1,2 or 8.
The release of 3-aminopyridine is followed by UV spectroscopy using UV-Vis-2401 PC-SHIMADZU apparatus. The drug solutions are analyzed at λ_{max} = 315 nm where ε = 4100 $L.cm^{-1}.mol^{-1}$ in pH = 1,2 and at λ_{max} = 289 nm where ε = 3420 $L.cm^{-1}.mol^{-1}$ in basic solutions (pH=8).

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