Supplementary Information (SI) for Sensors & Diagnostics.

Supporting Information

An origami colorimetric paper-based sensor for sustainable on-site and instrument-free analysis of nitrites

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Figure S1: General design of the NitriPad and schematic procedure for nitrites colorimetric detection on paper.

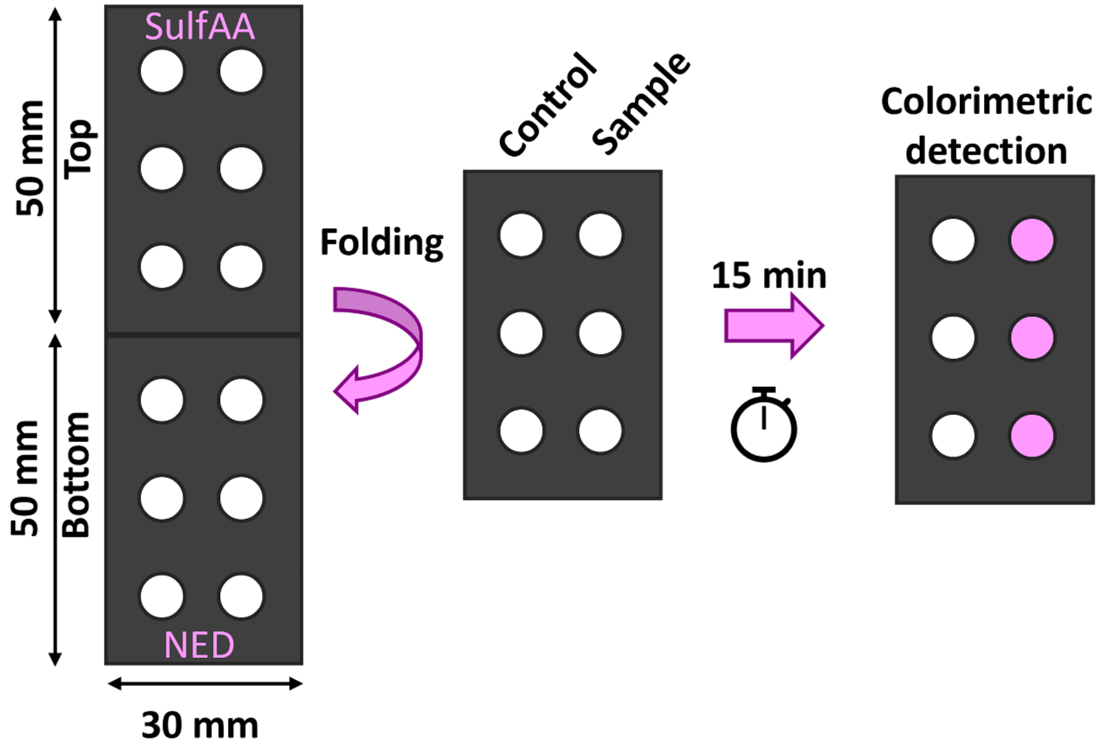


Figure S2: a) Image of $NaNO_2$ calibration curve on paper after 15 minutes of incubation in the dark, at RT and b) graphical elaboration of the colorimetric signals acquired with the OnePlus 6 smartphone

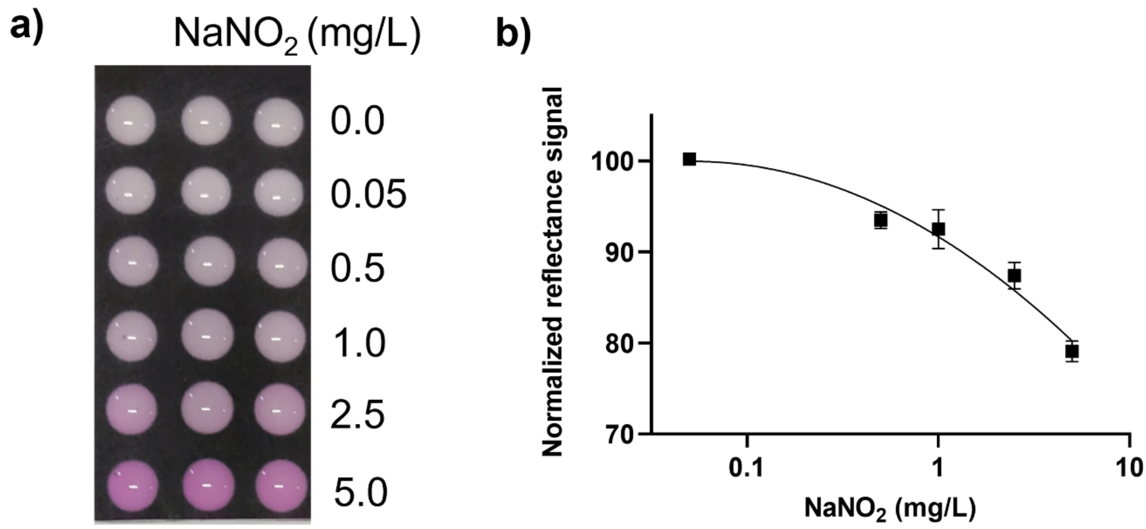


Figure S3: Reflectance signal of four paper sensors in which the mixed solution of SulfAA and NED is adsorbed into each well.

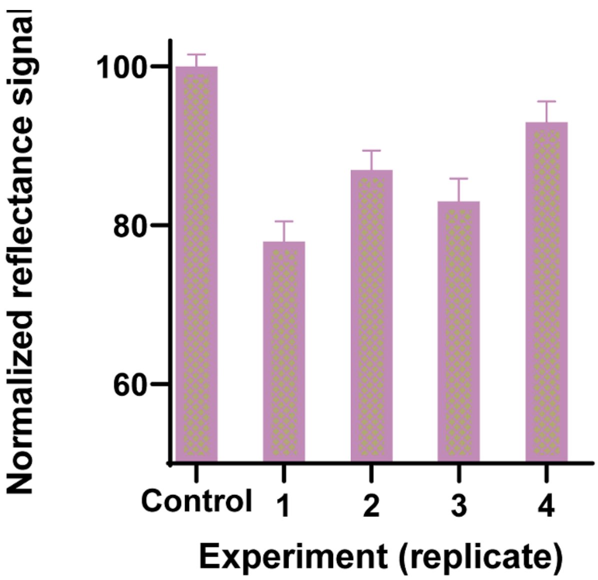
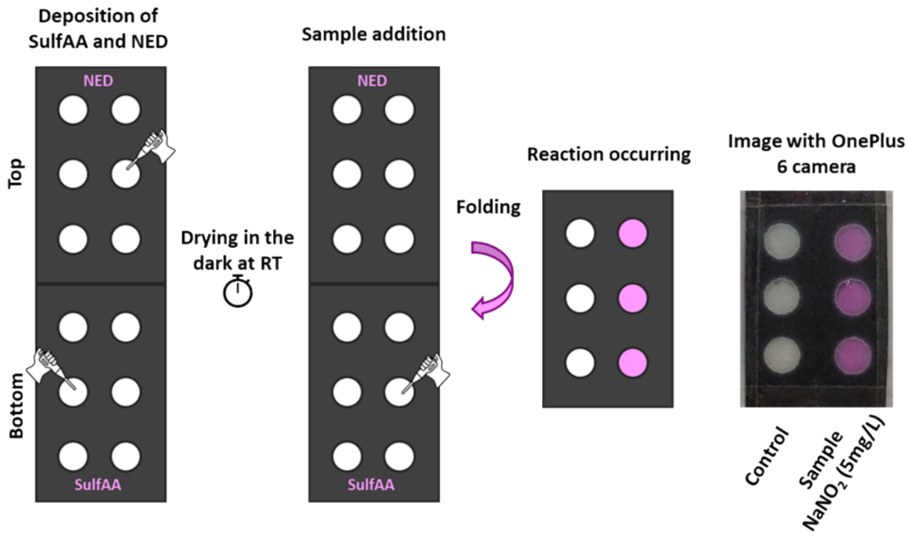
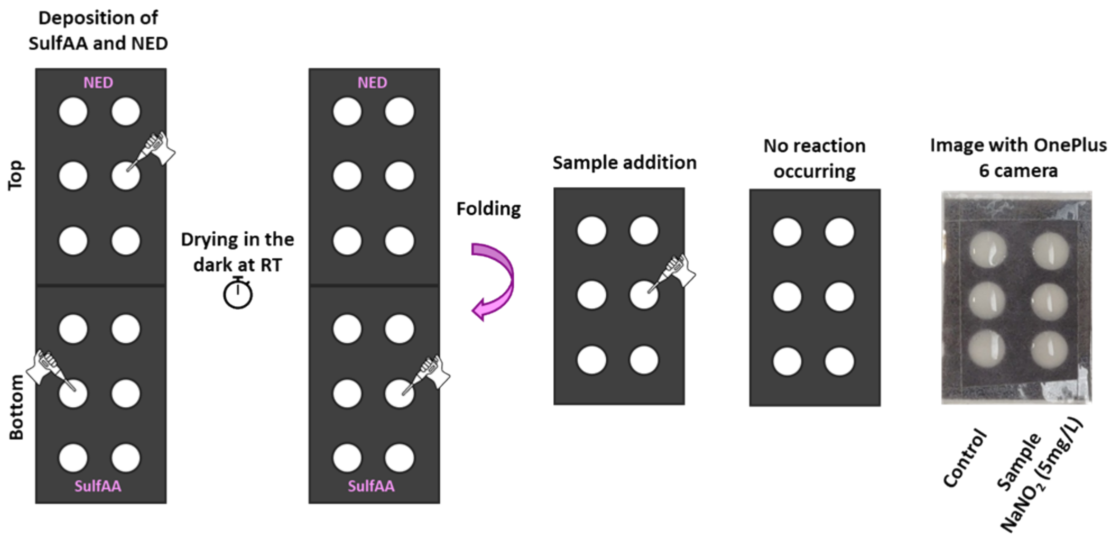


Figure S4: Schematic procedure for the creation of the three configurations A, B, and C, and relative images of the image of PADs acquired with a OnePlus6T smartphone camera after 15 minutes of incubation in the dark, at RT with 5.0 mg/L of $NaNO_2$.

Configuration A



Configuration B



Configuration C

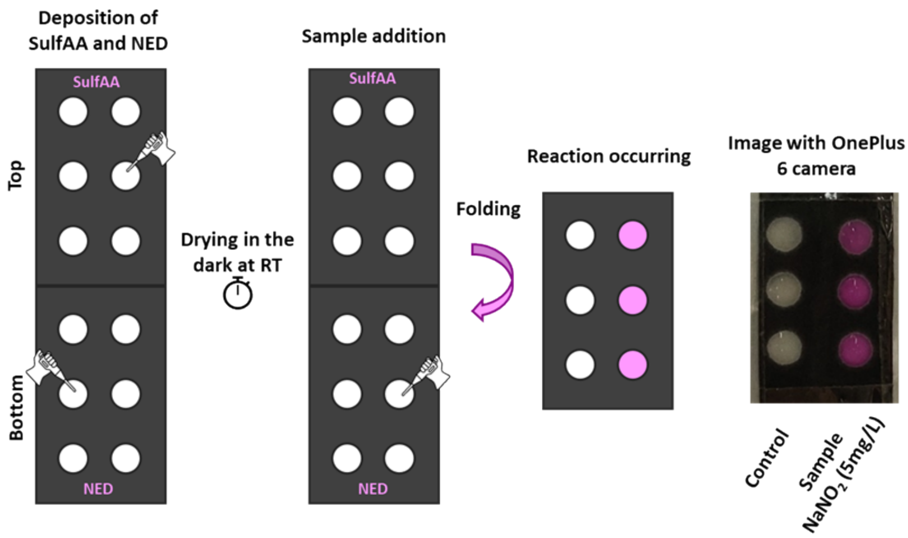


Figure S5: Reflectance signals obtained using the Configuration A and C of the origami colorimetric paper-based sensor and incubation with 5.0 mg/L of $NaNO_2$ for 0, 5, 10, 15, 20 and 30 min.

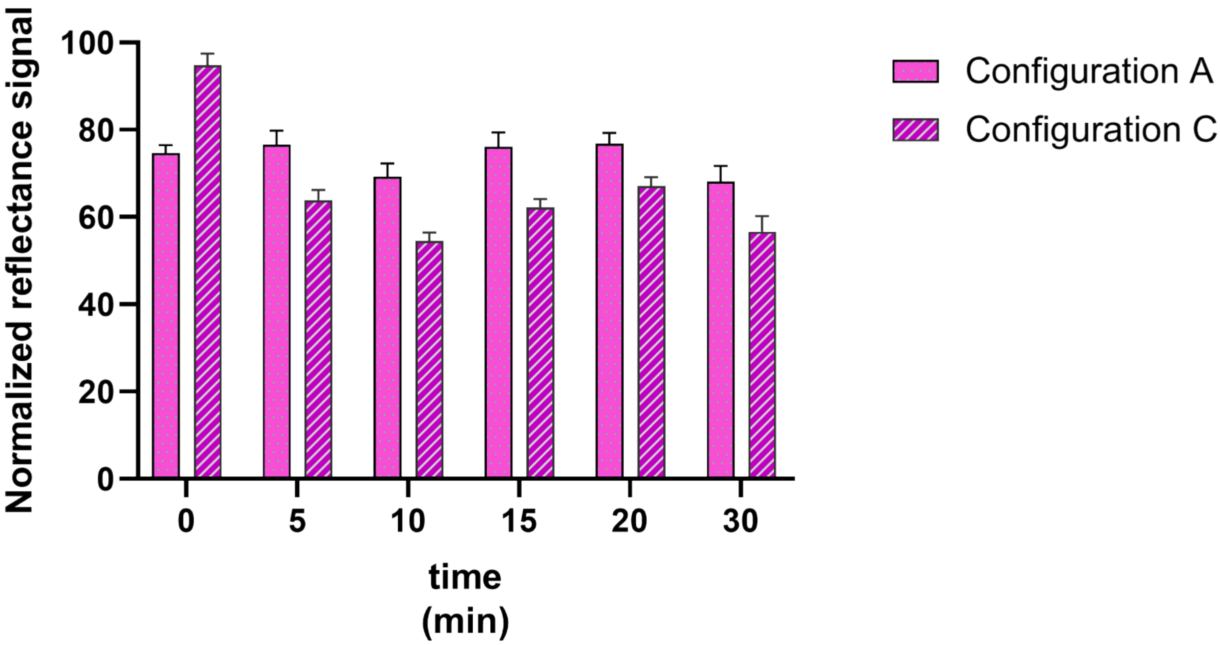


Figure S6: Optimization studies of the volumes of the Griess reagents and analyte (ratio 2:3) and the image of the NitriPad obtained after 15 minutes of incubation with $NaNO_2$ 0.0 and 5.0 mg/L and the normalized reflectance signal obtained after 0, 5, 10, 15, 20, and 30 minutes of incubation time.

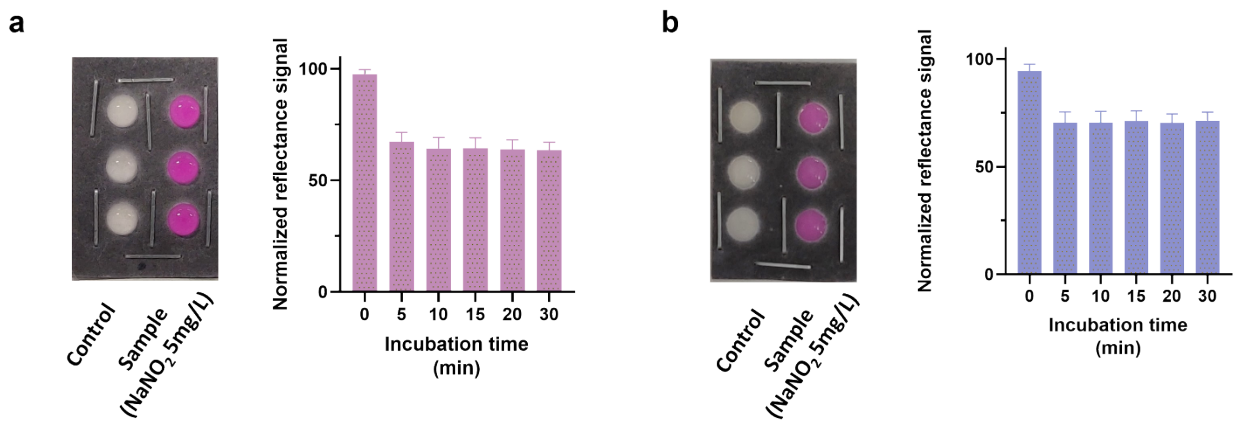
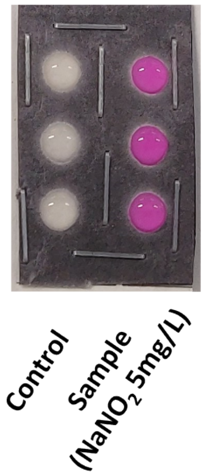


Figure S7: Optimization study of the ratio 1:1 of the Griess reagents:analyte and the image of the NitriPad after 15 minutes of incubation with $NaNO_2$ 0.0 and 5.0 mg/L and the normalized reflectance signal obtained after 0, 5, 10, 15, 20, and 30 minutes of incubation time.



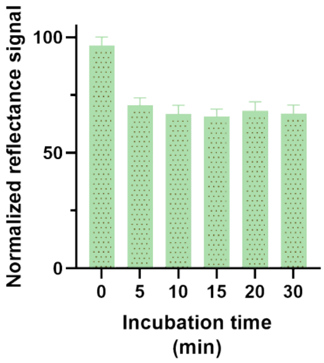


Figure S8: Image of the $NaNO_2$ calibration curve obtained with the NitriPad sensor after 15 min of incubation time.

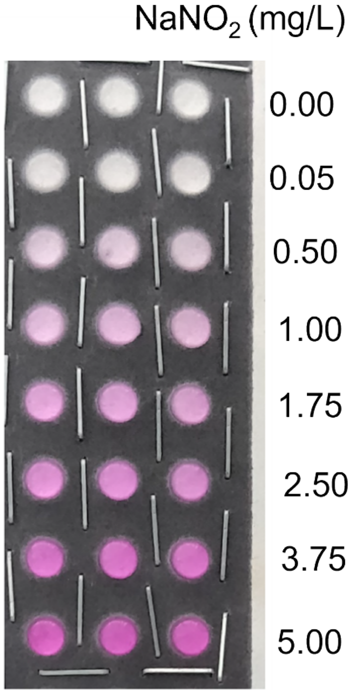


Table S1: Limit of detection (LOD) and limit of quantification (LOQ) obtained with the NitriPad sensor at different incubation times

Incubation time (min)	LOD $NaNO_2$ mg/L	LOQ $NaNO_2$ mg/L
0	/	0.73
5	0.09	0.43
10	0.53	1.82
15	0.40	1.67
20	0.79	3.76
30	0.39	1.22

Table S2. Nitrite concentrations in water sample obtained with the NitriPad and the commercial Nitrite/Nitrate Colorimetric Test (Roche) kit performed in 96-well microtiter plates and benchtop spectrophotometer.

Sample	**Nitrite (mg/L)**	
	NitriPad	**Nitrite/Nitrate Colorimetric Test kit**
Mean	0.60	0.58
Standard deviation	0.03	0.02
Number of measurements (*n*)	10	8

SUSTAINABILITY ASSESSMENT

Red principles

R1: Scope of application

An analytical method should be versatile, adaptable to various applications, and able to detect different types of analytes, and/or the same analyte in different samples. It needs to be robust against potential interferences, and it should be applied over a broad concentration spectrum.

R2: Limit Of Detection (LOD)

A suitable analytical method should have a high sensitivity, enabling the detection of trace amounts of a substance. The limit of detection represents the smallest quantity of analyte that can be reliably distinguished from the background noise. Thus, ensuring a low LOD is essential for the identification of analytes at minimal concentrations, and enhancing the overall reliability and applicability of the method across various sample types.

Table S3: Criteria for the assignment of the Red Principles scores according to Novak et al. for the efficiency of the NitriPad sensor for detecting nitrite ions. We compared our assay to previously published methods for the detection of nitrites, relying on paper-based devices. We reported here only the common parameters reported in all the compared methods.

Score	R1. Scope of Application	R2. LOD
100	sample type ≥ 7	μM ≤ 1
100 -75	7 < sample type ≤ 5	1 < μM ≤ 5
75 - 50	5 < sample type ≤ 3	5 < μM ≤ 10
50 - 25	3 < sample type < 1	10 < μM ≤ 20
25 - 0	≤ 1 sample type	μM ≥ 20

Green Principles

G1: Toxicity of reagents

A crucial aspect of the development of an analytical method is the toxicity of reagents. They must exhibit minimal toxicity, and it is advisable to select biodegradable, renewable, or natural reagents and materials. In this work, the safety data sheets of the reagents were evaluated to assess the safety of the materials that have been used.

G2: Amount of reagents and waste

In terms of sustainability, it is of utmost importance to reduce waste. Thus, the use of reduced volumes is preferable for the preparation of the devices and the use of samples.

G4: Direct impacts

The direct impacts parameter is aimed at studying the impact of the reagents used for the analysis on humans, animals, and the environment.

Table S4: Criteria for the assignment of green principle scores according to Novak et al.[1] for the assessment of sustainability of the NitriPad system. We used pictograms of the *"Globally Harmonized System of Classification and Labelling of Chemicals"*, and mL as a unit of quantification of the volumes used for the analysis.

Score	G1. Toxicity of reagents	G2. Amount of reagents and waste	G4. Direct impact
100	0 < pictograms ≤ 1	0 < ml ≤ 0.5	No hazardous activity
100 -75	1 < pictograms ≤ 2	0.5 < ml ≤ 1	Exceptionally low hazardous activity
75 - 50	2 < pictograms ≤ 5	1 < ml ≤ 2.5	low hazardous activities
50 - 25	5 < pictograms < 10	2.5 < ml ≤ 5	medium hazardous activities
25 - 0	≥ 10 pictograms	> 5 ml	Dangerous activities

Blue principles

B1: Cost-efficiency

The total analysis cost is an important parameter for the economic assessment of an analytical method. The aim is to minimize the cost of the analysis as much as possible by evaluating the cost of all materials, equipment, and reagents. Also, the qualification of the personnel is to be taken into account for these considerations.

B2: Time-efficiency

A desirable result is a short-time analysis, together with a reduction in the time of preparation of the experimental set-up and the reaction time. These parameters were taken into account for the evaluation of the time efficiency of the methods.

B3: Requirements

Requirements for the economic assessment of the method include the reduction of sample volumes, equipment, and trained personnel. We evaluated the detection systems, image and statistical analysis software used for each assay, and the need for any laboratory equipment.

B4: Operational simplicity

Ideally, an analytical method should be fully automated, miniaturized, and portable, enabling on-site usage for any user.

Table S5: Criteria for assigning the blue principles scores according to Nowak et al. for the economic assessment of the NitriPad system.

Score	B1 Cost Efficiency	B2 Time efficiency	B3. Requirements		B4. Operational Simplicity		
			B3.1 Sample consumption	B3.2 Other needs	B4.1 Portability	B4.2 Integration automation	B4.3 Miniaturization
100	Very low-cost	1 < min ≤ 5	0 < ml ≤ 0.05	Simple Accessories & tools (mobile, pipette, dark box, etc.)	Online analysis	High automation or simple, online data	Handheld
100-75	Low-cost	5 < min ≤ 15	0.05 < ml ≤ 0.1	Simple Equipment (glassware, dark box, basic equipment for manual preparation, mobile/camera/ccd, image processing, statistical software)	At line analysis, camp laboratory	High automation or simple	Portable
75-50	Medium cost	15 < min ≤ 30	0.1 < ml ≤ 1	Medium complexity instrumentation (scanner), statistical analysis, equipment for basic preparation	In lab analysis	Partially automation	Transportable
50-25	Expensive	30 < min ≤ 60	1 < ml ≤ 5	Complex instrumentation, equipment for preparation (spectrophotometer, statistical analysis, Refrigerator)	In lab analysis with sample storing, batch working, results obtained the same day of analysis	No automation	Bench
25 -	Extremely	≥ 60 min	> 5 ml	High complexity instrumentation	In lab analysis	No automatio	Complex

0	expensiv e			(luminometer, Statistical analysis software, other complex equipment	with sample storing, planned batch working, results obtain, days after the analysis	n	facilities

Table S6: Results from whiteness evaluation for our method and five NitriPad sensors for the detection of nitrites. The average values in percentage for the Red, Green, and Blue parameters of each method are reported.

Method number	R (%)	G (%)	B (%)	Whiteness (%)	Reference
1	47.5	73.8	93.8	71.7	This work
2	33.8	54.2	56.9	48.3	2
3	37.5	66.7	78.8	61.0	3
4	36.3	65.4	68.8	56.8	4
5	43.8	25.0	54.7	41.1	5
6	50.0	65.4	85.0	66.8	6

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